NEUTROPHIL-MEDIATED SKIN DISEASES: IMMUNOLOGY AND GENETICS

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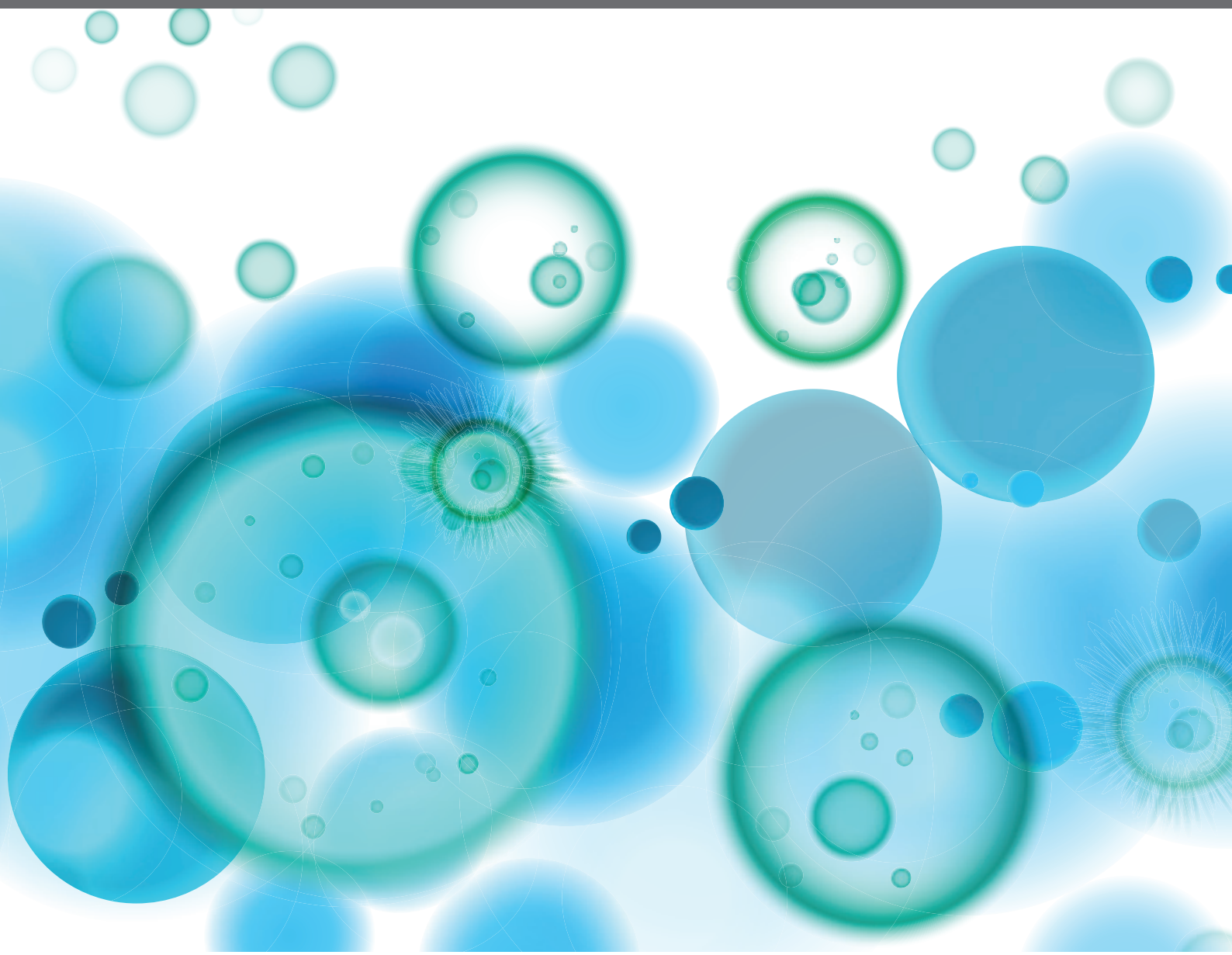

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NEUTROPHIL-MEDIATED SKIN DISEASES: IMMUNOLOGY AND GENETICS

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EDITORIAL
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Editorial: Neutrophil-Mediated Skin Diseases: Immunology and Genetics

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Keywords: neutrophil, skin—immunology, innate immunity, genetics, neutrophilic dermatoses (NDs), autoinflammation

Editorial on the Research Topic

Neutrophil-Mediated Skin Diseases: Immunology and Genetics

Neutrophils are involved in the effector phase of the host defense against micro-organisms and have a major role in the innate immune response but they also act in the modulation of the adaptive immunity as well as in orchestrating the response of the immune system to other triggers such as severe injury and trauma (Mortaz et al.). The deregulation of neutrophil function and their hyperactivity can lead to inflammation and tissue damage as seen in neutrophilic dermatoses that are a group of diseases due to accumulation of neutrophils in the skin and less frequently in internal organs (Marzano et al.). The systemic involvement, which may be sometimes severe, has led to coining the term "neutrophilic diseases." The prototype of neutrophilic diseases are pyoderma gangrenosum and Sweet's syndrome (Marzano et al.; Heath and Ortega-Loayza) but some authors suggest to include hidradenitis suppurativa as well (Tricarico et al.; Vossen et al.; Frew), though there is no full agreement on this point; for all these entities an important autoinflammatory component has been demonstrated in their pathogenesis. Moreover, the spectrum of neutrophilic diseases is broad; it comprises truly systemic diseases such as Behçet's disease (Leccese and Alpsoy), but also psoriasis where neutrophils play an important role in the pathophysiology (Le et al.; Wannick et al.) or the inflammatory immunological response of leprosy (Schmitz et al.). The present issue is focused on the interplay between immunology and genetics in neutrophil-mediated diseases, highlighting the close links with the group of autoinflammatory diseases. The latter are characterized by recurrent episodes of sterile inflammation in the affected organs with neutrophils involved as leading cells, and are due to mutations in genes regulating the innate immunity. The recognition of several monogenic diseases which can present with neutrophilic skin diseases, such as CAPS (cryopyrin-associated periodic syndromes), DIRA (deficiency of IL-1 receptor antagonist), DITRA (deficiency of IL-36 receptor antagonist), and PAPA (pyogenic sterile arthritis, pyoderma gangrenosum, acne), has led to an improved understanding of the possible mechanisms of polygenic non-mendelian inherited neutrophilic skin diseases (Marzano et al.; Heath and Ortega-Loayza; Tricarico et al.; Vossen et al.). An increasing body of evidence supports the role of pro-inflammatory cytokines like interleukin (IL)-1-beta, IL-17, and tumor necrosis factor (TNF)-alpha in the pathophysiology of neutrophilic diseases similarly to classic monogenic autoinflammatory diseases, suggesting common physiopathological mechanisms.

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Moreover, mutations of several genes involved in autoinflammatory diseases are likely to play a role in the pathogenesis of sporadic neutrophilic diseases, giving rise to regarding them as a spectrum of polygenic autoinflammatory conditions (Marzano et al.; Heath and Ortega-Loayza; Tricarico et al.; Vossen et al.). Indeed, mutations of *PSTPIP1* (proline-serine-threonine phosphatase interacting protein 1), the gene involved in PAPA, as well as of a number of other genes involved in classic autoinflammatory diseases have been demonstrated in both isolated and syndromic forms of pyoderma gangrenosum, whose prototype is PASH (pyoderma gangrenosum, acne, suppurative hidradenitis), as well as in neutrophilic diseases in general. At present, classic regimens such as systemic glucocorticosteroids and immunosuppressants are the mainstay of treatment while biologic drugs are reserved for refractory cases. We can thus hope that the precise elucidation of the immunology and genetics of neutrophil-mediated diseases will pave the way to pathogenesis-driven treatments and the development of new drugs specifically targeting the inflammatory pathways involved in those entities.

AUTHOR CONTRIBUTIONS

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

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ORIGINAL RESEARCH
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The Immunometabolomic Interface Receptor Hydroxycarboxylic Acid Receptor 2 Mediates the Therapeutic Effects of Dimethyl Fumarate in Autoantibody-Induced Skin Inflammation

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The drug dimethyl fumarate (DMF) is in clinical use for the treatment of psoriasis and multiple sclerosis. In addition, it has recently been demonstrated to ameliorate skin pathology in mouse models of pemphigoid diseases, a group of autoimmune blistering diseases of the skin and mucous membranes. However, the mode of action of DMF in inflammatory skin diseases has remained elusive. Therefore, we have investigated here the mechanisms by which DMF improves skin pathology, using the antibody transfer model of bullous pemphigoid-like epidermolysis bullosa acquisita (EBA). Experimental EBA was induced by transfer of antibodies against collagen VII that triggered the infiltration of immune cells into the skin and led to inflammatory skin lesions. DMF treatment reduced the infiltration of neutrophils and monocytes into the skin explaining the improved disease outcome in DMF-treated animals. Upon ingestion, DMF is converted to monomethyl fumarate that activates the hydroxycarboxylic acid receptor 2 (HCA_2). Interestingly, neutrophils and monocytes expressed *Hca2*. To investigate whether the therapeutic effect of DMF in EBA is mediated by HCA_2, we administered oral DMF to *Hca2*-deficient mice (*Hca2*$^{-/-}$) and wild-type littermates (*Hca2*$^{+/+}$) and induced EBA. DMF treatment ameliorated skin lesions in *Hca2*$^{+/+}$ but not in *Hca2*$^{-/-}$ animals. These findings demonstrate that HCA_2 is a molecular target of DMF treatment in EBA and suggest that HCA_2 activation limits skin pathology by inhibiting the infiltration of neutrophils and monocytes into the skin.

Keywords: pemphigoid disease, G protein-coupled receptor, immunomodulatory therapy, autoimmune blistering skin disease, neutrophils

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INTRODUCTION

Dimethyl fumarate (DMF) is an oral, immunomodulatory drug licensed for the treatment of multiple sclerosis (MS) and for moderate-to-severe plaque psoriasis. Upon oral ingestion, DMF is converted in the gut to monomethyl fumarate (MMF), which is the active principle of oral DMF treatment (1). Because of its overall favorable safety profile and its high efficacy, DMF has substantially improved the treatment of both MS and plaque psoriasis and has become a mainstay in the treatment of

both diseases (2, 3). The mode of action of DMF in both plaque psoriasis and MS is only poorly understood.

Diverse biochemical actions of DMF have been uncovered, indicating that DMF may exert multiple immunomodulatory effects possibly contributing to its therapeutic effects. Among others, MMF was demonstrated to covalently modify cysteinyl residues of proteins by addition of a 2-monomethyl succinyl group, thereby activating the antioxidant nuclear factor erythroid 2-related factor 2 (NRF2) and inhibiting the glycolytic enzyme glyceraldehyde 3-phosphate dehydrogenase (GAPDH) (4, 5). In addition, MMF is an agonist of the G protein-coupled receptor hydroxycarboxylic acid receptor 2 (HCA_2/GPR109A) (6). Recently, the agonism of MMF at HCA_2 has been revealed to contribute to its therapeutic effects in murine experimental autoimmune encephalitis (EAE), a model for MS (7). In this study, oral DMF treatment reduced the number of infiltrating neutrophils in the spinal cord, and MMF impaired the migration and adhesion of neutrophils in a HCA_2-dependent manner, indicating that the therapeutic effect of DMF in MS may be partially due to an inhibition of neutrophil recruitment into the CNS. The latter is a mechanism that has recently been suggested to be a key process in EAE and MS (8–11). HCA_2 is expressed on neutrophils, monocytes, macrophages, and Langerhans cells (12). Its natural ligands are butyrate, hydroxy butyrate, and nicotinic acid. Thus, it belongs to the group of G protein-coupled receptors for short chain fatty acids, which have been uncovered to modify the course of disease of several autoimmune, autoinflammatory, and allergic diseases (13).

Pemphigoid diseases are a group of autoimmune blistering skin diseases caused by autoantibody formation against different proteins at the dermal–epidermal junction and the consequent recruitment of neutrophils into the skin (14). A recent study showed that DMF is beneficial in a preclinical model of bullous pemphigoid-like epidermolysis bullosa acquisita (EBA) (15), a variant of pemphigoid disease caused by autoantibodies directed to type VII collagen in the dermal–epidermal adhesion complex (14). This finding has led to a currently running clinical trial examining the efficacy of DMF in the most common pemphigoid disease bullous pemphigoid. However, the mode of action of DMF in pemphigoid diseases has remained elusive. Therefore, we have investigated here the contribution of HCA_2 activation to the therapeutic effects of DMF in the antibody transfer mouse model of EBA ("experimental EBA"). Our study confirms the therapeutic effect of DMF in that model. Furthermore, we reveal that this therapeutic effect largely depends on the activation of HCA_2, thus, highlighting HCA_2 activation as new potential therapeutic principle in the treatment of pemphigoid diseases.

RESULTS

DMF Reduces the Antibody-Induced Inflammatory Cell Infiltration in Experimental EBA

To investigate the mode of action of DMF in inflammatory skin diseases, we induced EBA by transferring anti-collagen VII antibodies to mice. This mouse model reflects specifically the effector phase of the autoantibody-mediated skin disease. First, we set out to reproduce the protective effect of DMF that had been reported previously (15). We administered vehicle or DMF (50 mg/kg, twice daily, p.o.) to C57BL/6 mice starting 2 days prior to EBA induction. DMF significantly inhibited the precipitation of inflammatory skin lesions, thus, reducing disease severity at its peak by approximately 60% (**Figures 1A,B**).

To uncover the mode of action of DMF in EBA, we next characterized its effects on immune cell numbers in the lesional skin, peripheral blood, and lymphoid tissues by flow cytometry. In this profiling, we distinguished neutrophils as $CD45^+CD11b^+Ly6G^+$ cells and monocyte-derived cells as $CD45^+CD11b^+Ly6C^+$ cells. The latter are a heterogeneous population in skin comprised of monocyte-derived Langerhans cells, dendritic cells, and macrophages (16, 17). At disease onset (day 5 after EBA induction), the relative numbers of neutrophils and $CD11b^+Ly6C^{Lo}$ monocyte-derived cells were similar in the skin of vehicle- and DMF-treated mice (**Figures 1C,D**). However, at a more advanced disease stage (day 11 after EBA induction), DMF treatment diminished the relative number of neutrophils. In addition, the lesional skin of DMF-treated animals showed a trend toward lower relative numbers of $CD11b^+Ly6C^{Lo}$ monocyte-derived cells at day 11 after the first antibody transfer (**Figures 1C,D**). In blood, neither neutrophils nor $CD11b^+Ly6C^{Lo}$ monocytes were affected by DMF treatment (Figures S1 and S2 in Supplementary Material). While DMF treatment had no significant effect on relative numbers of $CD11b^+Ly6C^{Hi}$ monocytes in the peripheral blood and of $CD11b^+Ly6C^{Hi}$ monocyte-derived cells in the skin, it reduced $CD11b^+Ly6C^{Hi}$ monocytes in the spleen and in lymph nodes by day 11 after the induction of EBA (**Figures 2A,C**; Figure S2 in Supplementary Material). The relative numbers of $CD11b^+Ly6C^{Lo}$ monocytes and neutrophils were not affected by DMF treatment in lymphoid tissue (**Figures 2B,D**; Figure S1 in Supplementary Material).

In contrast to the partial reduction of myeloid cells, the relative number of CD3-$NK1.1^+$ natural killer cells that could not be detected in skin was unchanged upon EBA induction and not affected by DMF treatment in blood and lymphoid tissue (Figure S3 in Supplementary Material). Regarding the lymphoid cells, the relative numbers of $CD45^+\gamma\delta TCR^+$ T cells in the skin was higher in DMF-treated than in vehicle-treated animals on day 11 after EBA induction (**Figure 1E**). In secondary lymphoid tissues and in blood, DMF treatment had no effect on γδT cells (Figure S4 in Supplementary Material). Interestingly, the relative number of $CD3^+$ $\gamma\delta TCR^-$ αβT cells was increased in DMF-treated mice in spleen, but not in lymph nodes, blood, and skin (Figure S5 in Supplementary Material). The increased numbers of αβT cells in spleen could represent regulatory T cells that were shown to dampen disease progression in EBA (18). Overall, the data indicate that DMF modulates the numbers of neutrophils, $CD11b^+Ly6C^{Lo}$ monocyte-derived cells, and γδT cells in the skin.

DMF Treatment Increases CD62L Levels on Neutrophils and $CD11b^+Ly6C^{Lo}$ Monocytes

Having established that DMF treatment decreases the numbers of neutrophils and $CD11b^+Ly6C^{Lo}$ monocyte-derived cells in

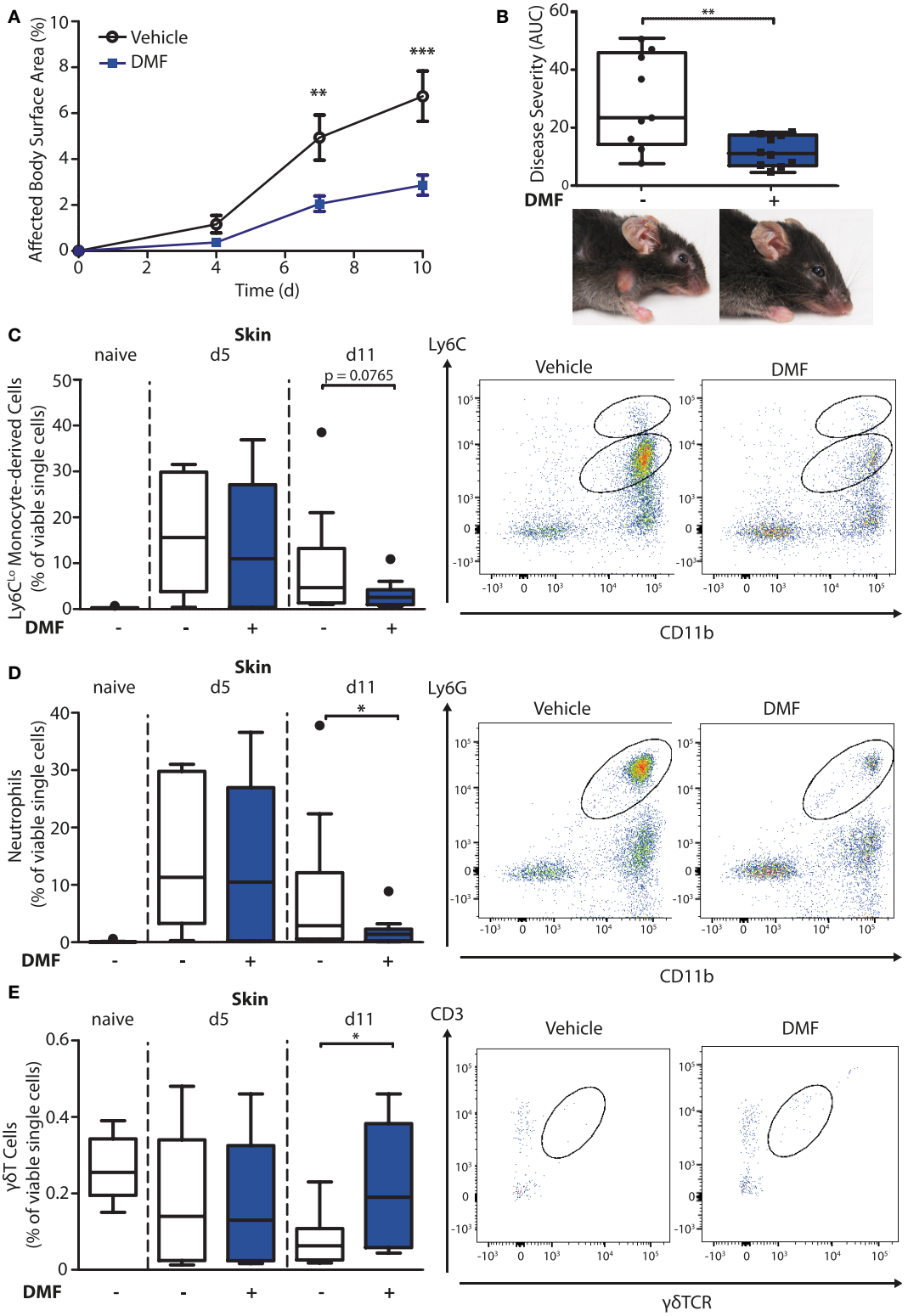

FIGURE 1 | Dimethyl fumarate (DMF) treatment diminishes disease severity of experimental epidermolysis bullosa acquisita (EBA) by reducing elevated numbers of pro-inflammatory cells in the skin. **(A)** Clinical course of antibody transfer EBA in wild-type mice that were treated with DMF (50 mg/kg, p.o., twice per day) or vehicle. Two-way ANOVA, $F(1/51) = 8.85$, $^{**}p < 0.01$; $^{***}p < 0.001$ ($n = 9$–14 mice, Bonferroni *post hoc* test). **(B)** Disease severity, calculated as area under the curve of the data in **(A)**, and clinical presentation. $^{**}p < 0.01$ (Mann–Whitney test). **(C–E)** Quantification of immune cell populations in ear skin of naive mice or of animals on day 5 (d5) and 11 (d11) after first antibody injection. The numbers of cells are shown in percent of viable cells for **(C)** $CD45^+CD11b^+Ly6C^{Lo}$ monocytes, **(D)** $CD45^+CD11b^+Ly6G^+$ neutrophils, and **(E)** $CD45^+CD3^+\gamma\delta TCR^+$ γδT cells. Representative dot plots of day 11 are shown. $^*p < 0.05$ ($n = 9$–14 mice, Mann–Whitney test).

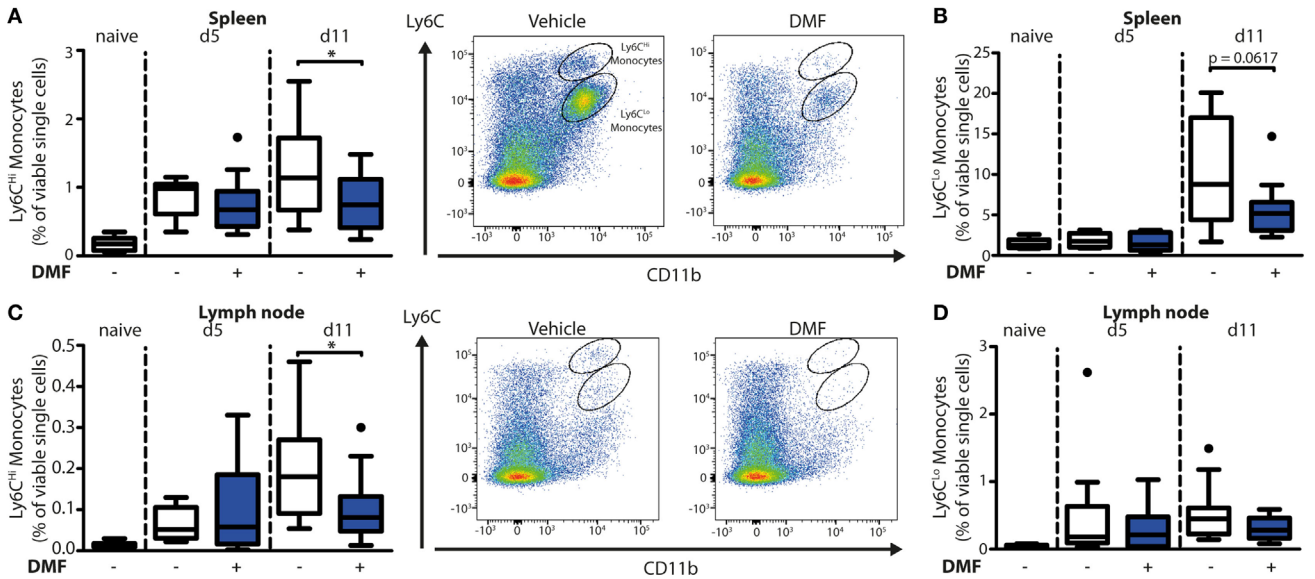

FIGURE 2 | Dimethyl fumarate (DMF) treatment of mice inhibits trafficking of Ly6C^{Hi} monocytes to secondary lymphoid tissues. Quantification of CD45$^+$CD11b$^+$Ly6C^{Hi} and CD45$^+$CD11b$^+$Ly6C^{Lo} monocyte populations in spleen **(A,B)** and lymph nodes **(C,D)** of naive mice or of animals on day 5 (d5) and 11 (d11) after first antibody injection. The animals received either vehicle or DMF (50 mg/kg, p.o., twice per day). The numbers of cells are shown in percent of viable single cells. Representative dot plots of day 11 are shown. *$p < 0.05$ (n = 9–14 mice, Mann–Whitney test).

skin lesions, we addressed whether these effects may be due to an inhibition of recruitment into the skin. Non-activated neutrophils and monocytes express high levels of CD62L (L-selectin) on their surface that is cleaved during activation and trans-endothelial migration. Consequently, low levels of CD62L are a marker of activated or migrating cells (19). Therefore, we determined the CD62L levels on immune cells in the blood on day 11 of the EBA model by flow cytometry. Neutrophils and CD11b$^+$Ly6C^{Lo} monocytes but not CD11b$^+$Ly6C^{Hi} monocytes (**Figure 3**) expressed higher levels of CD62L, thus being not activated or transmigrating, upon DMF treatment. This indicates that DMF lowers the infiltration of neutrophils and CD11b$^+$Ly6C^{Lo} monocytes and ameliorates EBA pathology by impairing the trans-endothelial migration. Furthermore, CD62L is essential for migration through high endothelial venules of secondary lymphoid organs (20). Indeed, upon DMF treatment, neutrophils and monocytes that entered lymphoid tissue had similar level of CD62L (Figure S6 in Supplementary Material).

Experimental EBA Increases HCA$_2$ Expression in the Blood and the Skin

To determine whether DMF may exert its therapeutic effects in EBA through activation of HCA$_2$ on infiltrating cell populations, we profiled the spatiotemporal dynamics of *Hca2* expression in the peripheral blood and in the skin in the course of experimental EBA. For this purpose, we employed the *Hca2*^{mRFP} (*Gpr109a*^{mRFP}) reporter mouse line, in which the *Hca2* locus directs the expression of the monomeric red fluorescent protein (mRFP) (21), and assayed mRFP expression by FACS. This approach revealed that significantly more immune cells in blood were mRFP$^+$ after EBA induction than in naïve mice (**Figure 4A**). In parallel, the relative numbers of mRFP$^+$ cells increased in the skin upon induction of EBA (**Figure 4B**). Nearly all neutrophils and CD11b$^+$Ly6C^{Lo} monocytes expressed the receptor, whereas only 5–20% of CD11b$^+$Ly6C^{Hi} monocytes were mRFP$^+$ (**Figure 4C**). Among T cells, we detected a small subpopulation of γδT cells that were mRFP$^+$, thus, providing a possible explanation for their responsiveness to DMF treatment. The increase of mRFP$^+$ cells in the blood and the skin in response to EBA induction is probably due to a rise of mRFP$^+$ neutrophils and CD11b$^+$Ly6C^{Lo} monocytes in blood (Figure S1 in Supplementary Material) and their infiltration into skin lesions (22). The percentage of mRFP$^+$ cells among immune cells in the blood and the skin remained stable even under DMF treatment (**Figures 4A,B**).

The Therapeutic Effect of DMF in EBA Is HCA$_2$-Dependent

After oral ingestion, DMF is converted to MMF that activates HCA$_2$ (6). The finding that HCA$_2$ is expressed by neutrophils and CD11b$^+$Ly6C^{Lo} monocyte-derived cells that respond to oral DMF treatment (**Figure 1**) is compatible with the idea that the receptor is required for the therapeutic efficacy of DMF. To directly test this concept, we investigated whether the therapeutic effect of DMF in EBA depends on HCA$_2$. For this purpose, we induced EBA in *Hca2*$^{-/-}$ mice and analyzed the course of disease in comparison to *Hca2*$^{+/+}$ littermates. While DMF treatment again reduced skin lesions in *Hca2*$^{+/+}$ mice throughout the entire period of observation (area under the curve, AUC) and on individual days, it lacked a therapeutic effect in *Hca2*$^{-/-}$ littermates (**Figures 5A–D**). Statistical analysis revealed that the effect of DMF depended on HCA$_2$ expression.

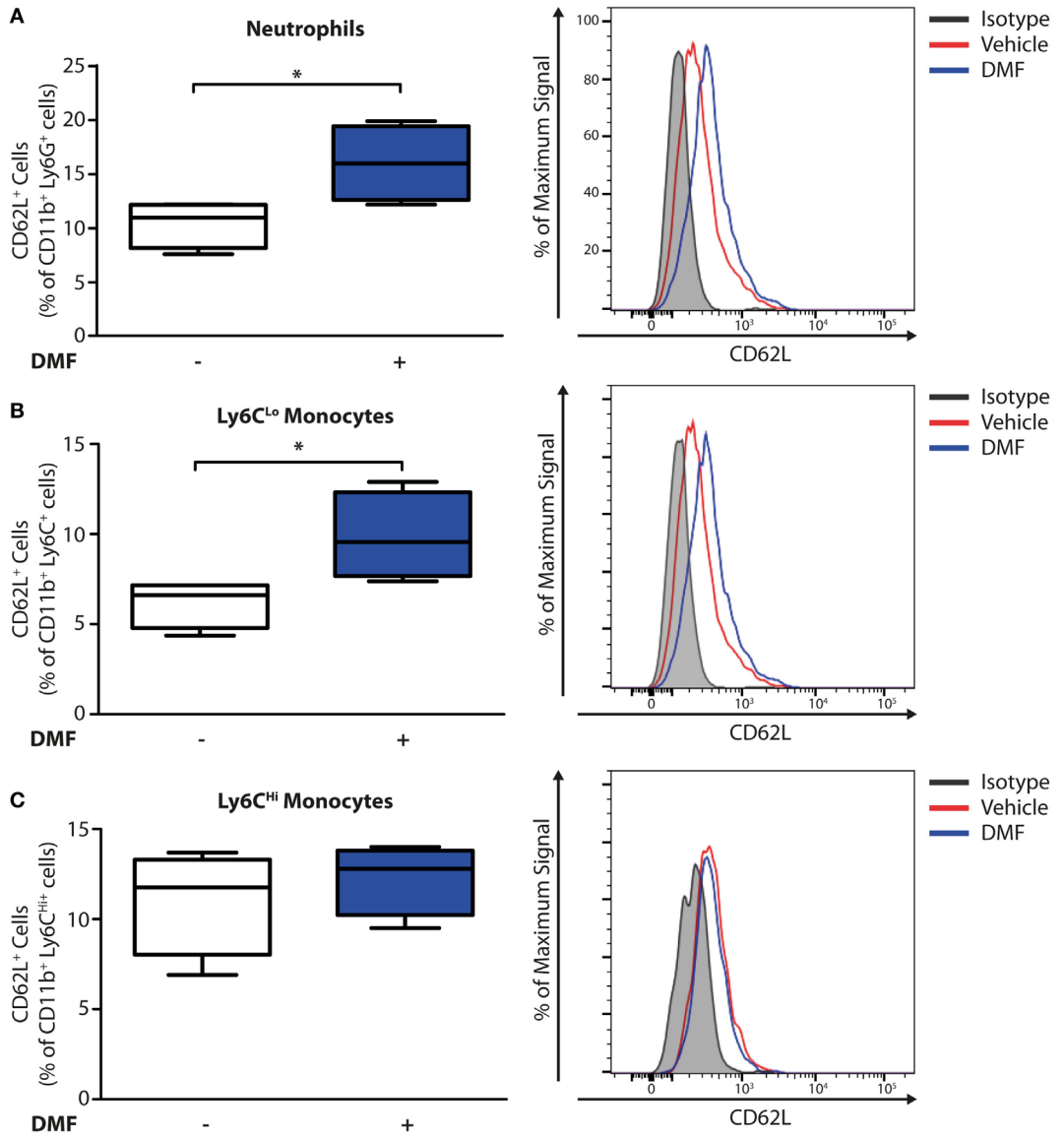

FIGURE 3 | Dimethyl fumarate (DMF) treatment of mice inhibits activation of blood neutrophils and Ly6C^{Lo} monocytes. Quantification of CD62L on immune cell populations in blood of wild-type mice on day 11 after first antibody injection. CD62L levels are decreased during activation and tissue infiltration. The numbers of CD62L$^+$ cells are shown in percent of **(A)** CD45$^+$CD11b$^+$Ly6G$^+$ neutrophils, **(B)** CD45$^+$CD11b$^+$Ly6C^{Lo} monocytes, and **(C)** CD45$^+$CD11b$^+$Ly6C^{Hi} monocytes. Representative histograms show isotype controls (gray) as well as anti-CD62L stained samples of vehicle- (red) and DMF-treated mice (blue). *$p < 0.05$ ($n = 4$ mice, Mann–Whitney test).

Interestingly, we also found that the severity of skin inflammation was significantly reduced in vehicle control-treated *Hca2*$^{-/-}$ mice compared to their vehicle-treated *Hca2*$^{+/+}$ littermates when compared as AUC of diseases activity (**Figure 5C**), suggesting that long-term HCA_2 deficiency could have an additional effect. However, at the end of the experiment (day 16 after EBA induction) the clinical score was similar between the genotypes in vehicle-treated groups (**Figure 5D**).

In fully developed lesions, histopathological inspection showed thickening of the inflamed epidermis and split formation in all groups (**Figure 5E**). Moreover, depositions of the injected anti-collagen VII-IgG and activated C3 factor of the complement cascade could be detected at the dermal–epidermal junction in all experimental groups providing evidence for the successful EBA induction (**Figure 5F**; Figure S7 in Supplementary Material).

In addition to activating HCA_2, DMF and its metabolite MMF stimulate the anti-oxidative transcription factor NRF2 (4). In lesional skin of *Hca2*$^{+/+}$ and *Hca2*$^{-/-}$ mice, DMF treatment increased the expression of *Nqo*1, a known target gene of NRF2 (Figure S8 in Supplementary Material), excluding the possibility that the lack of efficacy of DMF in *Hca2*$^{-/-}$ mice is due to differences in the tissue distribution of the active agent.

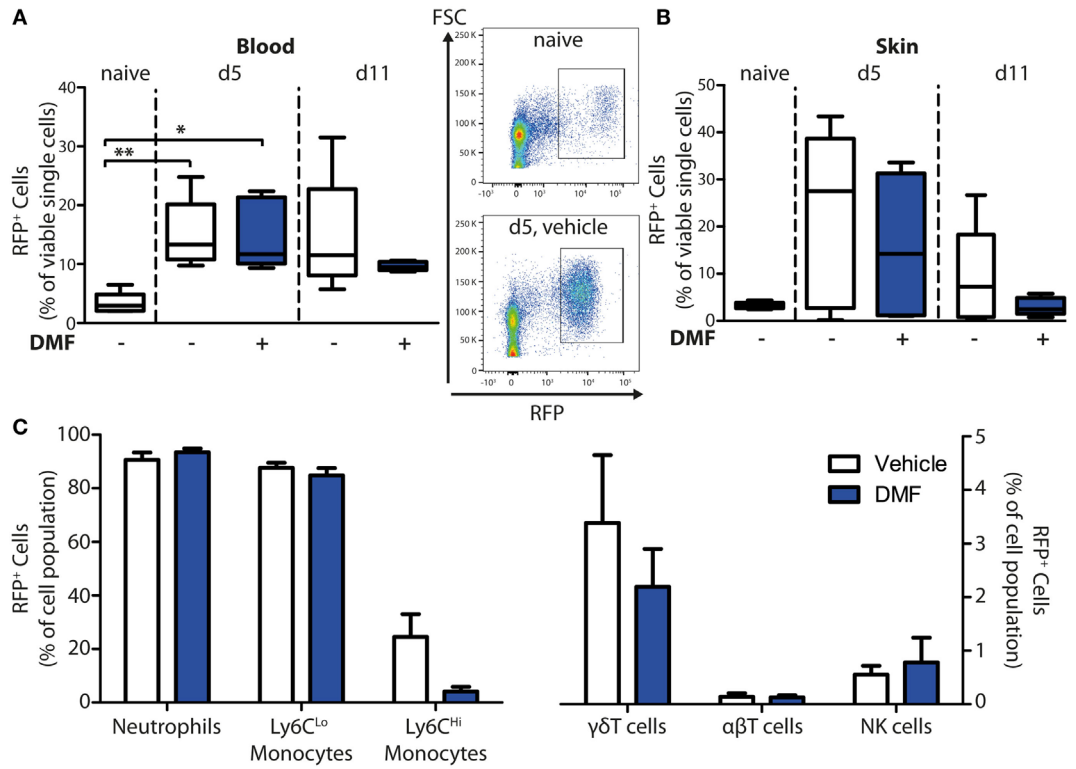

FIGURE 4 | HCA_2 expression increases in blood and skin upon induction of experimental epidermolysis bullosa acquisita. Quantification of monomeric red fluorescent protein (mRFP)$^+$ cells in blood and ear skin of *Hca2*^{mRFP} mice. The numbers of RFP$^+$ cells are shown in percent of viable cells for **(A)** blood and **(B)** skin of naive mice or of animals on day 5 (d5) and 11 (d11) after first antibody injection. Mice were treated with vehicle of dimethyl fumarate (DMF) (50 mg/kg, p.o., twice per day). *$p < 0.05$; **$p < 0.01$ ($n = 5$ mice, Kruskal–Wallis Test with Dunn's *post hoc* test). **(A)** Representative dot plots from naïve mice and animals at d5 (vehicle-treated group) are shown. **(C)** Quantification of mRFP$^+$ cells in immune cell populations of DMF- and vehicle-treated mice at d11. The numbers of mRFP$^+$ cells are expressed as percent of CD45$^+$CD11b$^+$Ly6G$^+$ neutrophils, CD45$^+$CD11b$^+$Ly6C^{Lo} monocytes, CD45$^+$CD11b$^+$Ly6C^{Hi} monocytes, CD45$^+$CD3$^+$γδTCR$^+$ γδT cells, CD45$^+$CD3$^+$γδTCR$^-$ αβT cells, and CD45$^+$CD3$^-$NK1.1$^+$ NK cells. Means ± SEM are depicted ($n = 5$ mice).

