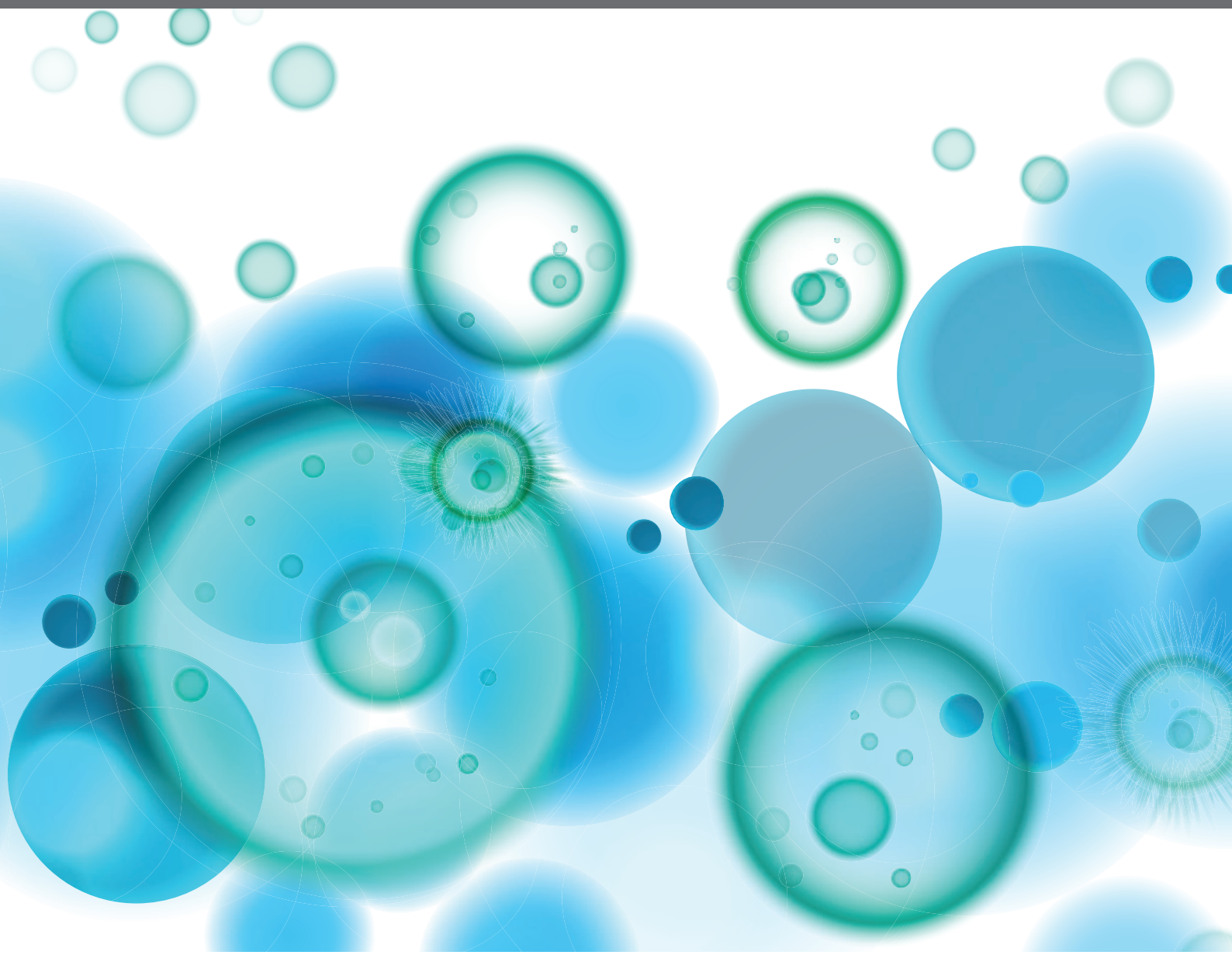
EBV INFECTION AND HUMAN PRIMARY IMMUNE DEFICIENCIES

EDITED BY: Isabelle Meyts and Jeffrey I. Cohen
PUBLISHED IN: Frontiers in Immunology



frontiers Research Topics

frontiers

ISSN 1664-8714
ISBN 978-2-88963-571-9
DOI 10.3389/978-2-88963-571-9

About Frontiers

Frontiers is more than just an open-access publisher of scholarly articles: it is a pioneering approach to the world of academia, radically improving the way scholarly research is managed. The grand vision of Frontiers is a world where all people have an equal opportunity to seek, share and generate knowledge. Frontiers provides immediate and permanent online open access to all its publications, but this alone is not enough to realize our grand goals.

Frontiers Journal Series

The Frontiers Journal Series is a multi-tier and interdisciplinary set of open-access, online journals, promising a paradigm shift from the current review, selection and dissemination processes in academic publishing. All Frontiers journals are driven by researchers for researchers; therefore, they constitute a service to the scholarly community. At the same time, the Frontiers Journal Series operates on a revolutionary invention, the tiered publishing system, initially addressing specific communities of scholars, and gradually climbing up to broader public understanding, thus serving the interests of the lay society, too.

Dedication to Quality

Each Frontiers article is a landmark of the highest quality, thanks to genuinely collaborative interactions between authors and review editors, who include some of the world's best academicians. Research must be certified by peers before entering a stream of knowledge that may eventually reach the public - and shape society; therefore, Frontiers only applies the most rigorous and unbiased reviews.
Frontiers revolutionizes research publishing by freely delivering the most outstanding research, evaluated with no bias from both the academic and social point of view. By applying the most advanced information technologies, Frontiers is catapulting scholarly publishing into a new generation.

What are Frontiers Research Topics?

Frontiers Research Topics are very popular trademarks of the Frontiers Journals Series: they are collections of at least ten articles, all centered on a particular subject. With their unique mix of varied contributions from Original Research to Review Articles, Frontiers Research Topics unify the most influential researchers, the latest key findings and historical advances in a hot research area! Find out more on how to host your own Frontiers Research Topic or contribute to one as an author by contacting the Frontiers Editorial Office: researchtopics@frontiersin.org

EBV INFECTION AND HUMAN PRIMARY IMMUNE DEFICIENCIES

Topic Editors:
Isabelle Meyts, KU Leuven, Belgium
Jeffrey I. Cohen, National Institutes of Health (NIH), United States

Citation: Meyts, I., Cohen, J. I., eds. (2020). EBV Infection and Human Primary Immune Deficiencies. Lausanne: Frontiers Media SA. doi: 10.3389/978-2-88963-571-9

Table of Contents

frontiers in Immunology

EDITORIAL
published: 07 February 2020
doi: 10.3389/fimmu.2020.00130

Editorial: EBV Infection and Human Primary Immune Deficiencies

Jeffrey I. Cohen[1]* *and Isabelle Meyts*[2]

[1] Laboratory of Infectious Diseases, National Institute of Allergy and Infectious Diseases, National Institutes of Health, Bethesda, MD, United States, [2] Department of Microbiology and Immunology, University Hospitals Leuven, Leuven, Belgium

Keywords: Epstein-Barr virus, immune deficiency, lymphoproliferative disease, hemophagocytic lymphohistiocytosis, B-cell lymphoma

Editorial on the Research Topic

EBV Infection and Human Primary Immune Deficiencies

Epstein-Barr virus (EBV) is a human herpes virus that infects nearly 95% of individuals worldwide and most persons are unaware that they are infected and never have disease associated with the infection. If infection is delayed until adolescence or early adulthood, most of these persons will develop infectious mononucleosis. Persons with certain congenital immunodeficiencies [e.g., X-linked lymphoproliferative disease 1 (XLP1)], acquired immunodeficiencies (e.g., AIDS), or iatrogenic immunodeficiencies (e.g., organ transplant recipients) can develop severe or even fatal EBV disease associated with primary infection or reactivation of the virus. These diseases include EBV B cell or T cell lymphoma, lymphoproliferative disease (LPD), hemophagocytic lymphohistiocytosis (HLH), or EBV smooth muscle tumors. Genetic disorders associated with severe EBV disease include those that predispose to infections with multiple viruses, bacteria, or fungi (e.g., GATA2 deficiency) or infection primarily associated with EBV alone (XLP1). These disorders primarily affect the function of T cells and NK cells which are important for immune surveillance against EBV-infected cells, rather than B cells that the virus infects, establishes latency in, and can drive to LPD. Identification of genetic disorders associated with EBV has furthered our knowledge of the role of the functions of cellular proteins important for signaling and effector activity of T cells and NK cells.

The collection of articles on EBV Infectious and Human Primary Immune Deficiencies begins with an overview of T cell responses to the virus by Long et al. This review emphasizes the importance of T cell responses to EBV during symptomatic and asymptomatic primary infection and during persistent infection. The authors also describe the contributions of tissue resident memory T cells, γδ T cells, and NKT cells for control of EBV infection. Latour and Winter provide an overview of immune deficiencies that predispose to EBV LPD. These include mutations in proteins that impair T cell proliferation, B cell-T cell interactions, and T cell and NK-cell cytotoxicity. Additional articles in this collection focus on specific immune deficiencies associated with severe EBV disease. Ghosh et al. report on IL-2 inducible kinase (ITK) deficiency which is critical for T cell signaling. Patients with defects in ITK can present with EBV-positive Hodgkin and non-Hodgkin lymphoma, LPD, and HLH. Panchal et al. review findings in patients with XLP1 who have loss-of-function mutations in SAP that present with B cell lymphoma, HLH, and/or dysgammaglobulinemia. SAP is an adapter protein important for activation of SLAM family members and signaling in T and NK cells. Patients with mutations in SAP have impaired T and NK cell function. Arjunaraja et al. report on B cell expansion with NF-κB and T cell anergy (BENTA) disease which is associated with gain-of-function mutations in CARD11. These patients have B cell lymphocytosis, reduced numbers of T and NK cells, low grade persistent EBV viremia, and constitutive activation of NF-κB. Caorsi et al. describe a patient with CD70 deficiency who presented

OPEN ACCESS

Edited by:
Andrew R. Gennery, Newcastle University, United Kingdom

Reviewed by:
Claire Booth, UCL Great Ormond Street Institute of Child Health, United Kingdom
Tomohiro Morio, Tokyo Medical and Dental University, Japan

***Correspondence:**
Jeffrey I. Cohen
jcohen@niaid.nih.gov

Specialty section:
This article was submitted to Primary Immunodeficiencies, a section of the journal Frontiers in Immunology

Received: *18 December 2019*
Accepted: *17 January 2020*
Published: *07 February 2020*

Citation:
Cohen JI and Meyts I (2020) Editorial: EBV Infection and Human Primary Immune Deficiencies. Front. Immunol. 11:130. doi: 10.3389/fimmu.2020.00130

with periodic fever, tonsillitis, cervical lymphadenitis, and EBV viremia. CD70 is expressed on antigen presenting cells (including B cells) and is the ligand for CD27 which is expressed on T cells; this interaction is important for cytotoxic T cell activation. Hoeger et al. report that nuclear factor kappa-light-chain-enhancer of activated B cells 1 (NF-κB1) haploinsufficiency is associated with common variable immunodeficiency-like B cell disease, recurrent pulmonary infections, and EBV LPD. NF-κB1 is important for NF-κB signaling in both cytotoxic T cells and in B cells. Carpier and Lucas review activated PI3Kδ syndrome (APDS) which is due to gain-of-function mutations in *PI3K3CD* or *PIK3R* or loss-of-function mutations in *PTEN*. These mutations result in constitutive activation of PI3K with senescent CD8 T cells and increased numbers of terminal effector CD8 T cells. Patients present with frequent sinopulmonary infections, EBV viremia, LPD, and lymphoma, as well as cytomegalovirus viremia and lymphadenitis. Kimura and Cohen describe chronic active EBV disease in which patients have high levels of EBV in circulating T or NK cells (or less commonly in B cells) which infiltrate the tissues and often result in EBV lymphoma or HLH. Some of these patients have somatic mutations in their EBV-positive T or NK cells, usually associated with driver mutations in genes such as *DDX3X* and *BCOR*.

Patients with genetic disorders associated with severe EBV can develop HLH. Marsh reports that patients with HLH present with fever, splenomegaly, reduced numbers of erythrocytes, leukocytes, or platelets, and often hepatitis. Cytotoxic T cells or NK cells from patients with HLH have impaired degranulation or cytotoxicity, and persistent hyperinflammation is present. HLH with severe EBV disease has been associated with mutations in *SH2D1A*, *BIRC4*, *CD27*, *ITK*, and *MAGT1*. While EBV-positive smooth muscle tumors were initially reported in solid organ transplant recipients or patients with AIDS, Magg et al. report that these tumors have been reported in immune deficiencies associated with EBV, including GATA2 or CARMIL2 deficiency, ataxia telangiectasia, and severe combined immune deficiency associated with mutations in *ADA*, *ZAP70*, or *IL2RG*. While hematopoietic stem cell transplantation (HSCT) has been used to correct many EBV-associated genetic disorders, many of these patients have severe viral infections prior to transplant which increases the morbidity associated with HSCT, and some may have relapses of EBV disease after HSCT. McLaughlin et al. report that the use of EBV-specific cytotoxic T cells either before HSCT to gain better control of infections, or after transplant to treat persistent EBV disease, has been effective. EBV-specific T cells derived from the HSCT donor or third-party HLA-matched cells have been effective.

The articles in this collection describe many of the genetic disorders associated with EBV; new disorders continue to be discovered. These diseases continue to inform us about the importance of interactions between T or NK cells and EBV-infected B cells and how the only human virus that establishes latency in B cells and induces B cell lymphoproliferation is controlled by our immune system. Better understanding of the role of individual T and NK cell proteins in controlling EBV may lead to improved immunologic-based treatments for both EBV disease as well as for cancer. In addition, identification of key proteins important for T cell and NK cell function could lead to novel targets for immune suppressive medications.

AUTHOR CONTRIBUTIONS

All authors listed have made a substantial, direct and intellectual contribution to the work, and approved it for publication.

FUNDING

This work was supported by the Intramural Research Program of the National Institute of Allergy and Infectious Diseases.

Conflict of Interest: The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

frontiers in Immunology

MINI REVIEW
published: 24 November 2017
doi: 10.3389/fimmu.2017.01658

Epstein–Barr Virus-Specific Immune Control by Innate Lymphocytes

*Christian Münz**

Viral Immunobiology, Institute of Experimental Immunology, University of Zürich, Zürich, Switzerland

Epstein–Barr virus (EBV) is a potent B cell transforming pathogen in humans. In most persistently EBV-infected individuals, potent cytotoxic lymphocyte responses prevent EBV-associated pathologies. In addition to comprehensive adaptive T cell responses, several innate lymphocyte populations seem to target different stages of EBV infection and are compromised in primary immunodeficiencies that render individuals susceptible to symptomatic EBV infection. In this mini-review, I will highlight the functions of natural killer, γδ T cells, and natural killer T cells during innate immune responses to EBV. These innate lymphocyte populations seem to restrict both lytic replication and transforming latent EBV antigen expression. The mechanisms underlying the recognition of these different EBV infection programs by the respective innate lymphocytes are just starting to become unraveled, but will provide immunotherapeutic strategies to target pathologies that are associated with the different EBV infection programs.

Keywords: natural killer cells, natural killer T cells, Vγ9Vδ2 T cells, lytic replication, infectious mononucleosis, NKG2D, CD27/CD70

OPEN ACCESS

Edited by:
Jeffrey I. Cohen, National Institutes of Health (NIH), United States

Reviewed by:
Silvia Clara Gilliani, University of Brescia, Italy
Tri Giang Phan, Garvan Institute of Medical Research, Australia

***Correspondence:**
Christian Münz
christian.muenz@uzh.ch

Specialty section:
This article was submitted to Primary Immunodeficiencies, a section of the journal Frontiers in Immunology

Received: *28 August 2017*
Accepted: *13 November 2017*
Published: *24 November 2017*

Citation:
Münz C (2017) Epstein–Barr Virus-Specific Immune Control by Innate Lymphocytes. Front. Immunol. 8:1658. doi: 10.3389/fimmu.2017.01658

INTRODUCTION ON INNATE LYMPHOCYTES

Epstein–Barr virus (EBV) is a common human γ-herpesvirus that persistently infects more than 90% of the human adult population. At the same time, it was the first human candidate tumor virus that was discovered (1, 2) and remains to date the only human pathogen that can readily transform human B cells into immortal continuously growing lymphoblastoid cell lines (LCLs) (3). Even so EBV contributes with 1–2% to the overall tumor burden in humans (4), the majority of infected individuals carry EBV for life without symptoms. This peaceful coexistence is thought to be maintained by cytotoxic lymphocytes, which massively expand during symptomatic primary EBV infection, called infectious mononucleosis (IM), can be used to treat some EBV-associated malignancies and are affected by primary or secondary immunodeficiencies that predispose for EBV-driven pathologies, such as human immunodeficiency virus-associated lymphomas (5–7).

Among these cytotoxic lymphocytes, adaptive $CD8^+$ T cell responses to EBV have been best characterized and single peptide epitope specificities against early lytic EBV antigens constitute in some individuals up to 40% of the massively expanded $CD8^+$ T cell compartment during IM (8). Much less is known about innate cytotoxic lymphocyte compartments during EBV infection, including natural killer (NK), natural killer T (NKT), and γδ T cells. Nevertheless, they can utilize the same eomesodermin-dependent perforin, granzymes, and death receptor ligands to eliminate EBV-infected cells by cytotoxicity (9). Furthermore, they exist at much higher frequencies than individual $CD8^+$ T cell clones at sites of primary EBV infection, like tonsils (more than 10^{10} more frequent), and therefore can more rapidly respond to pathogen encounter, ensuring the survival of the infected individual until specific T cells have been clonally expanded. However, their target

cell recognition is not directed against EBV protein-derived peptides presented on major histocompatibility complex (MHC) molecules, but instead they recognize infected targets with their germ line encoded receptors or invariant T cell receptors. Activation of innate lymphocytes depend on loss of MHC class I molecules from the surface, stress induced ligand upregulation, glycolipid presentation on non-classical MHC class I molecules, or mevalonate metabolite recognition in the context of butyrophilin (BTN) family members (10–14). As I will discuss below, these different target recognition mechanisms seem to be used to target different stages of EBV infection, thereby achieving a similarly comprehensive immune control over all EBV infection programs as T cells that target antigens of the different EBV life cycles. Thereby, primary immunodeficiencies that affect NK, NKT, or γδ T cells might manifest with different EBV-associated pathologies. A better understanding of which EBV pathology might be targeted by which innate lymphocyte compartment might enable us to utilize these innate cytotoxic lymphocytes in addition to classical T cells for respective immunotherapies.

NK CELLS IN THE PREVENTION OF SYMPTOMATIC PRIMARY EBV INFECTION

Natural killer cells are the preeminent cytotoxic innate lymphocytes, which have been originally described for their spontaneous cytotoxicity against infected and tumor targets (15–17). In particular, deficiencies in NK cells predispose in humans for herpesvirus-driven pathologies (18). It was indeed described early on that NK cells also expand during IM (19–22). IM symptoms are thought to be caused by the associated lymphocytosis of $CD8^+$ T cells, which primarily recognize lytic EBV antigens that are expressed during infectious virus production (23). Indeed, NK cells also preferentially recognize lytically EBV-replicating cells (22, 24, 25). Depletion of NK cells in mice with reconstituted human immune system components (HIS mice) increases viral loads and $CD8^+$ T cell lymphocytosis only for wild-type (wt), but not lytic EBV replication incompetent BZLF1-deficient EBV (25). The respective NK cell-depleted and wt EBV-infected HIS mice also develop more EBV-associated B cell lymphomas and need to be sacrificed due to weight loss 6 weeks after infection (25). HIS mice share an early differentiated NK cell compartment with newborns and young children (26). The respective $NKG2A^+$killer immunoglobulin-like receptor $(KIR)^-$ NK cells preferentially expand during IM and recognize lytically EBV-replicating cells (21, 22). Interestingly, these early differentiated NK cells are continuously lost during the first decade of life and get successively replaced by KIR^+ NK cell accumulation (22, 27). This coincides with an increased risk to develop IM when primary infection is delayed into adolescence (5). Recognition of lytic EBV replication might be mediated by the downregulation of MHC class I molecules and upregulation of NKG2D and DNAM-1 ligands on lytically EBV-replicating B cells (24, 28), tilting the balance of inhibitory, and activating NK cell receptor signaling toward activation. In contrast, EBV transformed B cells with the expression of all latent EBV antigens (LCLs) are only efficiently recognized by NK cells in the allogeneic MHC class I mismatched setting. This allows the recruitment of KIR^+ NK cells to the response and can be harnessed in mixed MHC class I mismatched human immune system reconstitution from two hematopoietic progenitor cell donors in HIS mice (29). Although NK cells in these mixed reconstituted HIS mice have a decreased cytotoxicity against MHC class I negative target cells and are therefore less licensed, they control EBV infection better by NK cells (29). This results presumably from NK cell recognition of the MHC class I mismatched EBV-infected B cells, recruiting KIR^+ NK cells to the innate immune response to EBV. Such allorecognition is currently being harnessed for NK cell-dependent immunotherapies of acute myeloid leukemias (30), but could also be harnessed against persistent infections that reactivate during bone marrow transplantation and home to the hematopoietic lineage. Thus, NK cells preferentially target lytic EBV replication, but might be therapeutically beneficial to target also other stages of EBV infection in the allogeneic setting.

γδ T CELLS AND THEIR RESTRICTION OF EBV LATENCY

Natural killer cells are by far not the only cytotoxic innate lymphocytes that react to EBV infection. In a subset of EBV-positive children (25–50%), Vγ9Vδ2 T cells are also expanded (31). These human T cells with an invariant γδ T cell receptor do not exist in mice and recognize pyrophosphate-containing molecules that are generated in the mevalonate metabolism (32). Interestingly, Vγ9Vδ2 T cell recognition of these phosphoantigens (pAgs) depends on the BTN 3A1 molecule (CD277), but how BTN3A1 supports pAg recognition, remains unclear (32). In addition, γδ T cells can utilize the NK cell receptor NKG2D for target cell recognition (32), which has previously been described to be important in lytic EBV replication recognition by NK cells (24, 28). Interestingly, these Vγ9Vδ2 T cells seem to preferentially recognize EBV-infected B cell lines that express the nuclear antigen 1 of EBV (EBNA1) as the sole viral protein, so-called EBV latency I (31). This latency I is found in Burkitt's lymphoma (BL), the most common childhood tumor in Sub-Saharan Africa and homeostatically proliferating EBV-infected memory B cells (33). Interestingly, such non-transformed EBV-infected memory B cells are thought to be the reservoir of EBV persistence (34), accumulate in the peripheral blood of IM patients (35), and might drive Vγ9Vδ2 T cell expansion in children, which sometimes have viral loads as high as IM patients (36). Indeed, BTN3A1 and NKG2D are required to expand Vγ9Vδ2 T cells with BL cell lines in donors who are susceptible for this expansion (31). Similarly, pAg stimulation of Vγ9Vδ2 T cells in HIS mice was able to prevent outgrowth of adoptively transferred EBV transformed LCLs *in vivo* (37). These activated Vγ9Vδ2 T cells also required their invariant T cell receptor and NKG2D for LCL recognition. In this study, Vγ9Vδ2 T cells seem to eliminate EBV transformed LCLs primarily by FasL- and TRAIL-mediated programmed cell death induction. Moreover, adoptive transfer of Vγ9Vδ2 T cells into HIS mice, in which EBV-associated lymphoma formation was induced by EBV infection, prevented tumorigenesis (38). Even 3 weeks after infection, adoptive transfer of activated Vγ9Vδ2 T cells was still able to reduce tumor burden substantially. These

data suggest that Vγ9Vδ2 T cells preferentially expand to EBV latency I-infected B cells, but, once activated, can also target other EBV latencies, including latency III carrying EBV transformed LCLs. However, it remains unclear why this Vγ9Vδ2 T cell expansion can only be achieved in some donors and how pAg presentation or mevalonate metabolism is regulated during the different EBV latency programs. Nevertheless, Vγ9Vδ2 T cells seem to complement NK cells by recognizing latent EBV infection, while the latter innate lymphocyte subset preferentially controls lytic EBV replication. A combination of both cytotoxic innate lymphocyte subsets could be beneficial to target EBV infection.

NKT CELL-MEDIATED IMMUNE CONTROL OF EBV-DRIVEN B CELL TRANSFORMATION

Similar to our lack of understanding of how EBV regulates the mevalonate metabolism for Vγ9Vδ2 T cell recognition, also NKT cell recognition of EBV-infected B and epithelial cells is poorly understood, even so cytotoxicity of $CD8^+$ NKT cells against EBV latency II Hodgkin lymphoma (HL) and nasopharyngeal carcinoma (NPC) cells was previously reported (39). NKT cells carry the invariant Vα24-Jα18/Vβ11 T cell receptor and recognize glycolipids that are presented on the non-classical MHC class I molecule CD1d (11). CD1d has been reported to be downregulated on fully EBV transformed LCLs (40). Nevertheless, EBV infection of primary human B cells and LCL outgrowth can be restricted by NKT cells, and restoring CD1d expression on LCLs allows NKT cells to recognize EBV latency III (40). These data suggest that during B cell infection and transformation CD1d ligands are produced and presented on CD1d that allow for NKT cell recognition. Therefore, NKT cells can also restrict EBV-induced tumorigenesis *in vivo* (39). In particular, $CD8^+$ NKT cells can directly lyse EBV positive HL and NPC cells and produce IFN-γ, which augments protective Th1 responses against EBV infection (39). $CD4^+$ NKT cells, which mainly produce IL-4 and bias immune responses toward Th2 polarization, do not seem to be able to control EBV on their own, but synergize with $CD8^+$ NKT cells for improved immune control (39). While NKT cells are reduced in the peripheral blood of HL patients (39), they seem to be enriched in the tumor tissue (41). The HL and NPC associated EBV latency II with expression of three EBV latent antigens, namely EBNA1 and the two latent membrane proteins 1 and 2 (LMP1 and 2), can also be found in germinal center (GC) B cells of healthy EBV carriers (42). Therefore, NKT cells might play a role in restricting EBV latency II in GC B cells and epithelial cells. The latter might, however, only occur during NPC tumorigenesis, because EBV seems to mainly induce lytic replication in epithelial cells of healthy EBV carriers (43).

PRIMARY IMMUNODEFICIENCIES THAT COMPROMISE EBV-SPECIFIC IMMUNE CONTROL

The above discussed studies seem to indicate that several human innate lymphocyte subsets target different stages of EBV infection with NK cells recognizing lytic replication, Vγ9Vδ2 T cells reacting to EBV latency I and maybe III, and NKT cells providing restriction of EBV latency II. Can further evidence for this differential targeting of EBV by innate lymphocytes be gleaned from primary immunodeficiencies that predispose for EBV-associated pathologies (7, 44) and compromise these innate lymphocyte compartments?

The selective loss of NK, NKT, or γδ T cells is rare in primary immunodeficiencies. Usually, the respective mutations affect multiple immune compartments like the GATA2 mutation that was later characterized in the original patient with susceptibility to herpesvirus infections and decreased NK cell activity (18, 45). This mutation results in low numbers of B, $CD4^+$ T, NK, dendritic, and monocytic cells. The associated uncontrolled EBV infection manifests in fulminant IM, hemophagocytic lymphohistiocytosis (HLH), and chronic active EBV (CAEBV). Similarly, mutations in the cytotoxic machinery (perforin, Munc13-4, and Munc18-2) that predispose for HLH and CAEBV affect all cytotoxic lymphocytes (46–48). Furthermore, the mutations in SLAM-associated protein (SAP) and X-linked inhibitor of apoptosis that result in X-linked lymphoproliferative diseases (XLP) 1 and 2 affect many lymphocytes and also result in fulminant IM and HLH (49–53), even so also NKT cell development is compromised in XLP1 patients (54, 55). Therefore, overall loss of cytotoxic lymphocyte control of EBV infection seems to result in uncontrolled IM, CAEBV, and HLH. However, other primary immunodeficiencies seem to be more selective, both with respect to clinical manifestation and loss of cytotoxic lymphocytes. In this regard, patients with mutations in IL-2 inducible T cell kinase (ITK) lack all NKT cells and present sometimes with HL (56–63). Similarly, CD70 deficiency predisposes for HL (64, 65), but so far only the deficiency of $CD8^+$ T cells in recognizing EBV transformed B cells has been characterized. While in four of the five patients with CD70 deficiency no information about NKT cell numbers were given (64), in one patient NKT cell numbers were at least fivefold decreased. Thus, it is tempting to speculate that primary immunodeficiences, resulting from ITK and CD70 mutations, more prominently predispose for loss of NKT cell-mediated innate immune control and thereby favor uncontrolled EBV latency II, as in HL.

Even so CD70 is so far the only known ligand of CD27, CD27 mutations predispose for a much larger spectrum of EBV-associated pathologies, including HLH and different EBV-associated lymphomas, and also increase the mortality of affected individuals (66–68). It has been speculated that this results from ligand-independent signaling events of CD27 that are compromised in addition to T and NK cell recognition of LCLs (44). In addition to CD27, mutations in the magnesium transporter MagT1 and the transcription factor NFκB1 compromise NK cell recognition and predispose for EBV-induced lymphoproliferations and lymphomas (28, 69–74). These have been suggested to compromise NKG2D, TNF receptor (e.g., CD27), and SLAM receptor family (SAP dependent) signaling (28, 73). These receptors are crucial costimulatory molecules and activating receptors on $CD8^+$ T and NK cells, respectively. More selective NK cell deficiencies have been reported for mutations in the minichromosome maintenance complex component

4 (MCM4) and the Fcγ receptor 3A (CD16) (75–78). Both types of mutations diminish or functionally impair the $CD56^{dim}CD16^{+}$ NK cell compartment, which contributes to the early differentiated $NKG2A^{+}KIR^{-}$ NK cells that were found to restrict lytic EBV replication (22, 25). In addition, they could mediate further restriction of lytic EBV replication by CD16-mediated antibody-dependent cellular cytotoxicity against late lytic viral glycoproteins. Patients with MCM4 and CD16 mutations present with EBV-induced lymphoproliferative diseases, including EBV-positive Castleman's disease in the case of CD16 mutations. These selective NK cell deficiencies could point toward ill controlled lytic EBV infection that stimulates these lymphoproliferations.

In contrast to NKT and NK cells, Vγ9Vδ2 T cells have not received much attention in the characterization of primary immunodeficiencies that predispose for EBV pathologies. However, from the above described pathways that are affected by these, several are predicted to affect also Vγ9Vδ2 T cell function. Downstream of the TCR signaling, which in the case of Vγ9Vδ2 T cells engages pAgs in the context of BTN3A1, ITK phosphorylates PLCγ1, which elicits Ca^{2+} flux and phosphatidylinositol-4,5-bisphosphate cleavage to release diacylglycerol that in turn activates the guanine nucleotide exchange factor RasGRP1, whose mutations also predispose for EBV-associated B cell lymphomas (79). PLCγ1 activation is also Mg^{2+} dependent and thereby influenced by MagT1 function. Thus, mutations in ITK, MagT1, and RasGRP1 affect T cell receptor signaling and predispose for EBV-associated pathologies. Furthermore, NKG2D is a prominent coreceptor on Vγ9Vδ2 T cells and elicits NFκB1-dependent gene transcription (31). NKG2D and NFκB1 are affected by primary immunodeficiencies with EBV pathologies that result from mutations in the magnesium transporter MagT1 and the transcription factor NFκB1, respectively (28, 73). Finally, cytotoxicity of Vγ9Vδ2 T cells is also affected by the perforin, Munc13-4, and Munc18-2 mutations. These considerations suggest that T cell receptor signaling, costimulation, and effector functions of Vγ9Vδ2 T cells are compromised in some primary immunodeficiencies that predispose for EBV pathologies.

Apart from these immunodeficiencies whose genes can be related to innate lymphocyte function, other more general

TABLE 1 | Primary immunodeficiencies that are associated with loss of immune control by innate lymphocytes and EBV-associated pathologies.

Affected protein	EBV-associated pathology	Affected innate lymphocytes	Reference
Cytotoxic machinery			
Perforin	CAEBV, HLH	NK, NKT, γδT	(46)
Munc13-4	CAEBV, HLH	NK, NKT, γδT	(47)
Munc18-2	CAEBV, HLH	NK, NKT, γδT	(48)
DNA-binding proteins			
GATA2	CAEBV, HLH	NK	(18, 45)
MCM4	EBV lymphoma	NK	(75, 76)
NF-κB1	EBV lymphoma	NK	(73, 74)
Costimulatory receptors and their ligands			
CD27	EBV lymphoma	NKT	(66–68)
CD70	EBV-positive Hodgkin's lymphoma	NKT	(64, 65)
CD16	EBV-positive Castleman's disease	NK	(77, 78)
NKG2D and TCR (because of MagT1 deficiency)	EBV lymphoma	NK, γδT	(69–72)
Signaling molecules			
SAP	EBV lymphoma, IM, HLH	NKT	(49–51, 54, 55)
ITK	EBV lymphoma	NKT	(56–63)
RasGRP1	EBV lymphoma	NKT	(79)
PI3K 110δ	EBV viremia	NK	(82)
Others			
XIAP	IM, HLH	NKT	(52, 53)
Coronin 1A	EBV lymphoma	NKT	(80)
CTP synthase 1	IM, EBV lymphoma	NKT	(81)

CAEBV, chronic active EBV; HLH, hemophagocytic lymphohistiocytosis; IM, infectious mononucleosis; NK, natural killer; EBV, Epstein–Barr virus; NKT, natural killer T; SAP, SLAM-associated protein; XIAP, X-linked inhibitor of apoptosis; ITK, inducible T cell kinase; MCM4, minichromosome maintenance complex component 4; PI3K, phosphatidylinositol-3-kinase.

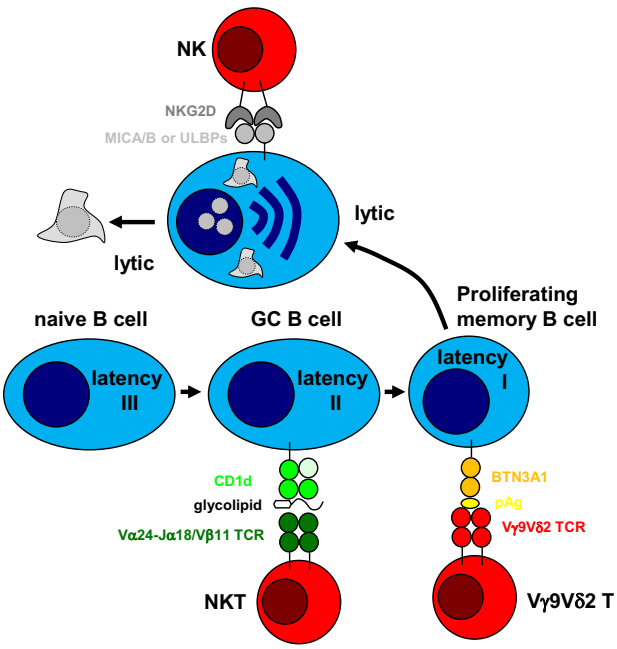


FIGURE 1 | Innate lymphocytes target different stages of Epstein–Barr virus (EBV) infection. EBV was suggested to drive B cell differentiation by expressing all eight latent EBV proteins (latency III) in tonsillar naïve B cells and rescuing germinal center (GC) B cells with the expression of three latent EBV proteins (latency II) toward memory B cells. In homeostatically proliferating memory B cells, only one latent EBV protein is expressed for viral genome maintenance (latency I). From this infected memory B cell pool, EBV can reactivate into virus producing lytic replication, most likely after B cell receptor engagement. Natural killer (NK) cells have been shown to preferentially recognize lytic EBV replication and NKG2D has been suggested as an activating receptor involved in this recognition after upregulation of its MICA/B and ULBP ligands. In a subgroup of infected individuals, Vγ9Vδ2 T cells can be stimulated by EBV latency I Burkitt's lymphoma cell lines and recognize these by mevalonate metabolite recognition in a butyrophilin (BTN) 3A1-dependent fashion. Finally, natural killer T (NKT) cells have been suggested to recognize EBV latency II in Hodgkin's lymphoma cell lines, presumably by recognizing glycolipid presentation on CD1d. Thus, cytotoxic innate lymphocytes can target different stages of EBV infection.

deficiencies like the mutations in the actin-binding protein coronin 1A and CTP synthase 1 are associated with NKT cell loss and EBV-associated lymphoproliferative diseases (80, 81). Furthermore, loss-of-function mutations in phosphatidylinositol-3-kinase subunit 110δ diminish NK cell killing and results in EBV viremia (82). Thus, primary immunodeficiences in the perforin machinery of cytotoxic lymphocytes, their costimulatory molecules, DNA-binding proteins that are required for their differentiation, and some less well-mechanistically understood gene products diminish innate lymphocyte activity and predispose for EBV-associated pathologies. These are summarized in **Table 1**.

CONCLUSION AND OUTLOOK

The above outlined arguments suggest a division of labor among innate lymphocytes in targeting different programs of EBV infection. While NK cells might preferentially eliminate lytically EBV replicating cells, and immunodeficiencies that affect them could primary result in lymphoproliferations, NKT cells might be superior in restricting Hodgkin's lymphoma and especially affected by ITK and CD70 deficiencies. Finally, Vγ9Vδ2 T cells might be able to target BL cells and LCLs. In combination, NK, NKT, and Vγ9Vδ2 T cells could therefore restrict EBV latencies I–III and lytic replication (**Figure 1**). This comprehensive immune control by innate lymphocytes might be especially important during early primary infection before protective $CD8^+$ T cell responses have been primed. A better understanding of how these innate lymphocyte subsets collaborate during primary EBV infection could provide insights why IM preferentially develops in adolescence and which subgroup of these are especially at risk. Furthermore, characterizing how NK, NKT, and Vγ9Vδ2 T cells recognize EBV-infected cells and which infection programs in virus-associated malignancies are especially susceptible to this recognition could suggest immunotherapeutic approaches against the respective tumors, harnessing these innate lymphocytes.

AUTHOR CONTRIBUTIONS

The author confirms being the sole contributor of this work and approved it for publication.

FUNDING

The research in my laboratory is supported by grants from the Swiss National Science Foundation (310030_162560 and CRSII3_160708), Cancer Research Switzerland (KFS-4091-02-2017), SPARKS (15UOZ01), Sobek Foundation, the Swiss MS Society, and the clinical research priority programs on Multiple sclerosis ($KFSP^{MS}$) and human hemato-lymphatic diseases ($KFSP^{HHLD}$) of the University of Zürich.

REFERENCES

1. Epstein MA, Achong BG, Barr YM. Virus particles in cultured lymphoblasts from Burkitt's lymphoma. *Lancet* (1964) 1:702–3. doi:10.1016/S0140-6736(64)91524-7
2. Epstein MA, Henle G, Achong BG, Barr YM. Morphological and biological studies on a virus in cultured lymphoblasts from Burkitt's lymphoma. *J Exp Med* (1964) 121:761–70. doi:10.1084/jem.121.5.761
3. Cesarman E. Gammaherpesviruses and lymphoproliferative disorders. *Annu Rev Pathol* (2014) 9:349–72. doi:10.1146/annurev-pathol-012513-104656
4. Cohen JI, Fauci AS, Varmus H, Nabel GJ. Epstein-Barr virus: an important vaccine target for cancer prevention. *Sci Transl Med* (2011) 3(107):107fs7. doi:10.1126/scitranslmed.3002878
5. Luzuriaga K, Sullivan JL. Infectious mononucleosis. *N Engl J Med* (2010) 362(21):1993–2000. doi:10.1056/NEJMcp1001116
6. Gottschalk S, Rooney CM, Heslop HE. Post-transplant lymphoproliferative disorders. *Annu Rev Med* (2005) 56:29–44. doi:10.1146/annurev.med.56.082103.104727
7. Cohen JI. Primary immunodeficiencies associated with EBV disease. *Curr Top Microbiol Immunol* (2015) 390(Pt 1):241–65. doi:10.1007/978-3-319-22822-8_10
8. Callan MF, Tan L, Annels N, Ogg GS, Wilson JD, O'Callaghan CA, et al. Direct visualization of antigen-specific $CD8^+$ T cells during the primary immune response to Epstein-Barr virus In vivo. *J Exp Med* (1998) 187(9):1395–402. doi:10.1084/jem.187.9.1395
9. Russell JH, Ley TJ. Lymphocyte-mediated cytotoxicity. *Annu Rev Immunol* (2002) 20:323–70. doi:10.1146/annurev.immunol.20.100201.131730
10. Vivier E, Raulet DH, Moretta A, Caligiuri MA, Zitvogel L, Lanier LL, et al. Innate or adaptive immunity? The example of natural killer cells. *Science* (2011) 331(6013):44–9. doi:10.1126/science.1198687
11. Chandra S, Kronenberg M. Activation and function of iNKT and MAIT cells. *Adv Immunol* (2015) 127:145–201. doi:10.1016/bs.ai.2015.03.003
12. Chien YH, Meyer C, Bonneville M. Gammadelta T cells: first line of defense and beyond. *Annu Rev Immunol* (2014) 32:121–55. doi:10.1146/annurev-immunol-032713-120216
13. Ljunggren HG, Karre K. Host resistance directed selectively against H-2-deficient lymphoma variants. Analysis of the mechanism. *J Exp Med* (1985) 162(6):1745–59. doi:10.1084/jem.162.6.1745
14. Correa I, Corral L, Raulet DH. Multiple natural killer cell-activating signals are inhibited by major histocompatibility complex class I expression in target cells. *Eur J Immunol* (1994) 24(6):1323–31. doi:10.1002/eji.1830240613
15. Herberman RB, Nunn ME, Lavrin DH. Natural cytotoxic reactivity of mouse lymphoid cells against syngeneic acid allogeneic tumors. I. Distribution of reactivity and specificity. *Int J Cancer* (1975) 16(2):216–29. doi:10.1002/ijc.2910160204
16. Kiessling R, Klein E, Wigzell H. "Natural" killer cells in the mouse. I. Cytotoxic cells with specificity for mouse Moloney leukemia cells. Specificity and distribution according to genotype. *Eur J Immunol* (1975) 5(2):112–7. doi:10.1002/eji.1830050208
17. Trinchieri G, Santoli D. Anti-viral activity induced by culturing lymphocytes with tumor-derived or virus-transformed cells. Enhancement of human natural killer cell activity by interferon and antagonistic inhibition of susceptibility of target cells to lysis. *J Exp Med* (1978) 147(5):1314–33. doi:10.1084/jem.147.5.1314
18. Biron CA, Byron KS, Sullivan JL. Severe herpesvirus infections in an adolescent without natural killer cells. *N Engl J Med* (1989) 320(26):1731–5. doi:10.1056/NEJM198906293202605
19. Williams H, McAulay K, Macsween KF, Gallacher NJ, Higgins CD, Harrison N, et al. The immune response to primary EBV infection: a role for natural killer cells. *Br J Haematol* (2005) 129(2):266–74. doi:10.1111/j.1365-2141.2005.05452.x
20. Dunmire SK, Grimm JM, Schmeling DO, Balfour HH Jr, Hogquist KA. The incubation period of primary Epstein-Barr virus infection: viral dynamics and immunologic events. *PLoS Pathog* (2015) 11(12):e1005286. doi:10.1371/journal.ppat.1005286
21. Hendricks DW, Balfour HH Jr, Dunmire SK, Schmeling DO, Hogquist KA, Lanier LL. Cutting edge: $NKG2C^{hi}CD57^+$ NK cells respond specifically to acute infection with cytomegalovirus and not Epstein-Barr virus. *J Immunol* (2014) 192(10):4492–6. doi:10.4049/jimmunol.1303211

22. Azzi T, Lunemann A, Murer A, Ueda S, Beziat V, Malmberg KJ, et al. Role for early-differentiated natural killer cells in infectious mononucleosis. *Blood* (2014) 124(16):2533–43. doi:10.1182/blood-2014-01-553024
23. Taylor GS, Long HM, Brooks JM, Rickinson AB, Hislop AD. The immunology of Epstein-Barr virus-induced disease. *Annu Rev Immunol* (2015) 33:787–821. doi:10.1146/annurev-immunol-032414-112326
24. Pappworth IY, Wang EC, Rowe M. The switch from latent to productive infection in Epstein-Barr virus-infected B cells is associated with sensitization to NK cell killing. *J Virol* (2007) 81(2):474–82. doi:10.1128/JVI.01777-06
25. Chijioke O, Muller A, Feederle R, Barros MH, Krieg C, Emmel V, et al. Human natural killer cells prevent infectious mononucleosis features by targeting lytic Epstein-Barr virus infection. *Cell Rep* (2013) 5(6):1489–98. doi:10.1016/j.celrep.2013.11.041
26. Strowig T, Chijioke O, Carrega P, Arrey F, Meixlsperger S, Ramer PC, et al. Human NK cells of mice with reconstituted human immune system components require preactivation to acquire functional competence. *Blood* (2010) 116(20):4158–67. doi:10.1182/blood-2010-02-270678
27. Sundstrom Y, Nilsson C, Lilja G, Karre K, Troye-Blomberg M, Berg L. The expression of human natural killer cell receptors in early life. *Scand J Immunol* (2007) 66(2–3):335–44. doi:10.1111/j.1365-3083.2007.01980.x
28. Chaigne-Delalande B, Li FY, O'Connor GM, Lukacs MJ, Jiang P, Zheng L, et al. $Mg2^+$ regulates cytotoxic functions of NK and CD8 T cells in chronic EBV infection through NKG2D. *Science* (2013) 341(6142):186–91. doi:10.1126/science.1240094
29. Landtwing V, Raykova A, Pezzino G, Beziat V, Marcenaro E, Graf C, et al. Cognate HLA absence in trans diminishes human NK cell education. *J Clin Invest* (2016) 126(10):3772–82. doi:10.1172/JCI86923
30. Velardi A, Ruggeri L, Mancusi A. Killer-cell immunoglobulin-like receptors reactivity and outcome of stem cell transplant. *Curr Opin Hematol* (2012) 19(4):319–23. doi:10.1097/MOH.0b013e32835423c3
31. Djaoud Z, Guethlein LA, Horowitz A, Azzi T, Nemat-Gorgani N, Olive D, et al. Two alternate strategies for innate immunity to Epstein-Barr virus: one using NK cells and the other NK cells and gammadelta T cells. *J Exp Med* (2017) 214(6):1827–41. doi:10.1084/jem.20161017
32. Chitadze G, Oberg HH, Wesch D, Kabelitz D. The ambiguous role of gammadelta T lymphocytes in antitumor immunity. *Trends Immunol* (2017) 38(9):668–78. doi:10.1016/j.it.2017.06.004
33. Hochberg D, Middeldorp JM, Catalina M, Sullivan JL, Luzuriaga K, Thorley-Lawson DA. Demonstration of the Burkitt's lymphoma Epstein-Barr virus phenotype in dividing latently infected memory cells in vivo. *Proc Natl Acad Sci U S A* (2004) 101(1):239–44. doi:10.1073/pnas.2237267100
34. Babcock GJ, Decker LL, Volk M, Thorley-Lawson DA. EBV persistence in memory B cells in vivo. *Immunity* (1998) 9(3):395–404. doi:10.1016/S1074-7613(00)80622-6
35. Babcock GJ, Decker LL, Freeman RB, Thorley-Lawson DA. Epstein-Barr virus-infected resting memory B cells, not proliferating lymphoblasts, accumulate in the peripheral blood of immunosuppressed patients. *J Exp Med* (1999) 190(4):567–76. doi:10.1084/jem.190.4.567
36. Jayasooriya S, de Silva TI, Njie-jobe J, Sanyang C, Leese AM, Bell AI, et al. Early virological and immunological events in asymptomatic Epstein-Barr virus infection in African children. *PLoS Pathog* (2015) 11(3):e1004746. doi:10.1371/journal.ppat.1004746
37. Xiang Z, Liu Y, Zheng J, Liu M, Lv A, Gao Y, et al. Targeted activation of human Vgamma9Vdelta2-T cells controls Epstein-Barr virus-induced B cell lymphoproliferative disease. *Cancer Cell* (2014) 26(4):565–76. doi:10.1016/j.ccr.2014.07.026
38. Zumwalde NA, Sharma A, Xu X, Ma S, Schneider CL, Romero-Masters JC, et al. Adoptively transferred Vgamma9Vdelta2 T cells show potent antitumor effects in a preclinical B cell lymphomagenesis model. *JCI Insight* (2017) 2(13):93179. doi:10.1172/jci.insight.93179
39. Yuling H, Ruijing X, Li L, Xiang J, Rui Z, Yujuan W, et al. EBV-induced human $CD8^+$ NKT cells suppress tumorigenesis by EBV-associated malignancies. *Cancer Res* (2009) 69(20):7935–44. doi:10.1158/0008-5472.CAN-09-0828
40. Chung BK, Tsai K, Allan LL, Zheng DJ, Nie JC, Biggs CM, et al. Innate immune control of EBV-infected B cells by invariant natural killer T cells. *Blood* (2013) 122(15):2600–8. doi:10.1182/blood-2013-01-480665
41. Hudnall SD, Betancourt E, Barnhart E, Patel J. Comparative flow immunophenotypic features of the inflammatory infiltrates of Hodgkin lymphoma and lymphoid hyperplasia. *Cytometry B Clin Cytom* (2008) 74(1):1–8. doi:10.1002/cyto.b.20376
42. Babcock JG, Hochberg D, Thorley-Lawson AD. The expression pattern of Epstein-Barr virus latent genes in vivo is dependent upon the differentiation stage of the infected B cell. *Immunity* (2000) 13(4):497–506. doi:10.1016/S1074-7613(00)00049-2
43. Hutt-Fletcher LM. The long and complicated relationship between Epstein-Barr virus and epithelial cells. *J Virol* (2017) 91(1):e01677–16. doi:10.1128/JVI.01677-16
44. Tangye SG, Palendira U, Edwards ES. Human immunity against EBV-lessons from the clinic. *J Exp Med* (2017) 214(2):269–83. doi:10.1084/jem.20161846
45. Mace EM, Hsu AP, Monaco-Shawver L, Makedonas G, Rosen JB, Dropulic L, et al. Mutations in GATA2 cause human NK cell deficiency with specific loss of the $CD56^{bright}$ subset. *Blood* (2013) 121(14):2669–77. doi:10.1182/blood-2012-09-453969
46. Katano H, Ali MA, Patera AC, Catalfamo M, Jaffe ES, Kimura H, et al. Chronic active Epstein-Barr virus infection associated with mutations in perforin that impair its maturation. *Blood* (2004) 103(4):1244–52. doi:10.1182/blood-2003-06-2171
47. Rohr J, Beutel K, Maul-Pavicic A, Vraetz T, Thiel J, Warnatz K, et al. Atypical familial hemophagocytic lymphohistiocytosis due to mutations in UNC13D and STXBP2 overlaps with primary immunodeficiency diseases. *Haematologica* (2010) 95(12):2080–7. doi:10.3324/haematol.2010.029389
48. Cohen JI, Niemela JE, Stoddard JL, Pittaluga S, Heslop H, Jaffe ES, et al. Late-onset severe chronic active EBV in a patient for five years with mutations in STXBP2 (MUNC18-2) and PRF1 (perforin 1). *J Clin Immunol* (2015) 35(5):445–8. doi:10.1007/s10875-015-0168-y
49. Coffey AJ, Brooksbank RA, Brandau O, Oohashi T, Howell GR, Bye JM, et al. Host response to EBV infection in X-linked lymphoproliferative disease results from mutations in an SH2-domain encoding gene. *Nat Genet* (1998) 20(2):129–35. doi:10.1038/2424
50. Nichols KE, Harkin DP, Levitz S, Krainer M, Kolquist KA, Genovese C, et al. Inactivating mutations in an SH2 domain-encoding gene in X-linked lymphoproliferative syndrome. *Proc Natl Acad Sci U S A* (1998) 95(23):13765–70. doi:10.1073/pnas.95.23.13765
51. Sayos J, Wu C, Morra M, Wang N, Zhang X, Allen D, et al. The X-linked lymphoproliferative-disease gene product SAP regulates signals induced through the co-receptor SLAM. *Nature* (1998) 395(6701):462–9. doi:10.1038/26683
52. Rigaud S, Fondaneche MC, Lambert N, Pasquier B, Mateo V, Soulas P, et al. XIAP deficiency in humans causes an X-linked lymphoproliferative syndrome. *Nature* (2006) 444(7115):110–4. doi:10.1038/nature05257
53. Speckmann C, Lehmberg K, Albert MH, Damgaard RB, Fritsch M, Gyrd-Hansen M, et al. X-linked inhibitor of apoptosis (XIAP) deficiency: the spectrum of presenting manifestations beyond hemophagocytic lymphohistiocytosis. *Clin Immunol* (2013) 149(1):133–41. doi:10.1016/j.clim.2013.07.004
54. Nichols KE, Hom J, Gong SY, Ganguly A, Ma CS, Cannons JL, et al. Regulation of NKT cell development by SAP, the protein defective in XLP. *Nat Med* (2005) 11(3):340–5. doi:10.1038/nm1189
55. Pasquier B, Yin L, Fondaneche MC, Relouzat F, Bloch-Queyrat C, Lambert N, et al. Defective NKT cell development in mice and humans lacking the adapter SAP, the X-linked lymphoproliferative syndrome gene product. *J Exp Med* (2005) 201(5):695–701. doi:10.1084/jem.20042432
56. Huck K, Feyen O, Niehues T, Ruschendorf F, Hubner N, Laws HJ, et al. Girls homozygous for an IL-2-inducible T cell kinase mutation that leads to protein deficiency develop fatal EBV-associated lymphoproliferation. *J Clin Invest* (2009) 119(5):1350–8. doi:10.1172/JCI37901
57. Linka RM, Risse SL, Bienemann K, Werner M, Linka Y, Krux F, et al. Loss-of-function mutations within the IL-2 inducible kinase ITK in patients with EBV-associated lymphoproliferative diseases. *Leukemia* (2012) 26(5):963–71. doi:10.1038/leu.2011.371
58. Stepensky P, Weintraub M, Yanir A, Revel-Vilk S, Krux F, Huck K, et al. IL-2-inducible T-cell kinase deficiency: clinical presentation and therapeutic approach. *Haematologica* (2011) 96(3):472–6. doi:10.3324/haematol.2010.033910
59. Mansouri D, Mahdaviani SA, Khalilzadeh S, Mohajerani SA, Hasanzad M, Sadr S, et al. IL-2-inducible T-cell kinase deficiency with pulmonary

manifestations due to disseminated Epstein-Barr virus infection. *Int Arch Allergy Immunol* (2012) 158(4):418–22. doi:10.1159/000333472
60. Ghosh S, Bienemann K, Boztug K, Borkhardt A. Interleukin-2-inducible T-cell kinase (ITK) deficiency – clinical and molecular aspects. *J Clin Immunol* (2014) 34(8):892–9. doi:10.1007/s10875-014-0110-8
61. Bienemann K, Borkhardt A, Klapper W, Oschlies I. High incidence of Epstein-Barr virus (EBV)-positive Hodgkin lymphoma and Hodgkin lymphoma-like B-cell lymphoproliferations with EBV latency profile 2 in children with interleukin-2-inducible T-cell kinase deficiency. *Histopathology* (2015) 67(5):607–16. doi:10.1111/his.12677
62. Cipe FE, Aydogmus C, Serwas NK, Tugcu D, Demirkaya M, Bicici FA, et al. ITK deficiency: how can EBV be treated before lymphoma? *Pediatr Blood Cancer* (2015) 62(12):2247–8. doi:10.1002/pbc.25648
63. Cagdas D, Erman B, Hanoglu D, Tavil B, Kuskonmaz B, Aydin B, et al. Course of IL-2-inducible T-cell kinase deficiency in a family: lymphomatoid granulomatosis, lymphoma and allogeneic bone marrow transplantation in one sibling; and death in the other. *Bone Marrow Transplant* (2017) 52(1):126–9. doi:10.1038/bmt.2016.185
64. Abolhassani H, Edwards ES, Ikinciogullari A, Jing H, Borte S, Buggert M, et al. Combined immunodeficiency and Epstein-Barr virus-induced B cell malignancy in humans with inherited CD70 deficiency. *J Exp Med* (2017) 214(1):91–106. doi:10.1084/jem.20160849
65. Izawa K, Martin E, Soudais C, Bruneau J, Boutboul D, Rodriguez R, et al. Inherited CD70 deficiency in humans reveals a critical role for the CD70-CD27 pathway in immunity to Epstein-Barr virus infection. *J Exp Med* (2017) 214(1):73–89. doi:10.1084/jem.20160784
66. van Montfrans JM, Hoepelman AI, Otto S, van Gijn M, van de Corput L, de Weger RA, et al. CD27 deficiency is associated with combined immunodeficiency and persistent symptomatic EBV viremia. *J Allergy Clin Immunol* (2012) 129(3):787–93.e6. doi:10.1016/j.jaci.2011.11.013
67. Salzer E, Daschkey S, Choo S, Gombert M, Santos-Valente E, Ginzel S, et al. Combined immunodeficiency with life-threatening EBV-associated lymphoproliferative disorder in patients lacking functional CD27. *Haematologica* (2013) 98(3):473–8. doi:10.3324/haematol.2012.068791
68. Alkhairy OK, Perez-Becker R, Driessen GJ, Abolhassani H, van Montfrans J, Borte S, et al. Novel mutations in TNFRSF7/CD27: clinical, immunologic, and genetic characterization of human CD27 deficiency. *J Allergy Clin Immunol* (2015) 136(3):703–12.e10. doi:10.1016/j.jaci.2015.02.022
69. Li FY, Chaigne-Delalande B, Kanellopoulou C, Davis JC, Matthews HF, Douek DC, et al. Second messenger role for $Mg2^{+}$ revealed by human T-cell immunodeficiency. *Nature* (2011) 475(7357):471–6. doi:10.1038/nature10246
70. Dhalla F, Murray S, Sadler R, Chaigne-Delalande B, Sadaoka T, Soilleux E, et al. Identification of a novel mutation in MAGT1 and progressive multifocal leucoencephalopathy in a 58-year-old man with XMEN disease. *J Clin Immunol* (2015) 35(2):112–8. doi:10.1007/s10875-014-0116-2
71. Patiroglu T, Haluk Akar H, Gilmour K, Unal E, Akif Ozdemir M, Bibi S, et al. A case of XMEN syndrome presented with severe auto-immune disorders mimicking autoimmune lymphoproliferative disease. *Clin Immunol* (2015) 159(1):58–62. doi:10.1016/j.clim.2015.04.015
72. Brigida I, Chiriaco M, Di Cesare S, Cittaro D, Di Matteo G, Giannelli S, et al. Large deletion of MAGT1 gene in a patient with classic Kaposi sarcoma, CD4 lymphopenia, and EBV infection. *J Clin Immunol* (2017) 37(1):32–5. doi:10.1007/s10875-016-0341-y
73. Boztug H, Hirschmugl T, Holter W, Lakatos K, Kager L, Trapin D, et al. NF-kappaB1 haploinsufficiency causing immunodeficiency and EBV-driven lymphoproliferation. *J Clin Immunol* (2016) 36(6):533–40. doi:10.1007/s10875-016-0306-1
74. Schipp C, Nabhani S, Bienemann K, Simanovsky N, Kfir-Erenfeld S, Assayag-Asherie N, et al. Specific antibody deficiency and autoinflammatory disease extend the clinical and immunological spectrum of heterozygous NFKB1 loss-of-function mutations in humans. *Haematologica* (2016) 101(10):e392–6. doi:10.3324/haematol.2016.145136
75. Eidenschenk C, Dunne J, Jouanguy E, Fourlinnie C, Gineau L, Bacq D, et al. A novel primary immunodeficiency with specific natural-killer cell deficiency maps to the centromeric region of chromosome 8. *Am J Hum Genet* (2006) 78(4):721–7. doi:10.1086/503269
76. Gineau L, Cognet C, Kara N, Lach FP, Dunne J, Veturi U, et al. Partial MCM4 deficiency in patients with growth retardation, adrenal insufficiency, and natural killer cell deficiency. *J Clin Invest* (2012) 122(3):821–32. doi:10.1172/JCI61014
77. de Vries E, Koene HR, Vossen JM, Gratama JW, Borne AE, Waaijer JL, et al. Identification of an unusual Fc gamma receptor IIIa (CD16) on natural killer cells in a patient with recurrent infections. *Blood* (1996) 88(8):3022–7.
78. Grier JT, Forbes LR, Monaco-Shawver L, Oshinsky J, Atkinson TP, Moody C, et al. Human immunodeficiency-causing mutation defines CD16 in spontaneous NK cell cytotoxicity. *J Clin Invest* (2012) 122(10):3769–80. doi:10.1172/JCI64837
79. Salzer E, Cagdas D, Hons M, Mace EM, Garncarz W, Petronczki OY, et al. RASGRP1 deficiency causes immunodeficiency with impaired cytoskeletal dynamics. *Nat Immunol* (2016) 17(12):1352–60. doi:10.1038/ni.3575
80. Moshous D, Martin E, Carpentier W, Lim A, Callebaut I, Canioni D, et al. Whole-exome sequencing identifies Coronin-1A deficiency in 3 siblings with immunodeficiency and EBV-associated B-cell lymphoproliferation. *J Allergy Clin Immunol* (2013) 131(6):1594–603. doi:10.1016/j.jaci.2013.01.042
81. Martin E, Palmic N, Sanquer S, Lenoir C, Hauck F, Mongellaz C, et al. CTP synthase 1 deficiency in humans reveals its central role in lymphocyte proliferation. *Nature* (2014) 510(7504):288–92. doi:10.1038/nature13386
82. Kuehn HS, Niemela JE, Rangel-Santos A, Zhang M, Pittaluga S, Stoddard JL, et al. Loss-of-function of the protein kinase C delta (PKCdelta) causes a B-cell lymphoproliferative syndrome in humans. *Blood* (2013) 121(16):3117–25. doi:10.1182/blood-2012-12-469544

Conflict of Interest Statement: The author declares that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

frontiers in Immunology

MINI REVIEW
published: 22 December 2017
doi: 10.3389/fimmu.2017.01869

GATA2 Deficiency and Epstein–Barr Virus Disease

*Jeffrey I. Cohen**

Medical Virology Section, Laboratory of Infectious Diseases, National Institute of Allergy and Infectious Diseases, National Institutes of Health, Bethesda, MD, United States

GATA2 is a transcription factor that binds to the promoter of hematopoietic genes. Mutations in one copy of the gene are associated with haploinsufficiency and reduced levels of protein. This results in reduced numbers of several cell types important for immune surveillance including dendritic cells, monocytes, CD4, and NK cells, as well as impaired NK cell function. Recently, GATA2 has been associated with several different presentations of severe Epstein–Barr virus (EBV) disease including primary infection requiring repeated hospitalizations, chronic active EBV disease, EBV-associated hydroa vacciniforme with hemophagocytosis, and EBV-positive smooth muscle tumors. EBV was found predominantly in B cells in each of the cases in which it was studied, unlike most cases of chronic active EBV disease in which the virus is usually present in T or NK cells. The variety of EBV-associated diseases seen in patients with GATA2 deficiency suggest that additional forms of severe EBV disease may be found in patients with GATA2 deficiency in the future.

Keywords: GATA2, Epstein–Barr, chronic active Epstein–Barr virus, infectious mononucleosis, hydroa vacciniforme, smooth muscle tumors

OPEN ACCESS

Edited by:
Stuart G. Tangye, Garvan Institute of Medical Research, Australia

Reviewed by:
Sabrin Albeituni, St. Jude Children's Research Hospital, United States
Claire Shannon-Lowe, University of Birmingham, United Kingdom

***Correspondence:**
Jeffrey I. Cohen
jcohen@niaid.nih.gov

Specialty section:
This article was submitted to Primary Immunodeficiencies, a section of the journal Frontiers in Immunology

Received: *09 September 2017*
Accepted: *08 December 2017*
Published: *22 December 2017*

Citation:
Cohen JI (2017) GATA2 Deficiency and Epstein–Barr Virus Disease. Front. Immunol. 8:1869. doi: 10.3389/fimmu.2017.01869

THE GATA FAMILY OF TRANSCRIPTION FACTORS

The GATA family of transcription factors consist of six proteins (GATA1 to GATA6) that contain two zinc finger-binding domains that bind to GATA sites on DNA (1, 2). GATA1 and GATA2 are important for hematopoiesis, with GATA1 important for development of red blood cells and platelets, and GATA2 for development of hematopoietic stem cells and progenitor cells. GATA3 is important for development of T cells. In contrast, GATA4 to GATA6 have critical roles in cardiac embryogenesis.

The GATA2 gene contains seven exons, five of which are translated. In addition to two zinc finger domains, the protein contains two transcriptional activation domains, a negative regulatory domain, and a nuclear localization signal (3). The protein undergoes a number of posttranslational modifications including phosphorylation, ubiquitination, SUMOylation, and acetylation (3). Mice that are homozygous knockouts for GATA2 die *in utero* due to a failure of hematopoiesis (4); in contrast, mice that are heterozygous for GATA-2 deficiency have reduced numbers of hematopoietic progenitor cells (5).

GATA2 is necessary for survival and renewal of hematopoietic stem cells and interacts with multiple transcription factors that regulate gene expression in hematopoietic stem cells. The quantity of GATA2 is critical for its activity, thus, reduced levels due to haploinsufficiency can have a profound phenotype. GATA2$^{+/-}$ mice have fewer functional hematopoietic stem cells and granulocyte–macrophage progenitors in the bone marrow and the cells are impaired for self-renewal (6, 7).

GATA2 DEFICIENCY PHENOTYPE AND MUTATIONS

GATA2 deficiency in humans, due to haploinsufficiency, has been associated with a wide array of diseases (8–11). These include hematologic disorders such as myelodysplastic syndrome, acute myelogenous leukemia, chronic myelomonocytic leukemia, aplastic anemia, as well as low numbers of monocytes, B cells, NK cells, dendritic cells, and neutrophils. Infectious complications include viral, bacterial, and fungal infections. Virus infections include human papillomavirus virus (HPV) infection that can transition to HPV-positive squamous cell carcinoma, or severe molluscum contagiosum, herpes simplex virus, varicella-zoster virus, cytomegalovirus, or Epstein–Barr virus (EBV) infection. Severe nontuberculous mycobacteria infections are commonly seen with GATA2 deficiency, while fungal infections include invasive aspergillosis, disseminated histoplasmosis, and recurrent candidiasis. Other complications reported in patients with GATA2 deficiency include pulmonary alveolar proteinosis, congenital lymphedema, panniculitis, erythema nodosum, venous thromboses, and deafness.

Many mutations have been detected in GATA (**Figure 1**), most of which are germ line, while somatic mutations have been reported in patients with leukemia (10). While most mutations have been reported in the coding region of the gene, mutations in regulatory regions such as in the enhancer region of intron 5 and the 5′ leader sequence result in reduced transcription (12). Mutations associated with disease are most often in one of the two zinc finger-binding domains; these include amino acid substitutions, frameshift mutations, and insertions and deletions. These result in either protein dysfunction or reduced transcription from one of the two alleles. Thus, deletion of one allele, mutations in non-coding regulatory regions of the gene, or mutations in one allele can result in haploinsufficiency due to reduced transcription, loss of protein expression, or expression of a non-functional protein. Most cases are due to *de novo* mutations while about one-third are inherited as an autosomal dominant condition. In some cases, mutations have not been identified, but transcription of only one of the two alleles has been demonstrated.

HEMATOLOGIC FINDINGS IN GATA2 DEFICIENCY IMPORTANT FOR CONTROL OF VIRUS INFECTIONS

The mechanism for the predilection of patients to severe EBV infections is almost certainly multifactorial (**Figure 2**). Absence or reduction in the numbers of dendritic cells with GATA2 insufficiency (13) can reduce recognition of EBV by the immune system. Dendritic cells are critical for presentation of EBV antigens to T cells (14) and EBV in turn inhibits dendritic cell maturation (15).

Reduction in the number of monocytes in patients with GATA deficiency (11, 16) reduces cytokine responses with reduced IFN-γ and IL-12. EBV inhibits MHC class I, class II, ICAM1, CD80, and CD86 expression on monocytes, which inhibit T cell proliferation and antigen presentation by the cells (17, 18). Patients with GATA2 deficiency have a decrease in the number of hematogones (precursor B cells) and B cells including naïve B cells, with a relative increase in the number of memory B cells (19, 20). B cells are important for antigen presentation and help to activate T cells.

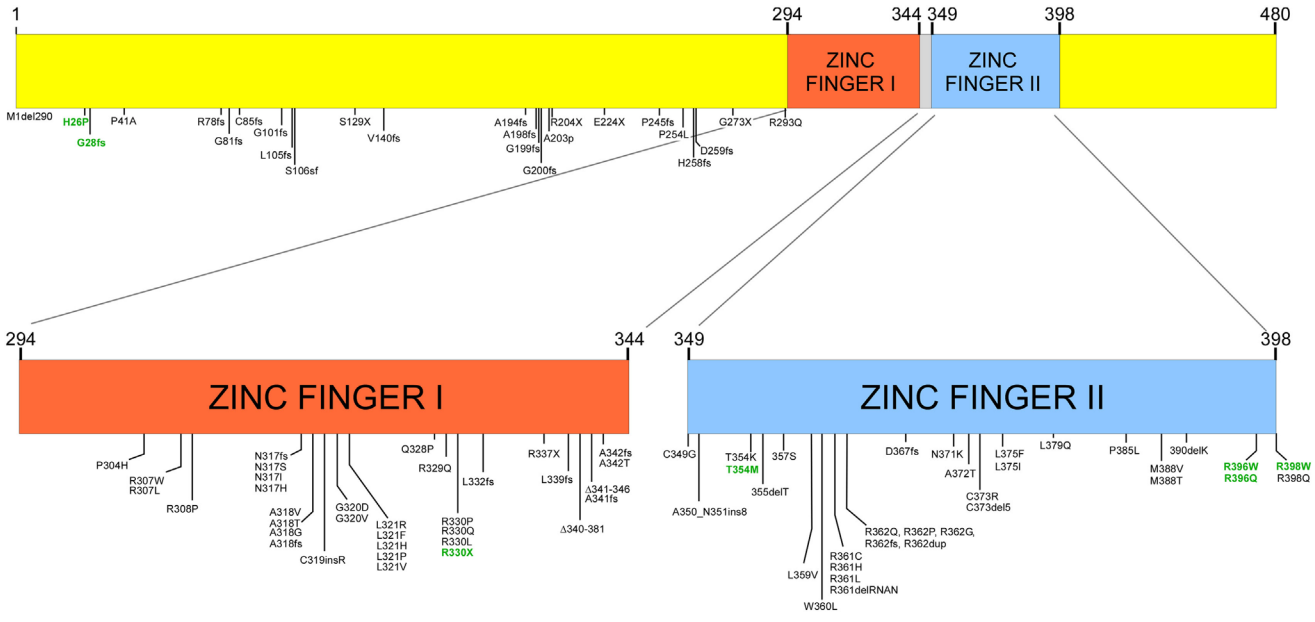


FIGURE 1 | Structure of GATA2 and mutations described with GATA2 insufficiency and in patients with severe Epstein–Barr virus (EBV) disease [adapted from data in Ref. (8)]. Numbers in green represent mutations associated with severe EBV disease.

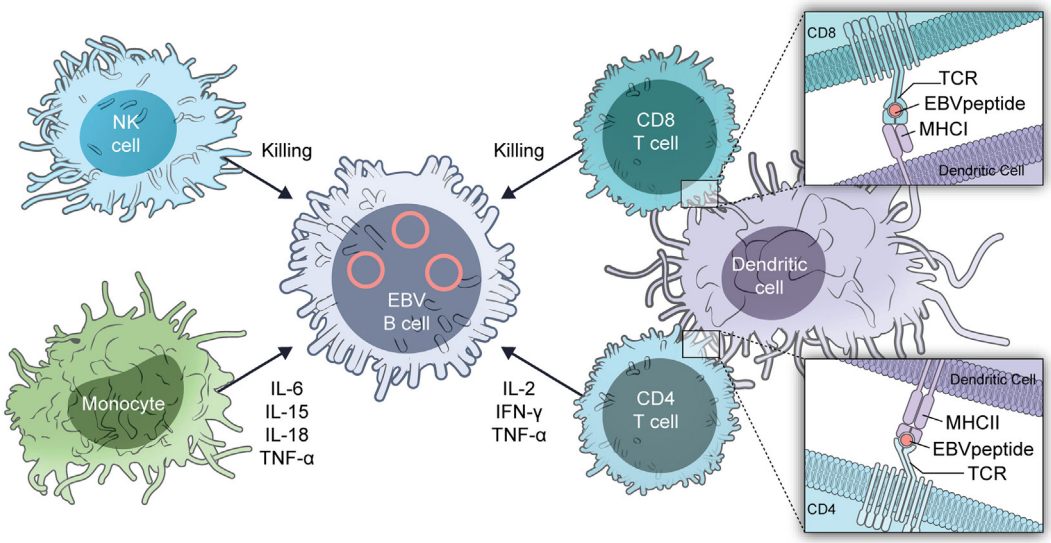


FIGURE 2 | Immune system cells important for controlling Epstein–Barr virus (EBV)-infected B cells.

GATA2 deficiency is associated with a reduction in the number of NK cells and reduced NK cell-mediated cytotoxicity (11, 16, 21). In addition, there is a reduction in the number of immature ($CD56^{bright}$) NK cells (21), while other NK cell markers indicative of maturity, such as killer cell immunoglobulin-like receptors, are increased (14). NK cells are critical for control of primary infection with EBV (22) as well as to inhibit transformation of B cells by the virus (23).

Like NK cells, CD8 T cells in patients with GATA2 deficiency are skewed toward a more mature, terminally differentiated phenotype (16). The reduction in naïve T cells may also impair control of EBV. These patients also have a reduction in the number of CD4 cells with an inverted CD4/CD8 ratio. Both CD4 and CD8 cells are important for control of EBV infection. During primary infection, there is a massive expansion of CD8 cells and these cells recognize both lytic and latent viral antigens (14). CD4 cells are important for control of primary infection, but there is less proliferation of CD4 than CD8 cells. During convalescence, up to 5% of CD8 cells are directed against EBV antigens, while less than 1% of CD4 cells recognize EBV proteins.

GATA2 may also affect herpesvirus latency. GATA2 controls the expression of at least two latency associated genes in human cytomegalovirus, UL144 and LUNA (24). GATA2 also increases expression of cellular IL-10 (25), which is important for CMV latency (26). At least two EBV latency associated genes, EBV-encoded RNA and latent membrane protein 1 (LMP1), upregulate cellular IL-10. Both CMV (27) and EBV (28) encode IL-10 homologs, which are important for immune evasion.

Studies of gene transcription in EBV-transformed B cells from patients with GATA2 deficiency due to haploinsufficiency compared with controls showed significant differences in expression of 102 genes (12). Several genes known to have an important role in EBV transformation, including NOTCH1, TRAF2, and TRAF3, were downregulated in EBV-transformed B cells derived from GATA2-deficient patients compared with controls. EBNA2, which is essential for EBV latency, is a functional homolog of a constitutive NOTCH receptor and LMP2, which also has a critical role in EBV latency, activates NOTCH to increase survival of B cells. Therefore, reduced expression of NOTCH1 in cells from patients with GATA2 deficiency may reduce the ability of the virus to maintain latency. LMP1, which is essential for EBV latency, interacts with TRAF2 and 3 to activate the NF-κB pathway and maintain latency. Thus, impaired expression of NOTCH1, TRAF2, and TRAF3 in cells from patients with GATA2 deficiency may reduce the ability of the virus to maintain latency. This could result in increased virus replication resulting in a higher viral load, more severe primary infection (infectious mononucleosis), and increased infection of additional cells resulting in more severe EBV disease.

EBV DISEASE ASSOCIATED WITH GATA2 DEFICIENCY

Patients with GATA2 deficiency may present with a variety of EBV-associated diseases. These patients may have severe complications with EBV infectious mononucleosis. Identical twins with GATA2 insufficiency, due to a stop codon (R330X) in one allele, presented with symptoms of infectious mononucleosis; one had three hospitalizations for anorexia and dehydration accompanied by anemia, fatigue, weight loss, and fever for 3 months with 20,600 copies of EBV DNA/ml of blood (29). Her course was complicated by numerous infections including *Neisseria meningitditis* bacteremia and Salmonella enteritis. Her sister also presented with EBV infectious mononucleosis complicated by fatigue, weight loss, anemia, thrombocytopenia, and hypotension with 8,900 copies of EBV DNA/ml of blood. Both sisters had reduced numbers of monocytes and CD4, CD8, B, and NK cells.

They had a persistently elevated EBV load in the blood after their symptoms of primary infection resolved. Both had a history of herpes stomatitis and severe warts and both did well after hematopoietic stem cell transplant. A third patient with a missense mutation (R396Q) in one allele of GATA2 presented with EBV infectious mononucleosis and was hospitalized twice, once for severe fatigue with headache and rash, and a second time for dehydration and malaise with 44,000 copies of EBV DNA/ml of blood. She had a history of herpes stomatitis and *Staphylococcus aureus* cellulitis and reduced numbers of monocytes and CD4, CD8, B, and NK cells. Both the bone marrow and a lymph node contained EBV-positive lymphocytes. She also had persistently elevated EBV DNA in the blood after resolution of her infectious mononucleosis symptoms.

A 29-year-old woman with GATA2 insufficiency with null allelic loss of one copy of the gene and a positive EBV IgM viral capsid antibody in the serum and cerebrospinal fluid, indicative of acute infection, developed a demyelinating polyradiculopathy (30). EBV DNA was found in the cerebrospinal fluid, but no EBV DNA was detected in a sural nerve biopsy. She had a history of recurrent pneumonia, severe varicella, and severe genital warts with cervical dysplasia. Her neurological disease responded to intravenous immunoglobulin and corticosteroid therapy.

A 22-year-old man with GATA2 insufficiency due to a missense mutation (T354M) in one allele of GATA2 presented with chronic active EBV disease (29). This is a very rare disorder characterized by infiltration of tissues with EBV-positive lymphocytes, a high level of EBV in the blood, and persistent or intermittent symptoms lasting 6 months or more. He had evidence for a primary EBV infection and persistent splenomegaly and pancytopenia and both the spleen and an adjacent lymph node showed EBV-positive B cells. He had 2,770 copies of EBV DNA/ml of blood. He had reduced numbers of monocytes and CD4, B, and NK cells, but normal numbers of CD8 cells. The patient also had *Mycobacterium abscessus* and died from his mycobacterial infection.

A 24-year-old woman who expressed only one allele of GATA2, but had no definite mutation in the gene, presented with EBV hydroa vacciniforme (29, 31). This EBV disorder is characterized by a vesicular rash and infiltration of the skin with EBV-infected lymphocytes in response to sun exposure; it may progress to a systemic disease with EBV-positive T or NK cell lymphoma. The patient had large skin lesions that were EBV-positive as well as EBV in the lung, intestine, skeletal muscle, and cerebrospinal fluid. There were 6.4 million copies of EBV DNA/ml of blood. Her course was complicated by multiple infections including *Mycobacterium avium* complex, histoplasmosis, and enterococcus bacteremia. She had reduced numbers of monocytes and CD4, B, and NK cells, but normal numbers of CD8 cells. She developed an EBV-positive T cell lymphoma of the lung, gastrointestinal tract, and skin as well as hemophagocytic lymphohistiocytosis. She did well after hematopoietic stem cell transplant.

Two patients with GATA2 insufficiency have been reported with EBV-positive smooth muscle tumors (29, 32, 33). One had a missense mutation (R398W) in GATA2 and EBV-positive leiomyosarcomas involving the posterior orbit, liver, colon, and uterus. She had a history of disseminated *M. avium* involving the skin, blood, and intestine, herpes simplex esophagitis, warts, and chronic myelomonocytic leukemia. She had reduced numbers of monocytes and CD4, B, and NK cells, but normal numbers of CD8 cells. She underwent hematopoietic stem cell transplant for the leukemia, but died of a respiratory tract infection. The second patient also had a missense in GATA2 (R396W) and an EBV-positive spindle cell tumor involving the liver with 3,350 copies of EBV DNA/ml of blood. A positron emission tomographic scan showed multiple metabolically active lesions in the liver, spleen, mediastinum, hilum, scapula, vertebrae, and pelvis. He had a history of recurrent pneumonia, *Mycobacterium szulgai* pneumonia, and *M. avium* in a bronchoalveolar lavage. He had reduced numbers of monocytes and CD4, CD8, B, and NK cells. He underwent hematopoietic stem cell transplant and his immunologic abnormalities resolved and his lesions resolved or remained stable.

A patient with a frameshift (G28fs) and missense mutation (H26P) in exon 2 of GATA2 had myelodysplastic syndrome, progressive pancytopenia, and an EBV-positive T-cell non-Hodgkin lymphoma of the nasopharynx (34). He died despite therapy with corticosteroids and rituximab.

STUDIES OF EBV DISEASE IN PATIENTS WITH GATA2 DEFICIENCY

Analysis of 51 patients with GATA2 deficiency showed that the median level of EBV DNA in the blood of EBV seropositive patients without EBV disease was 117 copies/ml, while the level was 14,750 copies/ml in persons with EBV disease (29). An additional patient has been reported with GATA2 deficiency, diffuse parenchymal lung disease, acute EBV infection and persistent viremia, but no other EBV complications (35). Patients with GATA2 deficiency and severe EBV disease have different patterns of EBV latency gene expression in their peripheral blood and some have expression of EBV BZLF1 indicating that the virus is undergoing lytic gene expression. Patients with severe EBV disease and GATA2 deficiency have high plasma levels of IP-10 (an interferon response gene) and TNF-α, and low levels of IL-1-β compared with normal controls. IP-10 and TNF-α are Th1 cytokines important for cellular immunity.

TREATMENT FOR EBV DISEASE ASSOCIATED WITH GATA2 DEFICIENCY

Definitive treatment for GATA2 deficiency requires hematopoietic stem cell transplant (36). In the largest series to date, 14 patients underwent non-myeloablative allogeneic hematopoietic stem cell transplant (31). Eight of the 14 were alive a median of 3.5 years later with reconstitution of their immune system. Survivors received peripheral blood stem cells from matched related, unrelated, or haploidentical related donors, or umbilical cord blood. The latter group had the lowest survival rate. Deaths were often due to sepsis, graft-versus-host disease, acute myelogenous leukemia, or acute respiratory distress syndrome. Transplantation can be especially challenging in this disease due

to concurrent infections and preexisting leukemia or transformation of myelodysplastic syndrome into leukemia. Cases of relapsed disease after hematopoietic stem cell transplant and graft rejection suggests that myeloablative conditioning may be preferred, although preexisting comorbidities including severe infections may make myeloablation challenging in these patients.

Antiviral therapy is generally ineffective for EBV disease associated with GATA2 deficiency. EBV malignancies are due to proliferation of latently infected lymphocytes or epithelial cells. The viral DNA replicates in these cells using the host cell polymerase, which is insensitive to antivirals; in contrast, lytic virus replication in epithelial cells, which occurs during virus shedding in healthy persons or in those with oral hairy leukoplakia is sensitive to antivirals.

Treatment of some patients with GATA2 insufficiency with IFN-α resulted in increased numbers of NK cells and/or function of the cells, but did not increase the number of $CD56^{bright}$ cells (21). Rituximab may have a role for EBV-positive B cell tumors, but in the absence of reconstitution of the immune system, the disease can recur with CD20-negative B cell tumors (37). Treatment with third party EBV-specific T cells might provide temporizing therapy prior to hematopoietic stem cell transplant (38).

FUTURE DIRECTIONS

GATA2 deficiency has been associated with a large number of diseases particularly hematologic and infectious diseases. At present, it is unclear why certain patients present with specific complications associated with GATA2 deficiency while others do not. While severe viral infections are significantly more common in persons with GATA2 null mutations (11), these infections were also associated with missense and regulatory mutations. However, within large families, different family members with the same mutation can have very different presentations (34). In our patients with severe EBV disease, a variety of mutations in GATA2 including missense mutations, stop codons, null allelic loss of one copy, and unialleleic gene expression were all observed. Some patients have high levels of EBV in the blood and complications of EBV, while others have levels of EBV that are seen in healthy controls. This does not appear to be specific to particular mutations in GATA2, but likely reflects the effects of polymorphisms, mutations, or epigenetic changes in other genes that might modify the phenotype of patients with GATA2 deficiency. This phenomenon of modifier genes has been well described in other genetic disorders (39) and with increasing resources and expertise in computation and bioinformatics, the role of modifier genes affecting GATA2 function may help to explain the different EBV phenotypes observed with GATA2 deficiency. In addition, environmental differences, the immune status of the patient, or coinfections at the time of primary EBV infection might also affect whether some patients with GATA2 deficiency develop severe EBV disease.

GATA2 deficiency is a relatively newly described disorder, and the wide array of severe EBV diseases observed in patients with this disorder suggest that additional presentations of EBV disease may be associated with GATA2 deficiency in the future. In addition, polymorphisms in the GATA2 gene or its regulatory elements might be associated with less severe presentations of EBV disease. Thus, additional studies of GATA2 should provide insights into the role of this gene in control of EBV and other infectious diseases.

AUTHOR CONTRIBUTIONS

The author confirms being the sole contributor of this work and approved it for publication.

ACKNOWLEDGMENTS

This work was supported by the Intramural Research Program of the National Institute of Allergy and Infectious Diseases. We thank Anita Mora for helping with the figures.

REFERENCES

1. Gao J, Chen YH, Peterson LC. GATA family transcriptional factors: emerging suspects in hematologic disorders. *Exp Hematol Oncol* (2015) 4:28. doi:10.1186/s40164-015-0024-z
2. Crispino JD, Horwitz MS. GATA factor mutations in hematologic disease. *Blood* (2017) 129:2103–10. doi:10.1182/blood-2016-09-687889
3. Vicente C, Conchillo A, García-Sánchez MA, Odero MD. The role of the GATA2 transcription factor in normal and malignant hematopoiesis. *Crit Rev Oncol Hematol* (2012) 82:1–17. doi:10.1016/j.critrevonc.2011.04.007
4. Tsai FY, Keller G, Kuo FC, Weiss M, Chen J, Rosenblatt M, et al. An early haematopoietic defect in mice lacking the transcription factor GATA-2. *Nature* (1994) 371:221–6. doi:10.1038/371221a0
5. Rodrigues NP, Janzen V, Forkert R, Dombkowski DM, Boyd AS, Orkin SH, et al. Haploinsufficiency of GATA-2 perturbs adult hematopoietic stem-cell homeostasis. *Blood* (2005) 106:477–84. doi:10.1182/blood-2004-08-2989
6. Ling KW, Ottersbach K, van Hamburg JP, Oziemlak A, Tsai FY, Orkin SH, et al. GATA-2 plays two functionally distinct roles during the ontogeny of hematopoietic stem cells. *J Exp Med* (2004) 200:871–82. doi:10.1084/jem.20031556
7. Rodrigues NP, Boyd AS, Fugazza C, May GE, Guo Y, Tipping AJ, et al. GATA-2 regulates granulocyte-macrophage progenitor cell function. *Blood* (2008) 112:4862–73. doi:10.1182/blood-2008-01-136564
8. Collin M, Dickinson R, Bigley V. Haematopoietic and immune defects associated with GATA2 mutation. *Br J Haematol* (2015) 169:173–87. doi:10.1111/bjh.13317
9. Hsu AP, McReynolds LJ, Holland SM. GATA2 deficiency. *Curr Opin Allergy Clin Immunol* (2015) 15:104–9. doi:10.1097/ACI.0000000000000126
10. Mir MA, Kochuparambil ST, Abraham RS, Rodriguez V, Howard M, Hsu AP, et al. Spectrum of myeloid neoplasms and immune deficiency associated with germline GATA2 mutations. *Cancer Med* (2015) 4:490–9. doi:10.1002/cam4.384
11. Spinner MA, Sanchez LA, Hsu AP, Shaw PA, Zerbe CS, Calvo KR, et al. GATA2 deficiency: a protean disorder of hematopoiesis, lymphatics, and immunity. *Blood* (2014) 123:809–21. doi:10.1182/blood-2013-07-515528
12. Hsu AP, Johnson KD, Falcone EL, Sanalkumar R, Sanchez L, Hickstein DD, et al. GATA2 haploinsufficiency caused by mutations in a conserved intronic element leads to MonoMAC syndrome. *Blood* (2013) 121:3830–7. doi:10.1182/blood-2012-08-452763
13. Dickinson RE, Griffin H, Bigley V, Reynard LN, Hussain R, Haniffa M, et al. Exome sequencing identifies GATA-2 mutation as the cause of dendritic cell, monocyte, B and NK lymphoid deficiency. *Blood* (2011) 118:2656–8. doi:10.1182/blood-2011-06-360313
14. Taylor GS, Long HM, Brooks JM, Rickinson AB, Hislop AD. The immunology of Epstein-Barr virus-induced disease. *Annu Rev Immunol* (2015) 33:787–821. doi:10.1146/annurev-immunol-032414-112326

15. Li L, Liu D, Hutt-Fletcher L, Morgan A, Masucci MG, Levitsky V. Epstein-Barr virus inhibits the development of dendritic cells by promoting apoptosis of their monocyte precursors in the presence of granulocyte macrophage-colony-stimulating factor and interleukin-4. *Blood* (2002) 99:3725–34. doi:10.1182/blood.V99.10.3725
16. Dickinson RE, Milne P, Jardine L, Zandi S, Swierczek SI, McGovern N, et al. The evolution of cellular deficiency in GATA2 mutation. *Blood* (2014) 123:863–74. doi:10.1182/blood-2013-07-517151
17. de Waal Malefyt R, Haanen J, Spits H, Roncarolo MG, te Velde A, Figdor C, et al. Interleukin 10 (IL-10) and viral IL-10 strongly reduce antigen-specific human T cell proliferation by diminishing the antigen-presenting capacity of monocytes via downregulation of class II major histocompatibility complex expression. *J Exp Med* (1991) 174:915–24. doi:10.1084/jem.174.4.915
18. Salek-Ardakani S, Arrand JR, Mackett M. Epstein-Barr virus encoded interleukin-10 inhibits HLA-class I, ICAM-1, and B7 expression on human monocytes: implications for immune evasion by EBV. *Virology* (2002) 304:342–51. doi:10.1006/viro.2002.1716
19. Chou J, Lutskiy M, Tsitsikov E, Notarangelo LD, Geha RS, Dioun A. Presence of hypogammaglobulinemia and abnormal antibody responses in GATA2 deficiency. *J Allergy Clin Immunol* (2014) 134:223–6. doi:10.1016/j.jaci.2014.01.041
20. Ganapathi KA, Townsley DM, Hsu AP, Arthur DC, Zerbe CS, Cuellar-Rodriguez J, et al. GATA2 deficiency-associated bone marrow disorder differs from idiopathic aplastic anemia. *Blood* (2015) 125:56–70. doi:10.1182/blood-2014-06-580340
21. Mace EM, Hsu AP, Monaco-Shawver L, Makedonas G, Rosen JB, Dropulic L, et al. Mutations in GATA2 cause human NK cell deficiency with specific loss of the CD56(bright) subset. *Blood* (2013) 121:2669–77. doi:10.1182/blood-2012-09-453969
22. Chijioke O, Landtwing V, Münz C. NK cell influence on the outcome of primary Epstein-Barr virus infection. *Front Immunol* (2016) 7:323. doi:10.3389/fimmu.2016.00323
23. Strowig T, Brilot F, Arrey F, Bougras G, Thomas D, Muller WA, et al. Tonsilar NK cells restrict B cell transformation by the Epstein-Barr virus via IFN-gamma. *PLoS Pathog* (2008) 4(2):e27. doi:10.1371/journal.ppat.0040027
24. Poole E, Walther A, Raven K, Benedict CA, Mason GM, Sinclair J. The myeloid transcription factor GATA-2 regulates the viral UL144 gene during human cytomegalovirus latency in an isolate-specific manner. *J Virol* (2013) 87:4261–71. doi:10.1128/JVI.03497-12
25. Poole E, McGregor Dallas SR, Colston J, Joseph RS, Sinclair J. Virally induced changes in cellular microRNAs maintain latency of human cytomegalovirus in $CD34^+$ progenitors. *J Gen Virol* (2011) 92:1539–49. doi:10.1099/vir.0.031377-0
26. Poole E, Sinclair J. Sleepless latency of human cytomegalovirus. *Med Microbiol Immunol* (2015) 204:421–9. doi:10.1007/s00430-015-0401-6
27. Spencer JV, Lockridge KM, Barry PA, Lin G, Tsang M, Penfold ME, et al. Potent immunosuppressive activities of cytomegalovirus-encoded interleukin-10. *J Virol* (2002) 76:1285–92. doi:10.1128/JVI.76.3.1285-1292.2002
28. Jochum S, Moosmann A, Lang S, Hammerschmidt W, Zeidler R. The EBV immunoevasins vIL-10 and BNLF2a protect newly infected B cells from immune recognition and elimination. *PLoS Pathog* (2012) 8:e1002704. doi:10.1371/journal.ppat.1002704
29. Cohen JI, Dropulic L, Hsu AP, Zerbe CS, Krogmann T, Dowdell K, et al. Association of GATA2 deficiency with severe primary Epstein-Barr virus (EBV) infection and EBV-associated cancers. *Clin Infect Dis* (2016) 63:41–7. doi:10.1093/cid/ciw160
30. Kazamel M, Klein CJ, Benarroch EE, Patnaik MM, Tracy JA. Subacute demyelinating polyradiculoneuropathy complicating Epstein-Barr virus infection in GATA2 haploinsufficiency. *Muscle Nerve* (2017) 57:150–6. doi:10.1002/mus.25581
31. Grossman J, Cuellar-Rodriguez J, Gea-Banacloche J, Zerbe C, Calvo K, Hughes T, et al. Nonmyeloablative allogeneic hematopoietic stem cell transplantation for GATA2 deficiency. *Biol Blood Marrow Transplant* (2014) 20:1940–8. doi:10.1016/j.bbmt.2014.08.004
32. Camargo JF, Lobo SA, Hsu AP, Zerbe CS, Wormser GP, Holland SM. MonoMAC syndrome in a patient with a GATA2 mutation: case report and review of the literature. *Clin Infect Dis* (2013) 57:697–9. doi:10.1093/cid/cit368
33. Parta M, Cuellar-Rodriguez J, Freeman AF, Gea-Banacloche J, Holland SM, Hickstein DD. Resolution of multifocal Epstein-Barr virus-related smooth muscle tumor in a patient with GATA2 deficiency following hematopoietic stem cell transplantation. *J Clin Immunol* (2017) 37:61–6. doi:10.1007/s10875-016-0360-8
34. Mutsaers PG, van de Loosdrecht AA, Tawana K, Bödör C, Fitzgibbon J, Menko FH. Highly variable clinical manifestations in a large family with a novel GATA2 mutation. *Leukemia* (2013) 27:2247–8. doi:10.1038/leu.2013.105
35. Svobodova T, Mejstrikova E, Salzer U, Sukova M, Hubacek P, Matej R, et al. Diffuse parenchymal lung disease as first clinical manifestation of GATA-2 deficiency in childhood. *BMC Pulm Med* (2015) 15:8. doi:10.1186/s12890-015-0006-2
36. Cuellar-Rodriguez J, Gea-Banacloche J, Freeman AF, Hsu AP, Zerbe CS, Calvo KR, et al. Successful allogeneic hematopoietic stem cell transplantation for GATA2 deficiency. *Blood* (2011) 118:3715–20. doi:10.1182/blood-2011-06-365049
37. Cohen JI, Jaffe ES, Dale JK, Pittaluga S, Heslop HE, Rooney CM, et al. Characterization and treatment of chronic active Epstein-Barr virus disease: a 28-year experience in the United States. *Blood* (2011) 117:5835–49. doi:10.1182/blood-2010-11-316745
38. Leen AM, Bollard CM, Mendizabal AM, Shpall EJ, Szabolcs P, Antin JH, et al. Multicenter study of banked third-party virus-specific T cells to treat severe viral infections after hematopoietic stem cell transplantation. *Blood* (2013) 121:5113–23. doi:10.1182/blood-2013-02-486324
39. Riordan JD, Nadeau JH. From peas to disease: modifier genes, network resilience, and the genetics of health. *Am J Hum Genet* (2017) 101:177–91. doi:10.1016/j.ajhg.2017.06.004

Conflict of Interest Statement: The author declares that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

frontiers in Immunology

MINI REVIEW
published: 22 December 2017
doi: 10.3389/fimmu.2017.01867

Chronic Active Epstein–Barr Virus Disease

*Hiroshi Kimura[1] and Jeffrey I. Cohen[2]**

[1] Department of Virology, Nagoya University Graduate School of Medicine, Nagoya, Japan, [2] Medical Virology Section, Laboratory of Infectious Diseases, National Institute of Allergy and Infectious Diseases, National Institutes of Health, Bethesda, MD, United States

Chronic active Epstein–Barr virus (CAEBV) disease is a rare disorder in which persons are unable to control infection with the virus. The disease is progressive with markedly elevated levels of EBV DNA in the blood and infiltration of organs by EBV-positive lymphocytes. Patients often present with fever, lymphadenopathy, splenomegaly, EBV hepatitis, or pancytopenia. Over time, these patients develop progressive immunodeficiency and if not treated, succumb to opportunistic infections, hemophagocytosis, multiorgan failure, or EBV-positive lymphomas. Patients with CAEBV in the United States most often present with disease involving B or T cells, while in Asia, the disease usually involves T or NK cells. The only proven effective treatment for the disease is hematopoietic stem cell transplantation. Current studies to find a cause of this disease focus on immune defects and genetic abnormalities associated with the disease.