DISCUSSION

In this study, we have addressed the mode of action of DMF in pemphigoid diseases, a group of prototypical organ-specific, autoantibody- and neutrophil-driven disorders (14). Using the antibody transfer EBA mouse model, a bullous pemphigoid-like disease, we first confirmed the therapeutic effect of DMF in experimental pemphigoid diseases and then profiled the therapeutic effect of DMF in these diseases on the cellular and molecular level. On the cellular level, DMF treatment curbed the infiltration of the skin with neutrophils and monocytes. On the molecular level, we show that HCA_2 is required for the therapeutic effect of DMF in experimental EBA. Upon DMF ingestion, HCA_2 is activated by MMF, the active metabolite of DMF (6, 21). Our data corroborate previous reports that neutrophils and CD11b$^+$Ly6c^{Lo} monocyte-derived cells express *Hca2* (23, 24). Interestingly, the numbers of these two cell populations in EBA skin lesions were reduced by DMF treatment. Apparently, this is due to a lower infiltration into the diseased skin because DMF treatment reduced the cleavage of CD62L that occurs during tissue infiltration of blood neutrophils and CD11b$^+$Ly6C^{Lo} monocytes. Indeed, by activating HCA_2 MMF is able to inhibit the adhesion and migration of neutrophils (7).

Could the inhibition of neutrophil and monocyte infiltration into the diseased skin be the mode of action by which DMF ameliorates EBA manifestations? In EBA and pemphigoid disease autoantibodies bind to the dermal–epidermal junction and trigger a complement activation that leads to the infiltration of neutrophils and monocytes (14, 25). Specifically, the complement factor C5a stimulates release of leukotriene B4 that seems to be a key chemoattractant of neutrophils in EBA (22). Ablating neutrophils or reducing leukotriene B4 synthesis ameliorated skin lesions in EBA. After infiltrating the skin, neutrophils release reactive oxygen species and seem to degrade the adhesion between dermis and epidermis (15, 26). Evidence for a functional role of monocyte-derived cells in EBA is still more circumstantial. Comparing the effect of two antibodies that deplete monocytes and neutrophils (anti-Ly6C/G) or only neutrophils (anti-Ly6G) suggests that the depletion of monocytes has an additional beneficial effect on experimental EBA (22, 26). Moreover, monocytes are able to execute subepidermal cleft formation in an *in vitro* model of the disease (25, 27). The roles of neutrophils and monocytes in pemphigoid diseases suggest that therapeutic principles targeting, like DMF, the recruitment of both neutrophils and

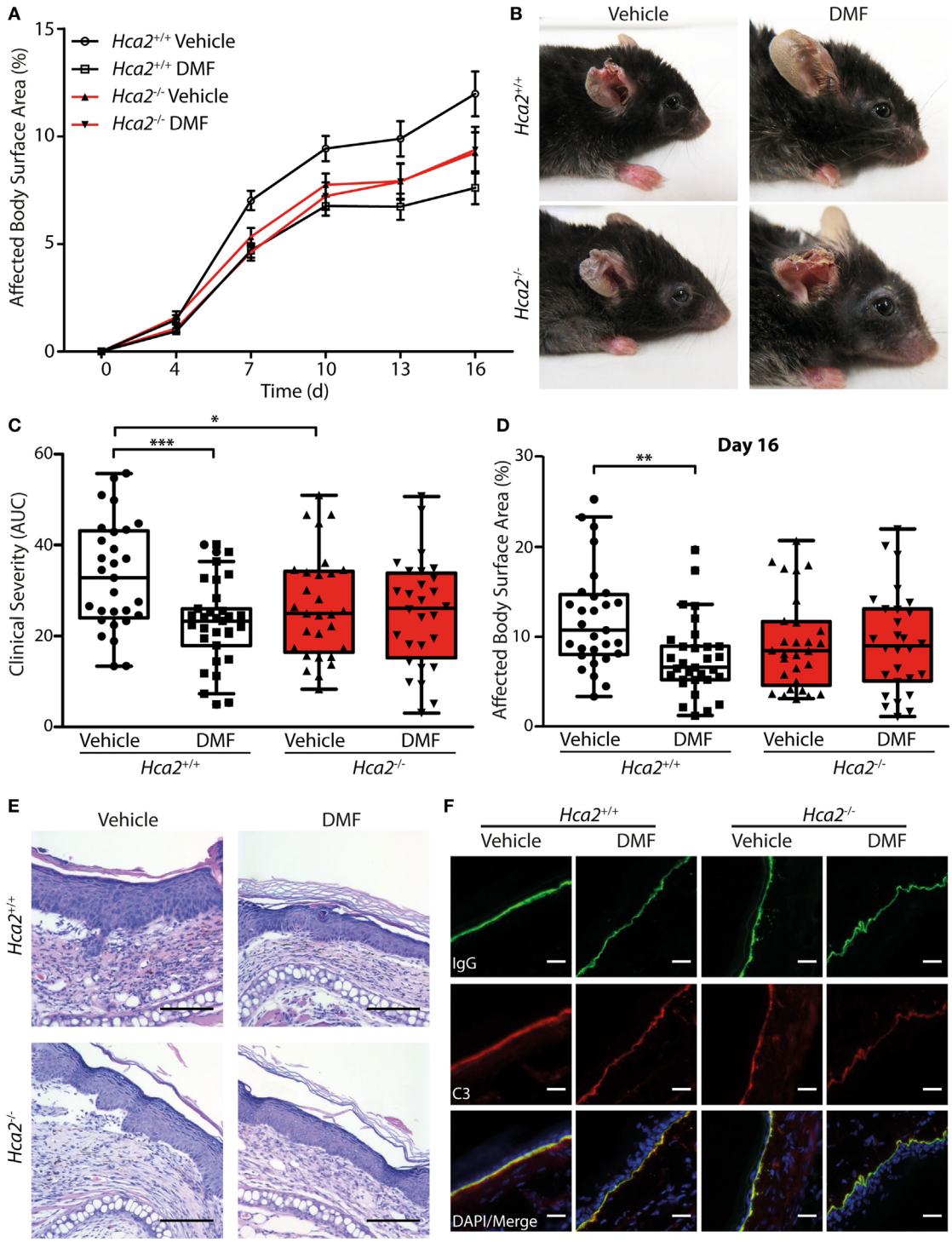

FIGURE 5 | Therapeutic dimethyl fumarate (DMF) effects in experimental epidermolysis bullosa acquisita (EBA) depend on HCA_2. **(A)** Clinical course of antibody transfer EBA in *Hca2*$^{+/+}$ and *Hca2*$^{-/-}$ mice that received oral vehicle or DMF treatment (50 mg/kg, p.o., twice per day). **(B)** Representative clinical presentation in the four experimental groups. **(C)** Clinical severity, calculated as area under the curve of the data in **(A)**. Two-way ANOVA, interaction between genotype and treatment $F(1/113) = 4.94$, $^{*}p < 0.05$; $^{***}p < 0.001$ ($n = 28$–31 mice, Bonferroni *post hoc* test) **(D)** Affected body surface area on day 16. ANOVA, interaction between genotype and treatment $F(1/113) = 5.75$, $^{**}p < 0.01$ ($n = 28$–31 mice, Bonferroni *post hoc* test). **(E)** Representative pictures of hematoxylin- and eosin-stained skin sections. Scale bar = 100 µm. **(F)** Representative immunohistochemical staining of anti-collagen VII-IgG and C3 depositions at the dermal–epidermal junction. Scale bar = 20 µm.

monocytes into the skin may be superior to strategies solely targeting neutrophils.

In our study, the number of γδT cells in skin was significantly higher in DMF-treated animals. γδT cells have previously been described to promote disease development. Ablating these cells with an anti-γδTCR antibody led to a reduced disease severity in EBA (28). In contrast, mice treated with DMF showed a milder clinical course at the time when γδT cells were elevated in the skin. Importantly, γδT cells are a heterogeneous cell population. Although some γδT cells produce pro-inflammatory cytokines, such as IL-17 and IFN-γ, other subpopulations, including IL-10 expressing γδT cells, have been described as regulatory cells required for the differentiation of Treg cells (29, 30). HCA_2^+ γδT cells have not been reported previously. Thus, a future study thoroughly characterizing this subpopulation and the effect of HCA_2 activation in these cells is warranted.

A key finding of our study is that in EBA the therapeutic DMF effect depends on HCA_2 as has previously been reported in a mouse model of MS (7). In addition to neutrophils, monocyte-derived cells, γδT cells, and some other cell types in the skin express HCA_2 and have been shown to be affected by HCA_2 activation. Unexpectedly, vehicle-treated *Hca2*-deficient mice showed reduced disease activity when the AUC was analyzed (**Figure 5C**). A basal phenotype of *Hca2*-deficient mice has not been described so far in other models of (skin) autoimmune diseases. Considering the fact that endogenous compounds, such as butyrate, nicotinic acid, and β-hydroxybutyrate, function as agonists of HCA_2, a basal activation of HCA_2 in some body compartments is possible, even in the absence of DMF treatment. Why such a basal HCA_2 activation should have the opposite effect of activation by DMF is unclear so far.

Apart from HCA_2 activation, DMF has been shown to act on the anti-oxidative NRF2 signaling pathway and on the glycolytic enzyme GAPDH (4, 5). By stimulating NRF2, DMF treatment induces a range of anti-oxidative and anti-inflammatory genes, an effect that is independent of HCA_2 (7). Whether HCA_2-independent effects contribute to the therapeutic effects of DMF in pemphigoid disease is unclear so far. In any case, identification of HCA_2 as an essential molecular target for EBA treatment suggests a strategy how to expand the therapeutic armamentarium for the treatment of autoimmune skin diseases. As a druggable G protein-coupled receptor, HCA_2 is activated by numerous compounds that await testing in pemphigoid disease and other autoimmune disorders (31).

MATERIALS AND METHODS

Mice

Hca2$^{-/-}$ mice were generated on the *C57BL/6* background, which is highly susceptible to the induction of passive EBA (32, 33). *Hca2*$^{-/-}$ and their respective littermate controls (*Hca2*$^{+/+}$) used for experiments were 8- to 12-week-old and all experimental groups were age- and sex-matched. To control for cage-specific effects, *Hca2*$^{-/-}$ and *Hca2*$^{+/+}$ mice and both treatment groups were housed together in individually ventilated cages on a 12–12 h light cycle with *ad libitum* access to food and water. All animal experiments were performed in accordance with Animal Protection Law and were approved by the Animal Research Ethics Board of the Ministry of the Environment, Kiel, Germany [Ethics approval V 242-79898-2015 (110-8-15)].

Generation of Anti-Collagen VII

To generate antibodies directed to murine collagen VII ("anti-collagen VII"), New Zealand White rabbits were immunized with 200 μg of a protein mixture containing three different recombinant proteins ("Col7A, B, and C") derived from the non-collagenous 1 domain of type VII collagen together with incomplete Freund's adjuvant, as described previously (33). IgGs were isolated from the serum of immunized rabbits by use of protein G, and afterward IgGs affinity purified with his-COL7 to specifically obtain rabbit anti-collagen VII IgG. To control for batch effects, all experiments were conducted using anti-COL7 IgG from at least two different batches.

Induction of Autoantibody Transfer ("Passive") EBA

Passive EBA was induced by i.p. injections of 75–100 μg affinity-purified anti-collagen VII IgG on day 0, 2, and 4 of the experiments. Disease severity was scored in a blinded fashion every 3 days for 16 days starting on day 4. The percentage of "affected skin" of the total body surface area of mice was assessed as described previously (18).

DMF Treatment

Dimethyl fumarate (Sigma-Aldrich) was prepared daily and suspended in 0.8% Methocel™/H_2O. Mice were treated with vehicle or DMF (50 mg/kg body weight) every 12 h by gavage.

Flow Cytometry

For the flow cytometric analysis of cells from blood, skin, spleen, and inguinal lymph nodes during EBA, mice were deeply anesthetized with ketamine (0.1 mg/g)/xylazin (0.015 mg/g) and killed. Blood was drawn from the right ventricle of the heart before spleen, inguinal lymph nodes, and ear skin were collected and stored on ice for further processing.

Cells were isolated from blood using the erythrocyte lysing buffer (Qiagen) in compliance with the manufacturer's instructions. Spleens and lymph nodes were homogenized using a 70-μm cell strainer (BD). Then, spleen cells underwent erythrocyte lysing as described above. Small pieces of ear skin were digested with liberase™ TL (1.2 mg/ml, Sigma) diluted in Iscove's Modified Dulbecco's Medium (Gibco) for 90 min at 37°C with constant agitation and subsequently ground on a 70-μm cell strainer. Single cells were resuspended in FACS buffer.

Using 2×10^6 cells per staining, blocking was performed with an anti-CD16/32 antibody (1:100, Mouse BD Fc Block). Surface antigens were stained with the appropriate antibodies (see **Table 1**) and a viability dye (eBioscience™ Fixable Viability Dye eFluor™ 780 or 660). Stained cells were analyzed using a FACS LSRII system and FACS DIVA software (BD Biosciences). Analysis was performed using FlowJo 10.3 software. For the gating strategy of viable and CD45 expressing cells, see Figure S9 in Supplementary Material.

TABLE 1 | Antibodies used for the flow cytometric analysis of immune cells.

Antibody	Clone	Supplier
Brilliant Violet 510™ anti-mouse CD45	30-F11	Biolegend
Brilliant Violet 650™ anti-mouse/human CD11b	M1/70	Biolegend
PE/Cy7 anti-mouse Ly-6C	HK1.4	Biolegend
PerCP/Cy5.5 anti-mouse Ly-6G	1A8	Biolegend
Brilliant Violet 421™ anti-mouse NK-1.1	PK136	Biolegend
FITC anti-mouse CD3	145-2C11	Biolegend
PerCP/Cy5.5 anti-mouse γδTCR	REA633	Miltenyi

Immunohistochemistry

For the detection of skin-bound complement factor C3 and anti-collagen VII antibodies, cryosections (10 µm) were fixed with acetone for 20 min at −20°C, washed and subsequently blocked with 1% BSA in PBS for 1 h. The sections were incubated with an anti-C3 antibody (1:400, clone 11H9, Hycult Biotech) overnight at 4°C. After 3 washing steps with PBS, the appropriate secondary antibodies (Alexa 488-labeled anti-rat IgG, 1:400, ThermoFisher; Cy3-labeled anti-rabbit IgG, 1:400, Jackson ImmunoResearch) and DAPI (2 µg/ml) were added and incubated for 1 h at room temperature. Then, the sections were embedded in Mowiol-DABCO. Images were acquired using a Leica DMI6000B fluorescence microscope.

Histology

Paraffin-embedded tissue sections (5 µm) were prepared on a microtome (Leica), dried overnight at room temperature and de-paraffinized using xylene and a descending alcohol dilution. The sections were incubated in 1% (v/v) acetic acid for 20 s before staining with hematoxylin (modified after Gill, Merck) for 10 min. Counter-staining with eosin Y (Merck) was carried out after two consecutive washes with warm tap water for 2 min. Then, the sections were dehydrated using an ascending alcohol series and two incubations with xylene before embedding in Eukitt embedding medium (Merck).

Quantitative Real-Time PCR

RNA of frozen lesional skin was isolated using the Navy Bullet Lysis Kit (Next Advance) in compliance with the manufacturer's instructions. cDNA synthesis was performed as previously described (7). The following primer sets were used: Ppib sense 5′-GGC TCC GTC GTC TTC CTT TT-3′, antisense 5′-ACT CGT CCT ACA GAT TCA TCT CC-3′, Nqo1 sense 5′-ATT CTC TGG CCG ATT CAG AGT G-3′, and antisense 5′-AGA CGG TTT CCA GAC GTT TCT T-3′.

Statistical Analysis

All data showing time courses of disease development are represented as the mean ± SEM. Bar graphs are shown as Box-Whisker plots according to Tukey. The statistical analysis was carried out using Prism (version 5.0, GraphPad Software, San Diego, USA) $*p < 0.05$, $**p < 0.01$, and $***p < 0.001$.

ETHICS STATEMENT

All animal experiments of this study were in accordance with the recommendations of the German Animal Protection Law and approved by the local animal ethics committee (Ministerium für Landwirtschaft, Umwelt und ländliche Räume, Kiel, Germany).

AUTHOR CONTRIBUTIONS

MW, JA, DZ, CS, and MS contributed to the conception and design of the study. MW analyzed cells by flow cytometry. MW and JA performed mouse experiments. JV contributed to sample preparation and immunohistochemical stainings. SO provided valuable tools and conceptual background. MW, JA, and MS wrote the first draft of the manuscript. All authors contributed to manuscript revision, read and approved the submitted version.

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SUPPLEMENTARY MATERIAL

The Supplementary Material for this article can be found online at https://www.frontiersin.org/articles/10.3389/fimmu.2018.01890/full#supplementary-material.

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REVIEW
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Update on Neutrophil Function in Severe Inflammation

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Neutrophils are main players in the effector phase of the host defense against micro-organisms and have a major role in the innate immune response. Neutrophils show phenotypic heterogeneity and functional flexibility, which highlight their importance in regulation of immune function. However, neutrophils can play a dual role and besides their antimicrobial function, deregulation of neutrophils and their hyperactivity can lead to tissue damage in severe inflammation or trauma. Neutrophils also have an important role in the modulation of the immune system in response to severe injury and trauma. In this review we will provide an overview of the current understanding of neutrophil subpopulations and their function during and post-infection and discuss the possible mechanisms of immune modulation by neutrophils in severe inflammation.

Keywords: neutrophils, infection, CD64, innate immunity, severe inflammation, trauma

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INTRODUCTION

Neutrophils are polymorphonuclear and phagocytic leukocytes that comprise the first line of host immune response against invading pathogens (1). They are also important effector cells during tissue injury-induced inflammation (2). Neutrophils have a high potency and efficacy to sense and eradicate microbial infections, and individuals with a neutrophil deficiency (such as neutropenia) are more susceptible to microbial and fungal infections (3).

Infections and their associated inflammatory mechanisms are accompanied by a rapid influx of neutrophils from the peripheral blood to the inflammatory site. There they engage and kill microorganisms and clear infections via a number of different mechanisms including chemotaxis, phagocytosis, release of reactive oxygen species (ROS), and granular proteins and the production and liberation of cytokines (4, 5). In addition to these well-established mechanisms, several reports have demonstrated the importance of neutrophil extracellular traps (NETs) in this process.

In addition to the pivotal role of neutrophils in innate immunity a large body of evidence has indicated the importance of neutrophils in the modulation of the adaptive immune response (6). Neutrophils are involved in immune regulation during both the innate and adaptive immune responses and are, therefore, considered as therapeutic targets in several diseases such as atherosclerosis (7, 8).

Although neutrophils have long been considered as a homogenous population with a conserved phenotype and function, recent evidence has demonstrated the presence of neutrophil heterogeneity with the identification of different functional phenotypes especially in cancer and inflammation (3, 9–11). Neutrophils show a spectrum of phenotypes and/or functional states. These are characterized by the expression of a wide range of cell-surface receptors that determine their function. These phenotypes seem to rapidly adapt to changes in environmental signals or triggers (12) and the expression profiles of neutrophil receptors can reflect the type and severity of the inflammatory response after severe injury (12). Since neutrophils are the main effector cells during the systemic inflammatory response (SIRS) to severe injury, neutrophil sub-phenotyping may provide both insight into disease mechanisms and be a useful risk assessment tool (12).

Although several neutrophil phenotypes exist with specialized functions, phenotypically homogenous populations with functional heterogeneity can be found in health and disease (10). However, it is unclear whether these cells originate from distinct bone marrow lineages or have undergone local differentiation (12, 13). These emerging properties of neutrophils provide us with new insight for further understanding of their roles in homeostasis and disease. We review here the roles and function of neutrophils in modulating the immune response during inflammation and summarize the mechanisms behind these processes.

DEFINITION, PROPERTIES, AND LIFE CYCLE OF NEUTROPHILS

Neutrophils are the most abundant circulating leukocyte population in the human immune system contributing about 50–70% of all circulating leukocytes in healthy adults (14). Neutrophils not only kill microorganisms through phagocytosis, degranulation, and the generation of NETs, but they also modulate the immune response by interacting with other immune cells such as lymphocytes and antigen presenting cells (APC) (6, 15). In addition, recent studies indicate that neutrophils show plasticity characterized by e.g., transdifferentiation to neutrophil-dendritic cell hybrids (16).

Neutrophil Life Cycle

Neutrophil generation from committed hemopoietic progenitor cells in the bone marrow is a highly controlled process that is regulated by different transcriptional factors such as C/EBP (17, 18). At the start of this process, a self-renewing hematopoietic stem cell (HSC) differentiates into a multipotent progenitor cell (MPP) which then, in turn, transforms into lymphoid-primed multipotent progenitor cells (LMPPs). LMPPs can finally give rise to granulocyte–monocyte progenitors (GMPs) (17). GMPs undergo neutrophil generation under the influence of various growth factors such as granulocyte colony-stimulating factor (G-CSF). This occurs in a step-wise process involving progression through promyelocyte, myelocyte, metamyelocyte, and finally band neutrophil stages during which developing neutrophils gradually acquire their mature phenotype [(16); **Figure 1**]. During these steps, it is thought that the expression of integrin α4β1 (VLA4) and CXCR4 (at least in mice) is downregulated and that expression of CXCR2 and Toll-like receptor 4 (TLR4) is increased. During this maturation neutrophils also attain their nuclear lobular morphology (19). The formation of granules inside the developing neutrophils starts between the myeloblast and promyelocyte stage and different granules are formed at different steps of the maturation process (20). A large pool of mature neutrophils is present in bone morrow from where they can be rapidly released into the circulation in response to infectious, inflammatory or tissue damage associated stimuli (21).

The number of neutrophils in the circulating blood is regulated by the CXCL12/CXCR4 axis in the mouse (22). Under normal conditions it is estimated that approximately 10^{11} mature neutrophils leave the bone marrow and enter the circulation each day (17, 21). Bone marrow stromal cells express the chemokine CXCL12, a ligand for CXCR4 which is thought to be expressed on bone marrow neutrophils and keeps them within the bone marrow (23). Although direct evidence of CXCR4 expression on human neutrophils in the bone marrow is lacking, the CXCR4 receptor antagonist plerixafor results in the mobilization of neutrophils into the blood (23, 24). Disruption of the CXCR4/CXCL12 balance such as that found in WHIM syndrome (Warts, Hypogammaglobulinemia, Immunodeficiency, and Myelokathexis syndrome) leads to deregulated neutrophil release into the circulation (24, 25). CXCR2 signaling can act as a functional CXCR4 antagonist to control neutrophil egress from the bone marrow into blood in mice (24, 25). This needs to be confirmed in man.

Neutrophil Access to Inflammatory Sites

Neutrophils quickly respond to inflammatory cues following infection or tissue damage and migrate to the inflamed/damaged area (26). Migration of neutrophils into the inflamed tissue, requires several steps that starts with adhesion to the endothelial surface followed by intravascular migration, extravasation and migration in the interstitium [(27); **Figure 2**].

Intravascular migration begins with neutrophil "tethering to" and "rolling on" the endothelium of blood vessels which is mediated by selectin molecules (21). Neutrophils then become activated by chemokines such as CXCL8 which trigger G-protein coupled receptors leading to a conformational change and activation of neutrophil integrin molecules such as VLA-4 (CD49D/CD29), MAC-1 (CD11b/CD18), and LFA-1 (CD11a/CD18) (21). This leads to an enhanced affinity for Ig-superfamily cell adhesion ligands (such as ICAM-1) expressed on the endothelium, which enables firm adherence of neutrophils to endothelial cells under flow conditions (17). Neutrophils then patrol the endothelial surface or migrate along a chemokine gradient to seek out the site of inflammation where they cross the endothelial layer in a processes generally referred to transendothelial migration or TEM (17).

Neutrophil extravasation through the endothelium occurs via either the paracellular or the transcellular route. The paracellular route involves leukocytes moving through endothelial cell junctions whilst the transcellular route involves neutrophil

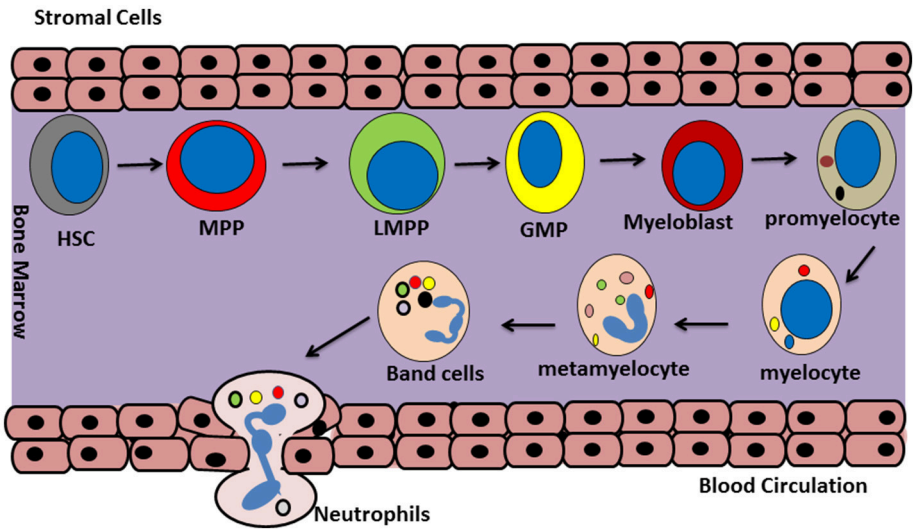

FIGURE 1 | Neutrophils generation. Granulopoiesis or neutrophil generation occur in the bone marrow. At the first step, a self-renewing hematopoietic stem cell (HSC) differentiate to a multipotent progenitor (MPP) cell. Then MPP differentiate to lymphoid-primed multipotent progenitors (LPMP), which give rise into granulocyte-monocyte progenitors (GMP). After that, GMP cells turn in to myeloblast and posses through a maturation process including promyelocyte, myelocyte, metamyelocyte, band cell, and finally will commit to generate the mature neutrophils.

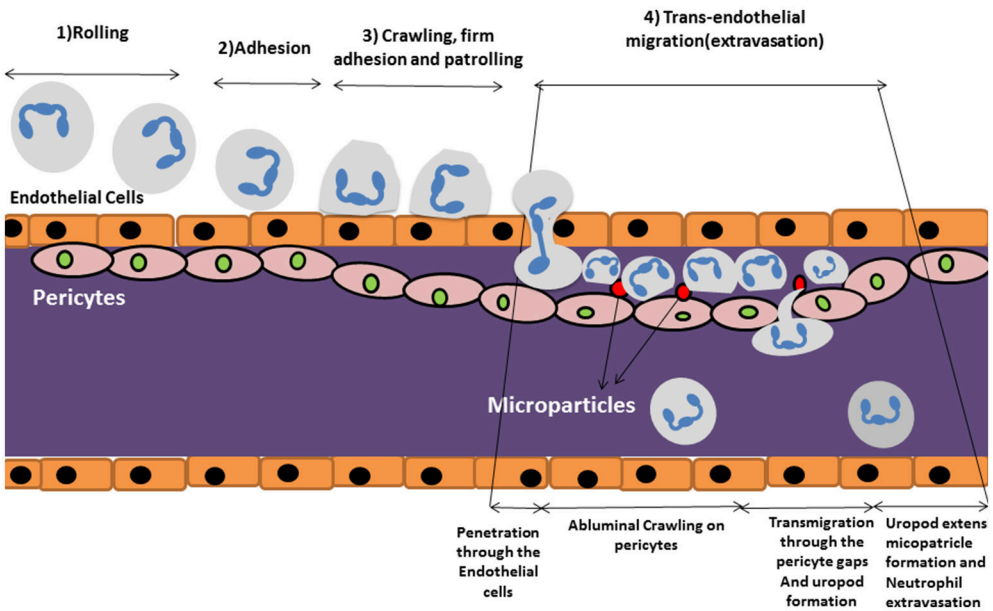

FIGURE 2 | Schematic review of neutrophil extravasation cascade. The process of neutrophil migration begins with neutrophil "tethering to" the endothelium of blood vessels in steps (1) rolling, (2) adhesion, and (3) crawling, firm adhesion and patrolling. (4) Trans endothelial migration occurs after approaching the site of inflammation where they cross the blood vessel wall in an extravasation step in which neutrophils travel along the endothelial basement membrane until finding a small gap between pericytes. They start migrating through the space by forming a protruding uropod which allows neutrophils to access to the inflamed area. Microparticle formation occurs following uropod formation which is shown to have pivotal role in controlling vascular permeability.

passage directly through the endothelial cell body (28). The paracellular route predominates in the majority of cases of neutrophil extravasation (29). Neutrophils travel along the endothelial basement membrane until they find a small gap between pericytes (27). Pericytes are contractile cells located at the abluminal site of microvessels and are responsible for controlling capillary permeability. Pericytes wrap around endothelial cells and cover 22–99% of the endothelial subcellular surface (30). These cells are also rich in pattern recognition receptors (PRRs) that enables them to sense inflammatory cues

and act accordingly (31). The cells facilitate the extravasation process of the neutrophils to the tissues.

Once the neutrophil finds a gap between pericytes, it starts migrating through the space by forming a protruding lamellapodium (32). Elongation and passage of the lamellapodium through the pericyte/endothelial membrane is mediated by integrins such as MAC1, LFA-1, and VLA-3, respectively (26, 33). In the last step, extravasating neutrophils shed their CD18 integrins via vesicles from their extended tail or uropod at the subendothelial layer enabling retraction of their extended tail (26). This allows access to the inflamed area where the activated neutrophil will initiate engagement with micro-organisms and the clearance of cell debris.

Extracellular Matrix Proteins and Neutrophils Activation

Neutrophils are strongly affected by their microenvironment including the presence of extracellular matrix (ECM). The effect of the ECM proteins such as collagen, laminin, fibronectin, and fibrinogen on inflammation has recently been explored and it is now evident that these proteins play a crucial role in providing signals that regulate different stages in neutrophil recruitment, transmigration and activation (1, 34–36).

The cytokine-induced respiratory burst in human neutrophils is dependent upon the interaction of ECM proteins with CD11/CD18 integrins (37). Subsequent studies have shown that the bovine neutrophil responses to IL-8 and platelet-activating factor (PAF) including intracellular calcium, actin polymerization, degranulation, adhesion, and oxidative burst changed dramatically after selective adhesion to different ECM proteins (38). Furthermore, the interaction between CD11b on neutrophils and the ECM protein fibrinogen, provided signals that enhanced the life span of neutrophils (39). ECM proteins also control neutrophil apoptosis indirectly by modulating tumor necrosis factor-alpha (TNF-α) expression within the local inflammatory milieu (39, 40). In contrast, the ECM proteolytic activity of neutrophils is critical for transmigration through the basement membrane (41).

Tissue damage can occur as a result of the neutrophils response to ECM protein signals in the inflammatory microenvironment (35). For example, in atherosclerosis the release of matrix modifying mediators such as neutrophil elastase (NE), myeloperoxidase (MPO), and defensins by activated neutrophils leads to the formation and development of atherosclerotic plaques (8, 42, 43). In the lung, the production and release of oxidants that results from the interaction of neutrophils with ECM proteins leads to injury and remodeling of the surrounding tissue matrix in COPD (44).

The interaction of ECM proteins with neutrophils also contributes to tumor metastasis. Chemokines produced by tumor cells activate microvascular endothelial cells inducing neutrophil adhesion and activation which is followed by the release of neutrophil oxidants and other matrix remodeling mediators. This results in a remodeling of the local microenviroment which facilitates the access of tumor cells to premetastatic sites (45). Greater research endeavors in this area may provide new therapeutic opportunities for the neutrophil-mediated inflammatory disorders.

Neutrophil Extracellular Traps (NETosis)

Neutrophils as the first line of immune defense against pathogens and they utilize various mechanisms to eliminate microbes include phagocytosis, ROS production as well as the generation and release of microbicidal molecules following degranulation (6). More recently, another distinct antimicrobial activity of neutrophils has been described called NETosis (46). In 2004 it was reported that neutrophils could eject nuclear chromatin that was decorated with antimicrobial peptides and enzymes including defensins and cathelicidins as well as NE and MPO (47). This externalized chromatin structure or NETs was capable of killing or suppressing fungal and bacterial proliferation (46). There is much evidence to support the role of NETs in blocking microbial dissemination. In addition, mice deficient in MPO production and with an absence of NETs are susceptible to greater fungal dissemination (48). Furthermore, bacterial strains with mutations in a NET-degrading nuclease do not disseminate (48, 49).

The importance of NETs in immune defense is highlighted by their conservation across vertebrate species (50–53). Lipopolysaccharide (LPS) or protein kinase C activators can rapidly trigger (within minutes) the formation of NETs under extreme conditions such as severe sepsis (54). NET formation is categorized as an innate immune process and can be triggered by downstream intracellular mediators such as ROS which activate MPO and NE leading to chromatin decondensation (46).

NETs are associated with several pathological and infectious conditions and have the potential to prime other immune cells leading to sterile inflammation (46). There is also evidence for a role in autoimmune and inflammatory disorders (46). However, the beneficial or detrimental role of NETs in immune defense is controversial and several factors in the local infectious or sterile microenvironment can determine the impact of NETs to potentiate or suppress inflammation (48).

There is debate regarding the concept that NETosis be considered as a specific form of programmed cell death. Malachowa et al suggest that formation of NETosis is an incidental phenomenon rather than a result of programmed cell death (55). Indeed, NETosis may be considered as a beneficial effect of neutrophil suicide or cellular death. Leben and colleagues showed that phagocytosis of *S. aureus* pHrodo™ beads by human neutrophil granulocytes correlated with NETosis process and was dependent on NADPH oxidase activation in contrast to other pathways of cell death (56). However, there are contradictory results regarding the induction of NETosis according to the stimuli used (57).

It is unlikely that NETosis is the major mechanism by which neutrophils control infection as this cytolytic process involves the release of numerous DAMPs which would prolong and intensify the inflammatory response (55, 57). In recent years the concept of NET formation without neutrophil cell death, referred to as non-cytolytic or "vital NETosis," has been introduced (57). This occurs via a ROS-independent mechanism (58) and is likely to be the normal manner by which NET factors are released.

NEUTROPHIL PHENOTYPES AND HETEROGENEITY

The hematopoietic system consists of different subsets of myeloid and lymphoid cells with different phenotypes and functions (11). Beyond the differences in embryonic origin, this heterogeneity can be dictated by several elements including ontogenic and environmental factors (59). The presence of neutrophil heterogeneity was considered controversial for a long time because of their limited transcriptional activity, limited lifetime, and inability for reverse transmigration (RT) to peripheral blood after tissue homing. These did not match features of other heterogeneous cell populations (11). However, neutrophils acquire distinct phenotypes within their local microenvironment depending upon the physiological and pathological cues present (60).

Neutrophil heterogeneity is has been linked to survival time, function, density, NET-releasing capability and receptor expression profiles (61). Furthermore, distinct neutrophil subsets have been described based on the expression of cell surface markers (15). For example, CD66b/CD33 represents low-density neutrophils (LDNs) within the neutrophilic myeloid-derived suppressor cell (MDSC) population (62, 63) and CD49d positive neutrophils have been found in atopic individuals (64). The roles of these neutrophil subtypes in disease pathophysiology is unknown although some subtypes may be harmful whereas other neutrophil subsets may be beneficial at the sites of chronic inflammation. The next section will describe some receptor molecules and properties that characterize neutrophil subsets present in homeostasis and under pathologic conditions.

Olfactomedin 4 (OLFM4) Positive Cells

The neutrophil granule protein olfactomedin 4 (OLFM4) defines two subtypes of neutrophils ($OLFM4^+$ and $OLFM4^-$) (11). These subtypes show no differences in apoptosis or antibacterial function *in vitro* and have an equal tendency for migration toward an inflamed area in response to inflammatory signals (65). OLFM4 gene knock-out mice exhibit reduced colonization of the gastric mucosa by *Helicobacter pylori (H. pylori)* which is associated with increased inflammatory cell infiltration, enhanced production of pro-inflammatory cytokines/chemokines such as IL-1β, IL-5, IL-12 p70, and MIP-1α and increased inflammatory response to *H. pylori* in gastric mucosa (66). It is speculated that OLFM4+ neutrophils localize to the NET of its parent cell during NETosis rather than increase NET formation *per se* and further studies are required to define the role of OLFM4+ neutrophils (39).

CD177 (NB1) Expressing Neutrophils

CD177 is a 55 kDa glycosyl-phosphatidylinositol-anchored receptor that is expressed on human circulating neutrophils (67). CD177 has an important role in neutrophil transmigration through the endothelium as it has a high affinity for the adhesion molecule, platelet endothelial cell adhesion molecule-1 (PECAM-1) (68, 69). CD177 activation also modulates human neutrophil migration in a β2 integrin-dependent manner (70).

CD177 is also associated with the expression of the serine protease PR3 (68, 69). In human neutrophils, CD177 is co-expressed with PR3 on the surface of neutrophils and together these promote extravasation of circulating neutrophils (69). In severe bacterial infection the circulating levels of CD177+ neutrophils is augmented probably due to the elevated co-expression of PR3 which facilitates increased neutrophil tissue infiltration (71).

The circulating levels of CD177+ neutrophils are increased in patients with anti-neutrophil cytoplasmic antibodies (ANCA)-dependent vasculitis (70). Enrichment of these cells was associated with an increased risk of relapse (72). However, in ANCA-dependent vasculitis the expression of membrane bound PR3 is enhanced in primed CD177 negative neutrophils suggesting that anti-PR3-mediated neutrophil recruitment is independent of the role of CD177 (71). Interestingly, CD177+ neutrophils are the functionally activated neutrophil population in inflammatory bowel disease and negatively regulate disease (73). The role of CD177 in neutrophil migration and IBD currently seems to be more consensual than in the airways.

CD63+ Neutrophils

Single-cell analysis identified a subset of neutrophils in the airway of cystic fibrosis (CF) patients that appeared to have undergone functional reprogramming and acquired profound differences to circulating neutrophils including reduced intracellular glutathione and augmentation of lipid raft assembly (74). These neutrophils expressed CD63+, a marker of NE-rich granules, on their cell surface. In addition, expression of key phagocytosis receptors including CD16 and CD14 was enhanced (75). This was in contrast to the reduced levels of CD80 and of the prostaglandin receptor CD294 on these cells. This subset of neutrophils may represent an important future therapeutic target for airways disease (74).

ICAM-1-Expressing Neutrophils

ICAM-1 (CD54) expressing neutrophils represent a population of tissue-experienced neutrophils that have migrated in a retrograde direction across endothelial cells and emerged again in the peripheral blood by reverse transmigration (75). This subpopulation of neutrophils are associated with chronic systemic inflammation (62). The function of these cells as well as their fate remain elusive.

$CD16^{bright}CD62L^{dim}$ Population and Immune Suppressive Neutrophils

A $CD16^{bright}CD62L^{dim}$ population of neutrophils was first described by Pillay and colleagues as a unique circulating population of myeloid derived suppressor cells (MDSC) (74, 76). MDSCs were originally identified in a murine model of cancer as a population of heterogeneous immature myeloid cells that suppress immune responses (77–79). Gabrilovic et al. in 2007 subsequently coined the term MDSCs to emphasis their heterogeneity (80).