Keywords: chronic active Epstein–Barr virus, Epstein–Barr virus lymphoma, infectious mononucleosis, hemophagocytosis, DDX3X

OPEN ACCESS

Edited by:
Stuart G. Tangye, Garvan Institute of Medical Research, Australia

Reviewed by:
Umaimainthan Palendira, Centenary Institute Australia, Australia
Claire Shannon-Lowe, University of Birmingham, United Kingdom

****Correspondence:***
Jeffrey I. Cohen
jcohen@niaid.nih.gov

Specialty section:
This article was submitted to Primary Immunodeficiencies, a section of the journal Frontiers in Immunology

Received: *09 September 2017*
Accepted: *08 December 2017*
Published: *22 December 2017*

Citation:
Kimura H and Cohen JI (2017) Chronic Active Epstein–Barr Virus Disease. Front. Immunol. 8:1867. doi: 10.3389/fimmu.2017.01867

INTRODUCTION

Primary infection of adolescents and young adults often results in infectious mononucleosis with fever, lymphadenopathy, and sore throat (1). Additional signs and symptoms include splenomegaly, hepatomegaly, lymphocytosis, and liver dysfunction. Fever and lymphadenopathy usually resolve within 2 weeks after onset but can persist for a month, or in rare cases even longer. EBV is present in circulating B cells, and the level of EBV DNA is elevated in the blood for the first month of the illness. Both the innate immune response (especially NK cells) and the acquired immune response (virus-specific CD4 and CD8 cells) have a critical role in clearing the infection (2).

Initial control of EBV in healthy persons involves NK cells that can kill virus-infected cells (3, 4) and secrete IFN-γ, which inhibits B cell proliferation, and monocytes, which release chemokines in response to virus infection (5). A large clonal or oligoclonal expansion of CD8 cells is observed during infectious mononucleosis (6). Most CD8 cells are directed to lytic antigens initially, and these cells rapidly undergo apoptosis (7). These patients have modestly elevated antibodies to EBV lytic antigens as well as antibodies to the EBV nuclear antigens (EBNAs), including EBNA1.

Rare patients who become infected with EBV, or reactivate EBV, develop disease that does not resolve. Some of these patients develop fulminant infectious mononucleosis and die within days or weeks of primary infection. Others develop a more chronic course with persistent or intermittent infectious mononucleosis-like symptoms including fever, persistent lymphadenopathy, splenomegaly, and EBV hepatitis. These patients are unable to control EBV infection and have infiltration of tissues by EBV positive T, NK, or less often B cells. They have markedly elevated levels of EBV

that persist in the blood. This entity is referred to as chronic active EBV (CAEBV) disease.

Some patients with CAEBV have been reported to have impaired NK cell (8) or T cell activity (9–13) against EBV-infected cells. In addition, reduced numbers of EBV-specific T cells have been described in patients with CAEBV disease (10). Unlike healthy persons with infectious mononucleosis, patients with CAEBV disease often have low numbers of EBV-specific CD8 cells (10). A recent study showed that patients with CAEBV or infectious mononucleosis have a decrease in the TCR-beta repertoire and expanded T cell clones in their peripheral blood compared with healthy carriers of EBV (14). Many have extremely high levels of antibodies to EBV lytic proteins and lack antibody to EBNA1 (13).

CAEBV DEFINITION AND FEATURES

Chronic active Epstein–Barr virus disease is usually defined as a chronic illness lasting at least 6 months, an increased EBV level in either the tissue or the blood, and lack of evidence of a known underlying immunodeficiency (15). Other authors, particularly when defining severe CAEBV disease, require both an elevated level of EBV in the blood as well as infiltration of tissues by EBV-positive lymphocytes (16). Recently, the duration of illness required for defining the disease has been shortened to 3 months (17). Former definitions required elevated levels of antibody to EBV viral capsid or early antigen in the blood (18); however, we have found that elevated levels of EBV DNA in the blood are more specific for CAEBV than elevated levels of EBV antibodies. Most laboratories now perform ELISA tests for EBV antibodies, and these are often less helpful than the previously used quantitative immunofluorescent assay using endpoint dilution of serum. It is important that DNA PCR is done using either whole blood or peripheral blood mononuclear cells, rather than plasma or serum which is much less sensitive for diagnosis of CAEBV disease.

Chronic active Epstein–Barr virus disease was originally reported in children during primary infection, but in recent years, perhaps with increasing recognition of the disease, CAEBV disease has been reported in adults as well (19). CAEBV disease may be indolent with episodic fever, lymphadenopathy, and viral hepatitis followed by periods that are nearly asymptomatic; however, during these asymptomatic periods, the Epstein–Barr viral load remains very elevated. Alternatively, the disease can have a persistent or even fulminant presentation with death occurring in a few weeks. CAEBV disease is more frequent in Asians and in persons from South and Central America and Mexico. In these patients, EBV is predominantly present in T cells (**Figure 1**) or NK cells (20). In contrast, patients from the United States with CAEBV more often have EBV in B or T cells (16). In most healthy persons, EBV is latent in B cells; however, EBV can sometimes be detected in T and NK cells in the tonsils (21), and virus has been detected in T cells in persons with HIV (22) and other lymphoproliferative diseases (23, 24). At present, it is unclear how the virus enters T and NK cells; these cells do not express CD21, the EBV receptor.

Epstein–Barr virus gene expression in patients with CAEBV disease varies. There are four patterns of EBV gene expression, ranging from type 0 with no viral proteins expressed, although EBV EBV-encoded RNA and BART RNAs are expressed, to type 3 with all the latent viral proteins expressed including the EBV nuclear antigens (EBNAs) 1, 2, 3A–C, and LP, and latent membrane proteins (LMP) 1 and 2. Type 1 latency involves expression of EBNA1 and no other proteins; with type 2 latency, EBNA1, LMP1, and LMP2 are expressed. Patients with infectious mononucleosis have type 3 latency, whereas healthy EBV carriers have type 0 latency. Type 1 latency is seen in Burkitt lymphoma and type 2 in nasopharyngeal carcinoma, Hodgkin lymphoma, peripheral T cell lymphoma, angioimmunoblastic T cell lymphoma, and extranodal NK/T cell lymphoma (25). Most patients with CAEBV disease express a limited number of EBV latency genes. Although many patients have been reported with a type 2 latency pattern (26, 27), other patterns of EBV gene expression have also been reported, including type 3 (28). Thus, patients with T and NK cell CAEBV have a latency pattern that resembles that seen in EBV-positive T cell and NK cell lymphomas. These findings are consistent with a recent study showing that the cellular gene expression profile in patients with NK cell CAEBV is similar to that in NK cell lymphoma (29).

Epstein–Barr virus can be clonal, oligoclonal, or polyclonal in peripheral blood mononuclear cells of patients with CAEBV

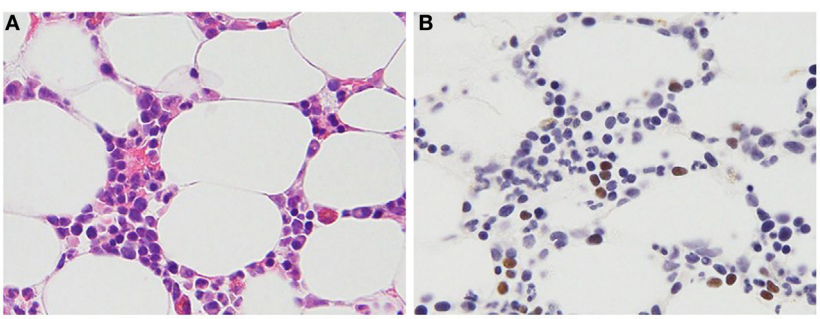


FIGURE 1 | Histopathologic features of a 47-year-old female patient with T cell chronic active Epstein–Barr virus (EBV) disease. **(A)** Hematoxylin and eosin stain. Small- to medium-sized lymphocytes without significant atypia infiltrate the bone marrow clot. **(B)** EBV-encoded RNA *in situ* hybridization. The brown staining lymphocytes are positive for EBV RNA.

disease. Clonality for CAEBV has been based on PCR of the T cell receptor genes (for T cell CAEBV) or IgH genes (for EBV B cell disease) (16) or on the terminal repeat structure of the EBV genome (20). In one study of 17 patients, most patients had clonal EBV (27). Clonality does not necessarily indicate a worse prognosis (20).

Cells from patients with CAEBV can express both T-helper (TH1) (e.g., interferon-γ, IL-1β, IL-2) and TH2 (IL-4, IL-10, IL-13) cytokines (30). This failure to express a predominantly antiviral TH1 pattern has been referred to as an "unbalanced cytokine profile." Patients with NK cell CAEBV disease were reported to have higher levels of IL-13 than those with T cell disease (27). Plasma levels of certain EBV microRNAs expressed from the BamH1 A fragment rightward transcript (BART) are higher in persons with CAEBV disease than in those with infectious mononucleosis or healthy controls (31). These findings suggest that these may be biomarkers useful for following these patients.

ETIOLOGY

Initial reports suggested that CAEBV disease may be due to an unusual strain of EBV that results in lytic replication, but is impaired for transformation (32, 33), or a strain with a deletion in the viral genome (34). However, a subsequent study by one of these groups (35) showed that the unaffected father of the patient with CAEBV disease and some healthy controls had the same lytic strain of the virus as the patient with CAEBV, indicating that the unusual strain of EBV was not the cause of the disease.

Several features of CAEBV suggest that there is likely a genetic etiology. First, the impaired cytotoxic activity of T or NK cells (cited above) suggests that the disease could be due to an immunodeficiency. Second, the increased rate of the disease in Asians or natives of Central or South America suggests that the genetic background may play a role in the disease.

One study reported CAEBV in family members (36); however most recent cases do not describe multiple family members with the disease (16, 37). Studies have not found a consistent cause for CAEBV disease. Patients with meeting the definition of CAEBV B cell disease were subsequently found to have compound heterozygous mutations in perforin (38), compound heterozygous mutations in Munc13-4 (39), homozygous or compound heterozygous mutations in Munc 18-2 (39, 40), a heterozygous gain-of-function mutation in phosphoinositide 3-kinase p110δ (41), a mutation in MAGT1 (42), a mutation in GATA2 (43), and homozygous mutations in *CTPS1* (44). In each of the patients tested, EBV was predominantly in B cells. At present, no single genetic defect has been associated with a large proportion of patients with CAEBV disease.

Recent comprehensive genetic analysis by whole-exome sequencing showed that germline mutations are rare in CAEBV, but somatic driver mutations are frequently found in EBV-infected cells (45). Driver mutations including DDX3X and other genes associated with hematologic malignancies have been shown to accumulate in EBV-infected T/NK cells. In a case in which serial samples were obtained, clonal evolution of EBV-infected cells was confirmed with branching mutations in DDX3X. Mutations in DDX3X are frequently seen in Burkitt lymphoma and extranodal NK/T cell lymphoma (46, 47). These results indicate that serial acquisition of mutations in EBV-infected NK or T cells have the potential to result in transformation of the cells and may contribute to lymphomagenesis in this disease.

Although no single genetic defect has been identified in CAEBV disease, a positive association with human leukocyte antigen (HLA) A26 and a negative association with B52 were observed (48). Interestingly, both the A26 and B52 alleles are frequently seen in East Asia and Mexico, where the prevalence of the disease is high. Associations with HLA loci have been reported in other EBV-associated malignancies that show geographically distinct distributions (49, 50).

CAEBV IN THE UNITED STATES

In the largest series of CAEBV reported in the United States, EBV was often detected in B cells in tissues from patients, with cases of T and NK cell disease less common (16). The age of onset ranged from 4 to 51 years (mean 19 years). Patients with T cell disease were younger (mean age 7 years) than those with B cell disease (mean age 23 years). Lymphadenopathy and splenomegaly were the most frequent signs and symptoms, followed by fever, hepatitis, hypogammaglobulinemia, pancytopenia, hemophagocytosis, and hepatomegaly. Less common symptoms included pneumonitis, central nervous system disease, and periphery neuropathy. Some patients had B cell lymphopenia, others had reduced numbers of NK cells, and some had low numbers of both cells. Deaths were most often due to progressive EBV lymphoproliferative disease or opportunistic infections.

CAEBV IN ASIA

T or NK cell CAEBV has a geographical predisposition, with most cases occurring in East Asians and some cases in Native American populations in the Western hemisphere (16). This distribution is analogous to that of extranodal NK/T cell lymphoma, also referred to as nasal NK/T-cell lymphoma. In Japan, nearly 60% of cases of CAEBV are T cell type, while 40% are NK cell type (37). EBV-infected T cells are variable: $CD4^+$ T cells, $CD8^+$ T cells, $CD4^+$ and $CD8^+$ T cells, $CD4^-$ and $CD8^-$ T cells, and γδ T cells have all been reported as the predominant cell type in individual patients with CAEBV. EBV-infected T or NK cells usually express cytotoxic molecules, such as perforin, granzyme, and T-cell intracytoplasmic antigen (TIA)-1 (51, 52), indicating that they have a cytotoxic cell phenotype.

The age at the onset of CAEBV in Asia ranged from 9 months to 53 years (mean, 11.3 years) (20). The signs and symptoms of CAEBV differ in frequency in the US and in Asia (**Table 1**). Typically in Asia, patients develop fever, hepatosplenomegaly, and lymphadenopathy; other common symptoms are thrombocytopenia, anemia, skin rash, diarrhea, and uveitis (20). The disease is sometimes complicated by hemophagocytic syndrome, coagulopathy, digestive tract ulcer/perforation, central nervous system involvement, myocarditis, interstitial pneumonia, multiorgan failure and sepsis (20). Interstitial pneumonia, calcifications in basal ganglia, and coronary aneurysms are occasionally seen without any symptoms. Some patients may have skin symptoms,

TABLE 1 | Comparison of signs and symptoms of CAEBV disease in the US and Japan.

Sign or symptom	US (%)[a]	Japan (%)[b]
Lymphadenopathy	79	40
Splenomegaly	68	73
Fever	47	93
Hepatitis	47	67
Pancytopenia	42	NR
Hypogammaglobulinemia	42	NR
Hepatomegaly	32	79
Hemophagocytosis	32	24
Interstitial pneumonia	26	5
CNS disease	21	9
Neuropathy	21	NR
Rash	21	26
Hypersensitivity to mosquito bite	0	13
Hydroa vacciniforme	5	10

NR, not reported.
[a]*Ref. (16).*
[b]*Ref. (20).*

such as hypersensitivity to mosquito bites and hydroa vacciniforme. Patients with severe mosquito bite allergy generally have EBV-infected NK cells, whereas those with hydroa vacciniforme often have EBV-infected γδ T cells (37). Patients with CAEBV sometimes develop T or NK cell neoplasms such as extranodal NK/T cell lymphoma, aggressive NK cell leukemia, and peripheral T cell lymphoma (37).

TREATMENT AND PROGNOSIS

In the absence of treatment, patients with CAEBV develop progressive cellular and humoral immunodeficiencies and develop opportunistic infections, hemophagocytosis, multi-organ failure, or EBV-positive B, T, or NK cell lymphomas (53). CAEBV is refractory to antiviral therapy, interferon, intravenous immunoglobulin, and conventional chemotherapy and thus has a poor prognosis. Many other treatments have been tried including immunosuppressive agents such as cyclosporine or corticosteroids, autologous EBV-specific cytotoxic T cells, rituximab in the case of B cell CAEV, and the combination of bortezomib and ganciclovir. In some cases, these other treatments have resulted in transient reductions in systemic symptoms with improvement in laboratory abnormalities; however, the disease eventually returns and patients succumb to their disease if they do not undergo hematopoietic stem cell transplantation.

The survival of patients with T cell-type CAEBV is significantly lower, compared with that of patients with NK cell-type CAEBV (20). Hematopoietic stem cell transplantation alone is a curative treatment for the disease, although the incidence of transplantation-related complications is high (54, 55).

AUTHOR CONTRIBUTIONS

All authors listed have made a substantial, direct, and intellectual contribution to the work and approved it for publication.

ACKNOWLEDGMENTS

This work was supported by the Intramural Research Program of the National Institute of Allergy and Infectious Diseases. We thank Dr. Seiichi Kato, Aichi Cancer Institute, Japan for the photomicrograph of the pathology slides.

REFERENCES

1. Balfour HH Jr, Holman CJ, Hokanson KM, Lelonek MM, Giesbrecht JE, White DR, et al. A prospective clinical study of Epstein-Barr virus and host interactions during acute infectious mononucleosis. *J Infect Dis* (2005) 192:1505–12. doi:10.1086/491740
2. Taylor GS, Long HM, Brooks JM, Rickinson AB, Hislop AD. The immunology of Epstein-Barr virus-induced disease. *Annu Rev Immunol* (2015) 33:787–821. doi:10.1146/annurev-immunol-032414-112326
3. Djaoud Z, Guethlein LA, Horowitz A, Azzi T, Nemat-Gorgani N, Olive D, et al. Two alternate strategies for innate immunity to Epstein-Barr virus: one using NK cells and the other NK cells and γδ T cells. *J Exp Med* (2017) 214:1827–41. doi:10.1084/jem.20161017
4. Chijioke O, Landtwing V, Münz C. NK cell influence on the outcome of primary Epstein-Barr virus infection. *Front Immunol* (2016) 7:323. doi:10.3389/fimmu.2016.00323
5. Gaudreault E, Fiola S, Olivier M, Gosselin J. Epstein-Barr virus induces MCP-1 secretion by human monocytes via TLR2. *J Virol* (2007) 81:8016–24. doi:10.1128/JVI.00403-07
6. Callan MF, Steven N, Krausa P, Wilson JD, Moss PA, Gillespie GM, et al. Large clonal expansions of CD8+ T cells in acute infectious mononucleosis. *Nat Med* (1996) 2:906–11. doi:10.1038/nm0896-906
7. Callan MF, Fazou C, Yang H, Rostron T, Poon K, Hatton C, et al. CD8(+) T-cell selection, function, and death in the primary immune response in vivo. *J Clin Invest* (2000) 106:1251–61. doi:10.1172/JCI10590
8. Joncas J, Monczak Y, Ghibu F, Alfieri C, Bonin A, Ahronheim G, et al. Brief report: killer cell defect and persistent immunological abnormalities in two patients with chronic active Epstein-Barr virus infection. *J Med Virol* (1989) 28:110–7. doi:10.1002/jmv.1890280211
9. Fujieda M, Wakiguchi H, Hisakawa H, Kubota H, Kurashige T. Defective activity of Epstein-Barr virus (EBV) specific cytotoxic T lymphocytes in children with chronic active EBV infection and in their parents. *Acta Paediatr Jpn* (1993) 35:394–9. doi:10.1111/j.1442-200X.1993.tb03079.x
10. Sugaya N, Kimura H, Hara S, Hoshino Y, Kojima S, Morishima T, et al. Quantitative analysis of Epstein-Barr virus (EBV)-specific CD8+ T cells in patients with chronic active EBV infection. *J Infect Dis* (2004) 190:985–8. doi:10.1086/423285
11. Tsuge I, Morishima T, Kimura H, Kuzushima K, Matsuoka H. Impaired cytotoxic T lymphocyte response to Epstein-Barr virus-infected NK cells in patients with severe chronic active EBV infection. *J Med Virol* (2001) 64:141–8. doi:10.1002/jmv.1029
12. Kimura H, Tsuge I, Imai S, Yamamoto M, Kuzushima K, Osato T, et al. Intact antigen presentation for Epstein-Barr virus (EBV)-specific CTL by a lymphoblastoid cell line established from a patient with severe chronic active EBV infection. *Med Microbiol Immunol* (1995) 184:63–8. doi:10.1007/BF00221388
13. Xing Y, Song HM, Wei M, Liu Y, Zhang YH, Gao L. Clinical significance of variations in levels of Epstein-Barr virus (EBV) antigen and adaptive immune response during chronic active EBV infection in children. *J Immunotoxicol* (2013) 10:387–92. doi:10.3109/1547691X.2012.758199
14. Liu S, Zhang Q, Huang D, Zhang W, Zhong F, Feng J, et al. Comprehensive assessment of peripheral blood TCRβ repertoire in infectious mononucleosis and chronic active EBV infection patients. *Ann Hematol* (2017) 96:665–80. doi:10.1007/s00277-016-2911-8
15. Kimura H. Pathogenesis of chronic active Epstein-Barr virus infection: is this an infectious disease, lymphoproliferative disorder, or immunodeficiency? *Rev Med Virol* (2006) 16:251–61. doi:10.1002/rmv.505
16. Cohen JI, Jaffe ES, Dale JK, Pittaluga S, Heslop HE, Rooney CM, et al. Characterization and treatment of chronic active Epstein-Barr virus

disease: a 28-year experience in the United States. *Blood* (2011) 117:5835–49. doi:10.1182/blood-2010-11-316745
17. Quintanilla-Martinez L, Ko YH, Kimura H, Jaffe ES. EBV-positive T-cell and NK-cell lymphoproliferative diseases of childhood. 4th ed. In: Swerdlow SH, Campo E, Harris NL, Jaffe ES, Pileri SA, Stein H, et al., editors. *WHO Classification of Tumours of Haematopoietic and Lymphoid Tissues*. Lyon, France: IARC Press (2017). p. 355–62.
18. Okano M, Kawa K, Kimura H, Yachie A, Wakiguchi H, Maeda A, et al. Proposed guidelines for diagnosing chronic active Epstein-Barr virus infection. *Am J Hematol* (2005) 80:64–9. doi:10.1002/ajh.20398
19. Arai A, Imadome K, Watanabe Y, Yoshimori M, Koyama T, Kawaguchi T, et al. Clinical features of adult-onset chronic active Epstein-Barr virus infection: a retrospective analysis. *Int J Hematol* (2011) 93:602–9. doi:10.1007/s12185-011-0831-x
20. Kimura H, Morishima T, Kanegane H, Ohga S, Hoshino Y, Maeda A, et al. Prognostic factors for chronic active Epstein-Barr virus infection. *J Infect Dis* (2003) 187:527–33. doi:10.1086/367988
21. Hudnall SD, Ge Y, Wei L, Yang NP, Wang HQ, Chen T. Distribution and phenotype of Epstein-Barr virus-infected cells in human pharyngeal tonsils. *Mod Pathol* (2005) 18:519–27. doi:10.1038/modpathol.3800369
22. Bekker V, Scherpbier H, Beld M, Piriou E, van Breda A, Lange J, et al. Epstein-Barr virus infects B and non-B lymphocytes in HIV-1-infected children and adolescents. *J Infect Dis* (2006) 194:1323–30. doi:10.1086/508197
23. Pallesen G, Hamilton-Dutoit SJ, Zhou X. The association of Epstein-Barr virus (EBV) with T-cell lymphoproliferations and Hodgkin's disease: two new developments in the EBV field. *Adv Cancer Res* (1993) 62:179–239. doi:10.1016/S0065-230X(08)60319-X
24. Calattini S, Sereti I, Scheinberg P, Kimura H, Childs RW, Cohen JI. Detection of EBV genomes in plasmablasts/plasma cells and non-B cells in the blood of most patients with EBV lymphoproliferative disorders by using immuno-FISH. *Blood* (2010) 116:4546–59. doi:10.1182/blood-2010-05-285452
25. Longnecker L, Kieff E, Cohen JI. Epstein-Barr virus. 6th ed. In: Knipe DM, Howley PM, Cohen JI, Griffith DE, Lamb RA, Martin MA, et al., editors. *Fields Virology*. Philadelphia: Lippincott Williams & Wilkins (2013). p. 1898–959.
26. Iwata S, Wada K, Tobita S, Gotoh K, Ito Y, Demachi-Okamura A, et al. Quantitative analysis of Epstein-Barr virus (EBV)-related gene expression in patients with chronic active EBV infection. *J Gen Virol* (2010) 91:42–50. doi:10.1099/vir.0.013482-0
27. Kimura H, Hoshino Y, Hara S, Sugaya N, Kawada J, Shibata Y, et al. Differences between T cell-type and natural killer cell-type chronic active Epstein-Barr virus infection. *J Infect Dis* (2005) 191:531–9. doi:10.1086/427239
28. Yoshioka M, Ishiguro N, Ishiko H, Ma X, Kikuta H, Kobayashi K. Heterogeneous, restricted patterns of Epstein-Barr virus (EBV) latent gene expression in patients with chronic active EBV infection. *J Gen Virol* (2001) 82:2385–92. doi:10.1099/0022-1317-82-10-2385
29. Washio K, Oka T, Abdalkader L, Muraoka M, Shimada A, Oda M, et al. Gene expression analysis of hypersensitivity to mosquito bite, chronic active EBV infection and NK/T-lymphoma/leukemia. *Leuk Lymphoma* (2017) 58:2683–94. doi:10.1080/10428194.2017.1304762
30. Ohga S, Nomura A, Takada H, Ihara K, Kawakami K, Yanai F, et al. Epstein-Barr virus (EBV) load and cytokine gene expression in activated T cells of chronic active EBV infection. *J Infect Dis* (2001) 183:1–7. doi:10.1086/317653
31. Kawano Y, Iwata S, Kawada J, Gotoh K, Suzuki M, Torii Y, et al. Plasma viral microRNA profiles reveal potential biomarkers for chronic active Epstein-Barr virus infection. *J Infect Dis* (2013) 208:771–9. doi:10.1093/infdis/jit222
32. Alfieri C, Ghibu F, Joncas JH. Lytic, nontransforming Epstein-Barr virus (EBV) from a patient with chronic active EBV infection. *Can Med Assoc J* (1984) 131:1249–52.
33. Schwarzmann F, von Baehr R, Jager M, Prang N, Böhm S, Reischl U, et al. A case of severe chronic active infection with Epstein-Barr virus: immunologic deficiencies associated with a lytic virus strain. *Clin Infect Dis* (1999) 29:626–31. doi:10.1086/598645
34. Schooley RT, Carey RW, Miller G, Henle W, Eastman R, Mark EJ, et al. Chronic Epstein-Barr virus infection associated with fever and interstitial pneumonitis. Clinical and serologic features and response to antiviral chemotherapy. *Ann Intern Med* (1986) 104:636–43. doi:10.7326/0003-4819-104-5-636
35. Alfieri C, Joncas JH. Biomolecular analysis of a defective nontransforming Epstein-Barr virus (EBV) from a patient with chronic active EBV infection. *J Virol* (1987) 61:3306–9.
36. Joncas JH, Ghibu F, Blagdon M, Montplaisir S, Stefanescu I, Menezes J. A familial syndrome of susceptibility to chronic active Epstein-Barr virus infection. *Can Med Assoc J* (1984) 130:280–4.
37. Kimura H, Ito Y, Kawabe S, Gotoh K, Takahashi Y, Kojima S, et al. EBV-associated T/NK-cell lymphoproliferative diseases in nonimmunocompromised hosts: prospective analysis of 108 cases. *Blood* (2012) 119:673–86. doi:10.1182/blood-2011-10-381921
38. Katano H, Ali MA, Patera AC, Catalfamo M, Jaffe ES, Kimura H, et al. Chronic active Epstein-Barr virus infection associated with mutations in perforin that impair its maturation. *Blood* (2004) 103:1244–52. doi:10.1182/blood-2003-06-2171
39. Rohr J, Beutel K, Maul-Pavicic A, Vraetz T, Thiel J, Warnatz K, et al. Atypical familial hemophagocytic lymphohistiocytosis due to mutations in UNC13D and STXBP2 overlaps with primary immunodeficiency diseases. *Haematologica* (2010) 95:2080–7. doi:10.3324/haematol.2010.029389
40. Cohen JI, Niemela JE, Stoddard JL, Pittaluga S, Heslop H, Jaffe ES, et al. Late-onset severe chronic active EBV in a patient for five years with mutations in STXBP2 (MUNC18-2) and PRF1 (perforin 1). *J Clin Immunol* (2015) 35:445–8. doi:10.1007/s10875-015-0168-y
41. Lucas CL, Kuehn HS, Zhao F, Niemela JE, Deenick EK, Palendira U, et al. Dominant-activating germline mutations in the gene encoding the PI(3)K catalytic subunit p110δ result in T cell senescence and human immunodeficiency. *Nat Immunol* (2014) 15:88–97. doi:10.1038/ni.2771
42. Li FY, Chaigne-Delalande B, Kanellopoulou C, Davis JC, Matthews HF, Douek DC, et al. Second messenger role for Mg2+ revealed by human T-cell immunodeficiency. *Nature* (2011) 475:471–6. doi:10.1038/nature10246
43. Cohen JI, Dropulic L, Hsu AP, Zerbe CS, Krogmann T, Dowdell K, et al. Association of GATA2 deficiency with severe primary Epstein-Barr virus (EBV) infection and EBV-associated cancers. *Clin Infect Dis* (2016) 63:41–7. doi:10.1093/cid/ciw160
44. Kucuk ZY, Zhang K, Filipovich L, Bleesing JJ. CTP synthase 1 deficiency in successfully transplanted siblings with combined immune deficiency and chronic active EBV infection. *J Clin Immunol* (2016) 36:750–3. doi:10.1007/s10875-016-0332-z
45. Okuno Y, Murata T, Ito Y, Sato Y, Kojima S, Ogawa S, et al. Comprehensive genetic study of chronic active EBV infection. *17th International Symposium on Epstein Barr Virus and Associated Diseases*. Zurich (2016). 114 p. Abstract number EBV2016-1149.
46. Jiang L, Gu ZH, Yan ZX, Zhao X, Xie YY, Zhang ZG, et al. Exome sequencing identifies somatic mutations of DDX3X in natural killer/T-cell lymphoma. *Nat Genet* (2015) 47:1061–6. doi:10.1038/ng.3358
47. Schmitz R, Young RM, Ceribelli M, Jhavar S, Xiao W, Zhang M, et al. Burkitt lymphoma pathogenesis and therapeutic targets from structural and functional genomics. *Nature* (2012) 490:116–20. doi:10.1038/nature11378
48. Ito Y, Suzuki R, Torii Y, Kawa K, Kikuta A, Kojima S. HLA-A*26 and HLA-B*52 are associated with a risk of developing EBV-associated T/NK lymphoproliferative disease. *Blood* (2013). Available from: http://www.bloodjournal.org/content/early/2011/11/16/blood-2011-10-381921/tab-e-letters
49. Hildesheim A, Apple RJ, Chen CJ, Wang SS, Cheng YJ, Klitz W, et al. Association of HLA class I and II alleles and extended haplotypes with nasopharyngeal carcinoma in Taiwan. *J Natl Cancer Inst* (2002) 94:1780–9. doi:10.1093/jnci/94.23.1780
50. Niens M, Jarrett RF, Hepkema B, Nolte IM, Diepstra A, Platteel M, et al. HLA-A*02 is associated with a reduced risk and HLA-A*01 with an increased risk of developing EBV+ Hodgkin lymphoma. *Blood* (2007) 110:3310–5. doi:10.1182/blood-2007-05-086934
51. Ohshima K, Suzumiya J, Shimazaki K, Kato A, Tanaka T, Kanda M, et al. Nasal T/NK cell lymphomas commonly express perforin and Fas ligand: important mediators of tissue damage. *Histopathology* (1997) 31:444–50. doi:10.1046/j.1365-2559.1997.2880887.x
52. Quintanilla-Martinez L, Kumar S, Fend F, Reyes E, Teruya-Feldstein J, Kingma DW, et al. Fulminant EBV(+) T-cell lymphoproliferative disorder following acute/chronic EBV infection: a distinct clinicopathologic syndrome. *Blood* (2000) 96:443–51.

53. Okano M. Overview and problematic standpoints of severe chronic active Epstein-Barr virus infection syndrome. *Crit Rev Oncol Hematol* (2002) 44:273–82. doi:10.1016/S1040-8428(02)00118-X
54. Gotoh K, Ito Y, Shibata-Watanabe Y, Kawada J, Takahashi Y, Yagasaki H, et al. Clinical and virological characteristics of 15 patients with chronic active Epstein-Barr virus infection treated with hematopoietic stem cell transplantation. *Clin Infect Dis* (2008) 46:1525–34. doi:10.1086/587671
55. Kawa K, Sawada A, Sato M, Okamura T, Sakata N, Kondo O, et al. Excellent outcome of allogeneic hematopoietic SCT with reduced-intensity conditioning for the treatment of chronic active EBV infection. *Bone Marrow Transplant* (2011) 46:77–83. doi:10.1038/bmt.2010.122

Conflict of Interest Statement: The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

frontiers in Immunology

REVIEW
published: 08 January 2018
doi: 10.3389/fimmu.2017.01902

Epstein–Barr Virus and Hemophagocytic Lymphohistiocytosis

*Rebecca A. Marsh**

Division of Bone Marrow Transplantation and Immune Deficiency, Cancer and Blood Diseases Institute, Cincinnati Children's Hospital Medical Center, Cincinnati, OH, United States

Epstein–Barr virus (EBV) is a ubiquitous virus that infects nearly all people worldwide without serious sequela. However, for patients who have genetic diseases which predispose them to the development of hemophagocytic lymphohistiocytosis (HLH), EBV infection is a life-threatening problem. As a part of a themed collection of articles on EBV infection and human primary immune deficiencies, we will review key concepts related to the understanding and treatment of HLH.

Keywords: Epstein–Barr virus, hemophagocytic lymphohistiocytosis, primary immunodeficiency, X-linked Lymphoproliferative Disease, Mononucleosis

OPEN ACCESS

Edited by:
Jeffrey I. Cohen, National Institutes of Health (NIH), United States

Reviewed by:
Thomas Gross, National Institutes of Health (NIH), United States
Shigeaki Nonoyama, National Defense Medical College, Japan

***Correspondence:**
Rebecca A. Marsh
rebecca.marsh@cchmc.org

Specialty section:
This article was submitted to Primary Immunodeficiencies, a section of the journal Frontiers in Immunology

Received: *06 October 2017*
Accepted: *13 December 2017*
Published: *08 January 2018*

Citation:
Marsh RA (2018) Epstein–Barr Virus and Hemophagocytic Lymphohistiocytosis. Front. Immunol. 8:1902. doi: 10.3389/fimmu.2017.01902

INTRODUCTION

Epstein–Barr virus (EBV) is a ubiquitous virus that infects nearly all people worldwide without serious sequela (1). However, EBV can cause serious disease complications in patients with primary immune deficiencies. In particular, for patients who have genetic diseases which predispose them to the development of hemophagocytic lymphohistiocytosis (HLH), EBV infection is often an immediately life-threatening problem due to the development of HLH. HLH is a syndrome of severe, life-threatening hyperinflammation (discussed below). Though it is difficult to quantify the association of EBV with HLH in North America and Europe, it is generally agreed that EBV is the most common infection observed to be associated with HLH. In one U.S. series, EBV was associated with HLH in approximately 1/3 of patients (2). For somewhat unclear reasons, EBV is highly associated with HLH in Asia, were it has been observed to be associated with HLH in almost 3/4 of patients in one report (3). It is important to note that HLH also develops in patients with genetic HLH in response to many other infections besides EBV, and it is also common for HLH to develop in these patients without an identified infectious trigger. Patients with X-linked lymphoproliferative disease type 1 (XLP1) are an exception to this statement. XLP1 is caused by mutations in *SH2D1A* (4–6), and HLH is these patients is nearly exclusively associated with EBV.

WHEN TO SUSPECT HLH IN PATIENTS WITH EBV

Hemophagocytic lymphohistiocytosis should be suspected when any of the characteristic signs and symptoms of HLH are present (**Table 1**), which include fever, splenomegaly, blood cytopenias, hepatitis and/or hepatomegaly, coagulopathy, central nervous system disturbances, and other more rare complications. However, the distinction between HLH and primary EBV infection can be very difficult, as patients with primary EBV infection may develop some of the hallmarks of HLH as part of natural infection. The timing and severity of these manifestations can help distinguish routine EBV infection from HLH complicating EBV infection. Most patients with routine EBV infection are non-toxic appearing. Fever typically dissipates over time, and

TABLE 1 | Commonly used diagnostic criteria for HLH, adapted from Henter et al. (8).

A diagnosis is consistent with HLH if 5/8 of the below criteria are met, or if the patient has a molecular diagnosis of genetic HLH (including: *PRF1, UNC13D, STX11, STXBP2, RAB27A, LYST, SH2D1A,* or *XIAP/BIRC4*)

1. Fever ≥38.5°C
2. Splenomegaly
3. Cytopenias (affecting at least 2 lineages)
   Hemoglobin <9 g/dL (in infants <4 weeks: hemoglobin <10 g/dL)
   Platelets <100 × 10^3/mL
   Neutrophils <1 × 10^3/mL
4. Hypertriglyceridemia (fasting, >265 mg/dL) and/or hypofibrinogenemia (<150 mg/dL)
5. Hemophagocytosis in bone marrow, spleen, lymph nodes, liver, or other tissue
6. Low or absent NK cell activity
7. Ferritin >500 ng/mL
8. Elevated sCD25 (soluble IL-2 receptor): >2,400 U/mL or elevated based on the laboratory-defined normal range

splenomegaly should gradually improve. Thrombocytopenia develops in up to approximately 50% of patients, and neutropenia develops in up to 80% of patients with EBV, but cytopenias are generally mild and largely resolve by 4 weeks following infection (7). Mild hepatitis is common with EBV infection and develops in 50–80% of cases, but elevations of liver enzymes are generally mild and improve within a few weeks (7). Jaundice is observed in some cases, but coagulopathy is not typical. By contrast, patients who develop HLH in association with EBV, in association with other triggers, or with spontaneous HLH, are generally ill appearing. Fevers are typically profound and do not improve. Cytopenias are generally life-threatening. Patients usually need transfusion support and are often initially evaluated for hematologic malignancies. Hepatitis can be severe; coagulopathy is common and acute liver failure necessitating a liver transplant can occur. Central nervous system involvement can be profound. Patients may develop focal or global deficits, seizures, and altered mental status. There are not firm clinical diagnostic thresholds that can distinguish routine primary EBV infection from HLH, but good clinical judgment can often help identify patients who are experiencing more severe manifestations of disease and warrant further evaluations for possible HLH. Blood levels of fibrinogen, triglycerides, ferritin, and soluble IL-2 receptor can be measured to help differentiate HLH in appropriate cases, as these markers are typically used to support or refute a diagnosis of HLH.

DIAGNOSING HLH

It is important to first recognize that HLH is a hyperinflammatory *syndrome*, which is *clinically diagnosed*. A clinical diagnosis of HLH should be suspected in patients with a variety of hyperinflammatory clinical presentations, such as patients who seem to be having a hyperinflammatory process in the setting of EBV infection. Most clinicians use the diagnostic criteria developed by the Histiocyte Society for the HLH-1994 and HLH-2004 clinical trials to help establish a clinical diagnosis of HLH (**Table 1**) (8). The "classic" clinical presentation is that of an infant or young child with unremitting fevers, pancytopenia, and hepatosplenomegaly. The patient may have a rash, jaundice, or bleeding problems. However, HLH can present at any age, and can present with a variety of other clinical manifestations including hepatitis or acute liver failure, or altered levels of consciousness or seizures if there is central nervous system involvement with HLH. Blood levels of the classic inflammatory markers ferritin and soluble IL-2 receptor are typically high in patients with HLH and are used to help make a diagnosis of HLH. Abnormalities in triglycerides (high) and fibrinogen (low) can also be supportive. Recent evidence strongly suggests that quantification of HLA-DR and other phenotypic markers on T cells can help distinguish patients with HLH (9). Of course, there are exceptions to these generalities, especially in the rare cases of isolated CNS disease in patients who lack any systemic illness. Newer markers of interferon gamma pathway activity or inflammasome activation such as CXCL9 and IL-18, respectively, are also starting to gain in use.

While the criteria in **Table 1** can be very useful while considering a diagnosis of HLH, they should be considered to be a guideline only. Some patients with HLH lack 5/8 criteria at presentation, or even throughout their clinical course. Particularly, many patients lack hemophagocytosis in marrow or tissue samples. Additionally, the NK cell function assay has been recently found to be inferior to newer diagnostic screening tests for genetic HLH (10) (discussed later). Another shortfall of the criteria are that some patients who meet 5/8 criteria are ultimately found to have disorders other than HLH, and physicians should be careful to consider alternative diagnoses such as malignancies, infections, auto-immune, and rheumatologic diseases (though these problems can of course be complicated by HLH).

PRIMARY VERSUS SECONDARY HLH

Once a clinical diagnosis of HLH is established, it is important to perform proper evaluations to check patients for genetic diseases which cause HLH (discussed below). HLH can be classified as "primary" HLH, in which case a patient has a proven genetic etiology or has repeatedly developed HLH or has a family history which supports that a genetic disease is very likely. These patients are typically infants or young children. HLH in patients who lack a known or strongly suspected genetic etiology can be classified as having "secondary" HLH. Patients with secondary HLH tend to be older, develop HLH in the setting of strong immunologic triggers such as infections (such as EBV) or malignancies, or in the setting of rheumatologic conditions. HLH that occurs in the setting of a rheumatologic disease is often termed macrophage activation syndrome. Sometimes, treating the underlying trigger of HLH in patients with secondary HLH will lead to resolution of HLH, but varying intensities of HLH-directed treatment are often needed.

GENETICS AND DEFECTS OF HLH

It was first recognized that HLH could have a hereditary basis in some patients in the 1950s (11, 12). Almost 50 years later, Stepp et al. reported that defects in perforin were responsible for HLH

in eight unrelated families (13). The last few decades have seen a tremendous advance in the basic scientific understanding of HLH through the discovery of many additional genetic causes of HLH (**Table 2**). It is now clear that many genetic causes of HLH essentially cripple cytotoxic lymphocyte granule-mediated cytotoxicity (**Figure 1**). An exception to this is that patients with X-linked lymphoproliferative disease type 2 (XLP2) due to mutations in *XIAP/BIRC4* appear to have normal cytotoxicity (14, 15), and instead may have dysregulated TNFR and inflammasome function (16). Very recently, activating mutations in *NLRC4* have been found to cause HLH, which also represents another exception to the rule as pathophysiology relates to inflammasome activation.

Many genetic forms of HLH are classified together as familial HLH (FHL). FHL2 is due to mutations in *PRF1*. FHL3-5 are due to mutations in *UNC13D, STX11*, and *STXBP2,* respectively (17–20). Related genetic diseases that are associated with HLH and also pigmentary disorders are Griscelli syndrome, due to mutations in *RAB27A*, Chediak–Higashi syndrome, due to mutations in *LYST*, and Hermansky–Pudlak syndrome type 2, due to mutations in *AP3B1* (21–23). Of note, patients with *RAB27A* mutations do not always have abnormal pigmentation. NK cells and T cells from patients with mutations in *UNC13D*, *STX11*, *STXBP2*, *RAB27A*, *LYST*, and *AP3B1* all fail to degranulate normally, because these proteins are critical for the process of normal cytotoxic granule trafficking, docking, or fusion with the outer cell membrane (**Figure 1**). The extrusion of cytotoxic granule contents from NK cells and T cells toward their intended target is an important method of elimination of virus infected cells or malignant cells, and also serves to regulate immune homeostasis (24). When the machinery required for this process is broken, intended target cells fail to die and continue to stimulate immune cells, which leads to continued activation and proliferation of immune cells, and a vicious hyperinflammatory cycle ensues.

NK cells and T cells from patients with *PRF1* mutations lack functional perforin. Perforin is normally contained within the cytotoxic granules of NK cells and T cells that were discussed above. Perforin is a unique protein that oligomerizes after release from cytotoxic granules and the complexes create pores in the surface membrane of the intended target cells, which allows cytotoxic granule contents to enter the target cell and ultimately results in target cell death (**Figure 1**) (25, 26). Lack of functional perforin results in the same pathophysiologic abnormality as other causes of FHL: defective cytotoxic lymphocyte granule-mediated cytotoxicity.

As mentioned above, there are other diseases which are associated with HLH that have different (and perhaps more complicated) mechanisms of disease. XLP1 is caused by mutations in *SH2D1A*, which leads to dysfunctional SLAM-associated protein (SAP) (4–6). SAP is a small SH2 domain-containing protein which is involved in signaling of the signaling lymphocytic activation molecule (SLAM) family of receptors. Lack of normal SAP function leads to several immunologic abnormalities including defective 2B4-mediated cytotoxicity, absence of invariant NKT cell development, defective T cell restimulation-induced cell death, and other humoral and cellular abnormalities (27–30). XLP2 is caused by mutations in X-linked inhibitor of apoptosis (XIAP)/baculoviral inhibitor of apoptosis repeat-containing 4 (BIRC4) (14). Defects in XIAP also lead to several immunologic abnormalities, but cytotoxicity is normal (14, 15). Many cells have an increased susceptibility to cell death, NOD2 signaling is defective, and TNF receptor signaling and inflammasome function are dysregulated (14–16, 31, 32). The development of HLH in these patients is multifactorial, but dysregulation of the NLRP3 inflammasome likely plays a key role in the development of disease. Patients with activating mutations in *NLRC4* also develop HLH, associated with constitutive activation of the NLRC4 inflammasome (33, 34). Thus far, there does not seem to be a strong association with EBV infection, as reported patients have not had an identified trigger of hyperinflammation (33–35). Still other genetic diseases that can be associated with EBV-associated HLH, or chronic active EBV or lymphoproliferative diseases, include ITK deficiency, CD27 deficiency, CD70 deficiency, and magnesium transporter 1 (MAGT1) deficiency which is called X-linked immunodeficiency with magnesium defect, EBV infection, and neoplasia or XMEN disease (36–45).

TABLE 2 | Genetic causes of hemophagocytic lymphohistiocytosis (HLH) and associated rapid flow cytometric screening tests.

Disease	Gene	Protein	Rapid screening test
Familial HLH 2	*PRF1*	Perforin	Perforin expression
Familial hemophagocytic lymphohistiocytosis (FHL) 3	*UNC13D*	Munc13-4	CD107a
FHL 4	*STX11*	Syntaxin 11	CD107a
FHL 5	*STXBP2*	Munc18-2	CD107a
X-linked lymphoproliferative disease type 1 (XLP1)	*SH2D1A*	Signaling lymphocytic activation molecule-associated protein (SAP)	SAP expression
X-linked lymphoproliferative disease type 2 (XLP2)	*XIAP/BIRC4*	X-linked inhibitor of apoptosis (XIAP)	XIAP expression, NOD2 Signaling, IL-18 levels
Griscelli syndrome	*RAB27A*	Rab27a	CD107a
Chediak–Higashi syndrome	*LYST*	LYST	CD107a
Hermansky–Pudlak syndrome type 2	*AP3B1*	AP3	CD107a
NLRC4 mutation	*NLRC4*	NLR family, CARD domain-containing protein 4 (NLRC4)	IL-18 levels
CD27 deficiency	*CD27*	CD27	
ITK deficiency	*ITK*	IL-2 Inducible T-Cell Kinase (ITK)	
X-linked immunodeficiency with magnesium defect, Epstein–Barr virus infection, and neoplasia disease (XMEN disease)	*MAGT1*	Magnesium transporter 1 (MAGT1)	

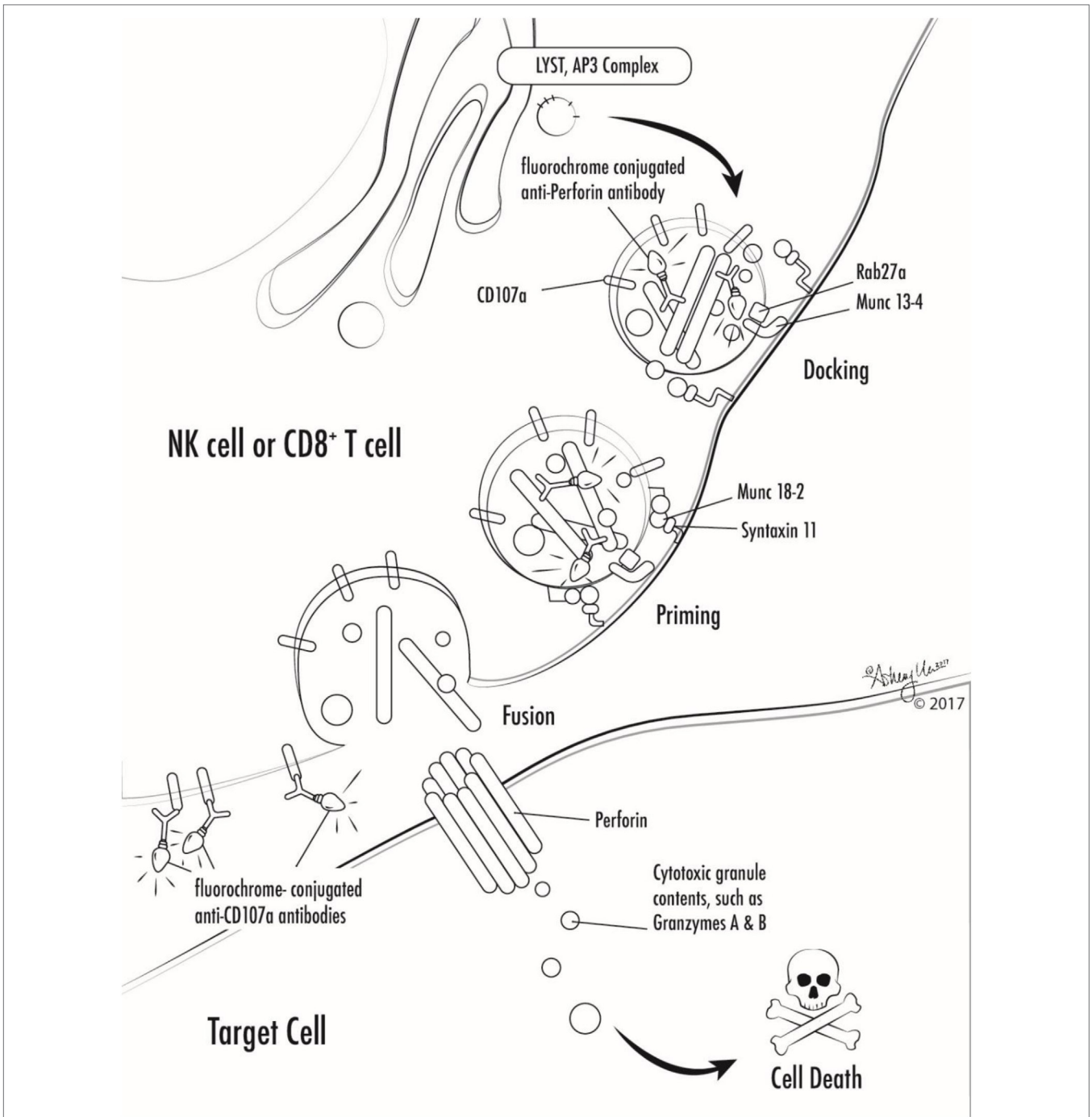


FIGURE 1 | Illustration of involvement of selected primary hemophagocytic lymphohistiocytosis proteins in cytotoxic lymphocyte degranulation and target cell killing. Antibodies against markers used in selected screening diagnostics are also shown (perforin and CD107a).

OTHER MANIFESTATIONS OF HLH-ASSOCIATED DISEASES

The FHL disorders are primarily associated with HLH but are often also associated with atypical hyperinflammatory syndromes that lack the full spectrum of HLH, as well as acute liver failure or isolated central nervous system disease in the absence of systemic inflammation. They may uncommonly be associated with atypical chronic active EBV infection, hypogammaglobulinemia, vasculitis, gastrointestinal inflammation, recurrent infections, and very rarely, lymphoproliferative complications (46–48). XLP1 is commonly associated with lymphoma or

hypogammaglobulinemia, and has more rare presentations that include aplastic anemia, vasculitis, and gastrointestinal inflammation. XLP2 is associated with a variety of other disease manifestations including atypical/mild HLH-like episodes, inflammatory bowel disease, recurrent infections, hypogammaglobulinemia, uveitis, fistulating skin disease, granulomatous hepatitis, granulomatous, and lymphocytic interstitial lung disease (14, 15, 49–52). CD70 deficiency, CD27 deficiency, ITK deficiency, and MAGT1 all share a strong predisposition to lymphoma.

SCREENING TESTS FOR GENETIC HLH

While the diagnosis of HLH is a clinical diagnosis based on clinical manifestations and laboratory findings, there are several specialized tests which can quickly screen patients for genetic forms of HLH (**Table 2**; **Figure 1**). Flow cytometric screening of perforin expression by NK cells and $CD8^+$ T cells serves as a quick screening test for perforin deficiency, and has been found to be highly sensitive (53). A flow cytometric assay to detect abnormal degranulation of NK cells is also available, which quantifies the surface upregulation of CD107a following exposure of NK cells to K562 target cells (or upregulation of CD107a on NK cells or T cells following other appropriate triggers). CD107a is normally expressed within cytotoxic granule membranes, and very little is found on the surface of NK cells or $CD8^+$ T Cells at rest. Hence, one can measure CD107a on the surface of NK cells before and following exposure to target cells as a marker of degranulation. This method has been shown to have good diagnostic accuracy for the detection of patients with mutations in the HLH genes associated with abnormal degranulation (*UNC13D*, *STX11*, *STXBP2*, *RAB27A*, *LYST*, and *AP3B1*) (54). Using both perforin and CD107a testing is more accurate for the identification of patients with genetic forms of HLH compared to traditional NK cell function testing (10). Of note, flow cytometric screening tests are also available to screen patients for XLP1 and XLP2 (55). XLP1 and XLP2 should be considered in male patients with HLH, and even in female patients in whom other genetic causes of HLH have been excluded, due to the observation that females with abnormal skewing of lionization toward XIAP-deficient cells can be symptomatic (56, 57). A functional screen for XIAP deficiency is available via evaluation of NOD2 signaling (58), and IL-18 levels can be helpful as a screening tool for patients with XIAP deficiency or NLRC4 mutations.

TREATMENT OF HLH, INCLUDING EBV–HLH

Once HLH has been diagnosed, therapy should be started as soon as possible. Of note, however, therapy should not be started until complete evaluations for lymphoma and leukemia have been performed. The treatment of HLH generally includes a variety of potent immunosuppressive regimens. In North America and most of Europe, a regimen of dexamethasone and etoposide has been the mainstay of treatment, based on the HLH-1994 and HLH-2004 study protocols (8, 59, 60). The HLH-2004 protocol incorporated the addition of cyclosporine, but there has been no clear benefit related to early administration of cyclosporine (60), and it should be noted that cyclosporine can be associated with notable complications including hypertension, renal injury, and posterior reversible encephalopathy syndrome. CNS HLH is typically treated with targeted therapy if patients are stable enough to undergo lumbar punctures and administration of intrathecal steroids and methotrexate. Approximately 50% of patients can be expected to achieve a complete response, and approximately 30% of patients will experience a partial response (59). The incidence of death prior to allogeneic hematopoietic cell transplant was observed to be 19–27% in the HLH 2004 and 1994 studies, respectively.

In France, an alternative regimen containing steroids and ATG has been used. Seventy-three percent of patients were reported to achieve a complete response with the regimen, and 24% of patients achieved a partial response (61). In general, either approach is appropriate, though there has been more widespread experience with dexamethasone and etoposide treatment. The dexamethasone and etoposide regimen offers avoidance of the risk of severe reactions that can be associated with ATG, and may be less T cell immunosuppressive. ATG offers avoidance of chemotherapy exposure and the risk of marrow suppression associated with etoposide. There has been a recent trial of a Hybrid Immunotherapy approach for HLH[1] and a European sister trial, but results are not yet available.

Many additional agents have been reported in single patients or small collections of patients. There have been several seemingly beneficial reports of anti-interleukin-1 directed therapies such as anakinra and canakinumab (62–71), and anti-tumor necrosis factor alpha-directed agents such as etanercept and infliximab (72–81), but there are no large data series on which to judge effectiveness. Likewise, plasma exchange has been reported in small numbers of patients, and while some authors report benefit (82, 83), it is difficult to draw conclusions about its effectiveness. More recently, newer agents are under formal investigation for the treatment of HLH. An anti-interferon gamma monoclonal antibody is actively being investigated in the U.S.A. and Europe.[2] Ruxolitinib is being trialed at a single center in North America for secondary HLH.[3] Both agents have strong mouse data to support their potential efficacy (84–86). Alemtuzumab is being trialed for up-front therapy in France[4] and has had reasonable success when used in the salvage setting (below) (87).

For cases of refractory HLH, "salvage" therapy is sometimes needed. Unfortunately, there are very little data on which to base decisions about salvage therapy. The Histiocyte Society Salvage Therapy Working Group recently reviewed the literature, and found that there was evidence for only three agents which had been used in HLH refractory to steroids and either etoposide or ATG: anakinra, ATG, and alemtuzumab, as well as a regimen

[1]https://clinicaltrials.gov/ct2/show/NCT01104025.
[2]https://clinicaltrials.gov/ct2/show/NCT01818492.
[3]https://clinicaltrials.gov/ct2/show/NCT02400463.
[4]https://clinicaltrials.gov/ct2/show/NCT02472054.

that combined liposomal doxorubicin with steroids and etoposide (88).

In patients with EBV–HLH, the addition of rituximab can be useful to deplete EBV-harboring B cells and improve HLH. In a retrospective multi-center study, Chellapandian et al. observed that rituximab (given with other HLH therapies) resulted in significant reductions in EBV load within 1 month of use and was also associated with significant decreases in ferritin levels (89).

In addition to these HLH-directed therapies, good supportive care, treatment of underlying triggers, anti-microbial prophylaxis, and close monitoring are usually needed. Antifungal prophylaxis, anti-pneumocystis jirovecii prophylaxis, anti-viral prophylaxis, and IVIG replacement during the active treatment period should all be considered. If patients have active virus infections that are associated with HLH such as EBV, CMV, adenovirus, influenza, etc., treatments targeting those infections should be initiated including rituximab for EBV, and anti-viral agents such ganciclovir, cidofovir, oseltamivir, and others as appropriate. The same is true for other infections such as histoplasmosis, tuberculosis, tick-borne diseases, etc. Routine monitoring of laboratory tests such as complete blood counts, liver panels, fibrinogen, and/or coagulation studies should be performed. Weekly or twice weekly monitoring of inflammatory markers such as soluble IL-2 receptor can help with identifying response to therapy or relapse of disease. Monitoring of ferritin can also be helpful, though it is often hindered by changes associated with blood transfusions, and is typically slow to normalize. Newer indicators of pathologic interferon gamma activity such as CXCL9 are gaining favor in use (90), and elevated levels of IL-18 have been found to be a good marker of XLP2/XIAP deficiency and disease activity in those patients (91). Markers of T cell activation such as HLA-DR can also be useful (9). For patients with EBV–HLH, EBV blood polymerase chain reaction (PCR) monitoring can be useful to monitor response to rituximab (89), and also watch for increasing viral loads following rituximab with B cell recovery. Persistently high EBV PCRs following rituximab in the setting of proven B cell depletion can suggest EBV infection of T and/or NK cells.

DEFINITIVE TREATMENT

For patients with secondary HLH, good medical management and follow-up following HLH resolution is needed. Depending on the underlying trigger of HLH in these patients, they may need indefinite care for management of chronic problems. For patients with proven or strongly suspected primary HLH, hematopoietic cell transplantation (HCT) is generally recommended. Historic outcomes of HCT using myeloablative regimens were poor due to high rates of toxicities that resulted in early deaths. Many groups reported survival of only 45–65% (59, 92–97). More recent reduced intensity conditioning (RIC) approaches have resulted in increased patient survival rates of 75% or higher (2, 98, 99). However, these approaches can be associated with the unique challenge of mixed donor and recipient chimerism, which somewhat limits this success of this approach. Patients with HLH do not require 100% donor chimerism for cure, but risk of HLH relapse increases as donor contribution to hematopoiesis (or cytotoxic lymphocyte development) decreases to less than 20–30% (100). Continued efforts to improve stable donor contribution to hematopoiesis will likely lead to increased success with RIC HCT approaches.

CONCLUSION

Hemophagocytic lymphohistiocytosis in response to EBV or otherwise remains a life-threatening problem for patients with genetic disorders that cause HLH. Discoveries made in recent decades have yielded extraordinary advances in our understanding of HLH. Early recognition and initiation of HLH-directed therapy remain key for patient survival. The next decade promises to yield even further advances in diagnostics and treatment breakthroughs which will continue to improve patient outcomes.

AUTHOR CONTRIBUTIONS

The author confirms being the sole contributor of this work and approved it for publication.

REFERENCES

1. Young LS, Rickinson AB. Epstein-Barr virus: 40 years on. *Nat Rev Cancer* (2004) 4(10):757–68. doi:10.1038/nrc1452
2. Marsh RA, Vaughn G, Kim MO, Li D, Jodele S, Joshi S, et al. Reduced-intensity conditioning significantly improves survival of patients with hemophagocytic lymphohistiocytosis undergoing allogeneic hematopoietic cell transplantation. *Blood* (2010) 116(26):5824–31. doi:10.1182/blood-2010-04-282392
3. Xu XJ, Wang HS, Ju XL, Xiao PF, Xiao Y, Xue HM, et al. Clinical presentation and outcome of pediatric patients with hemophagocytic lymphohistiocytosis in China: a retrospective multicenter study. *Pediatr Blood Cancer* (2017) 64(4). doi:10.1002/pbc.26264
4. Coffey AJ, Brooksbank RA, Brandau O, Oohashi T, Howell GR, Bye JM, et al. Host response to EBV infection in X-linked lymphoproliferative disease results from mutations in an SH2-domain encoding gene. *Nat Genet* (1998) 20(2):129–35. doi:10.1038/2424
5. Sayos J, Wu C, Morra M, Wang N, Zhang X, Allen D, et al. The X-linked lymphoproliferative-disease gene product SAP regulates signals induced through the co-receptor SLAM. *Nature* (1998) 395(6701):462–9. doi:10.1038/26683
6. Nichols KE, Harkin DP, Levitz S, Krainer M, Kolquist KA, Genovese C, et al. Inactivating mutations in an SH2 domain-encoding gene in X-linked lymphoproliferative syndrome. *Proc Natl Acad Sci U S A* (1998) 95(23):13765–70. doi:10.1073/pnas.95.23.13765
7. Jenson HB. Acute complications of Epstein-Barr virus infectious mononucleosis. *Curr Opin Pediatr* (2000) 12(3):263–8. doi:10.1097/00008480-200006000-00016
8. Henter JI, Horne A, Arico M, Egeler RM, Filipovich AH, Imashuku S, et al. HLH-2004: diagnostic and therapeutic guidelines for hemophagocytic lymphohistiocytosis. *Pediatr Blood Cancer* (2007) 48(2):124–31. doi:10.1002/pbc.21039
9. Ammann S, Lehmberg K, Zur Stadt U, Janka G, Rensing-Ehl A, Klemann C, et al. Primary and secondary hemophagocytic lymphohistiocytosis have different patterns of T-cell activation, differentiation and repertoire. *Eur J Immunol* (2017) 47(2):364–73. doi:10.1002/eji.201646686
10. Rubin TS, Zhang K, Gifford C, Lane A, Choo S, Bleesing JJ, et al. Perforin and CD107a testing is superior to NK cell function testing for

screening patients for genetic HLH. *Blood* (2017) 129(22):2993–9. doi:10.1182/blood-2016-12-753830
11. Reese AJ, Levy E. Familial incidence of non-lipoid reticuloendotheliosis (Letterer-Siwe disease). *Arch Dis Child* (1951) 26(130):578–81. doi:10.1136/adc.26.130.578
12. Farquhar JW, Claireaux AE. Familial haemophagocytic reticulosis. *Arch Dis Child* (1952) 27(136):519–25. doi:10.1136/adc.27.136.519
13. Stepp SE, Dufourcq-Lagelouse R, Le Deist F, Bhawan S, Certain S, Mathew PA, et al. Perforin gene defects in familial hemophagocytic lymphohistiocytosis. *Science* (1999) 286(5446):1957–9. doi:10.1126/science.286.5446.1957
14. Rigaud S, Fondaneche MC, Lambert N, Pasquier B, Mateo V, Soulas P, et al. XIAP deficiency in humans causes an X-linked lymphoproliferative syndrome. *Nature* (2006) 444(7115):110–4. doi:10.1038/nature05257
15. Marsh RA, Madden L, Kitchen BJ, Mody R, McClimon B, Jordan MB, et al. XIAP deficiency: a unique primary immunodeficiency best classified as X-linked familial hemophagocytic lymphohistiocytosis and not as X-linked lymphoproliferative disease. *Blood* (2010) 116(7):1079–82. doi:10.1182/blood-2010-01-256099
16. Yabal M, Muller N, Adler H, Knies N, Gross CJ, Damgaard RB, et al. XIAP restricts TNF- and RIP3-dependent cell death and inflammasome activation. *Cell Rep* (2014) 7(6):1796–808. doi:10.1016/j.celrep.2014.05.008
17. Feldmann J, Callebaut I, Raposo G, Certain S, Bacq D, Dumont C, et al. Munc13-4 is essential for cytolytic granules fusion and is mutated in a form of familial hemophagocytic lymphohistiocytosis (FHL3). *Cell* (2003) 115(4):461–73. doi:10.1016/S0092-8674(03)00855-9
18. zur Stadt U, Schmidt S, Kasper B, Beutel K, Diler AS, Henter JI, et al. Linkage of familial hemophagocytic lymphohistiocytosis (FHL) type-4 to chromosome 6q24 and identification of mutations in syntaxin 11. *Hum Mol Genet* (2005) 14(6):827–34. doi:10.1093/hmg/ddi076
19. zur Stadt U, Rohr J, Seifert W, Koch F, Grieve S, Pagel J, et al. Familial hemophagocytic lymphohistiocytosis type 5 (FHL-5) is caused by mutations in Munc18-2 and impaired binding to syntaxin 11. *Am J Hum Genet* (2009) 85(4):482–92. doi:10.1016/j.ajhg.2009.09.005
20. Cote M, Menager MM, Burgess A, Mahlaoui N, Picard C, Schaffner C, et al. Munc18-2 deficiency causes familial hemophagocytic lymphohistiocytosis type 5 and impairs cytotoxic granule exocytosis in patient NK cells. *J Clin Invest* (2009) 119(12):3765–73. doi:10.1172/JCI40732
21. Menasche G, Pastural E, Feldmann J, Certain S, Ersoy F, Dupuis S, et al. Mutations in RAB27A cause Griscelli syndrome associated with haemophagocytic syndrome. *Nat Genet* (2000) 25(2):173–6. doi:10.1038/76024
22. Barbosa MD, Nguyen QA, Tchernev VT, Ashley JA, Detter JC, Blaydes SM, et al. Identification of the homologous beige and Chediak-Higashi syndrome genes. *Nature* (1996) 382(6588):262–5. doi:10.1038/382262a0
23. Enders A, Zieger B, Schwarz K, Yoshimi A, Speckmann C, Knoepfle EM, et al. Lethal hemophagocytic lymphohistiocytosis in Hermansky-Pudlak syndrome type II. *Blood* (2006) 108(1):81–7. doi:10.1182/blood-2005-11-4413
24. de Saint Basile G, Menasche G, Fischer A. Molecular mechanisms of biogenesis and exocytosis of cytotoxic granules. *Nat Rev Immunol* (2010) 10(8):568–79. doi:10.1038/nri2803
25. Baran K, Dunstone M, Chia J, Ciccone A, Browne KA, Clarke CJ, et al. The molecular basis for perforin oligomerization and transmembrane pore assembly. *Immunity* (2009) 30(5):684–95. doi:10.1016/j.immuni.2009.03.016
26. Voskoboinik I, Dunstone MA, Baran K, Whisstock JC, Trapani JA. Perforin: structure, function, and role in human immunopathology. *Immunol Rev* (2010) 235(1):35–54. doi:10.1111/j.0105-2896.2010.00896.x
27. Cannons JL, Tangye SG, Schwartzberg PL. SLAM family receptors and SAP adaptors in immunity. *Annu Rev Immunol* (2011) 29:665–705. doi:10.1146/annurev-immunol-030409-101302
28. Nichols KE, Hom J, Gong SY, Ganguly A, Ma CS, Cannons JL, et al. Regulation of NKT cell development by SAP, the protein defective in XLP. *Nat Med* (2005) 11(3):340–5. doi:10.1038/nm1189
29. Pasquier B, Yin L, Fondaneche MC, Relouzat F, Bloch-Queyrat C, Lambert N, et al. Defective NKT cell development in mice and humans lacking the adapter SAP, the X-linked lymphoproliferative syndrome gene product. *J Exp Med* (2005) 201(5):695–701. doi:10.1084/jem.20042432
30. Snow AL, Marsh RA, Krummey SM, Roehrs P, Young LR, Zhang K, et al. Restimulation-induced apoptosis of T cells is impaired in patients with X-linked lymphoproliferative disease caused by SAP deficiency. *J Clin Invest* (2009) 119(10):2976–89. doi:10.1172/JCI39518
31. Damgaard RB, Nachbur U, Yabal M, Wong WW, Fiil BK, Kastirr M, et al. The ubiquitin ligase XIAP recruits LUBAC for NOD2 signaling in inflammation and innate immunity. *Mol Cell* (2012) 46(6):746–58. doi:10.1016/j.molcel.2012.04.014
32. Damgaard RB, Fiil BK, Speckmann C, Yabal M, Stadt UZ, Bekker-Jensen S, et al. Disease-causing mutations in the XIAP BIR2 domain impair NOD2-dependent immune signalling. *EMBO Mol Med* (2013) 5(8):1278–95. doi:10.1002/emmm.201303090
33. Canna SW, de Jesus AA, Gouni S, Brooks SR, Marrero B, Liu Y, et al. An activating NLRC4 inflammasome mutation causes autoinflammation with recurrent macrophage activation syndrome. *Nat Genet* (2014) 46(10):1140–6. doi:10.1038/ng.3089
34. Romberg N, Al Moussawi K, Nelson-Williams C, Stiegler AL, Loring E, Choi M, et al. Mutation of NLRC4 causes a syndrome of enterocolitis and autoinflammation. *Nat Genet* (2014) 46(10):1135–9. doi:10.1038/ng.3066
35. Liang J, Alfano DN, Squires JE, Riley MM, Parks WT, Kofler J, et al. Novel NLRC4 mutation causes a syndrome of perinatal autoinflammation with hemophagocytic lymphohistiocytosis, hepatosplenomegaly, fetal thrombotic vasculopathy, and congenital anemia and ascites. *Pediatr Dev Pathol* (2017) 20(6):498–505. doi:10.1177/1093526616686890
36. Huck K, Feyen O, Niehues T, Ruschendorf F, Hubner N, Laws HJ, et al. Girls homozygous for an IL-2-inducible T cell kinase mutation that leads to protein deficiency develop fatal EBV-associated lymphoproliferation. *J Clin Invest* (2009) 119(5):1350–8. doi:10.1172/JCI37901
37. Linka RM, Risse SL, Bienemann K, Werner M, Linka Y, Krux F, et al. Loss-of-function mutations within the IL-2 inducible kinase ITK in patients with EBV-associated lymphoproliferative diseases. *Leukemia* (2012) 26(5):963–71. doi:10.1038/leu.2011.371
38. Stepensky P, Weintraub M, Yanir A, Revel-Vilk S, Krux F, Huck K, et al. IL-2-inducible T-cell kinase deficiency: clinical presentation and therapeutic approach. *Haematologica* (2011) 96(3):472–6. doi:10.3324/haematol.2010.033910
39. van Montfrans JM, Hoepelman AI, Otto S, van Gijn M, van de Corput L, de Weger RA, et al. CD27 deficiency is associated with combined immunodeficiency and persistent symptomatic EBV viremia. *J Allergy Clin Immunol* (2012) 129(3):787–93.e6. doi:10.1016/j.jaci.2011.11.013
40. Salzer E, Daschkey S, Choo S, Gombert M, Santos-Valente E, Ginzel S, et al. Combined immunodeficiency with life-threatening EBV-associated lymphoproliferative disorder in patients lacking functional CD27. *Haematologica* (2013) 98(3):473–8. doi:10.3324/haematol.2012.068791
41. Alkhairy OK, Perez-Becker R, Driessen GJ, Abolhassani H, van Montfrans J, Borte S, et al. Novel mutations in TNFRSF7/CD27: clinical, immunologic, and genetic characterization of human CD27 deficiency. *J Allergy Clin Immunol* (2015) 136(3):703–12.e10. doi:10.1016/j.jaci.2015.02.022
42. Abolhassani H, Edwards ES, Ikinciogullari A, Jing H, Borte S, Buggert M, et al. Combined immunodeficiency and Epstein-Barr virus-induced B cell malignancy in humans with inherited CD70 deficiency. *J Exp Med* (2017) 214(1):91–106. doi:10.1084/jem.20160849
43. Izawa K, Martin E, Soudais C, Bruneau J, Boutboul D, Rodriguez R, et al. Inherited CD70 deficiency in humans reveals a critical role for the CD70-CD27 pathway in immunity to Epstein-Barr virus infection. *J Exp Med* (2017) 214(1):73–89. doi:10.1084/jem.20160784
44. Li FY, Chaigne-Delalande B, Kanellopoulou C, Davis JC, Matthews HF, Douek DC, et al. Second messenger role for Mg^{2+} revealed by human T-cell immunodeficiency. *Nature* (2011) 475(7357):471–6. doi:10.1038/nature10246
45. Li FY, Chaigne-Delalande B, Su H, Uzel G, Matthews H, Lenardo MJ. XMEN disease: a new primary immunodeficiency affecting Mg^{2+} regulation of immunity against Epstein-Barr virus. *Blood* (2014) 123(14):2148–52. doi:10.1182/blood-2013-11-538686
46. Katano H, Ali MA, Patera AC, Catalfamo M, Jaffe ES, Kimura H, et al. Chronic active Epstein-Barr virus infection associated with mutations in perforin that impair its maturation. *Blood* (2004) 103(4):1244–52. doi:10.1182/blood-2003-06-2171

47. Rohr J, Beutel K, Maul-Pavicic A, Vraetz T, Thiel J, Warnatz K, et al. Atypical familial hemophagocytic lymphohistiocytosis due to mutations in UNC13D and STXBP2 overlaps with primary immunodeficiency diseases. *Haematologica* (2010) 95(12):2080–7. doi:10.3324/haematol.2010.029389
48. Tesi B, Chiang SC, El-Ghoneimy D, Hussein AA, Langenskiold C, Wali R, et al. Spectrum of atypical clinical presentations in patients with biallelic PRF1 missense mutations. *Pediatr Blood Cancer* (2015) 62(12):2094–100. doi:10.1002/pbc.25646
49. Speckmann C, Lehmberg K, Albert MH, Damgaard RB, Fritsch M, Gyrd-Hansen M, et al. X-linked inhibitor of apoptosis (XIAP) deficiency: the spectrum of presenting manifestations beyond hemophagocytic lymphohistiocytosis. *Clin Immunol* (2013) 149(1):133–41. doi:10.1016/j.clim.2013.07.004
50. Basiaga ML, Weiss PF, Behrens EM. BIRC4 mutation: an important rare cause of uveitis. *J Clin Rheumatol* (2015) 21(8):444–7. doi:10.1097/RHU.0000000000000327
51. Pachlopnik Schmid J, Canioni D, Moshous D, Touzot F, Mahlaoui N, Hauck F, et al. Clinical similarities and differences of patients with X-linked lymphoproliferative syndrome type 1 (XLP-1/SAP deficiency) versus type 2 (XLP-2/XIAP deficiency). *Blood* (2011) 117(5):1522–9. doi:10.1182/blood-2010-07-298372
52. Steele CL, Dore M, Ammann S, Loughrey M, Montero A, Burns SO, et al. X-linked inhibitor of apoptosis complicated by granulomatous lymphocytic interstitial lung disease (GLILD) and granulomatous hepatitis. *J Clin Immunol* (2016) 36(7):733–8. doi:10.1007/s10875-016-0320-3
53. Abdalgani M, Filipovich AH, Choo S, Zhang K, Gifford C, Villanueva J, et al. Accuracy of flow cytometric perforin screening for detecting patients with FHL due to PRF1 mutations. *Blood* (2015) 126(15):1858–60. doi:10.1182/blood-2015-06-648659
54. Bryceson YT, Pende D, Maul-Pavicic A, Gilmour KC, Ufheil H, Vraetz T, et al. A prospective evaluation of degranulation assays in the rapid diagnosis of familial hemophagocytic syndromes. *Blood* (2012) 119(12):2754–63. doi:10.1182/blood-2011-08-374199
55. Gifford CE, Weingartner E, Villanueva J, Johnson JJ, Zhang K, Filipovich AH, et al. Clinical flow cytometric screening of SAP and XIAP expression accurately identifies patients with SH2D1A and XIAP/BIRC4 mutations. *Cytometry B Clin Cytom* (2014) 86(4):263–71. doi:10.1002/cytob.21166
56. Yang X, Hoshino A, Taga T, Kunitsu T, Ikeda Y, Yasumi T, et al. A female patient with incomplete hemophagocytic lymphohistiocytosis caused by a heterozygous XIAP mutation associated with non-random X-chromosome inactivation skewed towards the wild-type XIAP allele. *J Clin Immunol* (2015) 35(3):244–8. doi:10.1007/s10875-015-0144-6
57. Holle JR, Marsh RA, Holdcroft AM, Davies SM, Wang L, Zhang K, et al. Hemophagocytic lymphohistiocytosis in a female patient due to a heterozygous XIAP mutation and skewed X chromosome inactivation. *Pediatr Blood Cancer* (2015) 62(7):1288–90. doi:10.1002/pbc.25483
58. Ammann S, Elling R, Gyrd-Hansen M, Duckers G, Bredius R, Burns SO, et al. A new functional assay for the diagnosis of X-linked inhibitor of apoptosis (XIAP) deficiency. *Clin Exp Immunol* (2014) 176(3):394–400. doi:10.1111/cei.12306
59. Henter JI, Samuelsson-Horne A, Arico M, Egeler RM, Elinder G, Filipovich AH, et al. Treatment of hemophagocytic lymphohistiocytosis with HLH-94 immunochemotherapy and bone marrow transplantation. *Blood* (2002) 100(7):2367–73. doi:10.1182/blood-2002-01-0172
60. Bergsten E, Horne A, Arico M, Astigarraga I, Egeler RM, Filipovich AH, et al. Confirmed efficacy of etoposide and dexamethasone in HLH treatment: long term results of the cooperative HLH-2004 study. *Blood* (2017). doi:10.1182/blood-2017-06-788349
61. Mahlaoui N, Ouachee-Chardin M, de Saint Basile G, Neven B, Picard C, Blanche S, et al. Immunotherapy of familial hemophagocytic lymphohistiocytosis with antithymocyte globulins: a single-center retrospective report of 38 patients. *Pediatrics* (2007) 120(3):e622–8. doi:10.1542/peds.2006-3164
62. Bruck N, Suttorp M, Kabus M, Heubner G, Gahr M, Pessler F. Rapid and sustained remission of systemic juvenile idiopathic arthritis-associated macrophage activation syndrome through treatment with anakinra and corticosteroids. *J Clin Rheumatol* (2011) 17(1):23–7. doi:10.1097/RHU.0b013e318205092d
63. Durand M, Troyanov Y, Laflamme P, Gregoire G. Macrophage activation syndrome treated with anakinra. *J Rheumatol* (2010) 37(4):879–80. doi:10.3899/jrheum.091046
64. Kahn PJ, Cron RQ. Higher-dose anakinra is effective in a case of medically refractory macrophage activation syndrome. *J Rheumatol* (2013) 40(5):743–4. doi:10.3899/jrheum.121098
65. Loh NK, Lucas M, Fernandez S, Prentice D. Successful treatment of macrophage activation syndrome complicating adult Still disease with anakinra. *Intern Med J* (2012) 42(12):1358–62. doi:10.1111/imj.12002
66. Simon DW, Aneja R, Carcillo JA, Halstead ES. Plasma exchange, methylprednisolone, IV immune globulin, and now anakinra support continued PICU equipoise in management of hyperferritinemia-associated sepsis/multiple organ dysfunction syndrome/macrophage activation syndrome/secondary hemophagocytic lymphohistiocytosis syndrome*. *Pediatr Crit Care Med* (2014) 15(5):486–8. doi:10.1097/PCC.0000000000000098
67. Tayer-Shifman OE, Ben-Chetrit E. Refractory macrophage activation syndrome in a patient with SLE and APLA syndrome – successful use of PET-CT and anakinra in its diagnosis and treatment. *Mod Rheumatol* (2015) 25(6):954–7. doi:10.3109/14397595.2013.844403
68. Kelly A, Ramanan AV. A case of macrophage activation syndrome successfully treated with anakinra. *Nat Clin Pract Rheumatol* (2008) 4(11):615–20. doi:10.1038/ncprheum0919
69. Behrens EM, Kreiger PA, Cherian S, Cron RQ. Interleukin 1 receptor antagonist to treat cytophagic histiocytic panniculitis with secondary hemophagocytic lymphohistiocytosis. *J Rheumatol* (2006) 33(10):2081–4.
70. Rajasekaran S, Kruse K, Kovey K, Davis AT, Hassan NE, Ndika AN, et al. Therapeutic role of anakinra, an interleukin-1 receptor antagonist, in the management of secondary hemophagocytic lymphohistiocytosis/sepsis/multiple organ dysfunction/macrophage activating syndrome in critically ill children*. *Pediatr Crit Care Med* (2014) 15(5):401–8. doi:10.1097/PCC.0000000000000078
71. Miettunen PM, Narendran A, Jayanthan A, Behrens EM, Cron RQ. Successful treatment of severe paediatric rheumatic disease-associated macrophage activation syndrome with interleukin-1 inhibition following conventional immunosuppressive therapy: case series with 12 patients. *Rheumatology (Oxford)* (2011) 50(2):417–9. doi:10.1093/rheumatology/keq218
72. Gianella S, Schaer DJ, Schwarz U, Kurrer M, Heppner FL, Fehr J, et al. Retinal microangiopathy and rapidly fatal cerebral edema in a patient with adult-onset Still's disease and concurrent macrophage activation syndrome. *Am J Hematol* (2008) 83(5):424–7. doi:10.1002/ajh.21084
73. Henzan T, Nagafuji K, Tsukamoto H, Miyamoto T, Gondo H, Imashuku S, et al. Success with infliximab in treating refractory hemophagocytic lymphohistiocytosis. *Am J Hematol* (2006) 81(1):59–61. doi:10.1002/ajh.20462
74. Takahashi N, Naniwa T, Banno S. Successful use of etanercept in the treatment of acute lupus hemophagocytic syndrome. *Mod Rheumatol* (2008) 18(1):72–5. doi:10.3109/s10165-007-0006-z
75. Sellmer A, Stausbol-Gron B, Krag-Olsen B, Herlin T. Successful use of infliximab in macrophage activation syndrome with severe CNS involvement. *Scand J Rheumatol* (2011) 40(2):156–7. doi:10.3109/03009742.2010.508468
76. Makay B, Yilmaz S, Turkyilmaz Z, Unal N, Oren H, Unsal E. Etanercept for therapy-resistant macrophage activation syndrome. *Pediatr Blood Cancer* (2008) 50(2):419–21. doi:10.1002/pbc.21019
77. Cortis E, Insalaco A. Macrophage activation syndrome in juvenile idiopathic arthritis. *Acta Paediatr Suppl* (2006) 95(452):38–41. doi:10.1080/08035320600649713
78. Maeshima K, Ishii K, Iwakura M, Akamine M, Hamasaki H, Abe I, et al. Adult-onset Still's disease with macrophage activation syndrome successfully treated with a combination of methotrexate and etanercept. *Mod Rheumatol* (2012) 22(1):137–41. doi:10.1007/s10165-011-0477-9
79. Ideguchi H, Ohno S, Takase K, Hattori H, Kirino Y, Takeno M, et al. Successful treatment of refractory lupus-associated haemophagocytic lymphohistiocytosis with infliximab. *Rheumatology (Oxford)* (2007) 46(10):1621–2. doi:10.1093/rheumatology/kem205
80. Kikuchi H, Yamamoto T, Asako K, Takayama M, Shirasaki R, Ono Y. Etanercept for the treatment of intractable hemophagocytic syndrome with systemic lupus erythematosus. *Mod Rheumatol* (2012) 22(2):308–11. doi:10.1007/s10165-011-0500-1

81. Prahalad S, Bove KE, Dickens D, Lovell DJ, Grom AA. Etanercept in the treatment of macrophage activation syndrome. *J Rheumatol* (2001) 28(9):2120–4.
82. Bosnak M, Erdogan S, Aktekin EH, Bay A. Therapeutic plasma exchange in primary hemophagocytic lymphohistiocytosis: reports of two cases and a review of the literature. *Transfus Apher Sci* (2016) 55(3):353–6. doi:10.1016/j.transci.2016.09.015
83. Nakakura H, Ashida A, Matsumura H, Murata T, Nagatoya K, Shibahara N, et al. A case report of successful treatment with plasma exchange for hemophagocytic syndrome associated with severe systemic juvenile idiopathic arthritis in an infant girl. *Ther Apher Dial* (2009) 13(1):71–6. doi:10.1111/j.1744-9987.2009.00607.x
84. Das R, Guan P, Sprague L, Verbist K, Tedrick P, An QA, et al. Janus kinase inhibition lessens inflammation and ameliorates disease in murine models of hemophagocytic lymphohistiocytosis. *Blood* (2016) 127(13):1666–75. doi:10.1182/blood-2015-12-684399
85. Maschalidi S, Sepulveda FE, Garrigue A, Fischer A, de Saint Basile G. Therapeutic effect of JAK1/2 blockade on the manifestations of hemophagocytic lymphohistiocytosis in mice. *Blood* (2016) 128(1):60–71. doi:10.1182/blood-2016-02-700013
86. Jordan MB, Hildeman D, Kappler J, Marrack P. An animal model of hemophagocytic lymphohistiocytosis (HLH): CD8+ T cells and interferon gamma are essential for the disorder. *Blood* (2004) 104(3):735–43. doi:10.1182/blood-2003-10-3413
87. Marsh RA, Allen CE, McClain KL, Weinstein JL, Kanter J, Skiles J, et al. Salvage therapy of refractory hemophagocytic lymphohistiocytosis with alemtuzumab. *Pediatr Blood Cancer* (2013) 60(1):101–9. doi:10.1002/pbc.24188
88. Marsh RA, Jordan MB, Talano JA, Nichols KE, Kumar A, Naqvi A, et al. Salvage therapy for refractory hemophagocytic lymphohistiocytosis: a review of the published experience. *Pediatr Blood Cancer* (2017) 64(4). doi:10.1002/pbc.26308
89. Chellapandian D, Das R, Zelley K, Wiener SJ, Zhao H, Teachey DT, et al. Treatment of Epstein Barr virus-induced haemophagocytic lymphohistiocytosis with rituximab-containing chemo-immunotherapeutic regimens. *Br J Haematol* (2013) 162(3):376–82. doi:10.1111/bjh.12386
90. Buatois V, Chatel L, Cons L, Lory S, Richard F, Guilhot F, et al. Use of a mouse model to identify a blood biomarker for IFNgamma activity in pediatric secondary hemophagocytic lymphohistiocytosis. *Transl Res* (2017) 180:37–52.e2. doi:10.1016/j.trsl.2016.07.023
91. Wada T, Kanegane H, Ohta K, Katoh F, Imamura T, Nakazawa Y, et al. Sustained elevation of serum interleukin-18 and its association with hemophagocytic lymphohistiocytosis in XIAP deficiency. *Cytokine* (2014) 65(1):74–8. doi:10.1016/j.cyto.2013.09.007
92. Horne A, Janka G, Maarten Egeler R, Gadner H, Imashuku S, Ladisch S, et al. Haematopoietic stem cell transplantation in haemophagocytic lymphohistiocytosis. *Br J Haematol* (2005) 129(5):622–30. doi:10.1111/j.1365-2141.2005.05501.x
93. Ouachee-Chardin M, Elie C, de Saint Basile G, Le Deist F, Mahlaoui N, Picard C, et al. Hematopoietic stem cell transplantation in hemophagocytic lymphohistiocytosis: a single-center report of 48 patients. *Pediatrics* (2006) 117(4):e743–50. doi:10.1542/peds.2005-1789
94. Eapen M, DeLaat CA, Baker KS, Cairo MS, Cowan MJ, Kurtzberg J, et al. Hematopoietic cell transplantation for Chediak-Higashi syndrome. *Bone Marrow Transplant* (2007) 39(7):411–5. doi:10.1038/sj.bmt.1705600
95. Baker KS, Filipovich AH, Gross TG, Grossman WJ, Hale GA, Hayashi RJ, et al. Unrelated donor hematopoietic cell transplantation for hemophagocytic lymphohistiocytosis. *Bone Marrow Transplant* (2008) 42(3):175–80. doi:10.1038/bmt.2008.133
96. Cesaro S, Locatelli F, Lanino E, Porta F, Di Maio L, Messina C, et al. Hematopoietic stem cell transplantation for hemophagocytic lymphohistiocytosis: a retrospective analysis of data from the Italian Association of Pediatric Hematology Oncology (AIEOP). *Haematologica* (2008) 93(11):1694–701. doi:10.3324/haematol.13142
97. Ohga S, Kudo K, Ishii E, Honjo S, Morimoto A, Osugi Y, et al. Hematopoietic stem cell transplantation for familial hemophagocytic lymphohistiocytosis and Epstein-Barr virus-associated hemophagocytic lymphohistiocytosis in Japan. *Pediatr Blood Cancer* (2010) 54(2):299–306. doi:10.1002/pbc.22310
98. Cooper N, Rao K, Gilmour K, Hadad L, Adams S, Cale C, et al. Stem cell transplantation with reduced-intensity conditioning for hemophagocytic lymphohistiocytosis. *Blood* (2006) 107(3):1233–6. doi:10.1182/blood-2005-05-1819
99. Cooper N, Rao K, Goulden N, Webb D, Amrolia P, Veys P. The use of reduced-intensity stem cell transplantation in haemophagocytic lymphohistiocytosis and Langerhans cell histiocytosis. *Bone Marrow Transplant* (2008) 42(Suppl 2):S47–50. doi:10.1038/bmt.2008.283
100. Hartz B, Marsh R, Rao K, Henter JI, Jordan M, Filipovich L, et al. The minimum required level of donor chimerism in hereditary hemophagocytic lymphohistiocytosis. *Blood* (2016) 127(25):3281–90. doi:10.1182/blood-2015-12-684498

Conflict of Interest Statement: The author declares that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

The reviewer TG and handling editor declared their shared affiliation.

frontiers in Immunology

MINI REVIEW
published: 16 January 2018
doi: 10.3389/fimmu.2017.02005

Epstein–Barr Virus Susceptibility in Activated PI3Kδ Syndrome (APDS) Immunodeficiency

*Jean-Marie Carpier and Carrie L. Lucas**

Immunobiology Department, Yale University School of Medicine, New Haven, CT, United States

Activated PI3Kδ Syndrome (APDS) is an inherited immune disorder caused by heterozygous, gain-of-function mutations in the genes encoding the phosphoinositide 3-kinase delta (PI3Kδ) subunits p110δ or p85δ. This recently described primary immunodeficiency disease (PID) is characterized by recurrent sinopulmonary infections, lymphoproliferation, and susceptibility to herpesviruses, with Epstein–Barr virus (EBV) infection being most notable. A broad range of PIDs having disparate, molecularly defined genetic etiology can cause susceptibility to EBV, lymphoproliferative disease, and lymphoma. Historically, PID patients with loss-of-function mutations causing defective cell-mediated cytotoxicity or antigen receptor signaling were found to be highly susceptible to pathological EBV infection. By contrast, the gain of function in PI3K signaling observed in APDS patients paradoxically renders these patients susceptible to EBV, though the underlying mechanisms are incompletely understood. At a cellular level, APDS patients exhibit deranged B lymphocyte development and defects in class switch recombination, which generally lead to defective immunoglobulin production. Moreover, APDS patients also demonstrate an abnormal skewing of T cells toward terminal effectors with short telomeres and senescence markers. Here, we review APDS with a particular focus on how the altered lymphocyte biology in these patients may confer EBV susceptibility.

Keywords: Activated PI3Kδ Syndrome, PASLI, PI3K/AKT/mTOR, Epstein–Barr virus, immunodeficiency, B cell, T cell

Edited by:
Jeffrey I. Cohen, National Institutes of Health (NIH), United States

Reviewed by:
Shigeaki Nonoyama, National Defense Medical College, Japan
Kohsuke Imai, Tokyo Medical and Dental University, Japan

***Correspondence:**
Carrie L. Lucas
carrie.lucas@yale.edu

Specialty section:
This article was submitted to Primary Immunodeficiencies, a section of the journal Frontiers in Immunology

Received: *14 October 2017*
Accepted: *26 December 2017*
Published: *16 January 2018*

Citation:
Carpier JM and Lucas CL (2018) Epstein–Barr Virus Susceptibility in Activated PI3Kδ Syndrome (APDS) Immunodeficiency. Front. Immunol. 8:2005. doi: 10.3389/fimmu.2017.02005

INTRODUCTION

Epstein–Barr virus (EBV) is a gammaherpesvirus carried by ~95% of the world population. EBV has a tropism for oronasopharyngeal epithelial cells (site of lytic replication) and B lymphocytes (reservoir of latent virus) and is well controlled throughout life in most people. However, immunocompromised patients often show persistent EBV viremia, putting them at risk for B-cell transformation due to viral oncogenes. Indeed, the virus was first identified in a Burkitt's lymphoma in the 1960s (1) and is also associated with nasopharyngeal (2, 3) and gastric (4–7) cancer. Thus, inherited gene defects causing primary immunodeficiency diseases (PIDs) are often associated with recurrent or persistent EBV infections and related malignancies, and unraveling the genetic and molecular mechanisms underlying PIDs has led to better knowledge of the cellular and molecular components of the immune system that control herpesviruses. Here, we review the features of the recently described PID called **A**ctivated **P**I3K**δ** **S**yndrome (APDS) and discuss the immunological abnormalities that may confer susceptibility to EBV and elucidate the cellular and molecular immune mechanisms normally controlling EBV.

The Class IA phosphoinositide 3-kinase delta (PI3Kδ) complex is recruited to phosphotyrosines and catalyzes the phosphorylation of phosphatidylinositol-4,5-bisphosphate to generate

phosphatidylinositol-(3,4,5)-trisphosphate (PIP_3) that acts as a second messenger recruiting downstream signaling molecules. As a negative regulator of this signaling, the phosphatase PTEN can reverse this reaction and reduce levels of PIP_3. PI3Kδ is a heterodimer of the p110δ catalytic subunit and the p85α, p55α, or p50α regulatory subunit and is known to play a major role in cell survival, cell growth, and cell-cycle entry through downstream mediators including AKT and mTORC1 (8). Loss of PI3Kδ catalytic activity has been described in a single PID patient with severe disease, but EBV susceptibility was not reported (9). Gain-of-function (GoF) mutations in the *PIK3CD* or *PIK3R1* gene encoding p110δ or p85α, respectively, have been identified by us and others in PID patients with a disorder now known as PASLI Disease (**P**I3Kδ-**A**ctivating mutation causing **S**enescent T cells, **L**ymphadenopathy, and **I**mmunodeficiency), or APDS for short. In the following sections, we will briefly review the discovery of APDS and its genetic and molecular basis, the clinical and immunological features of APDS, and possible contributors to poor control of EBV in APDS patients.

GENETIC AND MOLECULAR BASIS OF APDS

Activated PI3Kδ Syndrome and causative *PIK3CD* mutations were initially described in two reports with a total of 26 patients in 14 unrelated families (10, 11). Prior to these initial reports, there had been one description of the most frequent mutation in *PIK3CD* (causing E1021K p110δ) in a single individual being studied for B-cell immunodeficiency, but no causative relationship was established (12). Shortly after discovery of APDS and underlying *PIK3CD* mutations, two additional reports with eight patients from six unrelated families with similar clinical findings described splice site mutations in *PIK3R1* as a second genetic cause for APDS (13, 14). Thus, APDS1 (or PASLI-CD) has been established to denote patients with *PIK3CD* mutations, and APDS2 (or PASLI-R1) denotes those with *PIK3R1* mutations. Another more recent phenocopy of APDS has been called APDS-like (APDS-L) and is caused by loss-of-function *PTEN* mutations (15, 16). Since the description of APDS in 2013, approximately 214 patients have been described with a spectrum of clinical features described below (10, 11, 13–41).

The PI3Kδ complex forms when p110δ and p85α bind at a 1:1 ratio. This constitutive complex remains stable due to tight binding interactions between the adaptor-binding domain (ABD) of p110δ and the inter-SH2 domain of p85α. To date, all activating APDS mutations affecting p110δ (E81K, G124D, N334K, R405C, C416R, E525K, E525A, R929C, E1021K, E1025G) and p85α (delE11, N564K) have been found or are expected to maintain some level of protein–protein interaction to form a hyperactive PI3Kδ complex, as free p110δ or p85α is unstable and would likely be degraded (**Figure 1A**). Each evaluated mutant has been found to hyperactivate signaling by disrupting inter- or intra-molecular inhibitory contacts, as observed for tumor-associated GoF mutations in the related *PIK3CA* (**Figure 1A**) (42, 43).

CLINICAL AND CELLULAR FEATURES OF APDS

The clinical spectrum of APDS1, APDS2, and APDS-L is largely overlapping and consists mostly of immunological abnormalities (**Table 1**), although growth retardation has also been reported APDS2 and, less frequently, APDS1 (10, 12–14, 17, 21, 24, 26, 27, 29–33, 37). Recurrent upper and lower respiratory tract infections are the most common clinical features affecting 98% of APDS patients and often resulting in progressive airway damage. APDS is associated with lymphoproliferative disease (71%), which commonly presents as lymphoid hyperplasia, splenomegaly, and/or lymphadenopathy. Autoinflammatory disease also occurs in 29% of cases. Importantly, recurrent infection with herpesviruses, such as EBV or cytomegalovirus (CMV), is observed in about 47% of cases but has not been associated with hemophagocytic lymphohistiocytosis (HLH). We hypothesize that HLH does not occur in APDS patients because, as described below, hyperactive PI3K drives polyclonal T-cell senescence, which limits homing, expansion, and survival of EBV-specific T cells and thereby prevents the cytokine storm that causes HLH (**Figure 1B**). EBV infection is found in 30% of APDS patients and represents an important risk factor for the development of B-cell lymphoma (occurring in 20% of EBV-infected APDS patients). However, the occurrence of EBV-negative lymphomas has overall been reported as higher (19%) than EBV-positive lymphomas (6%), which likely reflects the oncogenic potential of hyperactive PI3K signaling. Thus, intrinsically hyperactive PI3K (rather than EBV infection) appears to be the more dominant driver of B-cell transformation in APDS.

The susceptibility to infections displayed by APDS patients is associated with deficiencies in both T and B lymphocyte function, a feature that categorizes APDS as a combined immunodeficiency (**Table 1**). B-cell compartment abnormalities have been universally described in both APDS1 and APDS2. B-cell lymphopenia is found in 74% of patients and may be due to a developmental defect at the transitional stage, as IgD^+CD10^+ B cells are consistently increased in APDS patient blood (81%). Additionally, humoral defects have been observed in the majority of APDS patients, leading to poor vaccine responses in some patients. Serum concentrations of IgM are increased in 65% of cases, while IgA and at least one IgG isotype are decreased (68%). This phenotype suggests a defect in class-switch recombination (CSR), and *in vitro* studies have not yet provided a clear conclusion about whether this defect arises predominantly from B-cell-intrinsic or -extrinsic effects of PI3Kδ hyperactivation (11, 17, 22, 44). Although immunodeficiency is a major feature of APDS, expansion of CD8 T cells is commonly observed (70%) and, together with CD4 lymphopenia, explains the inverted CD4:CD8 ratios found in the disease (71%). In addition, the constitutive activation of PI3K is also linked to the progressive differentiation of T cells toward effector memory and terminally differentiated (T_{EMRA}) subtypes. Consistently, CD8 T cells from APDS patients exhibit normal degranulation activity (induced by anti-CD3 stimulation) and TNF/IFNγ production (11) with

reduced secretion of IL-2, weak proliferative responses, and enhanced restimulation-induced cell death (RICD) (10, 11, 14, 22).

Thus, APDS is characterized by a complex spectrum of clinical, immunological, and cellular features. Elucidation of the genetic and molecular defects has improved diagnosis and care of APDS

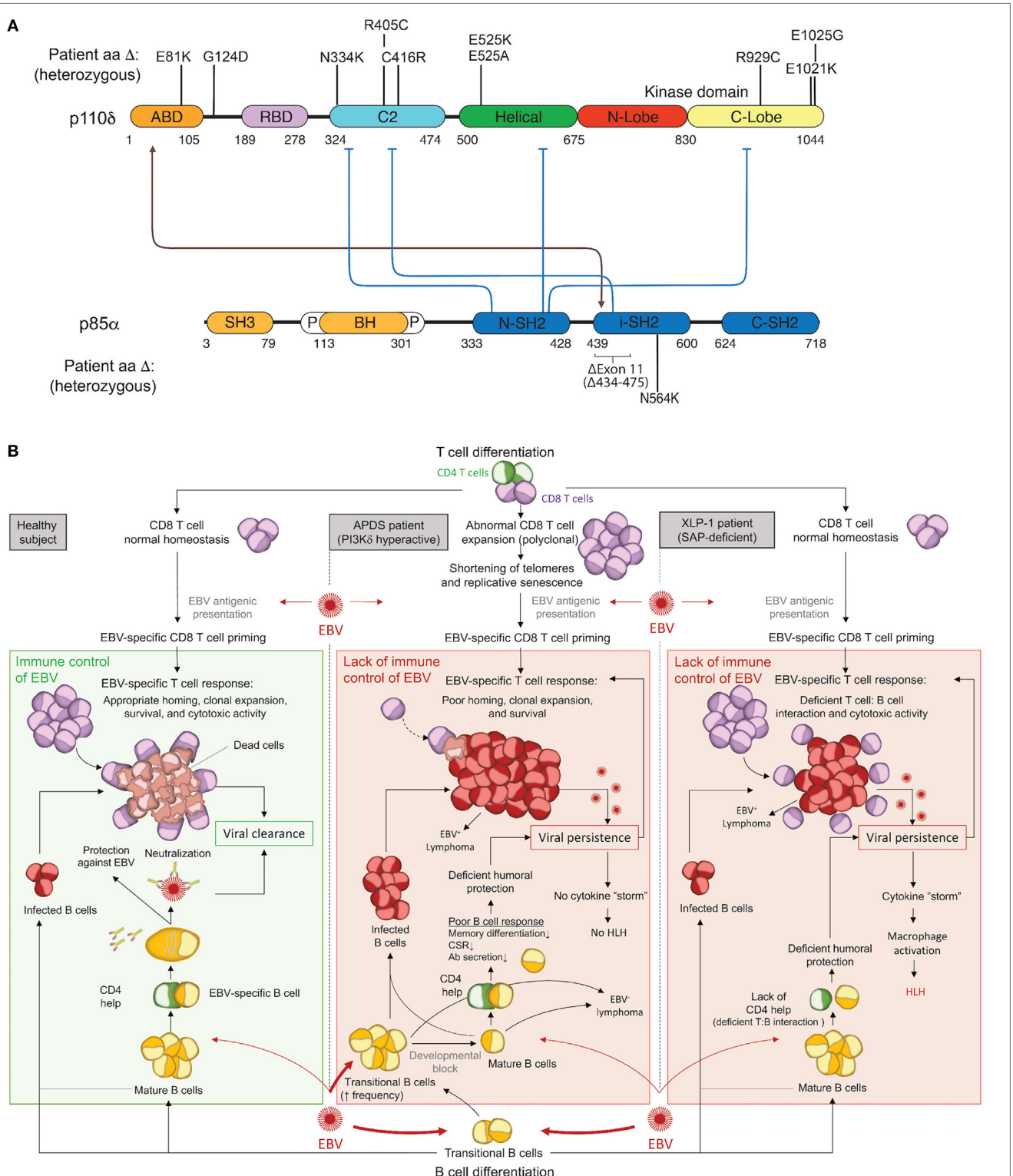


FIGURE 1 | Continued

FIGURE 1 | Activated PI3Kδ Syndrome (APDS) GoF mutations in the PI3Kδ complex and associated immune dysfunction responsible for Epstein–Barr virus (EBV) susceptibility. **(A)** Schematic representation of p110δ and p85α protein domains and APDS mutations reported in patients. The black line depicts the stabilizing interaction, and the blue lines show the inhibitory contacts within the PI3Kδ complex. ABD, adaptor-binding domain; BH, breakpoint-cluster region homology domain; P, proline-rich region; SH, SRC-homology domain; N, amino-terminal; i, inter; C, carboxy-terminal. **(B)** Schematic representation of the current understanding for the immune control of EBV in healthy subjects (left) and proposed hypothesis for EBV susceptibility in APDS (middle) and XLP1 (right) patients. APDS mutations cause abnormal polyclonal expansion of CD8 T cells that become senescent. Senescent CD8 T cells show an impaired EBV-specific response due to limited homing, expansion, and survival. In conjunction with CD8 T-cell defects, APDS patients exhibit an elevated frequency of transitional B cells, a major cell type for cell entry of EBV, and have defective humoral immunity that may further contribute to EBV susceptibility. In comparison, XLP1 patients, who are susceptible to EBV and develop HLH, are deficient in the SAP adaptor and exhibit defective EBV-specific T cell: B-cell interactions, causing a lack of CD4 help and a failure of CD8 T-cell cytotoxicity. As opposed to APDS, viral persistence in XLP1 patients causes a recurring stimulation/expansion of EBV-specific CD8 T cells and results in a cytokine storm underlying hemophagocytic lymphohistiocytosis (HLH). Antibodies depiction: taken from SMART (Servier Medical Art) licensed under a Creative Commons Attribution 3.0 Unported License.

patients (45). Because of the recurrent sinopulmonary infections, antibiotics are often given prophylactically, and immunoglobulin replacement is commonly used, although recurrent infections have been reported despite this treatment (15, 20, 26). Chemo- and/or radiotherapy are often used for lymphomas, a major cause of death in APDS patients (about 62% of deaths) (11, 14, 17–19, 24, 30, 31, 37). Beyond the treatment of these specific symptoms, hematopoietic stem-cell transplantation has proven beneficial for restoration of immune function in 67% of APDS patients receiving this therapy, which requires availability of an HLA-compatible donor and is particularly risky in the setting of EBV infection (10, 14, 15, 18, 24, 31, 34, 36). Identification of the genetic and molecular etiology of APDS has also led to more specific treatments, such as the use of the mTORC1 inhibitor (rapamycin) (10, 11, 23, 24, 26, 28, 34, 40) and specific p110δ inhibitors, which are currently being evaluated for APDS treatment in clinical trials.

EBV SUSCEPTIBILITY IN APDS PATIENTS

B-Cell Dysfunction

Epstein–Barr virus is usually acquired during childhood and is asymptomatic throughout life, while primary infection in young adulthood can (in ~30–70% of cases) cause infectious mononucleosis (IM) (46). Although control of EBV infection by the immune system has been mainly attributed to CD8 T cells and to a lesser extent to NK cells, a role for humoral immunity in protecting from EBV infection has recently been reevaluated with a focus on IM patients (46–48). Although a neutralizing antibody response against several viral proteins such as gp350, a particularly immunogenic EBV protein, is detectable in these patients (47), the peak of this antibody response occurs after disappearance of IM symptoms and clearance of the virus, and this delay has been attributed to B-cell dysfunction in acutely infected patients (46). Several vaccination strategies have focused on the gp350 protein (49–51) since it acts as a major mediator for entry of EBV into B cells through its interaction with CD21 (52). Interestingly, vaccination using recombinant gp350 in phase-I and-II trials correlated with a gp350-specific antibody response and showed a protective effect in IM development but not in asymptomatic EBV infections (50, 51). Thus, the role of neutralizing antibodies in protecting B cells from infection and lowering the extent of infection during primary exposure can be considered in asymptomatic individuals and especially in children who might carry maternal EBV-specific antibodies. This protection might also be crucial to prevent disease upon reexposure to EBV. As such, the defects in B-cell development and function observed in APDS patients might help explain their increased susceptibility to EBV.

Changes in B-cell differentiation and intrinsic B-cell dysregulation may also be relevant contributors to EBV susceptibility in APDS. The nature of the B-cell compartment primarily infected by EBV has been a matter of debate, and it was first proposed that $IgD^{-}CD27^{+}$ memory B cells are the major entry point (53). However, *in vitro* observations as well as data from IM patients suggested that primary infection of B cells occurs in naïve $IgD^{+}CD27$ cells, which then undergo differentiation in germinal center reactions, resulting in the emergence of class-switched memory B cells carrying EBV (54, 55). The observation that APDS patients exhibit an increased frequency of immature transitional $CD10^{+}$ B cells and have a low frequency of memory $CD27^{+}$ B cells (11) while remaining highly susceptible to EBV may support the possibility that EBV can also infect developing B cells. Indeed, several studies performed in mice have reported the ability of developing B cells to be infected by EBV (56) or the homologous γ-herpesvirus MHV68 (57, 58). The idea that transitional B cells might be a critical entry point and reservoir for EBV has been proposed before and fits with a model in which recurrent seeding of the developing B-cell compartment with EBV virions promotes establishment of long-term B-cell infection (57). In agreement with this hypothesis, depletion of transitional B cells in mice reduces EBV in the mature B-cell compartment (58). Therefore, it is possible that persistent EBV infection is facilitated in APDS patients by the predominant transitional B-cell compartment that would provide a pathologically increased reservoir of EBV, although additional studies are required to evaluate this hypothesis.

The EBV latency proteins LMP2a and LMP1 are thought to be key players in hijacking B-cell maturation by EBV since they mimic B-cell receptor and CD40 signaling, respectively (59, 60). LMP1 in particular is sufficient to transform several cell types, activates PI3K signaling, and promotes B-cell survival, growth, and proliferation programs (59–61). As p110δ is the main Class IA PI3K isoform expressed in EBV-positive B-cell lymphomas, this isoform might be a major target for LMP1 (62), and EBV-driven lymphomas in APDS may thus be facilitated in B cells expressing hyperactive forms of PI3Kδ. Moreover, several studies have demonstrated that PI3K inhibition reduces EBV reactivation (59, 63, 64), suggesting that the increased PI3Kδ activity displayed by APDS patients would favor a constitutive lytic program and may contribute to persistent viremia.

TABLE 1 | Summary of clinical and immunological features of APDS patients.

				Clinical features						Immunological features				
Reference	**Gene**	**Mutation[a]**	**Number of patients**	**Respiratory infections[b]**	**Lympho-proliferation[c]**	**EBV viremia**	**Other herpesviruses**	**B lymphoma**	**EBV + B lymphoma**	**Increased immature/ transitional B cells**	**Decreased IgA and/or IgG titers**	**Increased IgM titers**	**Defect in memory B cell[d]**	**Increased CD8 differentiation[e]**
Jou et al. (12)	*PIK3CD*	E1021K	1	1/1	n.d.	n.d.	1/1 (VZV)	n.d.	n.d.	n.d.	1/1	1/1	n.d.	n.d.
Angulo et al. (10)	*PIK3CD*	E1021K	17	17/17	10/17	1/17	4/17	1/17	n.d.	14/16	10/11	14/17	8/16	5/5
Lucas et al. (11)	*PIK3CD*	E1021K	3	3/3	3/3	3/3	1/2	1/3	1/3	3/3	2/3	2/3	2/2	2/2
	PIK3CD	E525K	5	5/5	3/5	5/5	4/5	1/5	1/5	5/5	3/5	0/5	3/5	1/1
	PIK3CD	N334K	1	1/1	1/1	1/1	0/1	0/1	0/1	1/1	1/1	1/1	1/1	1/1
Crank et al. (17)	*PIK3CD*	E1021K	1	1/1	1/1	0/1	0/1	1/1	0/1	1/1	1/1	1/1	n.d.	n.d.
	PIK3CD	C416R	2	2/2	2/2	1/2	1/2 (HSV)	2/2	0/2	2/2	1/2	2/2	n.d.	n.d.
Deau et al. (13)	*PIK3R1*	delE11	4	4/4	1/4	1/4	1/4 (CMV)	n.d.	n.d.	3/4	4/4	3/4	2/4	2/3
Kracker et al. (18)	*PIK3CD*	E1021K	8	8/8	6/8	0/8	0/8	2/8	0/8	0/1	5/8	7/8	2/2	n.d.
Lucas et al. (14)	*PIK3R1*	delE11	4	4/4	3/4	0/3	1/3 (CMV)	1/4	n.d.	n.d.	4/4	1/3	n.d.	Majority
Hartman et al. (19)	*PIK3CD*	E1021K	5	5/5	1/5	0/3	2/5 (HSV1, VZV)	n.d.	n.d.	n.d.	1/5	4/5	4/5	n.d.
Kannan et al. (20)	*PIK3CD*	E1021K	1	1/1	1/1	1/1	0/1	0/1	0/1	1/1	1/1	1/1	1/1	1/1
Lougaris et al. (21)	*PIK3R1*	delE11	4	4/4	4/4	n.d.	n.d.	n.d.	n.d.	2/2	4/4	4/4	3/3	n.d.
Elgizouli et al. (23)	*PIK3CD*	E1021K	5	5/5	5/5	1/5	1/5 (CMV)	0/5	0/5	2/4	5/5	1/5	2/4	n.d.
Elkaim et al. (24)	*PIK3R1*	delE11	36	36/36	22/36	8/36	6/35 (CMV), 2 (VZV)	10/36	1/36	14/15	27/35	18/31	11/19	10/10
Kuhlen et al. (29)	*PIK3R1*	delE11	1	1/1	1/1	0/1	1/1 (CMV)	n.d.	n.d.	n.d.	1/1	1/1	1/1	1/1
Martínez-Saavedra et al. (25)	*PIK3R1*	delE11	1	1/1	0/1	n.d.	n.d.	n.d.	n.d.	1/1	1/1	1/1	1/1	1/1
Olbrich et al. (26)	*PIK3R1*	delE11	2	1/2	2/2	2/2	2/2	n.d.	n.d.	1/1	2/2	2/2	2/2	1/1
Petrovski et al. (27)	*PIK3R1*	delE11	4	4/4	4/4	0/4	0/4	0/4	0/4	2/4	4/4	2/4	4/4	1/4
Rae et al. (28)	*PIK3CD*	E1021K	1	1/1	1/1	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	1/1	1/1
Tsujita et al. (15)	*PIK3CD*	E1021K	2	2/2	2/2	0/2	1/2 (HSV)	0/2	0/2	2/3	2/2	0/2	2/2	n.d.
	PIK3CD	E525A	3	2/3	2/3	0/3	1/3 (Herpes zoster)	0/3	0/3	3/3	3/3	1/3	0/3	n.d.
Bravo García-Morato et al. (30)	*PIK3R1*	delE11	2	2/2	2/2	1/2	1/2 (herpetic lesions)	1/2	0/2	1/1	2/2	1/2	0/1	1/1
Chiriaco et al. (22)	*PIK3CD*	E1021K	1	1/1	1/1	1/1	0/1	0/1	0/1	1/1	1/1	1/1	0/1	1/1
Coulter et al. (31)	*PIK3CD*	E1021K or E525K	50 + 3	51/53	39/53	14/53	49% including EBV + (human herpesvirus 6, VZV, HSV)	7/53	3/53	24/32	21/49	38/50	17/30	17/18

(*Continued*)

TABLE 1 | Continued

Reference	Gene	Mutation[a]	Number of patients	Clinical features						Immunological features				
				Respiratory infections[b]	Lympho-proliferation[c]	EBV viremia	Other herpesviruses	B lymphoma	EBV + B lymphoma	Increased immature/ transitional B cells	Decreased IgA and/or IgG titers	Increased IgM titers	Defect in memory B cell[d]	Increased CD8 differentiation[e]
Dulau et al. (35)	*PIK3CD*	E1021K	5	5/5	5/5	4/5	4/5 (CMV, HSV, VZV)	2/5	n.d.	4/5	3/5	4/5	5/5	n.d.
	PIK3CD	E525K	3	3/3	3/3	3/3	2/3 (CMV)	1/3	n.d.	3/3	2/3	1/3	2/3	n.d.
	PIK3CD	N334K	1	1/1	1/1	1/1	0/1	0/1	n.d.	1/1	1/1	1/1	0/1	n.d.
	PIK3CD	E1025G	1	1/1	1/1	1/1	1/1 (VZV)	0/1	n.d.	0/1	1/1	1/1	1/1	n.d.
Mettman et al. (41)	*PIK3CD*	E1021K	1	1/1	1/1	n.d.	n.d.	0/1	0/1	n.d.	0/1	1/1	1/1	n.d.
Goto et al. (40)	*PIK3CD*	E1021K	1	n.d.	1/1	1/1	1/1 (CMV)	0/1	0/1	1/1	1/1	1/1	1/1	1/1
Hauck et al. (37)	*PIK3R1*	delE11	3	3/3	2/3	1/3	0/3	1/3	1/3	0/2	2/3	2/3	n.d.	2/2
Wentink et al. (34)	*PIK3CD*	E1021K	9	9/9	3/9	2/9	n.d.	2/9	n.d.	Increased	5/11	5/11	Decreased	n.d.
	PIK3CD	E525K	1	1/1	1/1	0/1	n.d.	0/1	n.d.		0/1	0/1		n.d.
	PIK3CD	R929C	1	1/1	0/1	0/1	n.d.	0/1	n.d.		1/1	0/1		n.d.
	PIK3R1	N564K	1	1/1	0/1	0/1	n.d.	0/1	n.d.		0/1	0/1		n.d.
	PIK3R1	delE11	1	1/1	1/1	0/1	n.d.	0/1	n.d.		0/0	0/0		n.d.
Nademi et al. (36)	*PIK3CD*	E1021K	10	10/10	8/10	2/10	5/10	1/11	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
	PIK3R1	delE11	1	1/1	0/1	0/1	0/1		n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
Takeda et al (33)	*PIK3CD*	G124D	2	2/2	2/2	2/2	2/2 (Herpes zoster, labialis)	1/2	1/2	1/1	2/2	2/2	0/1	1/1
	PIK3CD	E81K	1	1/1	1/1	1/1	0/0	1/1	n.d.	0/1	0/0	0/0	1/1	0/0
Heurtier et al. (32)	*PIK3CD*	E81K	1	1/1	1/1	n.d.	n.d.	n.d.	n.d.	1/1	1/1	0/1	1/1	1/1
	PIK3CD	G124D	2	2/2	2/2	n.d.	n.d.	n.d.	n.d.	1/1	2/2	1/2	2/2	2/2
Rae et al. (38)	*PIK3CD*	R405C	1	1/1	0/1	0/1	0/1	0/1	0/1	n.d.	1/1	0/1	1/1	0/1
Saettini et al. (39)	*PIK3CD*	E1021K	1	1/1	1/1	1/1	0/1	0/1	0/1	1/1	1/1	0/1	1/1	1/1
			214	**98.1%**	**70.9%**	**29.5%**	**32.10%**	**18.80%**	**5.80%**	**80.7%**	**68.1%**	**65.3%**	**65.4%**	**70.3%**

[a]Frequencies of activating PI3Kδ mutations among APDS1 and APDS2 patients: E1021K, 58%; C416R, 1%; R405C, 0.5%; E525K, 6%; E525A, 1%; N334K, 1%; E81K, 1%; G124D, 2%; R929C, 0.5%; E1025G, 0.5%; delE11, 29%; N564K, 0.5%.

[b]Includes upper and lower respiratory tracts.

[c]Includes splenomegaly and lymphadenopathy.

[d]Assessment of cell counts, frequency or B-cell memory class switch.

[e]Frequencies of effector/memory cells, CD57 expression, telomere lengths.

n.d., not determined; CMV, cytomegalovirus; EBV, Epstein–Barr virus; HSV, herpes simplex virus; VZV, varicella zoster virus.

Thus, APDS patients harbor abnormal B cells that likely promote EBV susceptibility through several mechanisms. These may include, among others, poor anti-EBV antibody responses, increased transitional B cells serving as an EBV reservoir, and heightened cell-intrinsic PI3K signaling that may promote EBV-driven B-cell transformation and/or EBV reactivation.

T-Cell Dysfunction

T lymphocytes are a crucial immune cell type for control of EBV infection (65, 66). Substantial expansion of EBV-specific CD8 T cells has been observed in IM patients (67), and EBV control in healthy carriers has been correlated with the presence of functional EBV-specific CD8 T cells (68). However, the major arguments supporting a functional role for CD8 T cells in controlling EBV *in vivo* come from immunocompromised patients. Indeed, post-transplant lymphoproliferative disease (PTLD) is an important clinical concern in immunosuppressed transplant patients. In these patients, PTLD is caused by EBV-driven B-cell expansion and can be overcome by infusing EBV-specific cytotoxic T cells (69–72). Moreover, immunodeficiency syndromes, particularly HLH and X-linked lymphoproliferative diseases, have also provided valuable lessons and advanced our understanding of the role for CD8 T cells in EBV immunity (73, 74).

Monogenic causes of EBV-associated HLH have demonstrated that defective cytotoxicity machinery most commonly underlies disease (66, 75). However, these more general defects are not present in APDS patients, highlighting a more nuanced mechanism conferring EBV susceptibility when PI3K signaling is hyperactive. XLP1 patients deficient in the signaling lymphocytic activation molecule-associated protein (SAP) adaptor exhibit a very specific vulnerability to EBV viremia, and uncovering the genetic mutations responsible for disease contributed to defining crucial and non-redundant molecular pathways for EBV control by cytotoxic cells (76–79). Indeed, mutations in *SH2D1A* encoding SAP result in failure of T cell: B-cell interactions and inability to propagate 2B4- and NTBA-mediated signals promoting cytotoxicity and instead favor an inflammatory cytokine storm that drives HLH (77, 80–84). Although XLP1 and APDS patients fail to control EBV infection, both patient cohorts harbor EBV-specific T cells and their CD8 T cells show normal *in vitro* effector functions in response to SAP-independent stimuli (82, 85). Interestingly, positive signaling for cytotoxicity induced by receptors of the SLAM family (e.g., 2B4 and NTBA) that utilize the SAP adaptor involves PI3K/AKT activity (86, 87). Thus, both APDS and XLP1 share the feature of EBV susceptibility; however, unlike XLP1 patients, APDS patients are not susceptible to HLH. We hypothesize that hyperactive PI3K T-cell intrinsically drives polyclonal senescence and prevents a cytokine storm and HLH by limiting homing, expansion, and survival of EBV-specific T cells, as described further below (**Figure 1B**). Indeed, T cells from APDS patients exhibit enhanced stimulation-induced apoptosis (10), which is a feature shared with patients deficient in the anti-apoptotic factor XIAP who are susceptible to EBV and HLH (88, 89). Poor survival of EBV-reactive T cells may be a common underlying feature of EBV susceptibility in both XIAP deficiency and APDS, although the HLH phenotype in XIAP deficiency is poorly understood (90, 91).

The PI3K-driven expansion of effector CD8 T cells in APDS (11, 14) raises the question of why they cannot control EBV infection. The answer might come from the differentiation state of CD8 T cells since peripheral blood T cells in APDS patients are terminally differentiated with characteristics of senescence (92) (**Table 1**), including low IL-2 secretion, shortened telomeres, and poor proliferative capacity. Studies in mouse tumor models have similarly shown that senescent T cells exhibit *in vivo* defects including reduced survival, proliferation, IL-2 production, lymphoid homing, and tumor rejection (**Figure 1B**) (93, 94). Replicative senescence occurs when telomere erosion that occurs with each cell division reaches a critical point, leading to irreversible cell-cycle arrest through activation of the DNA damage response that is thought to protect from cellular transformation by preventing genomic instability and infinite proliferation (95). CD8 and CD4 T-cell immunosenescence has been observed in elderly individuals (96), and numerous studies demonstrate a high correlation between T-cell aging and persistent infections (e.g., CMV, EBV and HIV) (97–99) or the development of tumors (100, 101). A closer look at CMV-specific T cells has revealed a link between aging and increased frequency of CMV-specific CD8 T cells with a senescent phenotype (102, 103), suggesting that chronic antigen stimulation might drive T-cell senescence. Consistent with this hypothesis, the expression of the telomerase reverse transcriptase (TERT) that regulates the length of telomeres drastically declines in CD8 T cells after repeated antigen stimulation and acquisition of a senescent phenotype (104). Interestingly, overexpression of TERT increases the proliferative capacity of stimulated T cells (105), and using a pharmacological activator of TERT enhances CD8 T-cell-mediated control HIV infection *in vitro* (106).

Thus, immunosenescence represents a plausible contributor to defective EBV control in APDS patients, as CD8 T cells might not be able to clonally expand and mount a robust and specific response against EBV despite their prominent effector phenotype (11). While repeated EBV antigen stimulation seems to be an attractive hypothesis for driving T-cell immunosenescence in APDS, patients without active herpesviruses still have a high frequency of senescent T cells (**Table 1**), indicating that immunosenescence is likely not restricted to antigen-specific T cells. Instead, the hyperactivation of PI3K, a signaling pathway known to play multiple roles in survival, metabolism, cell growth, and cell-cycle progression (107–109), likely drives senescence by promoting exuberant *in vivo* CD8 T-cell proliferation (and resulting in clinical features of lymphoproliferation). Moreover, several studies have linked increased PI3K/AKT/mTORC1 activity with senescence in immortalized and primary cells (110–115). Interestingly, studies in cells with hyperactive PI3K signaling or mTORC1 inhibition with rapamycin have led to a model in which PI3K/AKT/mTORC1 signaling plays an early role in cell senescence induction without hyperproliferation as a prerequisite (110). While this latter set of data suggests that DNA damage is not a driving factor for PI3K-dependent senescence, other studies further proposed that PI3K/AKT contributes to reactive oxygen species production to cause irreparable chromosomal damage

and irreversible cell-cycle arrest (111, 116). Although it is clear that the PI3K pathway plays an important role in senescence, further investigation is required to fully understand senescence of CD8 T cells in APDS patients. As such, APDS provides an invaluable opportunity to study immunosenescence and roles for PI3K in its regulation in humans.

Thus, hyperactive PI3Kδ may drive CD8 T-cell growth, terminal differentiation, and immunosenescence, although the detailed molecular basis of T-cell senescence in APDS patients remains to be fully elucidated. This state is associated with altered CD8 T-cell functions, including decreased proliferation and increased TCR restimulation-induced cell death, that might contribute to failure of APDS patients to adequately control EBV.

CONCLUSION

Genomics has greatly advanced studies of PIDs (117, 118), shedding light on genes critical for human immunity. The recently solved PID called APDS highlights important roles for regulated PI3Kδ signaling in control of EBV through effects on B- and T-cell development and function.

AUTHOR CONTRIBUTIONS

JMC and CL prepared and wrote the minireview manuscript.

ACKNOWLEDGMENTS

The authors thank the patients and their families as well as referring clinicians and their teams. The authors also thank Mr. Andrew Takeda for discussions and feedback on the manuscript and Servier Medical Art for providing visual elements in **Figure 1** (https://smart.servier.com/smart_image/antibody-5/).

FUNDING

This work was supported by Yale University (grant number NHLBI R00HL125668) and the Anderson Postdoctoral Fellowship (Yale).

REFERENCES

1. Epstein MA, Achong BG, Barr YM. Virus particles in cultured lymphoblasts from Burkitt's lymphoma. *Lancet* (1964) 1:702–3. doi:10.1016/S0140-6736(64)91524-7
2. zur Hausen H, Schulte-Holthausen H, Klein G, Henle W, Henle G, Clifford P, et al. EBV DNA in biopsies of Burkitt tumours and anaplastic carcinomas of the nasopharynx. *Nature* (1970) 228:1056–8. doi:10.1038/2281056a0
3. Desgranges C, Wolf H, De-The G, Shanmugaratnam K, Cammoun N, Ellouz R, et al. Nasopharyngeal carcinoma. X. Presence of Epstein-Barr genomes in separated epithelial cells of tumours in patients from Singapore, Tunisia and Kenya. *Int J Cancer* (1975) 16:7–15. doi:10.1002/ijc.2910160103
4. Burke AP, Yen TS, Shekitka KM, Sobin LH. Lymphoepithelial carcinoma of the stomach with Epstein-Barr virus demonstrated by polymerase chain reaction. *Mod Pathol* (1990) 3:377–80.
5. Shibata D, Weiss LM. Epstein-Barr virus-associated gastric adenocarcinoma. *Am J Pathol* (1992) 140:769–74.
6. Tokunaga M, Land CE, Uemura Y, Tokudome T, Tanaka S, Sato E. Epstein-Barr virus in gastric carcinoma. *Am J Pathol* (1993) 143:1250–4.
7. Takada K. Epstein-Barr virus and gastric carcinoma. *Mol Pathol* (2000) 53:255–61. doi:10.1136/mp.53.5.255
8. Lucas CL, Chandra A, Nejentsev S, Condliffe AM, Okkenhaug K. PI3Kdelta and primary immunodeficiencies. *Nat Rev Immunol* (2016) 16:702–14. doi:10.1038/nri.2016.93
9. Zhang KJ, Husami A, Marsh R, Jordan MB. Identification of a phosphoinositide 3-kinase (PI-3K) p110δ (PIK3CD) deficient individual. *J Clin Immunol* (2013) 33:671–709. doi:10.1007/s10875-013-9869-2
10. Angulo I, Vadas O, Garcon F, Banham-Hall E, Plagnol V, Leahy TR, et al. Phosphoinositide 3-kinase delta gene mutation predisposes to respiratory infection and airway damage. *Science* (2013) 342:866–71. doi:10.1126/science.1243292
11. Lucas CL, Kuehn HS, Zhao F, Niemela JE, Deenick EK, Palendira U, et al. Dominant-activating germline mutations in the gene encoding the PI(3)K catalytic subunit p110delta result in T cell senescence and human immunodeficiency. *Nat Immunol* (2014) 15:88–97. doi:10.1038/ni.2771
12. Jou ST, Chien YH, Yang YH, Wang TC, Shyur SD, Chou CC, et al. Identification of variations in the human phosphoinositide 3-kinase p110delta gene in children with primary B-cell immunodeficiency of unknown aetiology. *Int J Immunogenet* (2006) 33:361–9. doi:10.1111/j.1744-313X.2006.00627.x
13. Deau MC, Heurtier L, Frange P, Suarez F, Bole-Feysot C, Nitschke P, et al. A human immunodeficiency caused by mutations in the PIK3R1 gene. *J Clin Invest* (2014) 124:3923–8. doi:10.1172/jci75746
14. Lucas CL, Zhang Y, Venida A, Wang Y, Hughes J, McElwee J, et al. Heterozygous splice mutation in PIK3R1 causes human immunodeficiency with lymphoproliferation due to dominant activation of PI3K. *J Exp Med* (2014) 211:2537–47. doi:10.1084/jem.20141759
15. Tsujita Y, Mitsui-Sekinaka K, Imai K, Yeh TW, Mitsuiki N, Asano T, et al. Phosphatase and tensin homolog (PTEN) mutation can cause activated phosphatidylinositol 3-kinase delta syndrome-like immunodeficiency. *J Allergy Clin Immunol* (2016) 138:1672–80.e1610. doi:10.1016/j.jaci.2016.03.055
16. Driessen GJ, IJspeert H, Wentink M, Yntema HG, van Hagen PM, van Strien A, et al. Increased PI3K/Akt activity and deregulated humoral immune response in human PTEN deficiency. *J Allergy Clin Immunol* (2016) 138:1744–7.e1745. doi:10.1016/j.jaci.2016.07.010
17. Crank MC, Grossman JK, Moir S, Pittaluga S, Buckner CM, Kardava L, et al. Mutations in PIK3CD can cause hyper IgM syndrome (HIGM) associated with increased cancer susceptibility. *J Clin Immunol* (2014) 34:272–6. doi:10.1007/s10875-014-0012-9
18. Kracker S, Curtis J, Ibrahim MA, Sediva A, Salisbury J, Campr V, et al. Occurrence of B-cell lymphomas in patients with activated phosphoinositide 3-kinase delta syndrome. *J Allergy Clin Immunol* (2014) 134:233–6. doi:10.1016/j.jaci.2014.02.020
19. Hartman HN, Niemela J, Hintermeyer MK, Garofalo M, Stoddard J, Verbsky JW, et al. Gain of function mutations of PIK3CD as a cause of primary sclerosing cholangitis. *J Clin Immunol* (2015) 35:11–4. doi:10.1007/s10875-014-0109-1
20. Kannan JA, Davila-Saldana BJ, Zhang K, Filipovich AH, Kucuk ZY. Activated phosphoinositide 3-kinase delta syndrome in a patient with a former diagnosis of common variable immune deficiency, bronchiectasis, and lymphoproliferative disease. *Ann Allergy Asthma Immunol* (2015) 115:452–4. doi:10.1016/j.anai.2015.08.009
21. Lougaris V, Faletra F, Lanzi G, Vozzi D, Marcuzzi A, Valencic E, et al. Altered germinal center reaction and abnormal B cell peripheral maturation in PI3KR1-mutated patients presenting with HIGM-like phenotype. *Clin Immunol* (2015) 159:33–6. doi:10.1016/j.clim.2015.04.014
22. Chiriaco M, Brigida I, Ariganello P, Di Cesare S, Di Matteo G, Taus F, et al. The case of an APDS patient: defects in maturation and function and decreased in vitro anti-mycobacterial activity in the myeloid compartment. *Clin Immunol* (2017) 178:20–8. doi:10.1016/j.clim.2015.12.008
23. Elgizouli M, Lowe DM, Speckmann C, Schubert D, Hulsdunker J, Eskandarian Z, et al. Activating PI3Kdelta mutations in a cohort of 669 patients with primary immunodeficiency. *Clin Exp Immunol* (2016) 183:221–9. doi:10.1111/cei.12706
24. Elkaim E, Neven B, Bruneau J, Mitsui-Sekinaka K, Stanislas A, Heurtier L, et al. Clinical and immunologic phenotype associated with activated phosphoinositide 3-kinase delta syndrome 2: a cohort study. *J Allergy Clin Immunol* (2016) 138:210–8.e219. doi:10.1016/j.jaci.2016.03.022
25. Martínez-Saavedra MT, García-Gomez S, Domínguez Acosta A, Mendoza Quintana JJ, Páez JP, García-Reino EJ, et al. Gain-of-function mutation in

PIK3R1 in a patient with a narrow clinical phenotype of respiratory infections. *Clin Immunol* (2016) 173:117–20. doi:10.1016/j.clim.2016.09.011
26. Olbrich P, Lorenz M, Cura Daball P, Lucena JM, Rensing-Ehl A, Sanchez B, et al. Activated PI3Kdelta syndrome type 2: two patients, a novel mutation, and review of the literature. *Pediatr Allergy Immunol* (2016) 27: 640–4. doi:10.1111/pai.12585
27. Petrovski S, Parrott RE, Roberts JL, Huang H, Yang J, Gorentla B, et al. Dominant splice site mutations in PIK3R1 cause hyper IgM syndrome, lymphadenopathy and short stature. *J Clin Immunol* (2016) 36:462–71. doi:10.1007/s10875-016-0281-6
28. Rae W, Ramakrishnan KA, Gao Y, Ashton-Key M, Pengelly RJ, Patel SV, et al. Precision treatment with sirolimus in a case of activated phosphoinositide 3-kinase delta syndrome. *Clin Immunol* (2016) 171:38–40. doi:10.1016/j.clim.2016.07.017
29. Kuhlen M, Hönscheid A, Loizou L, Nabhani S, Fischer U, Stepensky P, et al. De novo PIK3R1 gain-of-function with recurrent sinopulmonary infections, long-lasting chronic CMV-lymphadenitis and microcephaly. *Clin Immunol* (2016) 162:27–30. doi:10.1016/j.clim.2015.10.008
30. Bravo García-Morato M, García-Miñaúr S, Molina Garicano J, Santos Simarro F, Del Pino Molina L, López-Granados E, et al. Mutations in PIK3R1 can lead to APDS2, SHORT syndrome or a combination of the two. *Clin Immunol* (2017) 179:77–80. doi:10.1016/j.clim.2017.03.004
31. Coulter TI, Chandra A, Bacon CM, Babar J, Curtis J, Screaton N, et al. Clinical spectrum and features of activated phosphoinositide 3-kinase delta syndrome: a large patient cohort study. *J Allergy Clin Immunol* (2017) 139:597–606.e594. doi:10.1016/j.jaci.2016.06.021
32. Heurtier L, Lamrini H, Chentout L, Deau MC, Bouafia A, Rosain J, et al. Mutations in the adaptor-binding domain and associated linker region of p110delta cause activated PI3K-delta syndrome 1 (APDS1). *Haematologica* (2017) 102:e278–81. doi:10.3324/haematol.2017.167601
33. Takeda AJ, Zhang Y, Dornan GL, Siempelkamp BD, Jenkins ML, Matthews HF, et al. Novel PIK3CD mutations affecting N-terminal residues of p110delta cause activated PI3Kdelta syndrome (APDS) in humans. *J Allergy Clin Immunol* (2017). doi:10.1016/j.jaci.2017.03.026
34. Wentink M, Dalm V, Lankester AC, van Schouwenburg PA, Scholvinck L, Kalina T, et al. Genetic defects in PI3Kdelta affect B-cell differentiation and maturation leading to hypogammaglobulineamia and recurrent infections. *Clin Immunol* (2017) 176:77–86. doi:10.1016/j.clim.2017.01.004
35. Dulau Florea AE, Braylan RC, Schafernak KT, Williams KW, Daub J, Goyal RK, et al. Abnormal B-cell maturation in the bone marrow of patients with germline mutations in PIK3CD. *J Allergy Clin Immunol* (2017) 139:1032–5.e1036. doi:10.1016/j.jaci.2016.08.028
36. Nademi Z, Slatter MA, Dvorak CC, Neven B, Fischer A, Suarez F, et al. Hematopoietic stem cell transplant in patients with activated PI3K delta syndrome. *J Allergy Clin Immunol* (2017) 139:1046–9. doi:10.1016/j.jaci.2016.09.040
37. Hauck F, Magg T, Krolo A, Bilic I, Hirschmugl T, Laass M, et al. Variant PIK3R1 hypermorphic mutation and clinical phenotypes in a family with short statures, mild immunodeficiency and lymphoma. *Klin Padiatr* (2017) 229:113–7. doi:10.1055/s-0043-104218
38. Rae W, Gao Y, Ward D, Mattocks CJ, Eren E, Williams AP. A novel germline gain-of-function variant in PIK3CD. *Clin Immunol* (2017) 181:29–31. doi:10.1016/j.clim.2017.05.020
39. Saettini F, Pelagatti MA, Sala D, Moratto D, Giliani S, Badolato R, et al. Early diagnosis of PI3Kdelta syndrome in a 2 years old girl with recurrent otitis and enlarged spleen. *Immunol Lett* (2017) 190:279–81. doi:10.1016/j.imlet.2017.08.021
40. Goto F, Uchiyama T, Nakazawa Y, Imai K, Kawai T, Onodera M. Persistent impairment of T-cell regeneration in a patient with activated PI3K delta syndrome. *J Clin Immunol* (2017) 37:347–50. doi:10.1007/s10875-017-0393-7
41. Mettman D, Thiffault I, Dinakar C, Saunders C. Immunodeficiency-associated lymphoid hyperplasia as a cause of intussusception in a case of activated PI3K-delta syndrome. *Front Pediatr* (2017) 5:71. doi:10.3389/fped.2017.00071
42. Dornan GL, Siempelkamp BD, Jenkins ML, Vadas O, Lucas CL, Burke JE. Conformational disruption of PI3Kdelta regulation by immunodeficiency mutations in PIK3CD and PIK3R1. *Proc Natl Acad Sci U S A* (2017) 114:1982–7. doi:10.1073/pnas.1617244114
43. Burke JE, Williams RL. Synergy in activating class I PI3Ks. *Trends Biochem Sci* (2015) 40:88–100. doi:10.1016/j.tibs.2014.12.003
44. Compagno M, Wang Q, Pighi C, Cheong TC, Meng FL, Poggio T, et al. Phosphatidylinositol 3-kinase delta blockade increases genomic instability in B cells. *Nature* (2017) 542:489–93. doi:10.1038/nature21406
45. Garcelon N, Neuraz A, Benoit V, Salomon R, Kracker S, Suarez F, et al. Finding patients using similarity measures in a rare diseases-oriented clinical data warehouse: Dr. Warehouse and the needle in the needle stack. *J Biomed Inform* (2017) 73:51–61. doi:10.1016/j.jbi.2017.07.016
46. Panikkar A, Smith C, Hislop A, Tellam N, Dasari V, Hogquist KA, et al. Impaired Epstein-Barr virus-specific neutralizing antibody response during acute infectious mononucleosis is coincident with global B-cell dysfunction. *J Virol* (2015) 89:9137–41. doi:10.1128/jvi.01293-15
47. Bu W, Hayes GM, Liu H, Gemmell L, Schmeling DO, Radecki P, et al. Kinetics of Epstein-Barr virus (EBV) neutralizing and virus-specific antibodies after primary infection with EBV. *Clin Vaccine Immunol* (2016) 23:363–9. doi:10.1128/cvi.00674-15
48. Jayasooriya S, de Silva TI, Njie-jobe J, Sanyang C, Leese AM, Bell AI, et al. Early virological and immunological events in asymptomatic Epstein-Barr virus infection in African children. *PLoS Pathog* (2015) 11:e1004746. doi:10.1371/journal.ppat.1004746
49. Kanekiyo M, Bu W, Joyce MG, Meng G, Whittle JR, Baxa U, et al. Rational design of an Epstein-Barr virus vaccine targeting the receptor-binding site. *Cell* (2015) 162:1090–100. doi:10.1016/j.cell.2015.07.043
50. Moutschen M, Léonard P, Sokal EM, Smets F, Haumont M, Mazzu P, et al. Phase I/II studies to evaluate safety and immunogenicity of a recombinant gp350 Epstein-Barr virus vaccine in healthy adults. *Vaccine* (2007) 25:4697–705. doi:10.1016/j.vaccine.2007.04.008
51. Sokal EM, Hoppenbrouwers K, Vandermeulen C, Moutschen M, Léonard P, Moreels A, et al. Recombinant gp350 vaccine for infectious mononucleosis: a phase 2, randomized, double-blind, placebo-controlled trial to evaluate the safety, immunogenicity, and efficacy of an Epstein-Barr virus vaccine in healthy young adults. *J Infect Dis* (2007) 196:1749–53. doi:10.1086/523813
52. Martin DR, Marlowe RL, Ahearn JM. Determination of the role for CD21 during Epstein-Barr virus infection of B-lymphoblastoid cells. *J Virol* (1994) 68:4716–26.
53. Kurth J, Spieker T, Wustrow J, Strickler GJ, Hansmann LM, Rajewsky K, et al. EBV-infected B cells in infectious mononucleosis: viral strategies for spreading in the B cell compartment and establishing latency. *Immunity* (2000) 13:485–95. doi:10.1016/S1074-7613(00)00048-0
54. Thorley-Lawson DA. Epstein-Barr virus: exploiting the immune system. *Nat Rev Immunol* (2001) 1:75–82. doi:10.1038/35095584
55. Chaganti S, Heath EM, Bergler W, Kuo M, Buettner M, Niedobitek G, et al. Epstein-Barr virus colonization of tonsillar and peripheral blood B-cell subsets in primary infection and persistence. *Blood* (2009) 113:6372–81. doi:10.1182/blood-2008-08-175828
56. Lee EK, Joo EH, Song KA, Choi B, Kim M, Kim SH, et al. Effects of lymphocyte profile on development of EBV-induced lymphoma subtypes in humanized mice. *Proc Natl Acad Sci U S A* (2015) 112:13081–6. doi:10.1073/pnas.1407075112
57. Coleman CB, Nealy MS, Tibbetts SA. Immature and transitional B cells are latency reservoirs for a gammaherpesvirus. *J Virol* (2010) 84:13045–52. doi:10.1128/jvi.01455-10
58. Coleman CB, McGraw JE, Feldman ER, Roth AN, Keyes LR, Grau KR, et al. A gammaherpesvirus Bcl-2 ortholog blocks B cell receptor-mediated apoptosis and promotes the survival of developing B cells in vivo. *PLoS Pathog* (2014) 10:e1003916. doi:10.1371/journal.ppat.1003916
59. Liu X, Cohen JI. The role of PI3K/Akt in human herpesvirus infection: from the bench to the bedside. *Virology* (2015) 479-480:568–77. doi:10.1016/j.virol.2015.02.040
60. Hatton OL, Harris-Arnold A, Schaffert S, Krams SM, Martinez OM. The interplay between Epstein-Barr virus and B lymphocytes: implications for infection, immunity, and disease. *Immunol Res* (2014) 58:268–76. doi:10.1007/s12026-014-8496-1
61. Dawson CW, Tramountanis G, Eliopoulos AG, Young LS. Epstein-Barr virus latent membrane protein 1 (LMP1) activates the phosphatidylinositol 3-kinase/Akt pathway to promote cell survival and induce actin filament remodeling. *J Biol Chem* (2003) 278:3694–704. doi:10.1074/jbc.M209840200
62. Furukawa S, Wei L, Krams SM, Esquivel CO, Martinez OM. PI3Kdelta inhibition augments the efficacy of rapamycin in suppressing proliferation

of Epstein-Barr virus (EBV)+ B cell lymphomas. *Am J Transplant* (2013) 13:2035–43. doi:10.1111/ajt.12328
63. Goswami R, Gershburg S, Satorius A, Gershburg E. Protein kinase inhibitors that inhibit induction of lytic program and replication of Epstein-Barr virus. *Antiviral Res* (2012) 96:296–304. doi:10.1016/j.antiviral.2012.09.021
64. Oussaief L, Hippocrate A, Ramirez V, Rampanou A, Zhang W, Meyers D, et al. Phosphatidylinositol 3-kinase/Akt pathway targets acetylation of Smad3 through Smad3/CREB-binding protein interaction: contribution to transforming growth factor beta1-induced Epstein-Barr virus reactivation. *J Biol Chem* (2009) 284:23912–24. doi:10.1074/jbc.M109.036483
65. Taylor GS, Long HM, Brooks JM, Rickinson AB, Hislop AD. The immunology of Epstein-Barr virus-induced disease. *Annu Rev Immunol* (2015) 33:787–821. doi:10.1146/annurev-immunol-032414-112326
66. Cohen JI. Primary immunodeficiencies associated with EBV disease. *Curr Top Microbiol Immunol* (2015) 390:241–65. doi:10.1007/978-3-319-22822-8_10
67. Callan MF, Fazou C, Yang H, Rostron T, Poon K, Hatton C, et al. CD8(+) T-cell selection, function, and death in the primary immune response in vivo. *J Clin Invest* (2000) 106:1251–61. doi:10.1172/jci10590
68. Ning RJ, Xu XQ, Chan KH, Chiang AK. Long-term carriers generate Epstein-Barr virus (EBV)-specific CD4(+) and CD8(+) polyfunctional T-cell responses which show immunodominance hierarchies of EBV proteins. *Immunology* (2011) 134:161–71. doi:10.1111/j.1365-2567.2011.03476.x
69. Naik S, Nicholas SK, Martinez CA, Leen AM, Hanley PJ, Gottschalk SM, et al. Adoptive immunotherapy for primary immunodeficiency disorders with virus-specific T lymphocytes. *J Allergy Clin Immunol* (2016) 137:1498–505. e1491. doi:10.1016/j.jaci.2015.12.1311
70. Rooney CM, Smith CA, Ng CY, Loftin S, Li C, Krance RA, et al. Use of gene-modified virus-specific T lymphocytes to control Epstein-Barr-virus-related lymphoproliferation. *Lancet* (1995) 345:9–13. doi:10.1016/S0140-6736(95)91150-2
71. Rooney CM, Smith CA, Ng CY, Loftin SK, Sixbey JW, Gan Y, et al. Infusion of cytotoxic T cells for the prevention and treatment of Epstein-Barr virus-induced lymphoma in allogeneic transplant recipients. *Blood* (1998) 92:1549–55.
72. Doubrovina E, Oflaz-Sozmen B, Prockop SE, Kernan NA, Abramson S, Teruya-Feldstein J, et al. Adoptive immunotherapy with unselected or EBV-specific T cells for biopsy-proven EBV+ lymphomas after allogeneic hematopoietic cell transplantation. *Blood* (2012) 119:2644–56. doi:10.1182/blood-2011-08-371971
73. Palendira U, Low C, Bell AI, Ma CS, Abbott RJ, Phan TG, et al. Expansion of somatically reverted memory CD8+ T cells in patients with X-linked lymphoproliferative disease caused by selective pressure from Epstein-Barr virus. *J Exp Med* (2012) 209:913–24. doi:10.1084/jem.20112391
74. Palendira U, Rickinson AB. Primary immunodeficiencies and the control of Epstein-Barr virus infection. *Ann N Y Acad Sci* (2015) 1356:22–44. doi:10.1111/nyas.12937
75. Tangye SG, Palendira U, Edwards ES. Human immunity against EBV-lessons from the clinic. *J Exp Med* (2017) 214:269–83. doi:10.1084/jem.20161846
76. Tangye SG. XLP: clinical features and molecular etiology due to mutations in SH2D1A encoding SAP. *J Clin Immunol* (2014) 34:772–9. doi:10.1007/s10875-014-0083-7
77. Sayos J, Wu C, Morra M, Wang N, Zhang X, Allen D, et al. The X-linked lymphoproliferative-disease gene product SAP regulates signals induced through the co-receptor SLAM. *Nature* (1998) 395:462–9. doi:10.1038/26683
78. Coffey AJ, Brooksbank RA, Brandau O, Oohashi T, Howell GR, Bye JM, et al. Host response to EBV infection in X-linked lymphoproliferative disease results from mutations in an SH2-domain encoding gene. *Nat Genet* (1998) 20:129–35. doi:10.1038/2424
79. Nichols KE, Harkin DP, Levitz S, Krainer M, Kolquist KA, Genovese C, et al. Inactivating mutations in an SH2 domain-encoding gene in X-linked lymphoproliferative syndrome. *Proc Natl Acad Sci U S A* (1998) 95:13765–70. doi:10.1073/pnas.95.23.13765
80. Bottino C, Falco M, Parolini S, Marcenaro E, Augugliaro R, Sivori S, et al. NTB-A [correction of GNTB-A], a novel SH2D1A-associated surface molecule contributing to the inability of natural killer cells to kill Epstein-Barr virus-infected B cells in X-linked lymphoproliferative disease. *J Exp Med* (2001) 194:235–46. doi:10.1084/jem.194.3.235
81. Snow AL, Marsh RA, Krummey SM, Roehrs P, Young LR, Zhang K, et al. Restimulation-induced apoptosis of T cells is impaired in patients with X-linked lymphoproliferative disease caused by SAP deficiency. *J Clin Invest* (2009) 119:2976–89. doi:10.1172/jci39518
82. Hislop AD, Palendira U, Leese AM, Arkwright PD, Rohrlich PS, Tangye SG, et al. Impaired Epstein-Barr virus-specific CD8+ T-cell function in X-linked lymphoproliferative disease is restricted to SLAM family-positive B-cell targets. *Blood* (2010) 116:3249–57. doi:10.1182/blood-2009-09-238832
83. Nakajima H, Cella M, Bouchon A, Grierson HL, Lewis J, Duckett CS, et al. Patients with X-linked lymphoproliferative disease have a defect in 2B4 receptor-mediated NK cell cytotoxicity. *Eur J Immunol* (2000) 30:3309–18. doi:10.1002/1521-4141(200011)30:11<3309::AID-IMMU3309>3.0.CO;2-3
84. Qi H, Cannons JL, Klauschen F, Schwartzberg PL, Germain RN. SAP-controlled T-B cell interactions underlie germinal centre formation. *Nature* (2008) 455:764–9. doi:10.1038/nature07345
85. Dupré L, Andolfi G, Tangye SG, Clementi R, Locatelli F, Aricò M, et al. SAP controls the cytolytic activity of CD8+ T cells against EBV-infected cells. *Blood* (2005) 105:4383–9. doi:10.1182/blood-2004-08-3269
86. Aoukaty A, Tan R. Association of the X-linked lymphoproliferative disease gene product SAP/SH2D1A with 2B4, a natural killer cell-activating molecule, is dependent on phosphoinositide 3-kinase. *J Biol Chem* (2002) 277:13331–7. doi:10.1074/jbc.M112029200
87. Mikhalap SV, Shlapatska LM, Berdova AG, Law CL, Clark EA, Sidorenko SP. CDw150 associates with src-homology 2-containing inositol phosphatase and modulates CD95-mediated apoptosis. *J Immunol* (1999) 162:5719–27.
88. Rigaud S, Fondanèche MC, Lambert N, Pasquier B, Mateo V, Soulas P, et al. XIAP deficiency in humans causes an X-linked lymphoproliferative syndrome. *Nature* (2006) 444:110–4. doi:10.1038/nature05257
89. Aguilar C, Latour S. X-linked inhibitor of apoptosis protein deficiency: more than an X-linked lymphoproliferative syndrome. *J Clin Immunol* (2015) 35:331–8. doi:10.1007/s10875-015-0141-9
90. Hatton O, Phillips LK, Vaysberg M, Hurwich J, Krams SM, Martinez OM. Syk activation of phosphatidylinositol 3-kinase/Akt prevents HtrA2-dependent loss of X-linked inhibitor of apoptosis protein (XIAP) to promote survival of Epstein-Barr virus+ (EBV+) B cell lymphomas. *J Biol Chem* (2011) 286:37368–78. doi:10.1074/jbc.M111.255125
91. Carter BZ, Milella M, Tsao T, McQueen T, Schober WD, Hu W, et al. Regulation and targeting of antiapoptotic XIAP in acute myeloid leukemia. *Leukemia* (2003) 17:2081–9. doi:10.1038/sj.leu.2403113
92. Chou JP, Effros RB. T cell replicative senescence in human aging. *Curr Pharm Des* (2013) 19:1680–98. doi:10.2174/138161213805219711
93. Gattinoni L, Klebanoff CA, Palmer DC, Wrzesinski C, Kerstann K, Yu Z, et al. Acquisition of full effector function in vitro paradoxically impairs the in vivo antitumor efficacy of adoptively transferred CD8+ T cells. *J Clin Invest* (2005) 115:1616–26. doi:10.1172/jci24480
94. Gattinoni L, Powell DJ Jr, Rosenberg SA, Restifo NP. Adoptive immunotherapy for cancer: building on success. *Nat Rev Immunol* (2006) 6:383–93. doi:10.1038/nri1842
95. Campisi J. Aging, cellular senescence, and cancer. *Annu Rev Physiol* (2013) 75:685–705. doi:10.1146/annurev-physiol-030212-183653
96. Tarazona R, DelaRosa O, Alonso C, Ostos B, Espejo J, Peña J, et al. Increased expression of NK cell markers on T lymphocytes in aging and chronic activation of the immune system reflects the accumulation of effector/senescent T cells. *Mech Ageing Dev* (2000) 121:77–88. doi:10.1016/S0047-6374(00)00199-8
97. Ouyang Q, Wagner WM, Walter S, Müller CA, Wikby A, Aubert G, et al. An age-related increase in the number of CD8+ T cells carrying receptors for an immunodominant Epstein-Barr virus (EBV) epitope is counteracted by a decreased frequency of their antigen-specific responsiveness. *Mech Ageing Dev* (2003) 124:477–85. doi:10.1016/S0047-6374(03)00026-5
98. Sansoni P, Vescovini R, Fagnoni FF, Akbar A, Arens R, Chiu YL, et al. New advances in CMV and immunosenescence. *Exp Gerontol* (2014) 55:54–62. doi:10.1016/j.exger.2014.03.020
99. Dock JN, Effros RB. Role of CD8 T cell replicative senescence in human aging and in HIV-mediated immunosenescence. *Aging Dis* (2011) 2:382–97.
100. Poschke I, De Boniface J, Mao Y, Kiessling R. Tumor-induced changes in the phenotype of blood-derived and tumor-associated T cells of early stage breast cancer patients. *Int J Cancer* (2012) 131:1611–20. doi:10.1002/ijc.27410
101. Ye J, Peng G. Controlling T cell senescence in the tumor microenvironment for tumor immunotherapy. *Oncoimmunology* (2015) 4:e994398. doi:10.4161/2162402x.2014.994398

102. Pita-Lopez ML, Gayoso I, DelaRosa O, Casado JG, Alonso C, Muñoz-Gomariz E, et al. Effect of ageing on CMV-specific CD8 T cells from CMV seropositive healthy donors. *Immun Ageing* (2009) 6:11. doi:10.1186/1742-4933-6-11
103. Tu W, Rao S. Mechanisms underlying T cell immunosenescence: aging and cytomegalovirus infection. *Front Microbiol* (2016) 7:2111. doi:10.3389/fmicb.2016.02111
104. Valenzuela HF, Effros RB. Divergent telomerase and CD28 expression patterns in human CD4 and CD8 T cells following repeated encounters with the same antigenic stimulus. *Clin Immunol* (2002) 105:117–25. doi:10.1006/clim.2002.5271
105. Dagarag M, Evazyan T, Rao N, Effros RB. Genetic manipulation of telomerase in HIV-specific CD8+ T cells: enhanced antiviral functions accompany the increased proliferative potential and telomere length stabilization. *J Immunol* (2004) 173:6303–11. doi:10.4049/jimmunol.173.10.6303
106. Fauce SR, Jamieson BD, Chin AC, Mitsuyasu RT, Parish ST, Ng HL, et al. Telomerase-based pharmacologic enhancement of antiviral function of human CD8+ T lymphocytes. *J Immunol* (2008) 181:7400–6. doi:10.4049/jimmunol.181.10.7400
107. Appleman LJ, van Puijenbroek AA, Shu KM, Nadler LM, Boussiotis VA. CD28 costimulation mediates down-regulation of p27kip1 and cell cycle progression by activation of the PI3K/PKB signaling pathway in primary human T cells. *J Immunol* (2002) 168:2729–36. doi:10.4049/jimmunol.168.6.2729
108. Cappellini A, Tabellini G, Zweyer M, Bortul R, Tazzari PL, Billi AM, et al. The phosphoinositide 3-kinase/Akt pathway regulates cell cycle progression of HL60 human leukemia cells through cytoplasmic relocalization of the cyclin-dependent kinase inhibitor p27(Kip1) and control of cyclin D1 expression. *Leukemia* (2003) 17:2157–67. doi:10.1038/sj.leu.2403111
109. Fruman DA, Rommel C. PI3K and cancer: lessons, challenges and opportunities. *Nat Rev Drug Discov* (2014) 13:140–56. doi:10.1038/nrd4204
110. Astle MV, Hannan KM, Ng PY, Lee RS, George AJ, Hsu AK, et al. AKT induces senescence in human cells via mTORC1 and p53 in the absence of DNA damage: implications for targeting mTOR during malignancy. *Oncogene* (2012) 31:1949–62. doi:10.1038/onc.2011.394
111. Imai Y, Takahashi A, Hanyu A, Hori S, Sato S, Naka K, et al. Crosstalk between the Rb pathway and AKT signaling forms a quiescence-senescence switch. *Cell Rep* (2014) 7:194–207. doi:10.1016/j.celrep.2014.03.006
112. Chen Z, Trotman LC, Shaffer D, Lin HK, Dotan ZA, Niki M, et al. Crucial role of p53-dependent cellular senescence in suppression of Pten-deficient tumorigenesis. *Nature* (2005) 436:725–30. doi:10.1038/nature03918
113. Miyauchi H, Minamino T, Tateno K, Kunieda T, Toko H, Komuro I. Akt negatively regulates the in vitro lifespan of human endothelial cells via a p53/p21-dependent pathway. *EMBO J* (2004) 23:212–20. doi:10.1038/sj.emboj.7600045
114. Mavrakis KJ, Zhu H, Silva RL, Mills JR, Teruya-Feldstein J, Lowe SW, et al. Tumorigenic activity and therapeutic inhibition of Rheb GTPase. *Genes Dev* (2008) 22:2178–88. doi:10.1101/gad.1690808
115. Moral M, Segrelles C, Lara MF, Martínez-Cruz AB, Lorz C, Santos M, et al. Akt activation synergizes with Trp53 loss in oral epithelium to produce a novel mouse model for head and neck squamous cell carcinoma. *Cancer Res* (2009) 69:1099–108. doi:10.1158/0008-5472.can-08-3240
116. Nogueira V, Park Y, Chen CC, Xu PZ, Chen ML, Tonic I, et al. Akt determines replicative senescence and oxidative or oncogenic premature senescence and sensitizes cells to oxidative apoptosis. *Cancer Cell* (2008) 14:458–70. doi:10.1016/j.ccr.2008.11.003
117. Lucas CL, Lenardo MJ. Identifying genetic determinants of autoimmunity and immune dysregulation. *Curr Opin Immunol* (2015) 37:28–33. doi:10.1016/j.coi.2015.09.001
118. Lenardo M, Lo B, Lucas CL. Genomics of immune diseases and new therapies. *Annu Rev Immunol* (2016) 34:121–49. doi:10.1146/annurev-immunol-041015-055620

Conflict of Interest Statement: The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

frontiers in Immunology

REVIEW
published: 18 January 2018
doi: 10.3389/fimmu.2017.01978

Human NF-κB1 Haploinsufficiency and Epstein–Barr Virus-Induced Disease—Molecular Mechanisms and Consequences

*Birgit Hoeger[1,2], Nina Kathrin Serwas[1,2] and Kaan Boztug[1,2,3,4]**

[1] Ludwig Boltzmann Institute for Rare and Undiagnosed Diseases, Vienna, Austria, [2] CeMM Research Center for Molecular Medicine of the Austrian Academy of Sciences, Vienna, Austria, [3] Department of Pediatrics and Adolescent Medicine, Medical University of Vienna, Vienna, Austria, [4] Department of Pediatrics, St. Anna Kinderspital and Children's Cancer Research Institute, Medical University of Vienna, Vienna, Austria

Nuclear factor kappa-light-chain-enhancer of activated B cells 1 (NF-κB1)-related human primary immune deficiencies have initially been characterized as defining a subgroup of common variable immunodeficiencies (CVIDs), representing intrinsic B-cell disorders with antibody deficiency and recurrent infections of various kind. Recent evidence indicates that NF-κB1 haploinsufficiency underlies a variable type of combined immunodeficiency (CID) affecting both B and T lymphocyte compartments, with a broadened spectrum of disease manifestations, including Epstein–Barr virus (EBV)-induced lymphoproliferative disease and immediate life-threatening consequences. As part of this review series focused on EBV-related primary immunodeficiencies, we discuss the current clinical and molecular understanding of monoallelic *NFKB1* germline mutations with special focus on the emerging context of EBV-associated disease. We outline mechanistic implications of dysfunctional NF-κB1 in B and T cells and discuss the fatal relation of impaired T-cell function with the inability to clear EBV infections. Finally, we compare common and suggested treatment angles in the context of this complex disease.

Keywords: nuclear factor kappa-light-chain-enhancer of activated B cells 1, haploinsufficiency, common variable immunodeficiency, combined immunodeficiency, Epstein–Barr virus, lymphoproliferation, T cells, B cells

OPEN ACCESS

Edited by:
Jeffrey I. Cohen, National Institutes of Health (NIH), United States

Reviewed by:
Manfred Fliegauf, Universitätsklinikum Freiburg, Germany
Mark Ballow, University of South Florida, United States

****Correspondence:***
Kaan Boztug
kaan.boztug@rud.lbg.ac.at

Specialty section:
This article was submitted to Primary Immunodeficiencies, a section of the journal Frontiers in Immunology

Received: *31 October 2017*
Accepted: *20 December 2017*
Published: *18 January 2018*

Citation:
Hoeger B, Serwas NK and Boztug K (2018) Human NF-κB1 Haploinsufficiency and Epstein–Barr Virus-Induced Disease—Molecular Mechanisms and Consequences. Front. Immunol. 8:1978. doi: 10.3389/fimmu.2017.01978

Abbreviations: aHSCT, allogeneic hematopoietic stem cell transplantation; AKT, AKT serine/threonine kinase; ATM, ataxia telangiectasia mutated serine/threonine kinase; BAFF(-R), B-cell activating factor (receptor); BCL10, B-cell CLL/lymphoma 10; BCR, B-cell receptor; BIRC4, Baculoviral IAP-repeat containing protein 4; CARD11, caspase recruitment domain family member 11; CARMA, CARD-containing MAGUK protein 1; CD40L, CD40 ligand; CID, combined immunodeficiency; CTPS1, CTP synthase 1; CORO1A, Coronin 1A; CVID, common variable immunodeficiency; DD, death domain; EBV, Epstein–Barr virus; FCGR3A, Fc fragment of IgG receptor 3A; GATA2, GATA binding protein 2; GRR, glycine-rich region; IκB, inhibitor of NF-κB; IKK, inhibitor of NF-κB kinase; IL-1, interleukin-1; IL1R, interleukin-1 receptor; IRAK, interleukin-1 receptor-associated kinase 1; ITK, IL2-inducible T-cell kinase; LMP1, latent membrane protein 1; LPD, lymphoproliferative disease; LPS, lipopolysaccharide; LTβ(R), lymphotoxin β (receptor); MAGT1, magnesium transporter 1; MALT1, mucosa-associated lymphoid tissue lymphoma translocation protein 1; MAPK, mitogen-activated protein kinase; MCM4, minichromosome maintenance protein 4; NEMO, NF-κB essential modulator; NF-κB1/NF-κB2, nuclear factor kappa-light-chain-enhancer of activated B cells-1/-2; NIK, NF-κB inducing kinase; NLS, nuclear localization sequence; PID, primary immunodeficiency; PI3K, phosphatidylinositol 3-kinase; PIK3CD, phosphatidylinositol 3-kinase catalytic delta; PIK3R1, phosphatidylinositol 3-kinase receptor 1; PKC, protein kinase C; RASGRP1, rat-sarcoma guanyl releasing protein 1; RHD, rel-homology domain; SAP, signaling lymphocytic activation molecule-associated protein; *S. pneumoniae*, *Streptococcus pneumoniae*; STK4, serine/threonine protein kinase 4; TCR, T-cell receptor; TES1/TES2, transformation effector site 1/2; TIR, toll-like/interleukin-1 receptor; TLR, toll-like receptor; TRIF, TIR-domain containing adaptor-inducing interferon-β; TNFα, tumor necrosis factor-α; TNFR, tumor necrosis factor receptor; TRADD, tumor necrosis factor receptor type 1-associated DEATH domain protein; TRAF, tumor necrosis factor receptor-associated factor; WASP, Wiskott–Aldrich syndrome protein.

INTRODUCTION

Nuclear factor kappa-light-chain-enhancer of activated B cells (NF-κB) represents a key node in propagation of cellular signals, driving cell fate decisions toward proliferation, or apoptotic clearance. Due to their highly adaptable nature, immune cells in particular rely on NF-κB processes for their development, function and inflammatory responses. Both the innate and adaptive immune axes are critically dependent on functional NF-κB signaling networks (1). Owing to its name, NF-κB-derived programming of target genes particularly manages B-cell fates including their maturation, survival, differentiation and (T-cell independent) class switching processes (2, 3). Its importance for maintaining B-cell development and integrity has been underlined by studying murine knockout models (4) and the identification of human *NFKB1* and *NFKB2* gene defects with B-cell deficiency-related clinical manifestations initially classified as common variable immunodeficiencies (CVIDs) (5–18). Beyond its role in B-cell intrinsic processes, NF-κB1 defects presenting with recurrent or chronic Epstein–Barr virus (EBV) infection (6, 8) or fatal EBV-driven lymphoproliferative disease (7) suggest a broadened phenotypic spectrum, including combined immunodeficiency (CID) with B- and T-cell dysfunction (7). In addition, NF-κB1 has been shown to regulate human NK-cell maturation and effector function *in vitro*, with yet unclear consequences for human health (13). Together, these studies highlight the relevance of identifying human genetic defects for studying key processes in immune cell function and deciphering the spectrum of phenotypic consequences of newly emerging disorders. In this review article, we focus on discussing EBV-associated disease in the context of genetic predisposition to NF-κB1 dysfunction. A general review of NF-κB mechanisms in and beyond B cells and EBV pathogenesis has been discussed elsewhere (1–3, 19, 20) and is outlined here in comparing B- and T-cell functions related to the emergence of NF-κB-related EBV infectious disease.

NF-κB SIGNALING

Proteins of the NF-κB family of transcription factors are broadly expressed in cells of the immune system. The family consists of the two large precursor proteins NF-κB1 (p105) and NF-κB2 (p100), which can be processed to their smaller mature subunits p50 and p52, respectively, and their dimerization partners RelA (p65), RelB, and c-Rel (21). Upon NF-κB pathway activation, p105 and p100 are post-translationally processed for nuclear translocation of the resulting mature NF-κB dimers (22) (**Figure 1**). In resting state, NF-κB shows cytoplasmic localization. This is achieved through masking the nuclear localization sequence, either by binding of inhibitor of NF-κB (IκB) proteins to p50 dimers, or through *in-cis* binding of the C-terminal part of p105 (23).

Activation of the NF-κB pathway occurs through two distinct routes, the canonical and the non-canonical pathway, by distinct stimuli-receptor pairs (21, 24) as described in **Figure 1**. Specifically, in canonical signaling, binding of TNFα to TNF receptors results in the recruitment of the adaptor protein TRADD to the intracellular part of the TNFR which in turn activates the kinase RIP1 and subsequently IKK (25, 26). IL-1 or LPS binding to their respective receptors (IL-1 receptors and Toll-like receptors, respectively) results in the recruitment of adaptor proteins MyD88 and TRIF to the TIR (TLR/interleukin-1 receptor) domain. This induces IRAK1/4-dependent recruitment of IKK to the complex, and its subsequent activation through TRAF6 (27, 28). BCR and TCR signaling also result in the activation of IKK, which happens through the recruitment of the CARMA, BCL10, and MALT1 complex by PKCβ in B cells and PKCθ in T cells (29, 30). Stimulation of IKK (IκB-kinase)-α, IKKβ, and NEMO (IKKγ) induces phosphorylation and degradation of IκB (inhibitor of κB) proteins (23), with subsequent release of NF-κB dimers for nuclear translocation. By contrast, non-canonical NF-κB signaling induced by TNF receptor family ligands CD40L, BAFF or lymphotoxin-β (24) inhibits TRAF3-induced NIK degradation, leading to NIK kinase-dependent activation of primarily IKKα (31). Activated IKKα in turn phosphorylates NF-κB2/p100 which results in processing to its active form p52, and its nuclear translocation (32). Target genes are modulated by either activating or repressing interactions with κB DNA-binding sites (33). As the transactivation domain is provided by the Rel binding partners, NF-κB homodimers exert a repressive function. Substantial crosstalk between the canonical and non-canonical NF-κB signaling axes has been uncovered, forming the basis for a complex and tightly regulated signaling network that shapes cell-type and cell-state-specific functions (34).

In the modulation of immune-relevant processes, transcriptional activity of NF-κB is required during negative selection in T-cell development. This has been elucidated through the analysis of *Relb*$^{-/-}$ mice which develop spontaneous autoimmune dermatitis (35). Furthermore, NF-κB-mediated transcription is important for the development and maturation of NK and NKT cells in mice (36, 37). Most importantly, NF-κB transcriptional activity is required during B-cell development, maturation and survival. Already at the pre-BCR stage in B-cell development, NF-κB provides pro-survival signals (38). Deletion of IKK specifically from B cells results in reduced numbers of transitional and mature B cells in mice (39). Similarly, primary immunodeficiency (PID) diseases caused by mutations in the genes encoding family or pathway members of NF-κB predominantly present with B-cell deficiencies, as discussed below.

HUMAN GERMLINE MUTATIONS IN *NFKB1* AND THEIR CONSEQUENCES IN B- AND T-CELL FUNCTION

NF-κB1 Defects with CVID-Like Presentation

Common variable immunodeficiency denotes a heterogeneous group of B-cell disorders characterized by pronounced antibody deficiency and recurrent infections. It is considered the most prevalent symptomatic PID for which an underlying monogenic origin has been identified in only ~10% of cases (40–42). The first clinical manifestations of NF-κB1 haploinsufficiency and CVID-like presentation were reported in 2015 (5). Ever since, further cases of *NFKB1* defects with monoallelic inheritance have

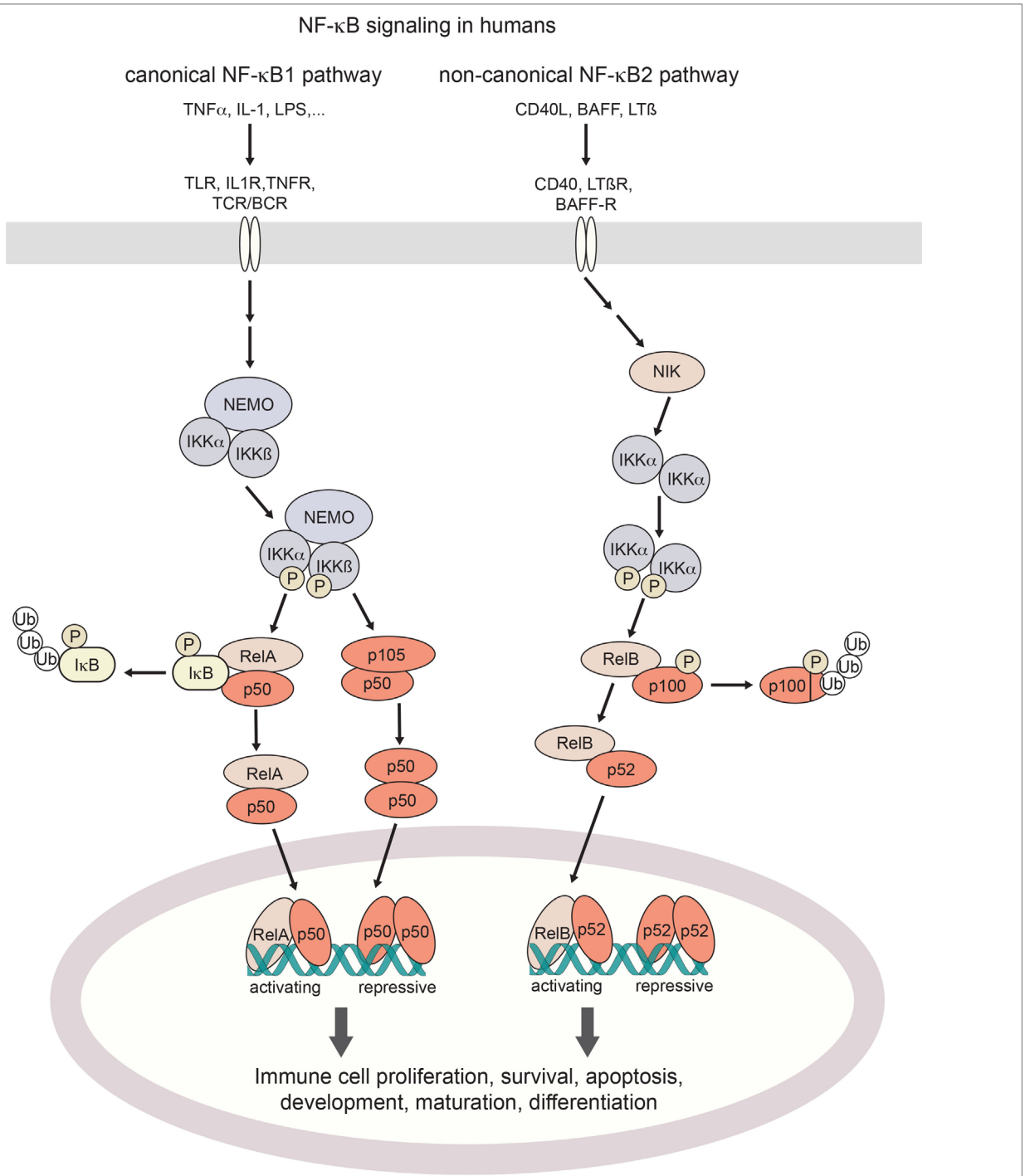


FIGURE 1 | Canonical and non-canonical NF-κB signaling in humans. Activation of the canonical NF-κB pathway is triggered by a broad range of proinflammatory cytokines such as TNFα or IL-1, bacterial pattern recognition molecules such as LPS, or antigen stimulation. Non-canonical signaling is triggered by TNF family receptors and their ligands, resulting in activation of NIK kinase activity. Both pathways cumulate in the activation of IKK (IκB-kinases) which phosphorylate inhibitory IκB binding partners for their poly-ubiquitination and proteosomal degradation (canonical axis) or the processing of p100 into its active form (non-canonical axis). Resulting NF-κB dimers translocate to the nucleus. Depending on their assembly into activating hetero- or repressive homo-dimeric conformations, NF-κB signaling regulates the expression of hundreds of target genes. TNF(R), tumor necrosis factor (receptor); IL-1(R), interleukin-1 (receptor); LPS, lipopolysaccharide; BAFF(-R), B-cell activating factor (receptor); LTβ(R), lymphotoxin β (receptor); TLR, toll-like receptor; TCR/BCR, T-cell/B-cell receptor; NIK, NF-κB inducing kinase; NEMO, NF-κB essential modulator; IKK, IκB kinase; IκB, Inhibitor of NF-κB; NF-κB, nuclear factor kappa-light-chain-enhancer of activated B cells.

been reported (6–13), broadening our understanding of NF-κB1 as critical factor in B-cell function. As appreciated from murine studies, mice deficient in NF-κB were shown defective in B-cell maturation, isotype switching, class-switch recombination, antibody response, and humoral immune response with increased susceptibility to infection, especially of the *S. pneumoniae* type (4, 43–46). T cells have been reported with abnormal proliferation responses. Intriguingly, development and reproduction of *Nfkb1*$^{-/-}$ mice is unaffected (43).

The initial report on NF-κB1 haploinsufficiency in humans describes three unrelated kindreds with 20 affected individuals presenting with CVID and/or hypogammaglobulinemia (5). Subsequent studies on patients haploinsufficient for NF-κB1 have reported an extended clinical phenotype and a predominant CVID-like presentation (6–13). Among the affected individuals, clinical manifestations were highly variable as summarized in **Table 1**. Immunoglobulins were mildly to severely decreased, and infections ranged from common sinusitis to progressive pulmonary disease. Autoimmune episodes were evident in some individuals, including autoimmune hypothyroidism, autoimmune anemia, cytopenias, and/or splenomegaly. Partly severe autoinflammatory conditions were apparent in a fraction of described patients (**Table 1**). A recent study on the consequences of NF-κB1 haploinsufficiency on the B-cell compartment revealed low numbers of peripheral B cells with normal T-cell counts, low numbers of CD27^{+} switched-memory B cells and an expansion of CD21^{lo} B cells (6). Furthermore, an impairment of early stages of B-cell differentiation upon monoallelic loss of *NFKB1* was detected, with partial maturational arrest at the pre-BI stage (6). Investigation of T-cell compositions in another study reported low CD4^{+} effector memory T-cell as well as T_h17 memory subsets in the respective individuals (9). Schipp and coworkers have observed decreased naïve CD4 and memory T-cell states, and increased double-negative T cells for an affected individual, with defective FasL-mediated apoptosis (8).

Overall, monoallelic mutations in *NFKB1* have been shown to impose B-cell dysfunction including immunoglobulin and antibody deficiencies often accompanied by autoimmune and also autoinflammatory responses. Detailed investigations of B-cell differentiation and function have been initiated based on the reported findings, and would benefit from further studies in human cell systems. As generally seen with monoallelic and especially haploinsufficient immunodeficiencies, penetrance of disease manifestation is below 100% and age of onset is variable in individuals with *NFKB1* mutations (5).

NF-κB1 Haploinsufficiency and Predisposition to EBV Infection and EBV-Lymphoproliferative Disease

The first notions of importance of NF-κB1 signaling in defense against EBV in humans were published in 2016. Schipp and coworkers reported a patient with a novel *NFKB1* frame-shift mutation that presented with chronic EBV infection in context of a complex and severe multi-system CVID-like disease (8). In 2017, two additional CVID patients with heterozygous *NFKB1* loss were reported with low-grade or reactivating EBV (see **Table 1**) (13).

Recently, we reported a novel case of NF-κB1 haploinsufficiency broadening the spectrum of disease manifestation by T-cell defects with severe EBV-associated lymphoproliferative disease (7). The patient presented with recurrent infections, autoimmunity manifestation, and with two severe episodes of EBV-associated lymphoproliferative disease (**Table 1**). In addition to low CD19^{+} B cells, reduced non-switched and switched memory B cells and low immunoglobulin levels, T-cell proliferation was impaired. Referring to NF-κB1 haploinsufficiency primarily known as B-cell disorder, the severe EBV-lymphoproliferative episodes were interpreted as a new feature of this disease and attributed to the apparent T-cell dysfunction (7).

Interestingly, EBV infections have not been reported among the common *NFKB2* mutations with functional p52 haploinsufficiency causing CVID (12, 16–18, 47–49). By contrast, an NF-κB2 mutant (p.R635*) with constitutive nuclear localization has been shown associated with multiple infections, including EBV viremia in one of three affected individuals (14).

Consequences of *NFKB1* Mutations on Protein Function

The comparison of the consequences of the various *NFKB1* mutations for protein integrity and function represents a first-line consideration of similarities and differences of NF-κB1-related disease manifestations. The distinct disease-associated monoallelic mutations in *NFKB1* that have been identified to date (6–13) span intronic and exonic alterations, non-sense mutations, splice-site donors and frameshift mutations, all resulting in NF-κB1 haploinsufficiency, as well as heterozygous missense mutations with functional haploinsufficiency (**Table 1**). Mapping these within the genetic locus and within the NF-κB1 protein domains depicts an accumulation of mutations especially in the Rel-homology domain (RHD) of p105/p50 (**Figure 2**). This domain is responsible for dimerization and DNA-binding abilities of the mature p50 subunit. This local accumulation includes a frameshift mutation with premature termination at position p.G165Afs*32 in the central part of the RHD, which has been associated with EBV lymphoproliferative disease (7), as well as further EBV-associated mutations in close proximity (p.R157*, p.I47Yfs*2) (8, 13) (**Figure 2**). Still, other mutations introducing early stop codons have been identified in the RHD domain lacking an association with EBV infection (5, 6, 9, 10, 12). In addition, mutation p.R157* that has been reported with EBV reactivation episodes (13) has been identified in unrelated patients without apparent EBV infection (8, 9, 11). Similarly, other clinical manifestations such as autoinflammatory syndromes do not correlate with clustering of mutations on protein domains (**Figure 2**). In conclusion, a rational genotype–phenotype relationship between NF-κB1-related disease manifestations cannot be found. Even though to date more than 55 *NFKB1* germline mutation carriers have been identified, larger case series should be established to perform meaningful phenotypic correlation studies. Also, the presence of modifying factors, such as secondary mutations and epigenetic alterations, could be addressed in such enlarged cohorts.

TABLE 1 | Clinical presentation of monoallelic loss of nuclear factor kappa-light-chain-enhancer of activated B cells 1 (NF-κB1) function. A comparison of phenotypes of affected individuals shows various occurrences and degrees of infections, autoimmunity and autoinflammatory syndromes, and varying manifestations of Epstein–Barr virus (EBV). Genetic information, diagnosis and clinical symptoms were extracted from the respective studies. Individuals are listed according to study reference, respective patient code, and type of mutation. Information about treatment and surgery is shown, if indicated in the respective publications. Asymptomatic mutation carriers are not listed.

Reference	Patient	G	Mutation	AoO	Diagn.	Symptoms			IgG (g/L)	IgA	IgM	Treatment	Surgery
						Infection	Autoimmunity/ Autoinflammation	Other					
(5)	FamNL1_16	♀	c.730+4A>G p.D191_K244 delinsE	29 years	Common variable immunodeficiency (CVID)	Respiratory tract infections, pansinusitis, atrophic gastritis, pneumonia	COPD	Pyoderma gangrenosum, squamous cell carcinoma, pulmonary insufficiency	2.76	0.30	0.16	IVIG, etanercept	n/a
	FamNL1_18	♀	c.730+4A>G p.D191_K244 delinsE	65 years	CVID	Sinopulmonary infections, *Campylobacter* enteritis	Splenomegaly, lymphadenopathy	Ischemic heart disease, lung adenocarcinoma, thrombocytopenia	2.49	0.13	0.53	n/a	n/a
	FamNL1_19	♀	n/a	46 years	–	Bronchitis, pneumonias (H. influenzae)	COPD	Coronary insufficiency, impaired pulmonary function	3.0	0.4	0.4	IVIG	n/a
	FamNL1_21	♂	c.730+4A>G p.D191_K244 delinsE	31 years	CVID	pneumonias, recurrent sinusitis, bronchiectasis	COPD	Lung fibrosis, respiratory insufficiency	6.0	1.26	0.4	IVIG	Appendectomy
	FamNL1_23	♀	n/a	n/a	–	Recurrent lower airway infections	COPD	Defective vaccination response	HGG	n/a	n/a	IVIG	n/a
	FamNL1_24	♀	n/a	n/a		Recurrent lower airway infections	COPD with pulmonary insufficiency	Defective vaccination response, mannose-binding lectin deficiency	HGG	n/a	n/a	IVIG	n/a
	FamNL1_25	♀	c.730+4A>G p.D191_K244 delinsE	52 years	CVID	Pulmonary infection	COPD	Respiratory insufficiency, pulmonary hypertension	4.6	1.0	0.4	IVIG	n/a
	FamNL1_34	♂	c.730+4A>G p.D191_K244 delinsE	44 years	CVID	Recurrent respiratory infections	Hypothyroidism	Alopecia areata, pyoderma gangrenosum	8.0	0.83	0.25	IVIG	Surgery for VSD
	FamNL1_36 [P.2 (6), P.4 (13)]	♀	c.730+4A>G p.D191_K244 delinsE	30 years	CVID	Pneumonia, recurrent sinusitis, otitis media, *Salmonella* enteritis	–	–	1.81	0.06	0.48	IVIG	n/a
	FamNL1_40	♀	c.730+4A>G p.D191_K244 delinsE	34 years	CVID	Recurrent upper airway infection, sporadic lower respiratory tract infections	COPD	–	5.1	<0.5	0.65	IVIG	n/a
	FamNL1_42	♀	c.730+4A>G p.D191_K244 delinsE	n/a	HGG	Recurrent paronychia, superficial skin infections	–	–	n/a	n/a	n/a	n/a	n/a

(*Continued*)

TABLE 1 | Continued

Reference	Patient	G	Mutation	AoO	Diagn.	Symptoms			IgG (g/L)	IgA	IgM	Treatment	Surgery
						Infection	Autoimmunity/ Autoinflammation	Other					
	FamNL1_48	♀	c.730+4A>G p.D191_K244 delinsE	15 years	HGG	Folliculitis, furunculosis	Thyroiditis	Aortic stenosis	3.72	0.59	0.74	Levothyroxine	n/a
	FamNL1_49	♀	c.730+4A>G p.D191_K244 delinsE	9 months	CVID	Giardiasis, recurrent respiratory tract infections, trichophyton skin infections	Thyroiditis	–	9.1[a]	1.41	0.11	IVIG	n/a
	FamNL1_57	♀	c.730+4A>G p.D191_K244 delinsE	39 years	CVID	Lymphocytic interstitial pneumonitis, recurrent sinopulmonary infections	–	Nodular regenerative hyperplasia, ongoing diarrhea, reticunodular and lymphocytic infiltrates, liver eosinophilic and lymphocytic infiltrates	1.3	<0.04	0.3	IVIG, IV-antibiotics, ursodeoxycholic acid	n/a
	FamNL1_62	♀	c.730+4A>G p.D191_K244 delinsE	30 years	CVID	Recurrent sinus infections	–	–	4.7	0.4	0.3	IVIG	n/a
	Fam089_I1	♂	c.835+2T>G p.K244_D279 delinsN	64 years	CVID	Severe pneumonia with pleural empyema	–	Multiple liver hemangiomas	1.42	0.08	0.4	IVIG	n/a
	Fam089_II2 [P.5 (13)]	♀	c.835+2T>G p.K244_D279 delinsN	16 years	CVID	Recurrent pulmonary infections, pneumonia	Hemolytic anemia, hepatomegaly, lymphadenopathy	Idiopathic thrombocytopenic purpura, infection-induced neutropenia, intermittent diarrhea, recurrent arthralgias	6.29	<0.3	<0.3	IVIG, rhG-CSF	n/a
	Fam089_III2	♀	c.835+2T>G p.K244_D279 delinsN	14 months	HGG	–	–	Transient hypogammaglobulinemia	1.94	<0.07	0.29	n/a	n/a
	FamNZ_I2	♀	c.465dupA p.A156Sfs*12	n/a	–	–	–	Alopecia, thrombocytopenia	9.9	0.58	0.8	n/a	n/a
	FamNZ_II1	♂	c.465dupA p.A156Sfs*12	2 years	CVID	–	Hemolytic anemia	Thrombocytopenia, neutropenia	5.17	0.07	0.53	IVIG	Splenectomy
	FamNZ_II2	♀	c.465dupA p.A156Sfs*12	20 years	CVID	Bronchiectasis, recurrent infections	–	Alopecia, marginal zone non-Hodgkin lymphoma, nodular regenerative hyperplasia	n/a	n/a	n/a	Rituximab, chlorambucil, IVIG	n/a
(6)	Patient 1	♂	c.1517delC p.A506Vfs*17	7 years	CVID	Recurrent respiratory infections, pneumonia, sinusitis	Thyroiditis, enteropathy	Gastric adenoma	1.6	0.14	0.14	IVIG, budesonide	n/a

(*Continued*)

TABLE 1 | Continued

Reference	Patient	G	Mutation	AoO	Diagn.	Symptoms			IgG (g/L)	IgA	IgM	Treatment	Surgery
						Infection	Autoimmunity/ Autoinflammation	Other					
(7)	Index patient	♀	c.494delG p.G165Afs*32	2 years	CID with EBV-LPD	Recurrent respiratory infections, recurrent EBV infection with EBV-LPD	Hepatosplenomegaly, esophagitis, cervical/anxillary/ supraclavicular lymphadenopathy	Bacterial parapharyngeal abscess, chronic abscess-forming inflammation, neutropenia, leukopenia, thrombocytopenia	2.32	<0.05	0.012	IVIG, antibiotics, corticosteroids, rituximab	Tonsillectomy, adenectomy
	Father	♂	c.494delG p.G165Afs*32	n/a	–	Non-respiratory tract infections	–	–	4.09	0.23	0.41	–	n/a
(8)	Patient 1	♀	c.139delA p.I47Yfs*2	n/a	CVID	Recurrent pneumonias, upper respiratory tract infection, fever, chronic otitis, mastoiditis, sinusitis, vulvovaginitis; recurrent viral (chronic EBV, recurrent herpes zoster, HPV38, HSV, RSV), bacterial (*Gardnerella* and group A *Streptococcus, C. braakii),* Candida (C. *lusitaniae*) infections	Hemolytic anemia, idiopathic thrombocytopenia, pancytopenia, autoantibodies, organ infiltration (liver, lung, spleen, kidney) with hepatosplenomegaly, pancolitis, generalized mucositis, recurrent aphthae and painful ulcers of mouth, esophagus, and genitalia	Recurrent idiopathic diarrhea, abdominal pain, bloody stools, ascites, intermittent proteinuria, latent hypothyreosis, multiple ovarian cysts, lack of calcium and vitamin D, recurrent headaches with vertigo, numbness and paresis of left arm and hand	4.85	<0.06	0.24	Steroids, mycophenolate, IVIG, mofetil, antibiotics, antiviral medication, calcium, vitamin D	Cholecystectomy, colectomy, mastoidectomy, ulcer excision, myringotomy, tympanostomy
	Patient 2	♀	c.469C>T p.R157*	n/a	CVID	Recurrent pneumonias *(S. pneumoniae),* upper respiratory tract infection, H. influenzae, *Salmonella* infections	Hemolytic anemia, idiopathic thrombocytopenia, leukopenia, splenomegaly	Recurrent diarrhea	10.9	<0.05	3.62	IVIG, steroids	n/a
(9)	F1.II-4	♀	c.200A>G p.H67R	n/a	Behçet-like; AB def	Upper respiratory tract infection	Recurrent monoarthritis, complex aphthae (mouth, genitalia), small vessel vasculitis	Periodic abdominal pain, chronic idiopathic diarrhea	HGG	n/a	n/a	IVIG	n/a
	F1.III-2	♀	c.200A>G p.H67R	n/a	Behçet-like; AB def	–	–	Benign kidney tumor	HGG	n/a	n/a	n/a	n/a
	F1.III-3	♂	c.200A>G p.H67R	n/a	Behçet-like; AB def	Upper respiratory tract infection	Complex aphthae (esophagus)	Rudimentary left kidney, febrile attacks	HGG	n/a	n/a	IVIG	n/a
	F1.III-6	♀	c.200A>G p.H67R	n/a	Behçet-like; AB def	Upper respiratory tract infection	Complex aphthae (mouth)	–	HGG	n/a	n/a	IVIG	n/a

(*Continued*)

TABLE 1 | Continued

Reference	Patient	G	Mutation	AoO	Diagn.	Symptoms			IgG (g/L)	IgA	IgM	Treatment	Surgery
						Infection	Autoimmunity/ Autoinflammation	Other					
	F1.III-7	♀	c.200A>G p.H67R	n/a	Behçet-like	Upper respiratory tract infection	Recurrent monoarthritis, hyperinflammatory response to tooth excision, complex aphthae (mouth, genitalia)	Febrile attacks, periodic abdominal pain, keratitis	n/a	n/a	n/a	n/a	n/a
	F1.III-8	♀	c.200A>G p.H67R	n/a	Behçet-like; AB def	Upper respiratory tract infection	Recurrent monoarthritis, microscopic colitis, complex aphthae (mouth)	Febrile attacks, periodic abdominal pain	HGG	n/a	n/a	IVIG	n/a
	F1.IV-2	♀	c.200A>G p.H67R	n/a	Behçet-like; AB def	Upper respiratory tract infection	Complex aphthae (mouth, genitalia)	Febrile attacks, periodic abdominal pain	HGG	n/a	n/a	n/a	n/a
	F2.II-3	♀	c.1659C>G p.I553M	n/a	CVID	Respiratory tract infection, autoimmune hypothyroiditis	Spondyloarthropathy oligoarthritis	Chronic idiopathic diarrhea, asthma	HGG	n/a	n/a	IVIG	n/a
	F2.III-2	♂	c.1659C>G p.I553M	n/a	CVID	Respiratory tract infection	Celiac disease, asthma	–	n/a	n/a	n/a	n/a	n/a
	F3.II-1	♂	c.469C>T p.R157*	n/a	FNC	Respiratory tract infection	Postoperative necrotizing cellulitis	–	n/a	n/a	n/a	n/a	Yes (unknown)
	F3.II-5	♂	c.469C>T p.R157*	n/a	FNC	–	Postoperative necrotizing cellulitis	–	n/a	n/a	n/a	n/a	Yes (unknown)
(10)	Patient 1	♂	c.1301-1G>A (intronic)	42 years	CVID	Pneumonias, chronic sinusitis, conjunctivitis, otitis, shingles	Lymphoid hyperplasia, aphthous ulcers, hypersplenism	Lung granulomas, enteropathy, neutropenia	Tx	<0.07	<0.11	n/a	Splenectomy
	Patient 2	♀	c.1301-1G>A (intronic)	19 years	CVID	Pneumonias, chronic sinusitis, conjunctivitis, otitis, shingles, *C. difficile* colitis	–	Morphea	<0.51	<0.05	<0.05	n/a	n/a
	Patient 3	♂	c.259-4A>G (intronic)	21 years	CVID	Pneumonias, empyema, chronic sinusitis	Bronchiectasis, hypothyroidism, vitiligo	–	Tx	<0.01	<0.05	n/a	Lobectomy
	Patient 4	♀	c.957T>A p.Y319*	19 years	CVID	MAI, pneumonia, lung abscesses, PML	Autoimmune hemolytic anemia, immune thrombocytopenia	Aplastic bone marrow	Tx	<0.07	<0.l7	n/a	Splenectomy
	Patient 5	♀	c.1375delT p.F459Lfs*26	7 years	CVID	Pneumonias, otitis, giardiasis, *C. difficile* colitis, HSV infection, cellulitis, MAI	Bronchiectasis	Enteropathy, osteopenia, poor growth	Tx	<0.02	<0.02	n/a	n/a
(11)	Patient 26	♀	n/a	n/a	CVID	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a

(*Continued*)

TABLE 1 | Continued

Reference	Patient	G	Mutation	AoO	Diagn.	Symptoms			IgG (g/L)	IgA	IgM	Treatment	Surgery
						Infection	Autoimmunity/ Autoinflammation	Other					
	Patient 27 [P.6 (13)]	♂	c.469C>T p.R157*	n/a	CVID	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a
	Patient 28	♀	n/a	n/a	CVID	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a
(12)	P9.1		c.904dupT p.S302Ffs*7	n/a	AB def	H. influenzae, pulmonary fibrosis	Autoimmune hemolytic anemia	–	n/a	n/a	n/a	Prednisol., rituximab	n/a
	P9.2		c.904dupT p.S302Ffs*7	n/a	AB def	–	Autoimmune hemolytic anemia, immune thrombocytopenia, autoimmune neutropenia	–	n/a	n/a	n/a	Prednisol., Ig	n/a
(13)	Patient 1	♂	c.1517delC p.A506Vfs*17	13 years	CVID	Recurrent pulmonary infections	Autoimmune thyroiditis, autoimmune enteropathy	Gastric adenoma	1.6	0.1	0.1	n/a	n/a
	Patient 2	♀	c.1365delT p.V456*	33 years	CVID	Pneumonias, necrotizing tonsillitis, periodontitis, infections (h. zoster, CMV viremia, intermittent low-grade EBV)	Autoimmune cytopenia, splenomegaly, lymphadenopathy, interstitial lung disease	Multiple liver hemangiomas	6.7	0.3	0.3	n/a	n/a
	Patient 3	♂	c.1365delT p.V456*	43 years	CVID	Recurrent sinusitis and otitis, pneumonia, *Salmonella* enteritis	Autoimmune cytopenia, arthritis, splenomegaly, vitiligo, lymphadenopathy, granulomatous lung disease	–	0.08	0.05	0.05	n/a	n/a
	Patient 6 [p.27 (11)]	♂	c.469C>T p.R157*	47 years	CVID	Chronic sinusitis, recurrent otitis, pneumonia, JC virus encephalitis, norovirus, EBV reactivation	Skin abscesses, atopic dermatitis, autoimmune enteropathy, nodular regenerative hyperplasia, splenomegaly, lymphadenopathy, thrombocytopenia	–	2.7	<0.06	0.21	n/a	n/a
	Patient 7	♀	c.295C>T p.Q99*	20 years	CVID	Recurrent bronchitis, sinusitis	Enteropathy, splenomegaly	Basal cell carcinoma, osteoporosis	n/a	n/a	n/a	n/a	n/a

G, gender; AoO, Age of onset (diagnosis); Diagn., diagnosis; COPD, chronic obstructive pulmonary disease; IVIG, intravenous immunoglobulin; HGG,hypogammaglobulinemia; VSD, ventricular septal defect; EBV-LPD, Epstein–Barr virus-associated lymphoproliferative disease; AB def, antibody deficiency; FNC, familial necrotizing cellulitis; prednisol., prednisolone; MAI, mycobacterium avium intracellulare; PML, progressive multifocal leukoencephalopathy; n/a, not applicable.