The $CD16^{bright}CD62L^{dim}$ population of neutrophils can mimic MDSCs and exhibit a suppressive function and suppress T-cell proliferation *in vitro* while remaining remarkably poor at

eliminating bacteria such as *Staphylococcus aureus (S. aureus)* (81). This suppressive immunophenotype of mature neutrophils is also detected in peripheral blood samples of cancer patients suggesting their involvement in antitumor immunity (82). The $CD16^{bright}CD62L^{dim}$ population will be discussed in greater detail in section Neutrophil Phenotypes After Trauma.

Pro-angiogenic Neutrophils

A subpopulation of CD11b+/Gr-1+ neutrophils in the mouse was first described in 2012 as being recruited to transplantation sites and having the ability to promote re-vascularization of the transplanted tissue (83). This population of neutrophils at least in the mouse express high levels of CXCR4 ($CXCR4^{hi}$). In an *in vivo* model they can deliver large amounts of MMP-9 to transplanted islets of Langerhans. This in turn, induced VEGF-dependent angiogenesis at the site of recruitment (84). These cells have yet to be found in humans *in vivo*.

Low Density Neutrophils (LDNs)

Low-density neutrophils (LDNs) comprise a population of neutrophils with a low buoyant density that are typically found in the mononuclear fraction upon density centrifugation. These cells include cells both segmented and banded nuclei as well as myelocyte-like progenitor cells (85). LDNs were first reported in systemic lupus erythematosus (SLE), rheumatoid arthritis and rheumatic fever (86). They are now also recognized as being elevated in cancer and are in some studies associated with tumor progression (87). The cells are responsible for the down-regulation of T-cell function via an arginase dependent mechanism such as found during the induction of materno-fetal tolerance (87).

In sepsis, LDNs are present and play a pivotal role in sepsis-induced immune suppression. In patients with sepsis, granulocyte-like MDSC which include a low density granulocyte (LDG) population, help drive T-cell dysfunction by the production of arginase 1 that enables the subsequent development of nosocomial infections (88).

LDNs from patients with SLE have a higher propensity to form NETs in a process referred to as NETosis (89) and to release pre-formed NETs. NETs can present auto-antigens to the immune system suggesting that LDNs, from SLE patients at least, can promote chronic inflammation leading to autoimmunity (89, 90). High CD66b+ LDN counts were also reported in the peritoneal cavity of patients with gastric cancer following abdominal surgery (91). These CD66b+ LDNs have the ability to form NETs and to selectively capture disseminated tumor cells (91).

The function of LDNs is dependent on the local microenvironment and the associated pathology (92). For example, in cancer LDNs have an immunosuppressive activity (93) whilst they generally possess a pro-inflammatory phenotype in autoimmunity disease (94, 95). In SLE, activated LDNs produce high levels of type 1 interferons (INFs) (95). LDNs with an activated phenotype have also been reported during leishmania infection (94, 96). However; LDNs play a major immunosuppressive role in sepsis which is associated with higher incidence of nosocomial infections (88). The characterization of these cells *in vivo* is difficult due to the lack of specific cell surface or molecular markers (97).

Tumor Associated Neutrophils (TANs)

Mature neutrophils that leave the bone marrow and are released into the circulation may migrate into tumors where they can be found as tumor associated neutrophils (TANs) (98). After infiltration to tumor sites, these neutrophils undergo profound phenotypic changes compared to their circulating counterparts (99).

In murine models of cancer, TANs showed two distinct populations referred to as N1 and N2 with pro- and anti-tumoral roles, respectively (100). Transcriptomic analysis showed that N1 and N2 neutrophils have distinct gene expression profiles and functional properties which are likely induced by the local tumor microenvironment (101). The role(s) of TANs in the tumor microenvironment in man remains unclear although TANs isolated from human lung tumors possess an activated phenotype ($CD62^{Low}CD54^{hi}$) with increased pro-inflammatory cytokine production. These TANs also induce T-cell proliferation and the production of IFN-γ (98).

In contrast, in colorectal cancer TANs produce arginase I and ROS thereby inhibiting proliferation and IFN-γ production by T-cells (102). These data suggest that these cells exhibit LDN-like properties (102). The numbers and functions of TANs are regulated by chemotherapy in human colorectal cancer which supports the hypothesis that they have a role in cancer progression (103). However, the degree to which TAN subsets are present in human cancers or whether different human cancers include different TAN subsets is unclear.

Despite the current consensus around the existence of neutrophil heterogeneity, there are still some challenges to be met. It is possible that the different observed properties of the neutrophil populations merely reflect the response to the local environment and differences result from their innate plasticity (93). Further studies are required to link the various phenotypic characteristics with cellular/tissue functions.

THE IMPORTANCE OF NEUTROPHILS IN INFLAMMATORY COMPLICATIONS FOUND AFTER TRAUMA

Trauma is the main case of mortality worldwide in people under the age of 50 (12). In 5% of cases, patients suffer from severe trauma. This clinical condition can ultimately lead to multiple organ dysfunction syndrome (MODS) in which the functionality of some organs such as liver, lung or kidney is markedly reduced (104). The main cause of post-traumatic complications is due to hyperactivation of the immune response (105). For example, a localized inflammatory reaction after trauma can be provoked by alarm signals (alarmins and other damage associated molecular patterns/DAMPS) which are secreted by healthy, damaged or necrotic cells (106).

Neutrophils are important effector cells in managing and regaining tissue homeostasis and in the maintenance of immune surveillance. Activation of neutrophils after trauma by alarm

signals evokes the development of a local inflammatory response. If this local inflammatory response becomes excessive this may lead to a SIRS and MODS.

MODS has a mortality rate of up to 50–80% (106). To control this disproportionate pro-inflammatory reaction and to restore the equilibrium, a compensatory anti-inflammatory response (CARS) may occur (105, 107). Alternatively, the pro-inflammatory and anti-inflammatory responses may counteract each other leading to a mixed antagonist response (MARS) (108, 109). Both CARS and MARS tune down the disproportionate immune activation (110) making the patient extremely susceptible to infection by microorganisms. This results in serious complications such as sepsis, septic shock and organ failure [(105); **Figure 3**].

Neutrophils play an important role in the pathophysiology of the deregulated immune response found in patients with trauma (111). Tissue damage leads to neutrophil activation and the production of ROS due to various triggers such as hypoxia and reperfusion injury in damaged tissue and the release of neutrophil chemoattractants and activators (112). The presence of neutrophil priming agents such as granulocyte-macrophage colony-stimulating factor (GM-CSF) or TNF-α in the peripheral blood, enhances neutrophil chemotaxis, extravasation and oxidative burst production (113). The spontaneous production of ROS by neutrophils is elevated in trauma patients and the uncontrolled ROS production by accumulated neutrophils in the vascular bed increases vascular permeability promoting organ failure (112).

Selectins and integrins mediate neutrophil transmigration toward the inflamed and/or damaged tissue. Neutrophils release L-selectin during migration and serum levels of L-selectin (sL-selectin) are associated with the degree of neutrophil activation. Maximum sL-selectin levels were observed 6 h after trauma (113, 114). The destructive effects of neutrophils within tissue is limited by neutrophil apoptosis. However, this process is delayed after trauma (115). Delayed neutrophil apoptosis leads to the accumulation of neutrophils, increased release of their cytotoxic products and the promotion of local tissue damage (105, 116).

Although neutrophils are activated during SIRS post-trauma, their responsiveness to the innate stimulus fMLP decreases. This is illustrated by the decreased expression of active FcγRII (CD32) induced by fMLP on neutrophils in poly trauma patients. Consequently, the low functionality of this most important Fcγ receptor on neutrophils probably involves the decrease of antibacterial function during CARS over the following days (117, 118). This may be due to the production of $CD16^{low}$ immature neutrophils (118). The recruitment of immature band forms of neutrophils from the bone marrow into the circulation is typically found in sepsis and SIRS (119). It is yet to be determined whether these young cells are dysfunctional or whether these cells are fully functional as is seen after LPS challenge (81). On the other hand, these immature neutrophils show lower expression of antibacterial receptors such as CD14 and MD-2 and have a reduced transmigration ability (118).

The endocrine system also modifies neutrophil changes after severe injury. Both trauma-induced cortisol and epinephrine strongly increase neutrophil release into circulating blood (120). Cortisol is also thought to extend the half-life of circulating neutrophils (120). Together with the reduced chemotaxis found after cortisol and epinephrine infusion these combined effects could account for increased susceptibility to infection observed after major trauma (120).

Neutrophil Phenotypes After Trauma

Acute inflammation is accompanied by the recruitment of neutrophils with different phenotypes into the circulation that are not present during homeostasis (12, 121). These "inflammatory" neutrophils have distinct characteristics such as enhanced expression of CD124 (IL-4Rα), CD15 (3- fucosyl-N-acetyl-lactosamine), and arginase in addition to a lower buoyant

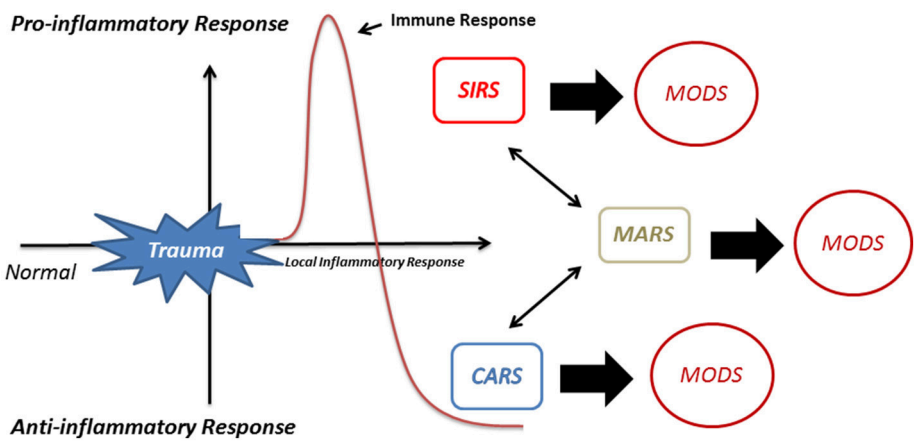

FIGURE 3 | Activation of immune response after trauma. Activation of neutrophils after trauma evokes the development of a local inflammatory response. If this local inflammatory response becomes excessive this may lead to a systemic inflammatory response (SIRS) and multiple organ dysfunction syndrome (MODS). To restore the equilibrium to a favorable state, a compensatory anti-inflammatory response (CARS) may occur or, alternatively, the pro-inflammatory and anti-inflammatory responses may counteract leading to a mixed antagonist response (MARS).

density and immunomodulatory properties (3). In 2012, Pillay et al. observed neutrophil subtypes in the circulation during experimental acute systemic inflammation evoked by systemic administration of 2 ng/kg LPS in human healthy volunteers (3). Based on the expression level of CD16 (FcγRIII) and CD62L (L-selectin), three different subsets of "inflammatory" neutrophils were observed: neutrophils with a conventional segmented nucleus ($CD16^{bright}/CD62L^{bright}$), neutrophils with a banded nucleus ($CD16^{dim}/CD62L^{bright}$), and CD62Ldim neutrophils ($CD16^{bright}/CD62L^{dim}$) with a higher number of nuclear lobes (hyper-segmented).

Banded neutrophils observed in acute inflammation are fully functional and are superior in killing *S. aureus* (81). In contrast, $CD62L^{dim}$ neutrophils were enriched by proteins involved in immune regulation (122) as these cells have immunosuppressive properties and inhibit T-cell proliferation (123). $CD16L^{dim}$ neutrophils also showed lower cell adhesion capacity and an extremely low capacity to contain bacteria in comparison to the two other subtypes (124). Chemotaxis toward end target chemoattractants was also decreased in this group which might result in reduced endothelial binding and extravasation to inflammatory sites (124). The origin of $CD62L^{dim}$ cells after trauma in humans is not clear but these cells do not represent aged cells (3).

Although it is commonly believed that increased nuclear segmentation occurs with increasing cellular age this is not really supported by experimental data. For example, in humans the hyper-segmented neutrophils seen in pernicious anemia result from defects in the DNA replication machinery. These hyper-segmented neutrophils appear in the circulation simultaneously with normal neutrophils (124). In addition, a study applying proteome profiling and *in vivo* kinetics of neutrophils following LPS challenge showed that hyper-segmented neutrophils have a similar age as normal segmented cells and take the same time to reach maturity and, as such, cannot be considered as aged cells (125). Furthermore, these hyper-segmented $CD62L^{dim}$ cells do not seem to originate from mature neutrophils but might be produced by a separate pathway compared to banded and normal segmented neutrophils in response to inflammation (123). These cells enter the bloodstream only during inflammation as a distinct neutrophil subset. It is, therefore, tempting to speculate that these cells have a specific goal of fine-tuning the acute immune response (96).

The Role of Neutrophils in Immune Dysfunction After Trauma and Inflammation

Neutrophils are involved in the deregulation of immune responses during trauma by several mechanisms including cleavage of essential cell surface receptors, modulation of the function of immune receptors, control of peripheral blood neutrophil numbers, and modulation of the adaptive immune response.

Cleavage of Essential Cell Surface Receptors

Neutrophil-derived serine proteases released by degranulation can mediate proteolytic cleavage of receptors on immune cells (126). During acute inflammation NE can cleave and downregulate the expression of the IL-8 receptors CXCR1 and 2 (127, 128). This, in turn, decreases the responsiveness to IL-8 and enhances the risk of pneumonia in trauma patients (129). Complement receptors may also be targeted by neutrophil proteases. Decreased levels of CR1/CD35 (130) and of C5aR/CD88 have been reported during inflammation which might result in a failure of neutrophil engagement with microorganisms (131). Cleavage of CD14 on the surface of monocytes can also be affected by NE which can lead to impaired TLR4-mediated recognition of lipopolysaccharide by monocytes (132). On the other hand, NE can also modulate adaptive immune responses by enhancing the shedding of the IL-2 and IL-6 receptors on T lymphocytes (133).

Desensitization of Immune Receptors

After severe injury and trauma, suppression of immune function may occur due to the desensitization of immune receptors on neutrophils (133). Trauma/severe injury leads to the release of endogenous danger signals (danger-associated molecular patterns; DAMPs) from necrotic tissue cells that can bind to PRRs on neutrophils (134). DAMPs, which alert the immune system in response to stress, resemble pathogen-associated molecular patterns (PAMPs) and can bind to their receptors on neutrophils (135). Finally, DAMPs released during trauma can induce both homologous and heterologous desensitization of immune receptors via internalization of PPRs which limit subsequent responses to microbial signals (136).

Regulation of Neutrophil Numbers

The number of neutrophils in the circulating blood is regulated by the CXCL12/CXCR4 axis in the mouse (24). Expression of the chemokine CXCL12 by bone marrow stromal cells provide a signal for neutrophils expressing the CXCL12 receptor CXCR4 to remain in the bone marrow (22). Conversely, attenuation of CXCR4 signaling leads to the release of neutrophils into the circulation. Disruption of the CXCR4/CXCL12 balance by inflammatory stimuli can increase neutrophil release into peripheral blood (24) or can lead to leukostasis in the bone marrow such as found in WHIM syndrome.

In human experimental endotoxemia, peripheral neutrophils exhibit a functional heterogeneity and different degrees of priming (122). Similar variations in neutrophil phenotypes are seen in the peripheral blood of patients during severe inflammation (137). A large number of immature or banded cells, suppressive neutrophils, myeloid-derived suppressor cells, and neutrophil progenitor cells can be detected (119). The presence of immature or banded neutrophils may be either a compensatory response due to depletion of mature neutrophils in bone marrow or it may be induced by the bacterial stimulus itself (123).

Modulation of the Adaptive Immune Response

Neutrophils play the major role in the paralysis of the immune system during CARS that may occur because of systemic inflammation (138). Immune paralysis is an immunosuppressed state in which immune responses are unable to recover despite the clearance of pathogens. This leads to a failure in the ability to control the primary infection and increased susceptibility to

secondary infections. Immune paralysis is the main cause of death in most sepsis patients (139).

Apart from their roles in innate immunity and direct anti-microbial defense, neutrophils can also modulate adaptive immune cells in severe inflammation (119). Neutrophils can modulate T-cell responses via different mechanisms. A T-cell found in an inflammatory microenvironment may be affected by neutrophil-derived chemokines and cytokines or by their released granular contents (140). NE can directly cleave receptors on the T-cell surface such as those for IL-2 and IL-6 (133). NE can also reduce the level of co-stimulatory molecules on dendritic cells and subsequently decrease T-cell maturation (141).

Macrophages shift toward an anti-inflammatory phenotype after phagocytosis of apoptotic neutrophils (142). MDSCs are a heterogonous population consisting of myeloid progenitors, immature neutrophils, macrophages and dendritic cells that expand during a wide range of pathological conditions such as cancer and inflammation (143). These cells are potent suppressors of various T-cell functions in mouse models. These cells can produce arginase-1 and thereby deplete arginine from the local microenvironment (144). Arginine is an essential amino acid and its depletion leads to cell cycle arrest of T-cells in the G0–G1 phase (145). A subset of neutrophils have been identified in the peripheral blood of patients with septic shock that can secrete arginase-1 and function as MDSCs (146).

In man, a systematic LPS challenge induced the release of a subtype of mature $CD62L^{dim}$ neutrophils with a hyper-segmented nuclear morphology into the circulation (123). These cells suppressed T-cell function via a Mac-1/(αMβ2)-dependent (CD11b/CD18-dependent) mechanism and delivery of hydrogen peroxide into the immunological synapse (123). A similar subset has been found after systemic treatment with G-CSF (147). However, the latter cells employ arginase rather than oxidants to suppress T-cell function *in vitro*.

Proteome profiling of L-selectin/CD62L low neutrophils showed that this subtype is enriched in proteins involved in immune regulation and exhibited a marked decrease in ribosomal proteins compared to immature banded neutrophils (3). This implied that the L-selectin low cells lost a significant part of their protein translational machinery (3).

INF-γ induces the expression PD-L1 by neutrophils which enables them to suppress lymphocyte proliferation and induce lymphocyte apoptosis (148). Blocking this PD-1/PD-L1 axis in a murine model of sepsis reversed immune dysfunction and improved survival (149). INF-γ also induced the expression of Fc gamma receptor (CD64) by induction of transcription factor STAT1 [(150); **Figure 4**].

Neutrophil subgroups also play a role in cancer immunity. $CD16^{high}CD62L^{dim}$ cells are more common in patients with head and neck squamous cell carcinoma (HNSCC). These cells produce NETs, displayed an activated phenotype and, in comparison to other subtypes, were more prone to migrate to tumor sites and perform anti-tumor immune functions including inhibition of proliferation and the induction of apoptosis in cancer cells. An increase in circulating $CD16^{high}CD62L^{dim}$ neutrophils was associated with increased NET formation and increased survival in HNSCC patients (151).

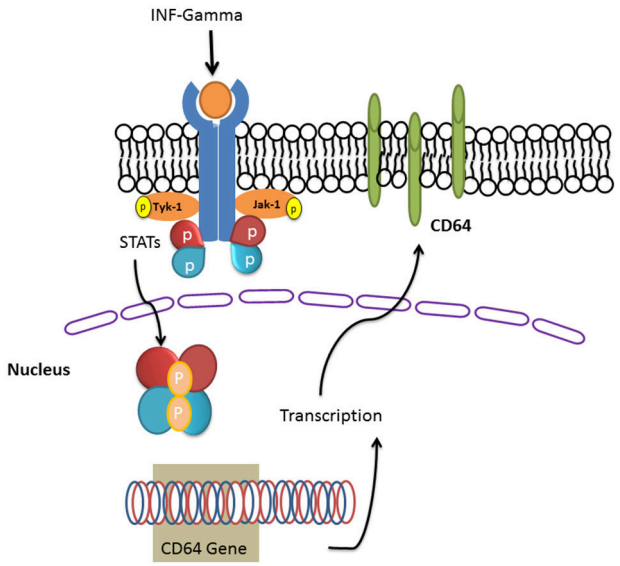

FIGURE 4 | INF-gamma induced the expression of Fc gamma receptor (CD64). IFN-γ induces its receptor and the downstream signaling pathways including JAKs and the STAT family of transcription factors. STAT dimers enhance the transcription of CD64 gene and the translated CD64 will be targeted in to the plasma membrane bilayer lipid.

THE APPLICATION OF NEUTROPHILS AS BIOMARKERS IN INFECTIOUS DISEASES

The neutrophil Fcγ-receptor I (FcγRI, CD64) has long been considered as a biomarker for infectious disease. It is a high affinity receptor that binds to the Fc part of the IgG heavy chain (152). CD64 is normally expressed at a very low level on the surface of resting neutrophils in healthy individuals (153) but its expression is markedly elevated within a few hours of bacterial infections (154). The expression can be elevated >10-fold which allows differentiation between resting and activated neutrophils (155, 156). This property of CD64 has been utilized as a diagnostic marker of infection (156) particularly in sepsis. The expression of this marker is very stable after blood collection and it requires only a small volume for assessment (156) making this a very attractive marker for infection monitoring.

The level of CD64 is moderately elevated in preterm newborn infants before changing to normal levels in the first month of life (157). However, a meta-analysis of the diagnostic accuracy of neutrophil CD64 in neonatal sepsis suggests that this alone cannot be used as a satisfactory marker for neonatal sepsis due to its relatively low sensitivity and specificity (158). However, neutrophil CD64 levels are a very sensitive diagnostic marker for early-onset clinical infection and pneumonia in newborns and can guide antibiotic therapy (159). In addition, circulating neutrophils in Erythema nodosum leprosum (ENL) patients expressed CD64 on their cell surface and this expression was correlated with disease severity (160).

Neutrophil gelatinase-associated lipocalin (NGAL) is another neutrophilic marker important for the early diagnosis of acute

kidney injury (161). NGAL is also used as a biomarker for inflammation in cardiovascular disease including atherosclerosis, heart failure as well as acute myocardial infarction (161).

A novel neutrophil derived inflammatory biomarker in CF patients has been recently introduced (162). This is a protein complex containing alpha-1 antitrypsin and CD16b (AAT:CD16b) that is released into the bloodstream from membranes of pro-inflammatory primed neutrophils. The plasma level of AAT:CD16b complex correlates with inflammatory status in CF patients and has been proposed as a biomarker for the diagnosis of CF exacerbations (162). In ulcerative colitis (UC), the presence of human neutrophil lipocalin (HNL), and MPO in colorectal perfusion fluids indicates intestinal neutrophil activation in UC (163).

CONCLUSION

Neutrophils are main players in the context of inflammatory complications during and after infections and tissue injury. The neutrophil compartment is heterogeneous and neutrophils with distinct properties have been identified. These cells exhibit a high plasticity and easily adapt to changes in microenvironment. Newly identified human neutrophil subsets can suppress T-cell activation and proliferation and their presence may provide a novel therapeutic and/or diagnostic avenue in chronic and acute infection as well as in cancers. In contrast, neutrophils also play the central role in the immune paralysis after severe inflammation and have a detrimental role in organ failure in post-injury events.

The paradox regarding the role of neutrophils in health and disease dictates that therapy should be targeted to the correct phenotypes to prevent off-target effects. It is now of paramount importance to identity the site of origin of different neutrophil phenotypes present in severe inflammation. The potential of neutrophil cell surface markers or their products to be used in diagnosis or in therapy has great potential but requires further study. In addition, the mechanism(s) by which neutrophils drive immune paralysis and subsequent tissue damage post-trauma are an important therapeutic target as there is great unmet medical need to control neutrophil activation in most inflammatory diseases.

AUTHOR CONTRIBUTIONS

EM and SA wrote the original draft of manuscript. SM helped with editing, literature collating and referencing. LK and IA revised and edited the manuscript.

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SYSTEMATIC REVIEW
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Hidradenitis Suppurativa: A Systematic Review Integrating Inflammatory Pathways Into a Cohesive Pathogenic Model

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Background: The pathogenesis of hidradenitis suppurativa (HS) is not fully understood. This systematic review examined the latest evidence for molecular inflammatory pathways involved in HS as a chronic inflammatory skin disease.

Methods: A systematic literature search was performed in PubMed/Medline and EMBASE from January 2013 through September 2017, according to the preferred reporting items for systematic reviews and meta-analyses (PRISMA). Findings on HS pathogenesis were also compared with those of other immune-mediated inflammatory diseases (IMIDs) in a non-systematic review. In addition, current therapeutic options for HS are briefly discussed on the basis of the findings for the inflammatory pathways involved in HS.

Results: A total of 32 eligible publications were identified by the systematic search; these were supplemented with three additional publications. The extracted data indicated that four key themes underlie the pathogenesis of HS and related syndromic conditions. First, nicastrin (*NCSTN*) and *PSTPIP1* mutations are directly associated with auto-inflammatory disease. Secondly, the up-regulation of several cytokines including tumor necrosis factor-α and T helper-17/interleukin-23 are connected to auto-inflammatory mechanisms in the pathogenesis of HS. Thirdly, the microbiome of lesional skin differs significantly vs. normal-appearing skin. Fourthly, HS risk is enhanced through physiological and environmental factors such as smoking, obesity, and mechanical friction. There is significant overlap between the pathogenesis of HS, its syndromic forms and other IMIDs, particularly with respect to aberrations in the innate immune response.

Conclusions: The evidence presented in this review supports HS as an auto-inflammatory skin disorder associated with alterations in the innate immune system. Based on these most recent data, an integrative viewpoint is presented on the pathogenesis of HS. Current management strategies on HS consist of anti-inflammatory therapies, surgical removal of chronic lesions, and lifestyle changes such as smoking cessation and weight loss. As large gaps remain in the understanding of the pathogenesis of HS, further research is warranted to ultimately improve the management and treatment of patients with HS and related syndromic conditions.

Keywords: acne inversa, pyoderma gangrenosum and acne, immune-mediated inflammatory disease, inflammatory bowel disease, auto-inflammation, nicastrin, PSTPIP1, obesity

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INTRODUCTION

Hidradenitis suppurativa (HS) is a chronic, recurrent, inflammatory follicular occlusive disease, that usually presents after puberty with painful, inflamed lesions, predominantly at inverse body sites such as the axillae, inguinal and anogenital regions (1). The physiological and psychological consequences of HS can profoundly reduce a patient's quality of life (1, 2). Prevalence estimates in North America and Europe range from <1 to 4% (3, 4).

The pathogenesis of HS is not fully understood. Current evidence highlights a complex multifactorial pathogenesis (5). A key triggering factor is the occlusion of the hair follicle, caused by keratosis and hyperplasia of the follicular epithelium leading to cyst development (6, 7). Subsequently, the cyst will rupture, causing a fierce immune response and inflammation that, depending on the severity, may progress to abscess and sinus tract development and scarring (6, 7). The name of the disease implies that sweating and bacterial infection are a fundamental part of the disease process. This is misleading and now considered a misnomer: no evidence has been found showing that HS is triggered by events in the apocrine or eccrine glands. Environmental risk factors reported to contribute to HS development include smoking and obesity (8). In addition, HS can occur with several co-morbid immune-mediated inflammatory diseases (IMIDs), notably inflammatory bowel disease (IBD) (9).

Clear evidence suggests the involvement of pro-inflammatory cytokines in immune dysregulation in HS, with elevated levels of tumor necrosis factor (TNF)-α, interleukin (IL)-1ß, IL-17 and interferon (IFN)-γ observed in HS lesions (5, 10, 11). Data also indicate the involvement of T helper (Th) cells, which accumulate in HS lesions, in the pathogenesis of HS (11, 12). In addition, studies have shown that antimicrobial peptides (AMPs) like cathelicidin (LL-37) and human β-defensin are increased in HS lesions compared with normal skin of HS patients (13). The use of TNF-α inhibitors such as adalimumab and infliximab have been associated with improvements in immune dysregulation in HS and support the importance of local molecular drivers in the pathogenesis of HS (1, 14, 15).

Furthermore, mutations in γ-secretase genes, whose gene products act on many substrates including Notch (16), suggest that Notch or other substrates of γ-secretase may play a role in the pathogenesis of HS. Interestingly, γ-secretase knock-out mice are characterized by a phenotype of multiple cutaneous cysts, a key feature of HS (17). To date it remains unclear whether the effects of Notch on follicle development or its immune role play a significant role in HS pathogenesis.

Rapidly evolving understanding in the auto-inflammatory arena is needed to improve awareness of HS, disease management, and ultimately improve patient outcomes. The aim of this systematic literature review was to summarize recent findings on the pathogenesis of HS and its syndromic forms, and to identify common pathways involved in HS pathogenesis and other IMIDs. Ultimately, we integrate the molecular pathways into a cohesive pathogenic model.

METHODS

A systematic review of recent original research was conducted according to the Preferred Reporting Items for Systematic review and Meta-Analyses Protocols (PRISMA-P) 2009 statement to identify the factors involved in the pathogenesis of HS (18).

A two-stage review process of PubMed/Medline and EMBASE databases was conducted using search strings designed to recognize studies reporting on auto-inflammatory disorders within the scope of HS (see **Supplementary Tables 1, 2**). The initial screening review identified studies published in English language from January 2007 through September 2017. After removing duplicate records, two reviewers independently screened the titles and abstracts in a double-blind manner and excluded those that did not meet the screening review inclusion criteria (**Supplementary Table 3**). Results were reviewed by a senior analyst for authentication and resolution of disagreements between the reviewers. Identified publications were reassessed according to the full-text review inclusion criteria; at this stage, the publication period was narrowed to January 2013 through September 2017 on the basis of the number of publications identified during screening; this was to ensure focus on the most recent data (**Supplementary Table 3**). Two reviewers, overseen by a senior analyst, independently assessed the remaining full-text copies of all relevant publications and any duplicate, low-quality or outdated publications were excluded. The bibliographies for all publications selected in the full-text review were also manually checked for relevant references.

Data were extracted from each relevant publication on study design (study setting, data source, study period), patient characteristics (sample size, mean age, sex, HS severity, disease location, notable comorbidities, smoking status), immunogenetic factors (genes, mutations or proteins involved), environmental factors (microbiological pathways, obesity, smoking, mechanical stress, sex, hormones), inflammatory pathway and cytokine status (pro-inflammatory, anti-inflammatory, proliferation, and growth factors).

In addition to the systematic review process, two informal literature searches were performed using the same databases, to facilitate discussion of the data. First, immunogenetic factors of HS are compared with other IMIDs such as Crohn's disease (CD), ulcerative colitis (UC), ankylosing spondylitis (AS), psoriasis and psoriatic arthritis (PsA), pyoderma gangrenosum (PG), and Behçet's disease. Second, actual therapeutic options for HS are briefly discussed on the basis of the inflammatory pathways involved in the pathogenesis of HS.

RESULTS

The initial search in PubMed and EMBASE identified a total of 3,580 records. Of these, 230 publications were screened for full-text review and 32 publications were selected for data extraction (**Figure 1**). Data from an additional three publications were also reviewed as they were considered to provide relevant information; two had been excluded during the systematic search

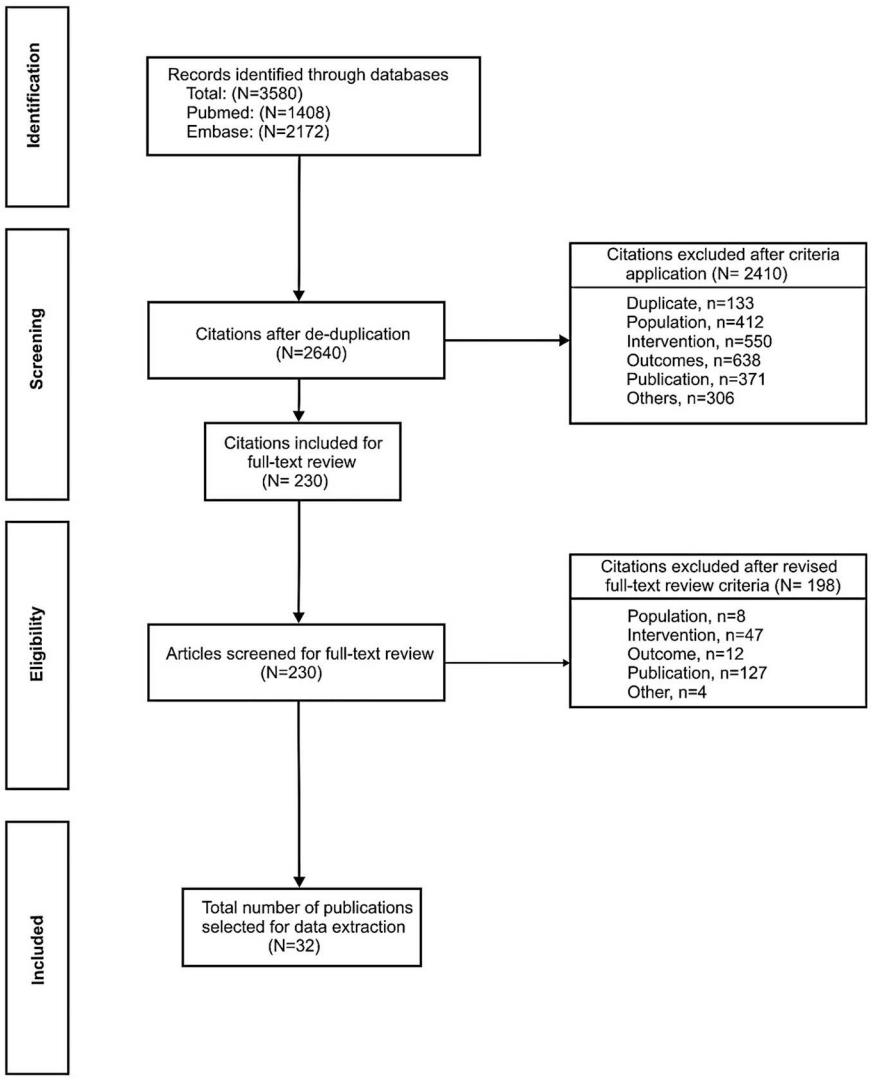

FIGURE 1 | PRISMA flow diagram of included studies. To supplement the 32 publications identified through the systematic process, data from three additional publications were also reviewed as they were considered to provide relevant information; two had been excluded during the systematic search on the basis of their publication type (editorial/letter) (19, 20) and one was published subsequently to the search end date (21).

on the basis of their publication type (editorial/letter) (19, 20) and one was published subsequently to the search end date (21).

The data extracted from these 35 publications were largely derived from four main lines of investigation in patients with HS and its syndromic forms: (1) genetic analyses (covered by 16 studies); (2) inflammatory marker levels (12 studies); (3) microbe analyses in lesions/expression of antimicrobial peptides (6 studies); and (4) contribution of physiological and environmental risk factors (11 studies) (**Table 1**).

Genetics

Loss of Function Mutations in γ-Secretase Complex Genes

Three studies identified by our review evaluated *NCSTN* gene mutations for the nicastrin protein subunit of γ-secretase in connection with HS pathogenesis (25, 26, 31). Genetic analyses in Chinese and Japanese families identified mutations in the *NCSTN* gene (c.647A>C, c.223G>A and c.582+1delG) carried by affected family members but not unaffected family members or healthy controls (25, 26). Meanwhile, an *in vitro* study in familial HS identified that mutations in *NCSTN* affect downstream signaling through Notch and/or phosphoinositide 3-kinase (PI3K) (31). However, NCSTN mutations in HS did not enhance cytokine production in LPS-stimulated peripheral blood mononuclear cells (20).

Mutations of Proline-Serine-Threonine Phosphatase Interacting Protein 1

Proline-serine-threonine phosphatase interacting protein 1 (PSTPIP1) is a cytoskeleton-associated adaptor protein, highly expressed in hemopoietic cells (29). The protein manifests its immunomodulatory effects through downregulation of CD2

TABLE 1 | Characteristics of included studies.

Factors associated with HS	Studies		Patients	Study location (n)	References
	Total, n	Per condition (n)	Per condition (n)		
Genetics	16	HS/AI (8) PG (2) PAPA (2) PAC (1) PASH (2) PAPASH (1) SAPHO, RA, AS, SPA (1)	HS/AI (368) PG (15) PAPA/PAPA-like (∼12)[α] PAC (1) PASH (8) PAPASH (1) SAPHO (71), RA (125), AS (67), SPA (35)	Europe (7) Asia (5) Middle East (2) North America (2)	(3, 19, 20, 22–34)
Inflammatory markers	12	HS/AI (10) PG (2) PASH (1)	HS/AI (351) PG (15) PASH (7)	Europe (9) North America (2) Asia (1)	(5, 28, 31, 33, 35–42)
Microbiome	6	HS/AI (6)	HS (297)	Europe (6)	(43–48)
Physiological and environmental risk	11	HS/AI (11)	HS (738)	Europe (10) Asia (1)	(21, 26, 43, 45–52)

[α]*Marcos et al. (27) used donated cell cultures (n = 2). Note that three publications were included in addition to the 32 identified by the systematic review (see text for details) (19, 20, 52). AI, acne inversa; AS, ankylosing spondylitis; HS, hidradenitis suppurativa; PAPA, pyogenic arthritis, pyoderma gangrenosum, and acne; PAC, pyoderma gangrenosum, acne and ulcerative colitis; PASH, pyoderma gangrenosum, acne and suppurative hidradenitis; PG, pyoderma gangrenosum; RA, rheumatoid arthritis; SAPHO, synovitis, acne, pustulosis, hyperostosis, and osteitis; SPA, seronegative spondyloarthropathy.*

(-triggered adhesion, regulation of c-Abl tyrosine kinase activity, and interaction with other immunity-related proteins including the Wiskott–Aldrich syndrome protein (WASp) (28) and pyrin, the familial Mediterranean fever (FMF) protein (29).

There is now evidence of mutations to the *PSTPIP1* gene in cases of pyoderma gangrenosum, acne and suppurative hidradenitis (PASH) and pyogenic arthritis, pyoderma gangrenosum, acne and suppurative hidradenitis (PAPASH) syndromes (19, 24). A p.E277D missense mutation was detected in the PASH case (24), whilst the patient with PAPASH had a heterozygous missense mutation (c.1213 C>T [p.Arg405Cys]) in exon 15 of *PSTPIP1*. Variations have also been reported in the *PSTPIP1* gene in other related syndromic conditions (22, 28, 29). First, a genetic analysis in a patient with a PAPA-like syndrome revealed a recessive inheritance pattern with a homozygous *PSTPIP1* mutation (c.773G>C and p.Gly258Ala), in contrast to a previously reported heterozygous polymorphism (22). Secondly, a patient with aggressive PG was found to have a novel *PSTPIP1-R405C* mutation (28). Data from this case study indicated that endogenous PSTPIP1 negatively regulates macrophage podosome formation and extracellular matrix degradation. Thirdly, a novel mutation in the *PSTPIP1* gene resulted in a case of pyoderma gangrenosum, acne and ulcerative colitis (PAC). The associated elevated IL-1β levels were responsive to the IL-1R antagonist anakinra (29). It is worth noting, however, that findings from a biochemical study suggested that *PSTPIP1* mutations associated with PAPA syndrome do not alter the negative regulatory role of PSTPIP1 in T-cell activation (27).