[a]*After IVIG.*

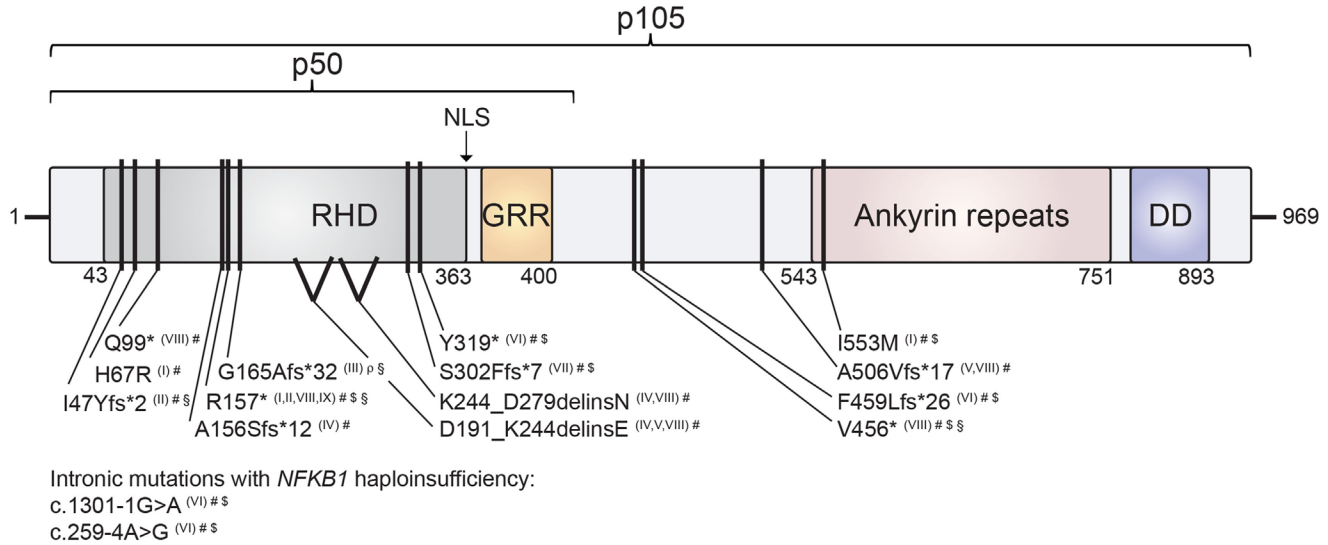


FIGURE 2 | Mapping of human nuclear factor kappa-light-chain enhancer of activated B cells 1 (NF-κB1) mutations on the protein's domain architecture. Comparison of identified NF-κB1 mutations and their clinical presentation reveals no obvious genotype–phenotype correlation. Individual mutations in either p105 or p105/p50 protein subunits of NF-κB1 are depicted by black bars. Stop codons are depicted by asterisks. NF-κB1 haploinsufficiency results from intronic mutations, non-sense mutations and frameshift mutations with premature truncations. Similarly, two identified splice-site mutations result in NF-κB1 haploinsufficiency due to loss of mutated precursors. Missense exchange p.I553M results in enhanced degradation of both p105 and p50, while p.H67R leads to reduced nuclear entry of the affected mutant p50 and, hence, to functional haploinsufficiency. NLS, nuclear localization sequence; RHD, Rel homology domain; GRR, glycine-rich region; DD, death domain. NF-κB1 mutations are referenced according to Roman numbering: (I) Kaustio et al. (9); (II) Schipp et al. (8); (III) Boztug et al. (7); (IV) Fliegauf et al. (5); (V) Lougaris, Moratto et al. (6); (VI) Maffucci et al. (10); (VII) Rae et al. (12); (VIII) Lougaris, Patrizi et al. (13) (IX) Keller et al. (11). Clinical presentations are indicated by the following symbols: #, common variable immunodeficiencies (CVID) (incl. autoimmunity); $, autoinflammation; §, chronic EBV/EBV-lymphoproliferation; ρ, combined immunodeficiency (CID).

MOLECULAR MECHANISMS OF NF-κB1 DEFECTS AND EBV-INDUCED DISEASE

Epstein–Barr virus infections pose a huge challenge to immunocompromised individuals, given the virus' wide prevalence in the adult population and its concomitant risk to induce life-threatening lymphoproliferative and malignant disease (50). EBV belonging to the herpesvirus family, was the first human tumor virus associated with B-cell and T-cell lymphoproliferative disease and related lymphoma (20, 50). Its mechanisms and implications for human health have been discussed by numerous studies [see, for example, reviews authored by Hatton and coworkers (19) or Thorley-Lawson (20)]. EBV primarily infects B cells and drives their proliferation by expressing a small number of latency genes that mimic growth, transcription, and anti-apoptotic markers, followed by a lytic replication phase to produce infectious virus (20). Successfully cleared by cytotoxic T and NK cells that interfere with different stages of infected B cells in healthy individuals (20, 51–53), immunodeficient humans fail to manage and eliminate these. As a consequence of cytotoxic T-cell dysfunction, accumulation of EBV-infected autoreactive B cells in target organs induces a concomitant infiltration of autoreactive T cells that results in harsh autoimmune episodes (54). EBV-associated disease is, thus, a phenomenon of infected B cells but their compromised elimination results from mainly T-cell intrinsic mechanisms.

As recent cases of NF-κB1 haploinsufficiency have been shown associated with EBV infection (8, 13) and EBV-lymphoproliferative disease with T-cell dysfunction (7), we compare NF-κB1 haploinsufficiency with other EBV-associated PIDs, discuss combined immunodeficiencies related to NF-κB by shared signaling pathways but without sign of EBV-associated disease, and outline the consequences of NF-κB1 dysfunction for T-cell integrity resulting in a proposed impairment of EBV clearance.

Comparison of EBV-Associated PIDs and What to Learn from Them

Epstein–Barr virus-driven lymphoproliferative disease is commonly understood as consequence of impaired cytotoxic T- or NK-cell function. Several PIDs have been clearly associated with EBV-induced disease and reviewed on various occasions (55–59). The investigation of how these PIDs disturb T-cell function to affect virus elimination abilities is of critical relevance for understanding disease mechanisms.

Among the PIDs predisposing to severe infections, some predispose to a single, while others respond to a multitude of pathogens (60, 61). Specific EBV-associated disease has been reported for X-linked lymphoproliferative syndrome (XLP)-1 and -2 [BIRC4 and SAP deficiency, respectively (62, 63)], ITK (64), CD27 (65), CD70 (66, 67), CORO1A (68), RASGRP1 (69), and MAGT1 (X-MEN syndrome) (70) deficiencies, as summarized by

various studies (55, 56, 59, 71). Clinically, these manifest in CID with antibody deficiencies and autoimmune episodes, including lymphoproliferative disease (55). Among the PIDs associated with risk of infections of various kinds, mutations of ATM, WASP, STK4, PIK3CD, PIK3R1, CTPS1, CARD11, FCGR3A, MCM4, and GATA2 have been reported with severe EBV infections (59).

Although impaired B- and NK-cell function were shown involved in EBV-associated diseases, it is primarily the cytotoxic T cells that initiate an effective—or defective—EBV-directed immune response (57, 60, 61, 71). A related disease state manifests through either a loss of effective T cells in the circulation, or through defective T-cell function (57).

NF-κB1 plays a critical role in cytotoxic T-cell function. A comparison with EBV disease-associated proteins and their canonical pathways underlines the importance of NF-κB1 signaling in recognition of EBV-infected B cells and their targeting for elimination. As recently reviewed by Tangye and coworkers (55), defined EBV-associated PID genes do share common pathways upstream of NF-κB1 in this specific process. NF-κB1 has been shown a relevant effector of B-cell recognition by cytotoxic T cells (**Figure 3**). CD70, a surface marker on (EBV-infected) B cells, binds to the CD27 receptor on T cells which triggers an NF-κB1-mediated response for expansion of cytotoxic T cells directed against the infected target cells (66, 67, 72). Loss of both CD70 and CD27 have been reported with CID-like syndromes, including EBV-driven malignancy and life-threatening EBV infection, respectively (65–67). Similarly, CD48/2B4 receptor pairing by infected B cells in contact with cytotoxic T cells, respectively, may trigger NF-κB1 activation *via* the 2B4-binding partner SAP. Loss of SAP or its function has been similarly reported with EBV disease manifestation (62). Future identification of further immunodeficiency-causing genes related in these processes might shed additional light on NF-κB1-related mechanisms in EBV-directed cytotoxic T-cell response.

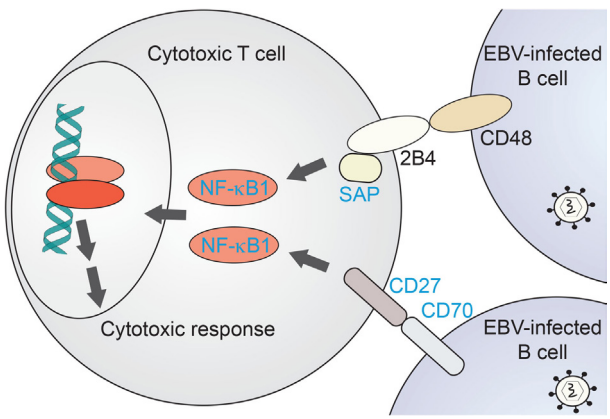


FIGURE 3 | NF-κB1-related immunodeficiencies affecting T-cell mediated clearance of EBV-infected B cells. Proposed scenario for NF-κB1 signaling induced in cytotoxic T cells downstream of B-cell recognition events. Proteins for which primary immunodeficiencies (PIDs) with EBV-associated disease have been identified are named in blue. NF-κB1, nuclear factor kappa-light-chain enhancer of activated B cells-1; SAP, SLAM-associated protein.

Combined Immunodeficiencies Associated with NF-κB Pathways

Among the immediate NF-κB pathway-associated immunodeficiencies, NF-κB1 haploinsufficiency itself has been reported with EBV-driven lymphoproliferative disease or recurrent EBV infection (7, 8, 13). In addition, a recent study reported a patient with a constitutively active mutant of NF-κB2 and EBV viremia (14). NF-κB-related immunodeficiencies harbor the potential to develop EBV-driven disease in a predisposing environment, and thus should be considered at risk. The hitherto identified deficiencies of upstream components of NF-κB have yet not been reported with EBV-related disease. These include homozygous deficiencies of IKK2, NIK, RELB, and X-linked deficiency of NEMO (see **Figure 1**). NEMO mutations were reported with susceptibility to pyrogenic and myco-bacterial infections, due to specific defects of NK-cell cytotoxicity. Selected affected patients presented with viral infections of molluscum contagiosum or human papillomavirus (73). Homozygous loss-of-function affecting NIK has been shown to affect various B cell compartments, with additional perturbation of NK cell and memory/follicular helper T-cell activity, but lacking evidence of EBV-linked episodes (74). Biallelic loss of *RELB* resulted in a CID with unresponsive T cells and impaired humoral immunity, presenting with respiratory infections but no report of EBV manifestation (75). Both NIK and RelB are crucial players in non-canonical NF-κB2-mediated signaling. NEMO deficiency was reported with severe CID-like characteristics including impaired B- and T-cell activation response through various stimuli, and generally absent regulatory and gamma-delta T cells. Alongside multi-pathogenic infections, viral infections were only reported for parainfluenza type 1 (76, 77).

Lastly, with the CD40/CD40L signaling system, an NF-κB-related mechanism itself is of relevance for successful EBV infection in B cells. CD40 expression by B cells and its engagement with its receptor CD40L on the surface of T lymphocytes is involved in the formation of memory B cells and Ig isotype switching (78). Homozygous mutations affecting either CD40 or CD40L have been shown to cause hyper-IgM syndrome (79–81). In EBV-infected B lymphocytes, CD40/CD40L signaling is induced by a mimicking mechanism, and critical for B-cell transformation (55).

Molecular Considerations of EBV-Driven Disease in NF-κB1 Haploinsufficiency

The persistent exposure to EBV that is present in over 90% of the human population causes a considerable threat to dysfunctional NF-κB signaling systems. Accumulating evidence enabled by studies on CD27, CD70, and SAP deficiencies points to NF-κB1 being involved in T-cell intrinsic mechanisms downstream of their recognition of EBV-infected B cells (see **Figure 3**). Due to the only recent discovery of EBV disease and T-cell dysfunctions in NF-κB1 haploinsufficiency, comprehensive studies of T-cell functionalities have not been systematically explored. For example, the T-cell related target gene expression sets modified by the various identified NF-κB1 haploinsufficient conditions should be addressed in a comparative manner. Similarly, it would

be necessary to explore detailed T-cell responses, including cytotoxicity and proliferation studies in larger cohorts, to evaluate genotype–phenotype relationships.

Numerous target genes have been found differentially expressed by the NF-κB signaling systems and summarized in their whole on a web resource at Boston University (http://www.bu.edu/nf-kb/gene-resources/target-genes/). Collectively, such affected targets include cytokines and chemokines, T-cell receptor and toll-like receptor subunits, T-cell activation and differentiation markers, cell adhesion molecules, stress response factors, growth factors and regulators of apoptosis, and numerous transcription factors and signaling molecules. Expression profiling would need to clarify which of these could contribute to T-cell-related EBV disease in NF-κB1 haploinsufficiency.

NF-κB signaling itself is critically relevant for EBV replication in B cells. EBV infects B cells by binding of glycoprotein gp350 and upregulation of latency genes such as LMP1 that mimics CD40 and B-cell receptor signaling (55). Subsequent induction of NF-κB signaling upregulates the B-cell reproductive machinery. Still, partial loss of the NF-κB signaling system through NF-κB1 haploinsufficiency has been shown associated with severe EBV proliferative disease (7). Due to the monoallelic nature of the disease, haploinsufficient NF-κB1 signaling might be sufficient for EBV to successfully reproduce in B cells, while at the same time the dysfunctional T cells are rendered unable to clear such infection. In addition, in case of disrupted canonical NF-κB1 signaling, the remaining NF-κB2 axis could sufficiently induce (infected) B-cell replication. EBV protein LMP1 has been shown to activate both the canonical and non-canonical NF-κB signaling networks by using specific domains termed TES1 and TES2, respectively (82). A recent report describes a patient with a heterozygous NF-κB2 precursor-skipping mutation that resulted in a constitutive presence of p52. The mutation was shown to cause CID with severe EBV infection (14). Through pathway crosstalk, NF-κB2 can inhibit NF-κB1 activation by direct interaction of respective subunits (82), and thus potentially lead to similar functional NF-κB1 defects.

Treatment Considerations for NF-κB- and EBV-Related Immune Deficiencies

Lymphoproliferation by EBV infection causes life-threatening autoimmune-like infiltration into target organs that demands aggressive treatment. Given this pressing urgency, the fatal correlation of EBV-associated disease occurring predominantly in immunodeficient individuals causes a discrepancy in the choice of treatment—simply spoken, a fully satisfactory therapy recommendation is still elusive. Virus-induced lymphocyte expansion is tackled by application of various immunosuppressants. Yet the use of immune-suppressing agents themselves additionally dampens the genetically compromised immune cell function. EBV infection and/or reactivation can be managed by combined application of rituximab and corticosteroids for depletion of (infected) B cells and infiltrating T cells (7). Allogeneic hematopoietic stem cell transplantation (aHSCT) should be considered in severe cases with recurring EBV disease (7). Still, such measures often fail to protect against viral infections (55).

In addition to unspecific B-cell depletion with CD20- or CD19-directed monoclonal antibodies, EBV-T cell-specific antibody therapy against viral proteins such as LMP1 is currently being discussed (52). Boosting immunity against EBV is similarly considered (54). This includes vaccination with the viral gp350 glycoprotein, or an elaborate T-cell boost by *in vitro* expansion of virus-specific T cells followed by re-infusion into the patient's circulation (54). In addition, administration of interleukin-7 was reported to expand virus-specific cytotoxic T cells (83).

Targeting virus expansion directly at its core would manage EBV-driven autoimmunity, especially when administered in combination with rituximab or other eliminators of infected B cells (54). Currently available antiviral therapeutics such as acyclovir only target the lytic/replicative state of viral infections, leaving the latently infected cells intact for breakout at a later stage (52, 54). Inhibitors of viral proteins such as LMP1 might prove beneficial in future studies, but are still in developmental stage. Drugs acting downstream of the EBV pathway, including PI3K, AKT and MAPK inhibitors, are in turn not specific in targeting only the virus-carrying host cells. Proteasome inhibitors are similarly non-specific. Zou and coworkers reported that bortezomib induced apoptosis in EBV-transformed B cells by interfering with NF-κB signals, prolonging survival in mice (84).

Proteasome inhibitors are as well being discussed in context of NF-κB deficiencies (85) and might hence be worth investigating in specific NF-κB1 disorders, especially in combination with EBV-induced disease. NF-κB1 dysfunction as consequence of accelerated degradation of mutated protein might be tackled by blocking the proteasome with specific inhibitors. On the other hand, proteasome inhibitors could also hinder IκB clearance and in turn inhibit NF-κB function, hence a dedicated study of affected NF-κB1 haploinsufficiency would be necessary to investigate a possible outcome. Drugs targeting the transcription machinery to restore or enhance *NFKB1* transcription, or to block IκB expression, are currently being discussed at a research level, though raise the concern of specificity. Targeted degradation of IκB seems an attractive novel approach. The recent advances in the development of degradation-directing therapeutics (86) might prove relevant for NF-κB-related disorders. When considering therapeutic induction or stabilization of NF-κB, it needs to be clarified to which extent excessively active NF-κB could increase the risk for hematological malignancies (87). Similar concerns apply for general kinase activators.

Among the reported drugs to interfere with NF-κB pathways, the chemotherapeutic etoposide has been reported to increase NF-κB (85, 88). Intriguingly, etoposide is part of the current HLH2004 protocol for treating hemophagocytic lymphohistiocytosis, a disease that is often triggered by EBV infection (89). In light of chemotherapy being considered for EBV-driven disease (52), a combined rationale in case of NF-κB dysfunction might prove beneficial. On the other hand, numerous agents have been shown to downregulate NF-κB activation by various mechanisms and are summarized by Yamamoto and Gaynor (85). These include anti-inflammatory steroids and glucocorticoids, such as prednisone and dexamethasone, as well as non-steroidal anti-inflammatory drugs, such as aspirin and sodium salicylate. Similarly, the common immunosuppressants cyclosporine A and

tacrolimus were reported to inhibit calcineurin and, thus, NF-κB pathways (85), although calcineurin can also exhibit positive modulatory activity for NF-κB (90).

On a larger scale, whether general or specific indications for invasive aHSCT are being met is only starting to be discussed in expert communities. Severe or recurrent disease manifestation with EBV-lymphoproliferative disease may represent an indication for such invasive treatment strategy, though owing to the lack of (published) data, it is currently not clear what the outcome of aHSCT in NF-κB1-mutant PID is with regard to long-term morbidity and survival. To define improved guidelines and assistance in therapeutic decisions, it will be necessary in the upcoming years to collect comprehensive data revealing phenotype–genotype relationships and long-term surveillance of this rather novel disease.

Altogether, EBV infectious disease and chronic EBV infections as a consequence of NF-κB1 (7, 8, 13) and also NF-κB2 (14) dysfunction deserve special consideration. Though initiated in early stages, personalized approaches for immune deficiencies with viral predisposition remain a challenge that will direct future considerations regarding cellular, genetic, immune, and drug therapies. The continuing urgent demand for virus-targeting therapeutics, and the increasing emergence of NF-κB-related immunodeficiencies within the past and coming years, will enable new lines of discussions.

CONCLUSION

Among the primary immunodeficiencies, haploinsufficiencies are considered inherently diverse in disease manifestation and penetrance (91). Similarly, CVIDs have been grouped based on a very heterogeneous collection of diseases (40–42). Monoallelic mutations in *NFKB1* causing genetic or functional NF-κB1 haploinsufficiency have recently been reported to not only account for CVID-like B-cell deficiency, but a rather complex immunodeficiency-like profile including the emergence of combined B- and T-cell dysfunction (5–13). As a consequence of affected T-cell integrity, NF-κB1 haploinsufficiency has been shown to coincide with recurrent EBV and life-threatening EBV-driven lymphoproliferative disease (7, 8, 13). The latter poses a challenge due to immediate urge and yet often unsatisfactory treatment options in immunodeficient individuals. Especially when facing a disease with broad clinical manifestation and highly individual genotype–phenotype presentation, defining tailor-made treatment options will be highly relevant. Based on the currently available clinical and immunological data, the causative relation between defined *NFKB1* mutations and EBV infections or associated disease remains unclarified. Larger cohorts and a broad investigation of cellular and genetic functionalities will be necessary to decipher this relationship. Comparison of EBV-associated mechanisms of immunodeficiencies and the NF-κB signaling system suggests that dysfunctional T-cell core processes underlie a particular vulnerability to EBV infection. It will, thus, be of urgent relevance to further investigate T-cell functions affected by NF-κB1 haploinsufficiencies, in order to understand this highly heterogeneous disorder and its relevance to EBV-associated lymphoproliferative disease.

AUTHOR CONTRIBUTIONS

BH performed literature research, designed the layout, and wrote the manuscript. NS performed literature research and wrote the manuscript. KB coordinated the study, performed literature research, and wrote the manuscript.

FUNDING

The authors thank for support by the European Research Council under the European Union's Seventh Framework Programme (FP7/2007-2013)/ERC Starting grant agreement 310857, and the Vienna Science and Technology Fund (WWTF) through project LS16-060 (both to KB).

REFERENCES

1. Vallabhapurapu S, Karin M. Regulation and function of NF-kappaB transcription factors in the immune system. *Annu Rev Immunol* (2009) 27:693–733. doi:10.1146/annurev.immunol.021908.132641
2. Gerondakis S, Siebenlist U. Roles of the NF-kappaB pathway in lymphocyte development and function. *Cold Spring Harb Perspect Biol* (2010) 2:a000182. doi:10.1101/cshperspect.a000182
3. Kaileh M, Sen R. NF-kappaB function in B lymphocytes. *Immunol Rev* (2012) 246:254–71. doi:10.1111/j.1600-065X.2012.01106.x
4. Gerondakis S, Grumont R, Gugasyan R, Wong L, Isomura I, Ho W, et al. Unravelling the complexities of the NF-kappaB signalling pathway using mouse knockout and transgenic models. *Oncogene* (2006) 25:6781–99. doi:10.1038/sj.onc.1209944
5. Fliegauf M, Bryant VL, Frede N, Slade C, Woon ST, Lehnert K, et al. Haploinsufficiency of the NF-kappaB1 subunit p50 in common variable immunodeficiency. *Am J Hum Genet* (2015) 97:389–403. doi:10.1016/j.ajhg.2015.07.008
6. Lougaris V, Moratto D, Baronio M, Tampella G, van der Meer JW, Badolato R, et al. Early and late B-cell developmental impairment in nuclear factor kappa B, subunit 1-mutated common variable immunodeficiency disease. *J Allergy Clin Immunol* (2017) 139:349–352e341. doi:10.1016/j.jaci.2016.05.045
7. Boztug H, Hirschmugl T, Holter W, Lakatos K, Kager L, Trapin D, et al. NF-kappaB1 haploinsufficiency causing immunodeficiency and EBV-driven lymphoproliferation. *J Clin Immunol* (2016) 36:533–40. doi:10.1007/s10875-016-0306-1
8. Schipp C, Nabhani S, Bienemann K, Simanovsky N, Kfir-Erenfeld S, Assayag-Asherie N. Specific antibody deficiency and autoinflammatory disease extend the clinical and immunological spectrum of heterozygous NFKB1 loss-of-function mutations in humans. *Haematologica* (2016) 101:e392. doi:10.3324/haematol.2016.145136
9. Kaustio M, Haapaniemi E, Goos H, Hautala T, Park G, Syrjanen J, et al. Damaging heterozygous mutations in NFKB1 lead to diverse immunologic phenotypes. *J Allergy Clin Immunol* (2017) 140(3):782–96. doi:10.1016/j.jaci.2016.10.054
10. Maffucci P, Filion CA, Boisson B, Itan Y, Shang L, Casanova JL, et al. Genetic diagnosis using whole exome sequencing in common variable immunodeficiency. *Front Immunol* (2016) 7:220. doi:10.3389/fimmu.2016.00220
11. Keller B, Cseresnyes Z, Stumpf I, Wehr C, Fliegauf M, Bulashevska A, et al. Disturbed canonical nuclear factor of kappa light chain signaling in B cells of patients with common variable immunodeficiency. *J Allergy Clin Immunol* (2017) 139:220–231e228. doi:10.1016/j.jaci.2016.04.043
12. Rae W, Ward D, Mattocks CJ, Gao Y, Pengelly RJ, Patel SV, et al. Autoimmunity/inflammation in a monogenic primary immunodeficiency cohort. *Clin Transl Immunol* (2017) 6:e155. doi:10.1038/cti.2017.38
13. Lougaris V, Patrizi O, Baronio M, Tabellini G, Tampella G, Damiati E, et al. NFKB1 regulates human NK cell maturation and effector functions. *Clin Immunol* (2017) 175:99–108. doi:10.1016/j.clim.2016.11.012

14. Kuehn HS, Niemela JE, Sreedhara K, Stoddard JL, Grossman J, Wysocki CA, et al. Novel nonsense gain-of-function NFKB2 mutations associated with a combined immunodeficiency phenotype. *Blood* (2017) 130(13):1553–64. doi:10.1182/blood-2017-05-782177
15. Chen K, Coonrod EM, Kumanovics A, Franks ZF, Durtschi JD, Margraf RL, et al. Germline mutations in NFKB2 implicate the noncanonical NF-kappaB pathway in the pathogenesis of common variable immunodeficiency. *Am J Hum Genet* (2013) 93:812–24. doi:10.1016/j.ajhg.2013.09.009
16. Brue T, Quentien MH, Khetchoumian K, Bensa M, Capo-Chichi JM, Delemer B, et al. Mutations in NFKB2 and potential genetic heterogeneity in patients with DAVID syndrome, having variable endocrine and immune deficiencies. *BMC Med Genet* (2014) 15:139. doi:10.1186/s12881-014-0139-9
17. Liu Y, Hanson S, Gurugama P, Jones A, Clark B, Ibrahim MA. Novel NFKB2 mutation in early-onset CVID. *J Clin Immunol* (2014) 34:686–90. doi:10.1007/s10875-014-0064-x
18. Lee CE, Fulcher DA, Whittle B, Chand R, Fewings N, Field M, et al. Autosomal-dominant B-cell deficiency with alopecia due to a mutation in NFKB2 that results in nonprocessable p100. *Blood* (2014) 124:2964–72. doi:10.1182/blood-2014-06-578542
19. Hatton OL, Harris-Arnold A, Schaffert S, Krams SM, Martinez OM. The interplay between Epstein-Barr virus and B lymphocytes: implications for infection, immunity, and disease. *Immunol Res* (2014) 58:268–76. doi:10.1007/s12026-014-8496-1
20. Thorley-Lawson DA. Epstein-Barr virus: exploiting the immune system. *Nat Rev Immunol* (2001) 1:75–82. doi:10.1038/35095584
21. Pereira SG, Oakley F. Nuclear factor-kappaB1: regulation and function. *Int J Biochem Cell Biol* (2008) 40:1425–30. doi:10.1016/j.biocel.2007.05.004
22. Cartwright T, Perkins ND, Wilson LC. NFKB1: a suppressor of inflammation, ageing and cancer. *FEBS J* (2016) 283:1812–22. doi:10.1111/febs.13627
23. Karin M, Ben-Neriah Y. Phosphorylation meets ubiquitination: the control of NF-[kappa]B activity. *Annu Rev Immunol* (2000) 18:621–63. doi:10.1146/annurev.immunol.18.1.621
24. Ghosh S, Hayden MS. New regulators of NF-kappaB in inflammation. *Nat Rev Immunol* (2008) 8:837–48. doi:10.1038/nri2423
25. Pobezinskaya YL, Kim YS, Choksi S, Morgan MJ, Li T, Liu C, et al. The function of TRADD in signaling through tumor necrosis factor receptor 1 and TRIF-dependent Toll-like receptors. *Nat Immunol* (2008) 9:1047–54. doi:10.1038/ni.1639
26. Ting AT, Pimentel-Muinos FX, Seed B. RIP mediates tumor necrosis factor receptor 1 activation of NF-kappaB but not Fas/APO-1-initiated apoptosis. *EMBO J* (1996) 15:6189–96.
27. Kawai T, Akira S. Signaling to NF-kappaB by Toll-like receptors. *Trends Mol Med* (2007) 13:460–9. doi:10.1016/j.molmed.2007.09.002
28. Cao Z, Xiong J, Takeuchi M, Kurama T, Goeddel DV. TRAF6 is a signal transducer for interleukin-1. *Nature* (1996) 383:443–6. doi:10.1038/383443a0
29. Sun Z, Arendt CW, Ellmeier W, Schaeffer EM, Sunshine MJ, Gandhi L, et al. PKC-theta is required for TCR-induced NF-kappaB activation in mature but not immature T lymphocytes. *Nature* (2000) 404:402–7. doi:10.1038/35006090
30. Su TT, Guo B, Kawakami Y, Sommer K, Chae K, Humphries LA, et al. PKC-beta controls I kappa B kinase lipid raft recruitment and activation in response to BCR signaling. *Nat Immunol* (2002) 3:780–6. doi:10.1038/ni823
31. Liao G, Zhang M, Harhaj EW, Sun SC. Regulation of the NF-kappaB-inducing kinase by tumor necrosis factor receptor-associated factor 3-induced degradation. *J Biol Chem* (2004) 279:26243–50. doi:10.1074/jbc.M403286200
32. Xiao G, Harhaj EW, Sun SC. NF-kappaB-inducing kinase regulates the processing of NF-kappaB2 p100. *Mol Cell* (2001) 7:401–9. doi:10.1016/S1097-2765(01)00187-3
33. Hoffmann A, Natoli G, Ghosh G. Transcriptional regulation via the NF-kappaB signaling module. *Oncogene* (2006) 25:6706–16. doi:10.1038/sj.onc.1209933
34. Oeckinghaus A, Hayden MS, Ghosh S. Crosstalk in NF-kappaB signaling pathways. *Nat Immunol* (2011) 12:695–708. doi:10.1038/ni.2065
35. Barton D, HogenEsch H, Weih F. Mice lacking the transcription factor RelB develop T cell-dependent skin lesions similar to human atopic dermatitis. *Eur J Immunol* (2000) 30:2323–32. doi:10.1002/1521-4141(2000)30:8<2323::AID-IMMU2323>3.0.CO;2-H
36. Sivakumar V, Hammond KJ, Howells N, Pfeffer K, Weih F. Differential requirement for Rel/nuclear factor kappa B family members in natural killer T cell development. *J Exp Med* (2003) 197:1613–21. doi:10.1084/jem.20022234
37. Samson SI, Memet S, Vosshenrich CA, Colucci F, Richard O, Ndiaye D, et al. Combined deficiency in IkappaBalpha and IkappaBepsilon reveals a critical window of NF-kappaB activity in natural killer cell differentiation. *Blood* (2004) 103:4573–80. doi:10.1182/blood-2003-08-2975
38. Siebenlist U, Brown K, Claudio E. Control of lymphocyte development by nuclear factor-kappaB. *Nat Rev Immunol* (2005) 5:435–45. doi:10.1038/nri1629
39. Pasparakis M, Schmidt-Supprian M, Rajewsky K. IkappaB kinase signaling is essential for maintenance of mature B cells. *J Exp Med* (2002) 196:743–52. doi:10.1084/jem.20020907
40. Ameratunga R, Brewerton M, Slade C, Jordan A, Gillis D, Steele R, et al. Comparison of diagnostic criteria for common variable immunodeficiency disorder. *Front Immunol* (2014) 5:415. doi:10.3389/fimmu.2014.00415
41. Yong PF, Thaventhiran JE, Grimbacher B. "A rose is a rose is a rose," but CVID is Not CVID common variable immune deficiency (CVID), what do we know in 2011? *Adv Immunol* (2011) 111:47–107. doi:10.1016/B978-0-12-385991-4.00002-7
42. Cunningham-Rundles C. The many faces of common variable immunodeficiency. *Hematology Am Soc Hematol Educ Program* (2012) 2012:301–5. doi:10.1182/asheducation-2012.1.301
43. Sha WC, Liou HC, Tuomanen EI, Baltimore D. Targeted disruption of the p50 subunit of NF-kappa B leads to multifocal defects in immune responses. *Cell* (1995) 80:321–30. doi:10.1016/0092-8674(95)90415-8
44. Snapper CM, Zelazowski P, Rosas FR, Kehry MR, Tian M, Baltimore D, et al. B cells from p50/NF-kappa B knockout mice have selective defects in proliferation, differentiation, germ-line CH transcription, and Ig class switching. *J Immunol* (1996) 156:183–91.
45. Hayden MS, Ghosh S. NF-kappaB in immunobiology. *Cell Res* (2011) 21:223–44. doi:10.1038/cr.2011.13
46. Bendall HH, Sikes ML, Ballard DW, Oltz EM. An intact NF-kappa B signaling pathway is required for maintenance of mature B cell subsets. *Mol Immunol* (1999) 36:187–95. doi:10.1016/S0161-5890(99)00031-0
47. Lougaris V, Tabellini G, Vitali M, Baronio M, Patrizi O, Tampella G, et al. Defective natural killer-cell cytotoxic activity in NFKB2-mutated CVID-like disease. *J Allergy Clin Immunol* (2015) 135:1641–3. doi:10.1016/j.jaci.2014.11.038
48. Lindsley AW, Qian Y, Valencia CA, Shah K, Zhang K, Assa'ad A. Combined immune deficiency in a patient with a novel NFKB2 mutation. *J Clin Immunol* (2014) 34:910–5. doi:10.1007/s10875-014-0095-3
49. Shi C, Wang F, Tong A, Zhang XQ, Song HM, Liu ZY, et al. NFKB2 mutation in common variable immunodeficiency and isolated adrenocorticotropic hormone deficiency: a case report and review of literature. *Medicine (Baltimore)* (2016) 95:e5081. doi:10.1097/MD.0000000000005081
50. Carbone A, Gloghini A, Dotti G. EBV-associated lymphoproliferative disorders: Classification and treatment. *Oncol* (2008) 13:577–85. doi:10.1634/theoncologist.2008-0036
51. Rickinson AB, Kieff E, Knipe DM, Howley PM, Griffin DE, Lamb RA, et al. editors. *Fields Virology*. 5th ed. Philadelphia, PA, USA: Lippincott Williams & Wilkins (2007). p. 2655–700.
52. Cai Q, Chen K, Young KH. Epstein-Barr virus-positive T/NK-cell lymphoproliferative disorders. *Exp Mol Med* (2015) 47:e133. doi:10.1038/emm.2014.105
53. Khanna R, Moss DJ, Burrows SR. Vaccine strategies against Epstein-Barr virus-associated diseases: lessons from studies on cytotoxic T-cell-mediated immune regulation. *Immunol Rev* (1999) 170:49–64. doi:10.1111/j.1600-065X.1999.tb01328.x
54. Pender MP. CD8+ T-cell deficiency, Epstein-Barr virus infection, vitamin D deficiency, and steps to autoimmunity: a unifying hypothesis. *Autoimmune Dis* (2012) 2012:189096. doi:10.1155/2012/189096
55. Tangye SG, Palendira U, Edwards ES. Human immunity against EBV-lessons from the clinic. *J Exp Med* (2017) 214:269–83. doi:10.1084/jem.20161846
56. Parvaneh N, Filipovich AH, Borkhardt A. Primary immunodeficiencies predisposed to Epstein-Barr virus-driven haematological diseases. *Br J Haematol* (2013) 162:573–86. doi:10.1111/bjh.12422
57. Shabani M, Nichols KE, Rezaei N. Primary immunodeficiencies associated with EBV-Induced lymphoproliferative disorders. *Crit Rev Oncol Hematol* (2016) 108:109–27. doi:10.1016/j.critrevonc.2016.10.014

58. Lucas CL, Kuehn HS, Zhao F, Niemela JE, Deenick EK, Palendira U, et al. Dominant-activating germline mutations in the gene encoding the PI(3)K catalytic subunit p110delta result in T cell senescence and human immunodeficiency. *Nat Immunol* (2014) 15:88–97. doi:10.1038/ni.2771
59. Cohen JI. Primary immunodeficiencies associated with EBV disease. *Curr Top Microbiol Immunol* (2015) 390:241–65. doi:10.1007/978-3-319-22822-8_10
60. Dropulic LK, Cohen JI. Severe viral infections and primary immunodeficiencies. *Clin Infect Dis* (2011) 53:897–909. doi:10.1093/cid/cir610
61. Picard C, Al-Herz W, Bousfiha A, Casanova JL, Chatila T, Conley ME, et al. Primary immunodeficiency diseases: an update on the classification from the International Union of Immunological Societies Expert Committee for Primary Immunodeficiency 2015. *J Clin Immunol* (2015) 35:696–726. doi:10.1007/s10875-015-0201-1
62. Coffey AJ, Brooksbank RA, Brandau O, Oohashi T, Howell GR, Bye JM, et al. Host response to EBV infection in X-linked lymphoproliferative disease results from mutations in an SH2-domain encoding gene. *Nat Genet* (1998) 20:129–35. doi:10.1038/2424
63. Rigaud S, Fondaneche MC, Lambert N, Pasquier B, Mateo V, Soulas P, et al. XIAP deficiency in humans causes an X-linked lymphoproliferative syndrome. *Nature* (2006) 444:110–4. doi:10.1038/nature05257
64. Huck K, Feyen O, Niehues T, Ruschendorf F, Hubner N, Laws HJ, et al. Girls homozygous for an IL-2-inducible T cell kinase mutation that leads to protein deficiency develop fatal EBV-associated lymphoproliferation. *J Clin Invest* (2009) 119:1350–8. doi:10.1172/JCI37901
65. Salzer E, Daschkey S, Choo S, Gombert M, Santos-Valente E, Ginzel S, et al. Combined immunodeficiency with life-threatening EBV-associated lymphoproliferative disorder in patients lacking functional CD27. *Haematologica* (2013) 98:473–8. doi:10.3324/haematol.2012.068791
66. Izawa K, Martin E, Soudais C, Bruneau J, Boutboul D, Rodriguez R, et al. Inherited CD70 deficiency in humans reveals a critical role for the CD70-CD27 pathway in immunity to Epstein-Barr virus infection. *J Exp Med* (2017) 214:73–89. doi:10.1084/jem.20160784
67. Abolhassani H, Edwards ES, Ikinciogullari A, Jing H, Borte S, Buggert M, et al. Combined immunodeficiency and Epstein-Barr virus-induced B cell malignancy in humans with inherited CD70 deficiency. *J Exp Med* (2017) 214:91–106. doi:10.1084/jem.20160849
68. Moshous D, Martin E, Carpentier W, Lim A, Callebaut I, Canioni D, et al. Whole-exome sequencing identifies Coronin-1A deficiency in 3 siblings with immunodeficiency and EBV-associated B-cell lymphoproliferation. *J Allergy Clin Immunol* (2013) 131:1594–603. doi:10.1016/j.jaci.2013.01.042
69. Salzer E, Cagdas D, Hons M, Mace EM, Garncarz W, Petronczki OY, et al. RASGRP1 deficiency causes immunodeficiency with impaired cytoskeletal dynamics. *Nat Immunol* (2016) 17:1352–60. doi:10.1038/ni.3575
70. Li FY, Chaigne-Delalande B, Su H, Uzel G, Matthews H, Lenardo MJ. XMEN disease: a new primary immunodeficiency affecting Mg2+ regulation of immunity against Epstein-Barr virus. *Blood* (2014) 123:2148–52. doi:10.1182/blood-2013-11-538686
71. Palendira U, Rickinson AB. Primary immunodeficiencies and the control of Epstein-Barr virus infection. *Ann N Y Acad Sci* (2015) 1356:22–44. doi:10.1111/nyas.12937
72. Lens SM, Tesselaar K, van Oers MH, van Lier RA. Control of lymphocyte function through CD27-CD70 interactions. *Semin Immunol* (1998) 10:491–9. doi:10.1006/smim.1998.0154
73. Orange JS, Jain A, Ballas ZK, Schneider LC, Geha RS, Bonilla FA. The presentation and natural history of immunodeficiency caused by nuclear factor kappaB essential modulator mutation. *J Allergy Clin Immunol* (2004) 113:725–33. doi:10.1016/j.jaci.2004.01.762
74. Willmann KL, Klaver S, Dogu F, Santos-Valente E, Garncarz W, Bilic I, et al. Biallelic loss-of-function mutation in NIK causes a primary immunodeficiency with multifaceted aberrant lymphoid immunity. *Nat Commun* (2014) 5:5360. doi:10.1038/ncomms6360
75. Sharfe N, Merico D, Karanxha A, Macdonald C, Dadi H, Ngan B, et al. The effects of RelB deficiency on lymphocyte development and function. *J Autoimmun* (2015) 65:90–100. doi:10.1016/j.jaut.2015.09.001
76. Pannicke U, Baumann B, Fuchs S, Henneke P, Rensing-Ehl A, Rizzi M, et al. Deficiency of innate and acquired immunity caused by an IKBKB mutation. *N Engl J Med* (2013) 369:2504–14. doi:10.1056/NEJMoa1309199
77. Nielsen C, Jakobsen MA, Larsen MJ, Muller AC, Hansen S, Lillevang ST, et al. Immunodeficiency associated with a nonsense mutation of IKBKB. *J Clin Immunol* (2014) 34:916–21. doi:10.1007/s10875-014-0097-1
78. Schonbeck U, Libby P. The CD40/CD154 receptor/ligand dyad. *Cell Mol Life Sci* (2001) 58:4–43. doi:10.1007/PL00000776
79. Korthauer U, Graf D, Mages HW, Briere F, Padayachee M, Malcolm S, et al. Defective expression of T-cell CD40 ligand causes X-linked immunodeficiency with hyper-IgM. *Nature* (1993) 361:539–41. doi:10.1038/361539a0
80. Allen RC, Armitage RJ, Conley ME, Rosenblatt H, Jenkins NA, Copeland NG, et al. CD40 ligand gene defects responsible for X-linked hyper-IgM syndrome. *Science* (1993) 259:990–3. doi:10.1126/science.7679801
81. Ferrari S, Giliani S, Insalaco A, Al-Ghonaium A, Soresina AR, Loubser M, et al. Mutations of CD40 gene cause an autosomal recessive form of immunodeficiency with hyper IgM. *Proc Natl Acad Sci U S A* (2001) 98:12614–9. doi:10.1073/pnas.221456898
82. Gewurz BE, Towfic F, Mar JC, Shinners NP, Takasaki K, Zhao B, et al. Genome-wide siRNA screen for mediators of NF-kappaB activation. *Proc Natl Acad Sci U S A* (2012) 109:2467–72. doi:10.1073/pnas.1120542109
83. Nanjappa SG, Kim EH, Suresh M. Immunotherapeutic effects of IL-7 during a chronic viral infection in mice. *Blood* (2011) 117:5123–32. doi:10.1182/blood-2010-12-323154
84. Zou P, Kawada J, Pesnicak L, Cohen JI. Bortezomib induces apoptosis of Epstein-Barr virus (EBV)-transformed B cells and prolongs survival of mice inoculated with EBV-transformed B cells. *J Virol* (2007) 81:10029–36. doi:10.1128/JVI.02241-06
85. Yamamoto Y, Gaynor RB. Therapeutic potential of inhibition of the NF-kappaB pathway in the treatment of inflammation and cancer. *J Clin Invest* (2001) 107:135–42. doi:10.1172/JCI11914
86. Lai AC, Crews CM. Induced protein degradation: an emerging drug discovery paradigm. *Nat Rev Drug Discov* (2017) 16:101–14. doi:10.1038/nrd.2016.211
87. Gasparini C, Celeghini C, Monasta L, Zauli G. NF-kappaB pathways in hematological malignancies. *Cell Mol Life Sci* (2014) 71:2083–102. doi:10.1007/s00018-013-1545-4
88. Wang CY, Guttridge DC, Mayo MW, Baldwin AS Jr. NF-kappaB induces expression of the Bcl-2 homologue A1/Bfl-1 to preferentially suppress chemotherapy-induced apoptosis. *Mol Cell Biol* (1999) 19:5923–9. doi:10.1128/MCB.19.9.5923
89. Henter JI, Horne A, Arico M, Egeler RM, Filipovich AH, Imashuku S, et al. HLH-2004: diagnostic and therapeutic guidelines for hemophagocytic lymphohistiocytosis. *Pediatr Blood Cancer* (2007) 48:124–31. doi:10.1002/pbc.21039
90. Frantz B, Nordby EC, Bren G, Steffan N, Paya CV, Kincaid RL, et al. Calcineurin acts in synergy with PMA to inactivate I kappa B/MAD3, an inhibitor of NF-kappa B. *EMBO J* (1994) 13:861–70.
91. Rieux-Laucat F, Casanova JL. Immunology. Autoimmunity by haploinsufficiency. *Science* (2014) 345:1560–1. doi:10.1126/science.1260791

Conflict of Interest Statement: The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

frontiers in Immunology

CASE REPORT
published: 29 January 2018
doi: 10.3389/fimmu.2017.02015

CD70 Deficiency due to a Novel Mutation in a Patient with Severe Chronic EBV Infection Presenting As a Periodic Fever

*Roberta Caorsi[1†], Marta Rusmini[2†], Stefano Volpi[1], Sabrina Chiesa[1], Claudia Pastorino[1], Angela Rita Sementa[3], Paolo Uva[4], Alice Grossi[2], Edoardo Lanino[5], Maura Faraci[5], Francesca Minoia[1], Sara Signa[1], Paolo Picco[1], Alberto Martini[1], Isabella Ceccherini[2] and Marco Gattorno[1]**

[1] Clinica Pediatria e Reumatologia, Istituto Giannina Gaslini, Genova, Italy, [2] Division of Human Genetics, Istituto Giannina Gaslini, Genova, Italy, [3] Pathology Unit, Istituto Giannina Gaslini, Genova, Italy, [4] Centre for Advanced Studies, Research and Development in Sardinia (CRS4), Science and Technology Park Polaris, Pula, Italy, [5] Bone Marrow Transplantation Unit, Istituto Giannina Gaslini, Genova, Italy

OPEN ACCESS

Edited by:
Isabelle Meyts,
KU Leuven, Belgium

Reviewed by:
Maria Pia Cicalese,
Scientific Institute San Raffaele (IRCCS), Italy
Sylvain Latour,
Centre national de la recherche scientifique (CNRS), France

***Correspondence:**
Marco Gattorno
marcogattorno@gaslini.org

†These authors have contributed equally to this work.

Specialty section:
This article was submitted to Primary Immunodeficiencies, a section of the journal Frontiers in Immunology

Received: 05 September 2017
Accepted: 28 December 2017
Published: 29 January 2018

Citation:
Caorsi R, Rusmini M, Volpi S, Chiesa S, Pastorino C, Sementa AR, Uva P, Grossi A, Lanino E, Faraci M, Minoia F, Signa S, Picco P, Martini A, Ceccherini I and Gattorno M (2018) CD70 Deficiency due to a Novel Mutation in a Patient with Severe Chronic EBV Infection Presenting As a Periodic Fever. Front. Immunol. 8:2015. doi: 10.3389/fimmu.2017.02015

Primary immunodeficiencies with selective susceptibility to EBV infection are rare conditions associated with severe lymphoproliferation. We followed a patient, son of consanguineous parents, referred to our center for recurrent periodic episodes of fever associated with tonsillitis and adenitis started after an infectious mononucleosis and responsive to oral steroid. An initial diagnosis of periodic fever, aphthous stomatitis, pharyngitis, cervical adenitis syndrome was done. In the following months, recurrent respiratory infections and episodes of keratitis were also observed, together with a progressive reduction of immunoglobulin levels and an increase of $CD20^+$ cells. Cell sorting and EBV PCR showed 25,000 copies for 100,000 leukocytes with predominant infection of B lymphocytes. Lymph node's biopsy revealed reactive lymphadenopathy with paracortical involvement consistent with a chronic EBV infection. Molecular analysis of *XIAP*, *SHA2D1A*, *ITK*, and *CD27* genes did not detect any pathogenic mutation. The patients underwent repeated courses of anti-CD20 therapy with only a partial control of the disease, followed by stem cell transplantation with a complete normalization of clinical and immunological features. Whole exome sequencing of the trio was performed. Among the variants identified, a novel loss of function homozygous c. 163-2A>G mutation of the *CD70* gene, affecting the exon 2 AG-acceptor splice site, fit the expected recessive model of inheritance. Indeed, deficiency of both CD27, and, more recently, of its ligand CD70, has been reported as a cause of EBV-driven lymphoproliferation and hypogammaglobulinemia. Cell surface analysis of patient-derived PHA-T cell blasts and EBV-transformed lymphoblastoid cell lines confirmed absence of CD70 expression. In conclusion, we describe a case of severe chronic EBV infection caused by a novel mutation of CD70 presenting with recurrent periodic fever.

Keywords: CD70 deficiency, periodic fever, aphthous stomatitis, pharyngitis, cervical adenitis syndrome, periodic fever, Ebstein–Barr virus, hematopoietic stem cell transplantation

A number of different conditions are associated with the chronic activation of an EBV infection. Chronic active EBV (CABEV) was first described in 1975 (1), to describe a condition characterized by the presence of chronic symptoms of EBV infection in the absence of malignancy, autoimmunity, or a known immunodeficiency (2–4). This condition is heterogeneous from both the clinical and immunological point of view: in fact, while in some patients, EBV is nearly only detected in T or NK cells [more frequently in the Asian population (1–4)], in other patients, it is mostly detected in B cells [more common in the Caucasian population (5)].

However, during the past years, it became evident that a relevant proportion of chronic EBV infections were secondary to genetic defects leading to a selective susceptibility to EBV-induced diseases. X-linked diseases caused by mutations in *SH2D1A* (6) (XLP), *XIAP* (7), and MAGT1 (8) or autosomal recessive diseases caused by mutation in ITK (9), CORO1A (10), and FCGR3A (11) are such an example. In this condition, patients develop various degrees of lymphoproliferation and immunodeficiency, with hemophagocytic lymphohistiocytosis, hypogammaglobulinemia, and/or lymphoid malignancy secondary to chronic EBV infection as part of the clinical picture. Biallelic mutations of CD27, a tumor necrosis factor (TNF) receptor superfamily member expressed on cells of adaptive immunity and NK cells cause an EBV-associate lymphoproliferative disease with hypogammaglobulinemia (12–14). More recently, an autosomal recessive deficiency of CD70, the ligand of CD27, has been associated to a combined immunodeficiency with EBV-induced B-cell malignancy in humans (15, 16).

Though presenting with a wide heterogeneity, most of the patients with chronic EBV infection share severe clinical manifestations with early onset and poor prognosis: common immunosuppressive and antiviral therapies are usually not effective, and most of the patients not treated with bone marrow transplantation die due to lymphoid malignancies (5). Here, we describe the genetic characterization of a patient with a severe chronic EBV infection due to a novel mutation of CD70, whose initial clinical picture resembled a periodic fever syndrome.

The patient, born to consanguineous parents, presented at the age of 15 months with a not-complicated infectious mononucleosis followed by the onset of recurrent episodes of fever associated with exudative tonsillitis, adenitis, splenomegaly, and sweating, lasting 3–5 days and treated with NSAIDS or, in some occasions, with antibiotics. Blood examination revealed neutrophilic leukocytosis and elevation of acute phase reactants, while serum immunoglobulins were within the normal range. An autoinflammatory condition, consistent with periodic fever, aphthosis stomatitis, pharyngitis, cervical adenitis (PFAPA) syndrome was suspected and on-demand steroidal treatment was suggested with a prompt response. In the following months, the child continued to present periodic fever episodes with a more clear association with respiratory viral and bacterial infections and more frequent use of antibiotics. Three episodes of anterior uveitis were also observed. The patient presented several destructive dental caries (**Figure 1A**) and hyper sensibility to mosquitoes' bites was reported.

At the age of 3, immunologic tests revealed a reduction in the level of serum immunoglobulins and a reduction of both T (in particular $CD3^+CD8^+$ cells) and B lymphocytes populations (**Table 1**). Quantitative PCR for EBV DNA revealed 21,935 copies for 100,000 leukocytes with prevalence of infection in the B cells (**Table 2**). Whole body positron emission tomography revealed a retroperitoneal formation of about 35 mm with an increased metabolism. At biopsy, staining was compatible with reactive lymphadenopathy with paracortical involvement associated with EBV infection (**Figure 1B**). No signs of lymphoma were observed. The cytofluorimetric characterization of the immunophenotype enlightened the presence of a mixed lymphocyte population composed of polyclonal T and B lymphocytes.

Most common genetic conditions possibly associated with chronic EBV infection and hypogammaglobulinemia were ruled out by molecular analysis of the coding sequence of target genes (*SHA2D1A*, *XIAP*, *BAFF-R,* and *ICOS*). In addition, the cytofluorimetric analysis of perforin, CD107, and 2B4 receptor was normal (data not shown). In light of these findings, a severe chronic EBV infection was suspected.

Taking into consideration, the overall satisfactory general conditions and in light of the prevalent involvement of $CD20^+$ lymphocytes, after informed consent approved by G. Gaslini ethical board, treatment with rituximab (375 mg/m^2/dose) was started with a good clinical response and a dramatic reduction of viral load (**Figure 1C**). The patient was followed longitudinally with the indication to repeat rituximab whenever the viral load exceeded 20,000 copies/100,000 lymphomonocytic cells and/or the re-appearance of fever and other manifestations associated with EBV. During the following 2 years, the patient received only two administrations of rituximab, in association with i.v. immunoglobulin substitutive treatment every 6 weeks. The patient presented a general wellbeing with a regular growth, without severe infections, and with persistent control of the viral load and of the number of CD20 cells (**Figure 1C**). However, 3 years after the diagnosis a clear progression of the

TABLE 1 | lymphocytes population detected in the patient.

Lymphocytes' population	Percentage of lymphocytes (absolute count/mmc)
CD3+	76 % (4071)
CD3+ CD4+	42.5 % (2246)
CD3+ CD8+	26.2 % (134)
CD19+	15.4 % (814)
CD20+	15 % (800)
CD3– CD16+ CD56+	7 % (370)

TABLE 2 | EBV DNA quantitative PCR in different lymphocytes populations.

Lymphocytes' population	EBV DNA quantitative PCR	% of infected cells
CD3+ CD8+	8100 copies / 1812200 cells	0.4 %
CD3+ CD4+	10700 copies / 4584300 cells	0.2 %
CD3– CD16+ CD56+	1700 copies / 367000 cells	0.5 %
CD19+	386540 copies / 4500000 cells	8.6 %

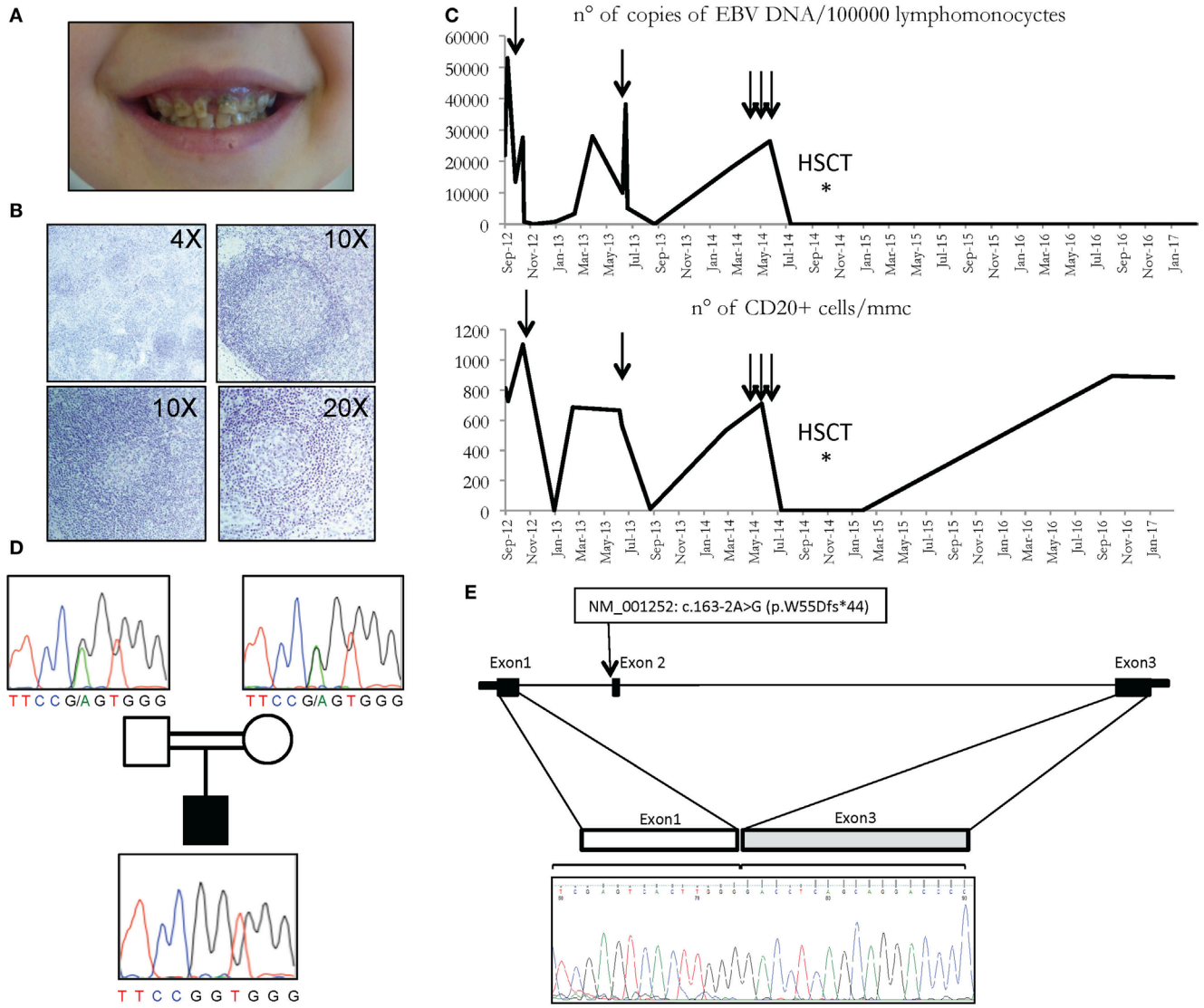


FIGURE 1 | **(A)** Multiple caries in CD70 deficient patient. **(B)** Histologic analysis of patient's lymph node showing follicular hyperplasia with paracortical expansion (top left), germinal centers with normal appearance (top right), follicular lysis (lower panels). **(C)** Copies of EBV DNA (above) and number of CD20 cells (below) detected in the patient. Arrows represent the infusions of rituximab. **(D)** Family pedigree with the c.163-2A>G variant of the *CD70* gene, heterozygous in carriers, and homozygous in the patient. **(E)** *CD70* genomic region and cDNA sequence of the first 3 exons in the patient is depicted, revealing the exon 2 skipping in the electropherogram at the bottom.

disease was observed, with subsequent elevations of the viral load and an increase in the frequency and severity of respiratory tract infections requiring, in some cases, prolonged hospitalization. In this period, the patient received three consecutive administrations of rituximab, with only a transient clinical response. On these bases, hematopoietic stem cell transplantation (HSCT) was planned.

The conditioning regimen consisted of Thiotepa (8 mg/kg, day −7), Fludarabine (40 mg/m^2/day, from day −6 to day −3), and Treosulfan (14 gr/m^2 from day −6 to day −4). Rabbit anti-thymocyte globulin (ATG, 30 mg/kg from day −4 to −2), micophenolyc acid (30 mg/kg from day 0), and cyclosporine (1 mg/kg until day −2 and 3 mg/kg from day −1) were administered as Graft-versus-Host Disease (GvHD) prophylaxis, whereas a single dose of rituximab (200 mg/sqm) was administered on day 0 for prevention of EBV-related lymphoprolipherative disease.

A total of 2.88 × 10^8/kg mononuclear bone marrow cells were infused from an 8/10, EBV positive HLA-matched unrelated female donor (1 antigenic HLA-C mismatch in host-versus-graft direction, 1 bidirectional HLA-DQB1 mismatch). Neutrophils and platelets engraftment occurred on day +24 and +18 after HSCT, respectively. At first evaluation, chimerism analysis revealed a mixed pattern (88% donor cell), shown to be of full donor origin, which persisted at all subsequent evaluations. The early post-transplant phase was complicated by mild mucositis, multiple CMV reactivations successfully treated by Foscarnet and/or Gancyclovir, grade 2 acute GvHD (grade 2) responsive to

corticosteroids. Immunosuppression was progressively tapered and eventually discontinued 16 months after HSCT in the absence of any manifestation of chronic GvHD. Blood EBV DNA never positivized after HSCT, and specific antibodies were detected since month +9. After 2 years and 6 months from HSCT, the patient persists in good health in the absence of any sign of the disease.

Having excluded some of the most common genetic causes of genetic susceptibility to EBV infections, and in light of the severity of the clinical picture, and the consanguinity of the parents (**Figure 1D**), a whole exome sequencing (WES) approach was undertaken in the patients and their parents. Variants were prioritized with a custom pipeline to identify the genetic cause of patient's condition. In particular, only variants either unreported or already reported in the general population with a frequency lower than 1% were considered. Moreover, synonymous, intronic, and UTR variants were excluded, in addition to splicing variants not specifically affecting the donor and acceptor splice sites. Among the homozygous variants, thus identified (Table S1 in Supplementary Material), a splicing variant of the CD70 gene, fitting the expected recessive model of inheritance, with the parents being heterozygous for the same mutation (**Figure 1D**), was further investigated. In particular, the variant c.163-2A>G affects the exon 2AG-acceptor splice site of the *CD70* gene (NM_001252). To analyze the effect of the mutation on the transcription of the gene, we sequenced the complementary DNA, revealing the skipping of exon 2 (**Figure 1E**).

Flow cytometry analysis of CD70 expression of patient's PHA-T cell blasts at 7 and 23 days of stimulation failed to detect even low amounts of CD70 protein. In contrast, expression of CD70 was detected on a fraction of PHA-stimulated T cells from healthy donors (**Figure 2A**). Similarly, CD70 was not detected on EBV-trasformed lymphoblastoid cells lines (EBV-LCL) derived from the patient, in contrast to EBV-LCL from healthy donors that expressed high levels of CD70 on their surface (**Figure 2B**). After HSCT, the expression of CD70 in patient's PHA-T cell blasts at 12 days of culture was comparable to healthy donor (**Figure 2C**). These data demonstrate that c.163-2A>G mutation causes exon skipping and absence of CD70 protein expression in patient's cells.

CD70 is the ligand of the TNF superfamily member CD27 and is expressed by antigen-presenting cells upon triggering of CD40 and toll-like receptors (TLR), by T cells upon TCR activation,

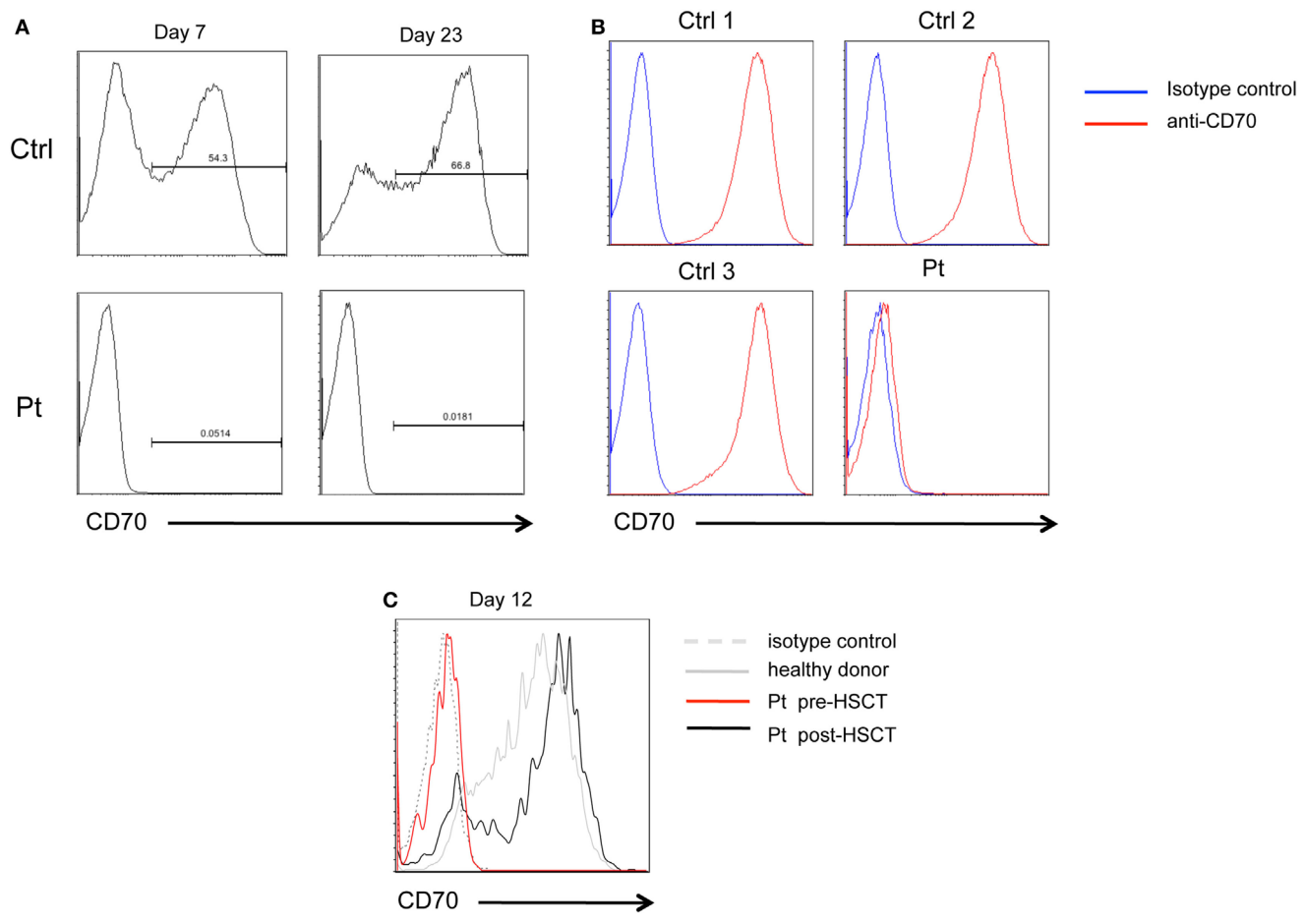


FIGURE 2 | CD70 expression. **(A)** FACS histograms showing CD70 expression detected with anti-CD70 antibody on PHA-stimulated $CD3^+$ T cell blasts from patient (Pt) or healthy donor (Ctrl), at day 7 and 23 of culture. **(B)** FACS histograms showing CD70 expression on lymphoblastoid cell lines derived from the Pt or three healthy donors (Ctrl1, Ctrl2, and Ctrl3) detected with anti-CD70 antibody. **(C)** FACS histograms showing CD70 expression on PHA-stimulated $CD3^+CD4^+$ T cell blasts from Pt pre-HSCT (red line), Pt post-HSCT (black line), or healthy donor (dashed gray line), at day 12 of culture.

CD28 cross-linking, and cytokine exposure, and constitutively by thymic epithelial cells (17). CD27–CD70 binding provides a costimulatory signal for CD4 and CD8 activation (18, 19), and studies in mice have provided evidences for its role in memory expansion and protection upon reinfection (20, 21). Inborn errors of *CD27* are a well-known cause of persistent symptomatic EBV viremia and hypogammaglobulinemia, thus making *CD70* mutations the likely cause of our patient's phenotype.

While our characterization of this novel immune defect was on-going, two groups reported the association of *CD70* mutations with combined immunodeficiency in a total of five patients affected by EBV-associated Hodking's lymphoma and hypogammaglobulinemia (15, 16). The groups demonstrated that CD70 deficiency (16) causes a reduction of *in vitro*-generated EBV-specific cytotoxic T cell activity and to a decreased expression of 2B4 and NKG2D, receptors implicated in controlling EBV infection, on memory $CD8^+$ T cells, consistent with their impaired capability to kill EBV-infected cells (15).

Four of the five reported patients suffered by EBV-associated Hodgkin's lymphoma with an onset at 2.5, 3 (two patients), and 17 years of age and hypogammaglobulinemia. The initial presentation was a severe varicella infection with pneumonia in one case, encephalitis of unknown cause in another one, and HL in the other three cases. Our patient uniquely presented with a subtle history of recurrent fever and inflammatory symptoms was similar to PFAPA syndrome. Of note, the patient reported by Izawa et al. following treatment for HL, also presented with recurrent fever and lymphoadenopathy, while P1 in Abolhassani et al. presented oral aphthous ulcers.

In conclusion, we report an early diagnosed case of CD70 deficiency with an onset of periodic fever, suggesting that in EBV positive patients with signs of PFAPA syndrome molecular analysis of *CD70* gene should be performed.

MATERIALS AND METHODS

Informed Consent

Experiments and molecular genetic analysis were performed, following informed consent and approval by the institute review board. The family gave permission for publication of clinical and laboratory data and photographic images.

Cell Culture

Whole blood samples were collected from the patient and healthy donors. Peripheral blood mononuclear cells (PBMC) were isolated by Ficoll-Paque density gradient from blood samples using standard procedures. Expansion of T cell blasts were obtained by incubating PBMCs for 48 h with 1 μg/ml of phytohemagglutinin (PHA) (Sigma-Aldrich) in RPMI supplemented with 10% FBS serum, 1% penicillin, and 1% streptomycin. After 2 days, PHA-blasts were maintained in culture with 100 U/ml IL-2.

EBV-trasformed cell lines were generated from the patient and control healthy donors with standard technique.

Lymph Node Histology

Lymph nodes from diagnostic biopsies were fixed in 4% formalin, paraffin embedded and sectioned for H&E staining with standard techniques. Images were recorded using a Zeiss Axioskop plus microscope mounting a digital microscopy camera AxioCam ICc5.

Flow Cytometry

Cell staining and phenotype analyses of blast T cells and cell lines were performed according to standard flow cytometry methods.

Expression of CD70 on PHA-T cell blasts pre- and post-HSCT and lymphoblastoid cell lines derived from the patient and four healthy donors was evaluated by flow cytometry. Anti-CD70 PE monoclonal antibody (clone: Ki-24, isotype: mouse IgG3, k, BD Biosciences), PeCy7 mouse anti-human CD3 (clone: SK7, isotype: mouse BALB/c IgG1, k, BD Biosciences), APC mouse anti-human CD4 (clone: RPA-T4, isotype: mouse IgG, k, BD Biosciences), and PC7 mouse anti-human CD19 (clone: J3-119, isotype: IgG1, mouse, Beckman Coulter) were used.

WES and Sanger Sequencing Validation

Molecular analysis of *SHA2D1A*, *XIAP*, *BAFF-R,* and *ICOS* genes was performed through Sanger sequencing in a CLIA certified laboratory.

Whole exome sequencing consisted of several steps. Exome capture was performed on genomic DNA using the Nextera Rapid Capture Expanded Exome Kit (Illumina Inc., San Diego, CA, USA) according to manufacturer instructions. The enriched libraries were sequenced on the Hiseq3000 instrument with 100 bp paired-end reads. This approach achieved an 86× average coverage over the 62Mb of target regions sequenced, with more than 95% regions covered. Data analysis has been performed using an analysis pipeline implemented in Orione (22). Briefly, paired-end sequence reads were aligned to the human genome (hg19) with BWA-MEM [v.0.7.9a (23)]. Initial mappings were processed using the GATK framework [version 2.8.1 (24)], according to the GATK best practices recommendations (25, 26). Variants were classified as known or novel based on dbSNP146 and annotated using KGGSeq (27).

Annotations included positions in UCSC, RefGene, GENCODE and ENSEMBL transcripts, OMIM and ClinVar annotations, potential false positive signals, allele frequency in dbSNP, ESP6500, 1000 Genome Project (release 05/2013), and ExAC, functional predictions for the amino acid changes according to different models (SIFT, Polyphen2, LRT, MutationTaster, MutationAssessor, and FATHMM) retrieved from dbNSFP v 2.9 (database of human nonsynonymous SNPs and their functional predictions) (28).

Homozygous missense and splicing mutations at ±2bp were taken into account and selected based on their allele frequency (variants unreported or reported with a frequency of <1% in the general population were selected). The identified CD70 variant was validated by Sanger sequencing both in the proband and in his parents. PCR products were purified by ExoSAP-IT (GE Healthcare) and directly sequenced using Big Dye v1.1 and an ABI3130 automated sequencer (Applied Biosystems, Foster City, CA, USA).

CD70 Transcript Characterization

Total RNA from patient's T cells and from three-unrelated controls was isolated by a commercial RNA purification kit (RNeasy Mini kit, Qiagen, GmbH, Germany) and 1 μg of total RNA was reverse transcribed by iScript cDNA synthesis kit (Bio-Rad Laboratories) according to the manufacturer's protocol. DNA amplification followed by Sanger sequencing was carried out on

the cDNA, thus obtained using CD70 specific primers designed on exon 1 (5′- GTGATCTGCCTCGTGGTGT-3′) and exon 3 (5′-AGGCAATGGTACAACCTTGG-3′).

ETHICS STATEMENT

All subjects gave written informed consent in accordance with the Declaration of Helsinki. The protocol was approved by the Regional Ethical Committee.

AUTHOR CONTRIBUTIONS

RC, EL, MF, FM, SS, PP, AM, and MG clinically followed the patient. MR, PU, AG and IC performed genetic analysis. ARS performed pathological studies. SC and CP performed *in vitro* studies. RC, MR, and SV wrote the manuscript and prepared the figures. SC contributed to the writing and prepared the figures. IC and MG supervised all experiments and edited the manuscript.

FUNDING

This work was funded by The INSAID Project (E-Rare 3 Program, grant no. 9003037603).

SUPPLEMENTARY MATERIAL

The Supplementary Material for this article can be found online at http://www.frontiersin.org/articles/10.3389/fimmu.2017.02015/full#supplementary-material.

REFERENCES

1. Horwitz CA, Henle W, Henle G, Schmitz H. Clinical evaluation of patients with infectious mononucleosis and development of antibodies to the R component of the Epstein-Barr virus-induced early antigen complex. *Am J Med* (1975) 58(3):330–8. doi:10.1016/0002-9343(75)90599-9
2. Tobi M, Morag A, Ravid Z, Chowers I, Feldman-Weiss V, Michaeli Y, et al. Prolonged atypical illness associated with serological evidence of persistent Epstein-Barr virus infection. *Lancet* (1982) 1(8263):61–4. doi:10.1016/S0140-6736(82)90210-0
3. Joncas JH, Ghibu F, Blagdon M, Montplaisir S, Stefanescu I, Menezes J. A familial syndrome of susceptibility to chronic active Epstein-Barr virus infection. *Can Med Assoc J* (1984) 130(3):280–4.
4. DuBois RE, Seeley JK, Brus I, Sakamoto K, Ballow M, Harada S, et al. Chronic mononucleosis syndrome. *South Med J* (1984) 77(11):1376–82. doi:10.1097/00007611-198411000-00007
5. Cohen JI, Jaffe ES, Dale JK, Pittaluga S, Heslop HE, Rooney CM, et al. Characterization and treatment of chronic active Epstein-Barr virus disease: a 28-year experience in the United States. *Blood* (2011) 117(22):5835–49. doi:10.1182/blood-2010-11-316745
6. Coffey AJ, Brooksbank RA, Brandau O, Oohashi T, Howell GR, Bye JM, et al. Host response to EBV infection in X-linked lymphoproliferative disease results from mutations in an SH2-domain encoding gene. *Nat Genet* (1998) 20(2):129–35. doi:10.1038/2424
7. Rigaud S, Fondanèche MC, Lambert N, Pasquier B, Mateo V, Soulas P, et al. XIAP deficiency in humans causes an X-linked lymphoproliferative syndrome. *Nature* (2006) 444(7115):110–4. doi:10.1038/nature05257
8. Li FY, Lenardo MJ, Chaigne-Delalande B. Loss of MAGT1 abrogates the Mg2+ flux required for T cell signaling and leads to a novel human primary immunodeficiency. *Magnes Res* (2011) 24(3):S109–14. doi:10.1684/mrh.2011.0286
9. Huck K, Feyen O, Niehues T, Rüschendorf F, Hübner N, Laws HJ, et al. Girls homozygous for an IL-2-inducible T cell kinase mutation that leads to protein deficiency develop fatal EBV-associated lymphoproliferation. *J Clin Invest* (2009) 119(5):1350–8. doi:10.1172/JCI37901
10. Moshous D, Martin E, Carpentier W, Lim A, Callebaut I, Canioni D, et al. Whole-exome sequencing identifies coronin-1A deficiency in 3 siblings with immunodeficiency and EBV-associated B-cell lymphoproliferation. *J Allergy Clin Immunol* (2013) 131(6):1594–603. doi:10.1016/j.jaci.2013.01.042
11. Grier JT, Forbes LR, Monaco-Shawver L, Oshinsky J, Atkinson TP, Moody C, et al. Human immunodeficiency-causing mutation defines CD16 in spontaneous NK cell cytotoxicity. *J Clin Invest* (2012) 122(10):3769–80. doi:10.1172/JCI64837
12. Alkhairy OK, Perez-Becker R, Driessen GJ, Abolhassani H, van Montfrans J, Borte S, et al. Novel mutations in TNFRSF7/CD27: clinical, immunologic, and genetic characterization of human CD27 deficiency. *J Allergy Clin Immunol* (2015) 136(3):703–712.e10. doi:10.1016/j.jaci.2015.02.022
13. van Montfrans JM, Hoepelman AI, Otto S, van Gijn M, van de Corput L, de Weger RA, et al. CD27 deficiency is associated with combined immunodeficiency and persistent symptomatic EBV viremia. *J Allergy Clin Immunol* (2012) 129(3):787–793.e6. doi:10.1016/j.jaci.2011.11.013
14. Salzer E, Daschkey S, Choo S, Gombert M, Santos-Valente E, Ginzel S, et al. Combined immunodeficiency with life-threatening EBV-associated lymphoproliferative disorder in patients lacking functional CD27. *Haematologica* (2013) 98(3):473–8. doi:10.3324/haematol.2012.068791
15. Abolhassani H, Edwards ES, Ikinciogullari A, Jing H, Borte S, Buggert M, et al. Combined immunodeficiency and Epstein-Barr virus-induced B cell malignancy in humans with inherited CD70 deficiency. *J Exp Med* (2017) 214(1):91–106. doi:10.1084/jem.20160849
16. Izawa K, Martin E, Soudais C, Bruneau J, Boutboul D, Rodriguez R, et al. Inherited CD70 deficiency in humans reveals a critical role for the CD70-CD27 pathway in immunity to Epstein-Barr virus infection. *J Exp Med* (2017) 214(1):73–89. doi:10.1084/jem.20160784
17. Croft M. The role of TNF superfamily members in T-cell function and diseases. *Nat Rev Immunol* (2009) 9(4):271–85. doi:10.1038/nri2526
18. Nolte MA, van Olffen RW, van Gisbergen KP, van Lier RA. Timing and tuning of CD27-CD70 interactions: the impact of signal strength in setting the balance between adaptive responses and immunopathology. *Immunol Rev* (2009) 229(1):216–31. doi:10.1111/j.1600-065X.2009.00774.x
19. Borst J, Hendriks J, Xiao Y. CD27 and CD70 in T cell and B cell activation. *Curr Opin Immunol* (2005) 17(3):275–81. doi:10.1016/j.coi.2005.04.004
20. Munitic I, Kuka M, Allam A, Scoville JP, Ashwell JD. CD70 deficiency impairs effector CD8 T cell generation and viral clearance but is dispensable for the recall response to lymphocytic choriomeningitis virus. *J Immunol* (2013) 190(3):1169–79. doi:10.4049/jimmunol.1202353
21. Allam A, Swiecki M, Vermi W, Ashwell JD, Colonna M. Dual function of CD70 in viral infection: modulator of early cytokine responses and activator of adaptive responses. *J Immunol* (2014) 193(2):871–8. doi:10.4049/jimmunol.1302429
22. Cuccuru G, Orsini M, Pinna A, Sbardellati A, Soranzo N, Travaglione A, et al. Orione, a web-based framework for NGS analysis in microbiology. *Bioinformatics* (2014) 30(13):1928–9. doi:10.1093/bioinformatics/btu135
23. Li H, Durbin R. Fast and accurate long-read alignment with Burrows-Wheeler transform. *Bioinformatics* (2010) 26(5):589–95. doi:10.1093/bioinformatics/btp698
24. McKenna A, Hanna M, Banks E, Sivachenko A, Cibulskis K, Kernytsky A, et al. The genome analysis toolkit: a MapReduce framework for analyzing next-generation DNA sequencing data. *Genome Res* (2010) 20(9):1297–303. doi:10.1101/gr.107524.110
25. DePristo MA, Banks E, Poplin R, Garimella KV, Maguire JR, Hartl C, et al. A framework for variation discovery and genotyping using next-generation DNA sequencing data. *Nat Genet* (2011) 43(5):491–8. doi:10.1038/ng.806

26. Van der Auwera GA, Carneiro MO, Hartl C, Poplin R, Del Angel G, Levy-Moonshine A, et al. From FastQ data to high confidence variant calls: the Genome Analysis Toolkit best practices pipeline. *Curr Protoc Bioinformatics* (2013) 43:111011–33. doi:10.1002/0471250953.bi1110s43
27. Li MX, Gui HS, Kwan JS, Bao SY, Sham PC. A comprehensive framework for prioritizing variants in exome sequencing studies of Mendelian diseases. *Nucleic Acids Res* (2012) 40(7):e53. doi:10.1093/nar/gkr1257
28. Liu X, Jian X, Boerwinkle E. dbNSFP v2.0: a database of human non-synonymous SNVs and their functional predictions and annotations. *Hum Mutat* (2013) 34(9):E2393–402. doi:10.1002/humu.22376

Conflict of Interest Statement: The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

frontiers in Immunology

MINI REVIEW
published: 08 February 2018
doi: 10.3389/fimmu.2018.00198

Impaired Control of Epstein–Barr Virus Infection in B-Cell Expansion with NF-κB and T-Cell Anergy Disease

*Swadhinya Arjunaraja[1], Pamela Angelus[2,3], Helen C. Su[2] and Andrew L. Snow[1]**

[1] Department of Pharmacology and Molecular Therapeutics, Uniformed Services University of the Health Sciences, Bethesda, MD, United States, [2] Laboratory of Clinical Immunology and Microbiology, National Institute of Allergy and Infectious Diseases, National Institutes of Health, Bethesda, MD, United States, [3] Clinical Research Directorate/Clinical Monitoring Research Program, Leidos Biomedical Research, Inc., National Cancer Institute at Frederick, Frederick, MD, United States

B-cell *e*xpansion with *N*F-κB and *T*-cell *a*nergy (BENTA) disease is a B-cell-specific lymphoproliferative disorder caused by germline gain-of-function mutations in *CARD11*. These mutations force the CARD11 scaffold into an open conformation capable of stimulating constitutive NF-κB activation in lymphocytes, without requiring antigen receptor engagement. Many BENTA patients also suffer from recurrent infections, with 7 out of 16 patients exhibiting chronic, low-grade Epstein–Barr virus (EBV) viremia. In this mini-review, we discuss EBV infection in the pathogenesis and clinical management of BENTA disease, and speculate on mechanisms that could explain inadequate control of viral infection in BENTA patients.

Keywords: Epstein–Barr virus, B-cell expansion with NF-κB and T-cell anergy, CARD11, NF-κB, primary immune deficiency

OPEN ACCESS

Edited by:
Jeffrey I. Cohen, National Institutes of Health (NIH), United States

Reviewed by:
Tri Giang Phan, Garvan Institute of Medical Research, Australia
Kaan Boztug, CeMM Research Center for Molecular Medicine (OAW), Austria

****Correspondence:***
Andrew L. Snow
andrew.snow@usuhs.edu

Specialty section:
This article was submitted to Primary Immunodeficiencies, a section of the journal Frontiers in Immunology

Received: *14 October 2017*
Accepted: *23 January 2018*
Published: *08 February 2018*

Citation:
Arjunaraja S, Angelus P, Su HC and Snow AL (2018) Impaired Control of Epstein–Barr Virus Infection in B-Cell Expansion with NF-κB and T-Cell Anergy Disease. Front. Immunol. 9:198. doi: 10.3389/fimmu.2018.00198

INTRODUCTION

Epstein–Barr virus (EBV) is a ubiquitous human herpesvirus that establishes life-long infection in ~90% of individuals (1). Primary EBV infection in childhood is usually asymptomatic in immunocompetent hosts, while acquisition of EBV during adolescence can result in infectious mononucleosis (IM) that usually resolves within days to weeks. However, EBV infections can also trigger lymphoproliferative disease, lymphoma, fulminant infectious mononucleosis (FIM), and/or hemophagocytic lymphohistiocytosis (HLH) in genetically or iatrogenically immunocompromised patients (2–4). These conditions clearly suggest that EBV has co-evolved with its host under constant immune surveillance to ensure that virus–host homeostasis is maintained (5, 6).

The transmission, circulation, and persistence of EBV in the human host have been reviewed extensively (1, 4, 7–9). EBV initially establishes lytic infection in both B lymphocytes and epithelial cells of the oropharynx. The EBV lytic gene program ensures both viral replication and evasion from early detection by either natural killer (NK) cells or $CD8^+$ T cells (10). EBV then switches to a latent infection program that expands the pool of infected B cells considerably (11). This is achieved in part through the expression of key latent membrane proteins 1 and 2A, which mimic constitutive CD40 and B cell receptor signaling, respectively. Evidence suggests that this "growth" program of latency (i.e., latency III) provides the proliferative and pro-survival signals necessary to drive the EBV-infected B cell through a germinal center-like reaction and eventually into the memory B cell pool,

all without requiring cognate antigen recognition. At the same time, latent proteins such as LMP2 and EBNA3A/B/C contain potent immunodominant class I MHC peptide epitopes for $CD8^+$ T cell recognition, ensuring the destruction of most EBV-infected B cells at this stage (3). $CD8^+$ T (and NK) cell-mediated killing is also aided by the robust upregulation of ligands for both NKG2D and signaling lymphocyte activation molecule (SLAM) family receptors on EBV-infected B cells, which participate in signaling for cytotoxic functions (see below) (5). Surviving EBV^+ memory B cells remain quiescently infected without expressing viral antigens (latency 0) for the lifetime of the individual, with occasional rounds of viral reactivation thought to occur as these cells traffic back through the oropharynx (12). EBV-specific cellular immunity maintains tight control throughout these cycles (5).

In this manner, EBV has evolved an elegant strategy for ensuring initial colonization followed by persistent, benign infection in the immunocompetent host, in which a sizable portion of the memory $CD8^+$ T cell pool (~2–5%) is dedicated to maintaining EBV-specific immunosurveillance (3, 13). Indeed, cytolytic killing of EBV-infected B cells by effector $CD8^+$ T cells occurs during acute IM, comprising up to 50% of the $CD8^+$ T cell compartment (4, 14). EBV-specific $CD4^+$ T cells are also important for robust $CD8^+$ cellular immunity and can also participate directly in cytotoxic killing of infected B cells (15, 16). During asymptomatic infection, NK cells help to restrict viral load by inhibiting their replication and can reduce the likelihood of EBV transformation of B cells (10, 17, 18). Furthermore, other innate effectors such as invariant natural killer T cells are also known to play a role in killing of infected B cells and can limit EBV transformation of B cells *in vitro* (19).

The advent of next-generation sequencing technology has enabled us to characterize primary immune deficiency (PID) states in humans caused by mutations in single immune-related genes that predispose them to certain pathogens. Indeed, several PIDs have now been recognized for their specific susceptibility to uncontrolled EBV infection and associated disease, sometimes referred to collectively as "EBV-opathies" (5, 20–22). In this review, we focus our attention on the incidence and severity of EBV infection in a recently characterized PID known as *B*-cell *e*xpansion with *N*F-κB and *T*-cell *a*nergy (BENTA). Mechanistic insights into possible immunological shortcomings surrounding EBV infections in BENTA patients are provided below.

BENTA DISEASE

Our group discovered a B-cell-specific lymphoproliferative congenital human disorder termed BENTA (23). BENTA disease is caused by heterozygous, germline-encoded gain-of-function mutations in the gene *CARD11*, which encodes a lymphocyte-specific scaffold protein (CARD11) also known as CARMA1. The CARD11 protein bridges the antigen receptor ligation in B or T cells with multiple downstream signaling pathways such as canonical NF-κB, c-Jun N-terminal kinase (JNK), and mechanistic target of rapamycin (mTOR) (24–26). Subsequent to antigen receptor ligation in lymphocytes, CARD11 is phosphorylated to facilitate BCL10 and MALT1 binding to form the CARD11–BCL10–MALT1 (CBM) complex, which further nucleates the dynamic signalosomes that activate inhibitor of κB kinase (IKK) and culminate in NF-κB translocation into the nucleus (27–30) to activate the canonical NF-κB pathway. The NF-κB family of transcription factors is critical for the induction of genes involved in cell survival, proliferation, and immune effector functions (31). GOF mutations in CARD11 render the protein in an open, active state irrespective of antigen receptor engagement, resulting in constitutive NF-κB activation (31–33).

To date, 16 different BENTA patients have been identified and definitively diagnosed, with five distinct *CARD11* mutations. Polyclonal B cell lymphocytosis in early childhood is a hallmark of BENTA disease, often accompanied by splenomegaly and lymphadenopathy (23, 34–40). Immunologic phenotyping reveals the remarkable accumulation of both $CD10^+CD24^{hi}CD38^{hi}$ transitional and IgM^+IgD^+ mature naïve polyclonal B cells, even though T cell numbers frequently fall within the normal range (**Table 1**). Many BENTA patients also present with several signs of primary immunodeficiency despite the absence of any autoimmune disease symptoms. Recurrent ear and sinopulmonary infections are common in all patients, with other opportunistic viral infections such as molluscum contagiosum, BK virus, and EBV observed in some patients. In most patients, inadequate antibody responses against T-cell independent pneumococcal and meningococcal polysaccharide-based vaccines are noted. Some patients also show poor responses to T-cell-dependent vaccines such as Varicella Zoster virus and measles. Poor humoral immune responses in these patients are also reflected in very low frequencies of circulating class-switched and memory B cells, as well as low levels of IgM and IgA in the serum. Impaired humoral immunity in BENTA is evidenced by intrinsic defects in plasma cell differentiation and antibody secretion upon stimulation of naïve patient B cells *in vitro*, despite normal proliferation and enhanced survival (41). The hyporesponsiveness of BENTA patient T cells to *in vitro* stimulation, including poor proliferation and reduced IL-2 secretion, may also contribute to defective class-switched Ab responses (23, 35).

Eight out of 16 patients are seropositive for EBV (**Table 1**). While EBV viral load is generally undetectable in healthy carriers, almost all BENTA patients (7/8) exposed to EBV are demonstrably viremic as measured by their DNA copy number (**Table 1**). However, EBV viral loads in BENTA patients are not nearly as high as seen in chronic active EBV (CA-EBV) patients and other PIDs (46). These data suggest that EBV-specific immunity is impaired in BENTA patients, but pales in comparison to other PIDs such as X-linked lymphoproliferative syndrome (XLP) or MAGT1/CD27/CD70/ITK or Coronin1A deficiency diseases featuring exquisite susceptibility to severe EBV infection and disease (5, 20–22). In the next section, we speculate on why gain-of-function CARD11 mutations might confer susceptibility to moderate EBV viremia in BENTA disease.

MECHANISMS UNDERLYING BENTA SUSCEPTIBILITY TO EBV

Too Many B Cells, Too Few T/NK Cells?

The consequences of distorted antigen receptor signaling in the presence of GOF CARD11 mutations reverberate throughout

TABLE 1 | Phenotypic analysis of BENTA patients.

	Age[b]	EBV VCA-IgG	EBV LOAD	CD8$^+$ T		CD4/CD8 ratio	NK		CD3$^+$ NKT		CD19$^+$ B	
			Log10 IU/ml	%	#/μl	Ratio	%	#/μl	%	#/μl	%	#/μl
Healthy control	**>18 years**	Neg	UD	11.2–34.8	178–853	1.17–5.17	6.2–34.6	126–729	2.2–12.4	29–299	3–19	59–329
ref ranges	**6–18 years**	Neg	UD	18–35	330–1,100	1.34–1.72	3–22	70–480	0.49–15[c]	12–350[c]	6–27	110–860
				BENTA patients								
G123S												
P4	**11**	**Pos**	**3.35**	**19**	**868**	**0.51**	**4.9**	**224**	**2.7**	**123**	**62.2**	**2,843**
P7	**43**	**Pos**	**UD**	**2**	**300**	**5**	**ND**	**ND**	**ND**	**ND**	**52**	**7,900**
P14	**20**	**Pos**	**4.6**	**3.3**	**200**	**1.5**	**ND**	**ND**	**Low**	**Low**	**83**	**5,000**
G123D												
P6	**15**	**Pos**	**2.07**[a]	**3.3**	**2,853**	**0.64**	**3.5**	**3,026**	**1.6**	**1,383**	**89.4**	**77,286**
H234L Δ235-8												
P10	80	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
P11	**57**	**Pos**	**3.13**	**31.7**	**970**	**1.11**	**8.9**	**272**	**10**	**306**	**20**	**612**
P12	**32**	**Pos**	**4.45**	**16.3**	**535**	**2.48**	**6.5**	**213**	**6**	**197**	**32.5**	**1,066**
P13	6	ND	Neg	13.7	507	2.55	4.1	152	3.6	133	43.3	1,602
C49Y												
P5	21	ND	Neg	10.7	322	1.8	2	60	2.2	66	66.4	1,938
P8	53	ND	Neg	8	152	3.37	10	190	ND	ND	48	912
P9	20	ND	Neg	11	418	2.6	6	228	ND	ND	50	1,900
P15	**43**	**Pos**	**2.07**	**14.6**	**298**	**1.28**	**4.7**	**96**	**1.6**	**33**	**56**	**1,142**
P16	**16**	**Pos**	**3.13**	**20.7**	**271**	**2.12**	**3.8**	**50**	**2.9**	**38**	**21.3**	**279**
E134G												
P1	55	Neg	Neg	34.1	1,449	1.09	5.6	238	15.5	659	18.9	803
P2	13	Neg	Neg	10.2	585	1.67	4.1	235	1.6	92	65.4	3,754
P3	11	Neg	Neg	10.6	409	1.99	3.6	139	2.1	81	61.9	2,389

Values in blue and red color indicate lower and higher range, respectively, compared with adult (>18 years) or pediatric (6–18 years) healthy control range (42, 43) as specified in the subheader column. Adult ranges are derived from the NIH Clinical Center (Department of Laboratory Medicine). EBV seropositive carriers are in bold. Several patients have been described in published reports, including P1–P4 (23, 44), P6 (35), P7 (38), P5, P8, and P9 (36), P14 (37), and P16 (36). Other patients (P10–P13, P15) have been evaluated at the NIH Clinical Center but have not been published to date.

ND, not determined; UD, undetectable; Pos, positive; Neg, negative; EBV, Epstein–Barr virus; NK, natural killer; BENTA, B-cell expansion with NF-κB and T-cell anergy.

[a]*Measurement taken while P6 was 14 years old, but for reasons unknown his EBV load was undetectable at age 15.*

[b]*Patient's age at the time of measurement.*

[c]*Measurement range for 5- to 16-year-old healthy controls (45).*

the lymphoid lineage in BENTA patients. Indeed, the size and makeup of lymphocyte compartments may influence EBV status in certain patients. Most notably, constitutive, canonical NF-κB activity induced by GOF CARD11 signaling in B cells drives excessive B cell accumulation in BENTA patients and may predispose them to malignant transformation as additional mutations are acquired over time. In fact, two patients in our cohort developed B-cell tumors in adulthood (P1 and P11), although neither was associated with EBV infection. Transgenic expression of a constitutively active form of IKKβ (caIKKβ) promotes the survival of mature murine B cells *in vivo*, though it is not sufficient to induce lymphomagenesis (47). Indeed, NF-κB-induced tumor suppressor genes such as A20 and IκB provide important negative feedback on NF-κB signaling, which must be overcome to promote lymphomagenesis (48). This negative feedback remains intact in primary BENTA B cells and may explain why only a fraction of BENTA B cells exhibit p65 nuclear localization at any given time (23).

Epstein–Barr virus itself is not likely a contributing factor for B-cell lymphocytosis in BENTA, as EBV-negative patients also have high B cell numbers. Although NF-κB actively represses lytic infection (49), the proliferation of latently infected EBV$^+$ B cells relies on NF-κB signaling through viral proteins such as LMP1 and LMP2A, which mimic CD40 and BCR signaling, respectively (5). Hence, constitutive NF-κB activity in BENTA patient B cells could better enable EBV to expand the pool of latently infected B cells. Perhaps this could manifest in increased viral reactivation and viremia as CARD11-dependent NF-κB activity oscillates in infected BENTA B cells. Regardless, the presence of EBV may increase the risk of B cell malignancy later in life, simply given the increased size of target B cell compartment. An expanded pool of naïve B cells may simply support an increased level of lytic infection at any given time in EBV-infected BENTA patients, contributing to consistently higher viral loads. In support of this notion, there is an unconfirmed case of EBV-driven Hodgkin lymphoma in the maternal grandfather of patient P16.

Could relative reductions in the T and NK cell compartments also compromise immunity to EBV? In addition to the critical role served by CD8$^+$ T cells, CD4$^+$ T cells, NK cells, and NKT cells are also implicated in clearing EBV infection (16, 17, 19). As shown in **Table 1**, the absolute number of CD8$^+$ T cells and the ratio of CD4$^+$/CD8$^+$ T cells are within normal range in most patients reported thus far. Nevertheless, the low number of NK/NKT cells observed in certain patients (e.g., P14, P15, and P16) could contribute to persistent EBV viremia in those individuals. Patient P5 also has a lower percentage and absolute number of NK cells,

presenting a potential culprit for her frequent susceptibility to other viral infections.

Impaired T/NK Cell Function

A more likely explanation for uncontrolled EBV infection concerns impaired T cell function described in BENTA disease. *In vitro*, we observed poor proliferation and reduced IL-2 secretion from BENTA patient T cells stimulated with anti-CD3/anti-CD28 antibodies. This "anergic" response correlated with defects in TCR-mediated MAPK signaling and Ca^{++} flux (23, 35). Although the biochemical mechanisms remain nebulous, these defects are almost certainly linked to constitutive canonical NF-κB activation induced by GOF CARD11 signaling. Indeed, Krishna et al showed that restricting expression of constitutively active IKKβ (caIKKβ) to murine T cells also rendered them hyporesponsive to TCR/CD28 stimulation, marked by proximal TCR signaling defects and attenuated responses to bacterial infection (50). The authors connected some of these defects to enhanced expression of the transcriptional repressor Blimp-1, which has been shown to promote T cell exhaustion. Although we have not measured Blimp-1 in BENTA T cells, we recently characterized a profound, intrinsic defect in patient B cell differentiation linked to failed induction of Blimp-1 (41). Clearly much more work is required to understand how elevated NF-κB activation perturbs seemingly independent pathways downstream of TCR signaling. Regardless, it is tempting to speculate that any flaw in PLCγ1-mediated Ca^{++} flux underlies poor T cell-dependent control of EBV, as observed in more dramatic fashion in both ITK and MAGT1 deficiency (51–55).

Inactivation of CARD11 has also been shown to inhibit NK cell development and function (56). Although it is not clear how GOF CARD11 signaling may affect NK cells in BENTA, poor IL-2 secretion by anergic T cells upon stimulation could certainly weaken the NK cell response to viral infection. As mentioned earlier, some patients also display lower frequencies of NK and NKT cells, although EBV viremia is observed in several patients with normal NK/NKT counts. Thus, the increased susceptibility of BENTA patients to EBV could be linked to both excessive polyclonal B cell lymphocytosis and hyporesponsive T cells/NK cells that help in combating the viral infection.

Dysregulation of Key Receptor–Ligand Signals Required for EBV Control

The removal of latently infected B cells by cytotoxic T and NK cells requires several receptor–ligand interactions for recognition, cell–cell conjugation, and cytolysis, some of which may be weakened in BENTA patients (**Figure 1**). For example, cognate engagement of EBV-infected B cells by T cells requires

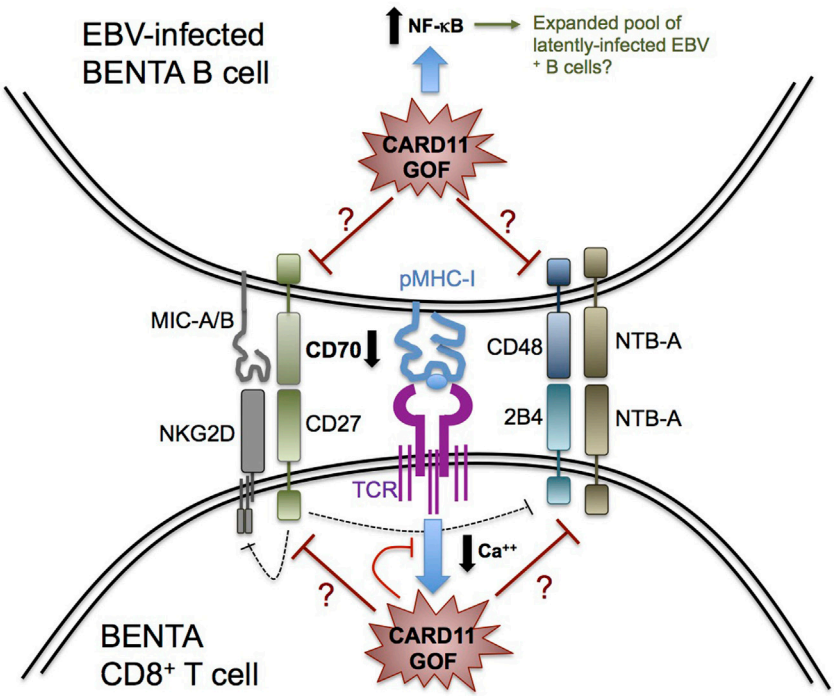


FIGURE 1 | Possible determinants of impaired Epstein–Barr virus (EBV) control in *B*-cell *e*xpansion with *N*F-κB and *T*-cell *a*nergy (BENTA) disease. Schematic diagram summarizing key receptor–ligand interactions that govern $CD8^+$ T cell recognition and killing of EBV-infected B cells, based on our knowledge of primary immune deficiencies featuring enhanced susceptibility to EBV-driven disease. CARD11 GOF signaling could perturb several molecular signals required for optimal cytolysis of EBV-infected B cells, including signaling lymphocyte activation molecule receptors (2B4, NTB-A), NKG2D, and CD27. For example, decreased CD70 expression on BENTA B cells could impair CD27 signaling and contribute to reduced NKG2D or 2B4 expression on BENTA T cells. Alternatively, attenuated TCR signaling (e.g., reduced Ca^{++} flux) likely contributes to BENTA patient T cell hyporesponsiveness, which could disrupt generation of $CD8^+$ effector T cells with optimal cytotoxic function. Finally, elevated NF-κB signaling in B cells could accelerate the expansion of latently infected EBV^+ B cells, contributing to detectable viremia as the virus continuously reactivates.

SAP-dependent signaling through two receptors belonging to the SLAM family: 2B4 and NTB-A (57–60). Whereas most SLAM receptors participate in homotypic interactions in *trans*, 2B4 recognizes a distinct ligand on target cells known as CD48. The expression of both NTB-A and CD48 is dramatically upregulated on the B cell surface upon EBV infection, which promotes T cell and NK cell activation during asymptomatic infection and acute infection, respectively (5, 61). Recruitment of SAP, a small SH2 adaptor protein, to the cytoplasmic tails of NTB-A and 2B4 upon ligand binding is required for conveying downstream signals that ensure strong T:B cell conjugation and B cell killing (62). In this regard, SAP deficiency in XLP-1 patients makes them exquisitely susceptible to severe, uncontrolled EBV infection due to debilitated 2B4 and NTB-A signaling, presenting as FIM/HLH (63, 64). Based on our published RNA-Seq data, BENTA B cells activated *in vitro* with polyclonal stimuli display normal expression of CD48 and NTB-A compared with healthy human donors (41). Whether perturbed 2B4 and/or NTB-A signaling in BENTA patient T cells may influence EBV predisposition remains unclear, but warrants further investigation.

Upon activation, the C-type lectin-like receptor NKG2D is also expressed on NK cells and $CD8^+$ T cells and plays a major role in cytotoxic elimination of transformed and virally infected cells (65). The importance of NKG2D in EBV immunity was recently revealed by the discovery of X-linked immunodeficiency with magnesium defect, EBV infection, and neoplasia (XMEN) disease, caused by deficiency of the magnesium transporter MAGT1. Although NK cell and $CD8^+$ T cell numbers are normal in XMEN patients, reduced intracellular Mg^{++} abrogates NKG2D receptor expression on activated NK cells and $CD8^+$ T cells, which compromises cytolytic responses against EBV^+ B cells (51, 66–68). Similarly, GOF CARD11 signaling may diminish NKG2D expression on BENTA NK cells or $CD8^+$ T cells, perhaps linked to a Ca^{++} flux defect similar to that noted in XMEN T cells (54). On the other hand, there is no evidence for transcriptional upregulation of NKG2D receptor ligands MICA, MICB, and ULBPs on activated BENTA B cells compared with healthy control B cells. Simultaneous engagement of SAP-dependent 2B4 and SAP-independent NKG2D signaling is indispensable for $CD8^+$ T cell-mediated killing of EBV-infected B cells (69), explaining why neither receptor (NKG2D or 2B4) can compensate for the absence of the other to maintain normal CTL activity in XMEN and XLP-1 patients. If constitutive CARD11 signaling indirectly impedes NKG2D or 2B4 signaling in BENTA $CD8^+$ T cells/NK cells, this could jeopardize their ability to completely control EBV infection.

CD27, a costimulatory molecule belonging to the tumor necrosis factor receptor superfamily, is constitutively expressed on memory B cells and most T cells. CD27 engagement in B cells is known to play a key role in B cell activation and immunoglobulin synthesis (70). Our recent *in vitro* studies with BENTA B cells revealed an intrinsic defect in plasma cell differentiation and antibody production that correlated with poor induction of several genes related to plasma cell commitment, including CD27 (41), although CD27 expression is readily detected on patient T cells (data not shown). CD27 interacts with the ligand CD70, expressed transiently on activated B cells, T cells, and dendritic cells. EBV infection upregulates CD70 expression to greater levels on B cells (20). Recently described human patients with CD27 or CD70 deficiency present with similar disease phenotypes, including hypogammaglobulinemia, reduced memory B cells, increased viral infection, and EBV-induced lymphoproliferation and lymphoma. Heightened susceptibility to EBV-driven disease in these patients, despite normal numbers of T and NK cells, highlights a critical, non-redundant role for CD27–CD70 interactions in driving Ab responses and ensuring optimal cellular control of EBV (44, 71–74). Intriguingly, we recently discovered a significant reduction in CD70 expression on activated BENTA B cells *in vitro* compared with healthy control B cells (data not shown). Thereby, an impaired CD27–CD70 signaling axis in BENTA could significantly contribute to both specific Ab deficiency and impaired priming and function of EBV-specific $CD8^+$ T cells. The latter could also be related to decreased NKG2D and 2B4 expression on memory $CD8^+$ T cells, similar to CD70-deficient patients (44). Further exploration of a potential CD27-CD70 signaling deficit in BENTA patients is therefore warranted to elucidate a plausible mechanism to explain the inability of BENTA T and NK cells to fully contain EBV.

CLINICAL MANAGEMENT OF EBV IN BENTA PATIENTS

Assuming B cell lymphocytosis may predispose BENTA patients to greater risk of B cell malignancy later in life, patients are monitored closely for any evidence of B cell clonal outgrowth, using flow cytometry and Ig heavy chain rearrangement analysis. EBV viral load is also measured regularly, as increases in detectable viremia may reflect further debilitation of $CD8^+$ T cell and NK cell function and could theoretically contribute to B cell transformation. However, viral loads in most EBV^+ BENTA patients remain comparatively low relative to CA-EBV and other PIDs (46).

To the best of our knowledge, only one patient (P6) was actively treated for EBV-related complications (35). This patient was hospitalized at age 4 with acute EBV infection, featuring profound adenopathy and splenomegaly, as well as immune thrombocytic purpura. Lymph node biopsies revealed substantial polyclonal B cell accumulation in follicular and parafollicular areas, mixed with moderate numbers of $CD8^+$ and $CD4^+$ T cells. At this time, years before the causative *CARD11* mutation was discovered, the patient was treated aggressively with intravenous immunoglobulin, rituximab, corticosteroids, and acyclovir. Symptoms resolved with treatment, and plasma EBV load was rendered undetectable by PCR. $CD4^+/CD8^+$ T cell ratio, which had dropped significantly during acute EBV infection, also recovered once infection was cleared. Following elective splenectomy 3 years later, his circulating B cell, T cell, and NK cell counts increased dramatically. This phenomenon has been observed in other patients following spleen removal (40) and likely reflects the loss of an important secondary lymphoid tissue niche for excess lymphocytes. Nevertheless, B cell counts in this patient remained 5–10 times higher than those noted in other BENTA patients. To control lymphocytosis, P6 was treated with methotrexate for 4 years until his lymphocyte count was reduced below $80 \times 10^3/\mu l$ (35).

This distinctive case provides an illustrative example of successful treatment for acute EBV infection and may represent a blueprint for care if EBV viremia or lymphocytosis increases rapidly in any BENTA patient. Unlike CA-EBV patients, BENTA patients should not require more radical clinical interventions, such as hematopoietic stem cell transplantation or administration of autologous cytotoxic T cells to combat EBV infection (46). In the future, pharmacological inhibitors of NF-κB activation may be an attractive therapeutic tool for reducing B cell numbers in BENTA patients but must be approached with caution to avoid exacerbating underlying T/NK cell immunodeficiency. Inhibitors of MALT1 protease, which dampen canonical NF-κB activity without completely blocking it, may be a more attractive option and have recently yielded promising results for treatment of B cell lymphoma and autoimmune disease (75–78). Future clinical management will ultimately be guided by more basic research into possible aforementioned mechanisms that might explain impaired CTL and NK cell function and compromised EBV control in BENTA disease.

CONCLUSION

Although the current cohort of patients remains small, impaired control of EBV infection has emerged as a recurring problem in BENTA disease. In contrast to PIDs involving severe EBV-related complications (e.g., fulminant hepatitis and HLH) and complete deficiency of aforementioned receptors and signaling proteins required for EBV immunity, lower viremia in BENTA patients likely reflects attenuation, but not complete abrogation, of T and NK cell functions (**Figure 1**). Further research is required to connect CARD11 GOF signaling mechanistically to these moderate functional defects, which may involve aberrant signaling through NF-κB as well as other downstream signaling nodes, including JNK and mTORC1. Indeed, severe atopic disease observed in patients carrying CARD11 loss-of-function mutations can be attributed to defects in both NF-κB and mTORC1 activation in T cells (79), although none of these patients have presented with significant EBV infections. Continued identification and careful characterization of additional patients harboring novel CARD11 variants should yield further insights into how CARD11 signaling ultimately governs the immune response against EBV.

AUTHOR CONTRIBUTIONS

SA wrote the manuscript. PA assembled data for **Table 1**. HS edited the manuscript and provided clinical perspective. AS supervised the project and edited the manuscript.

ACKNOWLEDGMENTS

The authors thank all patients and their families for participating in research described in this review. They thank Dr. Thomas Fleisher (NIH Clinical Center) for supporting diagnostic flow cytometry on BENTA patients seen at NIH. They also thank all of our collaborating physicians for referring patients and providing helpful information, including Drs. Marylin Desjardins and Bruce Mazer at McGill University Health Centre and Dr. Ian Morison at the University of Otago, New Zealand.

FUNDING

This work was supported with funding from the National Institutes of Health (1R21AI109187 to AS) and the Intramural Research Program of the NIH, National Institute of Allergy and Infectious Diseases. This project has also been funded in whole or in part with federal funds from the National Cancer Institute, National Institutes of Health, under Contract No. HHSN261200800001E. The content of this publication does not necessarily reflect the views or policies of the Department of Health and Human Services, nor does mention of trade names, commercial products, or organizations imply endorsement by the U.S. Government.

REFERENCES

1. Cohen JI. Epstein-Barr virus infection. *N Engl J Med* (2000) 343(7):481–92. doi:10.1056/NEJM200008173430707
2. Cohen JI. Epstein-Barr virus vaccines. *Clin Transl Immunol* (2015) 4(1):e32. doi:10.1038/cti.2014.27
3. Hislop AD, Taylor GS, Sauce D, Rickinson AB. Cellular responses to viral infection in humans: lessons from Epstein-Barr virus. *Annu Rev Immunol* (2007) 25:587–617. doi:10.1146/annurev.immunol.25.022106.141553
4. Taylor GS, Long HM, Brooks JM, Rickinson AB, Hislop AD. The immunology of Epstein-Barr virus-induced disease. *Annu Rev Immunol* (2015) 33:787–821. doi:10.1146/annurev-immunol-032414-112326
5. Tangye SG, Palendira U. Human immunity against EBV-lessons from the clinic. *J Exp Med* (2017) 214(2):269–83. doi:10.1084/jem.20161846
6. Young LS, Rickinson AB. Epstein-Barr virus: 40 years on. *Nat Rev Cancer* (2004) 4(10):757–68. doi:10.1038/nrc1452
7. Chen MR. Epstein-Barr virus, the immune system, and associated diseases. *Front Microbiol* (2011) 2:5. doi:10.3389/fmicb.2011.00005
8. Odumade OA, Hogquist KA, Balfour HH Jr. Progress and problems in understanding and managing primary Epstein-Barr virus infections. *Clin Microbiol Rev* (2011) 24(1):193–209. doi:10.1128/CMR.00044-10
9. Ohga S, Nomura A, Takada H, Hara T. Immunological aspects of Epstein-Barr virus infection. *Crit Rev Oncol Hematol* (2002) 44(3):203–15. doi:10.1016/S1040-8428(02)00112-9
10. Azzi T, Lünemann A, Murer A, Ueda S, Béziat V, Malmberg KJ, et al. Role for early-differentiated natural killer cells in infectious mononucleosis. *Blood* (2014) 124(16):2533–43. doi:10.1182/blood-2014-01-553024
11. Thorley-Lawson DA. EBV persistence – introducing the virus. *Curr Top Microbiol Immunol* (2015) 390(Pt 1):151–209. doi:10.1007/978-3-319-22822-8_8
12. Kuppers R. B cells under influence: transformation of B cells by Epstein-Barr virus. *Nat Rev Immunol* (2003) 3(10):801–12. doi:10.1038/nri1201
13. Rickinson AB, Callan MF, Annels NE. T-cell memory: lessons from Epstein-Barr virus infection in man. *Philos Trans R Soc Lond B Biol Sci* (2000) 355(1395):391–400. doi:10.1098/rstb.2000.0579
14. Callan MF, Fazou C, Yang H, Rostron T, Poon K, Hatton C, et al. CD8(+) T-cell selection, function, and death in the primary immune response in vivo. *J Clin Invest* (2000) 106(10):1251–61. doi:10.1172/JCI10590
15. Long HM, Haigh TA, Gudgeon NH, Leen AM, Tsang CW, Brooks J, et al. CD4+ T-cell responses to Epstein-Barr virus (EBV) latent-cycle antigens and the recognition of EBV-transformed lymphoblastoid cell lines. *J Virol* (2005) 79(8):4896–907. doi:10.1128/JVI.79.8.4896-4907.2005
16. Amyes E, Hatton C, Montamat-Sicotte D, Gudgeon N, Rickinson AB, McMichael AJ, et al. Characterization of the CD4+ T cell response to Epstein-Barr virus during primary and persistent infection. *J Exp Med* (2003) 198(6):903–11. doi:10.1084/jem.20022058
17. Chijioke O, Landtwing V, Munz C. NK cell influence on the outcome of primary Epstein-Barr virus infection. *Front Immunol* (2016) 7:323. doi:10.3389/fimmu.2016.00323

18. Strowig T, Brilot F, Arrey F, Bougras G, Thomas D, Muller WA, et al. Tonsilar NK cells restrict B cell transformation by the Epstein-Barr virus via IFN-gamma. *PLoS Pathog* (2008) 4(2):e27. doi:10.1371/journal.ppat.0040027
19. Chung BK, Tsai K, Allan LL, Zheng DJ, Nie JC, Biggs CM, et al. Innate immune control of EBV-infected B cells by invariant natural killer T cells. *Blood* (2013) 122(15):2600–8. doi:10.1182/blood-2013-01-480665
20. Palendira U, Rickinson AB. Primary immunodeficiencies and the control of Epstein-Barr virus infection. *Ann N Y Acad Sci* (2015) 1356:22–44. doi:10.1111/nyas.12937
21. Parvaneh N, Filipovich AH, Borkhardt A. Primary immunodeficiencies predisposed to Epstein-Barr virus-driven haematological diseases. *Br J Haematol* (2013) 162(5):573–86. doi:10.1111/bjh.12422
22. Yang X, Nishida N, Zhao X, Kanegane H. Advances in understanding the pathogenesis of Epstein-Barr virus-associated lymphoproliferative disorders. *Iran J Allergy Asthma Immunol* (2015) 14(5):462–71.
23. Snow AL, Xiao W, Stinson JR, Lu W, Chaigne-Delalande B, Zheng L, et al. Congenital B cell lymphocytosis explained by novel germline CARD11 mutations. *J Exp Med* (2012) 209(12):2247–61. doi:10.1084/jem.20120831
24. Blonska M, Lin X. CARMA1-mediated NF-kappaB and JNK activation in lymphocytes. *Immunol Rev* (2009) 228(1):199–211. doi:10.1111/j.1600-065X.2008.00749.x
25. Hamilton KS, Phong B, Corey C, Cheng J, Gorentla B, Zhong X, et al. T cell receptor-dependent activation of mTOR signaling in T cells is mediated by Carma1 and MALT1, but not Bcl10. *Sci Signal* (2014) 7(329):ra55. doi:10.1126/scisignal.2005169
26. Thome M, Charton JE, Pelzer C, Hailfinger S. Antigen receptor signaling to NF-kappaB via CARMA1, BCL10, and MALT1. *Cold Spring Harb Perspect Biol* (2010) 2(9):a003004. doi:10.1101/cshperspect.a003004
27. McCully RR, Pomerantz JL. The protein kinase C-responsive inhibitory domain of CARD11 functions in NF-kappaB activation to regulate the association of multiple signaling cofactors that differentially depend on Bcl10 and MALT1 for association. *Mol Cell Biol* (2008) 28(18):5668–86. doi:10.1128/MCB.00418-08
28. Paul S, Traver MK, Kashyap AK, Washington MA, Latoche JR, Schaefer BC, et al. T cell receptor signals to NF-kappaB are transmitted by a cytosolic p62-Bcl10-Malt1-IKK signalosome. *Sci Signal* (2014) 7(325):ra45. doi:10.1126/scisignal.2004882
29. Qiao Q, Yang C, Zheng C, Fontán L, David L, Yu X, et al. Structural architecture of the CARMA1/Bcl10/MALT1 signalosome: nucleation-induced filamentous assembly. *Mol Cell* (2013) 51(6):766–79. doi:10.1016/j.molcel.2013.08.032
30. Sommer K, Guo B, Pomerantz JL, Bandaranayake AD, Moreno-García ME, Ovechkina YL, et al. Phosphorylation of the CARMA1 linker controls NF-kappaB activation. *Immunity* (2005) 23(6):561–74. doi:10.1016/j.immuni.2005.09.014
31. Gerondakis S, Siebenlist U. Roles of the NF-kappaB pathway in lymphocyte development and function. *Cold Spring Harb Perspect Biol* (2010) 2(5):a000182. doi:10.1101/cshperspect.a000182
32. Jattani RP, Tritapoe JM, Pomerantz JL. Cooperative control of caspase recruitment domain-containing protein 11 (CARD11) signaling by an unusual array of redundant repressive elements. *J Biol Chem* (2016) 291(16):8324–36. doi:10.1074/jbc.M115.683714
33. Jattani RP, Tritapoe JM, Pomerantz JL. Intramolecular interactions and regulation of cofactor binding by the four repressive elements in the caspase recruitment domain-containing protein 11 (CARD11) inhibitory domain. *J Biol Chem* (2016) 291(16):8338–48. doi:10.1074/jbc.M116.717322
34. Arjunaraja S, Snow AL. Gain-of-function mutations and immunodeficiency: at a loss for proper tuning of lymphocyte signaling. *Curr Opin Allergy Clin Immunol* (2015) 15(6):533–8. doi:10.1097/ACI.0000000000000217
35. Brohl AS, Stinson JR, Su HC, Badgett T, Jennings CD, Sukumar G, et al. Germline CARD11 mutation in a patient with severe congenital B cell lymphocytosis. *J Clin Immunol* (2014) 5(1):32–46. doi:10.1007/s10875-014-0106-4
36. Buchbinder D, Sassoon A. A case of bad Carma! *Blood* (2017) 129(12):1737. doi:0.1182/blood-2016-12-756007
37. Buchbinder D, Stinson JR, Nugent DJ, Heurtier L, Suarez F, Sukumar G, et al. Mild B-cell lymphocytosis in patients with a CARD11 C49Y mutation. *J Allergy Clin Immunol* (2015) 136(3):819–21.e1. doi:10.1016/j.jaci.2015.03.008
38. Casey TP. Chronic lymphocytic leukaemia in a child presenting at the age of two years and eight months. *Australas Ann Med* (1968) 17(1):70–4. doi:10.1111/imj.1968.17.1.70
39. Outinen T, Syrjänen J, Rounioja S, Saarela J, Kaustio M, Helminen M. Constant B cell lymphocytosis since early age in a patient with CARD11 mutation: a 20-year follow-up. *Clin Immunol* (2016) 165:19–20. doi:10.1016/j.clim.2016.02.002
40. Darte JM, McClure PD, Saunders EF, Weber JL, Donohue WL, et al. Congenital lymphoid hyperplasia with persistent hyperlymphocytosis. *J Exp Med* (1971) 284(8):431–2.
41. Arjunaraja S, Nosé BD, Sukumar G, Lott NM, Dalgard CL, Snow AL. Intrinsic plasma cell differentiation defects in B cell expansion with NF-kappaB and t cell anergy patient B cells. *Front Immunol* (2017) 8:913. doi:10.3389/fimmu.2017.00913
42. Huck K, Feyen O, Ghosh S, Beltz K, Bellert S, Niehues T. Memory B-cells in healthy and antibody-deficient children. *Clin Immunol* (2009) 131(1):50–9. doi:10.1016/j.clim.2008.11.008
43. Shearer WT, Rosenblatt HM, Gelman RS, Oyomopito R, Plaeger S, Stiehm ER, et al. Lymphocyte subsets in healthy children from birth through 18 years of age: the Pediatric AIDS Clinical Trials Group P1009 study. *J Allergy Clin Immunol* (2003) 112(5):973–80. doi:10.1016/j.jaci.2003.07.003
44. Abolhassani H, Edwards ES, Ikinciogullari A, Jing H, Borte S, Buggert M, et al. Combined immunodeficiency and Epstein-Barr virus-induced B cell malignancy in humans with inherited CD70 deficiency. *J Exp Med* (2017) 214(1):91–106. doi:10.1084/jem.20160849
45. Schatorjé EJ, Gemen EF, Driessen GJ, Leuvenink J, van Hout RW, de Vries E. Paediatric reference values for the peripheral T cell compartment. *Scand J Immunol* (2012) 75(4):436–44. doi:10.1111/j.1365-3083.2012.02671.x
46. Cohen JI, Jaffe ES, Dale JK, Pittaluga S, Heslop HE, Rooney CM, et al. Characterization and treatment of chronic active Epstein-Barr virus disease: a 28-year experience in the United States. *Blood* (2011) 117(22):5835–49. doi:10.1182/blood-2010-11-316745
47. Sasaki Y, Derudder E, Hobeika E, Pelanda R, Reth M, Rajewsky K, et al. Canonical NF-kappaB activity, dispensable for B cell development, replaces BAFF-receptor signals and promotes B cell proliferation upon activation. *Immunity* (2006) 24(6):729–39. doi:10.1016/j.immuni.2006.04.005
48. Lim KH, Yang Y, Staudt LM. Pathogenetic importance and therapeutic implications of NF-kappaB in lymphoid malignancies. *Immunol Rev* (2012) 246(1):359–78. doi:10.1111/j.1600-065X.2012.01105.x
49. Brown HJ, Song MJ, Deng H, Wu TT, Cheng G, Sun R. NF-kappaB inhibits gammaherpesvirus lytic replication. *J Virol* (2003) 77(15):8532–40. doi:10.1128/JVI.77.15.8532-8540.2003
50. Krishna S, Xie D, Gorentla B, Shin J, Gao J, Zhong X-P. Chronic activation of the kinase IKKbeta impairs T cell function and survival. *J Immunol* (2012) 189(3):1209–19. doi:10.4049/jimmunol.1102429
51. Brigida I, Chiriaco M, Di Cesare S, Cittaro D, Di Matteo G, Giannelli S, et al. Large deletion of MAGT1 gene in a patient with classic Kaposi sarcoma, CD4 lymphopenia, and EBV infection. *J Clin Immunol* (2017) 37(1):32–5. doi:10.1007/s10875-016-0341-y
52. Ghosh S, Bienemann K, Boztug K, Borkhardt A. Interleukin-2-inducible T-cell kinase (ITK) deficiency – clinical and molecular aspects. *J Clin Immunol* (2014) 34(8):892–9. doi:10.1007/s10875-014-0110-8
53. Linka RM, Risse SL, Bienemann K, Werner M, Linka Y, Krux F, et al. Loss-of-function mutations within the IL-2 inducible kinase ITK in patients with EBV-associated lymphoproliferative diseases. *Leukemia* (2012) 26(5):963–71. doi:10.1038/leu.2011.371
54. Readinger JA, Mueller KL, Venegas AM, Horai R, Schwartzberg PL. Tec kinases regulate T-lymphocyte development and function: new insights into the roles of Itk and Rlk/Txk. *Immunol Rev* (2009) 228(1):93–114. doi:10.1111/j.1600-065X.2008.00757.x
55. Li FY, Chaigne-Delalande B, Kanellopoulou C, Davis JC, Matthews HF, Douek DC, et al. Second messenger role for Mg2+ revealed by human T-cell immunodeficiency. *Nature* (2011) 475(7357):471–6. doi:10.1038/nature10246
56. Hara H, Wada T, Bakal C, Kozieradzki I, Suzuki S, Suzuki N, et al. The MAGUK family protein CARD11 is essential for lymphocyte activation. *Immunity* (2003) 18(6):763–75. doi:10.1016/S1074-7613(03)00148-1
57. Bottino C, Falco M, Parolini S, Marcenaro E, Augugliaro R, Sivori S, et al. NTB-A [correction of GNTB-A], a novel SH2D1A-associated surface molecule contributing to the inability of natural killer cells to kill Epstein-Barr

virus-infected B cells in X-linked lymphoproliferative disease. *J Exp Med* (2001) 194(3):235–46. doi:10.1084/jem.194.3.235
58. Nakajima H, Colonna M. 2B4: an NK cell activating receptor with unique specificity and signal transduction mechanism. *Hum Immunol* (2000) 61(1):39–43. doi:10.1016/S0198-8859(99)00170-6
59. Parolini S, Bottino C, Falco M, Augugliaro R, Giliani S, Franceschini R, et al. X-linked lymphoproliferative disease. 2B4 molecules displaying inhibitory rather than activating function are responsible for the inability of natural killer cells to kill Epstein-Barr virus-infected cells. *J Exp Med* (2000) 192(3):337–46. doi:10.1084/jem.192.3.337
60. Tangye SG, Cherwinski H, Lanier LL, Phillips JH. 2B4-mediated activation of human natural killer cells. *Mol Immunol* (2000) 37(9):493–501. doi:10.1016/S0161-5890(00)00076-6
61. Thorley-Lawson DA, Schooley RT, Bhan AK, Nadler LM. Epstein-Barr virus super induces a new human B cell differentiation antigen (B-LAST 1) expressed on transformed lymphoblasts. *Cell* (1982) 30(2):415–25. doi:10.1016/0092-8674(82)90239-2
62. Dupré L, Andolfi G, Tangye SG, Clementi R, Locatelli F, Aricò M, et al. SAP controls the cytolytic activity of CD8+ T cells against EBV-infected cells. *Blood* (2005) 105(11):4383–9. doi:10.1182/blood-2004-08-3269
63. Filipovich AH, Zhang K, Snow AL, Marsh RA. X-linked lymphoproliferative syndromes: brothers or distant cousins? *Blood* (2010) 116(18):3398–408. doi:10.1182/blood-2010-03-275909
64. Nakajima H, Cella M, Bouchon A, Grierson HL, Lewis J, Duckett CS, et al. Patients with X-linked lymphoproliferative disease have a defect in 2B4 receptor-mediated NK cell cytotoxicity. *Eur J Immunol* (2000) 30(11):3309–18. doi:10.1002/1521-4141(200011)30:11<3309::AID-IMMU3309>3.0.CO;2-3
65. Jamieson AM, Diefenbach A, McMahon CW, Xiong N, Carlyle JR, Raulet DH. The role of the NKG2D immunoreceptor in immune cell activation and natural killing. *Immunity* (2002) 17(1):19–29. doi:10.1016/S1074-7613(02)00333-3
66. Chaigne-Delalande B, Li FY, O'Connor GM, Lukacs MJ, Jiang P, Zheng L, et al. Mg2+ regulates cytotoxic functions of NK and CD8 T cells in chronic EBV infection through NKG2D. *Science* (2013) 341(6142):186–91. doi:10.1126/science.1240094
67. Dhalla F, Murray S, Sadler R, Chaigne-Delalande B, Sadaoka T, Soilleux E, et al. Identification of a novel mutation in MAGT1 and progressive multifocal leucoencephalopathy in a 58-year-old man with XMEN disease. *J Clin Immunol* (2015) 35(2):112–8. doi:10.1007/s10875-014-0116-2
68. Patiroglu T, Haluk Akar H, Gilmour K, Unal E, Akif Ozdemir M, Bibi S, et al. A case of XMEN syndrome presented with severe auto-immune disorders mimicking autoimmune lymphoproliferative disease. *Clin Immunol* (2015) 159(1):58–62. doi:10.1016/j.clim.2015.04.015
69. Kwon HJ, Choi GE, Ryu S, Kwon SJ, Kim SC, Booth C, et al. Stepwise phosphorylation of p65 promotes NF-kappaB activation and NK cell responses during target cell recognition. *Nat Commun* (2016) 7:11686. doi:10.1038/ncomms11686
70. Borst J, Hendriks J, Xiao Y. CD27 and CD70 in T cell and B cell activation. *Curr Opin Immunol* (2005) 17(3):275–81. doi:10.1016/j.coi.2005.04.004
71. Alkhairy OK, Perez-Becker R, Driessen GJ, Abolhassani H, van Montfrans J, Borte S, et al. Novel mutations in TNFRSF7/CD27: clinical, immunologic, and genetic characterization of human CD27 deficiency. *J Allergy Clin Immunol* (2015) 136(3):703–12.e10. doi:10.1016/j.jaci.2015.02.022
72. Salzer E, Daschkey S, Choo S, Gombert M, Santos-Valente E, Ginzel S, et al. Combined immunodeficiency with life-threatening EBV-associated lymphoproliferative disorder in patients lacking functional CD27. *Haematologica* (2013) 98(3):473–8. doi:10.3324/haematol.2012.068791
73. van Montfrans JM, Hoepelman AI, Otto S, van Gijn M, van de Corput L, de Weger RA, et al. CD27 deficiency is associated with combined immunodeficiency and persistent symptomatic EBV viremia. *J Allergy Clin Immunol* (2012) 129(3):787.e–93.e. doi:10.1016/j.jaci.2011.11.013
74. Izawa K, Martin E. Inherited CD70 deficiency in humans reveals a critical role for the CD70-CD27 pathway in immunity to Epstein-Barr virus infection. *J Exp Med* (2017) 214(1):73–89. doi:10.1084/jem.20160784
75. Demeyer A, Staal J, Beyaert R. Targeting MALT1 proteolytic activity in immunity, inflammation and disease: good or bad? *Trends Mol Med* (2016) 22(2):135–50. doi:10.1016/j.molmed.2015.12.004
76. Lee CH, Bae SJ, Kim M. Mucosa-associated lymphoid tissue lymphoma translocation 1 as a novel therapeutic target for rheumatoid arthritis. *Sci Rep* (2017) 7(1):11889. doi:10.1038/s41598-017-12349-9
77. Saba NS, Wong DH, Tanios G, Iyer JR, Lobelle-Rich P, Dadashian EL, et al. MALT1 inhibition is efficacious in both naive and ibrutinib-resistant chronic lymphocytic leukemia. *Cancer Res* (2017) 77(24):7038–48. doi:10.1158/0008-5472.CAN-17-2485
78. Bardet M, Unterreiner A, Malinverni C, Lafossas F, Vedrine C, Boesch D, et al. The T-cell fingerprint of MALT1 paracaspase revealed by selective inhibition. *Immunol Cell Biol* (2017) 96(1):81–99. doi:10.1111/imcb.1018
79. Ma CA, Stinson JR, Zhang Y, Abbott JK, Weinreich MA, Hauk PJ, et al. Germline hypomorphic CARD11 mutations in severe atopic disease. *Nat Genet* (2017) 49(8):1192–201. doi:10.1038/ng.3898

Conflict of Interest Statement: The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

The handling editor declared a shared affiliation, although no other collaboration, with the authors PA and HS.

frontiers in Immunology

MINI REVIEW
published: 27 February 2018
doi: 10.3389/fimmu.2018.00368

Epstein–Barr Virus+ Smooth Muscle Tumors as Manifestation of Primary Immunodeficiency Disorders

*Thomas Magg[1], Tilmann Schober[1], Christoph Walz[2], Julia Ley-Zaporozhan[3], Fabio Facchetti[4], Christoph Klein[1] and Fabian Hauck[1]**

[1] Department of Pediatrics, Dr. von Hauner Children's Hospital, University Hospital, LMU Munich, Munich, Germany, [2] Faculty of Medicine, Institute of Pathology, LMU Munich, Munich, Germany, [3] Department of Radiology, University Hospital, LMU Munich, Munich, Germany, [4] Department of Molecular and Translational Medicine, Pathology Unit, University of Brescia School of Medicine, Spedali Civili Brescia, Brescia, Italy

OPEN ACCESS

Edited by:
Isabelle Meyts,
KU Leuven,
Belgium

Reviewed by:
Alexandra Freeman,
National Institutes of Health (NIH),
United States
Flore Rozenberg,
Université Paris Descartes, France
Cindy S. Ma,
Garvan Institute of Medical Research,
Australia

***Correspondence:**
Fabian Hauck
fabian.hauck@
med.uni-muenchen.de

Specialty section:
This article was submitted to
Primary Immunodeficiencies,
a section of the journal
Frontiers in Immunology

Received: 21 November 2017
Accepted: 09 February 2018
Published: 27 February 2018

Citation:
Magg T, Schober T, Walz C,
Ley-Zaporozhan J, Facchetti F,
Klein C and Hauck F (2018) Epstein–
Barr Virus+ Smooth Muscle Tumors
as Manifestation of Primary
Immunodeficiency Disorders.
Front. Immunol. 9:368.
doi: 10.3389/fimmu.2018.00368

Epstein–Barr virus positive (EBV+) smooth muscle tumors (SMTs) constitute a very rare oncological entity. They usually develop in the context of secondary immunodeficiency caused by human immunodeficiency virus infection or immunosuppressive treatment after solid organ transplantation. However, in a small fraction of predominantly pediatric patients, EBV+ SMTs may occur in patients with primary immunodeficiency disorders (PIDs), such as GATA2 and CARMIL2 deficiency. In secondary immunodeficiencies and when the underlying condition can not be cured, the treatment of EBV+ SMTs is based on surgery in combination with antiretroviral and reduced or altered immunosuppressive pharmacotherapy, respectively. Importantly, without definitive reconstitution of cellular immunity, long-term survival is poor. This is particularly relevant for patients with EBV+ SMTs on the basis of PIDs. Recently, allogeneic hematopoietic stem cell transplantation resulted in cure of immunodeficiency and EBV+ SMTs in a GATA2-deficient patient. We propose that in the absence of secondary immunodeficiency disorders patients presenting with EBV+ SMTs should be thoroughly evaluated for PIDs. Allogeneic hematopoietic stem cell transplantation should be taken into consideration, ideally in the setting of a prospective clinical trial.

Keywords: Epstein–Barr virus, smooth muscle tumor, primary immunodeficiency disorder, secondary immunodeficiency disorder, allogeneic hematopoietic stem cell transplantation, CARMIL2, GATA2

INTRODUCTION

Epstein–Barr virus (EBV) is a gamma 1 herpes virus that preferentially infects human epithelial cells of the oropharynx and B cells of the adaptive immune system to establish lifelong latency (1). Rarely, EBV can cause ectopic infections and has been found in NK, T, gastric epithelial, and smooth muscle cells as well (1, 2). In the majority of cases, primary EBV infection is asymptomatic. However, EBV infection can cause lymphoproliferative phenotypes ranging from common infectious mononucleosis to rare hemophagocytic lymphohistiocytosis (3). Additionally, EBV infection presents as chronic active infection and is associated with autoimmune disorders, such as multiple sclerosis (4, 5).

Epstein–Barr virus has an inherent capacity of immortalization and malignant transformation especially of its B cell target (6). In the laboratory, this is used to generate lymphoblastoid cell lines. *In vivo*, this can lead to post transplant lymphoproliferative disorder (PTLD) and malignant lymphoma, such as Hodgkin's lymphoma, Burkitt's lymphoma, and diffuse large B cell lymphoma (7–9).

While the underlying conditions for the more frequent lymphoproliferative phenotypes seem to be heterogeneous and combinatorial, especially the rare and severe phenotypes are associated with secondary immunodeficiency disorders (SIDs) or predisposing genotypes, such as hemizygous *SH2D1A* mutations that cause X-linked lymphoproliferative syndrome (1, 3).

Smooth muscle tumors (SMTs) represent a heterogeneous group of disorders with a broad pathological spectrum ranging from very common and benign uterine leiomyoma to malignant leiomyosarcoma. The latter is characterized by hypercellularity, nuclear atypia, high mitotic rate, and tumor cell necrosis (10). EBV+ SMTs are a distinct subset of SMTs and have often been named leiomyoma or leiomyosarcoma because of their close histological appearance to common SMTs. EBV+ SMTs are very rare and can be encountered at any age in the context of SIDs and in a small fraction of predominantly pediatric patients with primary immunodeficiency disorders (PIDs) (2, 11).

Here, we review the current knowledge on EBV+ SMTs in general and present it as an emerging manifestation of PIDs that might be targeted by allogeneic hematopoietic stem cell transplantation (alloHSCT).

PRESENTATION, PATHOGENESIS, AND TREATMENT OF EBV+ SMTs

Most EBV+ SMTs develop at any age in patients with SIDs due to uncontrolled human immunodeficiency virus infection (HIV EBV+ SMTs) and organ transplantation-associated immunosuppressive treatment (PT EBV+ SMTs) (2, 12). Additionally, they rarely present in pediatric patients with proven or suspected PID (PID EBV+ SMTs) (11). Overall the prevalence of EBV+ SMTs is estimated to be <1–5% for each patient group. Particularly, in the PT EBV+ SMTs group they present as late complications (median 48 months, range 5–348 months) (12, 13). The clinical manifestation of EBV+ SMTs is unspecific and mainly depends on the tumor localization, the tumor size, and the particular organ displacement and/or disruption (12, 13). The majority of EBV+ SMTs is located in the liver, but virtually every other organ can be affected and frequently the lungs, the gastrointestinal tract, the central nervous system, and the adrenal glands are involved (**Figures 1A,B**) (12, 13). Importantly, radiological imaging can not establish the diagnosis of EBV+ SMTs as there are no pathognomonic findings. EBV+ SMTs can occur at single or multiple sites

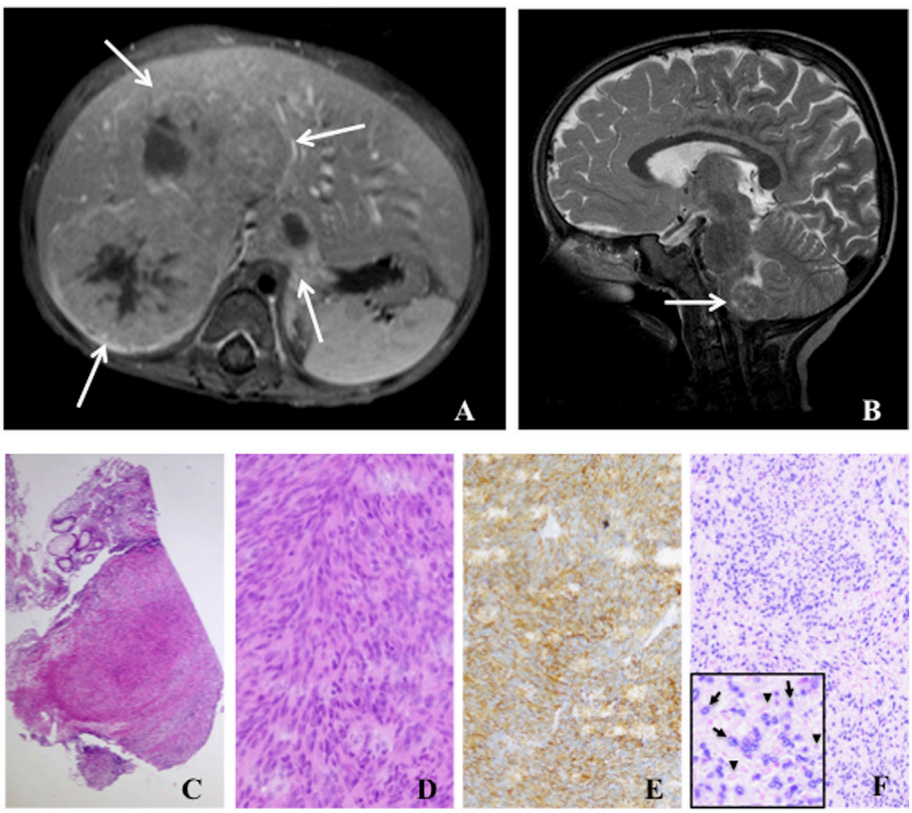


FIGURE 1 | Radiology and histology of Epstein–Barr virus positive (EBV+) smooth muscle tumors. **(A)** Abdominal magnetic resonance image (T1 fat-sat post contrast medium) shows solid liver tumors involving segments I and V–VIII (arrows). **(B)** Cranial magnetic resonance image (T2 sagittal) displays a tumor in the medulla oblongata (arrow). **(C)** Low-power (50×) examination of a colon biopsy shows a prominent nodular cellular proliferation in the mucosa and submucosa **(D)** High-power (400×) magnification displays fascicles of fusiform spindle cells with abundant eosinophilic cytoplasm and elongated or ovoid nuclei without significant atypia or mitoses suggesting a mesenchymal neoplasia of smooth muscle origin **(E)**. Immunohistochemistry for smooth muscle actin (200×) confirms the smooth muscle nature of the tumor **(F)**. EBV association is demonstrated by *in situ* hybridization for EBV-encoded RNA (EBER) in the same lesion (200×). Inlet displays positive cells with EBER in darkly stained basophilic nuclei (arrows) and negative cells with faint eosinophilic nuclei (arrowheads).

synchronously or metachronously, grow *per continuitatem* and do not metastasize (12, 13). They are believed to originate from myogenous venous wall cells and can be of recipient or donor origin in the setting of solid organ transplantation (12, 14, 15). Molecular genetic studies have shown that multiple EBV+ SMTs frequently constitute independent clones rather than metastases of a single tumor (16).

EBV+ SMTs have a spindle-shaped cell morphology with eosinophilic cytoplasm and elongated nuclei, but frequently lack histological atypia, elevated mitotic activity, and tumor necrosis (**Figures 1C,D**). Especially in association with HIV they can present with sarcoma-like histological features and frequently infiltrating lymphocytes and histiocytes can be detected. Expression of smooth muscle differentiation markers, such as smooth muscle actin, caldesmon, vimentin, and desmin, and transcriptional activity of EBV are demonstrated by immunohistochemistry and EBV-encoded small RNA (EBER) *in situ* hybridization (ISH), respectively (**Figures 1E,F**). Immunohistochemistry alone can lead to false negative results (17, 18). Importantly, histopathology findings do neither correlate with tumorbiology nor disease activity, but are mandatory to establish the diagnosis of EBV+ SMTs (11, 12).

In general in EBV+ SMTs, EBV achieves a latency type III-like pattern, i.e., cells are positive for EBV nuclear antigen 2 (EBNA2), EBNA3, and late membrane protein 1 (19–22). The majority of HIV EBV+ SMTs are positive for complement receptor 2 (CR2 or CD21) that is bound by EBV during B cell infection, while a substantial number of PT EBV+ SMTs and all analyzed cases of PID EBV+ SMTs are CD21 negative (21, 23, 24). Thus, the precise EBV entry mechanism into the tumor progenitor cells is unknown. It is possible that several entry routes exist. Also the molecular pathophysiology of tumorigenesis remains poorly understood. The activated mTOR/AKT-pathway seems to be involved and increased v-myc expression has been found (12, 25, 26).

The diagnosis of EBV+ SMTs is suspected in the context of SID and PID and, because there is no pathognomonic radiological morphology, biopsy based histopathology, immunohistochemistry, and EBER ISH are mandatory to formally establish the diagnosis (13).

The treatment of EBV+ SMTs is based on the principle of re-establishing efficient T cell immunity. In patients with HIV infection, appropriate antiretroviral treatment should be given. Patients with iatrogenic immunosuppression following organ transplantation may benefit from reduction of immunosuppressive treatment. It remains a matter of debate whether switching immunosuppression toward a mTOR inhibitor, such as sirolimus might lead to a more favorable outcome (27). Surgery should be performed whenever tumor masses compromise organ functions. Chemotherapy and radiotherapy can be applied but in general neither of these approaches is markedly improving the disease course (13). Prognostic data are derived from retrospective analyses of case records and show a five-year overall survival (OS) of approximately 50% for HIV EBV+ SMTs and PT EBV+ SMTs, while OS of PID EBV+ SMTs tends to be 0% (13). Especially, multiorgan involvement ($n = 33/68$, OS = 48.5%) and intracranial manifestations ($n = 7/68$, OS = 10%) are contributing to the dismal prognosis (13).

PIDs UNDERLYING EBV+ SMTs

The first description of PT EBV+ SMTs dates back to 1970, but it was not until 1995 that the first systematic studies on HIV EBV+ SMTs and PID EBV+ SMTs were published (2, 28, 29). Up to date only very few cases of PID EBV+ SMTs have been reported and a substantial proportion of these patients lack a precise molecular PID diagnosis (**Table 1**) (19–21, 30–37).

The first report by Mierau et al. dates to 1997 and describes a 14-year-old female with primary leiomyosarcoma of the brain in the context of common variable immunodeficiency (CVID) (31). The authors emphasize the need for proper histopathological work-up of unusual tumor entities in immunocompromised patients. In view of a positive family history, the authors conclude that EBV+ SMTs are caused by an inherited rather than acquired disorder (31).

In 1999, Tulbah and colleagues published another case of a child with a genetically undefined congenital immunodeficiency presenting with multifocal EBV+ SMTs located to the thyroid gland, liver, and lung, and stated that they are unaware of comparable cases (36).

The first report of a genetically proven PID, namely ataxia telangiectasia (AT), associated with EBV+ laryngeal leiomyosarcoma and jejunal cellular leiomyoma is published by Reyes et al. in 2002 (20). The authors conclude that EBV+ SMTs are related to the immunosuppressive consequences of AT and that searching for infectious causes is important as SMTs have been reported in AT without subsequent evaluation of underlying EBV infection (20).

In 2003, Monforte-Muñoz et al. published the case of an 8-year-old female with severe combined immunodeficiency (SCID) caused by adenosine deaminase (ADA) deficiency. The patient develops EBV+ SMTs in the gallbladder, spleen, pancreas, intestinal tract, and lung after alloHSCT. Additionally, the patient presents with EBV+ PTLD, pulmonary and gastric adenovirus, and large intestine cryptosporidum infections all of which are indicative of poor immune reconstitution and/or recurrences of the ADA-SCID (32). The authors state that the occurrence of EBV+ SMTs and EBV+ PTLD suggests a common pathogenesis that may have therapeutic and prognostic implications (32).

In 2006, Hatano et al. reported a 6-year-old male with an EBV+ SMT in the right bronchus that leads to atelectasis and abscess in the right upper and middle lobe (19). They find reduced numbers of T cells and impaired T cell proliferation after stimulation with phytohemagglutinin. As the patient has additional recurrent infections, they conclude that he has an undefined cellular immunodeficiency (19). We are currently investigating the precise molecular cause of the suspected PID.

In 2007, Atluri et al. published an *IL2RG* SCID patient who is treated with haploidentical alloHSCT and 8 years thereafter presents with renal and pulmonary EBV+ SMTs in the context of mixed donor T cell chimerism (30). Importantly, after donor lymphocyte infusion the EBV+ SMTs rest stable during a 2-year follow up and the authors conclude that EBV+ SMTs after partial immunoreconstitution may not require surgery or chemotherapy (30).

In 2012, Shaw et al. reported a 12-year-old female with quantitative classic NK cell deficiency presenting with bilateral adrenal

TABLE 1 | Epstein–Barr virus positive (EBV+) smooth muscle tumors (SMTs) in patients with primary immunodeficiency disorders (PIDs).

Publication	No. patient	Reported histology[a]	Reported type of PID (*Gene*)	Preceeding stem cell transplantation	SMT location	EBV viremia	PTLD	Treatment	Outcome
Mierau et al. (31)	1	Leiomyosarcoma	Common variable immunodeficiency	No	Brain	n.a.	No	Surgery	Tumor-free for 18 months
Tulbah et al. (36)	1	Leiomyosarcoma	Congenital T cell immunodeficiency	No	Thyroid, liver and lung	n.a.	No	Unclear	Lost to follow up
Reyes et al. (20)	1	Leiomyosarcoma	Ataxia telangiectasia (*ATM*)	No	Larynx, small bowel	n.a.	No	Surgery	Not reported
Monforte-Muñoz et al. (32)	1	Leiomyomatosis	Severe combined immunodeficiency (SCID) (*ADA*)	Yes	Gall bladder, liver, spleen, pancreas, intestinal tract and lung	n.a.	Yes	Unclear	Unclear
Hatano et al. (19)	1	Leiomyoma	Cellular and complement immunodeficiency	No	Lung	n.a.	No	Surgery	Tumor-free for >2 years
Atluri et al. (30)	1	Leiomyomatosis	SCID (*IL2RG*)	Yes	Lung, bilateral renal	Negative	Yes	Donor lymphocyte infusion	Tumor stable for >2 years
Vinh et al (37)	1	Leiomyosarcoma	GATA2 haploinsufficiency (*GATA2*)	No	Orbit, liver, colon, and uterus	n.a.	No	Surgery and stem cell transplantation	Died of post transplant viral infections
Shaw et al. (35)	1	Smooth muscle tumor	NK cell deficiency	No	Bilateral adrenal	n.a.	No	Surgery	Tumor-free for 26 months
Petrilli et al. (34)	1	Smooth muscle tumor	SCID (*ZAP70*)	No	Bilateral adrenal	n.a.	Yes	Surgery and stem cell transplantation	Died of EBV+ multifocal diffuse large B cell lymphoma five years after unsuccessful allogeneic hematopoietic stem cell transplantation (alloHSCT)
Parta et al. (33)	1	Smooth muscle tumor	GATA2 haploinsufficiency (*GATA2*)	No	Liver, vertebral	Positive	No	Stem cell transplantation	Cured with a three year follow up after alloHSCT
Schober et al. (21)	4	Smooth muscle tumor	CID (*CARMIL2*)	No	Gut, liver, lung, spleen, kidney, brain	Positive in 1/4	No	Surgery and chemotherapy	Died of EBV+ SMT-induced multi-organ failure

[a]*EBV+ SMTs were originally divided into EBV-associated leiomyomas and leiomyosarcomas, but current classification holds them all collectively as EBV+ SMTs.*

EBV+ SMTs that are treated by successful surgery with an event free follow up of 26 months (35). They perform intensive immunological analysis and document a marked deficiency of absolute numbers and cytotoxicity of $CD3^-CD16^+CD56^+$ NK cells at four separate timepoints over 18 months. The authors do not report an underlying genetic condition, but as NK cells are known to participate in protective EBV immunity, they speculate that the severe NK cell deficiency contributes to the development of EBV+ SMTs (35).

In 2014, Petrilli et al. reported a 7-year-old female with bilateral adrenal EBV+ SMTs and as the patient has recurrent respiratory tract infections, including tuberculosis, reduced immunoglobulins, and impaired T cell proliferation after mitogenic stimulation, they perform alloHSCT. Five years after unsuccessful alloHSCT, the patient develops lethal EBV+ multifocal diffuse large B cell lymphoma (34). The two different tumor entities are caused by independent EBV transformations and the EBV+ SMTs infiltrating lymphocytes are predominantly $CD3^+CD5^+CD8^+$ T cells (34). In 2016 and in collaboration with Petrilli and coworkers we find a homozygous autosomal recessive mutation in *ZAP70* (c.1765G > A, p.Val589Met) that could explain the patient's PID phenotype and would modify the diagnosis into (CID, Schober et al., unpublished data).

GATA2 haploinsufficiency is a recently identified polymorphic PID that manifests with a variety of infectious complications especially caused by mycobacteria, but as well by viral, bacterial, and fungal pathogens (38). In 2010, Vinh et al reported a 41-year-old female with multiple infections in the context of GATA2 haploinsufficiency and EBV+ SMTs located to the orbit, liver, colon, and uterus. The patient is treated with alloHSCT, but succumbs to post transplant viral infections (37).

In 2016, Parta et al. reported a 24-year-old male with GATA2 haploinsufficiency causing a polymorphic PID including EBV+ SMTs of the liver and possibly the spleen and the bones (33). They perform alloHSCT with a myeloablative conditioning regimen and peripheral blood hematopoietic stem cells from a matched sibling donor. After a 3-year follow up GATA2 haploinsufficiency and EBV+ SMTs are resolved and the authors conclude that at least in the context of GATA2 haploinsufficiency alloHSCT can lead to reconstitution of immunologic function and thereby cure of EBV-associated malignancy (33).

In 2017, our group reports four patients with EBV+ SMTs on the background of a novel CID caused by homozygous autosomal recessive *CARMIL2* mutations (21). Two of the patients initially are reported as cases of infantile myofibromatosis, but extensive immunobiological analyses reveal a profound regulatory T cell deficiency, defective CD28 co-signaling associated with impaired T cell activation, differentiation and function, as well as perturbed cytoskeletal organization associated with T cell polarity and migration disorders (21, 39, 40). Two patients decease before the PID diagnosis is established and the other two patients succumb to disease complications while being prepared for alloHSCT (21).

In summary, we are aware of 14 PID cases with EBV+ SMTs (**Table 1**). Twelve of them develop EBV+ SMTs as a primary PID manifestation and two of them develop the tumors after alloHSCT. Four of the cases are published without and ten with a genetic diagnosis confined to the *ATM*, *ADA*, *IL2RG*, *GATA2,* and *CARMIL2* genes. In one of the genetically undefined cases, we are able to retrospectively identify a mutation in the *ZAP70* gene. All of the genetically and immunologically defined PIDs impair T and/or NK cell immunity. Four of the reported cases are treated with alloHSCT in the presence of EBV+ SMTs and two of them decease because of viral infections or B cell lymphoma, while one develops stable disease after donor lymphocyte infusion and one is cured from PID and EBV+ SMTs.

CONCLUSION

EBV+ SMTs constitute very rare tumors seen in the context of SIDs caused by human immunodeficiency virus infection or immunosuppressive treatment after solid organ transplantation (11). The pathogenesis of EBV+ SMTs remains largely unknown, but it is evident that an immunocompromised host is a *conditio sine qua non* and that especially T and NK cell immunity is important to prevent the disease (11, 21, 35). Later, EBV+ SMTs emerge as possible manifestations of PIDs and up to now have been linked to mutations in *ATM*, *ADA*, *IL2RG*, *ZAP70*, *GATA2,* and *CARMIL2* (19–21, 30, 32–34, 37). Additionally, they have been found in genetically undefined PIDs and based on the clinical and immunological findings and our growing understanding of their pathogenesis these PIDs should at best be named CID and classic NK cell deficiency (31, 35, 36). At present, a particular molecular signaling or effector pathway has not been identified as a prerequisite to develop EBV+ SMTs. Given the multitude of CID causing gene defects, we thus propose an unbiased genetic work-up, such as whole exome sequencing to search for molecular PID causes in patients with EBV+ SMTs of unknown origin (41). In order to treat EBV+ SMTs, whenever possible, HIV infection should be addressed and post transplant immunosuppressive treatment should be reduced. AlloHSCT is a well-established curative treatment for CID and other PID and, therefore, seems a promising therapeutic approach for PID that is present with EBV+ SMTs (13, 33, 42).

Four major conclusions can be drawn at present. First, unusual SMTs should be screened for the presence of EBV preferentially by using EBER ISH. Second, in patients presenting with EBV+ SMTs without obvious SIDs, PIDs have to be considered strongly, necessitating appropriate investigation. Third, in PID patients presenting with solid tumors, EBV+ SMTs are a differential diagnosis. Fourth, PID patients manifesting with EBV+ SMTs might be treated with up front alloHSCT ideally in the setting of a prospective clinical trial yet to be initiated.

We envision that with increased awareness toward EBV+ SMTs as a manifestation of PIDs the rate of proper diagnosis of this association will increase and the outcome of curative alloHSCT will improve.

AUTHOR CONTRIBUTIONS

TM and FH wrote the article. TS, FF, and CK revised the article. CW provided histopathological images. JL-Z provided radiological images.

FUNDING

TM, TS, CK, and FH have been funded by the German Research Foundation (CRC 914 projects A4/A8, Gottfried-Wilhelm-Leibniz Program), the Care-for-Rare Foundation, the German Ministry of Education and Research (German PID-NET, Project A7), and the German Center for Infection Research (DZIF TTU07.909_00).

REFERENCES

1. Taylor GS, Long HM, Brooks JM, Rickinson AB, Hislop AD. The immunology of Epstein-Barr virus-induced disease. *Annu Rev Immunol* (2015) 33:787–821. doi:10.1146/annurev-immunol-032414-112326
2. McClain KL, Leach CT, Jenson HB, Joshi VV, Pollock BH, Parmley RT, et al. Association of Epstein-Barr virus with leiomyosarcomas in young people with AIDS. *N Engl J Med* (1995) 332:12–8. doi:10.1056/NEJM199501053320103
3. Veillette A, Perez-Quintero LA, Latour S. X-linked lymphoproliferative syndromes and related autosomal recessive disorders. *Curr Opin Allergy Clin Immunol* (2013) 13:614–22. doi:10.1097/ACI.0000000000000008
4. Fujiwara S, Kimura H, Imadome K, Arai A, Kodama E, Morio T, et al. Current research on chronic active Epstein-Barr virus infection in Japan. *Pediatr Int* (2014) 56:159–66. doi:10.1111/ped.12314
5. Pohl D, Krone B, Rostasy K, Kahler E, Brunner E, Lehnert M, et al. High seroprevalence of Epstein-Barr virus in children with multiple sclerosis. *Neurology* (2006) 67:2063–5. doi:10.1212/01.wnl.0000247665.94088.8d
6. Jha HC, Pei Y, Robertson ES. Epstein-Barr virus: diseases linked to infection and transformation. *Front Microbiol* (2016) 7:1602. doi:10.3389/fmicb.2016.01602
7. Rasche L, Kapp M, Einsele H, Mielke S. EBV-induced post transplant lymphoproliferative disorders: a persisting challenge in allogeneic hematopoetic SCT. *Bone Marrow Transplant* (2014) 49:163–7. doi:10.1038/bmt.2013.96
8. Rickinson AB. Co-infections, inflammation and oncogenesis: future directions for EBV research. *Semin Cancer Biol* (2014) 26:99–115. doi:10.1016/j.semcancer.2014.04.004
9. Maecker B, Jack T, Zimmermann M, Abdul-Khaliq H, Burdelski M, Fuchs A, et al. CNS or bone marrow involvement as risk factors for poor survival in post-transplantation lymphoproliferative disorders in children after solid organ transplantation. *J Clin Oncol* (2007) 25:4902–8. doi:10.1200/JCO.2006.10.2392
10. Miettinen M. Smooth muscle tumors of soft tissue and non-uterine viscera: biology and prognosis. *Mod Pathol* (2014) 27(Suppl 1):S17–29. doi:10.1038/modpathol.2013.178
11. Hussein K, Maecker-Kolhoff B, Donnerstag F, Laenger F, Kreipe H, Jonigk D. Epstein-Barr virus-associated smooth muscle tumours after transplantation, infection with human immunodeficiency virus and congenital immunodeficiency syndromes. *Pathobiology* (2013) 80:297–301. doi:10.1159/ 000351326
12. Jonigk D, Laenger F, Maegel L, Izykowski N, Rische J, Tiede C, et al. Molecular and clinicopathological analysis of Epstein-Barr virus-associated posttransplant smooth muscle tumors. *Am J Transplant* (2012) 12:1908–17. doi:10.1111/j.1600-6143.2012.04011.x
13. Hussein K, Rath B, Ludewig B, Kreipe H, Jonigk D. Clinico-pathological characteristics of different types of immunodeficiency-associated smooth muscle tumours. *Eur J Cancer* (2014) 50:2417–24. doi:10.1016/j.ejca.2014.06.006
14. Chaves NJ, Kotsimbos TC, Warren MA, McLean CA, Spelman DW, Williams TJ, et al. Cranial leiomyosarcoma in an Epstein-Barr virus (EBV)-mismatched lung transplant recipient. *J Heart Lung Transplant* (2007) 26:753–5. doi:10.1016/j.healun.2007.05.007
15. Somers GR, Tesoriero AA, Hartland E, Robertson CF, Robinson PJ, Venter DJ, et al. Multiple leiomyosarcomas of both donor and recipient origin arising in a heart-lung transplant patient. *Am J Surg Pathol* (1998) 22:1423–8. doi:10.1097/00000478-199811000-00014
16. Deyrup AT, Lee VK, Hill CE, Cheuk W, Toh HC, Kesavan S, et al. Epstein-Barr virus-associated smooth muscle tumors are distinctive mesenchymal tumors reflecting multiple infection events: a clinicopathologic and molecular analysis of 29 tumors from 19 patients. *Am J Surg Pathol* (2006) 30:75–82. doi:10.1097/01.pas.0000178088.69394.7b
17. Baumforth KR, Young LS, Flavell KJ, Constandinou C, Murray PG. The Epstein-Barr virus and its association with human cancers. *Mol Pathol* (1999) 52:307–22. doi:10.1136/mp.52.6.307
18. Murray PG, Swinnen LJ, Constandinou CM, Pyle JM, Carr TJ, Hardwick JM, et al. BCL-2 but not its Epstein-Barr virus-encoded homologue, BHRF1, is commonly expressed in posttransplantation lymphoproliferative disorders. *Blood* (1996) 87:706–11.
19. Hatano M, Takada H, Nomura A, Ohga S, Ohshima K, Saeki I, et al. Epstein-Barr virus-associated bronchial leiomyoma in a boy with cellular immunodeficiency. *Pediatr Pulmonol* (2006) 41:371–3. doi:10.1002/ppul.20375
20. Reyes C, Abuzaitoun O, De Jong A, Hanson C, Langston C. Epstein-Barr virus-associated smooth muscle tumors in ataxia-telangiectasia: a case report and review. *Hum Pathol* (2002) 33:133–6. doi:10.1053/hupa.2002.30214
21. Schober T, Magg T, Laschinger M, Rohlfs M, Linhares ND, Puchalka J, et al. A human immunodeficiency syndrome caused by mutations in CARMIL2. *Nat Commun* (2017) 8:14209. doi:10.1038/ncomms14209
22. Hussein K, Maecker-Kolhoff B, Klein C, Kreipe H. [Transplant-associated lympho proliferation]. *Pathologe* (2011) 32:152–8. doi:10.1007/s00292-010-1407-x
23. Purgina B, Rao UN, Miettinen M, Pantanowitz L. AIDS-related EBV-associated smooth muscle tumors: a review of 64 published cases. *Patholog Res Int* (2011) 2011:561548. doi:10.4061/2011/561548
24. Rougemont AL, Alfieri C, Fabre M, Gorska-Flipot I, Papp E, Champagne J, et al. Atypical Epstein-Barr virus (EBV) latent protein expression in EBV-associated smooth muscle tumours occurring in paediatric transplant recipients. *Histopathology* (2008) 53:363–7. doi:10.1111/j.1365-2559.2008.03086.x
25. Ong KW, Teo M, Lee V, Ong D, Lee A, Tan CS, et al. Expression of EBV latent antigens, mammalian target of rapamycin, and tumor suppression genes in EBV-positive smooth muscle tumors: clinical and therapeutic implications. *Clin Cancer Res* (2009) 15:5350–8. doi:10.1158/1078-0432.CCR-08-2979
26. Shen Q, Feng W, Long MS, Duan X, Jaijakul S, Arias CA, et al. Multicentric hepatic EBV-associated smooth muscle tumors in an AIDS patient: a case report, investigation of mTOR activation and review of the literature. *Int J Clin Exp Pathol* (2011) 4:421–9.
27. Tan CS, Loh HL, Foo MW, Choong LH, Wong KS, Kee TY. Epstein-Barr virus-associated smooth muscle tumors after kidney transplantation: treatment and outcomes in a single center. *Clin Transplant* (2013) 27:E462–8. doi:10.1111/ctr.12139
28. Pritzker KP, Huang SN, Marshall KG. Malignant tumours following immunosuppressive therapy. *Can Med Assoc J* (1970) 103:1362–5.
29. Lee ES, Locker J, Nalesnik M, Reyes J, Jaffe R, Alashari M, et al. The association of Epstein-Barr virus with smooth-muscle tumors occurring after organ transplantation. *N Engl J Med* (1995) 332:19–25. doi:10.1056/NEJM199501053320104
30. Atluri S, Neville K, Davis M, Robertson KA, Marshalleck FE, O'Malley DP, et al. Epstein-Barr-associated leiomyomatosis and T-cell chimerism after haploidentical bone marrow transplantation for severe combined immunodeficiency disease. *J Pediatr Hematol Oncol* (2007) 29:166–72. doi:10.1097/MPH.0b013e31803b95b3
31. Mierau GW, Greffe BS, Weeks DA. Primary leiomyosarcoma of brain in an adolescent with common variable immunodeficiency syndrome. *Ultrastruct Pathol* (1997) 21:301–5. doi:10.3109/01913129709021926
32. Monforte-Muñoz H, Kapoor N, Saavedra JA. Epstein-Barr virus-associated leiomyomatosis and posttransplant lymphoproliferative disorder in a child with severe combined immunodeficiency: case report and review of the literature. *Pediatr Dev Pathol* (2003) 6:449–57. doi:10.1007/s10024-003-8096-x
33. Parta M, Cuellar-Rodriguez J, Freeman AF, Gea-Banacloche J, Holland SM, Hickstein DD. Resolution of multifocal Epstein-Barr virus-related smooth muscle tumor in a patient with GATA2 deficiency following hematopoietic stem cell transplantation. *J Clin Immunol* (2017) 37:61–6. doi:10.1007/s10875-016-0360-8
34. Petrilli G, Lorenzi L, Paracchini R, Ubiali A, Schumacher RF, Cabassa P, et al. Epstein-Barr virus-associated adrenal smooth muscle tumors and disseminated diffuse large B-cell lymphoma in a child with common variable immunodeficiency: a case report and review of the literature. *Int J Surg Pathol* (2014) 22:712–21. doi:10.1177/1066896911399901

35. Shaw RK, Issekutz AC, Fraser R, Schmit P, Morash B, Monaco-Shawver L, et al. Bilateral adrenal EBV-associated smooth muscle tumors in a child with a natural killer cell deficiency. *Blood* (2012) 119:4009–12. doi:10.1182/blood-2011-10-385377
36. Tulbah A, Al-Dayel F, Fawaz I, Rosai J. Epstein-Barr virus-associated leiomyosarcoma of the thyroid in a child with congenital immunodeficiency: a case report. *Am J Surg Pathol* (1999) 23:473–6. doi:10.1097/00000478-199904000-00013
37. Vinh DC, Patel SY, Uzel G, Anderson VL, Freeman AF, Olivier KN, et al. Autosomal dominant and sporadic monocytopenia with susceptibility to mycobacteria, fungi, papillomaviruses, and myelodysplasia. *Blood* (2010) 115:1519–29. doi:10.1182/blood-2009-03-208629
38. Spinner MA, Sanchez LA, Hsu AP, Shaw PA, Zerbe CS, Calvo KR, et al. GATA2 deficiency: a protean disorder of hematopoiesis, lymphatics, and immunity. *Blood* (2014) 123:809–21. doi:10.1182/blood-2013-07-515528
39. Linhares ND, Freire MC, Cardenas RG, Pena HB, Bahia M, Pena SD, et al. Exome sequencing identifies a novel homozygous variant in NDRG4 in a family with infantile myofibromatosis. *Eur J Med Genet* (2014) 57:643–8. doi:10.1016/j.ejmg.2014.08.010
40. Linhares ND, Freire MC, Cardenas RG, Pena HB, Bahia M, Pena SD. "Exome sequencing identifies a novel homozygous variant in NDRG4 in a family with infantile myofibromatosis (Linhares et al., 2014)" turns out to be EBV+ leiomyomatosis caused by CARMIL2 mutations. *Eur J Med Genet* (2017) 61:106. doi:10.1016/j.ejmg.2017.02.002
41. Picard C, Bobby Gaspar H, Al-Herz W, Bousfiha A, Casanova JL, Chatila T, et al. International union of immunological societies: 2017 primary immunodeficiency diseases committee report on inborn errors of immunity. *J Clin Immunol* (2018) 38:96–128. doi:10.1007/s10875-017-0464-9
42. Cavazzana M, Touzot F, Moshous D, Neven B, Blanche S, Fischer A. Stem cell transplantation for primary immunodeficiencies: the European experience. *Curr Opin Allergy Clin Immunol* (2014) 14:516–20. doi:10.1097/ACI.0000000000000119

Conflict of Interest Statement: The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

frontiers in Immunology

REVIEW
published: 19 March 2018
doi: 10.3389/fimmu.2018.00556

Adoptive T Cell Therapy for Epstein–Barr Virus Complications in Patients With Primary Immunodeficiency Disorders

*Lauren P. McLaughlin[1,2], Catherine M. Bollard[1,3,4] and Michael D. Keller[1,3]**

[1] Center for Cancer and Immunology Research, Children's National Health System, The George Washington University, Washington, DC, United States, [2] Division of Oncology, Children's National Health System, Washington, DC, United States, [3] Division of Allergy and Immunology, Children's National Health System, Washington, DC, United States, [4] Division of Blood and Marrow Transplantation, Children's National Health System, Washington, DC, United States

Patients with primary immunodeficiency disorders (PID) have an increased risk from acute and chronic Epstein–Barr Virus (EBV) viral infections and EBV-associated malignancies. Hematopoietic stem cell transplantation (HSCT) is a curative strategy for many patients with PID, but EBV-related complications are common in the immediate post-transplant period due to delayed reconstitution of T cell immunity. Adoptive T cell therapy with EBV-specific T cells is a promising therapeutic strategy for patients with PID both before and after HSCT. Here we review the methods used to manufacture EBV-specific T cells, the clinical outcomes, and the ongoing challenges for future development of the strategy.

Keywords: primary immunodeficiency disorders, Epstein–barr virus, adoptive T cell therapy, immunotherapy, hematopoietic stem cell transplantation

OPEN ACCESS

Edited by:
Isabelle Meyts, KU Leuven, Belgium

Reviewed by:
Mark Ballow, University of South Florida, United States
Tomohiro Morio, Tokyo Medical and Dental University, Japan

***Correspondence:**
Michael D. Keller
mkeller@childrensnational.org

Specialty section:
This article was submitted to Primary Immunodeficiencies, a section of the journal Frontiers in Immunology

Received: *22 January 2018*
Accepted: *05 March 2018*
Published: *19 March 2018*

Citation:
McLaughlin LP, Bollard CM and Keller MD (2018) Adoptive T Cell Therapy for Epstein–Barr Virus Complications in Patients With Primary Immunodeficiency Disorders. Front. Immunol. 9:556. doi: 10.3389/fimmu.2018.00556

BACKGROUND

Epstein–Barr Virus (EBV) is a herpes virus that typically causes a mild to moderate self-limiting viral illness in healthy individuals. During primary infection, EBV establishes latency in B lymphocytes and oral epithelial cells. The level of B lymphocytes latently infected is maintained at a very low level through a potent cell-mediated immune response by EBV-specific T lymphocytes (1). However, individuals with moderate to severe forms of primary immunodeficiency disorders (PID) have weakened T-cell immunity with diminished immunosurveillance. PID patients are at risk from EBV-related complications which include acute and chronic infections and EBV-associated malignancies. EBV is also a frequent inciting factor for hemophagocytic lymphohistiocytosis (HLH) in PID with impaired cell-mediated cytotoxicity.

Hematopoietic stem cell transplantation (HSCT) has been used as curative approach for severe combined immunodeficiency (SCID) for over 50 years, and the approach is increasingly being used for other PIDs (2, 3). However, reconstitution of T cell immunity, needed to control both acquired viral infections reactivating viruses, is delayed for up to 6 months after transplantation. During this period patients remain extremely vulnerable to viral complications. While antiviral pharmacotherapy is available for many of the viruses that contribute to pre- and post-HSCT morbidity and mortality, their use is limited by toxicities and emerging resistance. Rituximab, a monoclonal antibody targeting CD20, has good efficacy against EBV. However, Rituximab targets not only the EBV-infected B cells, but also the healthy B cell compartments, which further weakens the immune system. Resistance to rituximab has also been described (4). Given these limitations, adoptive therapy with EBV-specific T cells has emerged as a promising therapeutic strategy for PID patients with EBV-related complications.

Adoptive therapy with viral-specific T cells (VSTs) has been used for over 20 years (5, 6). Earliest experience using cellular therapy for EBV-related post-transplant lymphoproliferative disease (PTLD) after HSCT included using unmanipulated donor lymphocyte infusions, which was often effective, but carried a high risk of graft-versus-host disease (GVHD) (7). Subsequently, VSTs have been developed that show safety and efficacy in treating EBV infections while minimizing the risk of GVHD (8–13). While previous reviews have primarily examined the use of all forms of VSTs for patients with PID (14, 15), this review focuses specifically on the development of and clinical use of EBV-specific T cells for patients with PID.

EBV-SPECIFIC T CELL GENERATION METHODS

Several methods have been developed to generate EBV-specific T cell products with minimally alloreactive T cells to decrease the risk of GVHD. These techniques include *ex vivo* expansion, multimer selection, and IFN-γ capture. To date, *ex vivo* expansion is the most commonly used method.

Many *ex vivo* expansion methods use EBV-transformed lymphoblastoid cell lines (LCL) as antigen presenting cells (APCs). LCLs are advantageous APCs as they express all 10 EBV latency antigens (type III latency), but also high levels of class I and II HLA and co-stimulatory molecules (16). Either activated monocytes or dendritic cells are used in the first stimulation, with LCLs used for subsequent stimulations. To further refine this technique, groups have developed methods for modifying LCL by either pulsing with synthetic peptide pools encompassing viral antigens, or transducing LCLs with adenovirus vectors that overexpress either latent membrane protein (LMP) 2 or LMP1 and LMP2. These strategies enhance T cell specificities for the less immunogenic EBV antigens LMP1 and LMP2 increasing their efficacy for EBV-related lymphomas that only express LMP1 and LMP2 (type II latency). While this method has proven to be safe and efficacious, it takes at least 8 weeks to generate a product suitable for clinical use as LCL take 3–4 weeks to manufacture. This has spurred the development of rapid *ex vivo* culture methods using a single stimulation with APC pulsed with synthetic peptide pools, or direct stimulation of PBMCs with synthetic peptide pools. These methods reduce the manufacturing time to 10–14 days. Rapid *ex vivo* culture methods have been used for multivirus specific T cells, but not for T cell products specific for EBV only.

Additional techniques, such as multimer selection or IFN-γ capture, can produce VSTs even more readily than rapid *ex vivo* culture (17–19). Multimer selection uses magnetically labeled peptide multimers to isolate T cells specific for the relevant peptide/MHC multimers. IFN-γ capture uses an immunomagnetic separation device to isolate T cells that produce IFN-γ when stimulated by viral antigens. Although these techniques produce a clinical grade product within 48 h, they require donors not only to be seropositive to the virus of interest, but also to have a detectable level of circulating virus specific T cells. Leukapheresis is typically needed to collect enough T cells for clinical use. While IFN-γ capture is not HLA-restricted and produces a polyclonal and polyfunctional product containing $CD4^+$ and $CD8^+$ T cells, multimer selection is an HLA-restricted process, and generally yields only $CD8^+$ T cells.

PREVIOUS CLINICAL USAGE OF EBV-SPECIFIC T CELLS FOR PID DISORDERS

Donor-Derived EBV-Specific T Cells

As PID is one of the most common non-malignant indications for referral to HSCT in pediatrics and is associated with high risk for viral complications, patients with PID constitute a sizeable proportion of patients in VST clinical trials (**Table 1**). A large, multi-center study with a median follow-up of 10 years treated 114 patients with EBV-specific T cells after HSCT, either for prophylaxis ($n = 101$) or treatment ($n = 13$) and included 13 patients with PID. All patients treated as prophylaxis had no subsequent EBV viremia, while three patients with active disease attained a complete response (CR) and three additional patients achieved a PR (10). Papadopoulou et al. included four patients with PID in their clinical trial of multivirus-specific T cells (CMV, EBV, AdV, HHV6, BK), two of whom received T cells for EBV-related complications and both of whom obtained a CR (20).

In a large retrospective review of 36 PID patients receiving VSTs, Naik et al. included four patients with IL2RG-SCID as well as patients with Wiscott–Aldrich and combined immunodeficiency disorder (CID) who received donor-derived-specific T cells for prophylaxis. All patients remained free of EBV viremia after receiving T cells (14). Additionally, one patient with HLH received donor-derived trivirus VSTs (CMV, EBV, Adv) for CMV and EBV viremia with clearance of both viruses.

Third Party EBV-Specific T Cells

To make cellular therapy more readily available, there is growing interest in establishing third-party banks of VSTs. Such T cell therapeutics produced from healthy donors are available for "off-the-shelf" use, eliminating the time and cost associated with custom-made products. These would be particularly beneficial in the setting of T-cell depleted transplantation, or when EBV-naive donors are the sole option for an EBV-seropositive patient, which would impart high risk of viral reactivation particularly in those with prior EBV-associated disease. While there is limited experience with third party banks to date, the results have been promising, particularly in patients with PID (**Table 2**).

Vickers et al. established a large third party bank of EBV-specific T cells to treat patients with PTLD and other EBV complications after HSCT or solid organ transplantation. To date, they have treated three patients with PIDs, including combined immune deficiency and chronic granulomatous disease (CGD). One patient had a CR, but the other two died from progressive disease (PD). At the time of publication, one additional patient with CGD had not undergone HSCT, but had EBV-specific T cells matched for use after transplantation (22). Two patients with CTP synthase 1 (CTPS1) deficiency have been treated with third party EBV-specific T cells for EBV-LPD and primary CNS lymphoma,

TABLE 1 | Previous clinical use of donor-derived EBV-specific T cells.

Reference	Primary immunodeficiency disorders diagnosis	Indication	Specificity	Generation method	Source	Cell Dose	Outcomes
Leen et al. (21)	SCID	Prophylaxis	EBV, AdV	Culture, lymphoblastoid cell lines (LCL) with Ad5f35 vector	Hematopoietic stem cell transplantation (HSCT) donor, peripheral blood	$1.35 \times 10^8/m^2$	Alive, no active infections
Papadopoulos et al. (7)	GATA2 deficiency	EBV, BK	CMV, EBV, AdV, HHV6, BK	Culture, peptide	HSCT donor, peripheral blood	$2 \times 10^7/m^2$	CR
	SCID variant	BK, EBV	CMV, EBV, AdV, HHV6, BK	Culture, peptide	HSCT donor, peripheral blood	$2 \times 10^7/m^2$	CR
	HLH	HHV6, BK; subsequent EBV reactivation	CMV, EBV, AdV, HHV6, BK	Culture, peptide	HSCT donor, peripheral blood	$1 \times 10^7/m^2$	HHV6: CR; BK: NR; EBV: CR
Heslop et al. (10)	XLP	Prophylaxis	EBV	Culture, LCL	HSCT donor, peripheral blood	$2 \times 10^7/m^2$	No viremia
	CID	Prophylaxis	EBV	Culture, LCL	HSCT donor, peripheral blood	$2.5 \times 10^7/m^2$	No viremia
	WAS	Prophylaxis	EBV	Culture, LCL	HSCT donor, peripheral blood	$2.5 \times 10^7/m^2$	No viremia
	XLP	Prophylaxis	EBV	Culture, LCL	HSCT donor, peripheral blood	$2 \times 10^7/m^2$	No viremia
	XLP-like	Prophylaxis	EBV	Culture, LCL	HSCT donor, peripheral blood	$2 \times 10^7/m^2$	No viremia
	WAS	EBV viremia	EBV	Culture, LCL	HSCT donor, peripheral blood	$2 \times 10^7/m^2$	CR
	SCAEBV/NK defect	EBV viremia	EBV	Culture, LCL	HSCT donor, peripheral blood	$1 \times 10^8/m^2$	CR
	SCAEBV	EBV viremia	EBV	Culture, LCL	HSCT donor, peripheral blood	$2 \times 10^7/m^2$	PR; died of progressive lymphoma
	SCAEBV	EBV viremia	EBV	Culture, LCL	HSCT donor, peripheral blood	$2 \times 10^7/m^2$	No further EBV reactivation
	XLP (SLAM mutation)	EBV viremia	EBV	Culture, LCL	HSCT donor, peripheral blood	$2 \times 10^7/m^2$	CR
	XLP	EBV viremia	EBV	Culture, LCL	HSCT donor, peripheral blood	$2 \times 10^7/m^2$	PR
	XLP	EBV viremia	EBV	Culture, LCL	HSCT donor, peripheral blood	$2 \times 10^7/m^2$	CR
	XLP	EBV viremia	EBV	Culture, LCL	HSCT donor, peripheral blood	$2 \times 10^7/m^2$	PR
Doubrovina et al. (12)	XLP	EBV-LPD	EBV	Culture, LCL	HSCT donor, peripheral blood	1×10^6/kg × 3 doses	PD; died
	ALPS	EBV-LPD	EBV	Culture, LCL	HSCT donor, peripheral blood	1×10^6/kg	NE; died
Naik et al. (14)	IL2RG-SCID	Prophylaxis	CMV, EBV, AdV	Culture, DC, and LCL with Ad5f35f-CMVpp65 vector	HSCT donor, umbilical cord	$1.5 \times 10^7/m^2$	No viremia
	IL2RG-SCID	Prophylaxis	CMV, EBV, AdV	Culture, DC, and LCL with Ad5f35f-CMVpp65 vector	HSCT donor, umbilical cord	$2.5 \times 10^7/m^2$	No viremia
	IL2RG-SCID	Prophylaxis	CMV, EBV, AdV	Culture, DC, and LCL with Ad5f35f-CMVpp65 vector	HSCT donor, umbilical cord	$1 \times 10^7/m^2$	No viremia
	IL2RG-SCID	Prophylaxis	CMV, EBV, AdV	Culture, DC, and LCL with Ad5f35f-CMVpp65 vector	HSCT donor, umbilical cord	$1 \times 10^7/m^2$	No viremia
	WAS	Prophylaxis	CMV, EBV, AdV	Culture, DC, and LCL with Ad5f35f-CMVpp65 vector	HSCT donor	$1 \times 10^7/m^2$	No viremia

(*Continued*)

TABLE 1 | Continued

Reference	Primary immunodeficiency disorders diagnosis	Indication	Specificity	Generation method	Source	Cell Dose	Outcomes
	CID	Prophylaxis	EBV	Culture, peptide	HSCT donor	$2.5 \times 10^7/m^2$	No viremia
	HLH (STXBP2)	CMV, EBV	CMV, EBV, AdV	Culture	HSCT donor	$1 \times 10^7/m^2 \times 2$ doses	CMV: CR; EBV: CR
	WAS	Prophylaxis	CMV, EBV, AdV	Culture	HSCT donor	$2 \times 10^7/m^2$	No viremia

SCID, severe combine immunodeficiency; EBV, Epstein–barr virus; AdV, adenovirus; CMV, cytomegalovirus; HHV6, human herpesvirus 6; CR, complete response, HLH, hemophagocytic lymphohistiocytosis; NR, no response; XLP, X-linked lymphoproliferative disease; CID, combined immune deficiency; WAS, Wiskott–Aldrich syndrome; SCAEBV, severe chronic active EBV; ALPS, autoimmune lymphoproliferative syndrome; DC, dendritic cell.

TABLE 2 | Previous clinical use of third party Epstein–Barr Virus (EBV)-specific T cells.

Reference	Primary immunodeficiency disorders diagnosis	Indication	Specificity	Generation method	Source	Cell dose	Outcomes
Vickers et al. (22)	Combined immunodeficiency disorder (CID)	PTLD	EBV	Culture, LCL	Third party	$1–2 \times 10^6$/kg/dose; 4 doses given weekly	CR
	CGD	PTLD	EBV	Culture, LCL	Third party	$1–2 \times 10^6$/kg/dose; 4 doses given weekly	PD; died
	CID	PTLD	EBV	Culture, LCL	Third party	$1–2 \times 10^6$/kg/dose; 4 doses given weekly	PD; died
Wynn et al. (23)	CTPS1 deficiency	Primary CNS lymphoma	EBV	Culture, LCL	Third party	2×10^6/kg/dose; 7 doses given weekly; 2 additional doses after re-emergence of EBV disease	CR
Doubrovina et al. (12)	Hemophagocytic lymphohistiocytosis (HLH)	EBV-LPD	EBV	Culture, LCL	Third party	1×10^6/kg x 3 doses	CR
Naik et al. (14)	ADA-severe combined immunodeficiency	EBV-LPD	CMV, EBV, AdV	Culture	Third party, pre-hematopoietic stem cell transplantation	$5 \times 10^6/m^2$	NR, died from EBV-LPD
	HLH	EBV	EBV	Culture	Third party	2×10^6/kg x 3 doses	PR; died of PTLD
	CTPS1 deficiency	EBV-LPD	EBV	Culture	Third party	2×10^6/kg x 2 doses	CR
Withers et al. (24)	SCAEBV	EBV	EBV	Culture	Third party	$2 \times 10^7/m^2$	EBV: NR; Died

CGD, chronic granulomatous disease; PTLD, post-transplant lymphoproliferative disease; PD, progressive disease; CTPS1, CTP synthase 1 deficiency; EBV-LPD, EBV-lymphoproliferative disease; PR, partial response.

and both had CRs after T cell therapy (14, 23). Third party EBV-specific T cells have also been used in HLH as discussed below.

SPECIAL CASES

Pretransplantation

As patients with PID are particularly vulnerable to chronic and refractory viral infections even prior to HSCT (25–27), another benefit of third party T cells is the ability to treat patients prior to transplant. This would not only minimize mortality associated with transplant, but could allow more patients to be referred to transplant. Naik et al. described two patients with PID (one with SCID and another with CTPS1 deficiency) with partially HLA-matched third-party T cells prior to HSCT for EBV-LPD, one of whom achieved a CR (14, 22, 23). A patient with ADA-SCID who developed EBV viremia and CMV colitis while on enzyme replacement therapy was treated with trivirus VSTs (CMV, EBV, AdV) that were 5/10 HLA matched with the patient, but no response was seen and the patient died of EBV-associated lymphoma. It was unclear if VST expansion may have been compromised in this case by the underlying inherent lymphotoxicity of ADA deficiency, as patients remain lymphopenic even in the setting of optimal enzyme replacement therapy. However, the patient with CTPS1 deficiency received 2 doses ($2 \times 10E^6$/kg/dose) of 9/10 HLA match EBV-specific T cells and attained a CR, and underwent subsequent HSCT without further viral complications.

HLH With EBV Viremia

Hemophagocytic lymphohistiocytosis is condition of hyperinflammation associated with immune dysregulation secondary to defects in cytotoxic T lymphocyte and NK cell function.

HLH can be either primary (associated with a known mutation) or secondary, and EBV viremia is a common trigger. In particular, familial HLH due to mutations in *STXBP2* and *PRF1* have been associated with chronic EBV viremia (28). Other PIDs have an increased risk of developing HLH, and several are associated with EBV viremia as well as including SAP deficiency, XIAP deficiency, ITK deficiency, and CD27 deficiency. While EBV typically infects B cells, EBV-related HLH is frequently associated with EBV-infected T cells, which presents therapeutic challenges (29). As familial HLH is universally fatal without HSCT as definitive treatment, these patients may benefit from EBV-directed cell therapy to restore EBV-specific immunity early after transplant.