Other Genes Implicated in HS

In a study of 139 unrelated patients with HS, single nucleotide polymorphisms of the *IL-12Rb1* gene coding for the IL-12Rb1 receptor subunit did not genetically predispose to HS (23). However, their carriage was directly associated with the phenotype of HS, indicating the importance of the IL-12/IL-23 pathway for the pathogenesis of HS. Findings from a case-control study of two independent and genetically diverse cohorts of patients with HS from Greece ($n = 163$) and Germany ($n = 98$) suggested that the copy number of the β-defensin gene cluster (DEFB) both confers susceptibility for HS and modulates the disease phenotype (30).

In a study involving 298 Han Chinese patients with a range of auto-inflammatory diseases (Synovitis, Acne, Pustulosis, Hyperostosis and Osteitis [SAPHO], rheumatoid arthritis, AS and seronegative spondyloarthropathy), an AS-associated single-nucleotide polymorphism (rs6908425 in *CDKAL1*) was associated with the risk of developing SAPHO syndrome (32).

A genetic analysis of auto-inflammation in PG (13 patients) and the syndromic form PASH (7 patients) identified mutations in a range of auto-inflammatory genes (*MEFV*, *NLRP3*, *NLRP12*, *NOD2*, *LPIN2,* and *PSTPIP1*), suggesting the involvement of inflammatory pathways such as NLRP inflammasomes, cystolic pattern recognition sensors, the innate immune system, and IL-1β signaling (33).

In addition to the genetic analyses, two biochemical studies implicated other proteins in the pathogenesis of HS. Microarray data from one study suggested altered sphingolipid metabolism in HS skin lesions compared with normal skin (3).

In a study of surgically excised skin or skin punch biopsies, HS skin lesions showed on average 25-fold higher lipocalin 2 (LCN2) mRNA expression levels compared with the skin of healthy donors (34).

Inflammatory Markers

There is increasing interest regarding the role markers of inflammation in patients with HS or other syndromic forms. A study in 14 patients with HS reported TNF-α-positive

inflammatory cells in the dermis of patients but not in healthy controls (35). A study comparing the presence of different inflammatory cytokines in wound fluid specimens demonstrated elevated levels of IFN-γ and TNF-β in HS lesions compared with samples from age-matched chronic wound patients (5).

A retrospective study of HS outpatient medical files found a significant association between C-reactive protein (CRP) levels and neutrophil count with HS disease severity (36). A second study reported elevated serum CRP levels in patients with HS compared with healthy volunteers (38). A number of studies have reported elevated mRNA and/or protein levels of interleukins in the skin or serum. Alterations in the skin have been reported for IL-1β (33), CXCL-8/IL-8 (33, 37), IL-17/IL-17A (33), IL-32 (42), and IL-36/IL-36α/IL-36β/IL-36γ (37, 41). Alterations in the serum have been reported for IL-1β (38), IL-6 (38), CXCL-8/IL-8 (38), IL-10 (38), IL-12p70 (38), and IL-17/IL-17A (38, 39).

Keratinocytes isolated from non-lesional skin of patients with HS exhibited a pro-inflammatory profile in addition to an enhanced production of AMPs such as hBD-2, psoriasin (S100A7), and calgranulin (S100A8) (44), indicating that the skin immune system is already activated in the steady state.

Microbiome

A number of studies investigated bacterial cultures from HS lesions and generated evidence implicating the involvement of microbes in disease pathogenesis. A histologic study of 42 patients with chronic HS identified bacterial aggregates (biofilms) in 67% of chronic lesion samples and in 75% of perilesional samples (47). The same author group conducted a case-control study of punch biopsy specimens and demonstrated that the microbiome in patients with HS differs significantly from that in healthy controls in both lesional and non-lesional skin (48). A microbial analysis of lesional vs. unaffected skin from 65 patients with HS identified anaerobic microbes in 83% lesions vs. 53% control samples, and the microbiome varied with disease severity (45). These bacteria were associated with low pathogenicity. An extensive prospective microbiological study identified two opportunistic bacterial pathogens associated with HS lesions (*S. lugdunensis* and anaerobic actinomycetes) (43). These pathogens can cause abscesses and severe infections. A cross-sectional study of 50 patients reported that bacterial colonization was correlated with severity and localization of HS lesions (46). Over two-thirds (68.8%) of patients with both aerobic and anaerobic bacteria had the most severe grade of HS (Hurley stage III).

Physiological and Environmental Risk Factors

Findings from the literature review supported the involvement of previously suggested physiological and environmental risk factors, such as smoking and obesity, in HS (36, 49, 51). A postal follow-up survey study ($n = 212$) found the chance of remission from HS may be improved in non-smokers vs. smokers, and in non-obese (body mass index [BMI] <30) vs. obese patients (49). In contrast, a retrospective study of inflammatory serum markers in HS outpatients found no association between smoking status and HS severity but smoking was associated with increased neutrophil counts (36). This study did find an association between increased BMI and HS severity, whereas there was no correlation between BMI and neutrophil counts.

Related to obesity, an analysis of 14 obese patients with HS described the role of mechanical stress (for example on the abdomen at the level of the waistband) in promoting the "Koebner phenomenon" in HS (51). The development of lesions at sites of traumatized but previously uninvolved skin highlights the importance of localized environmental factors in HS development. A hospital-based cross-sectional study conducted in the Netherlands reported a significantly higher average BMI in 106 patients with HS vs. 212 general dermatological patients (21). Among those patients identified as obese, bodyweight distribution was more peripheral in patients with HS than those without, consistent with enhanced friction due to overlapping skin folds.

Kromann and colleagues reported no clear effect of pregnancy or menopause on HS symptoms (49). However, in a cross-sectional survey based study, a substantial subset of women did experience HS-related alterations, with deterioration of HS around menses and amelioration of symptoms during pregnancy reported in 43% ($n = 80$) and 30% ($n = 29$) of the respondents, respectively (52).

Evidence for Shared Pathology With Other IMIDs

To consider the above findings in relation to the pathogenesis of other established IMIDs, an informal literature review was conducted. Inflammatory bowel disease (CD and UC), AS, psoriasis, PsA, PG and Behçet's disease are characterized by different pathogeneses but they also share common immunological, genetic and risk factors (**Table 2**).

Several cytokines are systemically-raised in many of these IMIDs, particularly those implicated in the Th1 and Th17 responses, including TNF-α, IL-12/23, IL-17, IL-12, IFN-γ, IL-1β, and the IL-1 family including IL-36 (33, 37, 60, 67, 73). Several of the inflammatory cytokines have also been shown to be upregulated in HS [e.g., IFN-γ (5), IL-2, TNF-α (33, 35) and TNF-β (5)] are produced by Th1 cells, implicating the Th1 response in the pathogenesis of HS. Furthermore, the IL-36 family, also found to be upregulated in HS (37, 41), plays an important role in the modulation of Th1 and Th17 immune responses.

Data also support the notion of shared genetic pathways of inflammation. For example, *NOD2* mutations in CD are identified in PG and PASH (33) but conflicting evidence concerns their association with psoriasis and PsA (63, 75). *MEFV* mutations are found in FMF as well as PG and PASH; co-occurrence of FMF and HS is not uncommon (33, 76). Other genes conferring susceptibility in CD, such as *OCTN,* are associated with PsA (63).

In addition to shared genetic factors, the overlap in risk factors observed for different IMIDs also highlights the similar mechanisms that account for them. For example, smoking may confer a protective role in the pathogenesis of UC, PG and Behçet's disease but increases susceptibility in CD, AK, psoriasis

TABLE 2 | Pathogenesis of established immune mediated inflammatory diseases in relation to hidradenitis suppurativa.

Disease	Disease overview	Key[α] genetic factor(s)	Key[α] cytokine profile	Biologics	Risk factors	References
HS	Inflammatory skin disease with genetic, immunological, and environmental background	γ-secretase (NCSTN), PSTPIP1	Th1, Th17 IL-1β, 6, CXCL/IL-8,12, 17, 23, IFN-γ, TNF-α	Anti-TNF-α inhibitors	Smoking, obesity, mechanical friction	(5, 19, 24–26, 31, 33, 36–38, 41, 49, 51)
IBD						
CD	Imbalance between gut microbiome and host immune system with genetic background	NOD2 (CARD15), ATG16L1, IRGM, FUT2, OCTN, TNFSF15, IL10, IL12B, IL23R, HLA, STAT3, JAK2, TNFSF15, MUC1	Th1, Th17 IL-1β, 6, 12, 17, 23, IFN-γ, TNF-α	Anti-TNF-α inhibitors	Smoking, diet, vitamin D deficiency, medications, enteric infections	(53–55)
UC	Imbalance between gut microbiome and host immune system with genetic background	HNF4A, CDH1, LAMB1, GNA12, SLC9A, TNFSF14, ECM1, IL10, IL12B, IL23R, HLA, STAT3, JAK2, TNFSF15, MUC1	Th2, Th17 IL-1β, 6, 12, 13, 17, 23 TNF-α	Anti-TNF-α inhibitors	Non-smoking, appendectomy, diet, vitamin D deficiency, medications, enteric infections	(55)
AS	Imbalance between gut microbiome and host immune system with genetic background	HLA-B27, HLA-B40, ERAP1/2, CARD9, IL12B. IL23R, IL27, STAT3, JAK2, TYK2	Th17 IL-6, 17, 22, 23, 26, IFN-γ, TNF-α	Anti-TNF-α inhibitors	Infection, smoking, testosterone	(56–62)
Psoriasis	Inflammatory skin disease with genetic and immunological background	PSOR1, HLA-C, ERAP1, LCE3D, IL12B, IL23R, TNFAIP3, ZNF313, TYK2,	Th1, Th17 IL-2, 17, 22, 23, 26, TNF-α, IFN–γ	Anti-TNF-α inhibitors, T cell targeted therapies	Obesity, infection	(63–68)
PsA	Inflammatory arthritis associated with psoriasis with genetic, immunological, and environmental background	HLA-B. HLA-C, OCTN IL12B, IL23R	Th1, Th17 IL17, 23, TNF-α	Anti-TNF-α inhibitors	Physical trauma, smoking, obesity, infection, heredity	
PG	Inflammatory, ulcerating, neutrophilic skin disease with genetic, immunological, and environmental background	MEFV, NLRP3, NLRP12, NOD2, LPIN2, PSTPIP1	IL-1β, 17, TNF-α	Anti-TNF-α inhibitors	Physical trauma, non-smoking, metabolic syndrome	(33, 69–71)
Behçet's disease	Multi-systemic, inflammatory, vasculitis with genetic, immunological, and environmental background	HLA-B5, ERAP1 IL10, IL12RB2, IL-23R,STAT4, CCR1-CCR3, KLRC4, TNFAIP3, FUT2	Th1, Th17 IL-6, 11, 17, 21, 22, 26, TNF-α, Chitinase3-like1, gp130/sIL−6Rb, sTNF-R1, sTNF-R2	Anti-TNF-α inhibitors, anti-IL1, INF-α	Non-smoking, obesity, infection	(69, 72–74)

[α]*Data summarize key genes and cytokines involved in the pathogenesis of these diseases but many other genes, cells types and mediators are involved. AS, ankylosing spondylitis; CD, Crohn's disease; HS, hidradenitis suppurativa; PG, pyoderma gangrenosum; PsA, psoriasis and psoriatic arthritis; UC, ulcerative colitis.*

and PsA (**Table 2**). With the exception of AK and conflicting evidence for Behçet's disease (56, 74), the pathogenesis of most IMIDs appears unrelated to sex-specific factors. Understanding the distinct and shared genetic, immunologic and risk factor profiles of IMIDs will aid the development of effective treatments to target the pathogenic mechanisms involved and modify the disease course.

It has previously been proposed that the link between HS and other conditions with demonstrated systemic pathology may be attributed to common genetic or environmental factors and/or shared inflammatory pathways (77).The data identified in this review demonstrate the significant overlap between the pathogenesis of HS and the aforementioned IMIDs. The most striking similarity among these diseases is that of aberrations in the innate immune response, particularly the IL-23/Th17 pathway (**Table 2**).

Integrated Viewpoint on HS Pathogenesis "Sequence of Events"

By identifying the latest publications on the pathogenesis of HS and evaluating it in the context of more established pathogenic mechanisms for known IMIDs, this review has collated substantial evidence that HS is a chronic immune-mediated auto-inflammatory disease with a multifactorial pathogenesis.

Four key themes have emerged from this review. First, genetic factors play a key role in causing HS. Mutations in a range of genes, including *NCSTN* mutations in the γ-secretase complex and *PSTPIP1* mutations, are directly associated with auto-inflammatory disease (26, 27, 29, 31). However, the majority of HS cases appear to be non-familial, suggesting the existence of separate subsets and the need for stratification within patients diagnosed with HS (25). Secondly, the up-regulation of cytokines

including TNF-α and a range of cytokines (predominantly Th17-related) are connected to auto-inflammatory mechanisms in the pathogenesis of HS (5, 35, 38). Thirdly, there is an alteration in the local microbiome of normal-appearing vs. lesional skin (43, 45, 47, 48). Data also suggest that bacterial aggregates are associated with inflammation of chronic HS lesions, and it is proposed that they most likely occur as a secondary event, possibly due to predisposing local anatomical changes such as sinus tracts (tunnels), keratinous detritus and dilated hair follicles (47). Finally, enhancement of HS risk occurs via a range of physiological and environmental factors such as smoking, obesity and mechanical friction (21, 36, 49, 51).

On the basis of the evidence reviewed here, we are able to take a cohesive view and to propose a three-stage sequence of events that contribute to the pathogenesis of HS. This integrated viewpoint is illustrated schematically in **Figure 2**.

The first event is follicular occlusion with subsequent dilation. This may be driven by endogenous factors in individuals harboring a genetic predisposition for an enhanced risk of infundibular keratinisation and cyst formation. Exogenous factors such as smoking, mechanical friction and metabolic

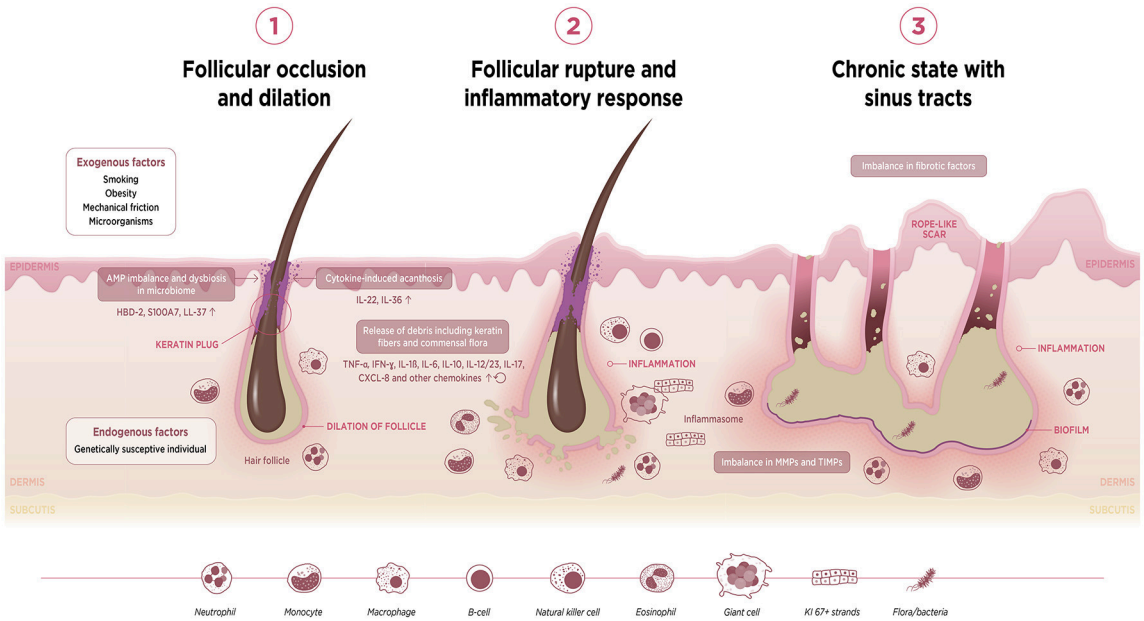

FIGURE 2 | Schematic diagram to illustrate postulated sequence of events underlying HS pathophysiology. AMP, antimicrobial protein; HBD, human beta-defensin; IFN, interferon; IL, interleukin; MMP, matrix metalloproteinase; TIMP, tissue inhibitor of metalloproteinase; TNF, tumor necrosis factor.

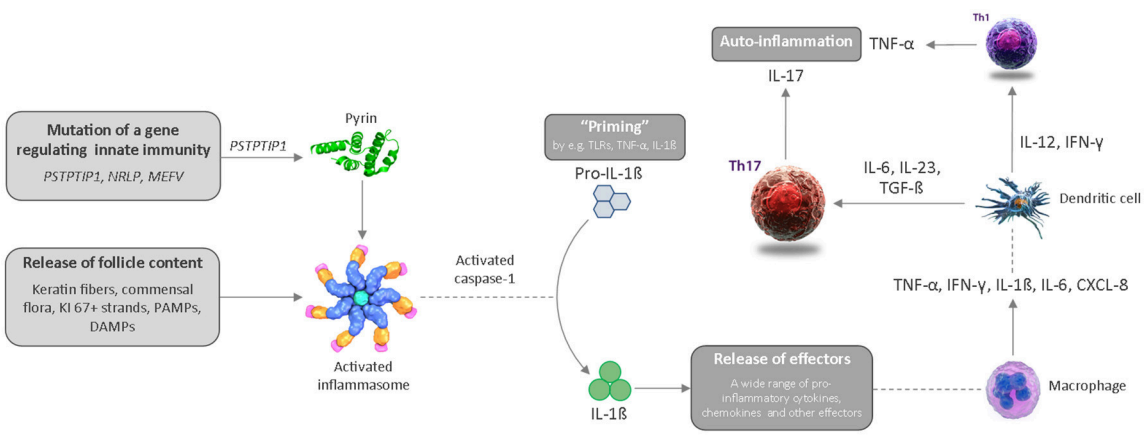

FIGURE 3 | Schematic diagram illustrating activation of the inflammasome in HS and related syndromes, which ultimately results in an auto-inflammatory immune response. CXCL, chemokine ligand; DAMP, danger-associated molecular pattern; IL, interleukin; IFN, interferon; PAMP, pathogen-associated molecular pattern; Th, T helper cell; TLR, toll-like receptor; TNF, tumor necrosis factor.

changes such as obesity—which is associated with acanthosis—also contribute to occlusion of the follicular isthmus. Furthermore, occlusion of the hair follicle may lead to a dysregulation of the homeostatic keratinocyte symbiosis and microbial dysbiosis, making the skin prone to a Th1/Th17-driven inflammatory disease.

The second event is rupture of the dilated follicle. The scattering of follicle content in the dermis including keratin fibers, commensal flora or pathogen- and damage-associated molecular patterns (PAMPs/DAMPs) triggers an acute and severe immune response. The anatomical location, i.e., the inverse body areas, and enhanced mechanical friction at these predilection sites facilitates the inward rupture and extension of inflammation. We argue that release of the follicular debris into the dermis results in simultaneous activation of multiple inflammatory pathways, particularly Th17/IL-23, the (NLRP) inflammasomes and innate receptors (toll-like receptors, TLRs such as TLR2). Activation of the inflammasome in HS and related syndromes including PASH and PAPA(SH) is illustrated schematically in **Figure 3**. This is accompanied by histological alterations with a diverse cell infiltrate characterized by the mixed participation of monocytes, neutrophils, multinucleated giant cells, B-cells, plasma cells, T-cells, and natural killer cells, leading to an erythematous nodule or fluctuating abscess.

The third event is chronic inflammation with sinus tract or tunnel formation. Following follicular rupture, sequestered proliferating Ki-67+ epithelial strands promote continuous activation of the immune system. The presence of epithelial strands in the dermis, in addition to an imbalance in matrix metalloproteinases (MMPs) and tissue inhibitors of metalloproteinase (TIMPs), and increased activity of fibrotic factors such as tissue growth factor (TGF)-ß 1-2-3, may lead to scarring and the development of sinuses/tunnels or fistulae, a hallmark of chronic HS. These intracutaneous (partly) epithelialized cavities provide an excellent habitat for biofilm-producing bacteria, which are able to continuously trigger inflammation with associated purulent drainage. Furthermore, we hypothesize that circulating pro-inflammatory cytokines and chemokines from chronic lesions may activate the immune system of the hair follicle in distant predilection sites.

Current Therapeutic Options for HS

HS management usually consists of the combination of both medical therapies and surgical interventions. The main treatment goal, to improve patients' quality of life, can be achieved by reducing the inflammation-related pain and purulent discharge, limiting the incidence and duration of flares, and removing chronic lesions using surgical techniques (78). A short overview of current treatment options including the therapeutic target and/or the suggested pathophysiological link(s) is depicted in **Table 3**. These data summarize anti-inflammatory therapies in addition to surgery and lifestyle changes such as smoking

TABLE 3 | Short overview of actual treatment options for hidradenitis suppurativa, based on Van Straalen et al. (78).

Treatment options[α]	Therapeutic target or suggested pathophysiological link	References[ß]
LIFESTYLE CHANGES		
Smoking cessation	Reduction of follicular acanthosis; less xenobiotic metabolism, e.g., via the aryl hydrocarbon receptor, with potential restoration of alterations in the immune response	(8, 49)
Weight loss	Improvement of the metabolic state, thereby reducing follicular acanthosis; less mechanical friction as a result of less overlapping skin folds with potential restoration of the local microbiome	(79)
LOCALLY ADMINISTERED AGENTS		
Clindamycin 1% lotion	Anti-inflammatory and antibacterial properties; for acute lesions	(80)
Resorcinol 15% cream	Removal of follicular plugging (prophylactic effect) and early rupture of an abscess due to its keratolytic properties; antiseptic properties	(81, 82)
Intralesional triamcinolone	Pan-cell inhibitor; for acute lesions to eradicate the inflammatory cell infiltrate	(83)
SYSTEMIC ANTIBIOTICS		
Tetracyclins; clindamycin and rifampicin; moxifloxacin, rifampicin, and metronidazole	Various modulations in the immune response, e.g., inhibition of neutrophilic migration and chemotaxis, inhibiting IL-1ß and TNF-α secretion, upregulation of IL-10, inhibition of the angiogenesis, and suppressing T-cell function; antibacterial effects	(80, 84–88)
BIOLOGICS		
Adalimumab, infliximab; ustekinumab; anakinra; MABp1	Monoclonal antibodies targeting TNF-α, IL-12/23p40, IL-1R, and IL-1α, respectively	(14, 89–92)
SMALL MOLECULE DRUGS		
Apremilast	Inhibits PDE-4 in various inflammatory cell types, thereby modulating several pro- and anti-inflammatory cytokines	(93, 94)
SURGERY		
Deroofing, excision	Removal of irreversibly damaged skin, i.e., sinus tracts or nodules/cysts recurring on fixed locations	(95, 96)

[α]*Data summarize the most important medical and surgical therapeutic options in addition to lifestyle changes. The majority of the remaining evidence to guide management decisions is based on case reports, case series with fewer than 10 patients, small cohort studies, and expert opinion, which are all not included in this overview.* [ß]*Based on highest level of evidence or largest cohort for each intervention. IL, interleukin; PDE-4, phosphodiesterase-4; TNF, tumor necrosis factor.*

cessation and weight loss. First-line treatment options include the use of antibiotics with anti-inflammatory properties, e.g., the tetracyclins and the combination of clindamycin and rifampicin (80, 87). The anti-TNF-α agents adalimumab and infliximab should be considered, respectively, as first- and second-choice biologics for moderate-to-severe HS after failure of systemic antibiotics (14, 90). Ustekinumab (anti-IL-12/23p40) is potentially effective in the treatment of HS (89), whereas the results of two randomized controlled trials investigating IL-17 antagonists are awaited (ClinicalTrials.gov Identifiers NCT02421172 and NCT03248531). Other promising treatment options are MABp1, targeting IL-1α for HS patients not eligible for adalimumab, and apremilast for patients with moderate HS (92, 94).

LIMITATIONS

This review was subject to certain limitations. PubMed/Medline and EMBASE were the only two databases used to identify eligible studies. Any studies published in journals not listed in PubMed/Medline and EMBASE are omitted from this review. The extent of recent published evidence relating to the pathogenesis of HS and related syndromic conditions is limited. Finally, the review of other IMIDs for comparison with HS was not systematic, and conclusions drawn from this informal review must be interpreted with this methodology in mind.

FUTURE RESEARCH

Large gaps still remain in the understanding of the pathogenesis of HS. Therefore, further research is warranted to ultimately improve the management and treatment of patients with this disease. Genetic research should aim to add more detail to the proposed mechanism by which loss of function of NCSTN or of other γ-secretase proteins causes familial HS and to better stratify patients with HS. Immunologic studies should focus on molecular drivers of tissue inflammation and injury in HS and the relationship between HS cytokine profile and disease activity. Microbiome research is needed to better characterize the disruption to the microbial ecosystem and to elucidate whether the disruption causes the disease or whether the disease causes the dysbiosis. High-throughput metagenomic methods can make this work possible. Finally, it will be important to focus research on the interaction of environmental factors and immunogenetic factors.

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SUPPLEMENTARY MATERIAL

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fimmu.2018.02965/full#supplementary-material

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GENERAL COMMENTARY
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Commentary: Hidradenitis Suppurativa: A Systematic Review Integrating Inflammatory Pathways Into a Cohesive Pathogenic Model

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A Commentary on

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We read with great interest the article "*Hidradenitis Suppurativa: A Systematic Review Integrating Inflammatory Pathways Into a Cohesive Pathogenic Model*" by Vossen et al. (1). The authors have admirably integrated data from genetic, cytokine, and microbiological studies in Hidradenitis Suppurativa (HS) into a three-phase pathogenic model of disease.

However, we have concerns that inherent bias in the collated data may introduce inaccuracies into the proposed model, and that highlighting the "*known unknowns*" in the pathogenesis of HS is needed to advance our knowledge of this disease.

CRITICAL EVALUATION OF GENETIC POLYMORPHISMS IN HS USING ACGM CRITERIA

Evidence for the role of gamma secretase sequence variants and notch signaling in HS is strong but incomplete (2). Critical evaluation of known sequence variants (2) has highlighted a *"likely pathogenic"* role for two of the three variants reported by Vossen et al. (1) (c.223G>A, c.582+1delG) and *"uncertain significance"* to the remaining variant (c.647A>C) as defined by American College of Genetic Medicine (ACGM) criteria (2). A recent systematic review (2) identified 17 of 41 variants in HS as of "*uncertain significance.*" Genome Wide Association Studies (GWAS) as well as functional and proteomic data linking identified sequence variants to the inflammatory mechanisms driving HS are currently not available. Both areas of research are vital in further evaluating the role of genetic variants in HS pathogenesis. It remains plausible that a complex polygenic model better describes observations in some cases of familial HS (2). This further emphasizes the importance of undertaking GWAS in this disorder.

DIFFERENCES IN NOTCH SIGNALING, THE TH17 AXIS AND EPIDERMAL DIFFERENTIATION IN HUMAN AND MURINE MODELS

Vossen et al. (1) correctly remark that the "*many substrates*" (1) of gamma secretase imply that Notch signaling may not be the sole causative pathway resulting in active disease in HS (2). In light

of this, one must heed caution in extrapolating the results of gamma secretase knockout mouse models to human disease, particularly given the known differences in Notch-AhR-IL-22-Th17 pathways between mice and humans (3, 4). This is especially pertinent given the role of the Th17 axis in HS (1, 5). Murine models of AhR stimulation demonstrate a concomitant elevation of IL-22 and IL-17A production whereas human IL-22 production is accompanied by IL-17A reduction due to expansion of IL22+ IL-17- CD4+ T cells (4). This additional layer of complexity may explain discrepancies in studies of lesional IL-17 levels in HS, but also highlights the caution required in interpreting immunological data from animal models in HS (6). Additionally, gamma-secretase-Notch pathway knockout mouse models demonstrate disturbed epidermal differentiation (both delayed and premature spinous differentiation), barrier function (leading to lethal phenotypes) and alopecia (7). Such manifestations are not seen in human cases of loss-of-function variants in the gamma-secretase-Notch pathway (2, 5, 6) implying the possibility of important differences in the roles of the gamma-secretase-Notch pathway in epidermal differentiation between murine and human models as previously discussed by van der Zee et al. (6).

MECHANICAL FRICTION AND BODY-FOLD OCCLUSION MAY INDIRECTLY DRIVE HS VIA INCREASING LOCALIZED MOISTURE AND PH ENCOURAGING PROTEOLYTIC ACTIVITY OF *PORPHYROMONAS* SP.

The follicular occlusion paradigm and the role of mechanical friction has been a long-standing component of HS pathophysiology (1). However, in other diseases such as acne, evidence is emerging that microcomedone formation may be secondary to inflammation (8) (mediated by IL-1α) bringing into question whether follicular occlusion is a primary or secondary phenomenon in HS (9). The role of occlusion and friction can be re-examined in light of insights into the microbiological contribution to HS pathogenesis. Obesity, heat and occlusion all have direct alterations to the cutaneous microbiome through elevation in pH and increases in moisture (10), both conditions which favor the proteolytic activity of implicated bacteria including *porphyromonas* sp. (10, 11), as well as the aberrant innate immune response to such bacteria hypothesized to be an inflammatory driver in select cases of HS (9, 11).

TREATMENT, SELECTION, AND ANALYTICAL BIAS IN HS CYTOKINE STUDIES

In examining the evidence from studies regarding inflammatory pathways in HS, many published studies suffer from treatment, selection, and analytical bias (12). The studies reporting alterations in serum IL-1β, IL-6, IL-8, IL-10, IL-12p70, TNF-α, and IL-17A were conducted on patients undergoing active treatment (including Adalimumab), whilst measurement of lesional IL-32 and IL-36, TNF-α and IL-17A was conducted 3–8 weeks after withdrawal of active treatment (11). Variations in the ratio of disease severity (measured by Hurley staging), BMI and proportion of smokers may also influence the results of these studies. Also, differences in analytical techniques (ELISA vs. electrochemiluminescence) may influence the accuracy of data (11). Future studies need to control or stratify for these potential confounders, or at a minimum acknowledge the potential influence upon results.

THE NEED FOR FURTHER MECHANISTIC STUDIES IN HS

Our understanding of the pathogenesis of HS is far from complete. Critical evaluation of mechanistic studies in HS is necessary in order to compile an accurate, reproducible model of disease. Identifying the major areas in which knowledge is deficient or mechanisms unclear is the only method or rectifying these areas of deficiency. For example, a major unknown in the pathogenesis of HS are the mechanism(s) underlying the development of sinus tracts. Vossen et al. (1) discuss the roles of proliferating epithelial strands, matrix metalloproteinases and TGF-B, however the exact mechanisms remain unclear. It is also unknown why some patients develop aggressive tract and scar formation and others do not (1, 9). The histologic features of early tract formation in HS are reminiscent of impaired wound healing and epithelial-mesenchyme transition (demonstrated by levels of TGF-β, MMP2, and ICAM-1) (9). Investigating these molecular mechanisms may help in the identification of patient at risk for aggressive tract formation or identify new therapies to prevent or ameliorate existing disease.

Vossen et al. (1) are to be commended for their efforts in synthesis of their pathogenic model for HS, however we wish to highlight the "*known unknowns*" in our understanding of this disease and the impact that bias in existing data may have in our current understanding of HS pathogenesis.

AUTHOR CONTRIBUTIONS

JF is responsible for the conception, design, writing, and review of this manuscript.

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REVIEW
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Insights Into the Pathogenesis of Sweet's Syndrome

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Sweet's syndrome, also known as Acute Febrile Neutrophilic Dermatosis, is a rare inflammatory condition. It is considered to be the prototype disease of neutrophilic dermatoses, and presents with acute onset dermal neutrophilic lesions, leukocytosis, and pyrexia. Several variants have been described both clinically and histopathologically. Classifications include *classic Sweet's syndrome, malignancy associated, and drug induced*. The cellular and molecular mechanisms involved in Sweet's syndrome have been difficult to elucidate due to the large variety of conditions leading to a common clinical presentation. The exact pathogenesis of Sweet's syndrome is unclear; however, new discoveries have shed light on the role of inflammatory signaling, disease induction, and relationship with malignancy. These findings include an improved understanding of inflammasome activation, malignant transformation into dermal infiltrating neutrophils, and genetic contributions. Continued investigations into effective treatments and targeted therapy will benefit patients and improve our molecular understanding of inflammatory diseases, including Sweet's syndrome.

Keywords: acute febrile neutrophilic dermatosis, neutrophilic dermatoses, malignancy, drug induced, autoinflammation, clonality, hematology

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INTRODUCTION

Sweet's syndrome (SS) was originally described as "acute febrile neutrophilic dermatosis" by Sweet, (1). His original report was based on the clinical-pathologic presentation of 8 women who presented with acute onset fever, leukocytosis and erythematous, tender plaques with dense neutrophilic infiltration in the dermis. These patients had no evidence of infection and had rapid response to systemic corticosteroids. As additional reports of this newly described pathologic entity surfaced, the syndrome was renamed to recognize Dr. Sweet (2). Subsequent to these initial accounts, thousands of cases have been described in literature. This led to a better understanding and recognition of a multitude of clinical variants and SS classifications. Unfortunately, due to the rarity of SS, epidemiologic information including incidence is unknown.

The traditional description of tender erythematous plaques and nodules remains the prototypical presentation. However, clinical variants including localized neutrophilic dermatosis of the dorsal hands, bullous, subcutaneous, cellulitic, and necrotizing lesions have been reported (3–7). Extracutaneous manifestations have also been reported including involvement with the central nervous system, internal organs and musculoskeletal system (8–10). Histopathologic variants include histiocytoid SS and SS with vasculitis which has been hypothesized to be a secondary reaction (11, 12).

SS is one pathologic entity within the broader neutrophilic dermatoses classification. Neutrophilic dermatoses include SS, pyoderma gangrenosum, neutrophilic eccrine hidradenitis, and Behçet's disease among others. Each disease has some overlapping pathophysiology with an autoinflammatory component made up of predominately neutrophilic infiltrate. Each entity is distinguished by disease chronicity, tissue involvement, and clinical appearance. Understanding the pathogenesis of SS is important from a diagnostic and therapeutic perspective. In a time of revolution in immunology and targeted therapy the pathways discovered in SS can have broader implications in additional autoinflammatory diseases as well as malignancy.

DISEASE CLASSIFICATIONS AND ASSOCIATIONS

SS has been associated with a multitude of diseases, malignancies and medications at varying frequencies (**Table 1**). Given the unpredictable nature of the disease, it has been difficult to reach conclusions regarding true associations and causations. The temporal relationships and frequency of concurrent processes has led to the recognition of several pathologic relationships. Some authors agree that there are three distinct variants which are important to distinguish, given differential work up and management recommendations. These three subtypes are Classic SS, Malignancy Associated SS, and Drug Induced SS and will be discussed individually and are summarized in **Table 1**.

Classic Sweet's Syndrome (Idiopathic Sweet's Syndrome)

Classic SS is responsible for most SS cases and has a predilection for women. Initial presentation most frequently occurs between age 30 and 60 years (517), but has been reported in multiple pediatric patients including neonates in the first 10 days of life (518). Although considered idiopathic, it has been reported in association with infections, pregnancy, and inflammatory and autoimmune disorders among others (**Table 1**) (13, 30, 330, 435).

Diagnostic criteria for classic SS was proposed by Su and Liu and updated by von den Driesch (254, 519). Diagnosis is based on fulfilling both major criteria and two of the four minor criteria which are presented in **Table 2**.

Drug Induced Sweet's Syndrome

The most commonly reported drug associations are Granulocyte-colony stimulating factor (G-CSF), Azathioprine, and All-trans retinoic acid (ATRA). Most other etiologies are infrequent (**Table 1**). Diagnostic criteria for drug induced SS was suggested by Walker and Cohen (250). It requires all five criteria summarized in **Table 3** be met to establish the diagnosis.

Malignancy-Associated Sweet's Syndrome

It has been suggested that the first reported case of malignancy associated SS was published by Costello 9 years prior to Sweet's disease defining paper (520). Malignancy, both solid tumor and hematologic, have been reported in a large proportion of SS cases (**Table 1**) (521). Specific SS characteristics may represent an increased risk of malignancy, including subcutaneous and histiocytoid histopathologic variants (522, 523). Diagnostic criteria for malignancy associated SS is the same as classic SS, except for the substitution of "an underlying malignancy" as a minor criterion rather than "an inflammatory disease, pregnancy, vaccination or infection" (254, 519).

PATHOGENESIS

Neutrophil Proliferation and Maturation

Just as the associated condition and etiology of SS varies considerably, the pathogenesis is multifactorial and likely non-uniform between subtypes of the disease. The inciting activator of SS, especially classic SS, has not been determined, although cases of hematologic malignancy and initiation of granulocyte colony stimulating factors (G-CSF), all-trans retinoic acid (ATRA), and fms-like tyrosine kinase-3 (FLT3) inhibitors offer a glimpse into one mechanism. G-CSF acts within the bone marrow, serum and tissue, causing neutrophil differentiation, maturation and activation. As a response to pathogens, G-CSF is a part of the innate immune system signaling which is maladaptively elevated in inflammatory states (524). In cases of classic SS, patients with an underlying infection or autoimmunity, the pathologic increase in colony stimulating factors may be the causative agent (525, 526). Endogenously elevated G-CSF levels have been reported in multiple cases of SS, with elevations in serum concentrations correlating with clinical disease severity (127, 524). *In vitro*, SS neutrophils have high rates of apoptosis when isolated. Conversely, when cultured with serum from SS patients, the apoptosis rate is significantly decreased and neutrophil survival is significantly greater (524). This serum enhanced survival suggests elevated G-CSF among other circulating factors contribute to the disease. Both solid tumor and hematologic malignancies can produce colony stimulating factors. In malignancy-associated SS, this paraneoplastic phenomenon might represent an inciting factor in disease progression (127, 527–529). The frequency of drug-induced SS from the exogenous use of G-CSF further reinforces the causative role of G-CSF in SS (517, 530–533). After initiation of G-CSF therapy in SS associated with hematologic malignancies, it is theorized that G-CSF induces differentiation and maturation of leukemic cells which then home to the skin (55, 534). Similarly, ATRA induces the differentiation of promyelocytes in acute promyelocytic leukemia (APL). ATRA has been associated with developing SS in APL and the mature dermal neutrophils may be progeny from differentiated malignant cells. This is evidenced by sequential SS lesional biopsies showing gradual maturation of neutrophils in the dermis mirroring neutrophil maturation in the peripheral blood (181).