A series of 49 patients who were treated for EBV-LPD following HSCT with donor lymphocyte infusion and/or EBV-specific VST included three patients with PID: one with autoimmune lymphoproliferative syndrome, one with x-linked lymphoproliferative disease, and another had primary HLH. There was an overall response of 68% following EBV-specific VST infusion, including a CR in the patient with HLH who received third party EBV-specific T cells (12). Similarly, Papadopoulou et al. reported a patient with HLH initially treated with multivirus VSTs for HHV6 and BK viremia who subsequently developed EBV reactivation and attained complete clearance of EBV viremia without other EBV-directed therapy after T cell infusion (20). The review by Naik et al. included two patients with HLH and EBV viremia, one of whom attained a CR while the other died of progressive EBV-PTLD (14).

Severe Chronic Active EBV

Severe chronic active EBV (SCAEBV) infection is a lymphoproliferative disorder characterized by markedly high levels of EBV in blood and tissue that often presents with fever, lymphadenopathy, hepatic dysfunction, and thrombocytopenia (30). In SCAEBV, EBV can infect T and NK cells in addition to B cells. While the etiology of SCAEBV is often unknown, the underlying defect may represent a type of immunodeficiency. To date, HSCT has shown to be the most effective treatment for SCAEBV. As the primary problem in SCAEBV is an ineffective immune response to EBV, adoptive T cell therapy with EBV-specific T cells may be very advantageous in this patient population after HSCT. Heslop et al. included three patients with SCAEBV (one of whom had a known NK cell deficiency) in their trial of 114 patients receiving EBV-specific T cells. One patient was treated on the prophylactic arm and remained free of EBV viremia. Of the two patients with active disease at the time of T cell infusion, one patient attained a CR while the other died of progressive EBV-associated lymphoma (10). Withers et al. reported a patient with SCAEBV who received third party EBV-specific T cells but died Day + 14 after infusion of PD (24).

CHALLENGES AND FUTURE DIRECTIONS

In spite of the successes with EBV-specific T-cells, there remain limitations in this approach for treatment of PID. Resistance to EBV-specific cellular therapy has been described in several prior studies, which may in some cases relate to viral escape mutations. In one study, a mutation in EBNA-3B enabled viral escape post-T-cell therapy (31). The targeted EBV antigens differ slightly between trials, and further studies of the lability of targeted epitopes will be crucial to improve the efficacy of EBV-specific T-cell therapy, particularly in the third-party setting. Best methods of partial HLA matching of third-party EBV-specific T-cells is also unclear, particularly in the setting of rare HLA alleles that are not known to mediate recognition of immunodominant EBV epitopes. EBV-specific T-cells are also subject to inactivation or killing by immunosuppressive therapies, such as corticosteroids, which limits their use in the setting of GVHD following HCT. Genetic modification of antigen-specific T-cells to render them resistant to glucocorticoids and calcineurin inhibitors may enable treatment of PTLD in spite of immunosuppressive therapy (32, 33).

It is also unclear if adoptive T-cell therapy will be effective prior to HCT in forms of PID in which APCs are impaired or absent. In a recent study, defects in the costimulatory receptor CD70 resulted in EBV-associated disease (34). CD70 defects could theoretically impair the ability of allogeneic T-cells to lyse infected target cells. EBV-specific T-cells have not been explored in patients with EBV-driven HLH prior to HCT, and is unclear whether partial restoration of cytotoxicity would be of any benefit in this hyper-inflammatory disorder.

Currently, manufacturing of EBV-specific T-cells requires a facility with the ability to meet regulatory guidelines for production of immune effector cells for clinical use. Selection methods allow make use of automated-closed systems, but are expensive and yield low cell numbers. *Ex vivo* expansion yields higher cell numbers, but requires expertise in more than minimal product manipulation. In both settings, an EBV-seropositive donor would need to be identified for product manufacturing. Third party T-cells circumvent these limitations, but similarly are limited by cost of bank generation and regulatory hurdles that limit widespread availability. New multicenter trials using regional banks will improve accessibility to studies using EBV-specific T-cells. With the recent FDA approval of two chimeric antigen receptor T-cell products, it is hoped that the use of antigen-specific T-cells may similarly be approved in the near future, enabling widespread accessibility to these products.

ETHICS STATEMENT

This study was carried out in accordance with the recommendations of The National Institutes of Health. The referenced studies were approved by the local Institutional Review Boards. All subjects gave written informed consent in accordance with the Declaration of Helsinki.

AUTHOR CONTRIBUTIONS

LM prepared the first draft of this manuscript, while MK performed the literature review and compiled the data set of patients

included in **Tables 1** and **2**. MK and CB both reviewed and edited the final manuscript.

ACKNOWLEDGMENTS

We would like to thank the staffs of the Divisions of Allergy and Immunology, Oncology, and Blood and Marrow Transplantation, Children's National Medical Center, our collaborators at the Cell and Gene Therapy center at Baylor College of Medicine and other institutions, and the Children's Research Institute. We would like to thank the Lymphoma Research Foundation and the Jeffrey Modell Foundation for their support of LM and MK and this work.

FUNDING

This research was funded by grants from the National Institutes of Health (U54 HL081007 to CB; K23- HL136783-01 to MK), the National Cancer Institute (PO1 CA148600e02 to CB), and the Jeffrey Modell Foundation.

REFERENCES

1. Taylor GS, Long HM, Brooks JM, Rickinson AB, Hislop AD. The immunology of Epstein-Barr virus-induced disease. *Annu Rev Immunol* (2015) 33:787–821. doi:10.1146/annurev-immunol-032414-112326
2. Gennery AR, Slatter MA, Grandin L, Taupin P, Cant AJ, Veys P, et al. Transplantation of hematopoietic stem cells and long-term survival for primary immunodeficiencies in Europe: entering a new century, do we do better? *J Allergy Clin Immunol* (2010) 126(3):602–10.e1–11. doi:10.1016/j.jaci.2010.06.015
3. Worth AJ, Booth C, Veys P. Stem cell transplantation for primary immune deficiency. *Curr Opin Hematol* (2013) 20(6):501–8. doi:10.1097/MOH.0b013e328365a13b
4. Rezvani AR, Maloney DG. Rituximab resistance. *Best Pract Res Clin Haematol* (2011) 24(2):203–16. doi:10.1016/j.beha.2011.02.009
5. Walter EA, Greenberg PD, Gilbert MJ, Finch RJ, Watanabe KS, Thomas ED, et al. Reconstitution of cellular immunity against cytomegalovirus in recipients of allogeneic bone marrow by transfer of T-cell clones from the donor. *N Engl J Med* (1995) 333(16):1038–44. doi:10.1056/NEJM199510193331603
6. Riddell SR, Watanabe KS, Goodrich JM, Li CR, Agha ME, Greenberg PD. Restoration of viral immunity in immunodeficient humans by the adoptive transfer of T cell clones. *Science* (1992) 257(5067):238–41. doi:10.1126/science.1352912
7. Papadopoulos EB, Ladanyi M, Emanuel D, Mackinnon S, Boulad F, Carabasi MH, et al. Infusions of donor leukocytes to treat Epstein-Barr virus-associated lymphoproliferative disorders after allogeneic bone marrow transplantation. *N Engl J Med* (1994) 330(17):1185–91. doi:10.1056/NEJM199404283301703
8. Haque T, Taylor C, Wilkie GM, Murad P, Amlot PL, Beath S, et al. Complete regression of posttransplant lymphoproliferative disease using partially HLA-matched Epstein Barr virus-specific cytotoxic T cells. *Transplantation* (2001) 72(8):1399–402. doi:10.1097/00007890-200110270-00003
9. Haque T, Wilkie GM, Jones MM, Higgins CD, Urquhart G, Wingate P, et al. Allogeneic cytotoxic T-cell therapy for EBV-positive posttransplantation lymphoproliferative disease: results of a phase 2 multicenter clinical trial. *Blood* (2007) 110(4):1123–31. doi:10.1182/blood-2006-12-063008
10. Heslop HE, Slobod KS, Pule MA, Hale GA, Rousseau A, Smith CA, et al. Long-term outcome of EBV-specific T-cell infusions to prevent or treat EBV-related lymphoproliferative disease in transplant recipients. *Blood* (2010) 115(5):925–35. doi:10.1182/blood-2009-08-239186
11. Bollard CM, Gottschalk S, Torrano V, Diouf O, Ku S, Hazrat Y, et al. Sustained complete responses in patients with lymphoma receiving autologous cytotoxic T lymphocytes targeting Epstein-Barr virus latent membrane proteins. *J Clin Oncol* (2014) 32(8):798–808. doi:10.1200/JCO.2013.51.5304
12. Doubrovina E, Oflaz-Sozmen B, Prockop SE, Kernan NA, Abramson S, Teruya-Feldstein J, et al. Adoptive immunotherapy with unselected or EBV-specific T cells for biopsy-proven EBV+ lymphomas after allogeneic hematopoietic cell transplantation. *Blood* (2012) 119(11):2644–56. doi:10.1182/blood-2011-08-371971
13. Leen AM, Bollard CM, Mendizabal AM, Shpall EJ, Szabolcs P, Antin JH, et al. Multicenter study of banked third-party virus-specific T cells to treat severe viral infections after hematopoietic stem cell transplantation. *Blood* (2013) 121(26):5113–23. doi:10.1182/blood-2013-02-486324
14. Naik S, Nicholas SK, Martinez CA, Leen AM, Hanley PJ, Gottschalk SM, et al. Adoptive immunotherapy for primary immunodeficiency disorders with virus-specific T lymphocytes. *J Allergy Clin Immunol* (2016) 137(5):1498–505.e1. doi:10.1016/j.jaci.2015.12.1311
15. McLaughlin LP, Bollard CM, Keller M. Adoptive T cell immunotherapy for patients with primary immunodeficiency disorders. *Curr Allergy Asthma Rep* (2017) 17(1):3. doi:10.1007/s11882-017-0669-2
16. Hislop AD, Taylor GS, Sauce D, Rickinson AB. Cellular responses to viral infection in humans: lessons from Epstein-Barr virus. *Annu Rev Immunol* (2007) 25:587–617. doi:10.1146/annurev.immunol.25.022106.141553
17. Neudorfer J, Schmidt B, Huster KM, Anderl F, Schiemann M, Holzapfel G, et al. Reversible HLA multimers (Streptamers) for the isolation of human cytotoxic T lymphocytes functionally active against tumor- and virus-derived antigens. *J Immunol Methods* (2007) 320(1–2):119–31. doi:10.1016/j.jim.2007.01.001
18. Feuchtinger T, Lucke J, Hamprecht K, Richard C, Handgretinger R, Schumm M, et al. Detection of adenovirus-specific T cells in children with adenovirus infection after allogeneic stem cell transplantation. *Br J Haematol* (2005) 128(4):503–9. doi:10.1111/j.1365-2141.2004.05331.x
19. Feucht J, Joachim L, Lang P, Feuchtinger T. Adoptive T-cell transfer for refractory viral infections with cytomegalovirus, Epstein-Barr virus or adenovirus after allogeneic stem cell transplantation. *Klin Padiatr* (2013) 225(3):164–9. doi:10.1055/s-0033-1333749
20. Papadopoulou A, Gerdemann U, Katari UL, Tzannou I, Liu H, Martinez C, et al. Activity of broad-spectrum T cells as treatment for AdV, EBV, CMV, BKV, and HHV6 infections after HSCT. *Sci Transl Med* (2014) 6(242):242ra83. doi:10.1126/scitranslmed.3008825
21. Leen AM, Christin A, Myers GD, Liu H, Cruz CR, Hanley PJ, et al. Cytotoxic T lymphocyte therapy with donor T cells prevents and treats adenovirus and Epstein-Barr virus infections after haploidentical and matched unrelated stem cell transplantation. *Blood* (2009) 114(19):4283–92. doi:10.1182/blood-2009-07-232454
22. Vickers MA, Wilkie GM, Robinson N, Rivera N, Haque T, Crawford DH, et al. Establishment and operation of a good manufacturing practice-compliant allogeneic Epstein-Barr virus (EBV)-specific cytotoxic cell bank for the treatment of EBV-associated lymphoproliferative disease. *Br J Haematol* (2014) 167(3):402–10. doi:10.1111/bjh.13051
23. Wynn RF, Arkwright PD, Haque T, Gharib MI, Wilkie G, Morton-Jones M, et al. Treatment of Epstein-Barr-virus-associated primary CNS B cell lymphoma with allogeneic T-cell immunotherapy and stem-cell transplantation. *Lancet Oncol* (2005) 6(5):344–6. doi:10.1016/S1470-2045(05)70171-6
24. Withers B, Blyth E, Clancy LE, Yong A, Fraser C, Burgess J, et al. Long-term control of recurrent or refractory viral infections after allogeneic HSCT with third-party virus-specific T cells. *Blood Adv* (2017) 1:2193–205. doi:10.1182/bloodadvances.2017010223
25. Hutspardol S, Essa M, Richardson S, Schechter T, Ali M, Krueger J, et al. Significant transplantation-related mortality from respiratory virus infections within the first one hundred days in children after hematopoietic stem cell transplantation. *Biol Blood Marrow Transplant* (2015) 21(10):1802–7. doi:10.1016/j.bbmt.2015.06.015
26. Odek C, Kendirli T, Dogu F, Yaman A, Vatansever G, Cipe F, et al. Patients with primary immunodeficiencies in pediatric intensive care unit: outcomes and mortality-related risk factors. *J Clin Immunol* (2014) 34(3):309–15. doi:10.1007/s10875-014-9994-6
27. Pai SY, Logan BR, Griffith LM, Buckley RH, Parrott RE, Dvorak CC, et al. Transplantation outcomes for severe combined immunodeficiency, 2000–2009. *N Engl J Med* (2014) 371(5):434–46. doi:10.1056/NEJMoa1401177

28. Parvaneh N, Filipovich AH, Borkhardt A. Primary immunodeficiencies predisposed to Epstein-Barr virus-driven haematological diseases. *Br J Haematol* (2013) 162(5):573–86. doi:10.1111/bjh.12422
29. Kasahara Y, Yachie A. Cell type specific infection of Epstein-Barr virus (EBV) in EBV-associated hemophagocytic lymphohistiocytosis and chronic active EBV infection. *Crit Rev Oncol Hematol* (2002) 44(3):283–94. doi:10.1016/S1040-8428(02)00119-1
30. Cohen JI, Jaffe ES, Dale JK, Pittaluga S, Heslop HE, Rooney CM, et al. Characterization and treatment of chronic active Epstein-Barr virus disease: a 28-year experience in the United States. *Blood* (2011) 117(22):5835–49. doi:10.1182/blood-2010-11-316745
31. Gottschalk S, Ng CY, Perez M, Smith CA, Sample C, Brenner MK, et al. An Epstein-Barr virus deletion mutant associated with fatal lymphoproliferative disease unresponsive to therapy with virus-specific CTLs. *Blood* (2001) 97(4):835–43. doi:10.1182/blood.V97.4.835
32. Brewin J, Mancao C, Straathof K, Karlsson H, Samarasinghe S, Amrolia PJ, et al. Generation of EBV-specific cytotoxic T cells that are resistant to calcineurin inhibitors for the treatment of posttransplantation lymphoproliferative disease. *Blood* (2009) 114(23):4792–803. doi:10.1182/blood-2009-07-228387
33. Menger L, Gouble A, Marzolini MA, Pachnio A, Bergerhoff K, Henry JY, et al. TALEN-mediated genetic inactivation of the glucocorticoid receptor in cytomegalovirus-specific T cells. *Blood* (2015) 126(26):2781–9. doi:10.1182/blood-2015-08-664755
34. Abolhassani H, Edwards ES, Ikinciogullari A, Jing H, Borte S, Buggert M, et al. Combined immunodeficiency and Epstein-Barr virus-induced B cell malignancy in humans with inherited CD70 deficiency. *J Exp Med* (2017) 214(1):91–106. doi:10.1084/jem.20160849

Conflict of Interest Statement: The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

frontiers in Immunology

REVIEW
published: 04 April 2018
doi: 10.3389/fimmu.2018.00666

X-Linked Lymphoproliferative Disease Type 1: A Clinical and Molecular Perspective

Neelam Panchal[1], Claire Booth[1,2], Jennifer L. Cannons[3,4] and Pamela L. Schwartzberg[3,4]**

[1] Molecular and Cellular Immunology Section, Great Ormond Street Institute of Child Health, University College London, London, United Kingdom, [2] Department of Pediatric Immunology, Great Ormond Street Hospital for Children NHS Foundation Trust, London, United Kingdom, [3] National Human Genome Research Institute, National Institutes of Health, Bethesda, MD, United States, [4] National Institute of Allergy and Infectious Diseases, National Institutes of Health, Bethesda, MD, United States

X-linked lymphoproliferative disease (XLP) was first described in the 1970s as a fatal lymphoproliferative syndrome associated with infection with Epstein–Barr virus (EBV). Features include hemophagocytic lymphohistiocytosis (HLH), lymphomas, and dysgammaglobulinemias. Molecular cloning of the causative gene, *SH2D1A*, has provided insight into the nature of disease, as well as helped characterize multiple features of normal immune cell function. Although XLP type 1 (XLP1) provides an example of a primary immunodeficiency in which patients have problems clearing primarily one infectious agent, it is clear that XLP1 is also a disease of severe immune dysregulation, even independent of EBV infection. Here, we describe clinical features of XLP1, how molecular and biological studies of the gene product, SAP, and the associated signaling lymphocyte activation molecule family receptors have provided insight into disease pathogenesis including specific immune cell defects, and current therapeutic approaches including the potential use of gene therapy. Together, these studies have helped change the outcome of this once almost uniformly fatal disease.

Keywords: X-linked lymphoproliferative disease 1, Epstein–Barr virus, SAP (signaling lymphocyte activation molecule-associated protein), signaling lymphocytic activation molecule, primary immunodeficiency disease, hemophagocytic lymphohistiocytosis, hematopoietic stem cell transfer, gene therapy

OPEN ACCESS

Edited by:
Isabelle Meyts, KU Leuven, Belgium

Reviewed by:
Tri Giang Phan, Garvan Institute of Medical Research, Australia
Sylvain Latour, Centre National de la Recherche Scientifique (CNRS), France

****Correspondence:***
Claire Booth
c.booth@ucl.ac.uk;
Pamela L. Schwartzberg
pams@mail.nih.gov

Specialty section:
This article was submitted to Primary Immunodeficiencies, a section of the journal Frontiers in Immunology

Received: *20 November 2017*
Accepted: *19 March 2018*
Published: *04 April 2018*

Citation:
Panchal N, Booth C, Cannons JL and Schwartzberg PL (2018) X-Linked Lymphoproliferative Disease Type 1: A Clinical and Molecular Perspective. Front. Immunol. 9:666. doi: 10.3389/fimmu.2018.00666

INTRODUCTION

Epstein–Barr virus (EBV) is a highly prevalent human gamma herpes virus that is spread *via* saliva and primarily infects the oropharyngeal epithelium and B cells (1). Infection in children is usually very mild, whereas in teenager and adults, it can result in a picture of infectious mononucleosis (IM) with fevers, flu-like symptoms, and even systemic lymphoproliferative disease. Studies suggest that EBV has infected approximately 90% of adults. After infection, EBV remains latent in B cells for the remainder of the life of the host, and although most people remain asymptomatic, it can cause B cell and T cell lymphomas, Hodgkin lymphoma, and Burkitt's lymphoma in certain groups, particularly in immunocompromised patients (2).

A major and critical issue with EBV arises in the case of such immunocompromised individuals, including those presenting with monogenic deficiencies, where EBV infection leads to a wide range of clinical complications and acquired disease phenotypes (3). In this review article, we will explore the disease pathologies arising in patients with a rare form of primary immunodeficiency (PID),

X-linked lymphoproliferative disease type 1 (XLP1), which is perhaps the classic example of a PID associated with an inability to clear EBV (3–5).

EBV IN XLP DISEASE

Characterization of Early Cases

X-linked lymphoproliferative disease or Duncan's disease was described in the mid-1970s by Purtilo and colleagues in the Duncan kindred, where 6 out of 18 young males died of a lymphoproliferative disorder (6, 7). Three of these males had IM either immediately prior to or concurrent with the development of disease, which included fatal IM, hemophagocytic syndrome, and B cell malignancies, as well as humoral immune defects such as dysgammaglobulinemia. The observation of EBV-driven manifestations associated with a primary immune-deficiency catalyzed the recognition of XLP. Of note, two other contemporary reports also described families with males who succumbed to lymphoproliferative disorders and/or agammaglobulinemia associated with EBV infection, who may have had the same syndrome (8, 9).

Early investigations carried out by Purtilo and his team aimed to understand why EBV infection led to such aggressive and often fatal clinical phenotypes in these patients. In 1980, an XLP registry was established (7), which tracked presumed XLP patients with regard to disease onset and progression. The study revealed that the majority of patients had succumbed to IM due to extensive liver pathology and lymphoid infiltration of organs. However, those who did survive, as well as some EBV-negative male relatives, still progressed to develop dysgammaglobulinemia and/or B cell malignancies (10, 11). By 1995, over 270 boys were registered from over 80 kindreds (12); the overall mortality was reported as 75% with the majority of boys dying before 10 years of age, proving the severity of this condition.

The cloning of the gene, *SH2D1A,* responsible for this disease both revealed phenotypes in family members before they presented with the classic picture of EBV-induced pathology and allowed further molecular understanding of what is now called XLP type 1 (XLP1) (13–15). Clinical manifestations of XLP1 are now recognized to include a wider range of phenotypes associated with immune dysregulation even independent of EBV infection (5, 16). It should be noted that mutations in a second gene, *BIRC4*, encoding the X-linked inhibitor of apoptosis, XIAP, have been described in a subset of XLP patients who did not carry mutations in *SH2D1A* (now referred to as having XLP2) (17). However, XLP2 is more closely associated with EBV-driven hemophagocytic lymphohistiocytosis (HLH), as well as other clinical features not found consistently in XLP1 such as splenomegaly and colitis and will not be discussed further in this review (17, 18).

Clinical Features

The main clinical features of XLP1 remain HLH, dysgammaglobulinemia, and lymphoma but other described manifestations include aplastic anemia, vasculitis, chronic gastritis, and skin lesions (12, 19–24). HLH is the most common and lethal presentation, tending to occur early in childhood and associated with significant mortality, with a proportion of patients succumbing before hematopoietic stem cell transplant (HSCT) (16). HLH is a multisystem syndrome caused by hyperinflammation resulting in immune dysregulation, tissue damage, and often multiorgan failure. The main features are fever, cytopenias, and hepatosplenomegaly but involvement of other organs is often seen. Diagnostic criteria are available (25).

Up to 50% of patients demonstrate a range of humoral immune abnormalities, ranging from impaired vaccine responses to generalized hypo-gammaglobulinemia (10, 12, 16). These may be incidental findings during diagnostic workup or lead to recurrent infections, particularly respiratory infections. Almost a third of patients develop lymphoma with the most common form being abdominal B cell non-Hodgkin lymphoma in both EBV+ and EBV− patients; prognosis has dramatically improved over the decades due to improved chemotherapy protocols.

Analyses of mutations have revealed deletions, splice site, nonsense, and missense changes in *SH2D1A*, but so far, there has not been a clear correlation between mutations and the severity of phenotypes identified (16, 26). Patients can progress from one phenotype to another, and different clinical features are seen within members of the same family. However, in some cases, second-site reversions of missense and nonsense mutations have been found, which were associated with restored CD8 cell function in a small fraction of cells and less severe phenotypes (27).

It is important to highlight that up to 35% of patients have no evidence of previous EBV infection; many of these patients are diagnosed based on family history (16, 28, 29). In EBV− patients, XLP1 is associated with higher rates of dysgammaglobulinemia (51.8 vs 37.2% for EBV+) and lymphoma [25 vs 19.6% for EBV+, see Table 2 from Ref. (16)]. However, EBV-negative boys with XLP1 can still develop HLH, although less frequently than those with EBV infection (21.4 vs 51% for EBV+) (16), and the trigger is unknown. Thus, XLP1 must be thought of as a disorder of immune dysregulation not only triggered by EBV. Nonetheless, there are no reports in the literature of a specific pathogen other than EBV being linked to HLH or other clinical features, arguing that XLP1 patients are specifically susceptible to EBV rather than other pathogens.

The overall mortality of the disease has reduced significantly since first reports from the registry, from 75 to 29% (16), largely due to improved chemotherapy and HSCT protocols, as well as improved monitoring and supportive care (which will be discussed later in this review). However, patients diagnosed at birth through family history still risk significant mortality despite close monitoring, highlighting the severity of this PID.

MOLECULAR INSIGHT INTO PHENOTYPES OF XLP1

Improved description of patient cohorts combined with the evolution of molecular techniques has widened our understanding of XLP1. However, equally important has been the investigation of the genetic cause of XLP1 and how lymphocyte development and function are affected by mutations of *SH2D1A* (4, 30).

Cloning of the Gene and Studies of SAP-Mediated Signaling

In 1998, three groups identified a gene, now known as *SH2D1A*, that was mutated in patients with XLP. While two groups identified this gene by positional cloning (13, 31), a third group independently identified the same gene as encoding a small adaptor molecule that bound to the cytoplasmic tail of a T cell costimulatory protein, signaling lymphocyte activation molecule (SLAM) (14). Genetic mapping and sequencing revealed that this gene was mutated in samples from several XLP patients (14). The identification of *SH2D1A* has helped identify patients with the disease, but has also led to new insight into the signaling pathways regulated by SLAM family members and how they contribute to the pathophysiology of XLP1 (4, 5, 32).

The evaluation of the predicted gene product revealed that *SH2D1A* encodes a small (14 kDa/128 aa) protein that is now known as SAP, or SLAM-associated protein (14). Intriguingly, SAP consists almost entirely of a single Src Homology 2 (SH2) domain, a conserved protein interaction module that binds to phosphotyrosine-based motifs. SH2 domains are usually part of larger multi-domain proteins involved in signaling pathways, including adaptor molecules that contain multiple protein–protein and/or protein–lipid interaction domains and enzymes such as kinases and phosphatases that are regulated by intra- and intermolecular SH2-protein interactions (33). Further experiments demonstrated that the SH2 domain of SAP bound specific tyrosines on the intracellular tail of SLAM and related receptors (34, 35). However, these observations raised questions on how a single protein interaction domain could regulate signaling and how the disruption of SAP expression led to phenotypes associated with XLP1.

Although SAP was first identified by virtue of its association with SLAM, a costimulatory receptor that helps regulate interferon gamma cytokine production by T cells, SAP is now known to bind to a series of related receptors, the SLAM family, which include SLAM/CD150 (SLAMF1), LY9/CD229 (SLAMF3), 2B4/CD244 (SLAMF4), CD84 (SLAMF5), NTB-A/Ly108/CD352 (SLAMF6), and CRACC/CD319 (SLAMF7) (36). These receptors are encoded in a highly polymorphic gene cluster on human and mouse chromosome 1, variants of which have been associated with predispositions to autoimmunity (37). With the exception of 2B4, these receptors are self-ligands and are activated by homophilic interactions (30, 36). The SLAM family also has homology to the larger CD2 superfamily of immunoglobulin domain containing receptors, which include CD48/SLAMF2 (the ligand for 2B4/SLAMF4). SLAM receptors exhibit a broad expression on hematopoietic cells; however, several members are most highly expressed on B cells (38–41), a feature that likely contributes to some of the B cell-specific phenotypes of XLP1 (42). By contrast, although some B cell expression has also been reported (35, 43, 44), SAP is most highly expressed in T and NK cells and is therefore most likely to affect SLAM family function in these cells (14, 45). Several of the SLAM family members, including 2B4/SLAMF4, NTB-A/SLAMF6, and CRACC/SLAMF7, have been implicated as cytotoxic receptors in NK and CD8 cells (30).

Extensive work on SAP-mediated signaling pathways provided evidence that SAP serves as a molecular switch allowing SLAM family members to act as either activating receptors in the presence of SAP or inhibitory receptors in the absence of SAP (**Figure 1**) (30, 35, 36, 46, 47). Thus, when SAP is present, it can recruit the FYN tyrosine kinase, leading to further tyrosine phosphorylation of SLAM family members (48–50) and interactions with other signaling molecules, including RasGAP, Shc, Dok1, and Dok2 in the case of SLAM (51) and Vav1 and c-Cbl in the case of 2B4 and Ly108 (41, 45, 52). Notably, Fyn deficiency can phenocopy some features of SAP deficiency including defects in 2B4-mediated killing (50). SAP has also been shown to inhibit diacylglycerol kinase-α (DGKα), a molecule that negatively affects TCR signaling (53). However, when SAP is not expressed, the same tyrosines on SLAM family members bind a number of strong inhibitory molecules, including the tyrosine phosphatases SHP1 and SHP2, as well as the lipid phosphatase SHIP (35, 41, 46, 47, 54). These inhibitory molecules essentially block aspects of T and NK cell activation, development and function when SLAM family members are engaged in the absence of SAP. Accordingly, the tyrosine-based motif that SAP recognizes has been coined an "ImmunoTyrosine Switch Motif" or ITSM (35).

Early data provided evidence that NK and CD8 cells from XLP patients exhibited defective killing of EBV-infected B cells (55, 56); this was linked to impaired killing *via* 2B4/SLAMF4 and NTB-A/SLAMF6 (57–60). Intriguingly, some data demonstrated that in the absence of SAP, 2B4/SLAMF4 prevented the killing of EBV-infected cells, providing further evidence that the SLAM family could act as inhibitory receptors (58). Combined with the biochemical evidence for the inhibitory function of SLAM family receptors, these results provided insight into why XLP1 patients have specific susceptibility to EBV infection. More recently, T cells from XLP1 patients have been found to exhibit defects in reactivation-induced cell death (RICD), resulting from inhibitory signaling from NTB-A/SLAMF6. This defect has been proposed to contribute to lymphoproliferation seen in XLP1 (61).

Insight From Mouse Models

The generation and study of SAP-deficient mice (62–64) has provided insight into additional phenotypes associated with SAP deficiency, some of which have subsequently been confirmed in XLP1 patients. One of these is a lack of invariant NKT cells, a rare innate type of T lymphocyte that rapidly responds to infection and may be involved in tumor surveillance—this defect was recognized due to the connection with Fyn, which also affects NKT cell development in mice (65–67). Whether and how the absence of NKT cells contributes to manifestations of XLP1 remains less well understood, but it is now appreciated that XLP1 patients exhibit an absolute lack of iNKT cells, independent of EBV infection status. The critical role of SAP in iNKT development is supported by studies of *SH2D1A* carriers demonstrating random X-inactivation in T and B cells but non-random X-inactivation in iNKT cells, suggesting an absolute requirement of SAP for the development of this population (66).

Another major phenotype is the lack of long-term humoral (antibody) responses and memory B cells, which have been observed both in response to infection and to immunization in SAP-deficient mice (62, 64, 68–70). These phenotypes were T cell intrinsic and associated with impaired formation of germinal centers (GCs) (68, 70), the site where B cells undergo immunoglobulin

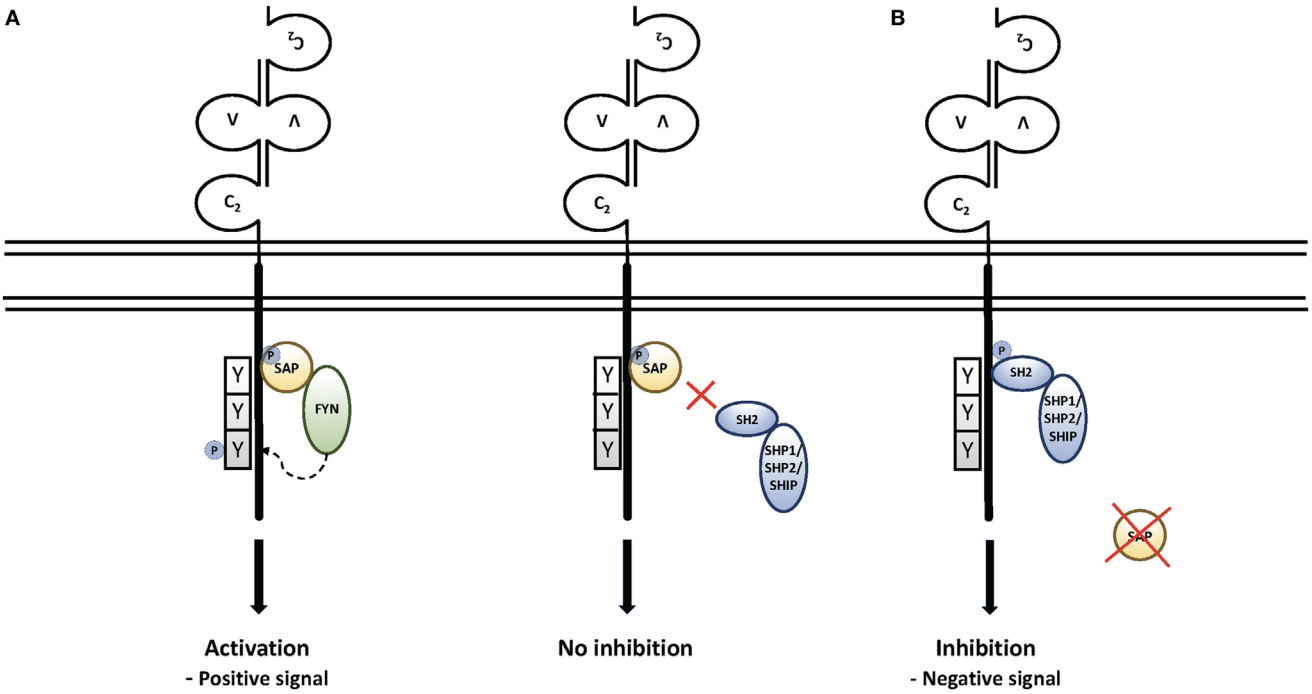


FIGURE 1 | Signaling through the signaling lymphocyte activation molecule (SLAM) family receptors: **(A)** upon engagement, SLAM family members recruit the SAP SH2 domain to immunotyrosine switch (ITSM) motifs on their intracellular tails. This leads to the recruitment of Src family kinases, further phosphorylation of SLAM family receptors, and recruitment of downstream signaling molecules. Recruitment of SAP also prevents recruitment of the phosphatases SHP1, SHP2, and the lipid phosphatase SHIP (50). **(B)** In the absence of SAP, the engagement of SLAM family receptors leads to SH2-mediated recruitment of SHP1, SHP2, and SHIP, which are associated with strong inhibitory signals that affect T and NK cell function and cell death, as well as NKT cell development.

gene class-switching and hypermutation in response to antigen in the context of contact-dependent signals from specific CD4 T helper lymphocytes, now known as follicular T helper (Tfh) cells (71). The GC is also the site where most memory B cells and long-lived plasma cells are derived. Subsequent evaluation of XLP1 patients revealed that they also lacked IgG^+ memory ($CD27^+$) B cells, and an autopsy confirmed a lack of GCs in lymph nodes from an XLP1 patient (72). Interestingly, in addition to the well-documented dysgammaglobulinemia in XLP1 patients, evidence of impaired responses to protein immunization had been reported (73). However, the characterization of SAP-deficient mice has provided a clearer picture of the nature of these humoral defects (74).

Further insight into these phenotypes came from intravital microscopy in mice, which revealed that SAP-deficient T cells exhibited impaired adhesion to B cells, a defect that was confirmed using *in vitro* flow-based cell conjugation assays (75). This defect was relatively specific, as that adhesion to antigen-presenting dendritic cells was less affected. The B cell specificity correlated with very high levels of the expression of multiple SLAM family members including SLAMF6 (Ly108/NTB-A), SLAMF5 (CD84), and CD48 (the ligand for 2B4) on activated B cells (38, 40, 42). In the absence of SAP, some of these ligands trigger an inhibitory response in SAP-deficient T cells, preventing full activation by and adhesion to B cells, likely by affecting TCR-induced inside-out signaling to integrins (76, 77).

Consistent with these observations, SAP-deficient T cells are initially activated normally by antigen-presenting dendritic cells in response to immunization and infection, but fail to form mature Tfh cells, a process now recognized to require B cell interactions (75, 78, 79). Indeed, insight into the critical role of Tfh cells in humoral immunity has been greatly advanced by studies of SAP-deficient mice. Such findings further suggested that defective adhesion to B cells was likely to contribute to the inability of SAP-deficient T cells to provide contact-dependent help for GC generation and long-term humoral immunity and thus the dysgammaglobulinemias seen in XLP1 (42, 75).

Moreover, the observation of defective interactions with B cells has provided mechanistic insight into other phenotypes of XLP1, many of which share a common feature of B cell involvement (**Figure 2**). SAP-deficient CD8 cells exhibit defective adhesion to and killing of activated B cell targets (39–41, 80), especially EBV-transformed cells, which express high levels of certain SLAM family members and CD48. Thus, the sensitivity to EBV may occur because EBV primarily infects B cells. Impaired immunosurveillance of B cell malignancies may contribute to the increased incidence of lymphomas, even in the absence of EBV infection (16, 42). Since defective CD8 and NK cell cytolysis have been linked to HLH, defects in killing EBV-infected B cells may trigger this phenotype as well (81), although the exact mechanism by which HLH develops in this population is yet to be elucidated. Moreover, since other hematopoietic cells also express SLAM

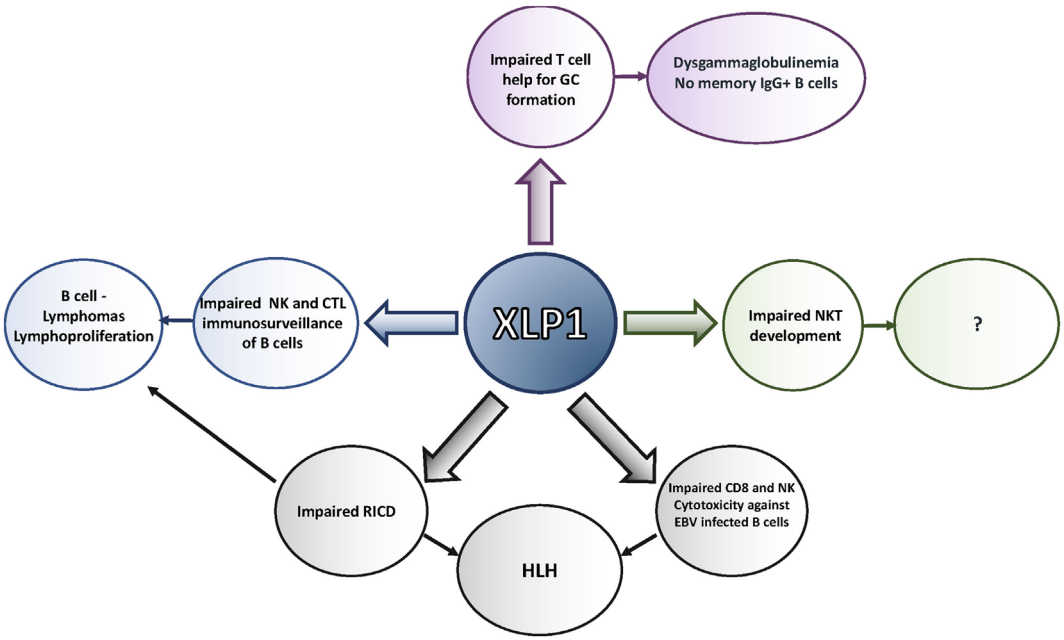


FIGURE 2 | Defects seen in X-linked lymphoproliferative disease (XLP)1. XLP1 is associated with multiple T and NK cellular defects, many of which may result from impaired interactions with B cells and other cells that express high levels of signaling lymphocyte activation molecule (SLAM) family members. These defects contribute to the phenotypes observed in the disease, including the inability to clear Epstein–Barr virus (EBV), lymphoproliferation and lymphoma, hemophagocytic lymphohistiocytosis (HLH), and antibody defects.

family members, defects may be extended to cytolysis of other hematopoietic targets; defects in NK cell cytolysis of multiple hematopoietic cell tumor lines that express SLAM family members have been observed (82). Nonetheless, it is also of note that NK cells deficient in SAP can kill non-hematopoietic cell targets better, perhaps accounting for the lack of increases in other types of cancer in XLP1 (83). Finally, the absence of NKT cells may result from impaired interactions between lymphocytes, as that NKT cells are not selected on the thymic stroma, but rather on double-positive thymocytes that express high levels of SLAM family members (84).

It is of note that the effects of SLAM family receptor mutations for the most part do not phenocopy those of SAP deficiency (85). Moreover, although polymorphisms of SLAM family members are associated with autoimmunity in lupus-prone mouse strains and humans (37), and with alterations in NKT cell numbers in NOD (non-obese diabetic) mice (86), to date, there have been no reports of immunodeficiency or EBV susceptibility associated with mutations of other SLAM family members in humans (36). Instead, many of the phenotypes of SAP deficiency appear to be related to inhibitory signals generated by SLAM family members in the absence of SAP (39, 76, 85), which are most strongly triggered by B cell interactions, either as targets (for cytolysis) or as antigen presentation (GC formation). Notably, unlike positive signaling, these inhibitory signals would not be expected to rely on the ability of SAP to recruit Fyn. Indeed, several phenotypes associated with SAP deficiency can be rescued by the expression of a mutant of SAP that cannot recruit Fyn but can still block the recruitment of inhibitory molecules. These include humoral defects, NK cell killing, and NK cell education (54, 70, 78, 83), although some phenotypes such as 2B4-mediated killing and NKT cell development may result from defects in both positive and negative signals since Fyn deficiency also impairs these processes (50, 87). Importantly, unlike positive signaling, these negative signals require the presence of SLAM family members to recruit phosphatases and manifest their inhibitory function (**Figure 1**). Thus, inhibiting interactions of the SLAM family members 2B4/SLAMF4 and NTB-A/SLAMF6 with their ligands actually improves cytolysis of B cells by SAP-deficient CD8 cells and NK cells (39, 41, 58). These observations suggest the intriguing possibility that blocking antibodies to SLAM family receptors might ameliorate some of the clinical manifestations of this disorder, raising the possibility of tailored SLAM family-based pharmacological approaches to XLP1 (see below). Support for this hypothesis can be found in murine genetic studies where mutations disrupting the expression of Ly108/SLAMF6 improved both the GC defect and NKT development in SAP-deficient mice (76).

CURRENT TREATMENT AND MANAGEMENT OPTIONS FOR XLP1

Given the severe morbidity and high rates of mortality in XLP1, it is strongly recommended that genetic screening and counseling be carried out in families with a history of XLP1 (5). Diagnosis is confirmed using flow cytometric analyses of SAP expression (88) followed by Sanger sequencing of the *SH2D1A* gene. Immunological status is assessed with focus on immunoglobulin levels and response to vaccines.

Currently, the only definitive treatment available for XLP1 patients is allogeneic HSCT (16). However, depending on clinical features, less aggressive treatments may be adopted, particularly if a suitable donor for transplant is not available. As many patients do not present with all symptoms simultaneously or at varying severity, there are a number of treatment options that target specific clinical phenotypes.

Treatment Approaches

Treatment of XLP1 is tailored to particular clinical symptoms and supportive care. However, close monitoring (e.g., of EBV viral loads) is important in this patient cohort to allow the prevention of recurrent infections, organ damage such as bronchiectasis, and permit early treatment of EBV infection and more serious complications. If there is evidence of EBV-driven disease, including HLH, treatment with a monoclonal anti-CD20 antibody (rituximab) can be used to deplete the B cell population harboring the virus (89). This approach is effective at reducing and often clearing the viremia but risks the effects of B cell depletion, including exacerbating long-term hypo-gammaglobulinemia. Antiviral agents are poorly effective against EBV but acyclovir has been used in some circumstances. Infection of T cells with EBV is also seen in XLP1 patients (unpublished data) and the use of rituximab in this situation may not be helpful.

Hemophagocytic lymphohistiocytosis is treated according to standardized protocols (HLH 94 and 2004) based on the use of dexamethasone, etoposide, and cyclosporin with the addition of intrathecal methotrexate and steroids if there is neurological involvement (90, 91). This is a highly suppressive regime and can be associated with significant toxicity. The protocol follows different stages, starting with an intense period of treatment initially, with reducing doses of steroids and frequency of etoposide over time if a response is seen. Re-intensification of therapy is occasionally required. This protocol aims to achieve remission of HLH, usually prior to moving swiftly to HSCT, but the mortality associated with this presentation is still over 60% (16). Other immunosuppressive agents have been used to control HLH, either in combination with steroids or as rescue therapy, including ATG (anti-thymocyte globulin) in combination with etoposide in the HIT (hybrid ImmunoTherapy)-HLH trial (NCT01104025), or Alemtuzumab (Campath/anti CD52 antibody). In addition, newer biologics are now available, and some are being tested in HLH including Toculizumab (anti-IL6R antibody). An anti-interferon gamma monoclonal antibody (Novimmune NI-0501) is now in trial in the USA and Europe with results eagerly awaited. The JAK1/2 inhibitor Ruxolitinib has shown promise in preclinical murine studies and is now also moving toward the clinic (92).

These more targeted therapies could offer an improved toxicity profile, which may be extremely beneficial to help transition patients rapidly to HSCT with as little organ damage and infectious complications as possible and thereby afford better outcomes post transplant. Lymphoma is also treated according to standardized protocols, and again mortality associated with this presentation has reduced over the years.

Patients with dysgammaglobulinemia or recurrent infections may benefit from immunoglobulin replacement therapy which can be delivered *via* intravenous route every few weeks, or subcutaneously every week, which is usually performed at home. Other manifestations of dysregulation such as aplastic anemia or vasculitis may respond to steroid therapy or other immunosuppressive agents.

Stem Cell Transplantation

Bone marrow (BM) or HSC transplantation (which includes the transfer of BM, mobilized $CD34^+$ cells from peripheral blood or umbilical cord-derived $CD34^+$ cells) is currently the only definitive treatment for XLP1; survival for untransplanted patients is below 20% (16). However, success is dependent on the availability of an appropriate donor who is human leukocyte antigen matched (16). There are a number of factors to consider prior to HSCT, including the disease status, previous treatments, and the type of pre-conditioning regimen. An EBV-positive donor is preferred in patients with EBV-driven disease.

Several studies have evaluated the clinical outcomes of patients undergoing HSCT using either myeloablative-conditioning regimens or reduced-intensity-conditioning (RIC) regimens (16, 93, 94). These studies revealed similar overall patient survival rates post transplantation between RIC and myeloablative protocols, with both averaging ~80% (16, 94). However, success rates drop depending on the presence of active HLH at the time of transplant (falling to 50%) and in the context of a mismatched donor (16). From this large cohort, all patients who died post HSCT had evidence of HLH.

Thus, although the survival in XLP1 has improved significantly over time, it remains a potentially fatal condition. The decision to undertake an HSCT in an asymptomatic patient requires intensive discussion with the family to understand both risks and benefits, especially when a mismatched donor is the available choice. However, many families are faced with severe complications at presentation, such as HLH or lymphoma, which necessitate a rapid move to HSCT.

Potential Future Therapies

SLAM Family Inhibitors

In the absence of SAP, the recruitment of phosphatases and other inhibitory signaling molecules convert SLAM family members into inhibitory receptors (4). This is particularly relevant for SLAMF4/2B4/CD244 and SLAMF6/NTB-A, which strongly inhibit CD8 and NK cell killing of B cell targets in the absence of SAP. Preventing SLAMF4/2B4 and/or SLAMF6/NTB-A engagement, either through genetic knockouts of these receptors in mice or through the use of blocking antibodies with human cells, can rescue phenotypes associated with SAP deficiency, including the defective killing of B cell targets, the absence of GC formation, defective NKT cell development, NK cell education, and impaired RICD (39, 41, 61, 76, 83). Limiting the homophilic interactions of SLAM family receptors (or in the case of SLAMF4/2B4, interactions with its ligand, CD48) in XLP1 patients may therefore prevent lymphoproliferation and other phenotypes of XLP1. *In vitro* experiments have provided evidence that blocking antibodies against CD48 and NTB-A rescue killing of EBV-infected targets, supporting the concept of humanized blocking antibodies as a potentially useful therapy (39). Alternatively, peptide(s) or small molecules with a

high affinity for the different SLAM receptors might block SLAM family interactions and the initiation of a negative signal.

Other potential therapeutic approaches include the use of small molecule inhibitors of signaling pathways affected by SAP and SLAM family members. The inhibition of SHP1/SHP2 rescued cytolysis of B cell targets *in vitro* using murine cells (41). Other data suggest that the inhibition of DGKα, another negative regulator of T cell activation that is affected by SAP, can rescue certain phenotypes associated with SAP deficiency, including RICD and hyperproliferative responses to lymphochoriomeningitis virus in mice (53, 95). However, none of these approaches are curative, and toxicity may be a major issue, particularly for long-term treatment.

Gene Therapy

Over the last few years, there have been great strides developing effective and safe hematopoietic stem cell gene therapy as a viable alternative to BM transplantation for a number of PIDs. Gene therapy also offers the advantages of reduced toxicity from conditioning as, in general, less chemotherapy is required and the use of autologous cells removes the risk of graft versus host disease which causes significant morbidity and mortality post HSCT (96, 97). Although several first-generation gene therapy trials were marred by the integration of gammaretroviral vectors near proto-oncogenes leading to leukemia and myelodysplasia, newer self-inactivating (16) retroviruses and lentiviruses have been developed that use internal mammalian promoters to drive transgene expression. Numerous clinical trials are underway using these later generation vectors, and no insertional events have been reported to date.

A proof of concept for gene therapy for XLP1 was established using such a second-generation lentiviral vector containing the human elongation factor 1 alpha promoter to drive codon-optimized SAP gene expression (98). This study utilized a SAP-deficient murine model into which gene-corrected hematopoietic progenitor cells were infused following lethal irradiation. The transfer of gene-corrected cells led to the restoration of NK and CD8 T cell cytotoxicity, NKT development, as well as GC formation and function upon immunological challenge. However, although no adverse effects of SAP expression at the stem cell level were seen in these studies, SAP is a tightly regulated signaling protein that is predominately expressed in T cells (14, 45), and the use of a ubiquitous human promoter that can drive expression in all hematopoietic cells may not be optimal.

An alternative approach to more directly address the T cell-dependent clinical manifestations of XLP1 is to develop a therapeutic strategy using gene-corrected autologous patient T cells. Murine studies utilizing gene-modified T cell transfers into *Sh2d1a*$^{-/y}$ mice demonstrated the correction of Tfh cell function, the restoration of GCs, and the improvement in baseline immunoglobulin levels (Panchal et al., in press). In addition, the correction of CD8^{+} T cell function was shown using an *in vivo* tumor model. These data provide a strong case that adoptive T gene therapy may be a useful therapeutic option.

Gene Editing

Along with developments in gene therapy, the latter part of this decade has seen great advancements in the use of gene-editing platforms for therapeutic benefits (96, 99–101). Zinc finger nucleases have been established to be effective in eliminating CCR5 expression on T cells from HIV-infected individuals in order to prevent viral spread (102, 103). Transcription activator-like effector nucleases and CRISPR/Cas9 nuclease systems have been used for TCR knockdowns as part of CAR-T cell therapy, to produce an "off the shelf" donor T cell product for the treatment of CD19^{+} B cell leukemias (104, 105). Gene-editing platforms hold great promise for the effective correction of endogenous genes using corrected DNA copies as donor templates, utilizing the cell's own DNA repair machinery. This approach may be particularly beneficial for monogenic diseases such as XLP1 that can present with point mutations in the gene. Gene editing also offers a resolution to the issue of gene regulation and the risk of overexpression in anomalous hematopoietic compartments and could significantly improve the safety profile of genetically engineered cellular therapy (100, 106). However, this type of therapy needs to be custom-designed to repair the genetic defect of each patient and may not be useful for patients with gene deletions. Potential off-target effects also need to be carefully evaluated. Nonetheless, such approaches hold high potential for the treatment of PIDs.

SUMMARY

Over the last 30 years, the outcome for patients with XLP1 has significantly improved, mainly due to improvements in the treatment of clinical manifestations such as HLH and lymphoma. Survival post HSCT has also improved, but mortality associated with active disease at the time of transplant and mismatched donor settings remains significant. As our understanding of the molecular and cellular pathology in XLP1 continues to expand, novel treatments, including gene therapy, will continue to be developed, hopefully leading to even greater improved outcomes for patients with this devastating disease.

AUTHOR CONTRIBUTIONS

NP, CB, JC, and PS all contributed to the writing and editing of this manuscript. NP and PS generated the figures.

ACKNOWLEDGMENTS

The authors would like to thank the patients, clinicians, and scientists who have contributed to our knowledge of XLP1 and apologize to those we have not fully cited due to space. The authors also thank members of the Schwartzberg and Booth laboratories for their assistance in this work.

FUNDING

This work was supported in part by intramural funding from the National Human Genome Research Institute and the National Institutes of Allergy and Infectious Diseases, National Institutes of Health (PS and JC), and the Great Ormond Street Hospital Children's Charity (CB and NP).

REFERENCES

1. Cohen JI. Epstein–Barr virus infection. *N Engl J Med* (2000) 343(7):481–92. doi:10.1056/NEJM200008173430707
2. Thorley-Lawson DA, Gross A. Persistence of the Epstein–Barr virus and the origins of associated lymphomas. *N Engl J Med* (2004) 350(13):1328–37. doi:10.1056/NEJMra032015
3. Worth AJ, Houldcroft CJ, Booth C. Severe Epstein–Barr virus infection in primary immunodeficiency and the normal host. *Br J Haematol* (2016) 175(4):559–76. doi:10.1111/bjh.14339
4. Tangye SG. XLP: clinical features and molecular etiology due to mutations in SH2D1A encoding SAP. *J Clin Immunol* (2014) 34(7):772–9. doi:10.1007/s10875-014-0083-7
5. Zhang K, Wakefield E, Marsh R. Lymphoproliferative disease, X-linked. In: Adam MP, Ardinger HH, Pagon RA, Wallace SE, Bean LJH, Stephens K, et al., editors. *GeneReviews(R)*. Seattle: University of Washington, Seattle University of Washington (1993).
6. Purtilo DT, Cassel CK, Yang JP, Harper R. X-linked recessive progressive combined variable immunodeficiency (Duncan's disease). *Lancet* (1975) 1(7913):935–40. doi:10.1016/S0140-6736(75)92004-8
7. Purtilo DT. Immunopathology of X-linked lymphoproliferative syndrome. *Immunol Today* (1983) 4(10):291–7. doi:10.1016/0167-5699(83)90139-1
8. Bar RS, DeLor CJ, Clausen KP, Hurtubise P, Henle W, Hewetson JF. Fatal infectious mononucleosis in a family. *N Engl J Med* (1974) 290(7):363–7. doi:10.1056/NEJM197402142900704
9. Provisor AJ, Iacuone JJ, Chilcote RR, Neiburger RG, Crussi FG. Acquired agammaglobulinemia after a life-threatening illness with clinical and laboratory features of infectious mononucleosis in three related male children. *N Engl J Med* (1975) 293(2):62–5. doi:10.1056/NEJM197507102930202
10. Grierson H, Purtilo DT. Epstein–Barr virus infections in males with the X-linked lymphoproliferative syndrome. *Ann Intern Med* (1987) 106(4):538–45. doi:10.7326/0003-4819-106-4-538
11. Harrington DS, Weisenburger DD, Purtilo DT. Malignant lymphoma in the X-linked lymphoproliferative syndrome. *Cancer* (1987) 59(8):1419–29. doi:10.1002/1097-0142(19870415)59:8<1419::AID-CNCR2820590807>3.0.CO;2-P
12. Seemayer TA, Gross TG, Egeler RM, Pirruccello SJ, Davis JR, Kelly CM, et al. X-linked lymphoproliferative disease: twenty-five years after the discovery. *Pediatr Res* (1995) 38(4):471–8. doi:10.1203/00006450-199510000-00001
13. Coffey AJ, Brooksbank RA, Brandau O, Oohashi T, Howell GR, Bye JM, et al. Host response to EBV infection in X-linked lymphoproliferative disease results from mutations in an SH2-domain encoding gene. *Nat Genet* (1998) 20(2):129–35. doi:10.1038/2424
14. Sayos J, Wu C, Morra M, Wang N, Zhang X, Allen D, et al. The X-linked lymphoproliferative-disease gene product SAP regulates signals induced through the co-receptor SLAM. *Nature* (1998) 395(6701):462–9. doi:10.1038/26683
15. Nichols KE, Ma CS, Cannons JL, Schwartzberg PL, Tangye SG. Molecular and cellular pathogenesis of X-linked lymphoproliferative disease. *Immunol Rev* (2005) 203:180–99. doi:10.1111/j.0105-2896.2005.00230.x
16. Booth C, Gilmour KC, Veys P, Gennery AR, Slatter MA, Chapel H, et al. X-linked lymphoproliferative disease due to SAP/SH2D1A deficiency: a multicenter study on the manifestations, management and outcome of the disease. *Blood* (2011) 117(1):53–62. doi:10.1182/blood-2010-06-284935
17. Rigaud S, Fondanèche MC, Lambert N, Pasquier B, Mateo V, Soulas P, et al. XIAP deficiency in humans causes an X-linked lymphoproliferative syndrome. *Nature* (2006) 444(7115):110–4. doi:10.1038/nature05257
18. Pachlopnik Schmid J, Canioni D, Moshous D, Touzot F, Mahlaoui N, Hauck F, et al. Clinical similarities and differences of patients with X-linked lymphoproliferative syndrome type 1 (XLP-1/SAP deficiency) versus type 2 (XLP-2/XIAP deficiency). *Blood* (2011) 117(5):1522–9. doi:10.1182/blood-2010-07-298372
19. Rougemont AL, Fournet JC, Martin SR, de Saint-Basile G, Latour S, Primeau MN, et al. Chronic active gastritis in X-linked lymphoproliferative disease. *Am J Surg Pathol* (2008) 32(2):323–8. doi:10.1097/PAS.0b013e318141fca1
20. Talaat KR, Rothman JA, Cohen JI, Santi M, Choi JK, Guzman M, et al. Lymphocytic vasculitis involving the central nervous system occurs in patients with X-linked lymphoproliferative disease in the absence of Epstein–Barr virus infection. *Pediatr Blood Cancer* (2009) 53(6):1120–3. doi:10.1002/pbc.22185
21. Loeffel S, Chang CH, Heyn R, Harada S, Lipscomb H, Sinangil F, et al. Necrotizing lymphoid vasculitis in X-linked lymphoproliferative syndrome. *Arch Pathol Lab Med* (1985) 109(6):546–50.
22. Kanegane H, Ito Y, Ohshima K, Shichijo T, Tomimasu K, Nomura K, et al. X-linked lymphoproliferative syndrome presenting with systemic lymphocytic vasculitis. *Am J Hematol* (2005) 78(2):130–3. doi:10.1002/ajh.20261
23. Dutz JP, Benoit L, Wang X, Demetrick DJ, Junker A, de Sa D, et al. Lymphocytic vasculitis in X-linked lymphoproliferative disease. *Blood* (2001) 97(1):95–100. doi:10.1182/blood.V97.1.95
24. Mejstríková E, Janda A, Hrusák O, Bucková H, Vlcková M, Hancárová M, et al. Skin lesions in a boy with X-linked lymphoproliferative disorder: comparison of 5 SH2D1A deletion cases. *Pediatrics* (2012) 129(2):e523–8. doi:10.1542/peds.2011-0870
25. Freeman HR, Ramanan AV. Review of haemophagocytic lymphohistiocytosis. *Arch Dis Child* (2011) 96(7):688–93. doi:10.1136/adc.2009.176610
26. Sumegi J, Seemayer TA, Huang D, Davis JR, Morra M, Gross TG, et al. A spectrum of mutations in SH2D1A that causes X-linked lymphoproliferative disease and other Epstein–Barr virus-associated illnesses. *Leuk Lymphoma* (2002) 43(6):1189–201. doi:10.1080/10428190290026240
27. Palendira U, Low C, Bell AI, Ma CS, Abbott RJ, Phan TG, et al. Expansion of somatically reverted memory CD8$^+$ T cells in patients with X-linked lymphoproliferative disease caused by selective pressure from Epstein–Barr virus. *J Exp Med* (2012) 209(5):913–24. doi:10.1084/jem.20112391
28. Gilmour KC, Cranston T, Jones A, Davies EG, Goldblatt D, Thrasher A, et al. Diagnosis of X-linked lymphoproliferative disease by analysis of SLAM-associated protein expression. *Eur J Immunol* (2000) 30(6):1691–7. doi:10.1002/1521-4141(200006)30:6<1691::AID-IMMU1691>3.0.CO;2-K
29. Sumegi J, Huang D, Lanyi A, Davis JD, Seemayer TA, Maeda A, et al. Correlation of mutations of the SH2D1A gene and Epstein–Barr virus infection with clinical phenotype and outcome in X-linked lymphoproliferative disease. *Blood* (2000) 96(9):3118–25.
30. Cannons JL, Tangye SG, Schwartzberg PL. SLAM family receptors and SAP adaptors in immunity. *Annu Rev Immunol* (2011) 29:665–705. doi:10.1146/annurev-immunol-030409-101302
31. Nichols KE, Harkin DP, Levitz S, Krainer M, Kolquist KA, Genovese C, et al. Inactivating mutations in an SH2 domain-encoding gene in X-linked lymphoproliferative syndrome. *Proc Natl Acad Sci U S A* (1998) 95(23):13765–70. doi:10.1073/pnas.95.23.13765
32. Cannons JL, Schwartzberg PL. SAP and lessons learned from a primary immunodeficiency. *J Immunol* (2017) 199(5):1531–3. doi:10.4049/jimmunol.1701007
33. Pawson T, Nash P. Protein–protein interactions define specificity in signal transduction. *Genes Dev* (2000) 14(9):1027–47.
34. Poy F, Yaffe MB, Sayos J, Saxena K, Morra M, Sumegi J, et al. Crystal structures of the XLP protein SAP reveal a class of SH2 domains with extended, phosphotyrosine-independent sequence recognition. *Mol Cell* (1999) 4(4):555–61. doi:10.1016/S1097-2765(00)80206-3
35. Shlapatska LM, Mikhalap SV, Berdova AG, Zelensky OM, Yun TJ, Nichols KE, et al. CD150 association with either the SH2-containing inositol phosphatase or the SH2-containing protein tyrosine phosphatase is regulated by the adaptor protein SH2D1A. *J Immunol* (2001) 166(9):5480–7. doi:10.4049/jimmunol.166.9.5480
36. Wu N, Veillette A. SLAM family receptors in normal immunity and immune pathologies. *Curr Opin Immunol* (2016) 38:45–51. doi:10.1016/j.coi.2015.11.003
37. Chan AY, Westcott JM, Mooney JM, Wakeland EK, Schatzle JD. The role of SAP and the SLAM family in autoimmunity. *Curr Opin Immunol* (2006) 18(6):656–64. doi:10.1016/j.coi.2006.09.010
38. Cannons JL, Qi H, Lu KT, Dutta M, Gomez-Rodriguez J, Cheng J, et al. Optimal germinal center responses require a multistage T cell:B cell adhesion process involving integrins, SLAM-associated protein, and CD84. *Immunity* (2010) 32(2):253–65. doi:10.1016/j.immuni.2010.01.010
39. Hislop AD, Palendira U, Leese AM, Arkwright PD, Rohrlich PS, Tangye SG, et al. Impaired Epstein–Barr virus-specific CD8$^+$ T-cell function in X-linked lymphoproliferative disease is restricted to SLAM family-positive B-cell targets. *Blood* (2010) 116(17):3249–57. doi:10.1182/blood-2009-09-238832
40. Palendira U, Low C, Chan A, Hislop AD, Ho E, Phan TG, et al. Molecular pathogenesis of EBV susceptibility in XLP as revealed by analysis of

female carriers with heterozygous expression of SAP. *PLoS Biol* (2011) 9(11):e1001187. doi:10.1371/journal.pbio.1001187
41. Zhao F, Cannons JL, Dutta M, Griffiths GM, Schwartzberg PL. Positive and negative signaling through SLAM receptors regulate synapse organization and thresholds of cytolysis. *Immunity* (2012) 36(6):1003–16. doi:10.1016/j.immuni.2012.05.017
42. Schwartzberg PL, Mueller KL, Qi H, Cannons JL. SLAM receptors and SAP influence lymphocyte interactions, development and function. *Nat Rev Immunol* (2009) 9(1):39–46. doi:10.1038/nri2456
43. Morra M, Barrington RA, Abadia-Molina AC, Okamoto S, Julien A, Gullo C, et al. Defective B cell responses in the absence of SH2D1A. *Proc Natl Acad Sci U S A* (2005) 102(13):4819–23. doi:10.1073/pnas.0408681102
44. Menard L, Cantaert T, Chamberlain N, Tangye SG, Riminton S, Church JA, et al. Signaling lymphocytic activation molecule (SLAM)/SLAM-associated protein pathway regulates human B-cell tolerance. *J Allergy Clin Immunol* (2014) 133(4):1149–61. doi:10.1016/j.jaci.2013.10.051
45. Veillette A, Zhang S, Shi X, Dong Z, Davidson D, Zhong MC. SAP expression in T cells, not in B cells, is required for humoral immunity. *Proc Natl Acad Sci U S A* (2008) 105(4):1273–8. doi:10.1073/pnas.0710698105
46. Tangye SG, Lazetic S, Woollatt E, Sutherland GR, Lanier LL, Phillips JH. Cutting edge: human 2B4, an activating NK cell receptor, recruits the protein tyrosine phosphatase SHP-2 and the adaptor signaling protein SAP. *J Immunol* (1999) 162(12):6981–5.
47. Li C, Iosef C, Jia CY, Han VK, Li SS. Dual functional roles for the X-linked lymphoproliferative syndrome gene product SAP/SH2D1A in signaling through the signaling lymphocyte activation molecule (SLAM) family of immune receptors. *J Biol Chem* (2003) 278(6):3852–9. doi:10.1074/jbc.M206649200
48. Chan B, Lanyi A, Song HK, Griesbach J, Simarro-Grande M, Poy F, et al. SAP couples Fyn to SLAM immune receptors. *Nat Cell Biol* (2003) 5(2):155–60. doi:10.1038/ncb920
49. Latour S, Roncagalli R, Chen R, Bakinowski M, Shi X, Schwartzberg PL, et al. Binding of SAP SH2 domain to FynT SH3 domain reveals a novel mechanism of receptor signalling in immune regulation. *Nat Cell Biol* (2003) 5(2):149–54. doi:10.1038/ncb919
50. Bloch-Queyrat C, Fondanèche MC, Chen R, Yin L, Relouzat F, Veillette A, et al. Regulation of natural cytotoxicity by the adaptor SAP and the Src-related kinase Fyn. *J Exp Med* (2005) 202(1):181–92. doi:10.1084/jem.20050449
51. Latour S, Gish G, Helgason CD, Humphries RK, Pawson T, Veillette A. Regulation of SLAM-mediated signal transduction by SAP, the X-linked lymphoproliferative gene product. *Nat Immunol* (2001) 2(8):681–90. doi:10.1038/90615
52. Kim HS, Das A, Gross CC, Bryceson YT, Long EO. Synergistic signals for natural cytotoxicity are required to overcome inhibition by c-Cbl ubiquitin ligase. *Immunity* (2010) 32(2):175–86. doi:10.1016/j.immuni.2010.02.004
53. Baldanzi G, Pighini A, Bettio V, Rainero E, Traini S, Chianale F, et al. SAP-mediated inhibition of diacylglycerol kinase alpha regulates TCR-induced diacylglycerol signaling. *J Immunol* (2011) 187(11):5941–51. doi:10.4049/jimmunol.1002476
54. Dong Z, Davidson D, Pérez-Quintero LA, Kurosaki T, Swat W, Veillette A. The adaptor SAP controls NK cell activation by regulating the enzymes Vav-1 and SHIP-1 and by enhancing conjugates with target cells. *Immunity* (2012) 36(6):974–85. doi:10.1016/j.immuni.2012.03.023
55. Sullivan JL, Byron KS, Brewster FE, Purtilo DT. Deficient natural killer cell activity in X-linked lymphoproliferative syndrome. *Science* (1980) 210(4469):543–5. doi:10.1126/science.6158759
56. Harada S, Bechtold T, Seeley JK, Purtilo DT. Cell-mediated immunity to Epstein–Barr virus (EBV) and natural killer (NK)-cell activity in the X-linked lymphoproliferative syndrome. *Int J Cancer* (1982) 30(6):739–44. doi:10.1002/ijc.2910300610
57. Nakajima H, Cella M, Bouchon A, Grierson HL, Lewis J, Duckett CS, et al. Patients with X-linked lymphoproliferative disease have a defect in 2B4 receptor-mediated NK cell cytotoxicity. *Eur J Immunol* (2000) 30(11):3309–18. doi:10.1002/1521-4141(200011)30:11<3309::AID-IMMU3309>3.0.CO;2-3
58. Parolini S, Bottino C, Falco M, Augugliaro R, Giliani S, Franceschini R, et al. X-linked lymphoproliferative disease. 2B4 molecules displaying inhibitory rather than activating function are responsible for the inability of natural killer cells to kill Epstein–Barr virus-infected cells. *J Exp Med* (2000) 192(3):337–46. doi:10.1084/jem.192.3.337
59. Tangye SG, Phillips JH, Lanier LL, Nichols KE. Functional requirement for SAP in 2B4-mediated activation of human natural killer cells as revealed by the X-linked lymphoproliferative syndrome. *J Immunol* (2000) 165(6):2932–6. doi:10.4049/jimmunol.165.6.2932
60. Bottino C, Falco M, Parolini S, Marcenaro E, Augugliaro R, Sivori S, et al. NTB-A [correction of GNTB-A], a novel SH2D1A-associated surface molecule contributing to the inability of natural killer cells to kill Epstein–Barr virus-infected B cells in X-linked lymphoproliferative disease. *J Exp Med* (2001) 194(3):235–46. doi:10.1084/jem.194.3.235
61. Snow AL, Marsh RA, Krummey SM, Roehrs P, Young LR, Zhang K, et al. Restimulation-induced apoptosis of T cells is impaired in patients with X-linked lymphoproliferative disease caused by SAP deficiency. *J Clin Invest* (2009) 119(10):2976–89. doi:10.1172/JCI39518
62. Czar MJ, Kersh EN, Mijares LA, Lanier G, Lewis J, Yap G, et al. Altered lymphocyte responses and cytokine production in mice deficient in the X-linked lymphoproliferative disease gene SH2D1A/DSHP/SAP. *Proc Natl Acad Sci U S A* (2001) 98(13):7449–54. doi:10.1073/pnas.131193098
63. Wu C, Nguyen KB, Pien GC, Wang N, Gullo C, Howie D, et al. SAP controls T cell responses to virus and terminal differentiation of TH2 cells. *Nat Immunol* (2001) 2(5):410–4. doi:10.1038/87713
64. Yin L, Al-Alem U, Liang J, Tong WM, Li C, Badiali M, et al. Mice deficient in the X-linked lymphoproliferative disease gene sap exhibit increased susceptibility to murine gammaherpesvirus-68 and hypo-gammaglobulinemia. *J Med Virol* (2003) 71(3):446–55. doi:10.1002/jmv.10504
65. Chung B, Aoukaty A, Dutz J, Terhorst C, Tan R. Signaling lymphocytic activation molecule-associated protein controls NKT cell functions. *J Immunol* (2005) 174(6):3153–7. doi:10.4049/jimmunol.174.6.3153
66. Nichols KE, Hom J, Gong SY, Ganguly A, Ma CS, Cannons JL, et al. Regulation of NKT cell development by SAP, the protein defective in XLP. *Nat Med* (2005) 11(3):340–5. doi:10.1038/nm1189
67. Pasquier B, Yin L, Fondanèche MC, Relouzat F, Bloch-Queyrat C, Lambert N, et al. Defective NKT cell development in mice and humans lacking the adapter SAP, the X-linked lymphoproliferative syndrome gene product. *J Exp Med* (2005) 201(5):695–701. doi:10.1084/jem.20042432
68. Crotty S, Kersh EN, Cannons J, Schwartzberg PL, Ahmed R. SAP is required for generating long-term humoral immunity. *Nature* (2003) 421(6920):282–7. doi:10.1038/nature01318
69. Hron JD, Caplan L, Gerth AJ, Schwartzberg PL, Peng SL. SH2D1A regulates T-dependent humoral autoimmunity. *J Exp Med* (2004) 200(2):261–6. doi:10.1084/jem.20040526
70. Cannons JL, Yu LJ, Jankovic D, Crotty S, Horai R, Kirby M, et al. SAP regulates T cell-mediated help for humoral immunity by a mechanism distinct from cytokine regulation. *J Exp Med* (2006) 203(6):1551–65. doi:10.1084/jem.20052097
71. Victora GD, Nussenzweig MC. Germinal centers. *Annu Rev Immunol* (2012) 30:429–57. doi:10.1146/annurev-immunol-020711-075032
72. Ma CS, Pittaluga S, Avery DT, Hare NJ, Maric I, Klion AD, et al. Selective generation of functional somatically mutated IgM^+CD27^+, but not Ig isotype-switched, memory B cells in X-linked lymphoproliferative disease. *J Clin Invest* (2006) 116(2):322–33. doi:10.1172/JCI25720
73. Ochs HD, Sullivan JL, Wedgwood RJ, Seeley JK, Sakamoto K, Purtilo DT. X-linked lymphoproliferative syndrome: abnormal antibody responses to bacteriophage phi X 174. *Birth Defects Orig Artic Ser* (1983) 19(3):321–3.
74. Ma CS, Nichols KE, Tangye SG. Regulation of cellular and humoral immune responses by the SLAM and SAP families of molecules. *Annu Rev Immunol* (2007) 25:337–79. doi:10.1146/annurev.immunol.25.022106.141651
75. Qi H, Cannons JL, Klauschen F, Schwartzberg PL, Germain RN. SAP-controlled T-B cell interactions underlie germinal centre formation. *Nature* (2008) 455(7214):764–9. doi:10.1038/nature07345
76. Kageyama R, Cannons JL, Zhao F, Yusuf I, Lao C, Locci M, et al. The receptor Ly108 functions as a SAP adaptor-dependent on-off switch for T cell help to B cells and NKT cell development. *Immunity* (2012) 36(6):986–1002. doi:10.1016/j.immuni.2012.05.016
77. Chu C, Wang Y, Zhang X, Ni X, Cao J, Xu W, et al. SAP-regulated T cell-APC adhesion and ligation-dependent and -independent Ly108–CD3zeta interactions. *J Immunol* (2014) 193(8):3860–71. doi:10.4049/jimmunol.1401660
78. McCausland MM, Yusuf I, Tran H, Ono N, Yanagi Y, Crotty S. SAP regulation of follicular helper CD4 T cell development and humoral immunity

is independent of SLAM and Fyn kinase. *J Immunol* (2007) 178(2):817–28. doi:10.4049/jimmunol.178.2.817
79. Crotty S. Follicular helper CD4 T cells (TFH). *Annu Rev Immunol* (2011) 29:621–63. doi:10.1146/annurev-immunol-031210-101400
80. Dupré L, Andolfi G, Tangye SG, Clementi R, Locatelli F, Aricò M, et al. SAP controls the cytolytic activity of $CD8^+$ T cells against EBV-infected cells. *Blood* (2005) 105(11):4383–9. doi:10.1182/blood-2004-08-3269
81. Sepulveda FE, de Saint Basile G. Hemophagocytic syndrome: primary forms and predisposing conditions. *Curr Opin Immunol* (2017) 49:20–6. doi:10.1016/j.coi.2017.08.004
82. Dong Z, Cruz-Munoz ME, Zhong MC, Chen R, Latour S, Veillette A. Essential function for SAP family adaptors in the surveillance of hematopoietic cells by natural killer cells. *Nat Immunol* (2009) 10(9):973–80. doi:10.1038/ni.1763
83. Wu N, Zhong MC, Roncagalli R, Pérez-Quintero LA, Guo H, Zhang Z, et al. A hematopoietic cell-driven mechanism involving SLAMF6 receptor, SAP adaptors and SHP-1 phosphatase regulates NK cell education. *Nat Immunol* (2016) 17(4):387–96. doi:10.1038/ni.3369
84. Griewank K, Borowski C, Rietdijk S, Wang N, Julien A, Wei DG, et al. Homotypic interactions mediated by SLAMf1 and SLAMf6 receptors control NKT cell lineage development. *Immunity* (2007) 27(5):751–62. doi:10.1016/j.immuni.2007.08.020
85. Chen S, Cai C, Li Z, Liu G, Wang Y, Blonska M, et al. Dissection of SAP-dependent and SAP-independent SLAM family signaling in NKT cell development and humoral immunity. *J Exp Med* (2017) 214(2):475–89. doi:10.1084/jem.20161312
86. Jordan MA, Fletcher JM, Pellicci D, Baxter AG. Slamf1, the NKT cell control gene Nkt1. *J Immunol* (2007) 178(3):1618–27. doi:10.4049/jimmunol.178.3.1618
87. Gadue P, Morton N, Stein PL. The Src family tyrosine kinase Fyn regulates natural killer T cell development. *J Exp Med* (1999) 190(8):1189–96. doi:10.1084/jem.190.8.1189
88. Marsh RA, Bleesing JJ, Filipovich AH. Flow cytometric measurement of SLAM-associated protein and X-linked inhibitor of apoptosis. *Methods Mol Biol* (2013) 979:189–97. doi:10.1007/978-1-62703-290-2_15
89. Milone MC, Tsai DE, Hodinka RL, Silverman LB, Malbran A, Wasik MA, et al. Treatment of primary Epstein–Barr virus infection in patients with X-linked lymphoproliferative disease using B-cell-directed therapy. *Blood* (2005) 105(3):994–6. doi:10.1182/blood-2004-07-2965
90. Bergsten E, Horne A, Aricó M, Astigarraga I, Egeler RM, Filipovich AH, et al. Confirmed efficacy of etoposide and dexamethasone in HLH treatment: long term results of the cooperative HLH-2004 study. *Blood* (2017) 130(25):2728–38. doi:10.1182/blood-2017-06-788349
91. Henter JI, Horne A, Aricó M, Egeler RM, Filipovich AH, Imashuku S, et al. HLH-2004: diagnostic and therapeutic guidelines for hemophagocytic lymphohistiocytosis. *Pediatr Blood Cancer* (2007) 48(2):124–31. doi:10.1002/pbc.21039
92. Das R, Guan P, Sprague L, Verbist K, Tedrick P, An QA, et al. Janus kinase inhibition lessens inflammation and ameliorates disease in murine models of hemophagocytic lymphohistiocytosis. *Blood* (2016) 127(13):1666–75. doi:10.1182/blood-2015-12-684399
93. Lankester AC, Visser LF, Hartwig NG, Bredius RG, Gaspar HB, van der Burg M, et al. Allogeneic stem cell transplantation in X-linked lymphoproliferative disease: two cases in one family and review of the literature. *Bone Marrow Transplant* (2005) 36(2):99–105. doi:10.1038/sj.bmt.1705016
94. Marsh RA, Bleesing JJ, Chandrakasan S, Jordan MB, Davies SM, Filipovich AH. Reduced-intensity conditioning hematopoietic cell transplantation is an effective treatment for patients with SLAM-associated protein deficiency/X-linked lymphoproliferative disease type 1. *Biol Blood Marrow Transplant* (2014) 20(10):1641–5. doi:10.1016/j.bbmt.2014.06.003
95. Ruffo E, Malacarne V, Larsen SE, Das R, Patrussi L, Wülfing C, et al. Inhibition of diacylglycerol kinase alpha restores restimulation-induced cell death and reduces immunopathology in XLP-1. *Sci Transl Med* (2016) 8(321):321ra7. doi:10.1126/scitranslmed.aad1565
96. Booth C, Gaspar HB, Thrasher AJ. Gene therapy for primary immunodeficiency. *Curr Opin Pediatr* (2011) 23(6):659–66. doi:10.1097/MOP.0b013e32834cd67a
97. Ghosh S, Gaspar HB. Gene therapy approaches to immunodeficiency. *Hematol Oncol Clin North Am* (2017) 31(5):823–34. doi:10.1016/j.hoc.2017.05.003
98. Rivat C, Booth C, Alonso-Ferrero M, Blundell M, Sebire NJ, Thrasher AJ, et al. SAP gene transfer restores cellular and humoral immune function in a murine model of X-linked lymphoproliferative disease. *Blood* (2013) 121(7):1073–6. doi:10.1182/blood-2012-07-445858
99. Porteus MH. Towards a new era in medicine: therapeutic genome editing. *Genome Biol* (2015) 16:286. doi:10.1186/s13059-015-0859-y
100. Booth C, Gaspar HB, Thrasher AJ. Treating immunodeficiency through HSC gene therapy. *Trends Mol Med* (2016) 22(4):317–27. doi:10.1016/j.molmed.2016.02.002
101. Candotti F. Advances of gene therapy for primary immunodeficiencies. *F1000Res* (2016) 5:Rev–310. doi:10.12688/f1000research.7512.1
102. Holt N, Wang J, Kim K, Friedman G, Wang X, Taupin V, et al. Human hematopoietic stem/progenitor cells modified by zinc-finger nucleases targeted to CCR5 control HIV-1 *in vivo*. *Nat Biotechnol* (2010) 28(8):839–47. doi:10.1038/nbt.1663
103. Cannon P, June C. Chemokine receptor 5 knockout strategies. *Curr Opin HIV AIDS* (2011) 6(1):74–9. doi:10.1097/COH.0b013e32834122d7
104. Georgiadis C, Qasim W. Emerging applications of gene edited T cells for the treatment of leukemia. *Expert Rev Hematol* (2017) 10(9):753–5. doi:10.1080/17474086.2017.1350575
105. Qasim W, Zhan H, Samarasinghe S, Adams S, Amrolia P, Stafford S, et al. Molecular remission of infant B-ALL after infusion of universal TALEN gene-edited CAR T cells. *Sci Transl Med* (2017) 9(374):eaaj2013. doi:10.1126/scitranslmed.aaj2013
106. Kohn DB, Kuo CY. New frontiers in the therapy of primary immunodeficiency: from gene addition to gene editing. *J Allergy Clin Immunol* (2017) 139(3):726–32. doi:10.1016/j.jaci.2017.01.007

Conflict of Interest Statement: The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

frontiers in Immunology

PERSPECTIVE
published: 08 May 2018
doi: 10.3389/fimmu.2018.00979

Interleukin-2-Inducible T-Cell Kinase Deficiency—New Patients, New Insight?

Sujal Ghosh[1], Ingo Drexler[2], Sanil Bhatia[1], Heiko Adler[3,4,5], Andrew R. Gennery[6] and Arndt Borkhardt[1]*

[1] Department of Pediatric Oncology, Hematology and Clinical Immunology, Medical Faculty, Center of Child and Adolescent Health, Heinrich-Heine-University, Düsseldorf, Germany, [2] Institute for Virology, Medical Faculty, Heinrich-Heine-Universität Düsseldorf, Düsseldorf, Germany, [3] Research Unit Lung Repair and Regeneration, Comprehensive Pneumology Center, Helmholtz Zentrum München—Deutsches Forschungszentrum für Gesundheit und Umwelt (GmbH), Munich, Germany, [4] University Hospital Grosshadern, Ludwig-Maximilians-Universität München, Munich, Germany, [5] German Center for Lung Research (DZL), Giessen, Germany, [6] Paediatric Immunology and HSCT, Newcastle University and Great North Children's Hospital, Newcastle upon Tyne, United Kingdom

OPEN ACCESS

Edited by:
Isabelle Meyts, KU Leuven, Belgium

Reviewed by:
Stuart G. Tangye, Garvan Institute of Medical Research, Australia
Shigeaki Nonoyama, National Defense Medical College, Japan
Pamela Lee Schwartzberg, National Institutes of Health (NIH), United States

***Correspondence:**
Sujal Ghosh
sujal.ghosh@med.uni-duesseldorf.de

Specialty section:
This article was submitted to Primary Immunodeficiencies, a section of the journal Frontiers in Immunology

Received: *23 October 2017*
Accepted: *20 April 2018*
Published: *08 May 2018*

Citation:
Ghosh S, Drexler I, Bhatia S, Adler H, Gennery AR and Borkhardt A (2018) Interleukin-2-Inducible T-Cell Kinase Deficiency—New Patients, New Insight? Front. Immunol. 9:979. doi: 10.3389/fimmu.2018.00979

Patients with primary immunodeficiency can be prone to severe Epstein–Barr virus (EBV) associated immune dysregulation. Individuals with mutations in the interleukin-2-inducible T-cell kinase (*ITK*) gene experience Hodgkin and non-Hodgkin lymphoma, EBV lymphoproliferative disease, hemophagocytic lymphohistiocytosis, and dysgammaglobulinemia. In this review, we give an update on further reported patients. We believe that current clinical data advocate early definitive treatment by hematopoietic stem cell transplantation, as transplant outcome in primary immunodeficiency disorders in general has gradually improved in recent years. Furthermore, we summarize experimental data in the murine model to provide further insight of pathophysiology in ITK deficiency.

Keywords: primary immunodeficiency, combined immunodeficiency, interleukin-2-inducible T-cell kinase, Epstein–Barr virus-related malignancies, lymphoproliferative disorders

INTRODUCTION

Epstein–Barr virus (EBV) is recognized to cause infectious mononucleosis. More than 90% of the global population carries the latent virus life-long and most individuals acquire the gammaherpesvirus by silent infection at young age. Several malignancies are associated with EBV and in the last decades patients with genetic defects of T cell signaling or cytotoxic pathway have demonstrated susceptibility to severe immune dysregulation upon EBV infection or reactivation. They usually present with fatal infectious mononucleosis, lymphoma and lymphoproliferative disease (LPD), hemophagocytic lymphohistiocytosis (HLH), and dysgammaglobulinemia (1, 2).

While many combined immunodeficiencies (e.g., defects of antigen receptor recombination *RAG1/2*) can lead to EBV immune dysregulation beside other infectious complications, there are diseases, which confer a higher propensity only of EBV associated disease. Several genes have been linked to EBV lymphoproliferation (*SH2D1A*, *STK4*, *CD27*, *CD70*, *LAT*, *RASGRP1*, *MAGT1*, *Coronin-1A*, and *CTPS1*) in recent years (2). Our group and others reported alterations in the interleukin-2-inducible T-cell kinase (*ITK*) gene in patients presenting with severe EBV associated dysregulation (3, 4). At least one decade earlier, murine studies had already shown that ITK is essential for various T cell functions, especially during a Th2 response. In this mini review, we update on clinical and immunological aspects in reported individuals and highlight the extensively investigated murine *itk*−/− model.

ITK DEFICIENCY—CLINICAL PRESENTATION AND DIAGNOSIS

The first patients were reported in 2009 by our group. Two sisters from consanguineous Turkish parents presented with EBV-driven lymphoproliferative disease (3). At age of 6 years, one developed pneumocystis pneumonitis, severe candida stomatitis, cytopenia, progressive hypogammaglobulinemia, and oligoclonal polymorphic B cell lymphoproliferation.

Eighteen months later, she presented with Hodgkin lymphoma (HL), which was successfully treated with chemotherapy. However, T lymphocytes were further declining and at age of 10 years the girl succumbed to pneumocystis pneumonia. The younger sister presented with pancytopenia and severely impaired hepatic function due to EBV-associated HL. Due to rapid clinical deterioration haploidentical peripheral blood stem cell transplantation (SCT) was performed as a salvage therapy, but unfortunately the patient died due to airway obstruction during aplasia. Genome-wide linkage analysis identified *ITK*, in which the causative homozygous R335W mutation was revealed. To date, we are aware of *ITK* mutations in 17 patients originating from Greece, India, Italy, Iran, Morocco, Pakistan, Palestine, and Turkey (16 patients described in **Table 1**) (5–12). These patients manifested between 2.5 months and 13 years of age and presented with fever, hepatosplenomegaly, lymphadenopathy, and EBV viremia. One patient was diagnosed at birth due to family history of disease in the older brother. Thirteen patients presented either with HL or with EBV-driven B cell lymphoproliferative disease (in some cases developing to Hodgkin or large B cell lymphoma), only two showed a classical non-HL histology. In a few patients, other viral infections including CMV and VZV were seen. Given the severe immune dysregulation, at least three patients developed autoimmune phenomena and two patients developed HLH.

The number of ITK patients is too few to deduce valid statistics. However, it appears that HLH occurs less frequent in ITK deficiency than, e.g., in SLAM-associated protein (SAP) deficiency (30%) (13).

As known from other disorders with EBV predisposition, pulmonary interstitial nodules were seen in most patients. Furthermore, progressive hypogammaglobulinemia and loss of CD4+ T cells was detected, in particular naive CD45RA+ CD4+ T cells were decreased. In parallel with other EBV prone disorders (e.g., SAP deficiency), peripheral NKT cells [determined as CD3+, T cell receptor (TCR) Vbeta11+, TCR Valpha24+] were decreased in ITK-deficient patients supporting observations in transformed cell lines that NKT cells might be essential for anti EBV immunity (14). However, there is some evidence that EBV infection itself might decrease the number of NKT cells in these patients, as normal numbers of NKT cells are demonstrated in EBV-naive patients, e.g. in patients with XIAP deficiency (15). Furthermore, there are disorders with a global lack of NKT cells, in which individuals are rather susceptible to Mycobacteria, but not to EBV infection (16).

Peak EBV viremia in ITK-deficient patients was quite heterogeneous in reported patients (10^4–10^8 copies/μg DNA). Unfortunately, we obtained incomplete information on serological phenotype at time of manifestation to predict the time between infection and clinical exacerbation; EBV-VCA-IgM was detected in one patient only. In contrast to one of the most similar immunological disorders—SAP deficiency—there is not a single reported EBV-VCA-IgG seronegative symptomatic EBV-LPD patient highlighting the paramount importance of EBV infection and maybe specificity in the disease setting. Interestingly the spectrum of histopathological diagnosis is quite variable in reported patients. Bienemann et al analyzed seven of the 16 patients presented here. In six events, a classic mixed-cellular HL histology was shown, while the other lymphoproliferative events were rather heterogeneous (polymorphic: three events, borderline polymorphic to monomorphic blast-rich B-cell LPD: two events, HL-like B-cell proliferation: two events and large B-cell lymphoma like LPD: two events). In contrast to many immunocompromised patients (who rather demonstrate latency type III), ITK-deficient patients had predominantly EBV latency type II and presented often with nodal and extranodal manifestations simultaneously (6). One patient with ITK deficiency differs from the other patients in several points. An 18-year-old male Turkish patient suffered from recurrent progressive pulmonary infections and bronchiectasis, but no lymphoproliferative disease. He remained EBV seronegative although PCR could detect a low EBV viral load of 1,000–2,000 copies/μl (11).

MANAGEMENT AND OUTCOME

As previously demonstrated in other EBV-LPD cases, a few patients with ITK deficiency were treated with Rituximab with some improvement. IgG substitution has conferred only temporary benefit, especially to partially ameliorate immune dysregulation manifesting as lymphoproliferation and autoimmunity; corticosteroids were not helpful in the reported cases. Eight patients died between 1 and 15 years after diagnosis (mostly due to malignancies), seven within 2 years from diagnosis. Nine patients did not receive definitive treatment. Most had a fatal outcome. Six patients died due to lymphoproliferation, while only two patients remained in remission after chemotherapy for HL. However, eight patients underwent hematopoietic SCT. Two patients died after HSCT. While one of the initial patients died during aplasia with hemorrhagic acute airway obstruction after receiving haploidentical PBSCT, another patient succumbed to severe graft-versus-host disease. Recently, three more patients have been reported at two different centers (Newcastle, UK and Paris, France), which have been presented orally at the Annual Meeting of the European Society for Immunodeficiencies in Edinburgh, September 2017. All three patients were diagnosed with Hodgkin-like lymphoma or diffuse B cell lymphoma like lymphoproliferation and were subject to HSCT. Remarkably, the Paris patient was treated with five courses of Rituximab and two injections of Brentuximab to achieve clinical remission before haploidentical T replete HSCT. We can learn from those cases that immunotherapy with Rituximab or Brentuximab can lead to partial or even complete remission and at least bridge to definitive cure. We strongly suggest that each patient should be carefully considered for early HSCT, once the diagnosis of ITK deficiency has been established.

TABLE 1 | Clinical and laboratory findings in *ITK*-deficient patients.

Origin Mutation	Patient 1 Turkey c.1003C>T: p.R335W	Patient 2 Turkey c.1003C>T: p.R335W	Patient 3 Palestine c.1764C>G: p.Y588X	Patient 4 Palestine c.1764C>G: p.Y588X	Patient 5 Palestine c.1764C>G: p.Y588X	Patient 6 Morocco c.86G>A: p.R29H	Patient 7 India c.1497delT: p.D500TfsX4	Patient 8 Iran c.468delT: p. L157Ffs*108	Patient 9 Turkey c.49C>T: p.Q17X	Patient 10 Italy/Greece Comp-het c.49C>T/c.922delG: p.Q17X A308Lfs*24	Patient 11 Turkey c.49C>T: p.Q17X	Patient 12 Turkey c.49C>T: p.Q17X	Patient 13 Turkey c.1003C>T: p.R335W	Patient 14 Turkey c.1003C>T: p.R335W	Patient 15 Pakistan c.626G>A: p.W209X	Patient 16 Pakistan c.626G>A: p.W209X
Sex	Female	Female	Female	Male	Male	Male	Female	Female	Male	Female	Female	Male	Male	Female	Male	Male
Age at diagnosis	5	6	4	5	3	11	6	13	18	5	6	2.5	7	3	4	Birth
Status	Died at age 10	Died at age 7 after HSCT	Died at age 6	Remission after Cx, age 12	a/w after HSCT, age 8	Died at age 26	Died after HSCT at age 8	Died at age 15	Unknown	a/w after HSCT age 5	Died at 8	Remission after Cx	a/w after HSCT age 8	Died at 3	a/w after HSCT age 4	a/w after HSCT age 1
Fever	+	+	+	+	+	+	+	+	+	n.a.	n.a	+	+	+	+	+
Lymphadenopathy	+	+	+	+	+	+	+	+	None	+	+	+	+	+	+	+
Hepatosplenomegaly	+	+	+	None	+	Unknown	None	+	None	+	+	+	+	+	+	None
Pulmonary involvement	+	None	None	+	+	+	+	+	Infections	None	+	None	+	+	+	+
Histology	B cell LPD Hodgkin	HL-like B cell LPD	HL	HL	HL	B cell LPD	B cell LPD, LBCL, LG	B cell LPD	None	HL	NHL	HL	LG, Burkitt	n.a.	HL-like LPD	DLBL-like LPD
Autoimmunity	None	None	None	Nephritis, thyroiditis	Thyroiditis	AIHA/ITP	None	None	None	None	None	None	None	None	None	None
HLH	None	(+)	+ (at relapse)	None	None	None	None	None	None	After HSCT	None	None	None	?	None	None
CD4+ cells	↓	↓	Normal	↓	Normal	↓	↓	↓	↓	n.i. (after Cx)	Normal	↘	↘	Normal	↓	Normal
CD8+ cells	Normal	↓	↑	↓	Normal	Normal	Normal	Normal	Normal	n.i. (after Cx)	Normal	Normal	Normal	Normal	↓	Normal
NKT cells	n.d.	↘	n.d	↘	n.d.	↘	↘	n.d.	↘	n.i. (after Cx)	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
Serology	VCA-G+, VCA-M–, EA-G+, EBNA-G–	VCA-G+, VCA-M–	VCA-G–, VCA-M–, EBNA+	VCA-G+, VCA-M–, EBNA-G–	n.d.	VCA-G+		VCA-G+	Negative	n.d.	VCA-G+, VCA-M–, EA-G–, EBNA-G–	VCA-G+, VCA-M–, EA-G–, EBNA-G–	VCA-G+, VCA-M–, EA-G–, EBNA-G+	VCA-G+, VCA-M+, EA-G+, EBNA-G+	n.d.	n.d.
Viral load at presentation	+ (n.q.)	10^3	10^5	10^3	10^5	+ (n.q.)	10^3	10^7	10^3	n.d.	10^4 CMV	10^4	None	n.a.	10^4	10^4
Peak viral load	10^7	10^4	Unknown	Unknown	Unknown	10^6	10^4	10^7	10^3	n.d.	10^4	10^4	n.a.	n.a.	10^4	10^4

AIHA, autoimmune hemolytic anemia; Cx, chemotherapy; HL, Hodgkin lymphoma; ITP, immune thrombocytopenia; LBCL, large B-cell lymphoma; LG, lymphomatoid granulomatosis; LPD, lymphoproliferative disease; n.d., not determined; n.q., not quantified; HLH, hemophagocytic lymphohistiocytosis; ITK, interleukin-2-inducible T-cell kinase.

↓, decreased; ↘, lower margin; ↑, increased.