Malignant Transformation

Investigations have shown neutrophilic clonality within SS lesions suggestive of either hematologic malignancy transformation into mature dermal neutrophils or localized non-malignant neutrophil stemming from a common dysfunctional progenitor (535, 536). Analysis with fluorescent *in situ* hybridization have shown the SS lesional neutrophils exhibit the same genetic abnormalities as the underlying

TABLE 1 | Conditions and medications coexisting in Sweet's Syndrome in descending order of referenced literature.

Classic SS				**Malignancy associated SS**		**Drug induced SS**	
Autoimmune and autoinflammatory conditions		**Infectious etiologies**		**Hematologic malignancies**			
Ulcerative Colitis	(13–29)	NTM	(30–42)	AML	(43–68)	G-CSF or GM-CSF	(55, 69–87)
Crohn's Disease	(66, 88–104)	HIV	(105–111)	MDS	(43, 112–134)	Azathioprine	(135–147)
Erythema nodosum	(128, 148–159)	TB	(160–166)	CML	(43, 44, 167–177)	ATRA*	(54, 178–188)
Sarcoidosis	(152, 154, 189–196)	URI	(197–200)	APL*	(178–187, 201)	Hydralazine	(202–207)
SLE	(208–214)	Hepatitis C Virus	(110, 194, 215, 216)	Multiple myeloma	(217–226)	Bortezomib	(218, 227–231)
Relapsing Polychondritis	(117, 119, 120, 124, 232–236)	Gastroenteritis	(237–241)	Hairy Cell leukemia	(37, 242–249)	TMP-SMX	(250–254)
Vasculitis	(114, 255–262)	Varicella Zoster Virus	(211, 263)	CLL	(264–269)	Tetracyclines	(270–274)
PG	(17, 28, 275, 276)	Cytomegalovirus	(277, 278)	Hodgkin's lymphoma	(279–281)	NSAID	(282–286)
MSO	(9, 287–289)	Hepatitis B Virus	(290, 291)	Non-Hodgkin's lymphoma	(292, 293)	Azacitidine	(294–299)
Behcet's disease	(300–305)	Parvovirus B19	(306, 307)	CNL	(308)	Vaccination	(108, 309–313)
Ankylosing spondylitis	(15, 91, 314)	Chlamydial Infection	(315, 316)	ALL	(46)	Oral Contraceptive	(317–319)
Rheumatoid arthritis	(164, 320–323)	Herpes Simplex Virus	(36, 324–326)	Juvenile MML	(327)	Lenalidomide	(328, 329)
SCLE	(330–332)	Bacterial Endocarditis	(333–335)	Juvenile CML	(336)	Ipilimumab	(337–340)
Subacute thyroiditis	(341)	Cellulitis	(342–344)	EATL	(345)	Imatinib	(170, 346, 347)
Hashimoto's thyroiditis	(348–350)	Capnocytophaga	(351)	DLBCL	(352)	Vemurafenib	(353, 354)
Autoimmune hepatitis	(278, 355)	Biliary sepsis	(356)	DHL	(357)	Furosemide	(358)
Bronchiolitis obliterans	(359, 360)	Dermatophyte	(361)	CTCL	(362)	Adalimumab	(15, 363)
Cryptogenic pneumonia	(364, 365)	Francisella tularensis	(366)	B cell lymphoma	(367)	Interferon β – 1b	(368, 369)
Multiple sclerosis	(368, 370)	Glandular Tularemia	(371)	**Solid Tumor Malignancies**		Isotretinoin	(372, 373)
Sjogren's syndrome	(164, 374)	Helicobacter pylori	(375)	Breast carcinoma	(268, 324, 376–380)	Sulfasalazine	(381, 382)
Unknown arthritis	(383–385)	HG anaplasmosis	(386)	Prostate Cancer	(133, 387–391)	Clindamycin	(392, 393)
Aseptic meningitis	(394)	Klebsiella cystitis	(395)	Oral SCC	(396–399)	Clozapine	(400, 401)
Autoimmune cholangitis	(265)	Pasteurella multocida	(402)	Cervical cancer	(165, 403, 404)	IL-2 therapy	(405)
Celiac disease	(406)	PCP	(57)	Gastric cancer	(407–410)	Abacavir	(411)
Cryptogenic cirrhosis	(275)	Coccidioidomycosis	(412)	Lung cancer	(217, 413–415)	APAP-codeine	(416)
Dermatomyositis	(417)	Salmonella typhimurium	(418)	Melanoma	(337–339)	Allopurinol	(419)
Dressler's Syndrome	(420)	Sporotrichosis	(421)	Ovarian carcinoma	(422, 423)	Dabrafenib/trametinib	(424)
FMF	(425)	**Other**		Testicular cancer	(426, 427)	Carbamazepine	(428)
Granuloma annulare	(377)	Pregnancy	(19, 429–438)	Bladder Cancer	(389, 439)	Decitabine	(440)
Grave's Disease	(441)	Trauma	(416, 442–450)	Thyroid Carcinoma	(451)	Diazepam	(452)
Hypothyroidism	(453)	Radiation therapy	(177, 398, 454–459)	Adrenal cortex carcinoma	(460)	Fluconazole	(461)
IHCP	(462)	Photoinduced	(463–465)	Merkel cell carcinoma	(466)	Gabapentin	(467)

(Continued)

TABLE 1 | Continued

Classic SS				Malignancy associated SS		Drug induced SS	
Autoimmune and autoinflammatory conditions		**Infectious etiologies**		**Hematologic malignancies**			
Myasthenia gravis	(468)	Chronic Lymphedema	(469–474)	Osteosarcoma	(475)	Infliximab	(476)
Pigmented villonodular synovitis	(477)	Fanconi Anemia	(478–481)	Pheochromocytoma	(482)	Ketoconazole	(483)
Pemphigus vulgaris	(484)	Polycythemia Vera	(485–489)	Tonsil cancer	(490)	Mesalamine	(491)
Still's disease	(492)	Myelofibrosis	(167, 459, 468, 493–497)	Liposarcoma	(498)	Hormonal IUD	(499)
Subacute necrotizing lymphadenitis	(500)	Other Immunodeficiency	(31, 501–503)	Gallbladder adenocarcinoma	(504)	Mitoxantrone	(370)
SAPHO	(505, 506)			Esophageal Adenocarcinoma	(507)	Nitrofurantoin	(508)
Autoimmune thyroiditis	(509)			Rectal adenocarcinoma	(510)	Norfloxacin	(388)
Connective tissue disorder	(511)					Ofloxacin	(90)
						Piperacillin and tazobactam	(265)
						Propylthiouracil	(512)
						Proton pump inhibitor	(378)
						Quinupristin and dalfopristin	(513)
						Ruxolitinib	(494)
						Ticagrelor	(514)
						Topotecan	(515)
						Vedolizumab	(516)
						Vorinostat	(297)

[*] *High proportion of reported Sweet's syndrome cases associated with acute promyelocytic leukemia also received ATRA.*

ALL, Acute lymphoblastic leukemia; AML, Acute myeloid leukemia; APAP, Acetaminophen ; APL, Acute promyelocytic leukemia; ATRA, All-trans retinoic acid; CGD, Chronic granulomatous Disease; CLL, Chronic lymphocytic leukemia; CML, Chronic myelogenous leukemia; CNL, Chronic neutrophilic leukemia; CTCL, Cutaneous T-cell lymphoma; CVID, Common Variable Immunodeficiency; DLBCL, Diffuse large B-cell lymphoma; DHL, Diffuse histiocytic lymphoma; EATL, Enteropathy-associated T cell lymphoma; FMF, Familial Mediterranean Fever; G-CSF, Granulocyte-colony stimulating factor; GM-CSF, Granulocyte macrophage-colony stimulating factor; HIV, Human immunodeficiency virus; HG, Human granulocytic; IHCP, Idiopathic hypertrophic cranial pachymeningitis; IL-2, Interleukin-2; IUD, Intrauterine device; MDS, Myelodysplastic syndrome; MML, myelomonocytic leukemia; MRSA, Methicillin-resistant Staphylococcus aureus; MSO, Multifocal sterile osteomyelitis; NSAIDs, Non-steroidal antiinflammatory drug; NTM, Non-tuberculous mycobacterium; PCP, Pneumocystis carinii; PG, Pyoderma Gangrenosum; SAPHO, Synovitis, acne, pustulosis, hyperostosis, and osteitis; SCC, Squamous cell carcinoma; SCLE, Subacute cutaneous lupus erythematosus; SLE, Systemic Lupus Erythematosus; TB, Tuberculosis; URI, Upper respiratory infection.

TABLE 2 | Diagnostic Criteria for Classic Sweet's Syndrome.

MAJOR CRITERIA
1. Abrupt onset of painful erythematous plaques or nodules
2. Histopathologic evidence of a dense neutrophilic infiltrate without evidence of leukocytoclastic vasculitis
MINOR CRITERIA
1. Fever >38°C
2. Associated with inflammatory disease or pregnancy or preceded by upper respiratory infection, gastrointestinal infection, or vaccination
3. Excellent response to treatment with systemic glucocorticoids or potassium iodide
4. Abnormal laboratory values at presentation (three of four of the following):
a. Erythrocyte sedimentation rate >20 mm/h
b. Positive C-reactive protein
c. >8,000 leukocytes per microliter
d. >70% neutrophils

TABLE 3 | Diagnostic Criteria for Drug Induced Sweet's Syndrome.

1. Abrupt onset of painful erythematous plaques or nodules
2. Histopathologic evidence of a dense neutrophilic infiltrate without evidence of leukocytoclastic vasculitis
3. Fever >38°
4. Temporal relationship between drug ingestion and clinical presentation, or temporally-related recurrence after oral challenge
5. Temporally-related resolution of lesions after drug withdrawal or treatment with systemic corticosteroids

malignant myeloblasts in serum and bone marrow, suggesting a clonal transformation into dysplastic neutrophils in the dermis (49, 55, 534, 537, 538). Recently, examination of the bone marrow and SS lesional tissue in a patient with concurrent acute myeloid leukemia (AML) with single nucleotide polymorphism array and next generation sequencing revealed FLT-3 gene mutations in infiltrating mature neutrophils and neoplastic progenitor cells (539). In one case series, FLT-3 mutations have been detected in 39% of patients with AML and SS and FLT-3 inhibitors are a known SS inducer (49, 540, 541). This gene encodes a receptor tyrosine kinase normally present on hematopoietic stem cells within the bone marrow and regulates myeloid progenitor cell proliferation, survival, and differentiation (542). In AML the FLT-3 mutations result in persistent activation. The identification of this mutation in dermal neutrophils and leukemic cells suggests a common progenitor origin.

Induction and Stimulus

Given the variety of underlying conditions including medications, infections, and malignancy associated with a similar clinicopathologic presentation in SS, one unifying hypothesis is that SS is a hypersensitivity reaction. Immune reaction to drugs, bacterial, viral, or tumor antigens may initiate a cytokine cascade resulting in SS (3). The efficacy of systemic corticosteroids and resolution of SS with treatment of underlying disease with antibiotics or chemotherapy supports this hypothesis, but there is a lack of evidence showing immune-complexes, immunoglobulins or changes in complement consistent with a hypersensitivity reaction (11, 519, 543).

Photoinduction and Koebner phenomenon have also been suggested as possible inciting etiologies in SS and may explain the distribution and localization to the skin (544). Photoinduction of SS has been documented and confirmed in select patients with experimental phototesting re-challenge (464, 545–549). While not fully elucidated, a proposed mechanism is founded on the immunomodulating effects of light. The most notable concept involves the pro-inflammatory potential of ultraviolet B in activating neutrophils and inducing the production of TNF-α and interleukin-8 (548, 550, 551). The formation of SS lesions in response to localized trauma has been demonstrated by lesions developing at sites of radiation therapy, surgery, burns, tattoos, and lymphedema (442–445, 454–457, 472, 474).

Cutaneous Localization

Localization of neutrophils to the dermis in SS is complex and theorized mechanisms are dependent on underlying etiology. Normal neutrophils require TNF-α activated endothelium which leads to neutrophil rolling and attachment via interdependent interactions with selectins, intercellular cell adhesion molecules (ICAM), and integrins (552). These surface linking molecules in concert with inflammatory molecules, including TNF-α and IL-1β, result in normal neutrophil extravasation into tissue. In hematologic malignancy, myeloid blast cells have increased expression of surface adhesion receptors and can induce non-activated endothelial cell adhesion to express receptors leading to accumulation of leukemic cells (553). These cells further promote recruitment, accumulation and tissue invasion by secreting inflammatory cytokines including TNF-α and IL-1β (553). Leukemia cutis, a paraneoplastic tissue invasion of leukemic cells, is well-recognized and has been coexistent in patients with SS and within SS lesions (554–556). Potential mechanisms include dysfunctional malignant cells activating adhesions and creating an inflammatory environment suitable for innocent bystander neutrophils to extravasate, creating SS lesions. Alternatively, cancer therapy, or paraneoplastic stimulatory factors may result in the maturation of leukemia cutis cells into the mature neutrophils within SS lesions. In non-malignant SS associated with other inflammatory conditions, a similar pathologic inflammatory environment could be responsible for localization and infiltration of neutrophils.

Dysfunctional Immune Mediators

The role of a dysfunctional innate immune response in SS is well-established, but evidence is emerging that the adaptive immune system has a significant role. In classic SS, lymphocytes, specifically Type 1 helper T cells (Th1), have been theorized to be responsible for neutrophil activation and localization. This is evidenced by elevated serum levels of Th1 cytokines including IL-1α, IL-1β, IL-2, and IFN-γ (557). Further investigation utilizing immunohistochemical stains has shown a significant presence of these Th1 cytokines and a relative reduction of

Type 2 helper T cell (Th2) markers in SS dermal lesions. This suggests hyperexpression of Th1 cells and a comparative suppression of Th2 cells (137, 558, 559). Th1 cells secrete TNF-α and INF-γ, which are potent neutrophil recruiters and activators. Proinflammatory T helper 17 (Th17) cells and related cytokines have also been identified as a pathologic agent in SS (559–562). The role of Th17 cells is most well studied in one of the most prevalent autoinflammatory diseases: psoriasis (563). Th17 produces multiple inflammatory molecules, including interleukin 17 (IL-17). IL-17 works synergistically with TNF α, IL-1β, and IFN-γ to create an inflammatory response and recruits and localizes neutrophils by inducing adhesion molecules, and chemoattractants such as IL-8 (564). Interactions with TNF α and IL-17 induces basement membrane remodeling via pericytes and neutrophils (565). In this SS driven remodeling process, matrix metalloproteinases (MMPs) are significantly upregulated. Upon inhibition of MMP-3, there is a reduction of neutrophil chemotaxis and extracellular matrix degradation (565). The production of G-CSF and GM-CSF are enhanced by IL-17, which leads to activation and proliferation of neutrophils (566, 567). Additional pro-inflammatory markers elevated in SS include: CD40/CD40 ligand, CD56, G-CSF, myeloperoxidase, IL-5, IL-8 IL-12, IL-13, L-selectin, MMP-2, MMP-9, Sialic acid-binding immunoglobulin-type lectin (Siglec) 5, Siglec 9, Transforming growing factor β (TGF-β), TIMP-1, TNF α, and VEGF (127, 524, 558–560, 562, 568, 569). Significant levels of CD56, a Natural killer cell marker, CD40/CD40 ligand, and IFN-γ may indicate the role of antigen presenting cells, as well as a cross-link between the robust innate and adaptive immune response in SS (570). Further evidence of adaptive immunity involvement is suggested by SS remission following treatment with therapies targeting adaptive cell processes including corticosteroids, cyclosporine, IVIG, rituximab, and vedolizumab (121, 132, 571–576). **Table 4** summarizes cytokines and inflammatory markers documented in SS. **Figure 1** shows the proposed multifactorial mechanism of disease.

TABLE 4 | Inflammatory and signaling molecules elevated within lesional dermis and serum.

Elevated in dermis	References	Elevated in serum	References
Interleukin-1β	(137, 559)	Interleukin-1α	(557)
Interleukin-4	(558)	Interleukin-1β	(557)
Interleukin-5	(558)	Interleukin-2	(557)
Interleukin-8	(559, 560, 562)	Interleukin-6	(127, 568)
Interleukin-10	(561)	Interferon γ	(557)
Interleukin-12	(558)	G-CSF	(127, 524, 568, 569)
Interleukin-13	(558)	TNF-α	(568)
Interleukin-17	(559, 560, 562)		
Interferon γ	(558)		
MMP-2	(559, 560, 562)		
MMP-9	(560, 562)		
Myeloperoxidase	(560, 562)		
Siglec 5	(559)		
Siglec 9	(559)		
TGF-β	(561)		
TNF-α	(559, 560, 562)		
TIMP-1	(559)		
VEGF	(560, 562)		

G-CSF, Granulocyte-colony stimulating factor; MMP, Matrix metalloproteinase; Siglec, Sialic acid-binding immunoglobulin-type lectin; TGF-β, Transforming growth factor-β; TNF-α, Tumor necrosis factor-α; VEGF, Vascular endothelial growth factor.

Genetic Contributions

There is a growing body of knowledge regarding the genetic contributions in neutrophilic dermatoses including SS. Genetic susceptibility to the SS variant, neutrophilic dermatosis of the dorsal hands, in HLA-B54 positive Japanese individuals has been reported (577). Additional evidence of genetic co-susceptibility and possible mechanisms of SS have been described in synovitis, acne, pustulosis, hyperostosis, osteitis (SAPHO) syndrome, chronic recurrent multifocal osteomyelitis (CRMO), and Majeed syndrome (289, 506, 578, 579). There have been several links between SS and Familial Mediterranean fever (FMF) (425, 580). FMF is an inherited disease in which mutations in the MEFV gene. The MEFV gene is the causative defect identified in FMF, and it is responsible for the expression of pyrin (581). In a non-pathologic state, pyrin, an intracellular pattern recognition receptor, forms the inflammasome complex in response to infections or changes in cellular homeostasis, leading to splicing and secretion of IL-1β (581, 582). Mutations to MEFV as seen in FMF and neutrophilic dermatoses leads to a pathogenic inflammatory response. FMF and SS have coexisted in the same patients and genetic analysis has revealed heterozygous mutations of MEFV in SS (425, 580).

Mutations in isocitrate dehydrogenase 1 (IDH1) have been identified as a possible connection to SS pathogenesis in malignancy (583). IDH1 catalyzes reactions leading to alterations in histones and DNA, causing differential gene expression (584). In myeloproliferative diseases mutations to IDH1 leads to epigenetic chaos as a result of DNA hypermethylation, which leads to abnormal transcription of numerous genes (583). Protein tyrosine phosphatase non-receptor type 6 (PTPN6) plays an essential role in the proliferation and signaling of cells within the immune system (585). Mutations leading to the disruption of normal function of PTPN6 have been identified in hematologic malignancies and neutrophilic dermatoses in mice models (586–590). Alteration of PTPN6 has also been identified in SS patients through DNA sequencing analysis (591). The evidence to date suggests that SS is a polygenic process but dysfunctional activation of the inflammasome and IL-1β pathway offers a unifying mechanism.

Model of Pathogenesis

The pathogenesis of SS is complex and multifactorial, the different components discussed do not provide a unifying pathway. The most complete model is within the subset of SS patients with hematologic malignancies. The pre-existing myeloid dysfunction and disruption

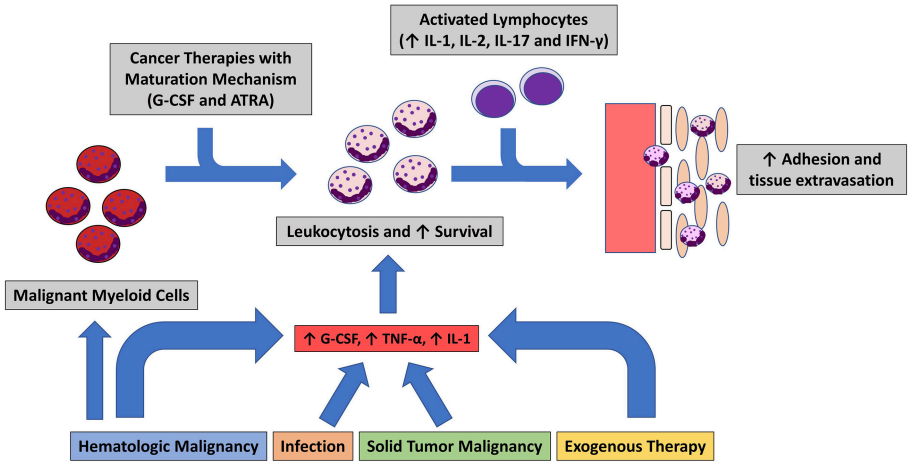

FIGURE 1 | Summary of the hypothesized of Sweet's Syndrome Pathogenesis. Inciting event leads to inflammatory state in which neutrophils mature and proliferate. Lymphocytic cytokine response leads to dermal localization.

in normal cytokine and stimulating factors provide the environment necessary for aberrant neutrophil activation and inflammation. When patients with hematologic malignancies undergoing treatment develop SS a proposed mechanism is transformation and maturation of dysfunction leukemic cells which continue to exhibit inappropriate activity. In classic SS and drug-induced SS, an inciting stimulus such as an antigen in an individual with a genetical predisposition likely creates a similar pro-inflammatory state resulting in SS. The rarity of SS and the lack of robust experimentation is a major restraint in understanding the disease pathogenesis.

TREATMENT APPROACHES

Management of SS is partially reliant on the underlying association, but given the severe presentation and possibility of non-modifiable etiology, prompt treatment is usually warranted (592). In drug induced SS, identification and removal of the offending agent is beneficial but does not negate the need for treatment. First line treatments for SS include corticosteroids and other agents such as potassium iodide or colchicine. Second line agents for SS include indomethacin, clofazimine, cyclosporin, and dapsone (592, 593). The effectiveness of these medications with differential mechanisms of action highlights the role of both adaptive and innate cells in the pathogenesis of SS (594–596). With advances in our understanding of the pathophysiology of neutrophilic dermatoses, especially the role of TNF-α and IL-1β, the use of targeted therapy with IL-1 and TNF-α inhibitors has been effective (323, 593, 597–603). There have been reports of several novel treatments for SS, including granulocyte and monocyte adsorption apheresis, but due to the rarity of SS and the effectiveness of established treatments there have been limited investigations into these alternative treatments (604).

CONCLUSIONS AND FUTURE DIRECTIONS OF RESEARCH

Over the last half century, SS has retained its defining characteristics while medical advances and scientific discovery have led to a better understanding of disease mechanisms and associations. The clinical similarity of SS with other neutrophilic driven autoinflammatory entities is challenging in clinical grounds as the diagnostic criteria is not applicable in atypical presentations or overlapping autoinflammatory dermatoses. Relations with medications, inflammatory diseases, and malignancy have been established and expanded on. Dermal neutrophil clonality and transformation of malignant myeloid progenitors into infiltrating neutrophils provides evidence for an etiology in myeloproliferative disease and offers insight into future directions of research. Investigations into immunologic signaling pathways have improved our understanding of the interrelationships between inflammation and disease pathogenesis. The involvement of IL-17, IL-1β, and inflammasome activation are of great interest in neutrophilic dermatoses including the utilization of targeted therapies. As this pathway is ubiquitous throughout inflammatory processes, an emphasis on better understanding its mechanism will be paramount to advances in not only SS but throughout medicine. As genetic analysis and gene profiling techniques are revolutionized and optimized, new discoveries on the role of genetic susceptibility, heritability, and more specific markers of neutrophilic dermatoses will be on the horizon

AUTHOR CONTRIBUTIONS

MH and AO-L conceived the idea for this work and performed the literature review on the subject. MH compiled the data with AO-L oversight. MH and AO-L wrote the manuscript and finalized the published version.

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REVIEW
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Neutrophils in Leprosy

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Leprosy is an infectious disease caused by the intracellular bacillus *Mycobacterium leprae* that mainly affects the skin and peripheral nerves. One of the most intriguing aspects of leprosy is the diversity of its clinical forms. Paucibacillary patients are characterized as having less than five skin lesions and rare bacilli while the lesions in multibacillary patients are disseminated with voluminous bacilli. The chronic course of leprosy is often interrupted by acute episodes of an inflammatory immunological response classified as either reversal reaction or erythema nodosum leprosum (ENL). Although ENL is considered a neutrophilic immune-complex mediated condition, little is known about the direct role of neutrophils in ENL and leprosy disease overall. Recent studies have shown a renewed interest in neutrophilic biology. One of the most interesting recent discoveries was that the neutrophilic population is not homogeneous. Neutrophilic polarization leads to divergent phenotypes (e.g., a pro- and antitumor profile) that are dynamic subpopulations with distinct phenotypical and functional abilities. Moreover, there is emerging evidence indicating that neutrophils expressing CD64 favor systemic inflammation during ENL. In the present review, neutrophilic involvement in leprosy is discussed with a particular focus on ENL and the potential of neutrophils as clinical biomarkers and therapeutic targets.

Keywords: leprosy, *Mycobacterium leprae*, erythema nodosum leprosum, inflammation, neutrophils

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INTRODUCTION

Leprosy is a millennial disease that continues to adversely impact the public health systems of endemic countries. The most commonly affected sites are the dermis and the peripheral nerves. Permanent disabilities are the direct consequence of the neurological damage caused by the *Mycobacterium leprae* infection, especially when the damage is left untreated in its early stages. During 2017, 150 countries reported 210,671 new cases of leprosy at a detection rate of 2.77/100,000 (1).

Leprosy severity is determined by the regulation of cell-mediated immunity, ranging anywhere from mild, presenting with a single, well-demarcated lesion (termed *tuberculoid*: TT), to severe, involving widespread, poorly-demarcated, raised, or nodular lesions (termed *lepromatous lepromatous*: LL). Biopsies of TT lesions reveal well-developed granulomatous inflammation associated with the marked presence of Langerhans cells ($CD1a^+$) and rare acid-fast bacilli. LL dermal lesions are characterized by the presence of numerous heavily-infected foamy macrophages, a sparse infiltrate of lymphoid cells, and the number of Langerhans cells is consistently low (2–4). The so-called borderline patients (BT, BB, and BL) are situated between the extremes of the TT and LL poles. These patients display a mixed unstable immune response whose characteristics are in accordance with their proximity to one pole or the other (5).

During disease evolution, 50% of LL and 5–10% of BL patients present a variety of dermatological inflammatory phenomena with systemic symptoms (6, 7), referred to as erythema nodosum leprosum (ENL, or Type 2 reaction). ENL together with reversal reaction are core aspects of leprosy that profoundly impact both the course of the disease and the development of nerve damage (8). Clinically, ENL patients demonstrate painful subcutaneous nodules on the apparently normal skin (**Figure 1**). More severe cases display systemic inflammation accompanied by neutrophilic leukocytosis, fever, and malaise similar to sepsis (9).

Histologically, ENL lesion has prominent neutrophilic infiltrate mainly lodged inside the deep layers of the dermis and subcutaneous tissue superimposed on chronic multibacillary leprosy (**Figure 2**). A cluster of foamy macrophages containing fragmented bacilli and a high number of Langerhans cells in dermis and epidermis are usual (4, 10–14). Eosinophils, lymphocytes and plasmocytes are also found together with neutrophils. It seems that with the evolution of the ENL lesions, the number of lymphocytes and plasmocytes increases, while the number of neutrophils and eosinophils decreases (11, 15–18). Vascular abnormalities (endothelial swelling, edema, and angiogenesis) are consistently observed in acute stage of ENL lesions and reduced after anti-reactional treatment (11, 18–20). The ulcerated form, called necrotizing ENL, demonstrates similar, though more intense, histological findings and leukocytoclastic vasculitis is observed (17, 21, 22) (**Figure 2**).

Currently, ENL is often designed as a neutrophilic immune complex-mediated disease (23). The cause of ENL is eminently complex. Immune complex deposits have been implicated in the cutaneous lesions of ENL (13, 24). It is primarily driven by an aberrant dermal immune response that is modified by genetic susceptibility (25) and various environmental stimuli (e.g., pregnancy, lactation, puberty, intercurrent infections, vaccination, and psychological stress) (26). Elevated levels of tumor necrosis factor (TNF)-α and other pro-inflammatory

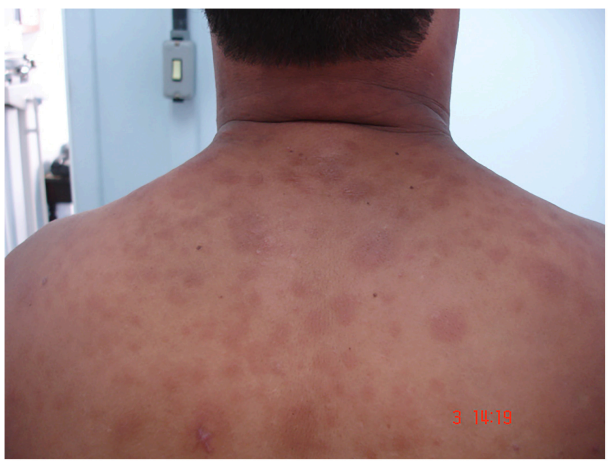

FIGURE 1 | Skin lesions of ENL patient. Image from Leprosy Laboratory collection.

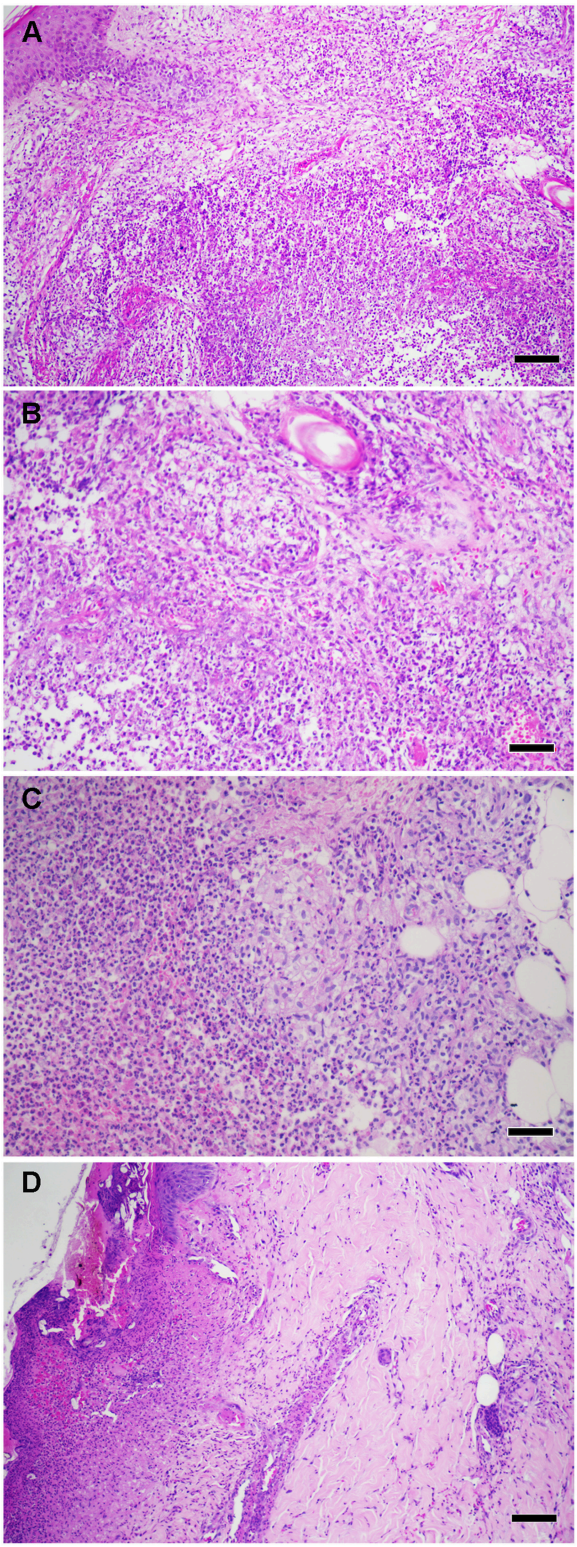

FIGURE 2 | (A) Histopathological aspects of ENL skin lesions. **(B)** High power showing foamy macrophages and neutrophil infiltrate. **(C)** High power showing a collection of neutrophils (microabscess) in deep dermis. **(D)** Necrotizing ENL, Epidermal ulceration with vasculitis. Hematoxylin and eosin staining (scale bars: 100 μm). Images from Leprosy Laboratory collection.

cytokines have been associated with ENL episodes while, in the opposite direction, TNF suppression leads to clinical improvement (27, 28). Learning more about the factors that ultimately trigger and/or sustain ENL could lead to the identification of strategies to control and, most importantly, prevent the associated inflammation.

A case can be made that the role played by neutrophils in leprosy has been largely overshadowed by several studies dedicated to the macrophages and Schwann cells targeted by *M. leprae* (29). In the blood of multibacillary leprosy patients (LL and BL), neutrophils and monocytes are loaded with the bacilli (30) and their clearance will only effectively occurs after 2–3 months of multidrug therapy (31). Novel aspects of neutrophilic biology reported in recent papers strongly indicate that, in ENL, neutrophils are active and not neutral, thus providing new insights into their participation in the disease. In the present review, we tried to highlight some of the potential gaps in knowledge among neutrophils in leprosy. Our focus was on attempting to identify the possible ways neutrophils might contribute to ENL-linked systemic inflammation. As a final concern, the potential of these cells as clinical biomarkers and therapeutic targets was highlighted.

SOME OLD AND NEW FINDINGS ON NEUTROPHIL BIOLOGY

Neutrophils have always been considered effector cells of innate immunity with a limited biosynthetic capacity. The primary role ascribed to these cells was as warriors against extracellular pathogens and in acute inflammation. These cells were classically characterized by their phagocytic ability, the release of lytic enzymes from their granules, and the production of reactive oxygen intermediates with a microbicidal potential. In the 1990s, however, this limited view was challenged by evidence that neutrophils actually survive much longer than initially believed (32) and have added ability to express genes encoding proinflammatory key mediators as components of the complement system, Fc receptors, chemokines, and cytokines (33).

Neutrophils are continuously generated in the bone marrow from its myeloid precursor. Daily production approximates 2×10^{11} cells. In humans, 50–70% of circulating leukocytes are neutrophils whereas, in mice, they range from 10 to 25%. This process is largely controlled by the granulocytic colony stimulating factor (G-CSF), produced in response to interleukin 17A (IL-17A). IL-17A is primarily synthesized by Th17 cells. But, innate immune cells, including γδ T cells, neutrophils, macrophages, innate lymphocyte cells (ILC), mast cells, and keratinocytes, have recently been found to be involved in IL-17 secretion (34). Other molecules—such as granulocytic–macrophage-colony stimulating factor (GM-CSF), IL-6, and KIT ligand (KITL, also known as KITLG)—likewise induce granulopoiesis. The production of this cytokine storm during the inflammatory responses results in overactive granulopoiesis and neutrophilia. During maturation, neutrophils undergo a number of stages referred to as either myeloblasts, pro-myelocytes, myelocytes, metamielocytes, band neutrophil, or, lastly, polymorphonuclear cells (segmented). Neutrophilic granules are formed sequentially during maturation of the pro-myeloid stage (35).

In the circulation, mature neutrophils have an average diameter of 7–10 μm, segmented nucleus, and enriched cytoplasmic granules and secretory vesicles. Three types of granules are formed during neutrophilic maturation, as follows: (i) azurophilic (or primary) containing myoloperoxidase (MPO); (ii) specific (or secondary) containing lactoferrin; and (iii) gelatinase (or tertiary) containing metalloproteinase 9 (MMP9, or gelatinase B). In humans, azurophilic granules are differentiated into defensin-poor and -rich ones (36).

Neutrophils have long been considered short, half-life cells in the circulation that normally survive approximately 1.5 h in mice and 8 h in humans (37, 38). Pillay et al. demonstrated that, under baseline conditions, the average life span of neutrophils in the circulation is 12.5 h in mice and 5.4 days in humans (37). During inflammation, neutrophils become activated and longevity increases, ensuring the presence of these cells at the inflammation site (32, 39). Endogenous products such as cytokines and growth factors together with bacterial products activate neutrophils. This increased half-life may allow neutrophils to perform more complex activities in the tissue. Examples may include: resolution of inflammation through the production of lipid mediators, modulation of the adaptive response, and reverse transmigration, which could involve the ability to exit the initial injury site and migrate to other tissues such as bone marrow (40).

Neutrophils eliminate pathogens through various intra- and extracellular mechanisms. When neutrophils encounter microorganisms, phagocytosis occurs followed by the formation of phagosomes. Microorganisms could be killed by NADPH oxygenase-dependent pathways (reactive oxygen species, ROS) or antimicrobial proteins (cathepsins, defensins, lactoferrin, and lysozyme) (35). These microbial proteins are released into either the phagosomes or the extracellular environment, acting against intra- or extracellular pathogens, respectively.

ROS production, i.e., oxidative burst, is considered a key component of the innate immune defense against bacterial and fungal infections (41). To the best of our knowledge the literature hasn't shown yet oxidative burst in leprosy neutrophils. However, oxidative stress was evaluated by measuring serum levels of malondialdehyde (MDA) and superoxide dismutase (SOD) activity and the results showed that leprosy patients present increased serum levels of MDA, MDA/SOD ratio together with a decreased SOD activity when compared to healthy controls suggesting oxidative stress in leprosy (42). In addition, it was demonstrated that the oxidative stress gradually increased along the spectrum from TT to LL (43).

An elegant strategy was used to measure the antimicrobial capacity of neutrophils to contain methicillin-resistant *Staphylococcus aureus* (MRSA). Leliefeld et al. (44) used a long-term neutrophil bacterial interaction in a 3D scaffold reminiscent of the *in vivo* environment. In such condition it was possible to evaluate the capacity for long-term intracellular containment of live bacteria. Neutrophils from chronic granulomatous disease (CGD) patients who lack a functional NADPH oxidase and healthy neutrophils under hypoxic conditions did not exhibit impaired bacterial containment and in that way this

containment was independent of ROS (44). Conversely, failure of the phagosomal acidification led to impaired intracellular containment of MRSA (44).

Activated neutrophils have the capacity to eliminate extracellular microorganisms after releasing neutrophilic extracellular traps (NETs) (45). NETs are composed of nuclear DNA in association with histones and granular proteins such as antimicrobial proteins and enzymes (MPO and neutrophil elastase). The functions of NETs include immobilizing pathogens to prevent them from disseminating and facilitating the subsequent phagocytosis of trapped microorganisms. Many pathogens, namely viruses, bacteria, parasites, and fungi can induce NETs. For this reason, the mechanisms for both the initiation and evasion of NETs by pathogens have been intensively studied (46). To our knowledge, no report exists in the literature regarding the ability or inability of *M. leprae* to induce NETs. In addition, there are two open questions: (1) Is there a connection between NET formation, neutrophil infiltration and ENL systemic inflammation? (2) Does the massive presence of neutrophils in the ENL lesions could generate necrotic areas? It is possible to observe a collection of neutrophils in ENL lesions forming a microabscess (**Figure 2**). The NETosis role in limiting the spread of necrotic tissues was demonstrated in acute abdominal inflammation where netting neutrophils create a barrier between necrotic and viables areas (47).

Although ROS production by neutrophils during infections is an important antimicrobial mechanism, the exacerbated production of ROS due to the massive involvement of neutrophils can lead to oxidative stress accompanied of cell death and necrosis (47). The large amounts of neutrophil-released enzymes could degrade cytokines but also modify glycan on the surrounding tissues (48). Alteration of glycan residues on IgG molecules is associated with high lupus disease activity (49). Moreover, the incomplete clearance of DNA-released material leads to systemic inflammation and autoantibody production (50).

Dias et al. (51) demonstrated that ENL patients displayed higher levels of human DNA–histone complexes than either BL/LL patients or healthy individuals. The increased levels of TLR-9 ligands and the TLR-9 *per se* in peripheral mononuclear cells was considered by the authors as a major innate immunity pathway activated during ENL (51). Meanwhile, the source of the DNA-histone complex has yet to be identified.

There is much evidence in support of the existence of neutrophilic subpopulations and their role in inflammation, infection, and tumor immunology. The neutrophils subsets have been characterized according to their phenotypic, functional, morphological, and physical characteristics under both homeostatic and pathophysiologic conditions (**Table 1**). A detailed description of all neutrophils subsets is beyond the scope of this work, but additional reviews can be found elsewhere (58). Nonetheless, it is not yet known whether these subpopulations are distinct subsets or rather represents the plastic development of their neutrophilic precursor.

Recruitment of leukocytes at a site of blood vessel growth is a crucial event for proper angiogenesis and subsequent

TABLE 1 | Studies of neutrophil subpopulations.

Neutrophil subsets	Findings	References
MRSA MOUSE MODEL OF INFECTION		
PMN-I (MRSA - resistant host)	$CD49d^{+}/CD11b^{-}$	(52)
	IL-12 and CCL3 production	
	Classically activated macrophages	
	Expression of TLR2, TLR4, TLR5, TLR8	
	Multi-lobular nucleus	
PMN-II (MRSA-sensitive hosts)	$CD49d^{-}/CD11b^{+}$	
	IL-10 and CCL2 production	
	Alternatively activated macrophages	
	Expression of TLR2, TLR4, TLR7, TLR9	
	Ring-shaped nucleus	
PMN-N (normal host)	$CD49d^{-}/CD11b^{-}$	
	No production of cytokines and chemokines	
	No effect on macrophage activation	
	Expression of TLR2, TLR4, TLR9	
	Round nucleus	
HEALTHY VOLUNTEERS CHALLENGE WITH I.V. LPS		
$CD16^{bright}/CD62L^{dim}$	Not found in healthy donors	(53)
	Hypersegmented neutrophils	(54)
	Rapid apoptosis rate (similar to normal)	(44)
	Higher expression of CD11b, CD11c and CD54	
	Inhibit T cell proliferation	
	Normal phagocytosis	
	Poor capacity to contain intracellular bacteria	
	Less chemotactic rate	
	Decreased adhesion	
$CD16^{dim}/CD62L^{bright}$	Not found in healthy donors	
	Banded neutrophils	
	Higher rate of survival	
	Higher NADPH oxidase activity	
	Higher acidification of phagosome	
	Contain intracellular bacteria	
	Enhanced adhesion	
	Higher chemotactic rate	
$CD16^{bright}/CD62L^{brigh}$	Phenotypically mature (normal)	
TUMOR ASSOCIATED NEUTROPHILS (TANS)		
N1	Pro-inflammatory properties	(55)
	TNF production	(56)
	High tumoral cytotoxicity	(57)
	High NET production	
	$ICAM1^{high}$	
	Hypersegmented nucleus	
N2	Anti-inflammatory	
	High production of arginase	
	Immature-like or segmented nuclei	
	Proangiogenic profile	
	Higher production of MMP-9 and VEGF	

MRSA, methicillin-resistant Staphylococcus aureus.

tissue perfusion. Pro-angiogenic neutrophils $CD11b^{+}$/$GR\text{-}1^{+}$/$CXCR4^{high}$ producing high levels of MMP9 were recruited into the tissue in response to VEGFA in a mouse model of non-vascularized transplant (59). These pro-angiogenic neutrophils have been shown to be essential in promoting neovascularization of transplanted pancreatic islands (59) and may be the same cells that are known to promote cancer cell survival (60).

Neutrophils with regulatory function were identified in several models. Lung neutrophils isolated from both bronchoalveolar lavage fluid and parenchyma of infected mice produced IL-10 and negatively regulating local lung inflammation during chronic phase of *M. tuberculosis* infection (61). Secreting IL-10 neutrophils reported in *Trypanosoma cruzi*-infected mice showed an IL-10-dependent suppressive phenotype *in vitro* inhibiting T-cell proliferation and IFN-γ production (62). However, these anti-inflammatory neutrophils may change into pro-inflammatory phenotypes ($IL\text{-}10^{low}$/$IL\text{-}12^{high}$) after interacting with Natural Killer T cells in a CD1d-dependent manner (63). It was showed that neutrophils (G-neutrophils) from G-CSF–treated human and mice donors could inhibit T cell activation both *in vitro* and *in vivo* in a model of experimental acute graft-versus-host disease (64, 65). The disease inhibition induced by G-Neutrophils is dependent on neutrophil IL-10 competence (66).

During infection with MRSA, distinct types of neutrophils have been identified in association with resistance and susceptibility to MRSA. Neutrophilic populations isolated from both resistant and susceptible MRSA, PMN-I, and PMN-II, respectively—were distinct from neutrophils isolated from healthy mice, PMN-N (**Table 1**). It is possible that these pro- and anti-inflammatory neutrophils may alter the course of the adaptive response by inducing M1 or M2 macrophages, respectively. It cannot be ruled out that these neutrophils may change their phenotypes during the course of inflammation to fit a particular aggressor and do not necessarily represent distinct lineages (52). To date, no work in the literature has identified any neutrophilic subpopulations in leprosy that might correlate with the TT leprosy-resistant or LL susceptible leprosy polar forms, TT vs. LL.

A myriad of neutrophilic subpopulations ($CD16^{bright}$/$CD62L^{dim}$, $CD16^{dim}$/ $CD62L^{bright}$, $CD16^{bright}$/$CD62L^{bright}$) has been identified in the circulation of human volunteers receiving lipopolysaccharide in contrast to the number in untreated individuals (53, 54) (**Table 1**). The subset $CD16^{bright}$/$CD62L^{dim}$ hypersegmented neutrophils displayed normal phagocytosis associated with a remarkably poor capacity to contain bacteria intracellularly. This defect in bacterial containment was associated with failure of acidification in the phagosomal compartment. On the other hand, $CD16^{dim}$/$CD62L^{bright}$ banded neutrophils were the only neutrophil subset that adequately contained MRSA (44).

Fridlender et al. (55) were the first to describe the existence of additional neutrophilic subsets nominated N1 e N2. The former is pro-inflammatory and the latter, anti-inflammatory (55). Via a murine model of cancer, the authors demonstrated the presence of tumor associated neutrophils (TANs), characterized by differential activation and phenotypical states. Neutrophils with an N1 phenotype possess a hypersegmented nucleus with pro-inflammatory and antitumor properties due to increased tumoral cytotoxicity, high NETs production, high ICAM1 expression, and production of inflammatory cytokines and chemokines like TNF. On the other hand, the N2 phenotype plays an opposite role and is classified as immunosuppressive and pro-tumoral mostly due to the elevated production of arginase like G-MDCs. These neutrophils usually possess an immature nucleus although some works have described them as being segmented. Another interesting fact about N2 neutrophils is their proangiogenic profile, which is driven by their capacity to produce elevated levels of MMP-9 and VEGF (57). Furthermore, it has been shown that TGF-β plays a critical role in neutrophilic polarization as a result of its ability to induce plasticity between a N1 subset into a N2 profile (55, 56). Other factors, namely angiotensin-II and type I IFNs, have recently been to shown to promote N1/N2 polarization too (58). Beyond phenotypic differences, it became clear by way of the transcriptomic approach that the N1 and 2 subsets represent distinct populations with diverse transcriptional signatures (56).

Interestingly, a new neutrophil subset with different densities has been the focus of several research projects. Associated with disease severity in some inflammatory disorders, a subset of low-density neutrophils (LDN) that co-localizes with peripheral blood mononuclear cells (PBMC) after density gradient separation has been reported (67). It is also noteworthy that, in cancer, this population was found to increase within tumor growth and be characterized by a morphologically homogeneous population that may contain band and segmented neutrophils (68, 69). Even though the origin and role of this subpopulation remain somewhat nebulous, some works have reported that LDNs display diverse profiles. The analyses of PBMC preparations from patients with Systemic Lupus Erythematous reveals that LDNs have an activated phenotype. In this scenario, LDN produce higher levels of such pro-inflammatory mediators as type I IFNs, IFN-γ, and TNF and are capable of modulating endothelial cell functions and increasing vascular damage (70). In addition, they are more disposed to form NETs that favor the chronic inflammation and disease severity (71, 72). While LDN have also been detected in many other pathologies like sepsis, HIV infection, malaria and also in tuberculosis (73–76), increasing our understanding of their surface marker patterns, cytokine expression, transcription factor regulators, and other trademarks of activation is of prime importance. Despite the uptick of studies describing the diversity of neutrophilic subpopulations, their distinct origins and plastic capacity remain unknown. New data need to be put forward that corroborate the existence of neutrophilic subpopulation in leprosy.

Under certain physiological conditions, the death of circulating neutrophils takes place in the liver, spleen, and bone marrow. Observed in old neutrophils, increased CXCR4 expression helps to direct them back to the bone marrow and subsequent elimination. CXCR4 is also involved in the down regulation of the newly-formed neutrophilic release into the marrow (39). Some studies suggest that terminal neutrophilic trafficking inside the intestinal tract also takes place to help

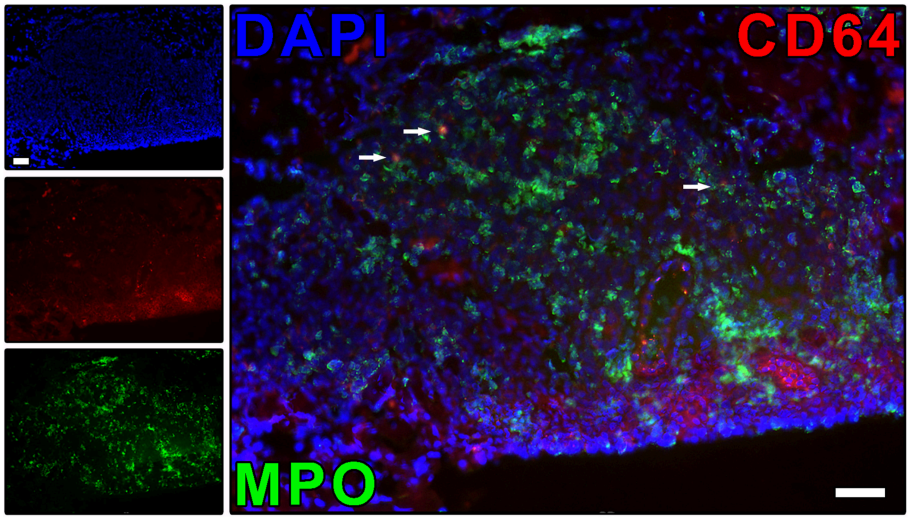

FIGURE 3 | ENL skin lesions present neutrophils expressing CD64. The protein expression of CD64 on neutrophil (MPO^+ cells) was evaluated by immunofluorescence. Skin lesion was stained for CD64 (red), myeloperoxidase (MPO; green), and nuclei (DAPI; blue). Images are representative of three independent samples of ENL patients. Co-localized areas of MPO^+CD64^+ cells were identified with arrows. The regulation of neutrophil CD64 expression has the potential to be useful in the ENL treatment, as well as to prevent ENL reactional episodes. Scale bars: 10 μm. Images from Leprosy Laboratory collection.

regulate the commensal flora (77). Both senescent neutrophils and those that die after fighting infection may also expire within the vasculature and be removed by Kupffer cells (resident liver macrophages) (78). Removal of neutrophils by both Kupffer and dendritic cells is mainly regulated by the IL-23/IL17/G-CSF axis. This cytokinetic axis stimulates neutrophilic production in the bone marrow and is down regulated by liver X receptor (LXR) (79). The mechanisms involved in the clearance of NETotic cells are not yet known. On the other hand, clearance of apoptotic cells is well-studied (50). Different cell types participates in the uptake of apoptotic bodies by employing different types of receptors. This process can be amplified or suppressed by different types of plasma proteins (80). The complement system, pentraxins, and collectins have been implicated in apoptotic cell clearance in circulation or in injured tissue (81, 82).

NEUTROPHILS IN LEPROSY

A number of studies in the 1970's addressed the neutrophilic functions in the different forms of leprosy (83–86). These studies used the nitro blue tetrazolium that measures neutrophilic activation through reduction of *in vitro*. Goihman-Yahr et al. (83, 85) found that, during reaction, there is spontaneous neutrophilic activation not witnessed in LL leprosy (83, 85). Moreover, neutrophils are equally well-activated by endotoxin and *M. leprae in vitro* (87) in all forms of leprosy. In sharp contrast, Sher et al. (86) found no spontaneous activation rate in neutrophils of TT, LL, or ENL patients. Even so, ENL sera activated neutrophils of healthy donors *in vitro*, suggesting that ENL sera contain a neutrophilic activation inductor (86). Another parameter assessed of neutrophilic activation was cell motility that was measured by three different assays (random migration, chemotaxis, and chemokinesis), all of which were defective in LL neutrophils whether in the absence or presence of ENL (86). Drutz et al. (88) reported no important differences among TT and LL patients and normal control subjects in the bactericidal and fungicidal functions of their phagocytic cells, including monocytes, macrophages, and neutrophils (88).

Circulating neutrophils from leprosy patients are loaded with *M. leprae* (30, 31) and there is apparently no sign of systemic inflammation. It remains unclear whether neutrophils are capable of killing the bacilli. Our group has reported that neutrophils isolated from LL patients with or without ENL released TNF and IL-8 subsequent to stimulation with *M. leprae ex vivo* (89). Besides, the apoptotic rate of ENL neutrophils is higher in comparation to that found in BL/LL patients and healthy volunteers (89). It has been previously demonstrated that apoptotic neutrophils infected or not with *M. tuberculosis* trigger a proinflammatory response in *M. tuberculosis*-infected macrophages through a caspase-1- and IL-1β-dependent mechanism (90). The biological responses of macrophages included an enhanced production of proinflammatory cytokines as well as an enhanced capacity to control the intracellular growth of *M. tuberculosis*. The interaction of apoptotic neutrophils and macrophages in leprosy has yet to be determined with certainty.

Our group has demonstrated that, during ENL, circulating and lesional neutrophils exclusively expresses CD64 (FcγRI) while leprosy patients without reaction, such as BL/LL, BT, and RR individuals, do not (9) (**Figure 3**). Besides, the higher CD64 levels on circulating neutrophils have been correlated with disease severity, pointing to CD64 as an early biomarker for ENL as well as a marker of severity (9). Since neutrophils function as biosensors, the proinflammatory microenvironment and/or fragments of the bacillus could induce the expression of CD64 on the neutrophil surface. The biological impact of

surface expression of CD64 in neutrophils needs to be better evaluated. Upregulation of CD64 *in vivo* has likewise been associated with enhanced neutrophilic functionality (91–93). At the onset of sepsis or septic shock, the CD64 expression rate in neutrophils is augmented (94). Fadlon et al. showed that the $CD64^+$ neutrophils bound to endothelial monolayers and $CD64^+$enriched neutrophils were 7 times more strongly adherent to endothelial monolayers than were CD64-depleted neutrophils (95).

Pentraxin-3 (PTX3), a protein present in the secondary neutrophilic granule, was originally identified as being induced by such primary inflammatory signals as TNF and interleukin 1β (IL-1β) (96, 97). Our group has shown evidence that PTX3 is released systemically and at the site of ENL lesions (98). Our research has also demonstrated that PTX3 serum levels correlated to the surface expression of CD64 in circulating neutrophils and that thalidomide treatment of ENL down regulated PTX3 levels (98). Interestingly, PTX3 serum levels were high in MB patients without reaction yet persistent in patients who developed ENL. In contrast, MB patients who developed RR had low levels of PTX3 prior to and at the onset of the event. These results indicate that high and persistent levels of PTX3 in MB patients may be associated with the occurrence of ENL while also identifying PTX3 as a potentially predictive ENL biomarker capable of differentiating it from an RR episode.

In recent years, several large-scale gene expression studies have been conducted to monitor the host response to pathogen. These study results could potentially serve as diagnostic tool to either distinguish disease-afflicted patients from healthy individuals or classify different forms of the same disease. Via microarray analyses, Lee et al. (99) compared LL -reaction free skin lesions to those of ENL patients. Their global gene expression profiles revealed the up-regulation of genes involved in cell-movement, including E-selectin and its ligands, both key molecules in mediating neutrophilic recruitment to inflammatory sites (99). Transcriptome profiles derived from ENL skin lesions have also recently detailed the participation of neutrophilic and endothelial cell-gene networks in the vasculitis resulting in tissue damage (100).

To date, no study has yet reported a gene expression signature based on leprosy whole blood. However, via the microarray, the global transcriptional profiles of PBMC revealed that there were 275 genes differentially expressed in RR and 517 differentially expressed in ENL (101). In addition, a granulocytic gene signature was identified in gene-expression arrays derived from ENL PBMCs (101). These data suggest that PBMC fractions of ENL patients may be contaminated with LDN subpopulation, as has been similarly described in autoimmune diseases. Nonetheless, the presence of LDN in light of their functional capacity and potential to contribute to the clinical manifestations of ENL remain basically unexplored.

Naranbhai et al. have recently mapped the quantitative trait loci (eQTL) expression in peripheral blood $CD16^+$ neutrophils from 101 healthy Europeans (102). The analyses found that leprosy and Crohn's disease, an autoimmune inflammatory bowel illness, showed a profound overlap in genetic architecture. The ancestral T allele of rs1981760 was associated with an increased susceptibility to MB leprosy. The authors observed a strong link between rs1981760-T and a reduced NOD2 expression in neutrophils in conjunction with a conversely elevated expression in monocytes. In addition, neutrophils stimulated with a NOD2 ligand, muramyl dipeptide supplemented with Pam3-CSK4, a synergistic agonist, express significantly higher levels of mRNA for IFNβ (102). These data demonstrate that rs1981760 affects NOD2 expression and the subsequent IFNβ responses to its ligand. Interestingly, eQTL in neutrophils are enriched for genes in the IFNβ network. These data suggest that type-1 interferons and neutrophils may be involved in leprosy such as has been previously shown in tuberculosis (103).

CONCLUSION

There are still large gaps in our understanding of the role of neutrophils in ENL and leprosy disease despite the large number of studies examining their immunological functions. Future works should aim to further determine the roles of neutrophils in host–mycobacterial interactions, particularly as relates to their early defensive posture and possible contribution to disease progression. The identification of subpopulations of neutrophils associated with the clinical forms of leprosy could provide novel insights of neutrophil function and reveal new targets in leprosy. The present review suggests the roles performed by neutrophils as both migratory and, for the first time, effector cells following chemo-attractants in the context of leprosy.

AUTHOR CONTRIBUTIONS

VS wrote the original draft of manuscript. IT, FP, JdS, and CdS helped with literature collating and referencing. IT, PP, AM, and ES revised and edited the manuscript.

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2D Visualization of the Psoriasis Transcriptome Fails to Support the Existence of Dual-Secreting IL-17A/IL-22 Th17 T Cells

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The present paradigm of psoriasis pathogenesis revolves around the IL-23/IL-17A axis. Dual-secreting Th17 T cells presumably are the predominant sources of the psoriasis phenotype-driving cytokines, IL-17A and IL-22. We thus conducted a meta-analysis of independently acquired RNA-seq psoriasis datasets to explore the relationship between the expression of *IL17A* and *IL22*. This analysis failed to support the existence of dual secreting IL-17A/IL-22 Th17 cells as a major source of these cytokines. However, variable relationships amongst the expression of psoriasis susceptibility genes and of *IL17A*, *IL22*, and *IL23A* were identified. Additionally, to shed light on gene expression relationships in psoriasis, we applied a machine learning nonlinear dimensionality reduction strategy (t-SNE) to display the entire psoriasis transcriptome as a 2-dimensonal image. This analysis revealed a variety of gene clusters, relevant to psoriasis pathophysiology but failed to support a relationship between *IL17A* and *IL22*. These results support existing theories on alternative sources of IL-17A and IL-22 in psoriasis such as a Th22 cells and non-T cell populations.

Keywords: IL17, IL22, machine learning, neutrophil, psoriasis, RNA-seq, T cell, transcriptome

INTRODUCTION

Psoriasis is a chronic inflammatory skin condition with nail and systemic manifestations that affects ∼3% of the general United States population. It is commonly associated with psoriatic arthritis and is likely linked to other comorbidities, such as cardiovascular disease and metabolic syndrome (1–4).

Of the many clinical variants, plaque psoriasis (psoriasis vulgaris) is the most common, accounting for ∼80–90% of cases (1, 5). It is also the most well-characterized histologically and genetically. Plaque psoriasis was initially proposed to be driven by hyperproliferative keratinocytes.

However, in 1890, neutrophil involvement was suggested after histologic evaluation revealed early neutrophil accumulation within the dermis and epidermis (i.e., microabscesses of Munro and pustules of Kogoj, respectively) (6).

Despite the clear existence of neutrophils in lesional skin, the role of the adaptive immune system in psoriasis pathophysiology became the main focus of the field after the T cell-targeting agent, cyclosporine, was shown to be an effective treatment (7–9). Thus, psoriasis researchers became very quickly focused on characterizing $CD4^+$ and $CD8^+$ T cell responses in normal and diseased human skin (10–12). Subsequently, experiments performed in animal models were also developed that supported the T cell-centric view of psoriasis. For example, it was demonstrated that a psoriasis-like phenotype could be induced following adoptive transfer of dysregulated $CD4^+$ T cells (13). With this knowledge came the development of the next generation of T cell-targeting therapeutics (alefacept, efalizumab) (14–17), which further corroborated the essential role of T cells in psoriasis pathophysiology.

At the time T cells became the focus of psoriasis, adaptive immune responses were typically divided into two types, T helper type 1 (Th1) and T helper type 2 (Th2) responses. In psoriasis, the absence of Th2-defining cytokines [interleukin (IL)-4, IL-5, and IL-10] (18) and the increased presence of Th1 cytokines (interferon gamma (IFN-γ), tumor necrosis factor (TNF) and IL-12) prompted researchers to classify psoriasis as a Th1-mediated disease (18). Soon thereafter, however, it became increasingly apparent that IL-17-secreting T cells (Th17 cells) played a major role in disease pathogenesis, not only in psoriasis, but also across a wide spectrum of animal models of autoimmunity (19–22).

Psoriasis is now thought to be a predominantly Th17-driven disease (23, 24) that is maintained by the key Th-17-supporting cytokine, IL-23 (25, 26). The dominant role of the IL-23/IL-17A axis in psoriasis is also evident by the overwhelming clinical success of newly developed IL-23/IL-17A axis-targeting biologics, which could induce near complete resolution of psoriasis, even in the most severely affected individuals (27–29). IL-22 is also a highly investigated cytokine involved in psoriasis

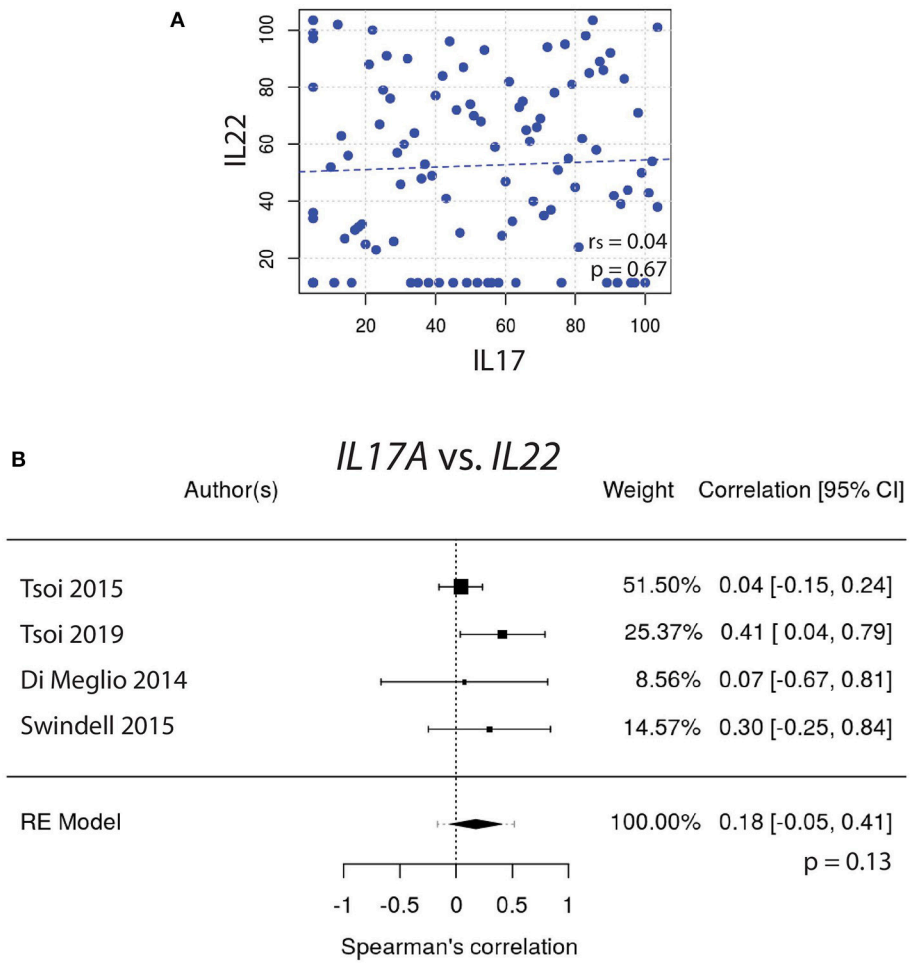

FIGURE 1 | (A) The expression of *IL17A* does not strongly correlate with the expression of *IL22* ($r_S = 0.04$). **(B)** Meta-analysis of four datasets further supports that *IL17A* and *IL22* expression do not strongly correlate [$r_S = 0.18$, with a confidence interval that crosses 0 (−0.05, 0.41)].

pathophysiology. It is thought to be the primary promoter of keratinocyte hyperproliferation (30, 31). The predominant view is that this cytokine is secreted by IL-17A/IL-22 dual-secreting Th17 cells (32).

However, the observed pathogenicity of IL-17A/IL-22 dual-secreting Th17 cells has never been formally demonstrated *in vivo*. In fact, the vast majority of evidence in support of these cells have come from animal studies and *in vitro* analysis of human T cells cultured under extreme polarizing conditions (32–35). Even when studied directly *ex vivo,* the dual secretion is usually seen only after non-physiologic T cell stimulation (36, 37). Since naturally processed autoimmune epitopes are difficult to identify (38), it is challenging to study cytokine secretion using more physiologic stimuli.

Thus, we sought evidence for the existence of dual secreting IL-17A/IL-22 Th17 cells within the psoriasis transcriptome. Weighted gene co-expression networks analysis (WGCNA) (39) have previously been used to analyze gene-gene correlations within RNA-Seq datasets. While this strategy has certain advantages, it is not ideally suited to explore gene relationships across multiple RNA-Seq datasets. Herein, we conduct meta-analyses of RNA-seq datasets to directly evaluate the current hypothesis that dual-secreting IL-17A/IL-22 Th17 cells are the dominant effector population in psoriasis. We also used this strategy to correlate the expression of *IL17A*, *IL22*, and *IL23A* with genes linked to psoriasis susceptibility identified through genome-wide association studies (GWAS). Finally, to explore the gene expression profile of *IL17A*, *IL22*, and *IL23A* in relation to other genes expressed in psoriatic plaques, we utilized a machine learning nonlinear dimensionality reduction strategy to visualize the entire psoriasis transcriptome as a 2-dimensional (2D) image. This allowed us to clearly visualize the relationship between *IL17A*, *IL22,* and *IL23A* and all other genes that are expressed in psoriatic skin.

MATERIALS AND METHODS

Human RNA-Seq

RNA-Seq FASTQ files of human normal and psoriasis lesional skin were downloaded from the NCBI Sequence Read Archive

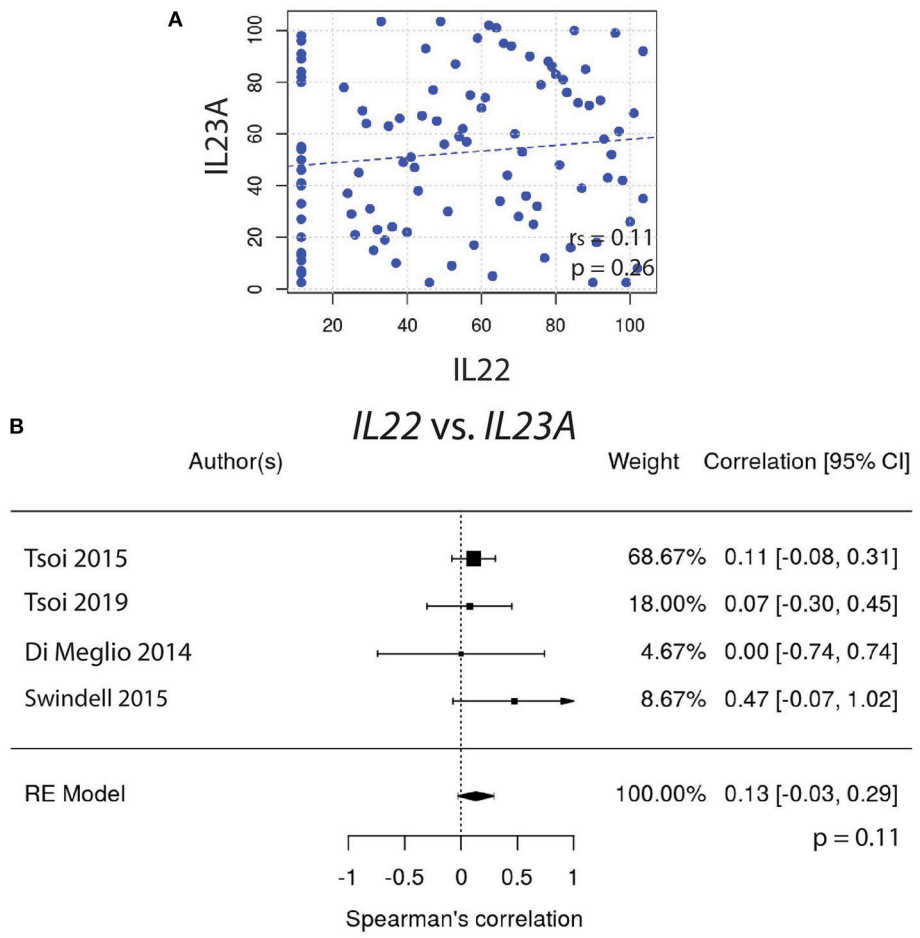

FIGURE 2 | (A) *IL22* gene expression does not correlate with *IL23A* ($r_S = 0.11$). **(B)** Meta-analysis confirms that *IL22* and *IL23A* do not strongly correlate [$r_S = 0.13$, with a confidence interval that crosses 0 (−0.03, 0.29)].

(http://www.ncbi.nlm.nih.gov/Traces/sra). Four total datasets were used: Three datasets (Accession numbers: SRP165679, SRP026042, SRP057087) and one dataset comprised of two combined experimental datasets published by the same research group (Accession numbers: SRP035988, SRP050971) (40–42).

Correlations

Correlation analyses of gene expressions were performed on read counts of each identified gene normalized with DESeq2 package (43). Values were subsequently log transformed and winsorized when necessary. Spearman's correlation coefficients were calculated (r_s) using the cor.test function in R (44). *P* values of the correlations were estimated by algorithm AS 89.

2D Visualization of the Psoriasis Transcriptome

We computed the gene pairwise distance using a formula, $1\text{-}r^2$, where r represents Pearson's correlation. A visual representation of the gene co-expression network was created using a dimensionality reduction technique, t-Distributed Stochastic Neighbor Embedding (t-SNE), calculated with Rtsne package (45).

Gene Selection

A Pubmed search was performed to identify genes linked to psoriasis through GWAS.

Genes selected for mapping included: *BTK, CD3E, CD4, CD8a, CD8b, CD19, CTSG*, *CXCL1*, *CXCL2*, *CXCL5*, *ELANE*, *ICAM1, IGH, IGK, IGL, IL1B, IL8*, *IL17A, IL22*, *IL23A*, *IL36A*, *IL36B*, *IL36G*, *IFNG*, *ITK, MPO*, *MS4A1* (CD20), *TNF*, *TRA* (TCRα), *TRB* (TCRβ), *TRD* (TCRδ) *TRG* (TCRγ).

Genes selected for meta-analysis included: *B3GNT2, CARD14, CARM1, CDKAL1, CTSG, CXCL1, CXCL5, CXCR2, DDX58, DEFB4A, ELANE, FBXL19, GJB2, HLAC, IFIH1, IL12B, IL17A, IL22, IL23A, IL36RN, IL4R, KLF4, KRT1, KRT5, KRT6A, KRT6B, KRT6C, KRT10, KRT14, KRT16, KRT17, KRT37, LCE3A, LCE3B, LCE3D, MPO, NFKBIA, NOS2, NOS3, PTPN22, RELB, RUNX3, SOCS1, STAT3, STAT5A, TNFAIP3, TNFRSF9, TNIP1, TRAF3IP2, TYK2, UBE2L3, VDR, VEGFA, VEGFB.*

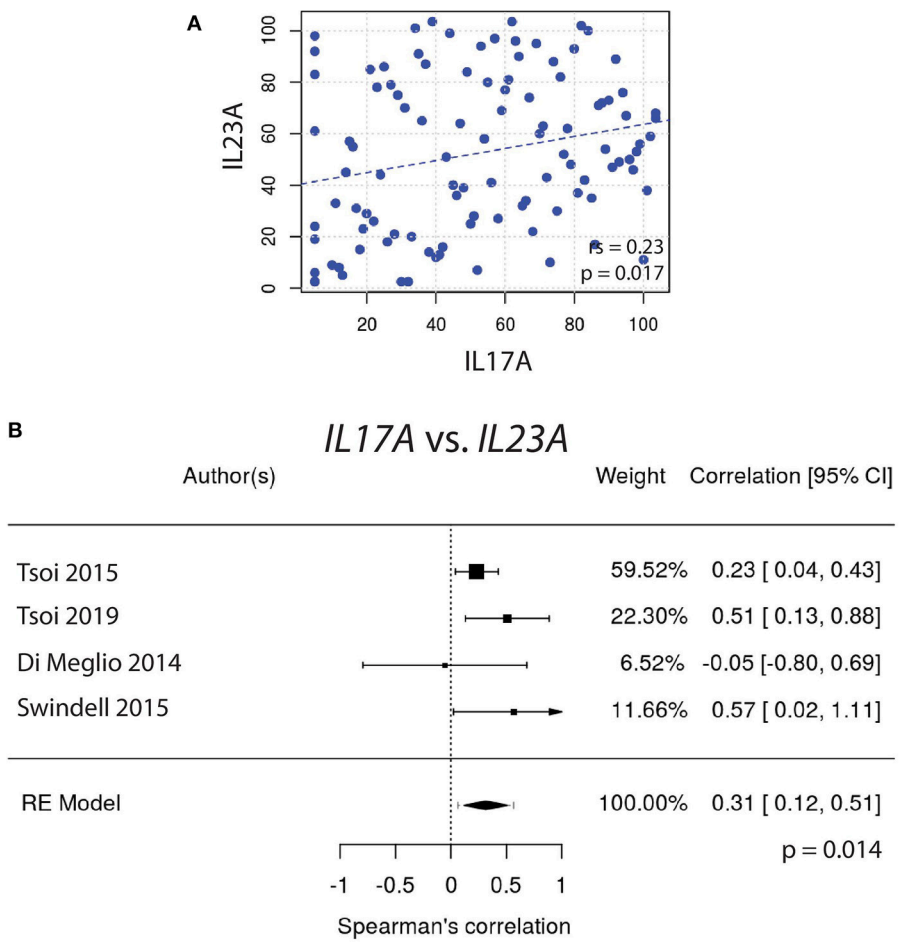

FIGURE 3 | (A) A significant Spearman correlation ($r_s = 0.23$, $p = 0.017$) is seen between *IL17A* and *IL23A* gene expression. **(B)** Meta-analysis supports a strong correlation between *IL17A* and *IL23A* [$r_s = 0.31$, (0.12, 0.51); $p = 0.0014$].

Meta-Analysis

Meta-analysis was completed using the R package "metafor" (46). A weighted random-effects model was used to estimate a summary effect size. To estimate between-study variance, a restricted maximum-likelihood estimator was applied. A weighted estimation with inverse-variance weights was used to fit the model.

RESULTS

The Expression of *IL17A* and *IL22* Do Not Strongly Correlate With One Another in Psoriatic Plaques

We hypothesized that if a significant amount of IL-17A and IL-22 is produced by IL-17A/IL-22 dual secreting Th17 cells in psoriasis, then the gene expression of these two cytokines should correlate with one another. In theory, their expression would be directly linked to the number of dual-secreting Th17 cells in a psoriasis plaque. Their gene expression should also correlate with *IL23A*, which activates and maintains Th17 cells.

To test this hypothesis, gene expression of *IL17A* vs. *IL22* was graphed and the Spearman's correlation coefficient (r_s) was calculated (**Figure 1A**). These correlative studies demonstrated that the expression of *IL22* does not strongly correlate with the expression of *IL17A* ($r_s = 0.04$, $p = 0.67$). To obtain a weighted average across all four independently acquired psoriasis datasets, a meta-analysis was performed and the resulting Forest plot (**Figure 1B**) demonstrated again that *IL17A* and *IL22* do not strongly correlate with one another [$r_s = 0.18$, with a confidence interval that crosses 0 (−0.05, 0.41)] (**Supplemental Figure 1**).