INTERLEUKIN-2-INDUCIBLE T-CELL KINASE

Interleukin-2-inducible T-cell kinase is one of five mammal TEC family kinases. All five proteins are involved in lymphocyte signaling and development (17). Years before the first patient with ITK deficiency was diagnosed, *ITK-SYK* translocations were found in individuals with T cell lymphoma (18). The *ITK* gene on chromosome 5q consists of 17 exons and 112 kbp, the protein (71 kDa) is formed by 620 amino acids. ITK is composed of an N-terminal pleckstrin homology (PH), a Tec homology (TH), an Src homology 3 (SH3), an Src homology 2 (SH2), and a C-terminal catalytic kinase domain (**Figure 1A**) (19). Upon activation of the TCR several phosphorylation events recruit ITK to the cell membrane (for details, see **Figure 1B**). ITK activates PLCγ1, generating inositol 1,4,5-trisphosphate (IP3), which leads to intracellular calcium release and diacylglycerol, which, *via* RASGRP and PKCδ, ultimately results in activation/induction of the NFκB, mTOR, and MAPK/ERK pathways.

Mutations were found in the kinase, SH2 and PH domain. Most patients demonstrated an autosomal-recessive trait, while in one individual a compound-heterozygous inheritance from

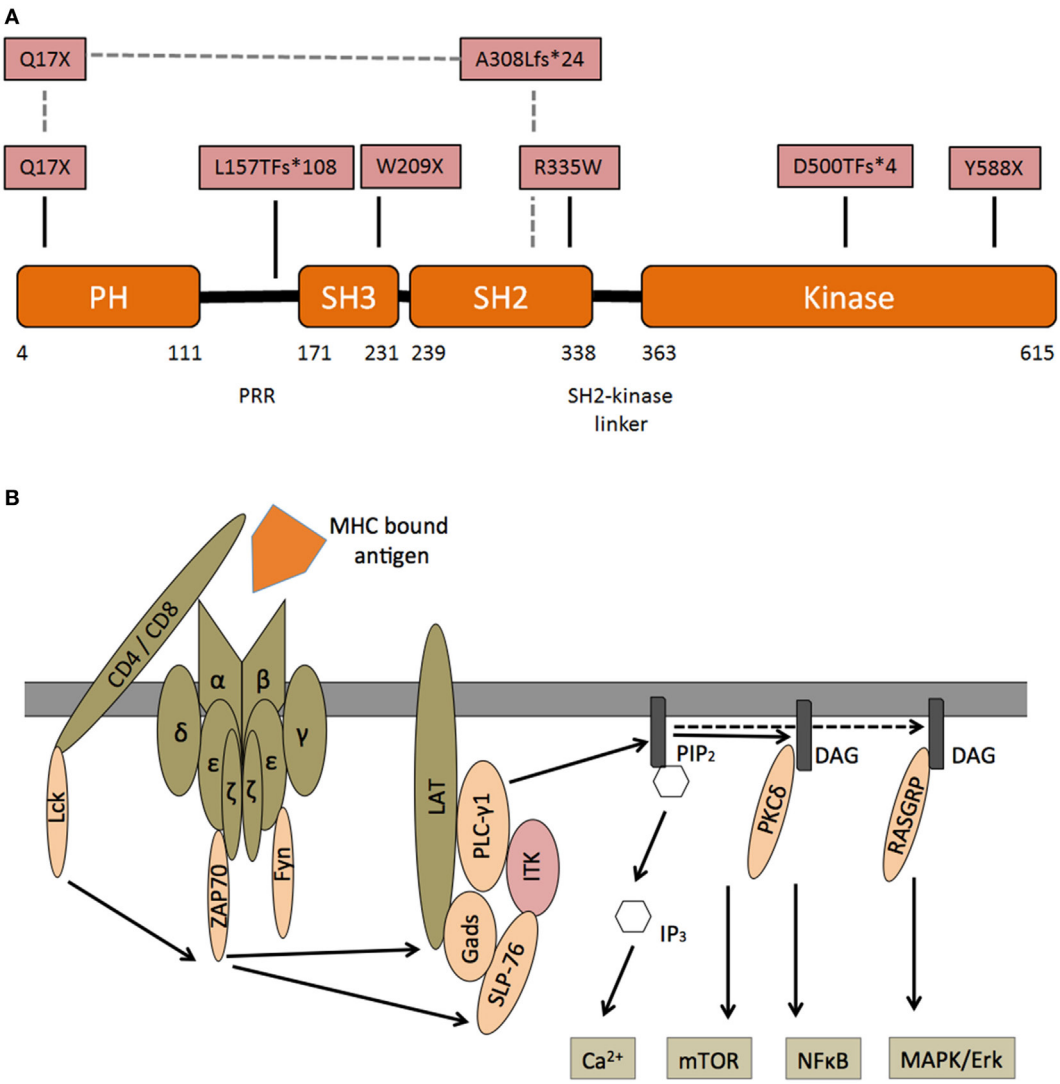


FIGURE 1 | Interleukin-2-inducible T-cell kinase (ITK)—structure and signaling—**(A)** domain organization of ITK and corresponding protein mutants in patients with ITK deficiency. N-terminal pleckstrin homology (PH), Tec homology, Src homology 3 (SH3), Src homology 2 (SH2), and C-terminal catalytic kinase domain. Pattern recognition receptors. In one patient, a compound heterozygous mutation is predicted to encode Q17X and A308LfS*24 mutant. **(B)** Following engagement of the T cell receptor (TCR) with an MHC bound foreign antigen, several intracellular signals are activated. Lck is recruited and phosphorylates immunoreceptor tyrosine-based activation motifs (ITAM) at the zeta chain of the TCR. ZAP70 binds double-phosphorylated ITAM residues and phosphorylates LAT, which is recruited to the TCR complex. Phosphorylated LAT recruits SLP76, which together with Itk, activates PLCγ1. Subsequently phosphatidylinositol 4,5-bisphosphate (PIP2) is catalyzed into inositol 1,4,5-trisphosphate (IP3), which leads to intracellular calcium release and diacylglycerol (DAG). DAG itself can recruit PKCδ and RASGRP, which induce the NFκB and MAPK/ERK pathways.

two different ethnicities (Greek–Italian) was revealed (5). Interestingly there are corresponding mutations in residues of the "sister" Tec kinase *BTK* (known to cause X-linked agammaglobulinemia), which are homologous to the mutations observed in our patients (9). Our group transformed Herpesvirus saimiri cell lines to reveal functional impairment in corresponding ITK mutations.

The alterations did not greatly change the amount of *ITK* mRNA expression, nevertheless immunoblot investigations showed several variants of endogenous ITK. Most importantly, we analyzed calcium response with flow cytometric flux studies and revealed a highly decreased release of calcium ions into the cytosol in most patients. With regards to functional complementation our group restored TCR-mediated calcium flux in murine *itk*−/− thymocytes by means of wild type ITK transduction.

Interestingly since the publication of our last review two new EBV prone diseases have been discovered in the proximity of ITK (with respect to location in the pathway). Linker for activation of T cells (LAT) is a transmembrane adapter molecule, which is phosphorylated after TCR triggering. It contains no intrinsic enzymatic activity and couples the TCR to downstream pathways as a scaffolding protein. PLCγ1 phosphorylation is highly dependent on the assembly of the LAT-SLP76 signalosome. However, the two initial reports on two kindreds with LAT deficiency show different phenotypes and ambiguous results (20, 21). One kindred presented with a typical (severe) immunodeficiency phenotype with failure to thrive and recurrent infections. The other report describes a family with infants with increased autoimmunity due to combined immunodeficiency with a higher number of residual T lymphocytes. All three siblings suffered from CMV and EBV infection before autoimmunity developed. Similar to our investigations in ITK-deficient cell lines, both of these new reports demonstrate decreased Ca2+ mobilization and other downstream signaling in LAT-deficient Jurkat cell lines (J.CaM2.5, ANJ3), and although, ITK phosphorylation of ITK, is reported to be dependent on LAT, it was not affected in J.CaM2.5. However, the same group (with the higher number of residual T lymphocytes and autoimmunity) had the opportunity to examine calcium flux in CD45RO patient lymphocytes, which was surprisingly within the range of healthy controls 21. Interestingly, all these patients had infectious (often CMV-relate) and autoimmune problems, rather than emerging lymphoproliferation. RASGRP1 is a guanine nucleotide exchange factor, which is downstream of the PLCγ1-mediated cleavage of phosphatidylinositol 4,5-bisphosphate. Mutations in RASGRP1 have also been associated with CD4 T lymphopenia, EBV-driven B cell lymphoma, and lymphoproliferative disease (22, 23).

Itk−/− MURINE PHENOTYPE

The murine *itk*−/− phenotype has been investigated for more than 25 years now, 15 years before the first patients were reported. Most studies had focused on Th1 skewing especially in infectious models; recent data further suggests that *itk*−/− CTLs harbor defects in expansion, degranulation and thus cytotoxicity. In the next chapter we will briefly summarize the *itk*−/− murine phenotype.

Itk−/− mice show an altered development of thymocytes with elevated numbers of innate single positive CD8+ (CD8SP) cells. These thymocytes parallel antigen-experienced T cells with a CD122+ CD44hiCXCR3+ phenotype and increased production of Eomesodermin and IFNγ, if stimulated. Similarly splenocytes (having decreased CD4 and CD8 expression in total) resemble a more differentiated phenotype (CD44+) mirroring peripheral CD8 cells of ITK-deficient patients (24–29). NKT cells have an impaired development, are dysfunctional and have a decreased survival in the periphery (30). Most studies, addressing the Th1 and Th2 paradigm suggest that ITK plays a role in a correct Th2 response (19). Upon TCR stimulation, itk−/− T cells have an impaired proliferation, less intracellular calcium release and a reduced production of effector cytokines (31).

Few epidemiological studies have observed asthma predisposition and variants in the *ITK* gene (32, 33). Several papers investigated the T lymphocytes dependent airway hyporesponsiveness in *itk*−/− mice. Pathophysiology of asthma usually involves pulmonary infiltration of Th2 cells. Due to an impaired Th2 response *itk*−/− mice show a reduced airway inflammation upon challenge with allergens (32, 34, 35). One group tried to administer an ITK inhibitor as a pharmacologic agent to suppress inflammation in already ovalbumin-induced hyperresponsive airways. Paradoxically, inhibition of ITK induced lymphoid hyperplasia, an observation they attributed to impaired cell death in the absence of cell death (32). Two studies have further focused on the impaired cell death in *itk*−/− mice, which might be at least one explanation for the lymphoproliferation seen in the patients. One study found reduced activation-induced cell death, evidenced by defective FasL upregulation upon activation and elevated T cell proliferation (36).

In recent years, Th17, Treg, and Th9 differentiation have been extensively addressed as well (37–39).

Infections show the impact of ITK on T cell differentiation and T cell effector function. In one of the first studies *itk*−/− mice on a BALB/c background failed to generate the usual Th2 response upon infection with *Leishmania major*, but rather mounted a Th1 dependent IFNγ response, which cleared the infection (31). In further studies *itk*−/− mice showed decreased granuloma formation after challenge with *Schistosoma mansoni* eggs or the nematode *Nippostrongylus brasiliensis*. Both helminths usually induce a Th2 response (31, 40). Upon *S. mansoni* infection compared to WT the size of granuloma and draining lymph nodes was significantly decreased and production of the Th2 cytokines IL-4, IL-5 and IL-10 was markedly reduced in *itk*−/− mice. Again, IFNγ levels were significantly higher suggesting Th1 skewing. If infected with *N. brasiliensis,* wild type BALB/c mice were able to fight the intestinal infection, while *itk*−/− mice showed a decrease in IL-4 and were incapable to clear the worm.

Toxoplasma gondii, on the other hand, promotes Th1 mediated-immunity. Although *itk*−/− mice do succumb to this infection, they are only slightly more susceptible to *T. gondii* than wild type mice (41). Serum IFN-y levels 5 days after infection and splenic IFN-y production upon stimulation after 30 days show similar values as wild type mice. Only few studies have addressed the CD8 T cell response in *itk*−/− mice. It was reported that, although *itk*−/− mice do mount protective responses to lymphocytic

choriomeningitis virus Armstrong, vaccinia virus, and vesicular stomatitis virus, viral clearance is delayed, most likely due to poor activation of CD8 T lymphocytes (42, 43). Given the clinical phenotype of the reported patients, a potential role for ITK in CTL function seems highly likely. Recently, the effect of ITK on cytotoxicity and degranulation of CTLs was demonstrated. ITK-deficient CTLs showed decreased expansion and a more naïve phenotype after activation. The authors revealed that in murine *itk*−/− deficient lymphocytes, early stages of cytotoxicity were intact, while defects in degranulation were the bigger concern (44).

As far as we know there has not been any study in which an infection model of the murine gammaherpesvirus 68 (MHV-68) has been investigated in itk−/− mice, although murine MHV-68 infection resembles human EBV infection quite a bit. MHV-68 spreads naturally by the respiratory route and is genetically related to EBV (45). Both EBV and MHV-68 have the ability to cause infectious mononucleosis. Following intranasal inoculation the virus causes an acute infection in the lungs and remains in a latent form within B cells. Depending on CD8 T cell function, MHV-68 can further infect other splenic B cells and circulate in other organs. MHV-68 infection has already been investigated in SAP deficient mice (*Sh2d1a*−/−) leading to hypogammaglobulinemia and organ damage (46, 47). Clinically, patients with SAP deficiency have shared features with patients with ITK deficiency, hence we decided to explore the natural course of MHV-68 infection in *itk*−/− mice in some preliminary experiments. B6 and *itk*−/− mice were intranasally infected with MHV-68. There was no difference in the lytic viral tire in lungs between B6 and *itk*−/− infected mice; furthermore, there was no difference in the splenic genomic viral load between B6 and *itk*−/− mice at day 17. Clinically the mice did not behave differently. Similarly to Sh2d1a−/− mice after 3 months in total *itk*−/− mice spleens were enlarged, and we could verify a Vbeta4 expansion in all infected mice, similar to other mouse models after MHV-68 infection. Interestingly, we saw a relative decrease in CD4 cells in *itk*−/− mice; on the other hand, CD8 numbers were similar in both groups. Most importantly we saw a bigger expansion of Vbeta4 cells within in the *itk*−/− group (own preliminary results). The expansion of this clone is line with reports in Sh2d1a−/− deficient mice, and further experiments are ongoing to evaluate a potential use of this model to investigate ITK deficiency.

SUMMARY

Since our last review the reported patient number with ITK deficiency has nearly doubled. All patients with previous EBV infection, developed EBV-associated malignancies, like Hodgkin and non-HL and lymphoproliferative diseases, while pulmonary involvement is one of the extranodal key features. Although the number of patients is limited, a curative treatment should be considered. In settings in which an HLA-matched donor is lacking, a haploidentical donor in conjunction with advanced T-depleting and adoptive T cell transfer strategies have improved the outcome. Immunotherapy with anti-CD20 or anti-CD30 can bridge to definitive cure. EBV-negative patients (without any viremia) have not been reported yet, so we are unaware of any problems in these individuals. However, an early transplant might improve outcome. Prospective data collection on HSCT in ITK deficiency and other EBV prone primary immunodeficiencies, as CD27 or CD70 deficiency is highly warranted.

AUTHOR CONTRIBUTIONS

All the authors wrote the manuscript and gathered data.

REFERENCES

1. Parvaneh N, Filipovich AH, Borkhardt A. Primary immunodeficiencies predisposed to Epstein-Barr virus-driven haematological diseases. *Br J Haematol* (2013) 162:573–86. doi:10.1111/bjh.12422
2. Tangye SG, Palendira U, Edwards ES. Human immunity against EBV-lessons from the clinic. *J Exp Med* (2017) 214:269–83. doi:10.1084/jem.20161846
3. Huck K, Feyen O, Niehues T, Ruschendorf F, Hubner N, Laws HJ, et al. Girls homozygous for an IL-2-inducible T cell kinase mutation that leads to protein deficiency develop fatal EBV-associated lymphoproliferation. *J Clin Invest* (2009) 119:1350–8. doi:10.1172/JCI37901
4. Ghosh S, Bienemann K, Boztug K, Borkhardt A. Interleukin-2-inducible T-cell kinase (ITK) deficiency – clinical and molecular aspects. *J Clin Immunol* (2014) 34:892–9. doi:10.1007/s10875-014-0110-8
5. Alme C, Satwani P, Alobeid B, Bhagat G, Kelly KM. Atypical clinical course in pediatric Hodgkin lymphoma: association with germline mutations in interleukin-2-inducible T-cell kinase. *J Pediatr Hematol Oncol* (2015) 37:507–8. doi:10.1097/MPH.0000000000000366
6. Bienemann K, Borkhardt A, Klapper W, Oschlies I. High incidence of Epstein-Barr virus (EBV)-positive Hodgkin lymphoma and Hodgkin lymphoma-like B-cell lymphoproliferations with EBV latency profile 2 in children with interleukin-2-inducible T-cell kinase deficiency. *Histopathology* (2015) 67:607–16. doi:10.1111/his.12677
7. Cagdas D, Erman B, Hanoglu D, Tavil B, Kuskonmaz B, Aydin B, et al. Course of IL-2-inducible T-cell kinase deficiency in a family: lymphomatoid granulomatosis, lymphoma and allogeneic bone marrow transplantation in one sibling; and death in the other. *Bone Marrow Transplant* (2017) 52:126–9. doi:10.1038/bmt.2016.185
8. Cipe FE, Aydogmus C, Serwas NK, Tugcu D, Demirkaya M, Bicici FA, et al. ITK deficiency: how can EBV be treated before lymphoma? *Pediatr Blood Cancer* (2015) 62:2247–8. doi:10.1002/pbc.25648
9. Linka RM, Risse SL, Bienemann K, Werner M, Linka Y, Krux F, et al. Loss-of-function mutations within the IL-2 inducible kinase ITK in patients with EBV-associated lymphoproliferative diseases. *Leukemia* (2012) 26:963–71. doi:10.1038/leu.2011.371
10. Mansouri D, Mahdaviani SA, Khalilzadeh S, Mohajerani SA, Hasanzad M, Sadr S, et al. IL-2-inducible T-cell kinase deficiency with pulmonary manifestations due to disseminated Epstein-Barr virus infection. *Int Arch Allergy Immunol* (2012) 158:418–22. doi:10.1159/000333472
11. Serwas NK, Cagdas D, Ban SA, Bienemann K, Salzer E, Tezcan I, et al. Identification of ITK deficiency as a novel genetic cause of idiopathic CD4+ T-cell lymphopenia. *Blood* (2014) 124:655–7. doi:10.1182/blood-2014-03-564930
12. Stepensky P, Weintraub M, Yanir A, Revel-Vilk S, Krux F, Huck K, et al. IL-2-inducible T-cell kinase deficiency: clinical presentation and therapeutic approach. *Haematologica* (2011) 96:472–6. doi:10.3324/haematol.2010.033910
13. Booth C, Gilmour KC, Veys P, Gennery AR, Slatter MA, Chapel H, et al. X-linked lymphoproliferative disease due to SAP/SH2D1A deficiency: a multicenter study on the manifestations, management and outcome of the disease. *Blood* (2011) 117:53–62. doi:10.1182/blood-2010-06-284935
14. Chung BK, Tsai K, Allan LL, Zheng DJ, Nie JC, Biggs CM, et al. Innate immune control of EBV-infected B cells by invariant natural killer T cells. *Blood* (2013) 122:2600–8. doi:10.1182/blood-2013-01-480665

15. Marsh RA, Madden L, Kitchen BJ, Mody R, McClimon B, Jordan MB, et al. XIAP deficiency: a unique primary immunodeficiency best classified as X-linked familial hemophagocytic lymphohistiocytosis and not as X-linked lymphoproliferative disease. *Blood* (2010) 116:1079–82. doi:10.1182/blood-2010-01-256099
16. Okada S, Markle JG, Deenick EK, Mele F, Averbuch D, Lagos M, et al. IMMUNODEFICIENCIES. Impairment of immunity to *Candida* and *Mycobacterium* in humans with bi-allelic RORC mutations. *Science* (2015) 349: 606–13. doi:10.1126/science.aaa4282
17. Horwood NJ, Urbaniak AM, Danks L. Tec family kinases in inflammation and disease. *Int Rev Immunol* (2012) 31:87–103. doi:10.3109/08830185.2012.670334
18. Hussain A, Yu L, Faryal R, Mohammad DK, Mohamed AJ, Smith CI. TEC family kinases in health and disease – loss-of-function of BTK and ITK and the gain-of-function fusions ITK-SYK and BTK-SYK. *FEBS J* (2011) 278:2001–10. doi:10.1111/j.1742-4658.2011.08134.x
19. Kosaka Y, Felices M, Berg LJ. Itk and Th2 responses: action but no reaction. *Trends Immunol* (2006) 27:453–60. doi:10.1016/j.it.2006.08.006
20. Bacchelli C, Moretti FA, Carmo M, Adams S, Stanescu HC, Pearce K, et al. Mutations in linker for activation of T cells (LAT) lead to a novel form of severe combined immunodeficiency. *J Allergy Clin Immunol* (2017) 139(634–42): e5. doi:10.1016/j.jaci.2016.05.036
21. Keller B, Zaidman I, Yousefi OS, Hershkovitz D, Stein J, Unger S, et al. Early onset combined immunodeficiency and autoimmunity in patients with loss-of-function mutation in LAT. *J Exp Med* (2016) 213:1185–99. doi:10.1084/jem.20151110
22. Platt CD, Fried AJ, Hoyos-Bachiloglu R, Usmani GN, Schmidt B, Whangbo J, et al. Combined immunodeficiency with EBV positive B cell lymphoma and epidermodysplasia verruciformis due to a novel homozygous mutation in RASGRP1. *Clin Immunol* (2017) 183:142–4. doi:10.1016/j.clim.2017.08.007
23. Salzer E, Cagdas D, Hons M, Mace EM, Garncarz W, Petronczki OY, et al. RASGRP1 deficiency causes immunodeficiency with impaired cytoskeletal dynamics. *Nat Immunol* (2016) 17:1352–60. doi:10.1038/ni.3575
24. Atherly LO, Lucas JA, Felices M, Yin CC, Reiner SL, Berg LJ. The Tec family tyrosine kinases Itk and Rlk regulate the development of conventional CD8+ T cells. *Immunity* (2006) 25:79–91. doi:10.1016/j.immuni.2006.05.012
25. Blomberg KE, Boucheron N, Lindvall JM, Yu L, Raberger J, Berglof A, et al. Transcriptional signatures of Itk-deficient CD3+, CD4+ and CD8+ T-cells. *BMC Genomics* (2009) 10:233. doi:10.1186/1471-2164-10-233
26. Broussard C, Fleischacker C, Horai R, Chetana M, Venegas AM, Sharp LL, et al. Altered development of CD8+ T cell lineages in mice deficient for the Tec kinases Itk and Rlk. *Immunity* (2006) 25:93–104. doi:10.1016/j.immuni.2006.05.011
27. Dubois S, Waldmann TA, Muller JR. ITK and IL-15 support two distinct subsets of CD8+ T cells. *Proc Natl Acad Sci U S A* (2006) 103:12075–80. doi:10.1073/pnas.0605212103
28. Nayar R, Enos M, Prince A, Shin H, Hemmers S, Jiang JK, et al. TCR signaling via Tec kinase ITK and interferon regulatory factor 4 (IRF4) regulates CD8+ T-cell differentiation. *Proc Natl Acad Sci U S A* (2012) 109:E2794–802. doi:10.1073/pnas.1205742109
29. Readinger JA, Mueller KL, Venegas AM, Horai R, Schwartzberg PL. Tec kinases regulate T-lymphocyte development and function: new insights into the roles of Itk and Rlk/Txk. *Immunol Rev* (2009) 228:93–114. doi:10.1111/j.1600-065X.2008.00757.x
30. Felices M, Berg LJ. The Tec kinases Itk and Rlk regulate NKT cell maturation, cytokine production, and survival. *J Immunol* (2008) 180:3007–18. doi:10.4049/jimmunol.180.5.3007
31. Fowell DJ, Shinkai K, Liao XC, Beebe AM, Coffman RL, Littman DR, et al. Impaired NFATc translocation and failure of Th2 development in Itk-deficient CD4+ T cells. *Immunity* (1999) 11:399–409. doi:10.1016/S1074-7613(00)80115-6
32. Sun Y, Peng I, Webster JD, Suto E, Lesch J, Wu X, et al. Inhibition of the kinase ITK in a mouse model of asthma reduces cell death and fails to inhibit the inflammatory response. *Sci Signal* (2015) 8:ra122. doi:10.1126/scisignal.aab0949
33. Graves PE, Siroux V, Guerra S, Klimecki WT, Martinez FD. Association of atopy and eczema with polymorphisms in T-cell immunoglobulin domain and mucin domain-IL-2-inducible T-cell kinase gene cluster in chromosome 5 q 33. *J Allergy Clin Immunol* (2005) 116:650–6. doi:10.1016/j.jaci.2005.05.004
34. Kannan AK, Sahu N, Mohanan S, Mohinta S, August A. IL-2-inducible T-cell kinase modulates TH2-mediated allergic airway inflammation by suppressing IFN-gamma in naive CD4+ T cells. *J Allergy Clin Immunol* (2013) 132:811–20. e1–5. doi:10.1016/j.jaci.2013.04.033
35. Mueller C, August A. Attenuation of immunological symptoms of allergic asthma in mice lacking the tyrosine kinase ITK. *J Immunol* (2003) 170: 5056–63. doi:10.4049/jimmunol.170.10.5056
36. Miller AT, Berg LJ. Defective Fas ligand expression and activation-induced cell death in the absence of IL-2-inducible T cell kinase. *J Immunol* (2002) 168:2163–72. doi:10.4049/jimmunol.168.5.2163
37. Gomez-Rodriguez J, Meylan F, Handon R, Hayes ET, Anderson SM, Kirby MR, et al. Itk is required for Th9 differentiation via TCR-mediated induction of IL-2 and IRF4. *Nat Commun* (2016) 7:10857. doi:10.1038/ncomms10857
38. Gomez-Rodriguez J, Sahu N, Handon R, Davidson TS, Anderson SM, Kirby MR, et al. Differential expression of interleukin-17A and -17F is coupled to T cell receptor signaling via inducible T cell kinase. *Immunity* (2009) 31:587–97. doi:10.1016/j.immuni.2009.07.009
39. Gomez-Rodriguez J, Wohlfert EA, Handon R, Meylan F, Wu JZ, Anderson SM, et al. Itk-mediated integration of T cell receptor and cytokine signaling regulates the balance between Th17 and regulatory T cells. *J Exp Med* (2014) 211:529–43. doi:10.1084/jem.20131459
40. Schaeffer EM, Yap GS, Lewis CM, Czar MJ, McVicar DW, Cheever AW, et al. Mutation of Tec family kinases alters T helper cell differentiation. *Nat Immunol* (2001) 2:1183–8. doi:10.1038/ni734
41. Schaeffer EM, Debnath J, Yap G, McVicar D, Liao XC, Littman DR, et al. Requirement for Tec kinases Rlk and Itk in T cell receptor signaling and immunity. *Science* (1999) 284:638–41. doi:10.1126/science.284.5414.638
42. Atherly LO, Brehm MA, Welsh RM, Berg LJ. Tec kinases Itk and Rlk are required for CD8+ T cell responses to virus infection independent of their role in CD4+ T cell help. *J Immunol* (2006) 176:1571–81. doi:10.4049/jimmunol.176.6.3842
43. Bachmann MF, Littman DR, Liao XC. Antiviral immune responses in Itk-deficient mice. *J Virol* (1997) 71:7253–7.
44. Kapnick SM, Stinchcombe JC, Griffiths GM, Schwartzberg PL. Inducible T cell kinase regulates the acquisition of cytolytic capacity and degranulation in CD8+ CTLs. *J Immunol* (2017) 198:2699–711. doi:10.4049/jimmunol.1601202
45. Sunil-Chandra NP, Arno J, Fazakerley J, Nash AA. Lymphoproliferative disease in mice infected with murine gammaherpesvirus 68. *Am J Pathol* (1994) 145:818–26.
46. Chen G, Tai AK, Lin M, Chang F, Terhorst C, Huber BT. Signaling lymphocyte activation molecule-associated protein is a negative regulator of the CD8 T cell response in mice. *J Immunol* (2005) 175:2212–8. doi:10.4049/jimmunol.175.4.2212
47. Yin L, Al-Alem U, Liang J, Tong WM, Li C, Badiali M, et al. Mice deficient in the X-linked lymphoproliferative disease gene sap exhibit increased susceptibility to murine gammaherpesvirus-68 and hypo-gammaglobulinemia. *J Med Virol* (2003) 71:446–55. doi:10.1002/jmv.10504

Conflict of Interest Statement: The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

frontiers in Immunology

REVIEW
published: 04 June 2018
doi: 10.3389/fimmu.2018.01103

Inherited Immunodeficiencies With High Predisposition to Epstein–Barr Virus-Driven Lymphoproliferative Diseases

Sylvain Latour[1,2,3]* *and Sarah Winter*[1,2,3]

[1] *Laboratory of Lymphocyte Activation and Susceptibility to EBV infection, INSERM UMR 1163, Paris, France,* [2] *Imagine Institute, Paris Descartes University, Sorbonne Paris Cité, Paris, France,* [3] *Equipe de Recherche Labéllisée, Ligue National contre le Cancer, Paris, France*

Epstein–Barr Virus (EBV) is a gamma-herpes virus that infects 90% of humans without any symptoms in most cases, but has an oncogenic potential, especially in immunocompromised individuals. In the past 30 years, several primary immunodeficiencies (PIDs) associated with a high risk to develop EBV-associated lymphoproliferative disorders (LPDs), essentially consisting of virus-associated hemophagocytic syndrome, non-malignant and malignant B-cell LPDs including non-Hodgkin and Hodgkin's types of B lymphomas have been characterized. Among them are *SH2D1A* (*SAP*), *XIAP*, *ITK*, *MAGT1*, *CD27*, *CD70*, *CTPS1*, *RASGRP1*, and *CORO1A* deficiencies. Penetrance of EBV infection ranges from 50 to 100% in those PIDs. Description of large cohorts and case reports has refined the specific phenotypes associated with these PIDs helping to the diagnosis. Specific pathways required for protective immunity to EBV have emerged from studies of these PIDs. SLAM-associated protein-dependent SLAM receptors and MAGT1-dependent NKG2D pathways are important for T and NK-cell cytotoxicity toward EBV-infected B-cells, while CD27–CD70 interactions are critical to drive the expansion of EBV-specific T-cells. CTPS1 and RASGRP1 deficiencies further strengthen that T-lymphocyte expansion is a key step in the immune response to EBV. These pathways appear to be also important for the anti-tumoral immune surveillance of abnormal B cells. Monogenic PIDs should be thus considered in case of any EBV-associated LPDs.

Keywords: immunodeficiencies, Epstein–Barr virus, lymphoproliferative disorders, genetic predisposition to disease, T lymphocytes, T lymphocyte activation

OPEN ACCESS

Edited by:
Isabelle Meyts,
KU Leuven, Belgium

Reviewed by:
Jeffrey I. Cohen,
National Institutes of Health (NIH), United States
Arndt Borkhardt,
Heinrich Heine Universität Düsseldorf, Germany
Stuart G. Tangye,
Garvan Institute of Medical Research, Australia

***Correspondence:**
Sylvain Latour
sylvain.latour@inserm.fr

Specialty section:
This article was submitted to Primary Immunodeficiencies, a section of the journal Frontiers in Immunology

Received: *15 February 2018*
Accepted: *02 May 2018*
Published: *04 June 2018*

Citation:
Latour S and Winter S (2018) Inherited Immunodeficiencies With High Predisposition to Epstein–Barr Virus-Driven Lymphoproliferative Diseases. Front. Immunol. 9:1103. doi: 10.3389/fimmu.2018.01103

The Epstein–Barr virus (EBV) is a gamma-herpes family virus that infects most of humans with a marked tropism for B lymphocytes. EBV is an oncogenic virus known to be the causative agent of several types of neoplasms, including B, T, and NK cell lymphomas, nasopharyngeal and gastric carcinomas, and Epstein–Barr virus-associated smooth muscle tumors (EBV-SMT). 110,000–200,000 cancer cases per year are attributable to EBV worldwide (1).

The reservoir of EBV is strictly human. After the age of 35 years, the incidence of the infection in the general population is more than 90%. The primary infection occurs in the oropharynx where EBV virions infect epithelial cells and B lymphocytes *via* the CD21 molecule. During the primary infection, EBV drives the activation and the expansion of latently infected B lymphoblasts (2, 3). These

proliferating B cells express EBV latent growth-transforming genes that establish EBV persistence (latency III program) and are mainly eliminated by specific $CD8^+$ T cells that strongly expand during the immune response. Innate cytotoxic lymphocytes like NK cells, γδ T cells, and iNKT cells, specifically early differentiated KIR-negative NK cells and Vγ9Vδ2 T cells, are also thought to play an important role in the early phase of the primary infection by recognition of lytically and latently EBV-replicating cells, respectively (2, 4, 5). Some EBV-infected B cells escape to the immune response by downregulating latent genes expression (latency 0 program) and acquire a memory phenotype, becoming invisible to the immune system and establishing a reservoir for EBV. Subsequent stimulations of these EBV-containing "reservoir" memory B cells will lead to reactivation of EBV from latency into the lytic cycle, thus promoting infections of new B cells and their expansion. Ultimately, EBV-transformed lymphoblasts can lead to lymphoma. In some very rare cases, EBV can also infect T cells and NK cells. This peculiar profile of infection is rather observed in Asian and South American populations and is associated with a chronic viremia, infiltration of organs with by EBV-positive lymphocytes, and life-threatening lymphoproliferative disorders (LPDs) including hemophagocytic syndrome or/and EBV-positive T/NK cell lymphoma. The mechanisms underlying the pathogenesis of this infection are not clearly known, as well as its genetic determinants that are thought to be oligogenic or polygenic (6, 7). This unusual EBV infection will not be covered in this review.

The first encounter with EBV usually happens during infancy and adolescence by oral transmission and is largely asymptomatic. However, in some immunocompetent individuals particularly during adolescence, primary infection causes infectious mononucleosis (IM), a self-limiting lymphoproliferative disease clinically characterized by fever, sore throat, body aches, swollen lymph nodes, and general fatigue (3). The lymphoproliferation consists of a robust and sustained expansion of $CD8^+$ T cells and infected B cells reflecting a strong immune response to the virus. Notably, $CD8^+$ EBV-specific T cells can represent more than 40% of circulating T cells in some subjects (8).

In immunocompromised individuals, reactivations of EBV and persistence of proliferating latent growth-transforming EBV-infected B cells are associated with severe pathologies that can have fatal outcome. Those include hemophagocytic lymphohistiocytosis (HLH), also termed virus-associated hemophagocytic syndrome, non-malignant B-cell LPDs, and B-cell lymphomas including Hodgkin's lymphomas and non-Hodgkin's lymphomas such as Burkitt's lymphoma and diffuse large B-cell lymphoma (DLBCL) (1). Such disorders defined as posttransplant lymproliferative disorders are often observed in patients with organ transplantation under immunosuppressive treatment. Similarly, HIV-infected patients with acquired immunodeficiency syndrome (AIDS) often experience lymphoproliferation disorders driven by EBV, that represent one of the most frequent cause of death in patients with AIDS (9). Those observations highlight that reactivations of EBV from latently EBV-infected B cells occur frequently in normal individuals throughout life and need to be tightly controlled by the adaptative immune response.

Beside acquired forms, several inherited combined immunodeficiencies (CIDs) leading to a particular susceptibility to EBV infection and to develop EBV-driven diseases have been identified over the last 20 years (10–12). Those genetic defects include mutations in *SH2D1A*, *ITK*, *MAGT1*, *CTPS1*, *CD27*, *CD70*, *CORO1A*, and *RASGRP1* (**Table 1**). In these genetically determined forms, the penetrance of the EBV susceptibility is high with more than 50% patients having presented at least one severe episode of EBV-driven LPD including Hodgkin and non-Hodgkin lymphomas (**Table 2**). However, a number of carriers of these gene defects can also experience other severe viral infections caused by CMV, VZV, HSV, HHV-6, or HPV. This is particularly true for CTPS1 and CORO1A deficiencies since patients often presented VZV and HPV infections, respectively. Bacterial infections, in particular recurrent lung infections are noticed in a number of patients and can be the initial clinical presentation. This may be related to the hypogammaglobulinemia and/or dysgammaglobulinemia associated with low number of $CD27^+$ memory B cells that are frequently observed in these defects. These phenotypes likely result or have been proven to result from abnormalities in T-cell help to B cells due to defects in T-helper cell maturation and/or activation. Intrinsic defects in B cell development and function may also contribute directly to the hypo/dysgammaglobulinemia, like in RASGRP1, CTPS1, CD27 deficiencies, for which there is clear evidence that these genes are directly involved in B-cell development and/or function. However, these B-cell defects do not to interfere with the ability to EBV to infect B cells and its oncogenic activity. $CD4^+$ T cell lymphopenia and/or decrease naïve T cells is also a frequent phenotype, which can be considered as an immunological hallmark of a T-cell defect (13, 14). NK cells and iNKT cells are also often found to be diminished or absent. Finally, several of these deficiencies are associated with very unique features, independently of the high risk to develop EBV-driven lymphoproliferations, helping to the diagnosis, such as autoimmunity, inflammatory bowel disease (IBD), lung involvement, and neurological disorders that develop in RASGRP1, XIAP-, ITK-, and CORO1A-deficiency, respectively (**Table 1**).

Over the last two decades, these inherited defects have represented very unique *in natura* models to decipher the immune response to EBV. Molecular and genetic characterization of these disorders and their study have revealed several unexpected and novel pathways required for an efficient immunity to EBV, but also more generally involved in cancer immune surveillance of B lymphocytes, and adaptative immune responses.

SH2D1A/SLAM-ASSOCIATED PROTEIN (SAP) DEFICIENCY

SH2D1A/SAP deficiency (also known as the X-linked lymphoproliferative syndrome type 1-XLP-1 or Purtilo syndrome) is caused by hemizygous mutations in the X-linked gene *SH2D1A* (15–19). More than 100 patients with hemizygous deleterious mutations in *SH2D1A* have been reported, but if considering the literature before the discovery of the gene, this is probably more than 200 cases (20–22). Clinical features of XLP-1 are EBV-driven fulminant or severe mononucleosis with all the clinical features of HLH including fulminant hepatitis, hepatic necrosis,

TABLE 1 | Lymphoma subtypes in primary immunodeficiencies associated with high susceptibility to develop EBV-driven lymphoproliferative diseases.

Mutated gene number of patients (*n*)	Age of onset (years)	EBV-associated diseases	Infections		Other clinical features	Immunological features			Outcome	Defective pathways/ functions
						T-cells	B-cells	NK-cells		
SH2D1A (XLP-1) *n* > 100	0.5–40	SIM/HLH 50–60% (neurological inv. 25%) B lymphoma 25–30% Vasculitis 3–4%	**EBV 60–70%** HHV-6 (1) HHV-7 (1) LCMV (1)	Lung infections 15% Diss. aspergillosis (1) *E. coli* sepsis (1)	Aplastic anemia 3–4% EBV-negative lymphoma EBV-negative vasculitis	Absence iNKT	Dys-γ, hypo-γ 50%	↓ NK	HSCT 10–40% Mortality 20–50%	SLAMR/SAP pathway (T and NK cytotoxicity and AICD)
XIAP (XLP-2) *n* > 100	0.5–40	SIM/HLH 50%	**EBV 35–40%** CMV 10–20% HHV-6 (1)		IBD 25–40% Inflammatory disorders 5% (uveitis, skin abcesses, etc.) Cholangitis 1–2%	↓ MAIT ↓ iNKT	Hypo-γ 15–20%		HSCT 10–30% Mortality 5–30%	Excess of apoptosis (AICD, TRAIL-R, Fas) NOD1/2 signaling/ function
ITK *n* = 13	2.5–18	LPD/B lymphoma 13/13 HLH 1/13 SMT 1/13	**EBV 13/13** CMV 1/13 VZV 1/13 BK-virus 2/13	Lung infections 9/13 PCP 2/13	Lung involvement 11/13 Kidney involvement 3/13 AI cytopenias 3/13	↓ $CD4^+$ 8/13 ↓ iNKT 5/5	Hypo-γ 8/13		HSCT 4/13 Death 7/13	TCR induced calcium flux T-cell proliferation
MAGT1 *n* = 11	3–45	LPD/B lymphoma 7/11	**EBV 11/11** HSV 2/11, VZV 1/11 JC virus (PML) 1/11 HHV-8 (KS) 1/11	Lung infections 9/11	AI cytopenias 3/11	↓ $CD4^+$ ↓ NKG2D	Dys-γ, hypo-γ 8/11 Lymphocytosis		HSCT 2/11 Death 3/11	NKG2D-dependent cytotoxicity
CORO1A *n* = 9	0.5–7	LPD/B lymphoma 5/9	**EBV 5/9** HPV 4/9, VZV 5/9 HSV 2/9 Parvovirus B19 1/9	Lung infections 7/9 Cutaneous leprosy 1/9 Visceral leishmaniasis 1/9	Neurological involvement (cognitive impairment) 3/9	↓ $CD4^+$ 8/9 ↓ MAIT 1/1 ↓ iNKT 1/1	High IgE level 4/5		HSCT 2/9 Death 4/9	Actin regulation T-cell survival NK cytotoxicity
CD27 *n* = 18	1–22	LPD/B lymphoma 12/18 T-cell lymphoma 1/18 SIM/HLH 5/18 Meningitis 1/18	**EBV 18/18** VZV 2/18 CMV 1/18	Lung infections 4/18 Gram-positive sepsis 1/18 Giardiasis 1/18	Aplastic anemia 1/18 Uveitis 5/18 Oral anal ulcers 5/18	↓ iNKT 3/10	Hypo-γ 13/18	↓ NK	HSCT 4/18 Death 3/18	CD27–CD70 pathway (T-cell proliferation) NK cytotoxicity
CTPS1 *n* = 12	0–5	LPD/B lymphoma 5/12 SIM 5/12	**EBV 11/12** VZV 6/12 Norovirus 3/12 CMV 2/12, HHV-6 2/12	Lung infections 7/12 Meningitis 3/12	Eczema 2/12	↓ MAIT ↓ iNKT	↓ IgG2 5/5 ↓ $CD27^+$ B-cells	↓ NK	HSCT 9/12 Death 3/12	*De novo* pyrimidine synthesis T- and B-cell proliferation
RASGRP1 *n* = 6	5–12	LPD/B lymphoma 4/6 SMT 2/6	**EBV 5/6** HSV 1/6 HPV (EV) 1/6 CMV 1/6	Lung infections 5/6 Diss. tuberculosis 1/6 LN tuberculosis 1/6 PCP 1/6	AI cytopenias 3/6 EBV-negative LPD 2/6	↓ $CD4^+$ 4/6 ↓ MAIT 2/2 ↓ iNKT 2/2	Hypo-γ 1/6 Hyper-γ 2/6 ↓ $CD27^+$ B-cells 2/4	↓ NK 3/6	HSCT 2/6 Death 2/6	MAPK pathway (ERK1/2, T-, B-cell proliferation) Actin/cytoskeleton dynamics NK cytotoxicity

(*Continued*)

TABLE 1 | Continued

Mutated gene number of patients (*n*)	Age of onset (years)	EBV-associated diseases	Infections	Other clinical features	Immunological features			Outcome	Defective pathways/ functions
					T-cells	B-cells	NK-cells		
CD70 *n* = 6	1–5	LPD/B-cell lymphoma 5/6	EBV 6/6 VZV 1/6	Lung infections 3/6	PFAPA 1/6 Hypersensitivity to mosquito bites 1/6	↓ MAIT 1/1 ↓ iNKT 1/1	Hypo-γ 5/6 ↓ CD27$^+$ B-cells 3/6	HSCT 1/6 All alive	CD27–CD70 pathway (T-cell proliferation) Decreased 2B4 and NKG2D on memory T CD8$^+$

SIM, severe infectious mononucleosis; HLH, hemophagocytic lymphohistiocytosis; LPD, lymphoproliferative disorder; inv., involvement; Diss., disseminated; PCP, pneumocytosis pneumoniae; IBD, inflammatory bowel disease; AI, autoimmunity; Dys-γ, dysgammaglobulinemia; Hypo-γ, hypogammaglobulinemia; KS, Kaposi sarcoma; PFAPA, perdiodic fever, aphtous stomatitis, phanyngitis and cervical adenitis; EV, epidermodysplasia verruciformis; SMT, smooth muscle tumor; PML, progressive multifocal leukoencephalopathy; HSCT, hematopoietic stem cell transplantation; LN, lymph node; EBV, Epstein–Barr virus; LCMV, lymphocytic choriomeningitis virus; CMV, cytomegalovirus; HHV, human herpes virus; VZV, varicella zoster virus; MAIT, mucosal-associated invariant T; AICD, activation-induced cell death; TCR, T-cell receptor; SAP, SLAM-associated protein; SLAMR, SLAM receptor.

bone marrow hypoplasia, neurological involvement in 20–30% of cases, hypogammaglobulinemia (50%), and B-cell lymphoma (25–30%) which often have abdominal localization. Few other rare phenotypes can be also observed such as vasculitis (2–5%), aplastic anemia (2–5%), and chronic gastritis (18, 23–26). All phenotypes can occur independently and some develop without any evidence of EBV infection (10–20%) or prior to EBV infection like HLH, hypogammaglobulinemia, lymphoma, aplastic anemia, and vasculitis (24–26). Some rare cases of T-cell lymphoma have also been reported (23, 27). However, a significant proportion of patients initially presented with combined variable immunodeficiency (CVID) associated with severe recurrent bacterial infections including disseminated aspergillosis and *E. coli* sepsis (28–31). Only five cases of other severe viral infections by HHV-6, HHV-7, and LCMV or HLH without EBV trigger have been reported in the recent literature (22, 26, 32, 33). This highlights that SAP has a very unique role in immunity to EBV, when it is not required for other viral infections except in very rare cases.

SH2D1A encodes a small adaptor protein of 128 amino acids named SAP, uniquely expressed in T and NK cells. SAP is only made of a unique SH2 domain that specifically binds to the intracytoplasmic domain of receptors of the SLAM family (SLAMF), which are self-ligands and are involved in homotypic cell–cell interactions with the exception of 2B4 (also known as CD244 or SLAMF4) that binds CD48 (34–36). On the one hand, SAP functions as a real adapter protein by its ability to recruit the tyrosine kinase FynT to SLAMF receptors, thus coupling those to downstream signaling pathways. On the other hand, SAP acts also as a blocker protein by inhibiting the recruitment of SH2-containing phosphatases to SLAMF (15, 35, 37–39). Early studies have documented NK-cell cytotoxicity defects toward EBV-infected B cells in XLP-1 patients (40, 41). Further investigations revealed that these defects involved two SLAM receptors, 2B4 and SLAMF6 (also known as NTB-A or Ly108) which have the capacity to trigger and activate NK- and CD8$^+$ T-cell cytotoxicity toward EBV-infected B cells or transformed B cells when SAP is normally expressed (42–46). B-cell lymphomas and EBV-infected B cells are known to express high levels of ligands for SLAMF-R, including CD48 the ligand of 2B4 (47). This expression might signal abnormal "dangerous" proliferating B cells to NK cells and T lymphocytes. Following studies demonstrated that the stimulatory function of 2B4 and SLAMF6, which is dependent of FynT was not only lost in the absence of SAP but also shifted toward an inhibition of other stimulatory pathways of cell cytotoxicity in NK- and CD8$^+$ T-cells (43, 48, 49). Additional findings in mice indicated that this inhibitory effect was independent of FynT, but depends of the blocking activity of SAP, and leads to decreased conjugate formation between CD8$^+$ T or NK cells and SLAMF-expressing target cells like B cells (50, 51). In this context, other activation pathways of cytotoxicity such as NKG2D may not compensate for the defective function of 2B4 and SLAMF6. The inhibitory activity of 2B4 and SLAMF6 in the absence of SAP may have a trans-inhibitory effect on activating receptors (such as NKG2D and/or killer cell activating receptors) when co-engaged at the cytotoxic synapse.

Importantly, NK-cell and CD8$^+$ T-cell cytotoxicity toward other APCs or target cells than B cells including non-hematopoietic

TABLE 2 | Lymphoma subtypes in primary immunodeficiencies associated with high susceptibility to develop EBV-driven lymphoproliferative diseases.

Mutated gene number of patients/total	Hodgkin lymphoma (subtype)	B-cell NHL			T-cell lymphoma
		DLBCL	Burkitt lymphoma	Not specified	
SH2D1A 25–30%		30–40%: abdominal (40–50%), cervical (30–40%), spinal (10–20%)	40–60%: abominal (50–60%), cervical (20%)	20–30%	1 patient (CNS)
ITK 9/13	6 patients (1 mixed cellularity)	1 patient	1 patient	1 patient	
MAGT1 5/11	2 patients	1 patient	1 patient	1 patient	
COR01A 4/9		4 patients			
CD27 6/18	3 patients (2 scleronodular and 1 mixed cellularity)			2 patients	1 patient
CTPS1 2/12				2 patients (CNS 2/2)	
RASGRP1 4/6	2 patients (1 scleronodular and 1 mixed cellularity)			2 patients	
CD70 4/6	4 patients (1 scleronodular and 1 mixed cellularity)				

CNS, central nervous system; NHL, non-Hodgkin lymphoma; DLBCL, diffuse large B-cell lymphoma; EBV, Epstein–Barr virus.

cells or/and SLAMF-negative target cells was preserved, and even augmented for NK-cell cytotoxicity (47, 52, 53). Recent studies in mice indicate that this excessive NK-cell cytotoxicity is dependent of a role of SAP and SLAMF6 in NK cell licensing/education (52). In the absence of SAP, NK-cell responsiveness toward SLAM-negative cells is increased. In XLP-1 patients, increased NK cytotoxicity toward the SLAMF-negative target cells K562 was documented (54), associated with increased CD16- and NKp46-dependent cytotoxicity (52). Thus, extra-hematopoietic manifestations associated with HLH seen in XLP-1 patients, like extensive liver damage may be caused by this increased NK-cell cytotoxicity toward non-hematopoietic cells.

Beside its function in NK- and $CD8^+$ T cell-cytotoxicity, SAP is also important to limit $CD8^+$ T-cell expansion. SAP-deficient T cells both in mice and human exhibited increased survival due to impaired activation-induced cell death (AICD), an important pathway involves in the contraction of the pool of Ag-specific T-cells during immune responses (55, 56). The positive role of SAP on AICD is dependent of SLAMF6 that delivers signals for the expression of the pro-apoptotic molecules Bim and FasL (55). In concert, these distinct defects certainly contribute to the inability of EBV-specific T cells to eliminate EBV-infected B cells and to their uncontrolled expansion and activity during primary infection leading to fulminant mononucleosis or HLH. The fact that patients also frequently develop non-EBV B cell lymphoma argues for an important role of the SLAM–SAP pathway in the immune surveillance of B cells.

XIAP DEFICIENCY

XIAP deficiency (also known as the X-linked lymphoproliferative syndrome type 2/XLP-2) is caused by hemizygous mutations in the *XIAP* gene coding the X-linked Inhibitor of Apoptosis Protein XIAP (54, 57). XIAP is an anti-apoptotic molecule member of the inhibitor of apoptosis protein family (IAP). XIAP is a potent inhibitor of program cell death, but it is also required for the function of (NOD)-like pattern recognition receptors (NLRs) NOD 1 and 2 and the regulation of the inflammasome activity of NLRP3 (58–61). More than 100 patients have been now reported with a XIAP deficiency. Initially reported in 2006 in a cohort of patients with a clinical phenotype close to that of SH2D1A-deficient patients (XLP-1), XIAP deficiency was further denominated XLP-2, since *XIAP* is located in the immediate vicinity of *SH2D1A* on the X chromosome (57). However, during the last 10 years, there were cumulative observations that the two diseases differ by many aspects, in particular, there is no evidence that they are functionally related. The main clinical phenotypes of the XIAP deficiency are the susceptibility to develop HLH in the context of EBV infection (36%), the recurrent splenomegaly corresponding to a minimal form of HLH (57%), and the IBD (26%) with features of Crohn's disease (57, 62, 63). The HLH is often less severe than in the XLP-1 deficiency with very rare neurological involvement (24). Some patients also developed variable auto-inflammatory symptoms like uveitis, arthritis, skin abscesses, erythema nodosum, and nephritis (62, 63). Thus, today although the susceptibility to EBV remains one important and severe clinical manifestation of the XIAP deficiency, more than half of the patients never experienced a peculiar EBV susceptibility (57, 62). XIAP-deficient patients also never experience lymphoma in contrast to patients with SAP deficiency, likely related to the anti-apoptotic function of XIAP, which may protect patients from cancer, and XIAP is now considered as a promising therapeutic target for cancer treatment (64).

T lymphocytes from XLP-2 patients have been shown to exhibit increased AICD in response to T-cell receptor (TCR) activation

and increased apoptosis to FAS/CD95 and TRAIL-R stimulations (54). On the other hand, monocytes from patients displayed impaired production of cytokines and chemokines (TNF-α, IL-10, IL-8, and MCP-1) to stimulation with NOD2 ligands (63, 65). Although the mechanisms of EBV-driven HLH in XIAP deficiency remains unclear, it is proposed that excessive AICD might compromise the expansion and proliferation of activated EBV-specific T cells like in CTPS1, CD27, or CD70 deficiencies (see below)(58). In this setting, accumulation of apoptotic cells and persistence of EBV-infected B cells could result in abnormal inflammation amplified by impaired inflammasome regulation and/or defective NOD1/2 activation of myeloid cell populations. In concert, T lymphocytes and myeloid defects may contribute to EBV-driven HLH in XIAP-deficient patients.

ITK DEFICIENCY

ITK deficiency is an autosomal recessive disorder caused by bi-allelic mutations in *ITK*. Until now, 13 patients from 8 families have been reported in the literature (66–72). All experienced EBV-associated recurrent non-malignant LPD or malignant B-cell lymphoproliferations including Hodgkin lymphoma in five, and one develop an EBV-SMT (69). B-cell lymphoproliferations have often a pulmonary localization and more than 50% also develop pulmonary infections. ITK (IL-2-inducible tyrosine kinase) is a well-characterized protein tyrosine kinase of the TEC/BTK family specifically expressed in T lymphocytes and NK cells. ITK is involved in TCR signaling by its capacity to phosphorylate and activate the PLC-γ1, a key enzyme that stimulates Ca^{++} fluxes through the production of IP3, both being critical second messengers of T-cell activation (73, 74). ITK has been also involved in CXCR4 signaling (75). Mice studies demonstrated the importance of ITK in immunity particularly in $CD4^+$ T-cell responses (73, 76). A recent report showed that ITK is also required for efficient $CD8^+$ T-cell responses in mouse (77). In the absence of ITK, $CD8^+$ T-cell expansion and maturation into cytolytic effector T cells is impaired leading to decreased $CD8^+$ T-cell cytotoxic responses. Very few studies have been made to characterize ITK-deficient T cells from patients, albeit-derived T-cell lines from ITK-deficient patients have been obtained and studied showing decreased Ca^{++} mobilization (67). Defective T-cell proliferation in response to TCR engagement was documented in one patient (69). Although the exact mechanism(s) underlying EBV susceptibility in ITK deficiency need to be established, the recent study of $CD8^+$ T cells from ITK-deficient mice (77), strongly supports that in T cells from ITK-deficient patients, TCR activation signals are impaired resulting in defective expansion and maturation of EBV-specific $CD8^+$ T cells like in CD70 and CD27 deficiencies (see below).

MAGT1 DEFICIENCY

MAGT1 deficiency also termed XMEN disease (for X-linked immunodeficiency, magnesium defect, EBV infection, and neoplasia syndrome) is caused by hemizygous mutations in *MAGT1*. To date, 11 male patients with MAGT1 deficiency have been identified and all developed susceptibility to EBV infection with chronic viremia and B-cell lymphomas including Hodgkin and DLBCL (78–82). In one patient, the initial clinical presentation was a HHV-8-associated Kaposi sarcoma (80). One particular trait of this disease is the B-cell lymphocytosis associated with a CD4 lymphopenia (83). *MAGT1* encodes a ubiquitously expressed transmembrane Mg^{++} transporter involved in the maintenance of free basal intracellular Mg^{++} pools. However, MAGT1 also associates with the *N*-oligosaccharyl transferase complex, and therefore may have a role in protein N-glycosylation (84). Following the discovery of the MAGT1 immunodeficiency, MAGT1-dependent Mg^{++} influx was documented in T cells upon TCR engagement. Importantly, this Mg^{++} mobilization was shown to be involved in PLC-γ1 activation and subsequent dependent Ca^{++} influx (78). Notably, all these events were markedly impaired in activated MAGT1-deficient T cells in response to TCR, but T-cell proliferation has been considered to be normal or diminished (85). Based on these findings, it was proposed that MAGT1 might be necessary to activate PLC-γ1 possibly by acting on ITK. In a recent study, intriguingly, Ca^{++} mobilization was found to be only moderately decreased and delayed in T cells from a patient carrier of a deletion encompassing *MAGT1* (80). Along these lines, an other recent report showed that MAGT1-deficient T cells from mice exhibited normal calcium flux upon TCR activation, while calcium flux was impaired in B cells in response to BCR stimulation (86). The discrepancy between these recent observations and earlier studies is not known. In any case, MAGT1 was further shown to be required for expression and function of NKG2D and its signaling adapter DAP10 (79). NKG2D is an activating NK-cell receptor expressed on NK cells, γδ T cells, and $CD8^+$ T cells, that recognizes MHC class I-homologous proteins induced by cellular stress in response to infection or neoplasia. Importantly, ligands of NKG2D are upregulated on EBV-infected B cells and EBV-associated lymphoproliferations (87, 88). Consistent with defective NKG2D expression, MAGT1-deficient $CD8^+$ T cells displayed impaired cytotoxic activity against autologous EBV-transformed B cells. Importantly, magnesium supplementation treatment *in vivo* and *in vitro* restored basal intracellular Mg^{++} concentration, NKG2D expression, cell cytotoxicity, and immunity to EBV in MAGT1-deficient patients (79). Defective N-glycosylation of NKG2D associated with increased ubiquitinylation leading to accelerated protein turnover might be considered as the main mechanism explaining the impaired NKG2D function. Thus, these observations highlight the essential role of the NKG2D pathway in immunity to EBV, albeit the exact role of MAGT1 in TCR signaling remains to be clarified.

CD27 DEFICIENCY

This deficiency is caused by bi-allelic mutations in *CD27* that encodes a protein belonging to the super family of TNF receptors (TNFSFR), also known as TNFSFR7. Until today, 18 patients have been reported and all developed EBV-associated LPDs including malignant B-cell proliferations, Hodgkin's lymphoma, B lymphoma, and few patients developed HLH triggered by EBV (89–92). Some patients also experienced inflammatory symptoms such as uveitis and oral ulcers. CD27 binds to CD70 (also named

TNFSF7), a member of the TNF superfamily ligands. CD27 is highly expressed by T cells including resting T cells and a small fraction of B cells corresponding to memory B cells (93–95). CD27 is a co-stimulatory molecule of T-cell activation and CD27–CD70 interactions in mice have been shown to enhance T-cell survival, effector functions, and memory T-cell expansion, in particular $CD8^+$ T cells during anti-viral immune responses (96, 97). Insights to the mechanism underlying the high susceptibility to EBV in CD27 deficiency was recently given by the identification of CD70-deficient patients (see below).

CD70 DEFICIENCY

Five patients with homozygous deleterious mutations in *CD70* have been reported in 2017 and one in 2018 (91, 98, 99). Five out of six patients developed EBV-Hodgkin's lymphoma and recurrent B-cell lymphoproliferations, and one initially presented clinical signs evoking a Behçet-like syndrome and had uncharacterized viral encephalitis. All patients had dysgammaglobulinemia and two patients developed other infections. By many aspects CD70-deficiency appears to be a phenocopy of the CD27 deficiency. In peripheral blood mononuclear cells, CD70 is only expressed by a very small fraction of B cells (91), but its expression is strongly upregulated on activated B cells and EBV-infected B cells during primary infection in tonsils of patients with IM (91). B-cell lymphomas and several other cancers such as solid carcinomas are also known to express CD70 (94). Izawa et al. demonstrated that CD70 expression on EBV-infected B cells drives the expansion of EBV-specific cytolytic T cells *via* a TCR-CD27-dependent co-stimulation. When CD70 is absent on EBV-infected B cells, or CD27 on T cells, EBV-specific T cells failed to expand leading to reduced cytotoxicity responses toward EBV-infected B cells (91). Decreased expression of 2B4 and NKG2D on memory $CD8^+$ T cells of CD70-deficient patients was also noticed and may also contribute to the inability of T cells to eliminate EBV-infected B cells (98). These findings demonstrate that the CD70–CD27 axis represents a key component of the protective immunity to EBV. Furthermore, the implication of the CD27–CD70 axis in anti-tumoral immune surveillance of abnormal B cells is supported by the observations that *CD70* is often found somatically mutated in B lymphomas, perhaps to escape to the immune surveillance (91).

CTPS1 DEFICIENCY

CTPS1 deficiency is an autosomal recessive immunodeficiency caused by a unique homozygous deleterious mutation in *CTPS1* with a founder effect in the population of the North West of England. Until now, 12 patients have been reported and all but 1 presented EBV susceptibility including severe infectious mononucleosis, LPD, and B-cell lymphoma, which was the initial clinical presentation in 40% of cases (100–102). Half of them also developed other viral infections including CMV, VZV, and HHV-6. Some also experienced recurrent bacterial infections with *Haemophilus influenza*, *Streptococcus pneumonia*, and/or *Neisseria meningitis*. *CTPS1* codes for the CTP synthetase or synthase 1, a key enzyme of the *de novo* synthesis of the CTP nucleotide, which is the limiting nucleotide in cells (103). CTP is a critical precursor in the metabolism of nucleic acids. CTP is produced by two pathways, a salvage pathway and a *de novo* synthesis pathway. The salvage pathway utilizes cytidine, a degradation product from nucleic acids. The "*de novo*" CTP synthesis is dependent of two enzymes CTPS1 and CTPS2, which catalyze ATP-dependent amination of UTP to CTP with ammonia (-NH3) transfer from hydrolyzed glutamine. In normal tissues, CTPS activity is rather low, while it is high in proliferating cells like cancer cells including lymphoma. Importantly, CTPS1 is very low in resting T cells and is rapidly and strongly upregulated in response to TCR stimulation (100). In CTPS1-deficient patients, the proliferation of T cells in response to TCR engagement is markedly impaired, while other T-cell responses including cytokines production, AICD, and cytotoxicity are not affected, and T cells normally proliferate in response to IL-2. Addition of CTP or cytidine in the culture medium restored T-cell proliferation of activated T cells. CTPS1 expression is also upregulated in activated B cells. However, the role of CTPS1 in B cells may be less important than in T cells as patients developed rather infections associated with a T-cell defect and the absence of CTPS1 has no effect on proliferation of EBV-infected B cells and their transformation by EBV. The discovery of the CTPS1 deficiency emphasizes the importance of T-cell expansion during anti-viral responses, specifically in primary infection to EBV, since 40% T cells of circulating can be specific to EBV (8).

CORO1A DEFICIENCY

Deficiency in the actin regulator CORO1A (Coronin-1A) has been identified in nine patients (104–108). Patients presented with severe infections, and five developed EBV-driven B cell lymphoma. Four patients had severe mucocutaneous-immunodeficiency manifestations including epidermodysplasia verruciformis-HPV (EV-HPV) (104, 108). Three patients also exhibited neurological abnormalities including autism-like symptoms. Patients with CORO1A deficiency are characterized by a profound T cell lymphopenia with strongly decreased or nearly absent naïve cells associated with defective thymic output. CORO1A-deficient T cells from patients showed increased spontaneous *in vitro* apoptosis, delayed ERK1/2 activation, increased filamentous actin, but normal or reduced T cell proliferation to mitogens and antigens and normal calcium flux and cytotoxicity. It is proposed that CORO1A deficiency is primarily a T-cell immunodeficiency caused by impaired thymic egress, migration, and survival of mature T cells, thereby affecting lymphocyte homeostasis, repertoire selection, and lineage commitment (109). CORO1A belongs to the family of Coronins that are evolutionarily conserved intracellular actin-binding proteins expressed at high level in most of leukocyte populations (110). In T cells, CORO1A has been shown to be a negative regulator of branched F-actin formation and be required for chemokine-mediated migration and lymphocyte survival given the fact that accumulation of F-actin is known to be toxic for cells (108, 111). However, CORO1A in T cells has been also involved in a variety of pathways including TCR and TGF-β signaling and immunologic synapse (IS) formation (112–115). Along these lines, defective NK cell degranulation was reported in one patient

in association with increased density of F-actin at the cytotoxic synapse (113). Neurological abnormalities found in patients are likely explained by the role of coronin-1A in neurodevelopment that has been reported in mice (116). Finally, Coronin 1A was recently shown to be required for neutrophil trafficking. These observations suggest that the immune defects in patients may not only be restricted to T cells (117). While it is not clearly established why patients with CORO1A deficiency are susceptible to EBV, it is very likely that the poor T-cell survival may result in defective expansion of EBV-specific $CD8^+$ T-cells leading to impaired control of EBV-infected B cells. Interestingly, four patients developed EV-HPV, which could bring closer CORO1A deficiency to primary immunodeficiencies (PIDs) associated with frequent and extensive HPV infections, especially those such as MST1/STK4 and DOCK8 deficiencies, that are characterized by CD4 lymphopenia, defective T cell migration, and/or abnormal F-actin polymerization and IS formation (118).

RASGRP1 DEFICIENCY

RASGRP1 deficiency is caused by bi-allelic mutations in *RASGRP1*. So far, three different null homozygous mutations have been identified in four patients having developed severe EBV-driven LPDs including two Hodgkin lymphoma. One had also an EBV-SMT (119–121). *RASGRP1* codes for a diacylglycerol-regulated guanidine exchange factor preferentially expressed in T and NK cells (122, 123), which acts as an activator of the small G protein RAS and the downstream RAF-MEK-ERK kinases cascade (also known as the MAP kinases pathway). In T lymphocytes, RASGRP1 is the main activator of the MAP kinases pathway (124, 125). RASGRP1-deficient T cells showed impaired ERK/MAPK activation and decreased T-cell proliferation in response to mitogens and antigens (119, 120). In the first report, RASGRP1-deficient T cells were also shown to have diminished cell cytotoxicity and migration capacity (120). NK cells also exhibited decreased cell cytotoxicity. A direct role of RASGRP1 in cytoskeletal dynamics during exocytosis of lytic granules in NK and T cells and in T-cell migration is suggested by its ability to interact with the dynein light chain DYNLL1 and to activate RhoA, respectively (120). This role may explain the impaired cytotoxic responses seen in RASGRP1-deficient T and NK cells. In a recent report by Winter et al., RASGRP1-deficient NK cells and $CD8^+$ T cells were nonetheless found to have normal degranulation when stimulated (119). The discrepancy between these studies is not known. However, in the different reports, low numbers of NK cells were consistently noticed in RASGRP1-deficient patients that might contribute to the decreased NK cell cytotoxicity. Winter et al. further analyzed the possible mechanisms underlying the EBV susceptibility in RASGRP1 deficiency and showed that RASGRP1-deficient T cells failed to expand normally (119). In particular, RASGRP1-deficient T cells had impaired CD27-dependent proliferation toward CD70-expressing EBV-transformed B cells, a critical pathway to expand EBV-specific T cells (see above). Interestingly, the impaired proliferation of activated RASGRP1-deficient T cells correlated with their inability to upregulate CTPS1 protein expression, suggestive of a role of RASGRP1/MAPK pathway in CTPS1 expression, but also in other factors involved in T-cell proliferation, as CTP or cytidine failed to restore impaired proliferation of RASGRP1-deficient T cells (119).

Two *RASGRP1* heterozygous compound mutations with no effect on RASGRP1 protein expression have been also recently identified in two siblings with multiple fungal, bacterial, and viral infections including EBV and CMV, and both also developed autoimmunity signs evoking autoimmune lymphoproliferative syndrome (126). Along these lines, RASGRP1-deficient mice developed autoimmune LPD resembling systemic lupus erythematous when getting older (127, 128) and RASGRP1 is considered as a risk locus for autoimmunity (129–131). T cells from the two patients showed defective TCR activation associated with impaired proliferation and AICD. However, complementation experiments with wild-type RASGRP1 were unsuccessful, leaving the possibility that other or additional genetic events contribute to this particular phenotype and/or these mutations do not behave as loss-of-function mutations. These observations could suggest a genotype–phenotype correlation.

PATHWAYS IN THE CONTROL OF EBV INFECTION

Several key pathways required for an efficient immunity to EBV have emerged from studies of these genetic disorders (11). Interestingly, these pathways are involved in the cell–cell interaction and cross-talk between T and B cells and appear to play a critical role in the immune surveillance of B cells by T cells, which is consistent with B cells as the privileged target of EBV infection and the reservoir of EBV. These pathways implicate pairs of receptor–ligand expressed by T and B cells, respectively (**Figure 1**). The best known and in depth studied is the SLAMR–SAP pathway, which is defective in the SAP deficiency/XLP-1 syndrome and mainly involves two SLAM receptors, 2B4 and NTB-A; however, other SLAMR such as CD229 may be also implicated as suggested from mice and human studies (47, 49). These pathways appear to be important in the recognition of EBV-infected B cells by T cells and in the activation of the T- and NK-cell cytotoxicity responses toward EBV-infected B cells. Another important pathway is dependent of the NKG2D receptor, well known to activate T- and NK-cell cytotoxic responses. In the absence of MAGT1, NKG2D expression on $CD8^+$ T cells and NK cells is impaired leading to defective killing of EBV-infected B cells (79). Nevertheless, it would be interesting to know whether EBV-specific T cells expand normally in MAGT1-deficient patients as NKG2D has been also involved in the survival and expansion of $CD8^+$ T cells during viral infections. The pair CD27–CD70 molecules forms a critical axis required for survival and expansion of EBV-specific T cells (91). The key role of T-cell expansion/proliferation to control EBV-infected B cells is also highlighted by the CTPS1 and RASGRP1 deficiencies, in which the capacity of T lymphocytes to proliferate in response to antigenic stimulation is specifically impaired.

Most of these deficiencies are characterized by a marked decreased or absence of invariant T cell populations iNKT and mucosal-associated invariant T (MAIT) cells, which raises the

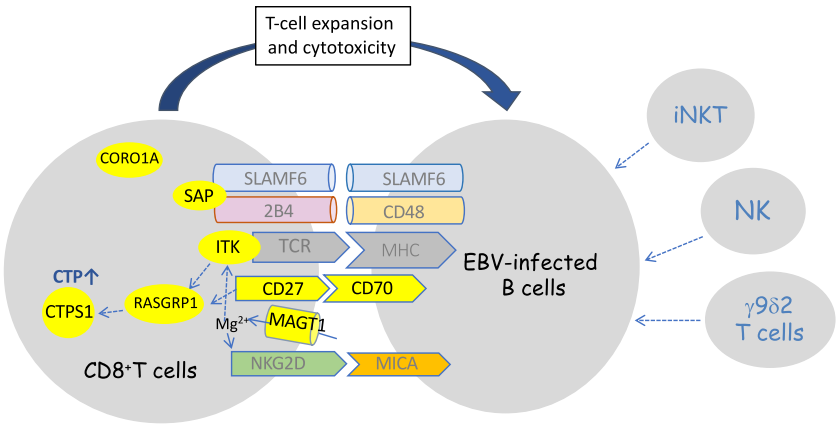


FIGURE 1 | Schematic representation of defective identified pathways in immunodeficiencies predisposing to high susceptility to Epstein–Barr virus (EBV)-driven B-cell lymphoproliferative diseases. Defective components in the pathways are in yellow. Three non-redundant pathways have been shown to be impaired: the SLAMF6/2B4/SLAM-associated protein (SAP) pathway, the CD70/CD27 pathway, and the NKG2D/MICA/MAGT1 pathway. At some point, several of these pathways may converge toward distal effector molecules such as RASGRP1 and CTPS1 that are required for expansion of T cells. The connection of CORO1A to these pathways is not known. Besides the essential role of $CD8^+$ T cells, NK and iNKT cells which are often decreased in these primary imunodeficiencies may play a role in the immune response against EBV, particularly during the early phase of the primary infection. Vγ9δ2 T cells are also thought to play an important role in the primary infection by recognition of latently EBV-replicating B cells.

question of the role of these cells in immunity to EBV. iNKT cells are characterized by innate-like properties including prompt activation and production of large amounts of cytokines. These cells have been involved in a variety of immune responses including anti-viral immunity. Mice models have provided the evidence that SAP, ITK, and RASGRP1 are directly implicated in the development/homeostasis/function of iNKT cells (132–137). Analysis of XIAP-deficient patients also indicated that these cellular defects can be secondary to the EBV infection, leading to cell exhaustion in relation with the peculiar high sensitivity of iNKT and MAIT cells to AICD (138). There are only limited studies that assessed the role of iNKT cells in EBV infection, though it was shown that iNKT cells, in particular $CD8^+$ iNKT cells have the capacity to directly lyse EBV-infected B cells expressing CD1d and to limit expansion of EBV-transformed B cells both *in vitro* and *in vivo* (139–141). However, RORC-deficient patients, who lack iNKT and MAIT cells are not particularly susceptible to EBV, indicating that these cells are not playing a critical role in immunity to EBV (142). Altered functions and decreased counts of NK cells are also often observed in a number of these immunodeficiencies. The role of NK cells might be particularly important during childhood, since KIR-negative early differentiated NK cells that expanded preferentially during IM and targeted lytically EBV-replicating B cells, progressively disappeared in the first decade of life (143). Recent data indicated that patients with severe combined immunodeficiency (SCID) who received bone marrow transplanted, although devoided of NK cells after immune reconstitution display an efficient immunity to EBV since none of them developed EBV-driven lymphoproliferation disorders, even 39 years after the transplantation (144). This suggests that NK cells are not essential for EBV immunity throughout the life. In any event, although they are not key components, accumulation of these different cellular defects might participate to the susceptibility to EBV in these genetic settings, particularly during childhood. CD4 T cell lymphopenia that is observed in several of these disease may also participate to EBV susceptibility, although the role of $CD4^+$ T cells in the control of EBV infection is not clearly established (3). Arguing against a relevant role of $CD4^+$ T cells, patients with MHC class II deficiency have a severe CD4 lymphopenia and do not develop EBV-driven LPDs and they have normal B cell counts. Although not really considered, some of these defects could also have intrinsic B-cell consequences that would further favor proliferation and/or lymphomagenesis of EBV-infected B cells in addition to the immune deficiency. In that respect, CD70 was shown to elicit reverse signaling involved in apoptosis of B cells and the recent study of MAGT1-deficient mice revealed a regulatory role of MAGT1 in B-cell development and proliferation that may explain the B lymphocytosis found in MAGT1-deficient patients (86, 145).

DIFFERENTIAL DIAGNOSIS

Given the importance of the $CD8^+$ T-cell response in immunity to EBV, it is not surprising that EBV-driven LPDs are also found in other PIDs associated with T-cell defects, but with a lower frequency. Herpes virus, in particular EBV, are often the trigger of HLH in patients with familial haemophagocytic lymphohistiocytosis (FHL), a group of diseases associated with impaired cytolytic activity of $CD8^+$ T cells and NK cells. These diseases are caused by gene defects in the perforin gene and in the components of lytic granule exocytosis machinery (146). Patients with CVID or CID, particularly those affecting T-cell survival, migration, and F-actin mobilization in T cells, can also develop EBV-associated disorders (12). Those include, among others, deficiencies in NFκB1 (147), MST1/STK4 (148, 149), WASP (150), DOCK8 (151), GATA2 (152), and gain-of-function mutations in *PIK3CD* known to cause activated PI3K-delta syndrome (153). Patients with hypomorphic mutations in genes involved in T-cell development such

as RAG1/2, DCLRE1C (ARTEMIS), or ZAP-70 can also experience EBV susceptibility (154–156). It should be considered that patients with the most severe T-cell defects will never present EBV problems since they develop very early-onset severe infections (other than EBV), requiring rapid bone marrow transplantation before they encounter EBV. At last, some of these immunodeficiencies (SCID) are also associated with a severe block in B-cell development, a cellular context that in all likelihood does not allow EBV infection establishment and dissemination.

THERAPEUTICS

So far, the only curative treatment for these PIDs is hematopoietic stem cell transplantation (HSCT). The first studies on large cohorts of XLP-1 and XLP-2 patients reported poor survival with or without HSCT (23, 157). This pejorative prognosis was partly linked to the use of full conditioning regimens associated with a high toxicity in patients (XIAP) and/or the absence of HLH remission at the time of HSCT. In the last few years, the use of reduced intensity conditionings and the development of new therapies like alemtuzumab (anti-CD52 antibody) has strongly improved the management of these diseases (33, 158, 159). Furthermore, rituximab (anti-CD20 antibody) has now a major role in the management of LPDs associated with these PIDs with patients being in remission after having received this treatment.

CONCLUDING REMARKS

Molecular characterization of familial forms of EBV susceptibility has provided over the last 20 years, novel diagnostic tools for these disorders. Analysis of large cohorts and case reports have underlined non-EBV phenotypes associated with these PIDs such as IBD, pulmonary involvement, neurological disorders, or other typical infections (such as HPV), that can further help to the diagnosis. However, there are still a number of patients with a high susceptibility to EBV, in whom the molecular/genetic basis of their disease is not known and remains to be determined. In the light of the knowledge gained throughout the studies of the genetically determined forms of EBV susceptibility, we can speculate that these uncharacterized forms are caused by defects in molecules/components involved in T–B cell interactions and required for T-cell cytolytic responses and/or T-cell expansion.

AUTHOR CONTRIBUTIONS

SL wrote the manuscript and made the **Figure 1**. SW compiled data for **Tables 1** and **2** and did the tables and participated to the writing of the manuscript.

FUNDING

SL is a senior scientist at the Centre National de la Recherche Scientifique-CNRS (France). SW is supported by Imagine Institut PhD program funded by the Fondation Bettencourt-Schueller. The work of the Laboratory of Lymphocyte Activation and Susceptibility to EBV headed by SL is supported by grants from Ligue Contre le Cancer-Equipe Labellisée (France), INSERM (France), the Agence Nationale de la Recherche, France (ANR-14-CE14-0028-01 (SL) and ANR-10-IAHU-01 (Imagine Institut)), the Rare Diseases Fondation (France), the Association François Aupetit (France), the Société Française de Lutte contre les Cancers et Leucémies de l'Enfant et de l'Adolescent (SFCE), AREMIG (France), Fédération Enfants et Santé (France), and the European Research Council (ERC-2009-AdG_20090506 no FP7-249816).

REFERENCES

1. Cohen JI, Fauci AS, Varmus H, Nabel GJ. Epstein-Barr virus: an important vaccine target for cancer prevention. *Sci Transl Med* (2011) 3:107fs107. doi:10.1126/scitranslmed.3002878
2. Hislop AD, Taylor GS, Sauce D, Rickinson AB. Cellular responses to viral infection in humans: lessons from Epstein-Barr virus. *Annu Rev Immunol* (2007) 25:587–617. doi:10.1146/annurev.immunol.25.022106.141553
3. Taylor GS, Long HM, Brooks JM, Rickinson AB, Hislop AD. The immunology of Epstein-Barr virus-induced disease. *Annu Rev Immunol* (2015) 33:787–821. doi:10.1146/annurev-immunol-032414-112326
4. Munz C. Epstein-Barr virus-specific immune control by innate lymphocytes. *Front Immunol* (2017) 8:1658. doi:10.3389/fimmu.2017.01658
5. Djaoud Z, Guethlein LA, Horowitz A, Azzi T, Nemat-Gorgani N, Olive D, et al. Two alternate strategies for innate immunity to Epstein-Barr virus: one using NK cells and the other NK cells and gammadelta T cells. *J Exp Med* (2017) 214:1827–41. doi:10.1084/jem.20161017
6. Fujiwara S, Kimura H, Imadome K-I, Arai A, Kodama E, Morio T, et al. Current research on chronic active Epstein-Barr virus infection in Japan. *Pediatr Int* (2014) 56:159–66. doi:10.1111/ped.12314
7. Kimura H, Cohen JI. Chronic active Epstein-Barr virus disease. *Front Immunol* (2017) 8:1867. doi:10.3389/fimmu.2017.01867
8. Callan MF, Tan L, Annels N, Ogg GS, Wilson JD, O'Callaghan CA, et al. Direct visualization of antigen-specific CD8+ T cells during the primary immune response to Epstein-Barr virus in vivo. *J Exp Med* (1998) 187:1395–402. doi:10.1084/jem.187.9.1395
9. Bibas M, Antinori A. EBV and HIV-related lymphoma. *Mediterr J Hematol Infect Dis* (2009) 1:e2009032. doi:10.4084/MJHID.2009.032
10. Veillette A, Perez-Quintero LA, Latour S. X-linked lymphoproliferative syndromes and related autosomal recessive disorders. *Curr Opin Allergy Clin Immunol* (2013) 13:614–22. doi:10.1097/ACI.0000000000000008
11. Tangye SG, Palendira U, Edwards ES. Human immunity against EBV-lessons from the clinic. *J Exp Med* (2017) 214:269–83. doi:10.1084/jem.20161846
12. Cohen JI. Primary immunodeficiencies associated with EBV disease. *Curr Top Microbiol Immunol* (2015) 390:241–65. doi:10.1007/978-3-319-22822-8_10
13. Fischer A. Recent advances in understanding the pathophysiology of primary T cell immunodeficiencies. *Trends Mol Med* (2015) 21:408–16. doi:10.1016/j.molmed.2015.04.002
14. Notarangelo LD. Combined immunodeficiencies with nonfunctional T lymphocytes. *Adv Immunol* (2014) 121:121–90. doi:10.1016/B978-0-12-800100-4.00004-0
15. Sayos J, Wu C, Morra M, Wang N, Zhang X, Allen D, et al. The X-linked lymphoproliferative-disease gene product SAP regulates signals induced through the co-receptor SLAM. *Nature* (1998) 395:462–9. doi:10.1038/26683
16. Coffey AJ, Brooksbank RA, Brandau O, Oohashi T, Howell GR, Bye JM, et al. Host response to EBV infection in X-linked lymphoproliferative disease results from mutations in an SH2-domain encoding gene. *Nat Genet* (1998) 20:129–35. doi:10.1038/2424
17. Nichols KE, Harkin DP, Levitz S, Krainer M, Kolquist KA, Genovese C, et al. Inactivating mutations in an SH2 domain-encoding gene in X-linked lymphoproliferative syndrome. *Proc Natl Acad Sci U S A* (1998) 95:13765–70. doi:10.1073/pnas.95.23.13765
18. Tangye SG. XLP: clinical features and molecular etiology due to mutations in SH2D1A encoding SAP. *J Clin Immunol* (2014) 34:772–9. doi:10.1007/s10875-014-0083-7

19. Gaspar HB, Sharifi R, Gilmour KC, Thrasher AJ. X-linked lymphoproliferative disease: clinical, diagnostic and molecular perspective. *Br J Haematol* (2002) 119:585–95. doi:10.1046/j.1365-2141.2002.03851.x
20. Purtilo DT, Sakamoto K, Barnabei V, Seeley J, Bechtold T, Rogers G, et al. Epstein-Barr virus-induced diseases in boys with the X-linked lymphoproliferative syndrome (XLP): update on studies of the registry. *Am J Med* (1982) 73:49–56. doi:10.1016/0002-9343(82)90923-8
21. Sumegi J, Huang D, Lanyi A, Davis JD, Seemayer TA, Maeda A, et al. Correlation of mutations of the SH2D1A gene and Epstein-Barr virus infection with clinical phenotype and outcome in X-linked lymphoproliferative disease. *Blood* (2000) 96:3118–25.
22. Kanegane H, Yang X, Zhao M, Yamato K, Inoue M, Hamamoto K, et al. Clinical features and outcome of X-linked lymphoproliferative syndrome type 1 (SAP deficiency) in Japan identified by the combination of flow cytometric assay and genetic analysis. *Pediatr Allergy Immunol* (2012) 23:488–93. doi:10.1111/j.1399-3038.2012.01282.x
23. Booth C, Gilmour KC, Veys P, Gennery AR, Slatter MA, Chapel H, et al. X-linked lymphoproliferative disease due to SAP/SH2D1A deficiency: a multicenter study on the manifestations, management and outcome of the disease. *Blood* (2011) 117:53–62. doi:10.1182/blood-2010-06-284935
24. Pachlopnik Schmid J, Canioni D, Moshous D, Touzot F, Mahlaoui N, Hauck F, et al. Clinical similarities and differences of patients with X-linked lymphoproliferative syndrome type 1 (XLP-1/SAP deficiency) versus type 2 (XLP-2/XIAP deficiency). *Blood* (2011) 117:1522–9. doi:10.1182/blood-2010-07-298372
25. Talaat KR, Rothman JA, Cohen JI, Santi M, Choi JK, Guzman M, et al. Lymphocytic vasculitis involving the central nervous system occurs in patients with X-linked lymphoproliferative disease in the absence of Epstein-Barr virus infection. *Pediatr Blood Cancer* (2009) 53:1120–3. doi:10.1002/pbc.22185
26. Gray PE, O'Brien TA, Wagle M, Tangye SG, Palendira U, Roscioli T, et al. Cerebral vasculitis in X-linked lymphoproliferative disease cured by matched unrelated cord blood transplant. *J Clin Immunol* (2015) 35:604–9. doi:10.1007/s10875-015-0194-9
27. Seemayer TA, Gross TG, Egeler RM, Pirruccello SJ, Davis JR, Kelly CM, et al. X-linked lymphoproliferative disease: twenty-five years after the discovery. *Pediatr Res* (1995) 38:471–8. doi:10.1203/00006450-199510000-00001
28. Morra M, Silander O, Calpe S, Choi M, Oettgen H, Myers L, et al. Alterations of the X-linked lymphoproliferative disease gene SH2D1A in common variable immunodeficiency syndrome. *Blood* (2001) 98:1321–5. doi:10.1182/blood.V98.5.1321
29. Eastwood D, Gilmour KC, Nistala K, Meaney C, Chapel H, Sherrell Z, et al. Prevalence of SAP gene defects in male patients diagnosed with common variable immunodeficiency. *Clin Exp Immunol* (2004) 137:584–8. doi:10.1111/j.1365-2249.2004.02581.x
30. Nistala K, Gilmour KC, Cranston T, Davies EG, Goldblatt D, Gaspar HB, et al. X-linked lymphoproliferative disease: three atypical cases. *Clin Exp Immunol* (2001) 126:126–30. doi:10.1046/j.1365-2249.2001.01599.x
31. Soresina A, Lougaris V, Giliani S, Cardinale F, Armenio L, Cattalini M, et al. Mutations of the X-linked lymphoproliferative disease gene SH2D1A mimicking common variable immunodeficiency. *Eur J Pediatr* (2002) 161:656–9. doi:10.1007/s00431-002-1083-9
32. Liu J, Tian W, Wang F, Teng W, Zhang Y, Tong C, et al. Maternal onset de novo SH2D1A mutation and lymphocytic choriomeningitis virus infection in a patient with X-linked lymphoproliferative disease type 1: a case report. *Mol Med Rep* (2015) 11:3291–4. doi:10.3892/mmr.2015.3173
33. Marsh RA, Bleesing JJ, Chandrakasan S, Jordan MB, Davies SM, Filipovich AH. Reduced-intensity conditioning hematopoietic cell transplantation is an effective treatment for patients with SLAM-associated protein deficiency/X-linked lymphoproliferative disease type 1. *Biol Blood Marrow Transplant* (2014) 20:1641–5. doi:10.1016/j.bbmt.2014.06.003
34. Veillette A. Immune regulation by SLAM family receptors and SAP-related adaptors. *Nat Rev Immunol* (2006) 6:56–66. doi:10.1038/nri1761
35. Veillette A, Dong Z, Perez-Quintero LA, Zhong MC, Cruz-Munoz ME. Importance and mechanism of 'switch' function of SAP family adapters. *Immunol Rev* (2009) 232:229–39. doi:10.1111/j.1600-065X.2009.00824.x
36. Cannons JL, Tangye SG, Schwartzberg PL. SLAM family receptors and SAP adaptors in immunity. *Annu Rev Immunol* (2011) 29:665–705. doi:10.1146/annurev-immunol-030409-101302
37. Latour S, Roncagalli R, Chen R, Bakinowski M, Shi X, Schwartzberg PL, et al. Binding of SAP SH2 domain to FynT SH3 domain reveals a novel mechanism of receptor signalling in immune regulation. *Nat Cell Biol* (2003) 5:149–54. doi:10.1038/ncb919
38. Latour S, Gish G, Helgason CD, Humphries RK, Pawson T, Veillette A. Regulation of SLAM-mediated signal transduction by SAP, the X-linked lymphoproliferative gene product. *Nat Immunol* (2001) 2:681–90. doi:10.1038/90615
39. Chan B, Lanyi A, Song HK, Griesbach J, Simarro-Grande M, Poy F, et al. SAP couples Fyn to SLAM immune receptors. *Nat Cell Biol* (2003) 5:155–60. doi:10.1038/ncb920
40. Harada S, Bechtold T, Seeley JK, Purtilo DT. Cell-mediated immunity to Epstein-Barr virus (EBV) and natural killer (NK)-cell activity in the X-linked lymphoproliferative syndrome. *Int J Cancer* (1982) 30:739–44. doi:10.1002/ijc.2910300610
41. Harada S, Sakamoto K, Seeley JK, Lindsten T, Bechtold T, Yetz J, et al. Immune deficiency in the X-linked lymphoproliferative syndrome. I. Epstein-Barr virus-specific defects. *J Immunol* (1982) 129:2532–5.
42. Tangye SG, Phillips JH, Lanier LL, Nichols KE. Functional requirement for SAP in 2B4-mediated activation of human natural killer cells as revealed by the X-linked lymphoproliferative syndrome. *J Immunol* (2000) 165:2932–6. doi:10.4049/jimmunol.165.6.2932
43. Parolini S, Bottino C, Falco M, Augugliaro R, Giliani S, Franceschini R, et al. X-linked lymphoproliferative disease. 2B4 molecules displaying inhibitory rather than activating function are responsible for the inability of natural killer cells to kill Epstein-Barr virus-infected cells. *J Exp Med* (2000) 192:337–46. doi:10.1084/jem.192.3.337
44. Bottino C, Falco M, Parolini S, Marcenaro E, Augugliaro R, Sivori S, et al. NTB-A [correction of GNTB-A], a novel SH2D1A-associated surface molecule contributing to the inability of natural killer cells to kill Epstein-Barr virus-infected B cells in X-linked lymphoproliferative disease. *J Exp Med* (2001) 194:235–46. doi:10.1084/jem.194.3.235
45. Dupre L, Andolfi G, Tangye SG, Clementi R, Locatelli F, Arico M, et al. SAP controls the cytolytic activity of CD8+ T cells against EBV-infected cells. *Blood* (2005) 105:4383–9. doi:10.1182/blood-2004-08-3269
46. Sharifi R, Sinclair JC, Gilmour KC, Arkwright PD, Kinnon C, Thrasher AJ, et al. SAP mediates specific cytotoxic T-cell functions in X-linked lymphoproliferative disease. *Blood* (2004) 103:3821–7. doi:10.1182/blood-2003-09-3359
47. Hislop AD, Palendira U, Leese AM, Arkwright PD, Rohrlich PS, Tangye SG, et al. Impaired Epstein-Barr virus-specific CD8+ T-cell function in X-linked lymphoproliferative disease is restricted to SLAM family-positive B-cell targets. *Blood* (2010) 116:3249–57. doi:10.1182/blood-2009-09-238832
48. Bloch-Queyrat C, Fondaneche MC, Chen R, Yin L, Relouzat F, Veillette A, et al. Regulation of natural cytotoxicity by the adaptor SAP and the Src-related kinase Fyn. *J Exp Med* (2005) 202:181–92. doi:10.1084/jem.20050449
49. Dong Z, Cruz-Munoz ME, Zhong MC, Chen R, Latour S, Veillette A. Essential function for SAP family adaptors in the surveillance of hematopoietic cells by natural killer cells. *Nat Immunol* (2009) 10:973–80. doi:10.1038/ni.1763
50. Dong Z, Davidson D, Perez-Quintero LA, Kurosaki T, Swat W, Veillette A. The adaptor SAP controls NK cell activation by regulating the enzymes Vav-1 and SHIP-1 and by enhancing conjugates with target cells. *Immunity* (2012) 36:974–85. doi:10.1016/j.immuni.2012.03.023
51. Zhao F, Cannons JL, Dutta M, Griffiths GM, Schwartzberg PL. Positive and negative signaling through SLAM receptors regulate synapse organization and thresholds of cytolysis. *Immunity* (2012) 36:1003–16. doi:10.1016/j.immuni.2012.05.017
52. Wu N, Zhong MC, Roncagalli R, Perez-Quintero LA, Guo H, Zhang Z, et al. A hematopoietic cell-driven mechanism involving SLAMF6 receptor, SAP adaptors and SHP-1 phosphatase regulates NK cell education. *Nat Immunol* (2016) 17:387–96. doi:10.1038/ni.3369
53. Palendira U, Low C, Chan A, Hislop AD, Ho E, Phan TG, et al. Molecular pathogenesis of EBV susceptibility in XLP as revealed by analysis of female carriers with heterozygous expression of SAP. *PLoS Biol* (2011) 9:e1001187. doi:10.1371/journal.pbio.1001187
54. Rigaud S, Fondaneche MC, Lambert N, Pasquier B, Mateo V, Soulas P, et al. XIAP deficiency in humans causes an X-linked lymphoproliferative syndrome. *Nature* (2006) 444:110–4. doi:10.1038/nature05257
55. Snow AL, Marsh RA, Krummey SM, Roehrs P, Young LR, Zhang K, et al. Restimulation-induced apoptosis of T cells is impaired in patients with