Similarly, *IL22* gene expression did not correlate well with *IL23A* (**Figures 2A,B**). In contrast, the expression of *IL17A* did correlate very well with *IL23A* (**Figure 3A**), a result that was consistent amongst a majority of datasets. The weighted average of this correlation across all psoriasis datasets was highly significant, [$r_s = 0.31$ (0.12, 0.51); $p = 0.0014$], with no

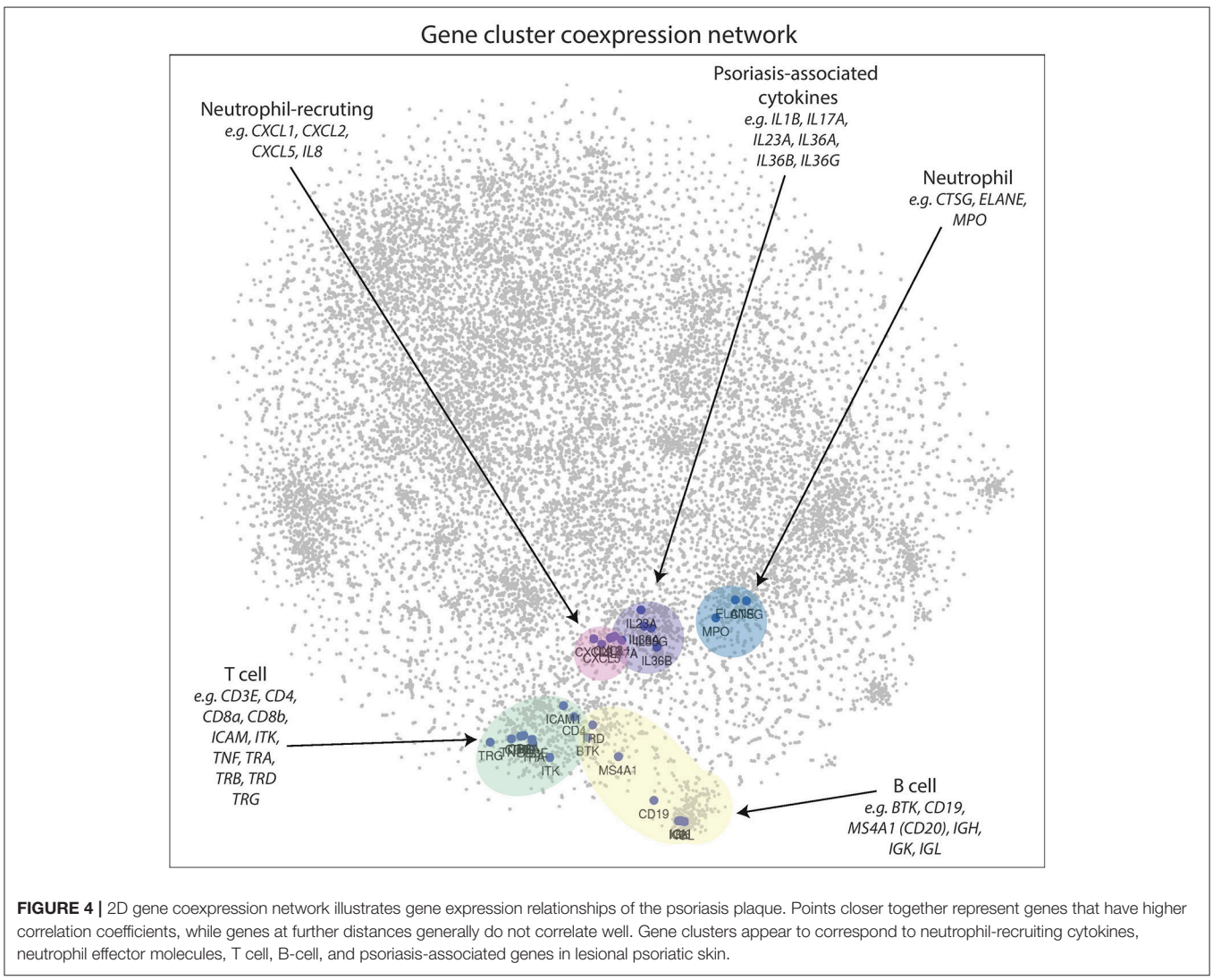

FIGURE 4 | 2D gene coexpression network illustrates gene expression relationships of the psoriasis plaque. Points closer together represent genes that have higher correlation coefficients, while genes at further distances generally do not correlate well. Gene clusters appear to correspond to neutrophil-recruiting cytokines, neutrophil effector molecules, T cell, B-cell, and psoriasis-associated genes in lesional psoriatic skin.

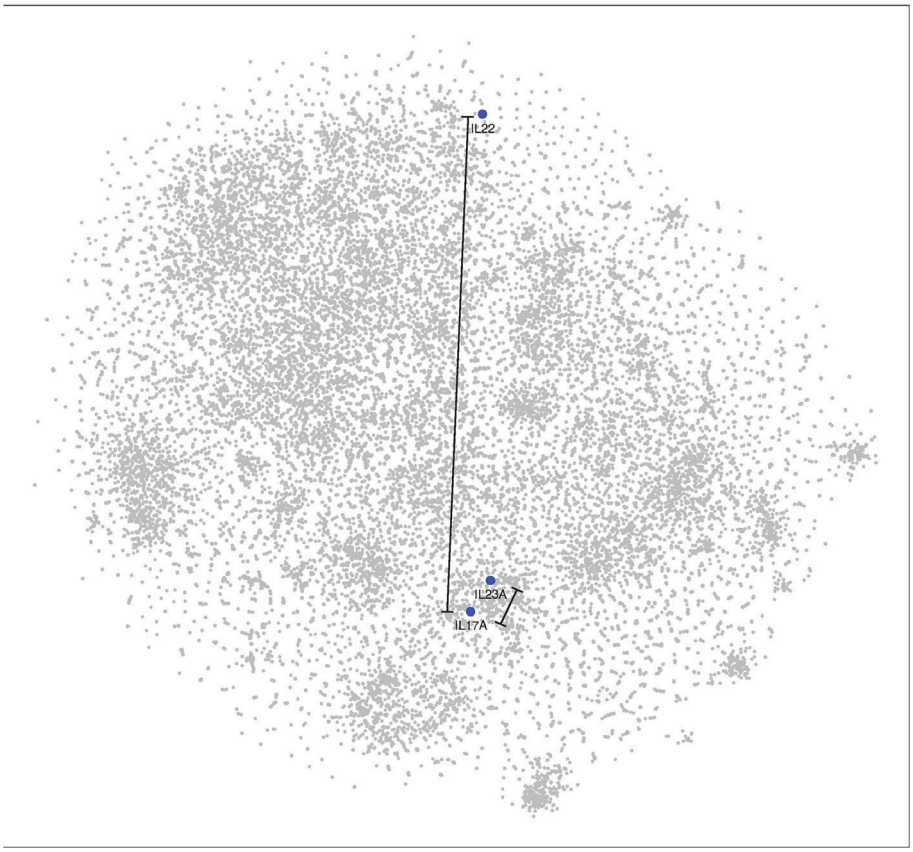

FIGURE 5 | *IL22* is located at a relatively great distance away from *IL17A* and *IL23A* in the psoriasis transcriptome, as illustrated in the 2D gene expression network map of psoriasis. These results suggest that *IL22* is not highly linked to either *IL17A* or *IL23A* in psoriasis pathophysiology.

evidence ($p = 0.33$) of any substantial residual heterogeneity (i.e., there was no remaining variability in effect sizes that was unexplained) (**Figure 3B**). Our data confirms the well-characterized dependency of IL-17A on IL-23A. However, IL-22 was not found to have a similar dependency on IL-23A, casting doubt on the theory that IL-17A and IL-22 are secreted mainly by the same dual-secreting cell.

2D Visualization of the Psoriasis Transcriptome Reveals T Cell, B Cell, Inflammatory Cytokines, Neutrophil-Recruiting, and Neutrophil Gene Clusters

To determine how genes expressed in psoriatic plaques are related to one another, correlation coefficients were calculated for all pairwise comparisons. The distances between each gene pair was calculated as described in the methods. The resulting distance matrix was then used to construct a 2D image using t-SNE.

In the 2D plot (**Figure 4**), genes that highly correlate with one another tend to be located in the same region, known as a cluster. Genes that do not cluster near each other do not correlate well. **Figure 4** clearly demonstrates that genes associated with B cells [*BTK, CD19, IGH, IGK, IGL, MS4A1* (CD20)], T cells (*CD3E, CD4, CD8a, CD8b, ICAM1, ITK, TRA TRB, TRD, TRG*), neutrophils (*CTSG, ELANE, MPO*), neutrophil-recruiting (*CXCL1, CXCL2, CXCL5, IL8*), and psoriasis-associated inflammatory cytokines (*IL1B, IL17A, IL23A, IL36A*, *IL36B, IL36G*) cluster well together in distinct groups, which supports this method as a means to visualize the entire psoriasis transcriptome.

IL22 Does Not Cluster With Other Inflammatory Cytokines Involved in Psoriasis, Including *IL17A*

With respect to other cytokines and chemokines involved in psoriasis pathophysiology, *IL22* is located peripherally at a relatively great distance away on the 2D plot of the psoriasis transcriptome (**Figure 5**). This supports our results from the meta-analyses and suggests that *IL22* does not correlate well with *IL23A*. Interestingly, *IL22* does not cluster well with any of the most commonly implicated cytokines in psoriasis

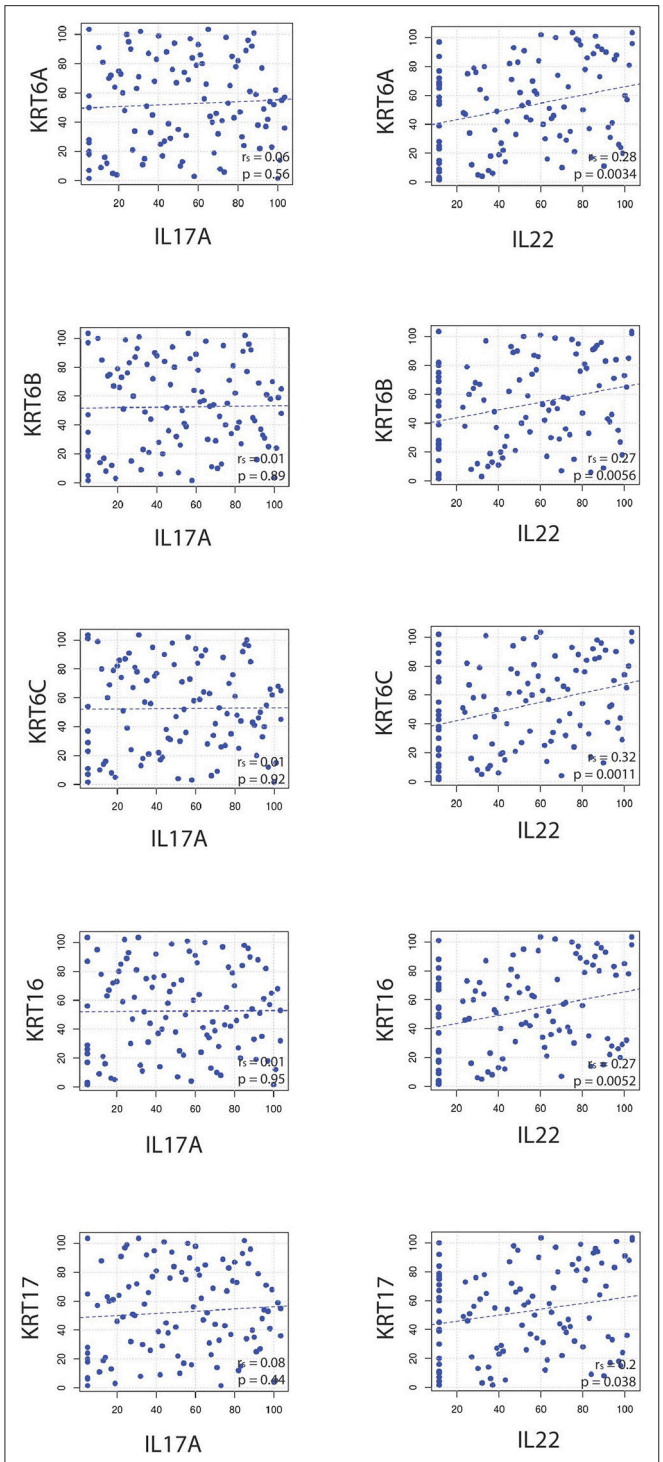

FIGURE 6 | Correlative studies demonstrate that *IL22* strongly correlates with commonly psoriasis-associated keratin genes (*KRT6A, KRT6B, KRT6C, KRT16, KRT17*), a finding that was not true for *IL17A*.

pathophysiology. In contrast, *IL17A* clusters together with *IL23A* and the other cytokines thought to be involved in psoriasis pathophysiology.

IL22 Correlates With Keratins

Several studies have demonstrated that IL-22 stimulates keratinocytes. There is a variety of evidence, including data obtained from *in vitro* studies with skin-like organoid cultures, that support IL-22 as the main cytokine responsible for epidermal hyperplasia, a hallmark of psoriasis (47, 48).

Because *IL22* failed to strongly correlate with *IL17A* and *IL23A* (**Figures 1A**, **2A**), the relationships between *IL22* and keratin genes were explored across the four independently acquired RNA-Seq psoriasis datasets. Again, Spearman's correlation coefficients (r_s) were calculated for each keratin gene's relationship with *IL17A*, *IL22*, and *IL23A*. These correlative studies demonstrate that the expression of *IL22* did indeed strongly correlate with the expression of the different keratin genes (**Figure 6**), especially *KRT6C* (keratin 6C) ($r_s = 0.32$, $p = 0.0011$). To obtain a weighted average across all four independent psoriasis datasets, a meta-analysis was performed and the resulting Forest plots (**Figure 7**) confirm the close relationship between *IL22* expression and keratin gene expression [*KRT6C*: $r_s = 0.34$, with a confidence interval that did not cross 0 (0.18–0.50)]. The weighted average of this correlation across all psoriasis datasets was highly significant ($p = 0.000025$), with no evidence ($p = 0.56$) of any substantial residual heterogeneity (i.e., there was no remaining variability in effect sizes that was unexplained). Additional genes that were found to positively correlate with *IL22* expression are listed in **Supplemental Figure 2**.

In contrast to *IL22*'s relationship with keratin gene expression, *IL17A* did not correlate well with the keratins (**Figure 6**), a finding that was confirmed by a meta-analysis across all four RNA-Seq datasets.

IL23A Correlates With Other Genes Besides *IL17A*

IL-23A is known for its ability to support Th17 T cells but it likely has a variety of functions independent of this role. To investigate this, *IL23A*'s ability to independently correlate with other immune-relevant genes was explored. **Figure 8** reveals that *IL23A* correlates with several genes unrelated to *IL17A*, a finding confirmed by meta-analyses across all psoriasis RNA-Seq datasets. Included in the analysis were genes identified by GWAS to be linked to psoriasis. Of these genes, *CARM1, KRT14, KRT37, TNFAIP3, UBE2L3* are elevated in psoriasis plaques compared to control healthy skin (**Table 1**). Thus, *IL23A* appears to be linked to other genes putatively involved in the pathophysiology of psoriasis that are unrelated to *IL17A*.

IL17A, *IL22*, and *IL23A* Expression Correlates With Psoriasis Susceptibility Genes

A variety of genes have been linked to psoriasis susceptibility through GWAS (49–55). **Table 2** demonstrates that many of these genes are differentially regulated in the setting of psoriasis. We thus sought to determine how the expression of genes located at psoriasis susceptibility loci correlated with the expression of *IL17A*, *IL22*, and *IL23A*, genes known to be

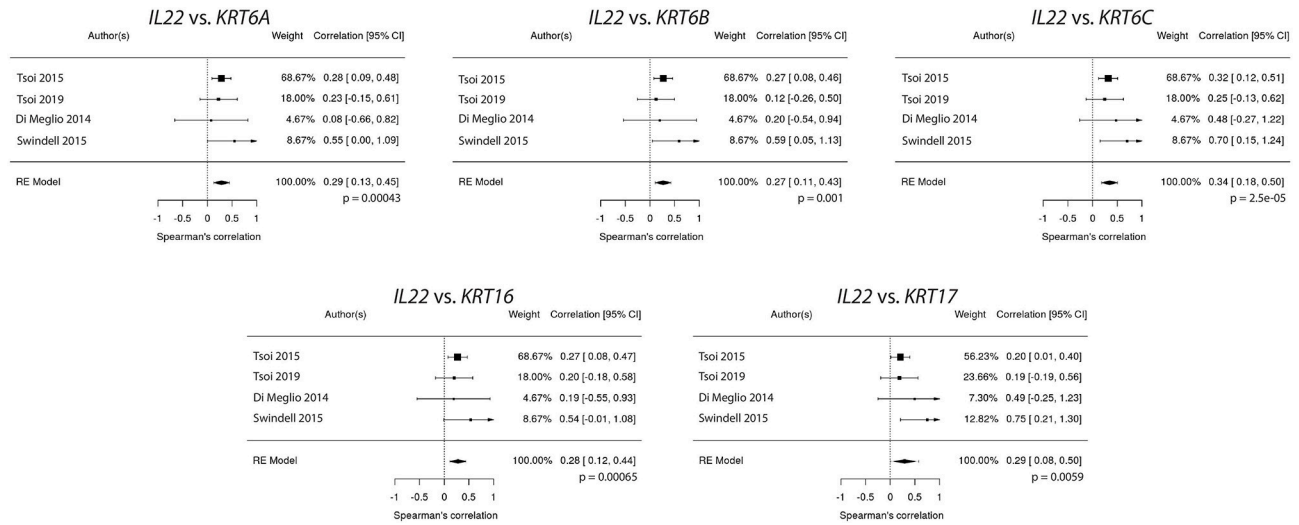

FIGURE 7 | Meta-analysis confirms a close relationship between *IL22* expression and keratin (*KRT6A, KRT6B, KRT6C, KRT16, KRT17*) expression.

linked to the pathophysiology of psoriasis. For this analysis, the expression of *IL17A*, *IL22*, and *IL23A* was plotted against the expression of each of the genes identified through GWAS studies. Spearman's correlation coefficients (r_s) were then calculated, which demonstrated a variety of significant correlations (**Table 2**) between GWAS-identified genes and *IL17A*, *IL22*, and *IL23A*. Correlation values between atopic dermatitis GWAS-identified genes and *IL17A*, *IL22* and *IL23A* expression in psoriasis samples were also obtained for comparison (**Table 3**). To obtain a weighted average across all four independent psoriasis data sets, meta-analysis was performed. The resulting Forest plots are depicted in **Figure 9**, which confirm the close relationship between *IL17A*, *IL22*, and *IL23A* and the different genes linked to psoriasis susceptibility. These results support a direct or indirect link between *IL17A*, *IL22*, and *IL23A* and these genes. Of note, the genes that significantly correlated with *IL17A*, *IL22*, and *IL23A* varied for each cytokine. These results will hopefully help investigators better understand the pathophysiology of psoriasis.

DISCUSSION

Investigators have employed numerous genetic strategies to characterize the immune response in the setting of psoriasis. Microarray and RNA-Seq have provided insight into the psoriatic transcriptome, identifying thousands of differentially expressed genes (40). However, differential expression alone does not necessarily mean that the gene is involved in psoriasis pathogenesis. For example, a gene that is normally downregulated in psoriatic T cells may actually appear falsely upregulated in psoriasis simply because there are more T cells in a psoriatic plaque. With the rising popularity of single cell sequencing, investigators are now focused on re-characterizing the psoriasis transcriptome at a greater cellular resolution, not previously obtained with whole tissue transcriptomics. However, single cell sequencing is also not without its drawbacks. Purifying immune cell populations from skin biopsy specimens can alter their transcriptome, especially for cells isolated by positive selection or flow cytometry. Furthermore, immune cells within the skin will undoubtedly have different purification yields. T cells in particular are especially difficult to analyze because once purified, they require additional non-physiologic *in vitro* stimulation with lectins or anti-CD3/anti-CD28 antibodies to identify their cytokine secretion profiles. How closely the garnered information from these studies will relate to *in vivo* cellular function remains unclear. Although each technique will yield important discoveries, none can perfectly decipher the *in vivo* pathogenic immune response.

With these limitations in mind, we have focused on developing new methods to characterize immune responses from whole tissue RNA-Seq (12, 62). We view this strategy to be an important complement to work currently being conducted by other investigators. The main advantage being that the data is not subject to experimentally-induced changes in gene expression. Its main disadvantage, however, is that it cannot discriminate between direct or indirect correlations between genes of interest.

In our current study, we utilize a machine-learning 2D visualization strategy, t-SNE, to characterize *IL17A*, *IL22*, and *IL23A* gene expression in the context of the entire psoriatic transcriptome. The 2D map of the psoriatic transcriptome revealed distinct gene clusters corresponding to common immune cell types (e.g., B cells, T cells, neutrophils).

Our data did not support the existence of a dual-secreting IL-17A/IL-22 Th17 cell as the major source of these cytokines in psoriasis. In fact, in the 2D model, these genes are located far from one another. As such, *IL22* correlated with several genes that did not appear to have a relationship with *IL17A*. In addition, a set of genes identified to be involved in psoriasis pathophysiology (*CARD14, CXCL5, CXCR2, DDX58, IFIH,*

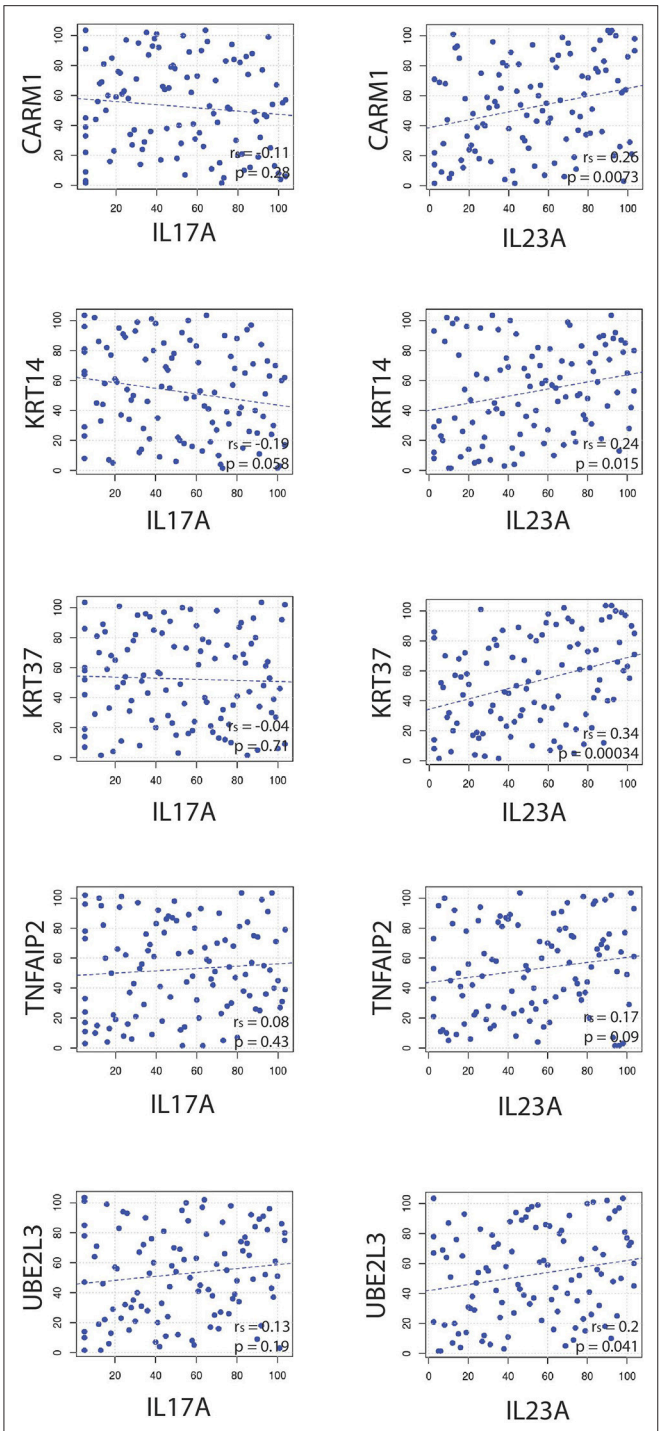

FIGURE 8 | Spearman correlations reveal that *IL23A* correlates with several genes that do not seem to be strongly related to *IL17A*.

PTPN22, and *TNFRSF9*) correlated with *IL17A* and *IL23A*, but did not correlate with *IL22*.

Though, IL-22 is commonly considered a hallmark Th17 cytokine (63), our results are in line with studies demonstrating

TABLE 1 | Expression of genes linked to psoriasis identified through GWAS and associated *IL23A* Spearman correlation and *p*-value and fold change increase in lesional skin and *p*-value.

Gene	*IL23A* Spearman correlation	*IL23A* Spearman *P*-value	Fold change increase in lesional skin	Fold change increase *P*-value
B3GNT2	−0.16	0.10537	1.001155781	0.979956721
CARD14	0.27	0.00682	2.389097546	1.33E−47
CARM1	0.26	0.00968	1.141422929	0.000834388
CDKAL1	−0.21	0.03518	0.8683625	3.52E−08
CTSG	−0.16	0.10656	0.655654701	4.50E-13
CXCL1	0.46	1.30E−06	83.27818074	2.62E−212
CXCL5	0.34	0.00056	26.29025755	2.84E−33
CXCR2	0.52	6.60E−08	6.492199066	1.40E−239
DDX58	0.33	0.00092	2.797571851	1.14E−69
DEFB4A	0.35	0.00034	1901.591548	2.62E−271
ELANE	−0.0041	0.96819	0.491347624	6.77E−10
FBXL19	0.34	0.00062	1.85489088	5.24E−28
GJB2	0.48	4.30E−07	20.36794869	0
HLAC	0.18	0.07342	1.194566942	0.001439986
IFIH1	0.42	1.40E−05	3.162866511	7.72E−104
IL12B	0.29	0.00397	29.88723345	3.40E−57
IL17A	0.24	0.01769	439.3348171	1.25E−65
IL22	0.1	0.31571	63.88350813	3.46E−30
IL23A	1	< 2E−16	3.760844628	8.35E−45
IL36RN	0.48	4.90E−07	7.305421524	1.16E−230
IL4R	0.51	6.60E−08	3.093054089	8.95E−181
KLF4	0.012	0.90338	0.667068955	3.81E−15
KRT1	−0.012	0.90464	1.206311292	0.001401592
KRT10	−0.062	0.54239	1.110563157	0.047606741
KRT14	0.22	0.02787	1.536561931	1.27E−11
KRT16	0.27	0.00767	45.90558693	0
KRT17	0.39	6.00E−05	4.076992497	2.38E−54
KRT37	0.3	0.00255	5.231342887	2.43E−46
KRT5	0.094	0.35243	1.303825713	1.61E−07
KRT6A	0.39	8.30E−05	24.37406248	0
KRT6B	0.29	0.00374	8.511861588	5.34E−107
KRT6C	0.25	0.01245	117.818558	3.29E−294
LCE3A	0.39	6.50E−05	184.4450577	6.18E−144
LCE3B	0.2	0.04403	67.28771597	2.14E−38
LCE3D	0.31	0.00208	36.65620978	0
MPO	−0.24	0.01605	1.474609904	0.0051495
NFKBIA	0.31	0.00184	1.194928571	6.14E−07
NOS2	0.64	1.20E−12	45.89643372	5.06E−181
NOS3	−0.024	0.81219	1.120615697	0.065873995
PTPN22	0.09	0.38043	2.512943835	2.58E−45
RELB	0.3	0.0023	1.63227558	3.66E−17
RUNX3	0.073	0.47497	0.879564111	0.003653355
SOCS1	0.41	3.10E−05	1.851306719	1.98E−17
STAT3	0.51	6.50E−08	2.199106208	7.64E−127
STAT5A	0.012	0.90974	0.825113995	1.27E−08
TNFAIP3	0.825113995	1.27E−08	1.053151539	0.237418045
TNFRSF9	0.24	0.01749	6.76780515	2.64E−126
TNIP1	0.4	4.20E−05	1.591537669	1.52E−33
TRAF3IP2	0.28	0.00626	1.27943256	1.21E−23
TYK2	0.084	0.41131	1.10633692	0.027521096
UBE2L3	0.22	0.03143	1.247192396	1.13E−20
VDR	0.36	0.00025	1.010394091	0.770645743
VEGFA	0.092	0.36524	1.300943308	1.11E−08
VEGFB	−0.12	0.22006	0.640896258	1.01E−36

TABLE 2 | Correlation (*R*-values) of *IL17A, IL22*, and *IL23A* with genes linked to psoriasis susceptibility through genome-wide association studies.

	***R*-value**		
Gene	***IL17A***	***IL22***	***IL23A***
CARD14	**0.18**	0.16	**0.3**
CARM1	−0.08	0.1	**0.26**
CDKAL1	−0.2	−0.15	**−0.21**
DDX58	**0.35**	0.06	**0.28**
DEFB4A	**0.32**	**0.3**	**0.4**
GJB2	**0.54**	**0.39**	**0.48**
HLAC	−0.07	−0.05	0.02
IFIH1	**0.37**	−0.02	**0.34**
IL12B	**0.47**	**0.28**	**0.32**
IL17A	**1**	0.18	**0.31**
IL22	0.18	**1**	0.13
IL23A	**0.31**	0.13	**1**
IL36RN	**0.5**	**0.4**	**0.35**
IL4R	**0.37**	**0.36**	**0.49**
KLF4	**−0.36**	−0.03	0
LCE3A	**0.21**	**0.26**	0.26
LCE3B	−0.03	**0.2**	**0.17**
LCE3D	0.08	**0.21**	**0.32**
NOS2	**0.49**	**0.31**	**0.47**
NOS3	0.04	0.08	0.1
PTPN22	**0.49**	0.09	**0.22**
RELB	0.03	**0.2**	**0.35**
RUNX3	**−0.18**	−0.04	0.13
SOCS1	**0.37**	**0.24**	**0.36**
STAT3	**0.32**	**0.45**	0.35
TNFAIP3	−0.04	0.03	**0.21**
TNFRSF9	**0.35**	0.02	**0.3**
TNIP1	0.23	**0.18**	0.37
TRAF3IP2	0.07	**0.28**	**0.24**
TYK2	0	0.06	0.14
UBE2L3	0.18	0.06	**0.17**
VDR	0.07	**0.17**	**0.4**
VEGFA	0.01	**0.23**	**0.2**
VEGFB	**−0.25**	**−0.18**	−0.21

Bolded cells $p < 0.05$.

TABLE 3 | Correlation (*R*-values) of *IL17A, IL22 and IL23A* in psoriasis with genes linked to atopic dermatitis susceptibility through genome-wide association studies (56–61).

	***R*-value**		
Gene	***IL17A***	***IL22***	***IL23A***
ADAMTS10	**−0.16**	−0.05	−0.10
C11orf30	**0.16**	−0.07	−0.03
LRRC32	**−0.18**	−0.04	−0.11
CARD11	0.10	0.05	0.15
CCDC80	**−0.24**	−0.05	**−0.22**
CLEC16A	0	0.12	**0.38**
CYP24A1	**0.32**	0.14	**0.32**
FLG	**−0.28**	**−0.16**	**−0.36**
GLB1	−0.14	−0.02	−0.02
GPSM3	−0.04	0	0.06
IL18R1	**0.23**	−0.05	0.02
IL18RAP	**0.23**	0.05	**0.27**
IL2	0.09	0.01	0.04
IL6R	−0.03	−0.10	0.11
KIF3A	0.14	−0.02	−0.01
IL13	0	**0.26**	0.06
NLRP10	−0.17	−0.11	**−0.19**
OR10A3	0.08	−0.08	0.08
OVOL1	**0.32**	**0.28**	**0.32**
PFDN4	0.01	−0.12	−0.06
PRR5L	**0.28**	−0.05	−0.06
RAD50	**0.20**	0.13	−0.01
TMEM232	0	0.11	−0.06
SLC25A46	0.06	0.03	−0.07
TNFRSF6B	−0.06	0.14	**0.28**
ZGPAT	0.03	0.12	**0.18**
ZNF652	**−0.38**	**−0.19**	**−0.42**

Bolded cells $p < 0.05$.

the existence of uniquely secreting IL-17 and IL-22 T cells or the existence of other cytokine-secreting phenotypes (48, 64–69), although these other studies usually relied upon non-physiologic *ex vivo* T cell stimulation. Another possibility is that other cell types, such as γδ T cells or mast cells, contribute to the IL-22 production in psoriasis (48, 65, 70). Even neutrophils have been implicated as major producers of IL-22 and IL-17A (71) and recent animal models have re-explored their role as effector cells in psoriasis pathophysiology (22, 72, 73). Indeed, there are numerous studies supporting a key function of these cells (71, 74–78). Single cell sequencing may provide information to verify the relationship between *IL17A* and *IL22* expression. Although it is possible that dual-secreting IL-17A/IL-22 Th17 cells exist, our results suggest that they are not a major source of IL-22.

Although we did not find evidence for a strong link between *IL22* with *IL17A* or *IL23A*, our results do support a strong correlation between the expression of *IL22* and the keratin genes, such as *KRT6A*, *KRT6B*, *KRT6C, KRT16*, *KRT17*, a finding in accord with IL-22's ability to induce epidermal hyperproliferation (48). IL-22 is clearly a major cytokine involved in psoriasis pathophysiology. In animal models, it has been demonstrated to simulate psoriasis-like epidermal changes (47, 79) and elevated levels of IL-22 positively correlate with disease severity in humans, as measured by Psoriasis Area Severity Index (PASI) scores (80–83).

CONCLUSION

Although dual-secreting T cells may exist, our results demonstrate that it is unlikely that the classical Th17 cells (IL-17A/ IL-22 dual-secreting T cells) play a universal role in

FIGURE 9 | Psoriasis susceptibility genes that positively correlate across *IL17A*, *IL22*, and *IL23A*, supporting a link between these cytokines and genes that have been linked to psoriasis through GWAS.

psoriasis pathophysiology. RNA-Seq analysis revealed that the expression of these cytokines seems to be largely unrelated to one another in the psoriasis transcriptome. However, the expression of *IL17A* did correlate with *IL23A* but, interestingly, unique relationships between *IL23A* and genes unrelated to *IL17A* were also established, supporting a broad function of IL-23.

Taken together, these results do not support the current dogma that IL-17A/IL-22 dual-secreting Th17 T cells are the major driver of psoriasis pathophysiology. In addition, our results support unique functions of IL-23 that are unrelated to its known role in supporting Th17 responses. Finally, we demonstrate that the expression of genes linked to psoriasis susceptibility also correlate with expression of either *IL17A*, *IL22*, or *IL23*. This supports the aforementioned cytokines' involvement in multiple avenues of psoriasis susceptibility.

2D mapping of inflammatory transcriptomes is an exciting innovative modality that may help us visualize relationships of all genes expressed in a disease process. When applied to gene expression relationships in psoriatic lesional skin, distinct clusters of cell lineage genes could be identified, supporting the presence of a complex crosstalk among separate cell lines in disease development. In the near future, single cell transcriptome analysis will provide additional insight into psoriasis pathogenesis. Identifying the cells responsible for the psoriasis phenotype will bring us one step closer to developing a cure for psoriasis.

DATA AVAILABILITY

Publicly available datasets were analyzed in this study. This data can be found here: "http://www.ncbi.nlm.nih.gov/Traces/sra".

AUTHOR CONTRIBUTIONS

EM, AAM, AIM, GL, MS, SR, and SH contributed to the conception and design of the study; LT and JG organized the database; AAM performed the statistical analysis; SL, JW, and CA performed data mining, SL and EM wrote the first draft of the manuscript; SL, IA, and EM wrote sections of the manuscript. All authors contributed to manuscript revision, read and approved the submitted version.

SUPPLEMENTARY MATERIAL

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fimmu.2019.00589/full#supplementary-material

Supplemental Figure 1 | Funnel plot representation demonstrating RNA-seq datasets analyzed in the meta-analysis of *IL17A* and *IL22*. All data points, representing individual data sets, fall within the 95% confidence interval. In our meta-analysis, the *p* value for residual heterogeneity did not reach significance ($p = 0.34$), indicating that all datasets are within the variation that is expected for this particular meta-analysis.

Supplemental Figure 2 | Additional genes that positively correlate with *IL22* expression.

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REVIEW
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An Integrated Approach to Unravel Hidradenitis Suppurativa Etiopathogenesis

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Hidradenitis suppurativa/acne inversa (HS) is a chronic inflammatory disease involving hair follicles that presents with painful nodules, abscesses, fistulae, and hypertrophic scars, typically occurring in apocrine gland bearing skin. Establishing a diagnosis of HS may take up to 7 years after disease onset. HS severely impairs the quality of life of patients and its high frequency causes significant costs for health care system. HS patients have an increased risk of developing associated diseases, such as inflammatory bowel diseases and spondyloarthropathies, thereby suggesting a common pathophysiological mechanism. Familial cases, which are around 35% of HS patients, have allowed the identification of susceptibility genes. HS is perceived as a complex disease where environmental factors trigger chronic inflammation in the skin of genetically predisposed individuals. Despite the efforts made to understand HS etiopathogenesis, the exact mechanisms at the basis of the disease need to be still unraveled. In this review, we considered all OMICs studies performed on HS and observed that OMICs contribution in the context of HS appeared as not clear enough and/or rich of useful clinical information. Indeed, most studies focused only on one aspect—genome, transcriptome, or proteome—of the disease, enrolling small numbers of patients. This is quite limiting for the genetic studies, from different geographical areas and looking at a few aspects of HS pathogenesis without any integration of the findings obtained or a comparison among different studies. A strong need for an integrated approach using OMICs tools is required to discover novel actors involved in HS etiopathogenesis. Moreover, we suggest the constitution of consortia to enroll a higher number of patients to be analyzed following common and consensus OMICs strategies. Comparison and integration with the findings present in the OMICs repositories are mandatory. In a theoretic pipeline, the Skin-OMICs profile obtained from each HS patient should be

compared and integrated with repositories and literature data by using appropriate InterOMICs approach. The final goal is not only to improve the knowledge of HS etiopathogenesis but also to provide novel tools to the clinicians with the eventual aim of offering a tailored treatment for HS patients.