X-linked lymphoproliferative disease caused by SAP deficiency. *J Clin Invest* (2009) 119:2976–89. doi:10.1172/JCI39518
56. Chen G, Tai AK, Lin M, Chang F, Terhorst C, Huber BT. Increased proliferation of CD8+ T cells in SAP-deficient mice is associated with impaired activation-induced cell death. *Eur J Immunol* (2007) 37:663–74. doi:10.1002/eji.200636417
57. Aguilar C, Latour S. X-linked inhibitor of apoptosis protein deficiency: more than an X-linked lymphoproliferative syndrome. *J Clin Immunol* (2015) 35:331–8. doi:10.1007/s10875-015-0141-9
58. Latour S, Aguilar C. XIAP deficiency syndrome in humans. *Semin Cell Dev Biol* (2015) 39:115–23. doi:10.1016/j.semcdb.2015.01.015
59. Vince JE, Wong WW, Gentle I, Lawlor KE, Allam R, O'Reilly L, et al. Inhibitor of apoptosis proteins limit RIP3 kinase-dependent interleukin-1 activation. *Immunity* (2012) 36:215–27. doi:10.1016/j.immuni.2012.01.012
60. Damgaard RB, Fiil BK, Speckmann C, Yabal M, zur Stadt U, Bekker-Jensen S, et al. Disease-causing mutations in the XIAP BIR2 domain impair NOD2-dependent immune signalling. *EMBO Mol Med* (2013) 5:1278–95. doi:10.1002/emmm.201303090
61. Krieg A, Correa RG, Garrison JB, Le Negrate G, Welsh K, Huang Z, et al. XIAP mediates NOD signaling via interaction with RIP2. *Proc Natl Acad Sci U S A* (2009) 106:14524–9. doi:10.1073/pnas.0907131106
62. Speckmann C, Lehmberg K, Albert MH, Damgaard RB, Fritsch M, Gyrd-Hansen M, et al. X-linked inhibitor of apoptosis (XIAP) deficiency: the spectrum of presenting manifestations beyond hemophagocytic lymphohistiocytosis. *Clin Immunol* (2013) 149:133–41. doi:10.1016/j.clim.2013.07.004
63. Aguilar C, Lenoir C, Lambert N, Begue B, Brousse N, Canioni D, et al. Characterization of Crohn disease in X-linked inhibitor of apoptosis-deficient male patients and female symptomatic carriers. *J Allergy Clin Immunol* (2014) 134:1131–41.e9. doi:10.1016/j.jaci.2014.04.031
64. Gu L, Zhang H, Liu T, Zhou S, Du Y, Xiong J, et al. Discovery of dual inhibitors of MDM2 and XIAP for cancer treatment. *Cancer Cell* (2016) 30:623–36. doi:10.1016/j.ccell.2016.08.015
65. Ammann S, Elling R, Gyrd-Hansen M, Duckers G, Bredius R, Burns SO, et al. A new functional assay for the diagnosis of X-linked inhibitor of apoptosis (XIAP) deficiency. *Clin Exp Immunol* (2014) 176:394–400. doi:10.1111/cei.12306
66. Huck K, Feyen O, Niehues T, Ruschendorf F, Hubner N, Laws HJ, et al. Girls homozygous for an IL-2-inducible T cell kinase mutation that leads to protein deficiency develop fatal EBV-associated lymphoproliferation. *J Clin Invest* (2009) 119:1350–8. doi:10.1172/JCI37901
67. Linka RM, Risse SL, Bienemann K, Werner M, Linka Y, Krux F, et al. Loss-of-function mutations within the IL-2 inducible kinase ITK in patients with EBV-associated lymphoproliferative diseases. *Leukemia* (2012) 26:963–71. doi:10.1038/leu.2011.371
68. Mansouri D, Mahdaviani SA, Khalilzadeh S, Mohajerani SA, Hasanzad M, Sadr S, et al. IL-2-inducible T-cell kinase deficiency with pulmonary manifestations due to disseminated Epstein-Barr virus infection. *Int Arch Allergy Immunol* (2012) 158:418–22. doi:10.1159/000333472
69. Serwas NK, Cagdas D, Ban SA, Bienemann K, Salzer E, Tezcan I, et al. Identification of ITK deficiency as a novel genetic cause of idiopathic CD4+ T-cell lymphopenia. *Blood* (2014) 124:655–7. doi:10.1182/blood-2014-03-564930
70. Cipe FE, Aydogmus C, Serwas NK, Tugcu D, Demirkaya M, Bicici FA, et al. ITK deficiency: how can EBV be treated before lymphoma? *Pediatr Blood Cancer* (2015) 62:2247–8. doi:10.1002/pbc.25648
71. Cagdas D, Erman B, Hanoglu D, Tavil B, Kuskonmaz B, Aydin B, et al. Course of IL-2-inducible T-cell kinase deficiency in a family: lymphomatoid granulomatosis, lymphoma and allogeneic bone marrow transplantation in one sibling; and death in the other. *Bone Marrow Transplant* (2017) 52:126–9. doi:10.1038/bmt.2016.185
72. Stepensky P, Weintraub M, Yanir A, Revel-Vilk S, Krux F, Huck K, et al. IL-2-inducible T-cell kinase deficiency: clinical presentation and therapeutic approach. *Haematologica* (2011) 96:472–6. doi:10.3324/haematol.2010.033910
73. Andreotti AH, Schwartzberg PL, Joseph RE, Berg LJ. T-cell signaling regulated by the Tec family kinase, Itk. *Cold Spring Harb Perspect Biol* (2010) 2:a002287. doi:10.1101/cshperspect.a002287
74. Liu KQ, Bunnell SC, Gurniak CB, Berg LJ. T cell receptor-initiated calcium release is uncoupled from capacitative calcium entry in Itk-deficient T cells. *J Exp Med* (1998) 187:1721–7. doi:10.1084/jem.187.10.1721
75. Fischer AM, Mercer JC, Iyer A, Ragin MJ, August A. Regulation of CXC chemokine receptor 4-mediated migration by the Tec family tyrosine kinase ITK. *J Biol Chem* (2004) 279:29816–20. doi:10.1074/jbc.M312848200
76. Schaeffer EM, Debnath J, Yap G, McVicar D, Liao XC, Littman DR, et al. Requirement for Tec kinases Rlk and Itk in T cell receptor signaling and immunity. *Science* (1999) 284:638–41. doi:10.1126/science.284.5414.638
77. Kapnick SM, Stinchcombe JC, Griffiths GM, Schwartzberg PL. Inducible T cell kinase regulates the acquisition of cytolytic capacity and degranulation in CD8(+) CTLs. *J Immunol* (2017) 198:2699–711. doi:10.4049/jimmunol.1601202
78. Li FY, Chaigne-Delalande B, Kanellopoulou C, Davis JC, Matthews HF, Douek DC, et al. Second messenger role for Mg2+ revealed by human T-cell immunodeficiency. *Nature* (2011) 475:471–6. doi:10.1038/nature10246
79. Chaigne-Delalande B, Li FY, O'Connor GM, Lukacs MJ, Jiang P, Zheng L, et al. Mg2+ regulates cytotoxic functions of NK and CD8 T cells in chronic EBV infection through NKG2D. *Science* (2013) 341:186–91. doi:10.1126/science.1240094
80. Brigida I, Chiriaco M, Di Cesare S, Cittaro D, Di Matteo G, Giannelli S, et al. Large deletion of MAGT1 gene in a patient with classic kaposi sarcoma, CD4 lymphopenia, and EBV infection. *J Clin Immunol* (2017) 37:32–5. doi:10.1007/s10875-016-0341-y
81. Dhalla F, Murray S, Sadler R, Chaigne-Delalande B, Sadaoka T, Soilleux E, et al. Identification of a novel mutation in MAGT1 and progressive multifocal leucoencephalopathy in a 58-year-old man with XMEN disease. *J Clin Immunol* (2015) 35:112–8. doi:10.1007/s10875-014-0116-2
82. Patiroglu T, Haluk Akar H, Gilmour K, Unal E, Akif Ozdemir M, Bibi S, et al. A case of XMEN syndrome presented with severe auto-immune disorders mimicking autoimmune lymphoproliferative disease. *Clin Immunol* (2015) 159:58–62. doi:10.1016/j.clim.2015.04.015
83. Li FY, Chaigne-Delalande B, Su H, Uzel G, Matthews H, Lenardo MJ. XMEN disease: a new primary immunodeficiency affecting Mg2+ regulation of immunity against Epstein-Barr virus. *Blood* (2014) 123:2148–52. doi:10.1182/blood-2013-11-538686
84. Cherepanova N, Shrimal S, Gilmore R. N-linked glycosylation and homeostasis of the endoplasmic reticulum. *Curr Opin Cell Biol* (2016) 41:57–65. doi:10.1016/j.ceb.2016.03.021
85. Li FY, Lenardo MJ, Chaigne-Delalande B. Loss of MAGT1 abrogates the Mg2+ flux required for T cell signaling and leads to a novel human primary immunodeficiency. *Magnes Res* (2011) 24:S109–14. doi:10.1684/mrh.2011.0286
86. Gotru SK, Gil-Pulido J, Beyersdorf N, Diefenbach A, Becker IC, Vogtle T, et al. Cutting edge: imbalanced cation homeostasis in MAGT1-deficient B Cells dysregulates B cell development and signaling in mice. *J Immunol* (2018) 200(8):2529–34. doi:10.4049/jimmunol.1701467
87. Pappworth IY, Wang EC, Rowe M. The switch from latent to productive infection in Epstein-Barr virus-infected B cells is associated with sensitization to NK cell killing. *J Virol* (2007) 81:474–82. doi:10.1128/JVI.01777-06
88. Obeidy P, Sharland AF. NKG2D and its ligands. *Int J Biochem Cell Biol* (2009) 41:2364–7. doi:10.1016/j.biocel.2009.07.005
89. van Montfrans JM, Hoepelman AI, Otto S, van Gijn M, van de Corput L, de Weger RA, et al. CD27 deficiency is associated with combined immunodeficiency and persistent symptomatic EBV viremia. *J Allergy Clin Immunol* (2012) 129:787–93.e6. doi:10.1016/j.jaci.2011.11.013
90. Alkhairy OK, Perez-Becker R, Driessen GJ, Abolhassani H, van Montfrans J, Borte S, et al. Novel mutations in TNFRSF7/CD27: clinical, immunologic, and genetic characterization of human CD27 deficiency. *J Allergy Clin Immunol* (2015) 136:703–12.e10. doi:10.1016/j.jaci.2015.02.022
91. Izawa K, Martin E, Soudais C, Bruneau J, Boutboul D, Rodriguez R, et al. Inherited CD70 deficiency in humans reveals a critical role for the CD70-CD27 pathway in immunity to Epstein-Barr virus infection. *J Exp Med* (2017) 214:73–89. doi:10.1084/jem.20160784
92. Salzer E, Daschkey S, Choo S, Gombert M, Santos-Valente E, Ginzel S, et al. Combined immunodeficiency with life-threatening EBV-associated lymphoproliferative disorder in patients lacking functional CD27. *Haematologica* (2013) 98:473–8. doi:10.3324/haematol.2012.068791

93. Klein U, Rajewsky K, Kuppers R. Human immunoglobulin (Ig)M+IgD+ peripheral blood B cells expressing the CD27 cell surface antigen carry somatically mutated variable region genes: CD27 as a general marker for somatically mutated (memory) B cells. *J Exp Med* (1998) 188:1679–89. doi:10.1084/jem.188.9.1679
94. Jacobs J, Deschoolmeester V, Zwaenepoel K, Rolfo C, Silence K, Rottey S, et al. CD70: an emerging target in cancer immunotherapy. *Pharmacol Ther* (2015) 155:1–10. doi:10.1016/j.pharmthera.2015.07.007
95. Tangye SG, Liu YJ, Aversa G, Phillips JH, de Vries JE. Identification of functional human splenic memory B cells by expression of CD148 and CD27. *J Exp Med* (1998) 188:1691–703. doi:10.1084/jem.188.9.1691
96. Hendriks J, Gravestein LA, Tesselaar K, van Lier RA, Schumacher TN, Borst J. CD27 is required for generation and long-term maintenance of T cell immunity. *Nat Immunol* (2000) 1:433–40. doi:10.1038/80877
97. Borst J, Hendriks J, Xiao Y. CD27 and CD70 in T cell and B cell activation. *Curr Opin Immunol* (2005) 17:275–81. doi:10.1016/j.coi.2005.04.004
98. Abolhassani H, Edwards ES, Ikinciogullari A, Jing H, Borte S, Buggert M, et al. Combined immunodeficiency and Epstein-Barr virus-induced B cell malignancy in humans with inherited CD70 deficiency. *J Exp Med* (2017) 214:91–106. doi:10.1084/jem.20160849
99. Caorsi R, Rusmini M, Volpi S, Chiesa S, Pastorino C, Sementa AR, et al. CD70 deficiency due to a novel mutation in a patient with severe chronic EBV infection presenting as a periodic fever. *Front Immunol* (2017) 8:2015. doi:10.3389/fimmu.2017.02015
100. Martin E, Palmic N, Sanquer S, Lenoir C, Hauck F, Mongellaz C, et al. CTP synthase 1 deficiency in humans reveals its central role in lymphocyte proliferation. *Nature* (2014) 510:288–92. doi:10.1038/nature13386
101. Kucuk ZY, Zhang K, Filipovich L, Bleesing JJ. CTP synthase 1 deficiency in successfully transplanted siblings with combined immune deficiency and chronic active EBV infection. *J Clin Immunol* (2016) 36:750–3. doi:10.1007/s10875-016-0332-z
102. Truck J, Kelly DF, Taylor JM, Kienzler AK, Lester T, Seller A, et al. Variable phenotype and discrete alterations of immune phenotypes in CTP synthase 1 deficiency: report of 2 siblings. *J Allergy Clin Immunol* (2016) 138:1722–5.e6. doi:10.1016/j.jaci.2016.04.059
103. Evans DR, Guy HI. Mammalian pyrimidine biosynthesis: fresh insights into an ancient pathway. *J Biol Chem* (2004) 279:33035–8. doi:10.1074/jbc.R400007200
104. Stray-Pedersen A, Jouanguy E, Crequer A, Bertuch AA, Brown BS, Jhangiani SN, et al. Compound heterozygous CORO1A mutations in siblings with a mucocutaneous-immunodeficiency syndrome of epidermodysplasia verruciformis-HPV, molluscum contagiosum and granulomatous tuberculoid leprosy. *J Clin Immunol* (2014) 34:871–90. doi:10.1007/s10875-014-0074-8
105. Shiow LR, Roadcap DW, Paris K, Watson SR, Grigorova IL, Lebet T, et al. The actin regulator coronin 1A is mutant in a thymic egress-deficient mouse strain and in a patient with severe combined immunodeficiency. *Nat Immunol* (2008) 9:1307–15. doi:10.1038/ni.1662
106. Moshous D, Martin E, Carpentier W, Lim A, Callebaut I, Canioni D, et al. Whole-exome sequencing identifies coronin-1A deficiency in 3 siblings with immunodeficiency and EBV-associated B-cell lymphoproliferation. *J Allergy Clin Immunol* (2013) 131:1594–603. doi:10.1016/j.jaci.2013.01.042
107. Punwani D, Pelz B, Yu J, Arva NC, Schafernak K, Kondratowicz K, et al. Coronin-1A: immune deficiency in humans and mice. *J Clin Immunol* (2015) 35:100–7. doi:10.1007/s10875-015-0130-z
108. Yee CS, Massaad MJ, Bainter W, Ohsumi TK, Foger N, Chan AC, et al. Recurrent viral infections associated with a homozygous CORO1A mutation that disrupts oligomerization and cytoskeletal association. *J Allergy Clin Immunol* (2016) 137:879–88.e2. doi:10.1016/j.jaci.2015.08.020
109. Moshous D, de Villartay JP. The expanding spectrum of human coronin 1A deficiency. *Curr Allergy Asthma Rep* (2014) 14:481. doi:10.1007/s11882-014-0481-1
110. Pieters J, Muller P, Jayachandran R. On guard: coronin proteins in innate and adaptive immunity. *Nat Rev Immunol* (2013) 13:510–8. doi:10.1038/nri3465
111. Foger N, Rangell L, Danilenko DM, Chan AC. Requirement for coronin 1 in T lymphocyte trafficking and cellular homeostasis. *Science* (2006) 313:839–42. doi:10.1126/science.1130563
112. Mueller P, Massner J, Jayachandran R, Combaluzier B, Albrecht I, Gatfield J, et al. Regulation of T cell survival through coronin-1-mediated generation of inositol-1,4,5-trisphosphate and calcium mobilization after T cell receptor triggering. *Nat Immunol* (2008) 9:424–31. doi:10.1038/ni1570
113. Mace EM, Orange JS. Lytic immune synapse function requires filamentous actin deconstruction by coronin 1A. *Proc Natl Acad Sci U S A* (2014) 111:6708–13. doi:10.1073/pnas.1314975111
114. Kaminski S, Hermann-Kleiter N, Meisel M, Thuille N, Cronin S, Hara H, et al. Coronin 1A is an essential regulator of the TGFbeta receptor/SMAD3 signaling pathway in Th17 CD4(+) T cells. *J Autoimmun* (2011) 37:198–208. doi:10.1016/j.jaut.2011.05.018
115. Siegmund K, Thuille N, Posch N, Fresser F, Baier G. Novel protein kinase C theta: coronin 1A complex in T lymphocytes. *Cell Commun Signal* (2015) 13:22. doi:10.1186/s12964-015-0100-3
116. Jayachandran R, Liu X, Bosedasgupta S, Muller P, Zhang CL, Moshous D, et al. Coronin 1 regulates cognition and behavior through modulation of cAMP/protein kinase A signaling. *PLoS Biol* (2014) 12:e1001820. doi:10.1371/journal.pbio.1001820
117. Pick R, Begandt D, Stocker TJ, Salvermoser M, Thome S, Bottcher RT, et al. Coronin 1A, a novel player in integrin biology, controls neutrophil trafficking in innate immunity. *Blood* (2017) 130:847–58. doi:10.1182/blood-2016-11-749622
118. Leiding JW, Holland SM. Warts and all: human papillomavirus in primary immunodeficiencies. *J Allergy Clin Immunol* (2012) 130:1030–48. doi:10.1016/j.jaci.2012.07.049
119. Winter S, Martin E, Boutboul D, Lenoir C, Boudjemaa S, Petit A, et al. Loss of RASGRP1 in humans impairs T-cell expansion leading to Epstein-Barr virus susceptibility. *EMBO Mol Med* (2018) 10(2):188–99. doi:10.15252/emmm.201708292
120. Salzer E, Cagdas D, Hons M, Mace EM, Garncarz W, Petronczki OY, et al. RASGRP1 deficiency causes immunodeficiency with impaired cytoskeletal dynamics. *Nat Immunol* (2016) 17:1352–60. doi:10.1038/ni.3575
121. Platt CD, Fried AJ, Hoyos-Bachiloglu R, Usmani GN, Schmidt B, Whangbo J, et al. Combined immunodeficiency with EBV positive B cell lymphoma and epidermodysplasia verruciformis due to a novel homozygous mutation in RASGRP1. *Clin Immunol* (2017) 183:142–4. doi:10.1016/j.clim.2017.08.007
122. Kortum RL, Rouquette-Jazdanian AK, Samelson LE. Ras and extracellular signal-regulated kinase signaling in thymocytes and T cells. *Trends Immunol* (2013) 34:259–68. doi:10.1016/j.it.2013.02.004
123. Hogquist K. RasGRP: the missing link for Ras activation in thymocytes. *Trends Immunol* (2001) 22:69. doi:10.1016/S1471-4906(00)01845-7
124. Roose JP, Mollenauer M, Gupta VA, Stone J, Weiss A. A diacylglycerol-protein kinase C-RasGRP1 pathway directs Ras activation upon antigen receptor stimulation of T cells. *Mol Cell Biol* (2005) 25:4426–41. doi:10.1128/MCB.25.11.4426-4441.2005
125. Warnecke N, Poltorak M, Kowtharapu BS, Arndt B, Stone JC, Schraven B, et al. TCR-mediated Erk activation does not depend on Sos and Grb2 in peripheral human T cells. *EMBO Rep* (2012) 13:386–91. doi:10.1038/embor.2012.17
126. Mao H, Yang W, Latour S, Yang J, Winter S, Zheng J, et al. RASGRP1 mutation in autoimmune lymphoproliferative syndrome-like disease. *J Allergy Clin Immunol* (2017). doi:10.1016/j.jaci.2017.10.026
127. Bartlett A, Buhlmann JE, Stone J, Lim B, Barrington RA. Multiple checkpoint breach of B cell tolerance in Rasgrp1-deficient mice. *J Immunol* (2013) 191:3605–13. doi:10.4049/jimmunol.1202892
128. Daley SR, Coakley KM, Hu DY, Randall KL, Jenne CN, Limnander A, et al. Rasgrp1 mutation increases naive T-cell CD44 expression and drives mTOR-dependent accumulation of Helios(+) T cells and autoantibodies. *Elife* (2013) 2:e01020. doi:10.7554/eLife.01020
129. Sun C, Molineros JE, Looger LL, Zhou XJ, Kim K, Okada Y, et al. High-density genotyping of immune-related loci identifies new SLE risk variants in individuals with Asian ancestry. *Nat Genet* (2016) 48:323–30. doi:10.1038/ng.3496
130. Zhou XJ, Nath SK, Qi YY, Sun C, Hou P, Zhang YM, et al. Novel identified associations of RGS1 and RASGRP1 variants in IgA nephropathy. *Sci Rep* (2016) 6:35781. doi:10.1038/srep35781
131. Qu HQ, Grant SF, Bradfield JP, Kim C, Frackelton E, Hakonarson H, et al. Association of RASGRP1 with type 1 diabetes is revealed by combined follow-up of two genome-wide studies. *J Med Genet* (2009) 46:553–4. doi:10.1136/jmg.2009.067140

132. Pasquier B, Yin L, Fondaneche MC, Relouzat F, Bloch-Queyrat C, Lambert N, et al. Defective NKT cell development in mice and humans lacking the adapter SAP, the X-linked lymphoproliferative syndrome gene product. *J Exp Med* (2005) 201:695–701. doi:10.1084/jem.20042432
133. Gadue P, Stein PL. NK T cell precursors exhibit differential cytokine regulation and require Itk for efficient maturation. *J Immunol* (2002) 169:2397–406. doi:10.4049/jimmunol.169.5.2397
134. Felices M, Berg LJ. The Tec kinases Itk and Rlk regulate NKT cell maturation, cytokine production, and survival. *J Immunol* (2008) 180:3007–18. doi:10.4049/jimmunol.180.5.3007
135. Shen S, Chen Y, Gorentla BK, Lu J, Stone JC, Zhong XP. Critical roles of RasGRP1 for invariant NKT cell development. *J Immunol* (2011) 187:4467–73. doi:10.4049/jimmunol.1003798
136. Nichols KE, Hom J, Gong SY, Ganguly A, Ma CS, Cannons JL, et al. Regulation of NKT cell development by SAP, the protein defective in XLP. *Nat Med* (2005) 11:340–5. doi:10.1038/nm1189
137. Das R, Bassiri H, Guan P, Wiener S, Banerjee PP, Zhong MC, et al. The adaptor molecule SAP plays essential roles during invariant NKT cell cytotoxicity and lytic synapse formation. *Blood* (2013) 121:3386–95. doi:10.1182/blood-2012-11-468868
138. Gerart S, Siberil S, Martin E, Lenoir C, Aguilar C, Picard C, et al. Human iNKT and MAIT cells exhibit a PLZF-dependent proapoptotic propensity that is counterbalanced by XIAP. *Blood* (2013) 121:614–23. doi:10.1182/blood-2012-09-456095
139. Chung BK, Tsai K, Allan LL, Zheng DJ, Nie JC, Biggs CM, et al. Innate immune control of EBV-infected B cells by invariant natural killer T cells. *Blood* (2013) 122:2600–8. doi:10.1182/blood-2013-01-480665
140. Yuling H, Ruijing X, Li L, Xiang J, Rui Z, Yujuan W, et al. EBV-induced human CD8+ NKT cells suppress tumorigenesis by EBV-associated malignancies. *Cancer Res* (2009) 69:7935–44. doi:10.1158/0008-5472.CAN-09-0828
141. Xiao W, Li L, Zhou R, Xiao R, Wang Y, Ji X, et al. EBV-induced human CD8(+) NKT cells synergise CD4(+) NKT cells suppressing EBV-associated tumours upon induction of Th1-bias. *Cell Mol Immunol* (2009) 6:367–79. doi:10.1038/cmi.2009.48
142. Okada S, Markle JG, Deenick EK, Mele F, Averbuch D, Lagos M, et al. IMMUNODEFICIENCIES. Impairment of immunity to *Candida* and *Mycobacterium* in humans with bi-allelic RORC mutations. *Science* (2015) 349:606–13. doi:10.1126/science.aaa4282
143. Azzi T, Lunemann A, Murer A, Ueda S, Beziat V, Malmberg KJ, et al. Role for early-differentiated natural killer cells in infectious mononucleosis. *Blood* (2014) 124:2533–43. doi:10.1182/blood-2014-01-553024
144. Vely F, Barlogis V, Vallentin B, Neven B, Piperoglou C, Ebbo M, et al. Evidence of innate lymphoid cell redundancy in humans. *Nat Immunol* (2016) 17:1291–9. doi:10.1038/ni.3553
145. Park GB, Kim YS, Lee HK, Song H, Cho DH, Lee WJ, et al. Endoplasmic reticulum stress-mediated apoptosis of EBV-transformed B cells by cross-linking of CD70 is dependent upon generation of reactive oxygen species and activation of p38 MAPK and JNK pathway. *J Immunol* (2010) 185:7274–84. doi:10.4049/jimmunol.1001547
146. Sepulveda FE, de Saint Basile G. Hemophagocytic syndrome: primary forms and predisposing conditions. *Curr Opin Immunol* (2017) 49:20–6. doi:10.1016/j.coi.2017.08.004
147. Boztug H, Hirschmugl T, Holter W, Lakatos K, Kager L, Trapin D, et al. NF-kappaB1 haploinsufficiency causing Immunodeficiency and EBV-driven lymphoproliferation. *J Clin Immunol* (2016) 36:533–40. doi:10.1007/s10875-016-0306-1
148. Nehme NT, Schmid JP, Debeurme F, Andre-Schmutz I, Lim A, Nitschke P, et al. MST1 mutations in autosomal recessive primary immunodeficiency characterized by defective naive T-cell survival. *Blood* (2012) 119:3458–68. doi:10.1182/blood-2011-09-378364
149. Abdollahpour H, Appaswamy G, Kotlarz D, Diestelhorst J, Beier R, Schaffer AA, et al. The phenotype of human STK4 deficiency. *Blood* (2012) 119:3450–7. doi:10.1182/blood-2011-09-378158
150. Du S, Scuderi R, Malicki DM, Willert J, Bastian J, Weidner N. Hodgkin's and non-Hodgkin's lymphomas occurring in two brothers with Wiskott-Aldrich syndrome and review of the literature. *Pediatr Dev Pathol* (2011) 14:64–70. doi:10.2350/10-01-0787-CR.1
151. Aydin SE, Kilic SS, Aytekin C, Kumar A, Porras O, Kainulainen L, et al. DOCK8 deficiency: clinical and immunological phenotype and treatment options – a review of 136 patients. *J Clin Immunol* (2015) 35:189–98. doi:10.1007/s10875-014-0126-0
152. Cohen JI. GATA2 deficiency and Epstein-Barr virus disease. *Front Immunol* (2017) 8:1869. doi:10.3389/fimmu.2017.01869
153. Coulter TI, Chandra A, Bacon CM, Babar J, Curtis J, Screaton N, et al. Clinical spectrum and features of activated phosphoinositide 3-kinase δ syndrome: a large patient cohort study. *J Allergy Clin Immunol* (2016) 139(2):597–606.e4. doi:10.1016/j.jaci.2016.06.021
154. Newell A, Dadi H, Goldberg R, Ngan BY, Grunebaum E, Roifman CM. Diffuse large B-cell lymphoma as presenting feature of Zap-70 deficiency. *J Allergy Clin Immunol* (2011) 127:517–20. doi:10.1016/j.jaci.2010.09.016
155. Schuetz C, Huck K, Gudowius S, Megahed M, Feyen O, Hubner B, et al. An immunodeficiency disease with RAG mutations and granulomas. *N Engl J Med* (2008) 358:2030–8. doi:10.1056/NEJMoa073966
156. Felgentreff K, Lee YN, Frugoni F, Du L, van der Burg M, Giliani S, et al. Functional analysis of naturally occurring DCLRE1C mutations and correlation with the clinical phenotype of ARTEMIS deficiency. *J Allergy Clin Immunol* (2015) 136:140–150.e7. doi:10.1016/j.jaci.2015.03.005
157. Marsh RA, Rao K, Satwani P, Lehmberg K, Muller I, Li D, et al. Allogeneic hematopoietic cell transplantation for XIAP deficiency: an international survey reveals poor outcomes. *Blood* (2013) 121:877–83. doi:10.1182/blood-2012-06-432500
158. Worth AJ, Nikolajeva O, Chiesa R, Rao K, Veys P, Amrolia PJ. Successful stem cell transplant with antibody-based conditioning for XIAP deficiency with refractory hemophagocytic lymphohistiocytosis. *Blood* (2013) 121:4966–8. doi:10.1182/blood-2013-01-478735
159. Ono S, Okano T, Hoshino A, Yanagimachi M, Hamamoto K, Nakazawa Y, et al. Hematopoietic stem cell transplantation for XIAP deficiency in Japan. *J Clin Immunol* (2017) 37:85–91. doi:10.1007/s10875-016-0348-4

Conflict of Interest Statement: The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

frontiers in Immunology

REVIEW
published: 18 September 2019
doi: 10.3389/fimmu.2019.02193

The T-cell Response to Epstein-Barr Virus–New Tricks From an Old Dog

Heather M. Long, Benjamin J. Meckiff and Graham S. Taylor**

Institute of Immunology and Immunotherapy, University of Birmingham, Birmingham, United Kingdom

Epstein-Barr virus (EBV) infects most people and establishes life-long infection controlled by the host's immune system. The genetic stability of the virus, deep understanding of the viral antigens and immune epitopes recognized by the host's T-cell system and the fact that recent infection can be identified by the development of symptomatic infectious mononucleosis makes EBV a powerful system in which to study human immunology. The association between EBV and multiple cancers also means that the lessons learned have strong translational potential. Increasing evidence of a role for resident memory T-cells and non-conventional γδ T-cells in controlling EBV infection suggests new opportunities for research and means the virus will continue to provide exciting new insights into human biology and immunology into the future.

Keywords: resident memory, nasopharyngeal carcinoma (NPC), tumor virus, DLBCL - Diffuse large B cell lymphoma, Burkitt lymphoma, gamma delta T cells, cytotoxic CD4 T cell, Hodgkin Lymphoma

OPEN ACCESS

Edited by:
Jeffrey I. Cohen,
National Institutes of Health (NIH),
United States

Reviewed by:
Rajiv Khanna,
QIMR Berghofer Medical Research Institute, Australia
Stuart G. Tangye,
Garvan Institute of Medical Research, Australia

****Correspondence:***
Heather M. Long
h.m.long@bham.ac.uk
Graham S. Taylor
g.s.taylor@bham.ac.uk

Specialty section:
This article was submitted to Primary Immunodeficiencies, a section of the journal Frontiers in Immunology

Received: *09 July 2019*
Accepted: *30 August 2019*
Published: *18 September 2019*

Citation:
Long HM, Meckiff BJ and Taylor GS (2019) The T-cell Response to Epstein-Barr Virus–New Tricks From an Old Dog. Front. Immunol. 10:2193. doi: 10.3389/fimmu.2019.02193

INTRODUCTION

Epstein-Barr virus (EBV) was first identified in 1964 in a biopsy from a patient with Burkitt Lymphoma (1). This gammaherpesvirus has co-evolved with humans for millennia and is a highly successful pathogen, infecting 90–95% of people worldwide who then carry the virus for life. EBV infection normally occurs in young children with few if any symptoms (2). However, if acquisition is delayed to adolescence then 25–75% of those infected develop infectious mononucleosis (IM). This is an acute syndrome characterized by a tetrad of symptoms: fever, fatigue, sore throat, and lymphadenopathy (3, 4). The acute symptoms of IM usually resolve by themselves, but serious rare complications may occur which include airway obstruction and splenic rupture (2). Longer term, a history of IM is associated with a raised incidence of Hodgkin lymphoma (HL) in the decade following infection and an increased risk of developing multiple sclerosis (5, 6).

EBV transmission occurs orally. Initial infection and replication of the virus most likely occurs in epithelial cells and locally infiltrating B-cells, resulting in high levels of virus shedding in the oropharynx (7–9). This lytic stage of viral replication is driven by up to 80 viral genes expressed in a temporally regulated manner (7). At the same time, the virus drives a proliferation of B-cells by activating its growth transforming programme. Here, viral gene expression comprises six Epstein-Barr Nuclear Antigens (EBNAs: EBNA1, EBNA2, EBNA3A, EBNA3B, EBNA3C, EBNA-LP), two latent membrane proteins (LMPs: LMP1, LMP2) and the viral anti-apoptotic protein BHRF1 (7, 10). This programme leads to the expansion of EBV-infected B cells in the oropharyngeal lymphoid tissue and the appearance of infected B cells in the blood. In some infected B-cells the virus downregulates its growth transforming program allowing the cells to enter the memory B-cell pool with the virus persisting as a truly latent infection lacking viral gene expression (11). These infected B-cells circulate between the blood and oropharyngeal lymphoid tissue and, in the latter, may occasionally switch into lytic cycle, releasing infectious virus for transmission to new hosts and for infection of new local B cells to maintain the viral reservoir (7).

EBV has potent growth transforming activity, as demonstrated *in vitro* by its ability to efficiently transform B-cells into immortalized lymphoblastoid cells lines (LCLs) (12). This property of the virus is used by laboratories worldwide to simply and reliably generate permanently growing B cell lines for research (13). The virus also has oncogenic potential, as demonstrated by its association with several malignancies that together total almost 200,000 cases of cancer each year worldwide (14). Nevertheless, the large majority of people infected by EBV do not suffer any long-term ill effects from the virus. This is due to the anti-viral immune response which, although unable to eliminate the virus, counters primary EBV infection and then maintains subsequent lifelong control to enable mutual co-existence of the virus and its host (8). Early control of EBV infection is associated with expansion of innate immune cells (primarily NK cells, described by Professor Munz in this review series) and of CD8+ and CD4+ T-cells specific for a broad range of EBV proteins expressed during the lytic and latent stages of viral infection (8). Over time, these T-cell responses decrease in magnitude but persist for the life time of the host. Low frequencies of latently EBV-infected B-cells can, nevertheless, be detected in the circulation (15) and infectious virus is periodically produced in the oropharynx and secreted in saliva (16, 17). Therefore, despite the exuberant primary immune response that occurs immediately after infection, and subsequent long-term immune surveillance, the virus is able to successfully persist for life. This apparent détente can, however, be broken if the balance between the virus and its host's immune response is disrupted. The clearest demonstration of this is in immunosuppressed patients, where loss of immune control of EBV can allow virus reactivation and the accumulation of EBV-transformed B cells, leading to post-transplant lymphoproliferative disease (PTLD) (18).

THE EBV-SPECIFIC T-CELL RESPONSE DURING SYMPTOMATIC PRIMARY INFECTION

Most work studying T-cell responses during primary infection has investigated people identified as having been recently infected with EBV through the overt symptoms of IM. The results of such studies are valuable but need to be interpreted with two caveats. First, in contrast to the vast majority of individuals who acquire EBV asymptomatically in early childhood, IM represents an atypical pathological state. Second, viral infection occurs several weeks prior to symptoms developing and samples being taken (19). On presentation, IM patients have unusually high numbers of atypical lymphocytes in the blood, the magnitude of which can resemble leukemia (20). Detailed analysis of blood from these patients shows that the majority of the expanded lymphocytes are EBV-specific T-cells (8). These largely comprise CD8+ T-cells specific for the EBV lytic cycle proteins with a clearly defined hierarchy. Most are specific for immediate early EBV lytic cycle proteins, a smaller number are specific for early proteins with few specific for late proteins (21–24). CD8+ T-cells specific for latent cycle proteins are also expanded but to a smaller degree. Of these, most are specific for the EBNA3A, 3B, and 3C proteins with a lower frequency of LMP2-specific T-cells also present (25, 26). Responses to the EBNA1 protein occur sporadically in IM patients bearing particular HLA alleles, such as HLA-B*3501; that are uncommon in the general population. In people with these alleles, however, the EBNA1-specific CD8 T-cell response is strong (27). The phenotype of the CD8+ T-cell response has been explored using HLA-class I tetramers. As might be expected the EBV-specific CD8+ T cells are proliferating and highly activated, expressing HLA-DR, CD38, and CD69 (28). They also express the CD45RO isoform, lack expression of the lymphoid homing markers CCR7 and CD62-L (26, 29), and are highly susceptible to apoptosis, likely due to low expression of the anti-apoptotic protein bcl-2 (30, 31). Given their extreme sensitivity to apoptosis *in vitro*, with significant cell death occurring within just a few hours, measurement of response size using directly *ex vivo* methods such as HLA tetramer staining provide the most accurate enumeration. Studies in IM patients using HLA tetramers report that CD8+ T-cells specific for individual EBV lytic and latent epitopes can account for 1–40 and 0.1–5% of total CD8+ T cells, respectively (25, 26, 28).

Regarding the EBV-specific CD4 T-cell response, during IM weak responses to lytic and latent cycle antigens are present with the former observed more frequently (32, 33). This early research utilized cytokine secretion assays to detect T-cells reactive to recombinant antigens or lysates of EBV-infected cells. As described above, the propensity of EBV-specific T-cells from IM patients to undergo apoptosis *in vitro* may have limited the sensitivity of this work. The recent development of HLA class II tetramers has allowed this obstacle to be overcome and the CD4+ T-cell response has now been accurately measured (34). *Ex vivo* HLA tetramer staining and flow cytometry has revealed that, although the overall size of the CD4+ T cell compartment does not appear to be expanded (3), the early EBV-specific CD4+ T-cell response is much stronger than previously appreciated. Responses to individual epitopes can reach as high as 1.5% of total CD4+ T-cells (34). Unlike the CD8+ T-cell response, for CD4+ T-cells the latent antigen responses numerically dominate the lytic. The exception is EBNA1, where CD4+ T cell responses are undetectable or low in the blood of patients with IM for several months before they develop (34, 35). Interestingly, this delayed appearance is in line with the previously documented but unexplained delay in EBNA1-specific IgG antibodies (36, 37). Akin to the CD8+ T cell response, EBV-specific CD4+ T cells express high levels of CD38 and CD45RO and lack the lymphoid homing markers CCR7 and CD62L (34). Considering the magnitude of the T-cell response to individual antigens, CD4+ T cells are smaller in number than CD8+ T-cells. However, the CD4+ T cell response is broader and targets more epitopes (8, 38–40). The breadth of the CD4+ T-cell response means that the total EBV-specific expansion is substantial and the overall activation status and phenotype of the total CD4+ T cell pool is altered within IM (34).

The functional profile of EBV-specific CD4+ T cells is consistent with Th1-like cells: most express T-bet and the predominant cytokine produced in *ex vivo* stimulation assays is IFNg. Some cells also produce TNFα and/or IL-2, either in

combination with IFNg or alone (41, 42). Early observations of raised perforin expression within the total CD4+ T cells of IM patients suggested that cytotoxic CD4+ T cells are present during primary EBV infection (43). Recent *ex vivo* HLA class II tetramer analysis has now shown that in fact the majority of activated EBV-specific CD4+ T cells express both perforin and granzyme B. Importantly, these cytotoxic proteins were not detected in co-existing influenza A-specific memory CD4+ T cells demonstrating that such expression was not due to non-specific bystander activation (42). Testing with EBV peptides has shown that some EBV-specific CD4+ T cells upregulate cell surface CD107a indicating degranulation and release of perforin and granzyme is possible (41). These observations strongly suggest that EBV-specific CD4+ T cells can exert cytotoxic function *in vivo*; if this is the case they could be highly effective against MHC-II positive EBV infected B cells.

Over time the symptoms of IM resolve with a concomitant decrease of both EBV DNA load and the frequency of EBV-specific CD8+ and CD4+ T-cells in the peripheral blood to values typical of life-long virus carriage (23, 34, 44, 45). All specificities decrease in magnitude although the predominant lytic antigen-specific CD8+ T-cell responses decline the most (25, 26, 34). The phenotype of the T-cells also changes. Activation marker expression decreases and anti-apoptotic proteins such as bcl-2 are upregulated (46). Latent antigen-specific CD8+ T-cells also begin to upregulate lymphoid homing markers allowing them to begin entering the tonsil, followed later and to a lesser extent by lytic antigen-specific CD8+ T cells (23).

THE EBV-SPECIFIC T CELL RESPONSE DURING ASYMPTOMATIC PRIMARY INFECTION

Although the above studies have informed our understanding of primary infection, most EBV infections occur in the absence of IM (2). Identifying newly-infected asymptomatic individuals is extremely challenging but has been achieved by several longitudinal studies that tracked EBV seronegative individuals over time. Together these studies provide an insight into the immunological events occurring in response to EBV infection in the absence of any clinical manifestation of disease. Early studies of newly infected infants showed no perturbations of the lymphoid compartment or febrile illness (47, 48). Newly infected African children had high levels of EBV DNA in the blood but no change in the overall size of the CD8 compartment. High frequencies of activated EBV-specific CD8+ T-cells could nevertheless be detected (49). Similarly, newly infected young adults also had high EBV DNA load in their blood but lacked lymphocytosis and the size of their T-cell compartment was unchanged (50). A recent prospective study of University students in the United Kingdom identified several individuals undergoing silent infection (51). Each had high EBV viral loads in the blood, reminiscent of IM, but no marked disturbance of total T cell or NK cell frequencies. Of three individuals with the highest viral loads, two had concurrent expansions of EBV-specific CD8+ T cells. In the third individual, EBV-specific T cells did not appear in the blood for several months until the peripheral viral load had decreased (51). Taken together, these studies strongly suggest that in asymptomatic individuals EBV infection elicits a virus-specific CD8+ T cell response. Although lower in magnitude than that seen in IM, this response is nevertheless sufficient to control the infection. The overall conclusion from this body of work is that the characteristic symptoms of IM result from the globally large expansions of highly-activated EBV-specific T-cells, which are predominantly CD8+.

THE EBV-SPECIFIC T-CELL RESPONSE DURING PERSISTENT INFECTION

EBV-specific T-cell responses are readily detected in the blood of healthy EBV carriers and are present at similar frequencies regardless of whether an individual experienced symptomatic or asymptomatic primary infection (8). CD8+ T-cell responses to lytic and latent cycle antigens are present, the former occurring at higher frequency. Individual lytic epitope-specific responses can account for up to 2% of the total CD8+ T-cell population. The lytic antigen hierarchy seen in IM patients is broadly maintained in memory: responses to immediate early antigens dominate those to early antigens and responses to late-expressed antigens are rare (22, 24). For latent antigen-specific responses, CD8+ T-cells targeting the EBNA3A, 3B, and 3C proteins are dominant. Fewer sub-dominant responses specific for EBNA1, EBNA2, and LMP2 are present; responses against EBNA-LP and LMP1 are rare (8). This general rule is, however, not observed in individuals possessing particular HLA types. Thus, individuals carrying HLA-B*3801 possess strong responses to an EBNA2 epitope and those carrying HLA-A*0203 possess strong responses to an epitope from EBNA-LP (30, 52). Phenotyping of HLA-I class tetramer-stained cells shows that the EBV-specific T-cell repertoire in persistently infected individuals contains resting antigen-experienced T-cells that are neither activated nor proliferating (30, 52). However, upon antigen challenge these cells exhibit potent effector functions including cytotoxicity and cytokine secretion (29, 52). Expression of lymphoid homing markers, such as CCR7 and CD62L, are variable but are generally expressed more frequently on T-cells specific for latent antigen compared to lytic antigens (52). The phenotype, functional profile and TCR clonotype composition of the virus-specific CD8+ T-cells is stable over many years (53, 54).

Compared to the CD8+ T cell response, the EBV-specific CD4+ T-cell response in healthy carriers is much smaller but the greater diversity of epitopes targeted by these cells in IM is maintained (34, 38–40, 55). Considering responses to individual epitopes, the CD4+ T-cell response is often 10-fold lower than the CD8+ T cell response to the same antigen (33, 34, 38, 39, 56). Across different antigens, latent antigen-specific responses outnumber lytic antigen-specific responses in magnitude. Lytic antigen-specific CD4+ T-cells are equally distributed against the immediate early, early and late antigens (39, 40); the heavy skewing exhibited by CD8+ T-cells is absent. HLA class II tetramer analysis shows that EBV-specific memory CD4+ T cells have the same phenotype regardless of whether they target latent

or lytic antigens. They do not express activation markers and are evenly distributed between the CCR7+ central memory and CCR7- effector memory subsets (34). Compared to CD4+ T-cells in IM patients, they no longer express perforin and granzyme, and upon *ex vivo* stimulation their cytokine polyfunctionality is increased with TNFa being the most predominant cytokine produced (41, 42, 57).

LOCATION, LOCATION, LOCATION: THE EMERGING IMPORTANCE OF RESIDENT T-CELL MEMORY

Much of our understanding of EBV-specific T cell immunity has come from studying circulating virus-specific T cells because sampling blood lymphocytes is convenient and minimally invasive. However, such analyses do not provide a complete picture of overall immune control. Recent studies in mice have highlighted the vital role of local immune responses, including virus-specific tissue resident memory T-cells (Trm cells), in providing long-term protective immunity against viral infection (58). Trm cells reside at sites of infection, where they enable rapid local immune responses against reactivation or secondary infection. Studying immunity in human tissues naturally presents a far greater challenge. Nevertheless, CD8+ and CD4+ Trm cells have been detected in a range of lymphoid and non-lymphoid tissues (59). These cells are transcriptionally distinct from circulating memory T cell populations (60).

Investigation of local EBV-specific T cell responses within tissues has been achieved by analysis of tonsils, which represent one of the major oropharyngeal sites of EBV infection and reactivation. In IM patients EBV loads in the tonsils are high yet EBV-specific CD8+ and CD4+ T cells are markedly lower in frequency at this site than in the blood of the same individual (23, 34, 42). Unlike lymph nodes, where T-cells may enter passively via afferent lymph vessels, entry into tonsils is dependent on transition across high endothelial venules (61). Expression of the lymphoid homing markers required for this process, CCR7 and CD62L, is highly downregulated on EBV-specific T cells in IM patients (26, 28, 34). This likely explains why EBV infection in the oropharynx is inefficiently targeted by virus-specific T cells during IM. As the symptoms of IM resolve, latent antigen-specific CD8+ T cells in the blood re-express CCR7 and CD62L and this coincides with their increasing frequency in the tonsil. At this time, some tonsillar latent antigen-specific CD8+ T cells express high levels of the activation marker CD38, consistent with encountering antigen at that site. In contrast, lytic antigen-specific CD8+ T cells remain CCR7-negative in the blood for several months after IM and their accumulation in the tonsils is correspondingly delayed. This delay may explain why lytic infection is not controlled in the throat of IM patients who continue to shed virus in saliva for many months after primary infection (23, 44).

In long-term EBV carriers the picture is reversed. Latent and lytic antigen-specific CD8+ T cells are higher in frequency in the tonsils than in the blood and the accumulated tonsillar EBV-specific CD8+ T cells no longer express the activation marker CD38. The degree of enrichment in the tonsils varies for latent vs. lytic antigen specific T-cells, with the former preferentially enriched compared to the latter (10-fold and 4-fold enrichment in the tonsil respectively) (23). This variable enrichment reflects the expression of CCR7 and CD62L on T-cells in the blood (26, 28) but other factors are likely involved. Thus, enrichment of EBV-specific CD8+ T-cells occurs only in the tonsils (and presumably other oropharyngeal lymphoid tissues) but not lymph nodes from other anatomical sites nor bone marrow (62, 63). The TCR repertoire of EBV-specific CD8+ T-cells within tonsils and blood of the same individual shows little difference (31) suggesting that tonsillar T-cell enrichment is not the result of selective recruitment or clonal expansion with the tonsil site.

Most EBV-specific CD8+ T cells in the tonsils of long-term EBV carriers express CD69 (64), one of the distinguishing markers of Trm cells (60). CD69 is a C-type lectin that mediates T cell retention in tissues and secondary lymphoid organs through sequestration of sphingosine-1-phosphate receptor (S1PR), a key molecule required for egress (65, 66). Although CD69 is also transiently expressed upon T cell activation (67) there is no concurrent raised expression of other cellular activation markers, including CD38 and HLA-DR, suggesting that CD69 expression in this context reflects active T cell retention rather than activation. In long-term EBV carriers, many tonsillar EBV-specific CD8+ T cells also express CD103 (αEβ7), an integrin that binds to E-cadherin and mediates retention at epithelial sites (23, 64). In contrast, this protein is not detected on the lower frequency EBV-specific CD8+ cells present in the tonsils of IM patients (23). Importantly, the CD103+ CD8+ subset of T cells from long-term EBV carrier tonsils show greater sensitivity *in vitro* to stimulation with cognate antigen (68). Elegant immunofluorescence microscopy of human tonsils has revealed that CD69+CD103+ CD8+ T cells preferentially localize at or near the tonsillar lymphoepithelial barrier (64). Although analysis of antigen specific T cells was not possible in this study, this observation suggests that EBV-specific CD8+ T cells of this phenotype may be retained in close proximity to the tonsillar epithelium, the region of the tonsils where EBV-positive B cells are predominantly found (69, 70).

BEYOND αβ: ACCUMULATING EVIDENCE OF A ROLE FOR γδ T-CELLS

All of the research described above has studied the αβ subset of T-cells and how these respond to EBV in primary and persistent infection. These T-cells express T-cell receptors composed of a heterodimeric α and β chain which enables them to recognize peptide epitopes presented by HLA molecules (8). A second subset of T-cells exists that express a different T-cell receptor formed from a heterodimeric γ and δ chain. These γδ T-cells can, in humans, be broadly divided into two groups based on the type of δ chain they express. T-cells expressing the Vδ2 chain are more abundant in the blood, where they comprise 90% of the circulating γδ T-cell pool (71). Cells expressing the Vδ1 chain

occur at low levels in the blood but comprise the bulk of γδ T-cells in tissues (72).

There is good evidence from mouse studies that γδ T-cells are involved in protection from herpesviruses such as murine cytomegalovirus (73, 74). A role for γδ T-cells in cancer is also suggested both by studies in mouse cancer models (75) and in humans by associations observed between intratumoural γδ T-cell frequency and prognosis (76). Several lines of evidence now show that γδ T-cells also play an important role in controlling EBV infection and transformation.

γδ T-CELLS IN INFECTIOUS MONONUCLEOSIS

Only a small number of studies have examined γδ T-cell during IM. Two studies, each analyzing 10 IM patients and a number of matched controls, showed an increased frequency of γδ T-cells in the blood by flow cytometry, with up to a 4-fold increase in absolute number (77, 78). The majority of γδ T-cells in IM patients were positive for the cell surface activation marker CD38 whereas this marker was absent from γδ T-cells in healthy donors (77) A larger study detected increased αβ and γδ TCR gene expression in the blood of IM patients by transcriptional analysis. Based on a deeper analysis of the transcriptome the authors suggest that both the Vδ1 and Vδ2 subsets are increased in IM (79). Because increases in RNA levels may not necessarily reflect changes in cell frequency the authors performed confirmatory flow cytometry analysis of a subset of patients which demonstrated a 3.4-fold increase in γδ T-cell frequency in IM (79). Note that the antibody used for this work was unable to differentiate between the two γδ T-cell subsets so the relative contribution each makes to the overall expansion of γδ T-cells in IM requires further investigation.

The above observational studies cannot determine whether the increases in γδ frequency that occur in IM are an indirect result of bystander activation or represent direct recognition of EBV infected cells. An experiment of nature suggests the latter may be the case. An EBV-negative recipient of a cord blood transplant, who acquired EBV 31 days after transplantation, experienced prolonged high-level EBV viremia yet did not develop any clinical manifestations of EBV-associated disease. This patient lacked detectable EBV-specific αβ T-cells by HLA class I tetramer staining and interferon-gamma ELISpot assays (80) but had large expansions of γδ T-cells that reached almost 50% of total T-cells. These cells were mostly Vδ1 T-cells but a smaller number of Vδ2 T-cells were also present. The Vδ1 T-cells were predominantly CD45RA- CD27+ central memory cells and, based on their expression of CD57, the authors concluded they were activated. Interestingly, the γδ T-cells were able to degranulate when exposed to an EBV+ve cell line *in vitro* suggesting direct recognition of EBV+ve cells was possible. In a separate study, an abundant Vδ1 T-cell clone isolated from a recipient of an allogeneic stem cell transplant killed autologous and allogeneic LCLs but not the EBV-ve Raji Burkitt Lymphoma (BL) cell line (81).

γδ T-CELLS AND EBV-ASSOCIATED NEOPLASMS

EBV is associated with malignancies arising in different cell backgrounds. Examples include BL, a tumor of B cells that occurs predominantly in Sub Saharan Africa, and nasopharyngeal carcinoma (NPC) an epithelial carcinoma that occurs at high incidence throughout South East Asia. γδ T-cells are altered in patients with these EBV+ve malignancies. Two papers studying patients with NPC both report that while the frequency of γδ T-cells in patients is unaltered their functional capacity is impaired. Following *in vitro* culture, peripheral blood mononuclear cells (PBMCs) from NPC patients yielded smaller numbers of γδ cells compared to control donors and were unable to kill an NPC cell line (82). When tested in cytotoxicity assays, γδ T-cells from patients lacked the ability to kill CNE-2, a tumor cell line established from an NPC patient (82). Interestingly, this deficit in NPC cell killing was found only in patients with active NPC since γδ T-cells from successfully-treated NPC patients exhibited the same level of cytotoxicity against CNE2 as those from control donors. Similarly, another research group found that Vγ9Vδ2 T-cells from NPC patients, but not healthy donors, were unable to lyse the NPC cell line HK1 or the control cell line K562 when tested directly *ex vivo* (83). In this study Vγ9Vδ2 T-cells from NPC patients also produced less interferon-gamma and TNFα than cells from control donors when exposed to HMBPP, a phosphoantigen that is a potent stimulator of Vγ9Vδ2 T-cell activity. Flow cytometry analysis showed that Vγ9Vδ2 T-cells in NPC patients were more highly differentiated, with a smaller proportion of central memory and higher proportion of terminally differentiated TEMRA cells. A study in BL patients in Ghana reports that patients have lower Vγ9Vδ2 T-cell frequency than healthy donors (84). Whether the functional capacity of Vγ9Vδ2 T-cell in BL patients is impaired is unknown.

In several of the above papers, γδ T-cells were reported to be capable of recognizing EBV+ LCLs (80–82), the NPC cell line HK1 (83) or the NPC cell line CNE2 (82); note that experiments using CNE2 need cautious interpretation as it has been shown to be contaminated with HeLa (85). These observations raise the question of which cellular targets render EBV+ve cells visible to γδ T-cells. In this regard it is important to point out that γδ T-cell recognition of target cells is complex and can involve the T-cell receptor and/or ligands for natural killer receptors they express, such as NKG2D (86, 87).

γδ T-CELL RECOGNITION OF EBV+VE CELLS

The BL line Daudi (88) is a reliable stimulator of Vγ9Vδ2 T-cells and has been used for many years for this purpose (89–99). These studies also often included other BL cell lines which in contrast showed lower or indeed no γδ T-cell stimulatory activity. Indeed, the BL line Raji, as well as LCLs, were often used as a negative controls in such work.

It is now known that Daudi overproduces endogenous non-peptidic phosphorylated metabolites due to upregulation of the mevalonate pathway (96) resulting in intracellular accumulation of IPP, a host phosphoantigen counterpart of HMBPP (100). Vγ9Vδ2 T-cell phosphoantigen sensing is dependent on: (i) target cell exposure to/accumulation of HMBPP and IPP and (ii) target cell expression of BTN3A1, which binds phosphoantigens via its intracellular B30.2 domain (101). It is currently unclear why most BL cell lines are unable to generate Vγ9Vδ2 T-cell lines when co-cultured with PBMCs, however, this potentially could reflect deficient expression of BTN3A1 or insufficient IPP production. These possibilities require further investigation.

Interestingly, being able to generate Vγ9Vδ2 T-cell lines and being able to be recognized by those same cells appear to be distinct properties. Thus, Daudi-stimulated Vγ9Vδ2 T-cells are able to recognize and kill multiple BL lines that themselves lack stimulatory activity for Vγ9Vδ2 T-cells (94). This difference between the ability to generate Vγ9Vδ2 T-cells and being sensitive to γδ T-cell recognition could be due to: (i) activated Vγ9Vδ2 T-cells possessing increased sensitivity to a lower level of phosphoantigens and/or BTN3A1 (ii) Daudi stimulated Vγ9Vδ2 T-cells being able to utilize alternative target recognition mechanisms that are independent of phosphoantigens. For example, Vγ9Vδ2 T-cells expressing NKg2D can recognize target cells expressing ULBP4, a protein upregulated upon EBV infection of B-cells (102).

Additional complexity is suggested by a recent report of a fundamental population-level difference in the response to BL lines (99). After co-culturing PBMCs from 24 donors with EBV+ve Akata BL cells for 10 days *in vitro*, Djaoud and colleagues found that 13 co-cultures had large expansions of Vγ9Vδ2 T-cells and NK cells. In contrast, the other 11 co-cultures had a large expansion of NK cells but only very small increases in Vγ9Vδ2 T-cells. Vγ9Vδ2 T-cell expansion was sensitive to an anti-BTN3A1 blocking antibody or mevastatin, an inhibitor of the mevalonate pathway that generates the intracellular phosphoantigens recognized by Vg9Vd2 T-cells. Interestingly, the authors reported that Vγ9Vδ2 T-cell expansion did not occur when the EBV-ve derivative of the Akata BL cell line was used instead. The authors extended their work to include a larger range of EBV+ve stimulator cells. These experiments used the EBV+ve Akata BL cell line as well as the EBV+ve BL cell lines Daudi and Kem-I (all three are type I BL lines expressing EBNA1), Sal (a Wp-restricted BL line that expresses EBNA1 but also EBNAs 3A, 3B, and 3C along with a truncated form of EBNA-LP) and Raji and Jijoye (the EBV+ve variants of these two lines express all EBV latent cycle proteins). Only the type I BL cells (Akata, Daudi, and Kem-I) stimulated Vγ9Vδ2 T-cell expansion (99). This result led the authors to conclude that Vγ9Vδ2 T-cell expansion was a property of type-I BL cells only. Independent confirmation of these results and testing of a larger range of B-cell lines is now needed to determine if Vγ9Vδ2 T-cell recognition is indeed limited solely to type I BL lines and to reveal the underpinning biological mechanisms responsible.

γδ T-CELLS IN MOUSE MODELS OF EBV

Further support for a role of Vγ9Vδ2 T-cells in the control of EBV comes from studies in mice. Growth of CNE2 cells injected subcutaneously into nude mice was slowed following intravenous administration of Vγ9Vδ2 T-cells (103). More advanced mouse models now exist including mice reconstituted with human immune components which, arguably, are better models in which to study EBV immunology (104). Work using an early form of this model, CB.17$^{scid/scid}$ mice [severe combined immunodeficient (SCID) mice] injected intraperitoneally with peripheral blood lymphocytes, showed increases in γδ T-cells following administration of irradiated Daudi cells. These mice were protected against subsequent challenge with non-irradiated Daudi cells but not non-irradiated Raji BL cells which developed into disseminated lymphoma (105).

Control of EBV-driven lymphoproliferations and lymphomas has been demonstrated by two studies that used more advanced mouse models. The first used EBV-transformed LCLs as targets of Vγ9Vδ2T-cells (106). In preliminary *in vitro* work, phosphoantigen-specific Vγ9Vδ2T-cells were unable to recognize LCLs, a result consistent with previous publications. However, when the same Vγ9Vδ2 T-cells were positively selected using magnetic beads they then recognized and killed LCL cells. The authors suggested that binding of the anti-γδ antibody-loaded beads to the Vγ9Vδ2 T-cell receptor may have activated the cells thereby allowing them to recognize LCLs. Cytotoxicity of the T-cells was inhibited by concanamycin A, a widely-used inhibitor of perforin-mediated cytotoxicity. A somewhat surprising observation, however, was that purified bcl-2 protein also inhibited cytotoxicity: how an extracellular protein was able to enter the LCLs to inhibit intracellular granzyme activity was not discussed.

Initial *in vivo* experiments, using immunodeficient Rag2$^{-/-}$ γc$^{-/-}$ mice injected with LCLs, then showed that adoptive transfer of magnetically enriched Vγ9Vδ2 T-cells could prevent LCL-induced lymphoproliferative disease and eliminated established LCL tumors (106). Extending the work to Rag2$^{-/-}$ γc$^{-/-}$ mice carrying human immune components (established by injection of PBMC) showed these mice carried human Vγ9Vδ2 T–cells and that LCL tumors were rejected after the mice were treated with pamidronate, a drug that stimulates Vγ9Vδ2 T-cells (106). The reasons why Vγ9Vδ2 T-cells in these mice did not require activation via bead selection in order to recognize and kill LCLs was not explored. Pamidronate-induced tumor rejection was, however, clearly Vγ9Vδ2 T-cell dependent. These results raise the interesting possibility that patients with PTLD could one day be treated using small molecular agents to activate their own Vγ9Vδ2 T-cells. Several ways to achieve this in the clinic can be envisaged (107) including using zoledronate, a drug licensed to treat bone diseases but which also exhibits potent Vγ9Vδ2 stimulatory activity (96).

Work by another group also suggests that Vγ9Vδ2 T-cells may be able to eliminate LCLs *in vivo* without requiring activation via bead selection. Here the model used was the Rag2$^{-/-}$

$\gamma c^{-/-}$ system but now using human cord blood to reconstituted the mice with human immune components (108). Spontaneous EBV-driven lymphoproliferations could be induced in the mice by infecting the cord blood with EBV immediately prior to injection. Adoptive transfer of Vγ9Vδ2 T-cells (generated *in vitro* but without magnetic bead selection) soon after reconstitution prevented lymphoma development. Delaying adoptive transfer of Vγ9Vδ2 T-cells until tumors were evident retarded tumor growth but did not eliminate them.

Finally, although far fewer studies have investigated Vδ1 T-cell recognition of EBV targets, the available data clearly shows marked differences exists between the Vδ1 and Vδ2 T-cell subsets. Thus, Vδ1 T-cells are not stimulated by Daudi BL cells but are instead stimulated by LCLs (109–111). Regarding other BL lines, in one study, Daudi, Raji, Ramos, BL41, and BL57 did not stimulate Vδ1 T-cells (111) whereas Raji was reported to be stimulatory by another (109). These disparate results may reflect the fact that Vδ1 cells employ a range of target recognition mechanisms (72).

Collectively the above results clearly show that both γδ T-cell subsets warrant far greater attention then has been the case in the past. Which subset will prove to be more important in controlling EBV remains to be determined. Indeed, the critical effector subset may vary as individuals progress from primary infection into the long term carrier state and, for a minority, develop an EBV+ cancer.

A ROLE FOR NATURAL KILLER T (NKT) CELLS IN CONTROLLING EBV?

A limited number of studies have examined the contribution of NKT cells to EBV immunity. NKT cells are a conserved population of innate-like T cells that express the semi-invariant Vα24-Jα18/Vβ11 T cell receptor. Unlike conventional αβ T cells, NKT cells recognize glycolipid antigens presented by the non-polymorphic MHC class I-like molecule, CD1d (112). Only one study has assessed the frequency of NKT cells in the blood during EBV infection (113). Using flow cytometry, no expansion of CD3+CD56+CD244+ NKT cells was detected in 11 acute IM patients compared to age-matched healthy carriers. A larger study to assess the frequency and function of Vα24-Jα18/Vβ11+ NKT cells is required to confirm this observation. Nevertheless, a possible role for NKT cells in immune control of EBV is supported by the following lines of evidence. (i) NKT cells are significantly lower in frequency in the blood of EBV+ HL and NPC patients than in healthy carriers (114). Furthermore, NKT cells derived from these patients were less functional upon stimulation with the synthetic glycolipid α-GalCer *in vitro*. (ii) In patients with primary immunodeficiency, individuals with genetic mutations that affect the NKT cell lineage are predisposed to develop EBV-associated disease (reviewed by Professor Munz in this series). Importantly, however, such mutations rarely affect NKT cells in isolation and most patients have coexisting defects in NK and/or T cell development and/or function. Therefore, deciphering the precise contribution of NKT cell deficiency to the lack of overall immune control of EBV in these patients is extremely challenging (115). (iii) The presence of NKT cells reduces EBV transformation of B cells *in vitro*. Upon infection of PBMCs with EBV, prior depletion of NKT cells led to both increased numbers of EBV-infected B cells and raised overall viral titers in the cultures (116). Interestingly CD1d expression was lost from the B cell surface during transformation, and NKT cells were subsequently unable to recognize fully transformed LCL cell lines. The authors therefore suggest that NKT cells may be important for early immune recognition of newly EBV-infected B cells, and exert their function prior to EBV-driven downregulation of CD1d (116). Note that similar downregulation of CD1d has been reported in KSHV and HSV-1 infected cells (117, 118), suggesting that evasion of NKT cell surveillance may be a common strategy of herpesviruses.

CONCLUSIONS

Research on EBV continues to reveal new tricks employed by the human T cell immune system to control infection with this ancient virus. Novel techniques have enabled deeper understanding of the targets, function and evolution of the CD8+ and CD4+ T cell responses, and the potential contributions of previously neglected unconventional T cell subsets are increasingly coming to light. Although it is challenging to obtain tissue samples from people, studying EBV-specific immunity at the site of infection is vital if we are to fully understand the interplay between the virus and its host. With this in mind, we note the current dearth of information on intra-tumoural EBV-specific immunity and highlight this area as a priority for future research.

AUTHOR CONTRIBUTIONS

All authors listed have made a substantial, direct and intellectual contribution to the work, and approved it for publication.

FUNDING

Work in HL's and GT's laboratories was supported by the Medical Research Council (grant MR/P013201), Bloodwise (Project Grants 14039 and 15021) and the Cancer Research UK Birmingham Center.

REFERENCES

1. Epstein MA, Achong BG, Barr YM. Virus particles in cultured lymphoblasts from Burkitt's lymphoma. *Lancet*. (1964) 1:702–3. doi: 10.1016/s0140-6736(64)91524-7
2. Balfour HH, Dunmire SK, Hogquist KA. Infectious mononucleosis. *Clin Transl Immunol*. (2015) 4:e33. doi: 10.1038/cti.2015.1
3. Balfour HH, Odumade OA, Schmeling DO, Mullan BD, Ed JA, Knight JA, et al. Behavioral, virologic, and immunologic factors associated with

acquisition and severity of primary Epstein-Barr virus infection in university students. *J Infect Dis*. (2013) 207:80–8. doi: 10.1093/infdis/jis646
4. Crawford DH, Macsween KF, Higgins CD, Thomas R, McAulay K, Williams H, et al. A cohort study among university students: identification of risk factors for Epstein-Barr virus seroconversion and infectious mononucleosis. *Clin Infect Dis*. (2006) 43:276–82. doi: 10.1086/505400
5. Hjalgrim H, Askling J, Rostgaard K, Hamilton-Dutoit S, Frisch M, Zhang J-S, et al. Characteristics of Hodgkin's lymphoma after infectious mononucleosis. *New Engl J Med*. (2003) 349:1324–32. doi: 10.1056/NEJMoa023141
6. Ascherio A, Munger KL. EBV and autoimmunity. *Curr Top Microbiol Immunol*. (2015) 390:365–85. doi: 10.1007/978-3-319-22822-8_15
7. Longnecker R, Kieff R, Cohen J I. Epstein-Barr virus. In: Knipe DM, Howley PM, Cohen JI, Griffeth DE, Lamb RA., Martin MA, et al. editors. *Fields Virology*. Philadelphia: Lippincott Williams & Wilkins. (2013) p. 1898–959.
8. Taylor GS, Long HM, Brooks JM, Rickinson AB, Hislop AD. The immunology of Epstein-Barr virus-induced disease. *Annu Rev Immunol*. (2015) 33:787–821. doi: 10.1146/annurev-immunol-032414-112326
9. Thorley-Lawson DA, Hawkins JB, Tracy SI, Shapiro M. The pathogenesis of Epstein-Barr virus persistent infection. *Curr Opin Virol*. (2013) 3:227–32. doi: 10.1016/j.coviro.2013.04.005
10. Kelly GL, Long HM, Stylianou J, Thomas WA, Leese A, Bell AI, et al. An Epstein-Barr virus anti-apoptotic protein constitutively expressed in transformed cells and implicated in burkitt lymphomagenesis: the Wp/BHRF1 link. *PLoS Pathog*. (2009) 5:e1000341. doi: 10.1371/journal.ppat.1000341
11. Babcock GJ, Hochberg D, Thorley-Lawson AD. The expression pattern of Epstein-Barr virus latent genes *in vivo* is dependent upon the differentiation stage of the infected B cell. *Immunity*. (2000) 13:497–506. doi: 10.1016/S1074-7613(00)00049-2
12. Neitzel H. A routine method for the establishment of permanent growing lymphoblastoid cell lines. *Hum Genet*. (1986) 73:320–6.
13. Hussain T, Mulherkar R. Lymphoblastoid cell lines: a continuous *in vitro* source of cells to study carcinogen sensitivity and DNA repair. *Int J Mol Cell Med*. (2012) 1:75–87.
14. Parkin DM. The global health burden of infection-associated cancers in the year 2002. *Int J Cancer*. (2006) 118:3030–44. doi: 10.1002/ijc.21731
15. Babcock GJ, Decker LL, Volk M, Thorley-Lawson DA. EBV persistence in memory B cells *in vivo*. *Immunity*. (1998) 9:395–404.
16. Perera RAPM, Samaranayake LP, Tsang CSP. Shedding dynamics of Epstein-Barr virus: a type 1 carcinogen. *Arch Oral Biol*. (2010) 55:639–47. doi: 10.1016/j.archoralbio.2010.06.009
17. Johnson KH, Webb C-H, Schmeling DO, Brundage RC, Balfour HH. Epstein–Barr virus dynamics in asymptomatic immunocompetent adults: an intensive 6-month study. *Clin Trans Immunol*. (2016) 5:e81. doi: 10.1038/cti.2016.28
18. Crombie JL, LaCasce AS. Epstein Barr virus associated B-cell lymphomas and iatrogenic lymphoproliferative disorders. *Front Oncol*. (2019) 9:109. doi: 10.3389/fonc.2019.00109
19. Dunmire SK, Grimm JM, Schmeling DO, Balfour HH, Hogquist KA. The incubation period of primary Epstein-Barr virus infection: viral dynamics and immunologic events. *PLoS Pathog*. (2015) 11:e1005286. doi: 10.1371/journal.ppat.1005286
20. Chhabra P, Law AD, Sharma U, Suri V, Sachdeva MS, Kumari S, et al. Epstein-Barr virus infection masquerading as acute leukemia: a report of two cases and review of literature. *Indian J Hematol Blood Transfus*. (2014) 30:26–8. doi: 10.1007/s12288-012-0207-2
21. Woodberry T, Suscovich TJ, Henry LM, Davis JK, Frahm N, Walker BD, et al. Differential targeting and shifts in the immunodominance of Epstein-Barr virus–specific CD8 and CD4 T cell responses during acute and persistent infection. *J Infect Dis*. (2005) 192:1513–24. doi: 10.1086/491741
22. Abbott RJM, Quinn LL, Leese AM, Scholes HM, Pachnio A, Rickinson AB. CD8+ T cell responses to lytic EBV infection: late antigen specificities as subdominant components of the total response. *J Immunol*. (2013) 191:5398–409. doi: 10.4049/jimmunol.1301629
23. Hislop AD, Kuo M, Drake-Lee AB, Akbar AN, Bergler W, Hammerschmitt N, et al. Tonsillar homing of Epstein-Barr virus-specific CD8+ T cells and the virus-host balance. *J Clin Invest*. (2005) 115:2546–55. doi: 10.1172/JCI24810
24. Pudney VA, Leese AM, Rickinson AB, Hislop AD. CD8+ immunodominance among Epstein-Barr virus lytic cycle antigens directly reflects the efficiency of antigen presentation in lytically infected cells. *J Exp Med*. (2005) 201:349–60. doi: 10.1084/jem.20041542
25. Catalina MD, Sullivan JL, Bak KR, Luzuriaga K. Differential evolution and stability of epitope-specific CD8(+) T cell responses in EBV infection. *J Immunol*. (2001) 167:4450–7. doi: 10.4049/jimmunol.167.8.4450
26. Hislop AD, Annels NE, Gudgeon NH, Leese AM, Rickinson AB. Epitope-specific evolution of human CD8(+) T cell responses from primary to persistent phases of Epstein-Barr virus infection. *J Exp Med*. (2002) 195:893–905. doi: 10.1084/jem.20011692
27. Blake N, Lee S, Redchenko I, Thomas W, Steven N, Leese A, et al. Human CD8+ T cell responses to EBV EBNA1: HLA class I presentation of the (Gly-Ala)-containing protein requires exogenous processing. *Immunity*. (1997) 7:791–802.
28. Callan MF, Tan L, Annels N, Ogg GS, Wilson JD, O'Callaghan CA, et al. Direct visualization of antigen-specific CD8+ T cells during the primary immune response to Epstein-Barr virus *in vivo*. *J Exp Med*. (1998) 187:1395–402. doi: 10.1084/jem.187.9.1395
29. Catalina MD, Sullivan JL, Brody RM, Luzuriaga K. Phenotypic and functional heterogeneity of EBV epitope-specific CD8+ T cells. *J Immunol*. (2002) 168:4184–91. doi: 10.4049/jimmunol.168.8.4184
30. Callan MF, Fazou C, Yang H, Rostron T, Poon K, Hatton C, et al. CD8(+) T-cell selection, function, and death in the primary immune response *in vivo*. *J Clin Invest*. (2000) 106:1251–61. doi: 10.1172/JCI10590
31. Soares MVD, Plunkett FJ, Verbeke CS, Cook JE, Faint JM, Belaramani LL, et al. Integration of apoptosis and telomere erosion in virus-specific CD8+ T cells from blood and tonsils during primary infection. *Blood*. (2004) 103:162–7. doi: 10.1182/blood-2003-06-1791
32. Precopio ML, Sullivan JL, Willard C, Somasundaran M, Luzuriaga K. Differential kinetics and specificity of EBV-specific CD4+ and CD8+ T cells during primary infection. *J Immunol*. (2003) 170:2590–8. doi: 10.4049/jimmunol.170.5.2590
33. Amyes E, Hatton C, Montamat-Sicotte D, Gudgeon N, Rickinson AB, McMichael AJ, et al. Characterization of the CD4+ T cell response to Epstein-Barr virus during primary and persistent infection. *J Exp Med*. (2003) 198:903–911. doi: 10.1084/jem.20022058
34. Long HM, Chagoury OL, Leese AM, Ryan GB, James E, Morton LT, et al. MHC II tetramers visualize human CD4+ T cell responses to Epstein-Barr virus infection and demonstrate atypical kinetics of the nuclear antigen EBNA1 response. *J Exp Med*. (2013) 210:933–49. doi: 10.1084/jem.20121437
35. Scherrenburg J, Piriou E, Nanlohy NM, van Baarle D. Detailed analysis of EpsteinBarr virus-specific CD4+ and CD8+ T cell responses during infectious mononucleosis. *Clin Exp Immunol*. (2008) 153:231–9. doi: 10.1111/j.1365-2249.2008.03699.x
36. Henle W, Henle G, Andersson J, Ernberg I, Klein G, Horwitz CA, et al. Antibody responses to Epstein-Barr virus-determined nuclear antigen (EBNA)-1 and EBNA-2 in acute and chronic Epstein-Barr virus infection. *Proc Natl Acad Sci*. (1987) 84:570–4. doi: 10.1073/pnas.84.2.570
37. Hille A, Klein K, Bäumler S, Grässer FA, Mueller-Lantzsch N. Expression of Epstein-Barr virus nuclear antigen 1,2A and 2B in the baculovirus expression system: serological evaluation of human antibodies to these proteins. *J Med Virol*. (1993) 39:233–41. doi: 10.1002/jmv.1890390311
38. Long HM, Haigh TA, Gudgeon NH, Leen AM, Tsang C-W, Brooks J, et al. CD4+ T-cell responses to Epstein-Barr Virus (EBV) latent-cycle antigens and the recognition of EBV-transformed lymphoblastoid cell lines. *J Virol*. (2005) 79:4896–907. doi: 10.1128/JVI.79.8.4896-4907.2005
39. Long HM, Leese AM, Chagoury OL, Connerty SR, Quarcoopome J, Quinn LL, et al. Cytotoxic CD4+ T cell responses to EBV contrast with CD8 responses in breadth of lytic cycle antigen choice and in lytic cycle recognition. *J Immunol*. (2011) 187:92–101. doi: 10.4049/jimmunol.1100590
40. Adhikary D, Behrends U, Boerschmann H, Pfünder A, Burdach S, Moosmann A, et al. Immunodominance of lytic cycle antigens in Epstein-Barr virus-specific CD4+ T cell preparations for therapy. *PLoS ONE*. (2007) 2:e583. doi: 10.1371/journal.pone.0000583

41. Lam JKP, Hui KF, Ning RJ, Xu XQ, Chan KH, Chiang AKS. Emergence of CD4+ and CD8+ Polyfunctional T cell responses against immunodominant lytic and latent EBV antigens in children with primary EBV infection. *Front Microbiol*. (2018) 9:416. doi: 10.3389/fmicb.2018.00416
42. Meckiff B, Ladell K, McLaren JE, Ryan GB, Leese AM, James E, et al. Primary EBV infection induces an acute wave of activated antigen-specific cytotoxic CD4+ T cells. *J Immunol*. (2019) 203:1276–87. doi: 10.4049/jimmunol.1900377
43. Appay V, Zaunders JJ, Papagno L, Sutton J, Jaramillo A, Waters A, et al. Characterization of CD4(+) CTLs ex vivo. *J Immunol*. (2002) 168:5954–8. doi: 10.4049/jimmunol.168.11.5954
44. Fafi-Kremer S, Morand P, Brion J-P, Pavese P, Baccard M, Germi R, et al. Long-term shedding of infectious Epstein-Barr virus after infectious mononucleosis. *J Infect Dis*. (2005) 191:985–9. doi: 10.1086/428097
45. Balfour HH, Holman CJ, Hokanson KM, Lelonek MM, Giesbrecht JE, White DR, et al. A prospective clinical study of Epstein-Barr virus and host interactions during acute infectious mononucleosis. *J Infect Dis*. (2005) 192:1505–12. doi: 10.1086/491740
46. Dunne PJ, Faint JM, Gudgeon NH, Fletcher JM, Plunkett FJ, Soares MVD, et al. Epstein-Barr virus-specific CD8(+) T cells that re-express CD45RA are apoptosis-resistant memory cells that retain replicative potential. *Blood*. (2002) 100:933–40. doi: 10.1182/blood-2002-01-0160
47. Biggar RJ, Henle G, Böcker J, Lennette ET, Fleisher G, Henle W. Primary Epstein-Barr virus infections in African infants. II. Clinical and serological observations during seroconversion. *Int J Cancer*. (1978) 22:244–50. doi: 10.1002/ijc.2910220305
48. Fleisher G, Henle W, Henle G, Lennette ET, Biggar RJ. Primary infection with Epstein-Barr virus in infants in the United States: clinical and serologic observations. *J Infect Dis*. (1979) 139:553–8. doi: 10.1093/infdis/139.5.553
49. Jayasooriya S, de Silva TI, Njie-jobe J, Sanyang C, Leese AM, Bell AI, et al. Early virological and immunological events in asymptomatic Epstein-Barr virus infection in African children. *PLoS Pathog*. (2015) 11:e1004746. doi: 10.1371/journal.ppat.1004746
50. Silins SL, Sherritt MA, Silleri JM, Cross SM, Elliott SL, Bharadwaj M, et al. Asymptomatic primary Epstein-Barr virus infection occurs in the absence of blood T-cell repertoire perturbations despite high levels of systemic viral load. *Blood*. (2001) 98:3739–44. doi: 10.1182/blood.v98.13.3739
51. Abbott RJ, Pachnio A, Pedroza-Pacheco I, Leese AM, Begum J, Long HM, et al. Asymptomatic primary infection with Epstein-Barr virus: observations on young adult cases. *J Virol*. (2017) 91:e00382–17. doi: 10.1128/JVI.00382-17
52. Hislop AD, Gudgeon NH, Callan MF, Fazou C, Hasegawa H, Salmon M, et al. EBV-specific CD8+ T cell memory: relationships between epitope specificity, cell phenotype, and immediate effector function. *J Immunol*. (2001) 167:2019–29. doi: 10.4049/jimmunol.167.4.2019
53. Lelic A, Verschoor CP, Ventresca M, Parsons R, Evelegh C, Bowdish D, et al. The polyfunctionality of human memory CD8+ T cells elicited by acute and chronic virus infections is not influenced by age. *PLoS Pathog*. (2012) 8:e1003076. doi: 10.1371/journal.ppat.1003076
54. Klarenbeek PL, Remmerswaal EBM, ten Berge IJM, Doorenspleet ME, van Schaik BDC, Esveldt REE, et al. Deep sequencing of antiviral T-cell responses to HCMV and EBV in humans reveals a stable repertoire that is maintained for many years. *PLoS Pathog*. (2012) 8:e1002889. doi: 10.1371/journal.ppat.1002889
55. Haigh TA, Lin X, Jia H, Hui EP, Chan ATC, Rickinson AB, et al. EBV latent membrane proteins. (LMPs) 1 and 2 as immunotherapeutic targets: LMP-specific CD4+ cytotoxic T cell recognition of EBV-transformed B cell lines. *J Immunol*. (2008) 180:1643–54. doi: 10.4049/jimmunol.180.3.1643
56. Leen A, Meij P, Redchenko I, Middeldorp J, Bloemena E, Rickinson A, et al. Differential immunogenicity of Epstein-Barr virus latent-cycle proteins for human CD4(+) T-helper 1 responses. *J Virol*. (2001) 75:8649–59. doi: 10.1128/jvi.75.18.8649-8659.2001
57. Ning RJ, Xu XQ, Chan KH, Chiang AKS. Long-term carriers generate Epstein-Barr virus (EBV)-specific CD4(+) and CD8(+) polyfunctional T-cell responses which show immunodominance hierarchies of EBV proteins. *Immunology*. (2011) 134:161–71. doi: 10.1111/j.1365-2567.2011.03476.x
58. Mueller SN, Mackay LK. Tissue-resident memory T cells: local specialists in immune defence. *Nat Rev Immunol*. (2016) 16:79–89. doi: 10.1038/nri.2015.3
59. Sathaliyawala T, Kubota M, Yudanin N, Turner D, Camp P, Thome JJC, et al. Distribution and compartmentalization of human circulating and tissue-resident memory T cell subsets. *Immunity*. (2013) 38:187–97. doi: 10.1016/j.immuni.2012.09.020
60. Kumar BV, Ma W, Miron M, Granot T, Guyer RS, Carpenter DJ, et al. Human tissue-resident memory T cells are defined by core transcriptional and functional signatures in lymphoid and mucosal sites. *Cell Rep*. (2017) 20:2921–34. doi: 10.1016/j.celrep.2017.08.078
61. Brandtzaeg P. Immunology of tonsils and adenoids: everything the ENT surgeon needs to know. *Int J Pediatr Otorhinolaryngol*. (2003) 67(Suppl 1):S69–76. doi: 10.1016/S0531-5131(03)00964-6
62. Remmerswaal EBM, Klarenbeek PL, Alves NL, Doorenspleet ME, van Schaik BDC, Esveldt REE, et al. Clonal evolution of CD8+ T cell responses against latent viruses: relationship among phenotype, localization, and function. *J Virol*. (2015) 89:568–80. doi: 10.1128/JVI.02003-14
63. Palendira U, Chinn R, Raza W, Piper K, Pratt G, Machado L, et al. Selective accumulation of virus-specific CD8+ T cells with unique homing phenotype within the human bone marrow. *Blood*. (2008) 112:3293–302. doi: 10.1182/blood-2008-02-138040
64. Woon HG, Braun A, Li J, Smith C, Edwards J, Sierro F, et al. Compartmentalization of total and virus-specific tissue-resident memory CD8+ T cells in human lymphoid organs. *PLoS Pathog*. (2016) 12:e1005799. doi: 10.1371/journal.ppat.1005799
65. Matloubian M, Lo CG, Cinamon G, Lesneski MJ, Xu Y, Brinkmann V, et al. Lymphocyte egress from thymus and peripheral lymphoid organs is dependent on S1P receptor 1. *Nature*. (2004) 427:355–60. doi: 10.1038/nature02284
66. Shiow LR, Rosen DB, Brdicková N, Xu Y, An J, Lanier LL, et al. CD69 acts downstream of interferon-alpha/beta to inhibit S1P1 and lymphocyte egress from lymphoid organs. *Nature*. (2006) 440:540–4. doi: 10.1038/nature04606
67. Cibrián D, Sánchez-Madrid F. CD69: from activation marker to metabolic gatekeeper. *Eur J Immunol*. (2017) 47:946–53. doi: 10.1002/eji.201646837
68. Woodberry T, Suscovich TJ, Henry LM, August M, Waring MT, Kaur A, et al. Alpha E beta 7 (CD103) expression identifies a highly active, tonsil-resident effector-memory CTL population. *J Immunol*. (2005) 175:4355–62. doi: 10.4049/jimmunol.175.7.4355
69. Hudnall SD, Ge Y, Wei L, Yang N-P, Wang H-Q, Chen T. Distribution and phenotype of Epstein-Barr virus-infected cells in human pharyngeal tonsils. *Mod Pathol*. (2005) 18:519–27. doi: 10.1038/modpathol.3800369
70. Kurth J, Spieker T, Wustrow J, Strickler GJ, Hansmann LM, Rajewsky K, et al. EBV-infected B cells in infectious mononucleosis: viral strategies for spreading in the B cell compartment and establishing latency. *Immunity*. (2000) 13:485–495.
71. Willcox CR, Davey MS, Willcox BE. Development and selection of the human Vγ9Vδ2+ T-cell repertoire. *Front Immunol*. (2018) 9:1501. doi: 10.3389/fimmu.2018.01501
72. Davey MS, Willcox CR, Baker AT, Hunter S, Willcox BE. Recasting human Vδ1 lymphocytes in an adaptive role. *Trends Immunol*. (2018) 39:446–59. doi: 10.1016/j.it.2018.03.003
73. Khairallah C, Netzer S, Villacreces A, Juzan M, Rousseau B, Dulanto S, et al. γδ T cells confer protection against murine cytomegalovirus (MCMV). *PLoS Pathog*. (2015) 11:e1004702. doi: 10.1371/journal.ppat.1004702
74. Sell S, Dietz M, Schneider A, Holtappels R, Mach M, Winkler TH. Control of murine cytomegalovirus infection by γδ T cells. *PLOS Pathogens*. (2015) 11:e1004481. doi: 10.1371/journal.ppat.1004481
75. Girardi M. Regulation of cutaneous malignancy by gamma delta T cells. *Science*. (2001) 294:605–9. doi: 10.1126/science.1063916
76. Gentles AJ, Newman AM, Liu CL, Bratman SV, Feng W, Kim D, et al. The prognostic landscape of genes and infiltrating immune cells across human cancers. *Nat Med*. (2015) 21:938–45. doi: 10.1038/nm.3909
77. De Paoli P, Gennari D, Martelli P, Cavarzerani V, Comoretto R, Santini G. Gamma delta T cell receptor-bearing lymphocytes during Epstein-Barr virus infection. *J Infect Dis*. (1990) 161:1013–1016. doi: 10.1093/infdis/161.5.1013
78. Hassan J, Feighery C, Bresnihan B, Whelan A. Elevated T cell receptor gamma delta + T cells in patients with infectious mononucleosis. *Br J Haematol*. (1991) 77:255–6. doi: 10.1111/j.1365-2141.1991.tb07990.x
79. Zhong H, Hu X, Janowski AB, Storch GA, Su L, Cao L, et al. Whole transcriptome profiling reveals major cell types in the cellular immune

response against acute and chronic active Epstein-Barr virus infection. *Sci Rep*. (2017) 7:17775. doi: 10.1038/s41598-017-18195-z
80. Farnault L, Gertner-Dardenne J, Gondois-Rey F, Michel G, Chambost H, Hirsch I, et al. Clinical evidence implicating gamma-delta T cells in EBV control following cord blood transplantation. *Bone Marrow Transplant*. (2013) 48:1478–9. doi: 10.1038/bmt.2013.75
81. Fujishima N, Hirokawa M, Fujishima M, Yamashita J, Saitoh H, Ichikawa Y, et al. Skewed T cell receptor repertoire of Vdelta1(+) gammadelta T lymphocytes after human allogeneic haematopoietic stem cell transplantation and the potential role for Epstein-Barr virus-infected B cells in clonal restriction. *Clin Exp Immunol*. (2007) 149:70–9. doi: 10.1111/j.1365-2249.2007.03388.x
82. Zheng BJ, Ng SP, Chua DTT, Sham JST, Kwong DLW, Lam CK, et al. Peripheral gamma delta T-cell deficit in nasopharyngeal carcinoma. *Int J Cancer*. (2002) 99:213–7. doi: 10.1002/ijc.10326
83. Puan KJ, Low JSH, Tan TWK, Wee JTS, Tan EH, Fong KW, et al. Phenotypic and functional alterations of Vgamma2Vdelta2 T cell subsets in patients with active nasopharyngeal carcinoma. *Cancer Immunol Immunother*. (2009) 58:1095–107. doi: 10.1007/s00262-008-0629-8
84. Futagbi G, Welbeck JE, Tetteh JKA, Hviid L, Akanmori BD. Selective activation of TCR-γδ+ cells in endemic Burkitt's lymphoma. *Malar J*. (2007) 6:69. doi: 10.1186/1475-2875-6-69
85. Chan SY-Y, Choy K-W, Tsao S-W, Tao Q, Tang T, Chung GT-Y, et al. Authentication of nasopharyngeal carcinoma tumor lines. *Int J Cancer*. (2008) 122:2169–71. doi: 10.1002/ijc.23374
86. Davey MS, Willcox CR, Hunter S, Kasatskaya SA, Remmerswaal EBM, Salim M, et al. The human Vδ2+ T-cell compartment comprises distinct innate-like Vγ9+ and adaptive Vγ9- subsets. *Nat Commun*. (2018) 9:1760. doi: 10.1038/s41467-018-04076-0
87. Davey MS, Willcox CR, Joyce SP, Ladell K, Kasatskaya SA, McLaren JE, et al. Clonal selection in the human Vδ1 T cell repertoire indicates γδ TCR-dependent adaptive immune surveillance. *Nat Commun*. (2017) 8:14760. doi: 10.1038/ncomms14760
88. Klein E, Klein G, Nadkarni JS, Nadkarni JJ, Wigzell H, Clifford P. Surface IgM-kappa specificity on a Burkitt lymphoma cell *in vivo* and in derived culture lines. *Cancer Res*. (1968) 28:1300–10.
89. Hebbeler AM, Cairo C, Cummings JS, Pauza CD. Individual Vgamma2-Jgamma1.2+ T cells respond to both isopentenyl pyrophosphate and Daudi cell stimulation: generating tumor effectors with low molecular weight phosphoantigens. *Cancer Immunol Immunother*. (2007) 56:819–29. doi: 10.1007/s00262-006-0235-6
90. Fisch P, Oettel K, Fudim N, Surfus JE, Malkovsky M, Sondel PM. MHC-unrestricted cytotoxic and proliferative responses of two distinct human gamma/delta T cell subsets to Daudi cells. *J Immunol*. (1992) 148:2 315–23.
91. Fisch P, Malkovsky M, Kovats S, Sturm E, Braakman E, Klein BS, et al. Recognition by human V gamma 9/V delta 2 T cells of a GroEL homolog on Daudi Burkitt's lymphoma cells. *Science*. (1990) 250:1269–73. doi: 10.1126/science.1978758
92. Fisch P, Malkovsky M, Braakman E, Sturm E, Bolhuis RL, Prieve A, et al. Gamma/delta T cell clones and natural killer cell clones mediate distinct patterns of non-major histocompatibility complex-restricted cytolysis. *J Exp Med*. (1990) 171:1567–79. doi: 10.1084/jem.171.5.1567
93. Davodeau F, Peyrat MA, Hallet MM, Gaschet J, Houde I, Vivien R, et al. Close correlation between Daudi and mycobacterial antigen recognition by human gamma delta T cells and expression of V9JPC1 gamma/V2DJC delta-encoded T cell receptors. *J Immunol*. (1993) 151: 1214–23.
94. Kaur I, de Jong J, Schell K, Hank J, Fisch P, Sondel PM. Human peripheral gamma delta T cells are stimulated by Daudi Burkitt's lymphoma and not by any other Burkitt's lymphoma tested. *Cell Immunol*. (1994) 156:54–61. doi: 10.1006/cimm.1994.1152
95. Kaur I, Voss SD, Gupta RS, Schell K, Fisch P, Sondel PM. Human peripheral gamma delta T cells recognize hsp60 molecules on Daudi Burkitt's lymphoma cells. *J Immunol*. (1993) 150:2046–55.
96. Gober H-J, Kistowska M, Angman L, Jenö P, Mori L, De Libero G. Human T cell receptor gammadelta cells recognize endogenous mevalonate metabolites in tumor cells. *J Exp Med*. (2003) 197:163–8. doi: 10.1084/jem.20021500
97. Fayen JD, Tykocinski ML. The expansion of human gammadelta T cells in response to Daudi cells requires the participation of CD4+ T cells. *Immunology*. (1999) 97:272–9. doi: 10.1046/j.1365-2567.1999.00761.x
98. Sturm E, Braakman E, Fisch P, Vreugdenhil RJ, Sondel P, Bolhuis RL. Human V gamma 9-V delta 2 T cell receptor-gamma delta lymphocytes show specificity to Daudi Burkitt's lymphoma cells. *J Immunol*. (1990) 145:3202–8.
99. Djaoud Z, Guethlein LA, Horowitz A, Azzi T, Nemat-Gorgani N, Olive D, et al. Two alternate strategies for innate immunity to Epstein-Barr virus: One using NK cells and the other NK cells and γδ T cells. *J Exp Med*. (2017) 214:1827–41. doi: 10.1084/jem.20161017
100. Morita CT, Jin C, Sarikonda G, Wang H. Nonpeptide antigens, presentation mechanisms, and immunological memory of human Vgamma2Vdelta2 T cells: discriminating friend from foe through the recognition of prenyl pyrophosphate antigens. *Immunol Rev*. (2007) 215:59–76. doi: 10.1111/j.1600-065X.2006.00479.x
101. Sandstrom A, Peigné C-M, Léger A, Crooks JE, Konczak F, Gesnel M-C, et al. The intracellular B30.2 domain of butyrophilin 3A1 binds phosphoantigens to mediate activation of human Vγ9Vδ2 T cells. *Immunity*. (2014) 40:490–500. doi: 10.1016/j.immuni.2014.03.003
102. Kong Y, Cao W, Xi X, Ma C, Cui L, He W. The NKG2D ligand ULBP4 binds to TCRgamma9/delta2 and induces cytotoxicity to tumor cells through both TCRgammadelta and NKG2D. *Blood*. (2009) 114:310–7. doi: 10.1182/blood-2008-12-196287
103. Zheng B-J, Chan K-W, Im S, Chua D, Sham JST, Tin P-C, et al. Anti-tumor effects of human peripheral γδ T cells in a mouse tumor model. *Int J Cancer*. (2001) 92:421–5. doi: 10.1002/ijc.1198
104. Münz C. Humanized mouse models for Epstein Barr virus infection. *Curr Opin Virol*. (2017) 25:113–8. doi: 10.1016/j.coviro.2017.07.026
105. Malkovska V, Cigel F, Storer BE. Human T cells in hu-PBL-SCID mice proliferate in response to Daudi lymphoma and confer anti-tumour immunity. *Clini Exp Immunol*. (1994) 96:158–65. doi: 10.1111/j.1365-2249.1994.tb06246.x
106. Xiang Z, Liu Y, Zheng J, Liu M, Lv A, Gao Y, et al. Targeted activation of human Vγ9Vδ2-T cells controls Epstein-Barr virus-induced B cell lymphoproliferative disease. *Cancer Cell*. (2014) 26:565–76. doi: 10.1016/j.ccr.2014.07.026
107. Hoeres T, Smetak M, Pretscher D, Wilhelm M. Improving the efficiency of Vγ9Vδ2 T-cell immunotherapy in cancer. *Front Immunol*. (2018) 9:800: doi: 10.3389/fimmu.2018.00800
108. Zumwalde NA, Sharma A, Xu X, Ma S, Schneider CL, Romero-Masters JC, et al. Adoptively transferred Vγ9Vδ2 T cells show potent antitumor effects in a preclinical B cell lymphomagenesis model. *JCI Insight*. (2017) 2:e93179. doi: 10.1172/jci.insight.93179
109. Häcker G, Kromer S, Falk M, Heeg K, Wagner H, Pfeffer K. V delta 1+ subset of human gamma delta T cells responds to ligands expressed by EBV-infected Burkitt lymphoma cells and transformed B lymphocytes. *J Immunol*. (1992) 149:3984–9.
110. Orsini DLM, Res PCM, Laar JMV, Muller LM, Soprano AEL, Kooy YMC, et al. A subset of Vδ1+ T cells proliferates in response to Epstein-Barr virus-transformed B cell lines *in vitro*. *Scand J Immunol*. (1993) 38:335–40. doi: 10.1111/j.1365-3083.1993.tb01735.x
111. Orsini DL, van Gils M, Kooy YM, Struyk L, Klein G, van den Elsen P, et al. Functional and molecular characterization of B cell-responsive V delta 1+ gamma delta T cells. *Eur J Immunol*. (1994) 24:3199–204. doi: 10.1002/eji.1830241243
112. Wolf BJ, Choi JE, Exle MA. Novel approaches to exploiting invariant NKT cells in cancer immunotherapy. *Front. Immunol.*. (2018) 9:384. doi: 10.3389/fimmu.2018.00384
113. Williams H, McAulay K, Macsween KF, Gallacher NJ, Higgins CD, Harrison N, et al. The immune response to primary EBV infection: a role for natural killer cells.. (2005) *Br J Haematol*. 129:266–74. doi: 10.1111/j.1365-2141.2005.05452.x
114. Yuling H, Ruijing X, Li L, Xiang J, Rui Z, Yujuan W, et al. EBV-induced human CD8+ NKT cells suppress tumorigenesis by EBV-associated malignancies.. (2009) *Cancer Res*. (2009) 69:7935–44. doi: 10.1158/0008-5472.CAN-09-0828
115. Tangye SG, Palendira U, Edwards ES. Human immunity against EBV—lessons from the clinic. *J Exp Med*. (2017) 214:269. doi: 10.1084/jem.20161846

116. Chung BK, Tsai K, Allan LL, Zheng DJ, Nie JC, Biggs CM, et al. Innate immune control of EBV-infected B cells by invariant natural killer T cells. *Blood*. (2013) 122:2600–8. doi: 10.1182/blood-2013-01-480665
117. Sanchez DJ, Gumperz JE, Ganem D. Regulation of CD1d expression and function by a herpesvirus infection. *J Clin Invest*. (2005) 115:1369–78. doi: 10.1172/JCI24041
118. Yuan W, Dasgupta A, Cresswell P. Herpes simplex virus evades natural killer T cell recognition by suppressing CD1d recycling. *Nat Immunol*. (2006) 7:835–42. doi: 10.1038/ni1364

Conflict of Interest Statement: The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

Advantages of publishing in Frontiers

OPEN ACCESS
Articles are free to read for greatest visibility and readership

FAST PUBLICATION
Around 90 days from submission to decision

HIGH QUALITY PEER-REVIEW
Rigorous, collaborative, and constructive peer-review

TRANSPARENT PEER-REVIEW
Editors and reviewers acknowledged by name on published articles

Frontiers
Avenue du Tribunal-Fédéral 34
1005 Lausanne | Switzerland

Visit us: www.frontiersin.org
Contact us: info@frontiersin.org | +41 21 510 17 00

REPRODUCIBILITY OF RESEARCH
Support open data and methods to enhance research reproducibility

DIGITAL PUBLISHING
Articles designed for optimal readership across devices

FOLLOW US
@frontiersin

IMPACT METRICS
Advanced article metrics track visibility across digital media

EXTENSIVE PROMOTION
Marketing and promotion of impactful research

LOOP RESEARCH NETWORK
Our network increases your article's readership