Keywords: hidradenitis suppurativa, genomics, transcriptomics, proteomics, OMICs, data integration, public repositories

INTRODUCTION

Hidradenitis suppurativa/acne inversa (HS) is a chronic-recurrent, inflammatory, debilitating skin disease that usually presents after puberty. It is hallmarked by painful, deep-seated, chronic, suppurating lesions most commonly located in the axillary, inguinal, anogenital, and infra-mammary areas (1, 2). Treatment strategies rely on both medical and surgical options. Medical treatment is founded on the use of antibiotics, such as tetracyclines, rifampicin and clindamycin, retinoids, and immunosuppressive agents. Anti-TNFα agents, notably adalimumab that is the only biologic agent approved for HS, are the mainstay of treatment in moderate-to-severe HS (3–5).

HS incidence in different countries ranges from 6 per 100.000 in Olmsted County (6) to 6.7 per 1,000 in Australia (7) to 1.8 per 100 in Denmark (8). This epidemiological variability may reflect differences both in the awareness of physicians and in susceptibility to HS in distinct populations. In fact, it has been shown that in the United States, African Americans are more susceptible to HS, even if the underlying causes are unknown (9, 10).

The idea that the disorder is primarily caused by an inflammation of apocrine sweat glands is nowadays rejected and follicular hyperkeratosis and perifolliculitis are regarded as the earliest events detected in HS skins (11, 12). Follicular hyperkeratosis probably engenders the occlusion of the terminal hair follicles, its dilation, and finally its rupture (12). It is thought that keratin, corneocytes, hair shaft, sebum products spilled from breached pilosebaceous units into the dermis (13) can act as danger-associated molecular patterns (DAMPs) activating an immune response in deep dermis sustained by CD3+ T cells (mainly CD4+, but also CD8+), B lymphocytes, macrophages and, more importantly, neutrophils (13). CD4+ T cells (T helper (Th)) and neutrophils are the main producers of IL-17 (14, 15) that, together with TNF-α, IL-1β, and IL-10, are the cytokines found consistently overexpressed in HS lesional and perilesional skin (16–19).

Very few data are available for the events of the "subclinical inflammation" phase (20) but the hypothesis of microfilm-forming microbes or skin pathogens as main drivers of HS inflammation is fading away. In fact, Ring et al. (21) showed by peptide nucleic acid (PNA)-FISH a paucity rather than an enrichment of bacterial aggregates in HF pre-clinical HS skin when compared with healthy controls. Next-generation sequencing (22) studies performed on skin microbiome of HS patients during flares showed the existence of a dysbiosis (21, 23) that could allow the development of a pathobiome or an augmented expression of virulence factors by otherwise harmless commensal bacteria (24, 25) probably driven by host inflammation, as shown in atopic dermatitis (26). It is still debated whether these bacteria maintain a vicious circle that amplifies and sustains skin inflammation or are the *primum movens* of the disease (27).

GENOMICS

Genetics of HS: γ-Secretase

Identification of English families where HS was transmitted as an autosomal dominant trait has shed light on the genetic basis of disease susceptibility (28). Still, in pedigrees with members from more generations affected, the percentage of first-degree relatives affected was 34%. This was, according to the authors, quite far from the 50% expected for a dominant disease but was incompatible with a multigenic trait transmission. Interestingly, some families showed more women affected than men, with a 3:1 female to male ratio that today is confirmed by several epidemiological studies (8, 9), whilst other ones showed a preferential male-to-male transmission predicting that one gene-one disease cannot be applied for HS. Authors stated that assessment of genetic transmission could have been complicated by reduced penetrance, unpredictable onset age, and variable clinical severity, leading to the fact that family members presenting mild clinical manifestations might have remained undiagnosed. In addition, a strong feeling of shame associated with the disorder may lead relatives to conceal their condition to the family (28).

Gao and colleagues analyzed a four generations Chinese family by linkage analysis using microsatellite markers mapping the genes for HS in a region of about 76 Mb at chromosome 1 (1p21.1 - 1q25.3) (29). Later on, Wang et al. (30), using the same strategy with Gao et al. analyzed two Chinese Han families identifying a region on chromosome 19q13 containing about 200 Refseq genes. By Sanger sequencing, Wang et al. found two different one-nucleotide deletions not found in 200 healthy controls in *PSENEN*, encoding for presenilin enhancer (PEN2). As *PSENEN* encodes for one of the four subunits of γ-secretase complex (31), they sequenced all γ-secretase genes in four families and found 1 frameshift mutation in *PSEN1* (14q24.2) and 3 in *NCSTN* (1q23.2). Notably, each family presented a different mutation and all the mutations caused haploinsufficiency of one γ-secretase following the non-sense mediated decay (NMD) of their mRNA. Since γ-secretase catalyzes the intramembrane proteolysis of Notch receptors (30), deficiency of which caused histological features of HS in several mice models (32–34),

Wang and collaborators concluded that HS is the results of an attenuated Notch signaling in the skin of patients with *NCSTN*, *PSENEN,* and *PSEN1* inactivating mutations (30).

A DNA variant affecting splicing was found later by Liu et al. (35) in the family analyzed by Gao and collaborators thus confirming the association of *NCSTN* mutations (and the chromosome region 1q23.2) with HS. *NCSTN* and *PSENEN* novel mutations segregating with the trait were found in families from UK (36), France (37), Japan (38) and one African-American family from the United States (39).

Interestingly, two studies on sequentially recruited patients showed that very few "sporadic" patients, i.e., patients that did not report a family history for HS, presented pathogenic DNA variants in the three morbid genes (40, 41). Deep sequencing of *NCSTN* was performed by Liu et al. (42) on 95 European and African-American HS patients enrolled in the Pioneer I and II clinical trials. The majority ($n = 57$) of patients had a family history of the disease but only one patient with a nonsense mutation (rs387906896; p. R117X) and one sporadic patient with a missense variant (rs147225198; p. A410V) were found, thus reinforcing the idea that mutations in γ-secretase genes are responsible for a small percentage of HS cases and are not sufficient alone to explain all HS phenotypes.

Reduced penetrance of *NCSTN* mutations has been shown once in a Japanese family analyzed by Nomura et al. (43) where the proband's 70-year-old sister carrying the missense variant p.Q568X had never manifested any sign of the disease probably because, unlike to the other affected family members, she claimed to have never smoked.

To date more than 30 mutations have been described in *NCSTN* in HS patients (44, 45), 15 mutations in *PSENEN* (46–48) and only one "likely pathogenic" mutation in *PSEN1* (44).

Interestingly mutations in *PSENEN* results in 3 different phenotypes: (1) HS, (2) Dowling-Degos Disease (DDD), or (3) HS and DDD (47, 49), whilst DDD is not associated with any mutations in *NCSTN*.

Even if the common idea is that HS is the result of a deficient NOTCH signaling in patients with mutations in γ-secretase genes, this claim has been weakened lately by different findings.

For instance, the "likely pathogenic" mutation PSEN1 c.725delC was shown to increase, not to diminish, NOTCH signaling in zebrafish (50). In addition, genomic variations in *TSPEAR* that decrease NOTCH signaling similarly to γ-secretase mutations, have been associated to a novel form of ectodermal dysplasia affecting tooth and hair follicles without any sign of skin inflammation typical of HS (51).

The mechanism by which *NCSTN, PSEN1,* and *PSENEN* mutations lead to HS has yet to be elucidated. This seems a rather complex mechanism as γ-secretase has more than 100 identified substrates (31, 52) and process 21 Receptor Tyrosine Kinases (RTKs) involved in important cellular processes such as cell cycle, survival, differentiation, and migration (53). Gamma-secretase deficiency could also regulate inflammation as it processes important cytokines receptors such as IL-1β R1/R2 and IL-6R (31).

Genetic of the HS: Other Genes

As shown in **Table 1** and depicted in **Figure 1**, in addition to the 3 genes that encode for the subunits of γ-secretase complex, other 8 genes are involved in HS.

Mutations in the connexin-26 gene (*GJB2*) on chromosome 13q11-q12 GJB2 gene, that encodes connexin-26 (Cx26), have recently been linked to HS. Mutations in this gene caused Keratitis-ichthyosis-deafness (KID) syndrome, a rare congenital disorder of the ectoderm that gives rise to keratitis, erythrokeratoderma and neurosensory deafness. HS has been reported in association with KID syndrome in a few cases with distinct Cx26 mutations such as D50N, A40V, G12R (55–57).

Cx26 is one of the main connexins in human skin and is normally restricted to hair follicles and eccrine sweat glands (58).

The mutations of Cx26 disturb the gap junctions, specialized channels that connect the cytoplasm of adjacent cells. These cellular structures are important for tissue homeostasis, growth and development and for cellular response to external stimuli (59).

The exact correlation between HS and Cx26 mutations and the interplay of gap junctions and inflammation remain to be elucidated; it is believed that HS might result from the hyperproliferative tendency of KID syndrome patients' epidermis, leading to follicular plugging, cyst formation, and rupture and spillage of keratin and glandular secretions into the subcutaneous tissue, causing an inflammatory response (55).

Recently, Higgins et al. (60) identified a germline missense mutation in fibroblast growth factor-receptor 2 (*FGFR2*) gene in exon 5 (c.G492C, p.K164N) in a patient with HS. *FGFR2* is normally expressed in keratinocytes, hair follicles and sebaceous gland. It is a tyrosine-protein kinase that plays an essential role in cell proliferation, differentiation, migration, and apoptosis, and in the regulation of embryonic development (61). Unfortunately, to date there are no functional and expression studies about this mutation. A predictive analysis with the help of several prediction algorithms has assessed that this mutation may have a pathological consequence on the impaired protein function. Considering that FGFR2 mutations are also associated with acne and that FGFR2 results in the activation of the HS-related PI3K/Akt pathway (caused by mutations in γ-secretase genes), exploration of this aspect could be relevant (62, 63).

Marzuillo et al. (64) identified mutations in inositol polyphosphate-5-phosphatase 1 (*OCRL1*) gene in HS patients. *OCRL1* encodes an inositol polyphosphate 5-phosphatase and is involved in regulating membrane trafficking and primary cilium formation. Mutations in *OCRL1* are associated with Dent disease 2 (DD2), a disorder characterized by proximal tubule dysfunction. In a case report Marzuillo et al. described 5 DD2 patients with *OCRL1* mutations and 4 of these patients were diagnosed as having HS.

Mutations in *OCRL1* drastically reduce the OCRL1 activity, causing an increase of phosphoinositol-4,5-bisphosphate (PI(4,5)P2) levels in the plasma membrane, a substrate of this enzyme. The correlation between HS and DD2 could just be due to an accumulation of PI(4,5)P2, able to increase susceptibility to cutaneous infections.

TABLE 1 | Summary of the genes involved in HS pathogenesis, including their encoding proteins, functions, and mutation category.

Gene	Encoding protein	Function	Mutation category
PSENEN	Presenilin enhancer protein 2	Essential subunit of the gamma-secretase complex, an endoprotease complex that catalyzes the intramembrane cleavage of integral membrane proteins such as Notch receptors, and Amyloid-beta Precursor Protein	Frameshift, nonsense, splicing, missense
PSEN1	Presenilin 1	Catalytic subunit of the gamma-secretase complex, an endoprotease complex that catalyzes the intramembrane cleavage of integral membrane proteins such as Notch receptors, and Amyloid-beta Precursor Protein	Frameshift
NCSTN	Nicastrin	Essential subunit of the gamma-secretase complex, an endoprotease complex that catalyzes the intramembrane cleavage of integral membrane proteins such as Notch receptors, and Amyloid-beta Precursor Protein	Missense, nonsense, frameshift, splice site
GJB2	Gap junction protein beta 2, Connexin-26	Member of the gap junction protein family specialized in cell-cell contacts that provide direct intracellular communication.	Missense
FGFR2	Fibroblast growth factor receptor	Member of the fibroblast growth factor receptor family that plays an essential role in the regulation of cell proliferation, differentiation, migration, and apoptosis, and in the regulation of embryonic development	Missense
OCRL1	Inositol polyphosphate 5-phosphatase	Involved in regulating membrane trafficking and primary cilium formation	Missense
TNF	Tumor necrosis factor	Multifunctional proinflammatory cytokine involved in the regulation of a wide spectrum of biological processes including cell proliferation, differentiation, apoptosis, lipid metabolism, and coagulation	Non coding variant that is associated with gene expression
IL-12Rb1	Interleukin-12 Receptor Subunit Beta-1	IL-12/IL-23 pathway. IL-12 is implicated in the differentiation of the Th-1 immune response and IL-23 is mediating T17 response, the latter priming chronic neutrophils influx	Missense
DEFB103	Defensin beta 3 (hBD3)	Play an important role in innate epithelial defense	Copy number variation
DEFB4	Defensin beta 2 (hBD2)	Play an important role in innate epithelial defense	Copy number variation
MYD88	Myeloid differentiation primary response protein MyD88	Plays a central role in the innate and adaptive immune response and it is involved in the Toll-like receptor and IL-1 receptor signaling pathways	Nonsense

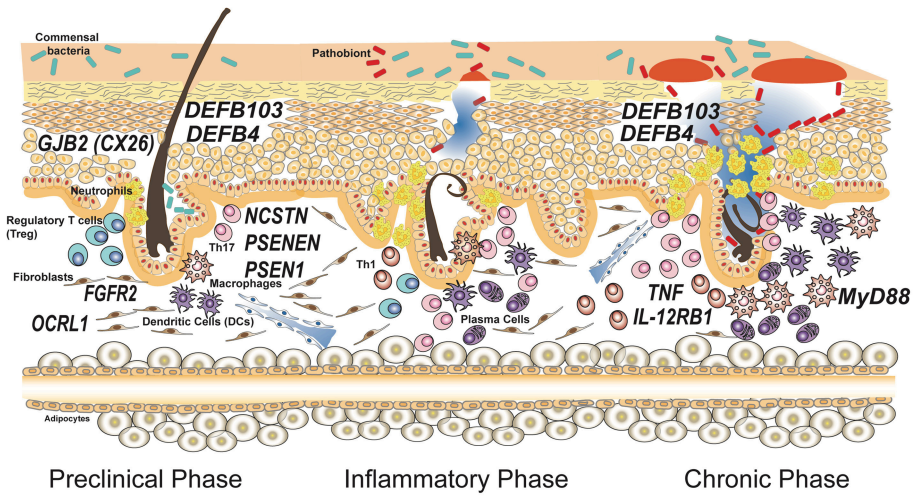

FIGURE 1 | Genes associated with susceptibility and progression of hidradenitis suppurativa. Susceptibility to the disease is caused by mutations in genes involved in keratinocytes homeostasis having a role in maintaining the integrity of the epithelial barrier. Common polymorphisms in genes encoding for proteins involved in the immune response have been associated to severity of the disease and influence the inflammatory and chronic phases. The disease model depicted is based on the most accepted model reported by Berna-Serna and Berna-Mestre (54) for hidradenitis suppurativa.

Considering evidence suggesting the central role of deranged immune response in the pathogenesis of HS, several genetic studies have focused the attention on genes encoding for protein of immune response.

In this context, Savva et al. (65) decided to investigate SNPs in tumor necrosis factor (*TNF*) and Toll-like receptor 4 (*TLR4*) genes, in DNA from 190 patients and 84 healthy controls. They found that only one SNP of the promoter region of the *TNF* gene

(-238 TNF gene polymorphism) is related both with susceptibility to HS and with the natural course of the disease; in fact, it is related to more frequent exacerbation and more severe disease. Regarding *TLR4* SNPs, they failed to identify the impact of these SNPs on susceptibility to HS (65).

Indeed, Giatrakos et al. (66) have hypothesized that the dysregulation of antigen-presentation could play a role in the pathogenesis of HS, in particular the IL-12/IL-23 pathway. Considering that both IL-12 and IL-23 receptors have a common subunit encoded by the *IL-12Rb1* gene and that there is an association between this gene and several autoimmune disorders, they decided to investigate the association between the risk for developing HS and SNPs in *IL-12Rb1*. Studying DNA from 139 patients and 113 healthy controls, they observed that SNPs in *IL-12Rb1* did not seem to play a role in the genetic predisposition; however, they found that these SNPs impacted considerably on the clinical phenotype of the disease; in fact, they are associated with more severe disease, extended skin involvement and earlier disease onset (66).

Of note, few times genetic findings contradicted common concepts in HS pathogenesis. This is true, for instance, for the study of copy number variation (CNVs) of β-defensin genes *DEFB103* and *DEFB4* (67). The idea that HS is caused by uncontrolled growth of skin microflora or by a bacterial pathogen colonizing the skin of the patients is testified by the common use of antibiotics as a first line treatment for the disease. Thus, researchers would have expected a deficiency in antimicrobial peptides production, but Giamarellos-Bourboulis and collaborators showed that an increased number of *DEFB103* and *DEFB4* genes, associated with augmented expression of β-defensin 2 and 3 proteins, is an important risk factor for HS susceptibility. However, patients with more copies of these genes were protected against a severe phenotype in terms of both age of initiation and number of affected sites (see **Figure 1**).

Recently, Agut-Busquet et al. (68) observed an association of Myeloid differentiation primary response gene 88 (*MYD88*) SNPs and susceptibility to severe HS, analyzing the DNA of 101 HS patients. This gene encodes a cytosolic adapter protein that plays a central role in the innate and adaptive immune response. This protein is involved in the Toll-like receptor and IL-1 receptor signaling pathway in the innate immune response (69). Agut-Busquet et al. found a significantly increased risk of developing severe HS (Hurley III) for the GG genotype of rs6853 in *MYD88* gene.

Genotype-Phenotype Correlation

Different authors have attempted to clinically classify HS in order to stratify patients for clinical trials and identify subpopulations prone to respond to specific therapies. Canoui-Poitrine et al. (70) identified 3 subtypes of disease ("axillary-mammary," "follicular," and "gluteal") by means of a latent class analysis on prospective clinical data of 618 consecutive patients, while 6 different phenotypes (regular type, frictional furuncle type, scarring folliculitis type, conglobata type, syndromic type, ectopic type) were suggested by Van der Zee and Jemec (71). Despite these efforts to distinguish different clinical categories of HS, establishing a clear genotype-phenotype correlation is not possible to date. However, several mutations affecting the components of the inflammasome cascade or the proteins that regulate inflammasome function have been described in syndromic HS patients. The two main syndromes including HS as a part of their cutaneous manifestations are PASH, a disorder presenting with the triad pyoderma, acne and HS (72–76), and PAPASH, a syndrome described by our group and characterized by the same triad of PASH and pyogenic arthritis (77) in whom genetic studies evaluating exons 10 and 11 of the *PSTPIP1* gene revealed a p.E277D previously unreported missense mutation.

PASH patients are generally young adults with a very early onset of the clinical manifestations of the syndrome, especially acne (72–74, 78, 79). For the first two reported PASH cases, it was hypothesized that the presence of alleles with a higher number of CCTG motif repeats close to the *PSTPIP1* promoter deregulated PSTPIP1 expression and predisposed to neutrophilic inflammation (72). This microsatellite may, therefore, be involved as a modifier gene, although it is probably not causal (80). The initial hypothesis was that PASH is a monogenic disorder, but nowadays its polygenic autoinflammatory nature has been confirmed (74, 81). An observational study of five PASH patients (74) showed that their nine gene mutations had already been entered in the database of single nucleotide polymorphisms and that seven were in the registry of hereditary autoinflammatory disorder mutations. Four of these five patients had genetic alterations typical of monogenic autoinflammatory diseases, and the only patient without any genetic changes had Crohn's disease, which is regarded as an autoinflammatory disease. Indeed, mutations of the *MEFV* (Mediterranean fever) gene have previously been associated with the typical clinical picture of recessive familial Mediterranean fever (FMF) and mutations of the *NOD2* (nucleotide-binding oligomerization domain-containing protein 2) gene are associated with an increased risk of developing Crohn's disease (82). A loss-of-function mutation in the *NCSTN* gene has been reported in one PASH patient (79). The nature and location of this mutation do not distinguish it from the reported HS mutations (83), thus supporting a close relationship between isolated HS and PASH.

TRANSCRIPTOMICS: DIFFERENTIAL GENE EXPRESSION IN HS

The impact of genetics in the susceptibility to hereditary and sporadic HS is not only limited to mutations impairing proteins known to be associated with the disease (i.e., those involved in the γ-secretase pathway); other genetic variations such as epigenetic changes, or variations in regulatory regions could play a role in HS susceptibility or in HS clinical phenotype modulation.

With this purpose, several studies analyzed the gene expression profiles in different anatomical districts (i.e., lesional skin, peripheral blood) of HS patients aimed at discovering novel actors possibly involved in the diseases or in its clinical modulation (see **Table 2**).

TABLE 2 | Overview of gene expression in lesional and non-lesional skin of HS patients, healthy controls, and subjects suffering from other skin diseases, such as psoriasis and atopic dermatitis.

Gene	Expression	Tissue	Technique	Number of subjects	References
Whole genome	50 probes differentially expressed (no validation), 10 putative disease-related pathways	Lesional skin, non-lesional skin whole blood	Affymetrix GeneChip. NO VALIDATION	27 (17 HS patients, 10 healthy donors)	(84)
Drosha, DGRC8, Dicer Exportin-5	Drosha ↓, DGRC8 ↓ in non lesional skin	Skin lesions and non-lesional skin	RT QPCR, IHC	28 (18 HS patients, 10 healthy controls)	(85)
miRNA-155-5p, miRNA-223-5p, miRNA-31-5p, miRNA-21-5p, miRNA-125b-5p, and miRNA-146	miRNA-155-5p ↑, miRNA-223-5p ↑, miRNA-31-5p ↑, miRNA-21-5p ↑, miRNA-146a ↑, miRNA-125b-5p ↓	Lesional and perilesional skin	RT QPCR	25 (15 HS patients, 10 healthy controls)	(86)
TRBP1, TRBP2, PACT, AGO1, AGO2, metadherin, SND1	TRBP1 ↓, PACT ↓, AGO1 ↓, AGO2↓, SND1 ↓	Lesional skin, peri-lesional skin psoriasis, healthy skin	RT QPCR	38 (18 HS patients, 10 psoriasis patients, 10 healthy controls)	(87)
IL-12, IL-23, IL-17	Il 12 ↑, IL17 ↑, IL-23 ↑	Lesional skin, healthy skin	RT QPCR, IHC	18 (10 patients with HS, 8 healthy controls)	(88)
IL-22, IL-20, IL-17A, IL-26, IFN-γ, IL-24, IL-1β, hBD1, hBD2, hBD3, S100A7, S100A8, S100A9	IL-22 ↓, IL-20 ↓, hBD1 ↓, hBD2 ↓, hBD3 ↓, S100A7 ↓, S100A8 ↓, S100A9 ↓	HS lesional skin vs. Psoriatic and atopic dermatitis lesional skin	RT QPCR	37 (8 healthy controls; 14 Psoriasis patients; 7 HS patients; 8 patients with atopic dermatitis)	(89)
IL-1β, IP-10, RANTES, hBD1, hBD2, hBD3, S100A7, S100A8, S100A9, RNAse7	IL-1β↑, IP-10↑, RANTES ↑, hBD1↓, S100A7↑	Keratinocytes isolated from hair follicles	RT QPCR	–	(90)
IL-17, IL-1β, TNF-α, NLRP3, IL1β, IL18	IL-17↑, IL-1β↑, TNF-α↑, NLRP3↑, IL1β↑, IL18 ↑	LESIONAL, non-lesional skin, uninvolved skin from the same patients.	RT QPCR, FC, enzyme-linked immunosorbent assays	54 (44 HS patients, 10 healthy controls)	(20)
IL32	IL32 ↑	Lesional skin and serum	RT QPCR, IHC, ELISA	36 (20 HS patients, 8 psoriasis patients, 8 atopic dermatitis patients)	(91)
IL36	IL36 ↑	Lesional skin and serum	RT QPCR, IHC, ELISA	38 (25 HS patients, 6 psoriasis patients, 7 healthy donors)	(92)
TLR2	TLR2 ↑	Skin lesions, CD68+ macrophages, CD209+ DCs	RT QPCR, IHC, FC	16 (9 HS patients, 7 healthy controls)	(93)
hBD3, RNAase 7, psoriasin (S100A7), dermicin (DCD)	hBD3 ↑	Lesional skin, healthy skin	RT QPCR	93 (36 HS patients 57 healthy controls)	(94)
GSE72702 expression profile of genes encoding sphingolipid-related enzymes from Gene Expression Omnibus database	Perilipin 1 ↑, S1P (sphingosine-1-phosphate) ↑, SMase, (sphingomyelinase) ↑; CerS2 (Ceramide synthase 2) ↓, SK2 (sphingosine kinase) ↓, SPT (serine palmitoyl CoA transferase) ↓	Skin inflammatory lesions, skin biopsies of healthy controls	*In silico* Microarray repository NOT VALIDATED	30 (17 HS patients; 13 healthy skin tissue)	(95)

↑, up-regulated in HS lesional skin; ↓, down-regulated in HS lesional skin.

Whole Genome Expression

To the best of our knowledge, the most complete gene expression profiling in HS patients has been performed by Blok et al. (84), who analyzed lesional skin and whole blood from 17 HS patients comparing their whole gene expression profile with 13 samples of healthy skins (from non lesional areas of HS patients) and whole blood from 10 healthy donors. The authors studied the whole genome expression using the Affymetrix GeneChip HT HG-U133+PM Array (Affymetrix, Santa Clara, CA, US). The first interesting finding is that no differences in NCSTN, PSEN1, and PSENEN gene expression have been found either at skin level or in whole blood from patients and controls. Blok et al. claim that the absence of differences in whole blood between HS patients and controls should be related to a possible post-transcriptional negative control of cytokines production due to augmented

serum level of tumor necrosis factor (TNF)-α as reported by Matusiak et al. (96).

When considering HS patients skin, Blok et al. identified 50 probes differentially expressed between lesional and non-lesional skin of HS patients as well as 10 pathways possibly involved in the disease (97); these pathways are (in order of statistical significance based on *p*-values): Granulocyte adhesion and diapedesis, agranulocyte adhesion and diapedesis, atherosclerosis signaling, hepatic fibrosis, primary immunodeficiency signaling, communication between innate, and adaptive immune cells, dendritic cell maturation, complement system, systemic lupus erythematosus signaling and leukocytes extravasation signaling.

The authors, in our opinion, did not exhaustively explain the findings obtained, just justifying the differences in gene expression based on the genetic background of HS patients. However, it should be underlined that Blok et al. acknowledged the limitation of their study related to the relatively small number of samples analyzed and overall to the lack of validation (both immunohistochemistry on *in situ* hybridization as well as RT-QPCR).

miRNA Regulatory Elements Expression

Another important aspect of gene expression regulation has been widely considered by Hessam et al. (85–87); in three independent studies, the authors analyzed miRNA expression profiles in inflammatory lesions from HS patients.

In the first study, the authors. (85) assessed, using RT QPCR, the expression of Drosha, Drosha co-factor DGRC8, Dicer and Exportin-5 in skin lesions and non-lesional skin from HS patients, skin lesions from patients with psoriasis and skin biopsies from healthy individuals. By finding a down-regulated gene expression of Drosha and DGRC8 just in non-lesional skin from HS patients, the authors hypothesized an early intervention of these miRNA regulators during the first, clinically and histologically not detectable, stages of inflammation, thus suggesting that when inflammation signs become observable only at that moment Dicer and Exportin-5 are involved.

In the second study (86), the expression of inflammation-related miRNA (namely miRNA-155-5p, miRNA-223-5p, miRNA-31-5p, miRNA-21-5p, miRNA-125b-5p, and miRNA-146) was evaluated through RT QPCR in lesional and perilesional skin of 15 HS patients and 10 healthy controls: the above-mentioned miRNA was shown as differentially expressed in HS patients as compared to controls, leading the authors to hypothesize a function in the modulation of the inflammatory response in the lesional skin of HS patients.

In the third study, Hessam et al. (87) enrolled HS and psoriasis patients as well as healthy controls analyzed the expression profile of RNA-induced silencing complex (98) components (specifically, transactivation-responsive RNAbinding protein-1 (TRBP1), TRBP2, protein activator (PACT) of the interferon-induced protein kinase R, Argonaute RISC Catalytic Component-1 (AGO1) and Component- 2 (AGO2), metadherin, and staphylococcal nuclease and Tudor domain-containing-1 (SND1)), also in this case using RT QPCR, in their inflamed tissues (skin biopsies). The authors concluded, after RISC component comparison between skin biopsies of HS and psoriasis patients and healthy controls, that all RISC components were differentially expressed thus highlighting a possible role in the modulation of skin inflammation in HS patients.

Indeed, the three studies of Hessam et al., also in this case with the limitation of the low number of individuals considered and the lack of information about ethnicity of patients and controls enrolled, possibly accounting for genetic differences, evidenced novel possible biomarkers correlating with local skin inflammation to be eventually considered in the follow-up of HS patients (4).

Cytokine Expression

Due to their widely accepted role in the modulation of inflammatory processes, cytokine-encoding genes have been extensively studied in the context of HS etiopathogenesis.

Schlapbach et al. (88) analyzed, using RT QPCR and validating their findings with immunohistochemistry, lesional skin of HS patients and compared IL-12, IL-23, and IL-17 gene expression with skin biopsies from healthy controls. The authors observed a specific expression of the IL-23/Th17 pathway in lesional skin, thus evidencing, as expected, a connection between the immune system and the inflammatory phenotype in the HS lesions.

Starting from the observation that IL-22 has been reported as correlated with chronic cutaneous diseases such as psoriasis, Wolk et al. (89) evaluated IL-22 encoding gene expression in HS patients. In their work, the authors showed diminished expression of IL-22 and IL-20, but not of IL-17A, IL-26, IFN-γ, IL-24, or IL-1β in HS lesional skin. Furthermore, a correlation between a shortage of IL-22 and IL-20 and reduced expression of antimicrobial peptides (hBD1, hBD2, hBD3, S100A7, S100A8, S100A9) has also been found in HS lesional skin. Wolk et al. concluded that IL-22, same as for other chronic skin diseases, could be another actor potentially involved in HS etiopathogenesis.

Hotz et al. (90) observed a significant increase in IL-1β, IP-10 secretion, and chemokine ligand 5 (CCL5/RANTES), either constitutively or on pattern recognition receptor stimulations, in keratinocytes isolated from hair follicles of patients with HS.

Using a multitasking experimental approach involving RT QPCR, flow cytometry and enzyme-linked immunosorbent assays, Kelly et al. (20), detected an augmented expression of genes encoding IL-17, IL-1β and TNF-α in biopsies of lesional skin from HS patients when compared to biopsies from non-lesional skin and uninvolved skin from the same patients. Moreover, the authors demonstrated an involvement of the inflammasome platform in HS lesions, being increased the expression of NLRP3, IL-1β, and IL-18. Finally, differential cytokine expression was detected in perilesional and non-lesional skin biopsies, leading the authors to hypothesize the presence of inflammation in HS patients present before the development of clinically evident lesions.

Thomi et al. (91) reported an increased expression of IL-36 encoding gene in skin biopsies and serum from HS patients, highlighting a local and systemic involvement of this cytokine, but the exact mechanism of action of IL-36 in HS pathogenesis has not been suggested.

In another independent study, the same authors (92) observed enhanced IL-32 gene expression in both lesional skin and serum from HS patients when compared to healthy controls or patients suffering from psoriasis and atopic dermatitis. Moreover, Thomi et al. identified the cells producing IL-32, namely natural killer cells, T cells, macrophages and dendritic cells localized at dermal level. The authors conclude that IL-32 could be a potential target for novel drug development.

At last, Jenei et al. (99) suggested after performing protein arrays that not only the microbiota and chemical content of human skin show three main topographical areas (dry, moist, oily/sebaceous), but probably in correlation to this, the immune and barrier characteristics of these topographical regions are also distinct, which can make these skin regions become prone to the development of "region-specific" inflammatory skin diseases, like HS on apocrine gland-rich areas and acne or rosacea.

Other Differentially Expressed Genes

Hunger et al. (93) aimed at exploring the function of TLR2 in the modulation of the clinical phenotype of HS patients, studies TLR2 encoding gene expression in skin lesions of HS patients. Using a multidisciplinary approach consisting in RT QPCR, immunohistochemistry and flow cytometry, the authors demonstrated an up-regulated TLR2 gene expression in HS patients skin lesions, also identifying CD68+ macrophages and CD209+ DCs as the cells expressing TLR2.

Hofmann et al. (94) published a seminal paper on defensins gene expression in the epithelium of HS patients. The authors analyzed through RT QPCR, the expression of HBD3, RNAase 7, psoriasin, and dermicin antimicrobial peptides encoding genes in lesional skin from HS patients (36 individuals) and skin biopsies from healthy controls (57 subjects). It has been observed a defective RNAase 7 expression (both at RNA and protein levels) in HS patients, while HBD3 expression (both RNA and peptide) was increased in HS patients but not in those with a more severe phenotype (Hurley grade III). The authors suggest that lack of antimicrobial peptide expression could predispose to major susceptibility to infections in skin lesions, while reduced HBD3 expression in severe HS cases could be related to a potential anti-inflammatory role.

Dany and Elston (95) using a microarray-based approach analyzed the expression of sphingolipid-related enzymes in skin inflammatory lesions of HS patients and skin biopsies of healthy controls. The authors observed an up-regulation of genes encoding ceramide and sphingomyelin generating enzymes as well as augmented expression of genes encoding enzymes catabolizing ceramide to sphingosine and those converting ceramide to galactosylceramide and gangliosides. Dany and Elston suggested that, based on the findings obtained and acknowledging the limitation due to the lack of evaluation of the sphingolipids generated by the evaluated enzymes, sphingolipid metabolism is modified in HS lesional skin. This study also suffers the absence of RT QPCR validation of the microarray results.

PROTEOMICS

Two studies on proteins being involved in HS development have been performed by Blok et al. (97) and Zouboulis et al. (100).

The authors analyzed sera from 17 patients with moderate to severe HS (based on Hurley scale), treated with ustekinumab, a monoclonal antibody directed against IL-12 and IL-23 and approved for the treatment of psoriasis. The clinical trial has been designed to understand if any proteomic marker was possibly involved in the successful (or not) treatment with the drug for 40 weeks follow-up. Blok et al. analyzed 1,129 proteins in the sera of HS patients at the beginning and the end of ustekinumab treatment.

Serum proteomic analysis revealed a different expression of 54 proteins in the 17 HS patients when compared to 10 healthy subjects. These 54 differentially expressed proteins, after accurate pathway analysis, resulted involved in inflammatory processes, cellular signaling related to immune processes and tissues architecture modulation. Moreover, among the 4 patients who achieved a good response after drug administration, all were characterized by up-regulated production of Leukotriene A4 Hydrolase (LTA4H), follicle-stimulating hormone (FSH), luteinizing hormone (LH), and human chorionic gonadotropin (HCG), firstly detected with protein array, then validated by ELISA. No effect of ustekinumab treatment has been observed when considering TNF-α, IL-17A, IL-17F.

At the end of their clinical the authors suggest that treatment with ustekinumab, a drug used for psoriasis, was somehow beneficial for HS patients, also proposing the dosage of LTA4H, together with the clinical evaluation using the Hidradenitis Suppurativa Clinical Response (HiSCR) score, for the prediction of the immunosuppressive drug in patients with mild or severe HS.

This work is of some interest in the field of serum markers possibly associated with HS and its treatment. What is strongly needed to unravel the molecular mechanisms at the basis of HS by means of proteome analysis in lesional, pre-lesional, and healthy skin in biopsies from mild to severe HS patients, as studied in the second preliminary study by Zouboulis et al. (100) in 8 HS patients involved and uninvolved skin and 8 gender-, age-, and skin location-matched female patients. The response to pharmacological treatment could be also considered but the main goal should be depicting what is happening at proteomic level in the skin of individuals with HS. Of course, the identification of serological markers related to the clinical conditions and drugs response of patients suffering from HS is also envisaged, since it is easy to be employed in their routine follow-up.

DATA INTEGRATION SKIN-OMICS

After several studies tackling HS pathogenesis using a single OMICs approach, the one of Hoffmann et al. (101) finally succeeded to integrate skin/serum transcriptomics and proteomics findings obtained in a limited number of HS patients ($n = 17$) with different degree of disease severity and healthy subjects ($n = 10$). The authors made comparisons between transcriptomic and proteomics profiles present in the main repositories or reported in previous articles (see those described above). This integrated approach, the first to our knowledge used until now to disclose the mechanisms at the basis of HS pathogenesis, provided interesting results and opened a new path to approach this complex disease.

Hoffmann et al. propose, based on integrated OMICs findings a novel pathogenic model for HS consisting of two distinct and subsequent stages, initiation with the well-known follicular obstruction and progression of the disease, being the latter characterized by a strong immune response to microbiota, thus adding a novel actor in HS etiopathogenesis.

The authors hypothesized that the differential genes and protein expression (i.e., enhanced expression of innate immune response, immunoglobulins, complements proteins, augmented interferon signature) could be due to the attempts of the immune system, both innate, and adaptive to react to microbiota present in HS patients skin; this is particularly evident if we consider the role of activated complement proteins in HS patients in the fight against commensal skin bacteria, being the main taxa (identified through literature search and metagenomic analysis) *Porphyromonas* and *Prevotella*. Moreover, it is suggested that the strong involvement of the skin-related immune system is a mechanism already observed in other cutaneous diseases that could share with HS the same immunologic mechanisms of response to skin dysbiosis.

Despite the novel approach used, the study of Hoffmann et al. suffers the important bias characterizing all OMICs studies performed to date: few patients analyzed, lack of correlation and integration with GWAS findings. In fact, the authors did not consider in their interesting integrate approach the genetic findings present in the literature, that could have contributed to identifying genetic causative variants in genes encoding the immune system actors involved in the response to dysbiosis, so missing validation of their findings by triple-checking their results with the genetic findings.

CONCLUSIONS

In this review, we collected all the information concerning the OMICs studies performed on HS patients aimed at unraveling the mechanisms at the basis of the disease or associated to clinical severity and/or the successful response to pharmacological treatment (including biological drugs).

The general picture of the OMICs contribution in the context of HS is not so clear and/or rich of clinical useful information, since most of the studies focused only on one aspect (genome, transcriptome, or proteome) of the disease, enrolling small numbers of patients (this is quite limiting for the genetic studies) from different geographical areas, looking just a few aspects of HS pathogenesis without any integration of the findings obtained or a comparison within studies.

In this sense just two articles [(97, 100): described above] constructively compared the transcriptomic and proteomic profiles of skin and serum from HS patients with previous data present in biological repositories. We do think that this is the right path to be followed to disclose the fine mechanisms at the basis of HS and its clinical course.

An integrated approach using OMICs tools is strongly required to study the full genome, the skin transcriptome and proteome (from lesional, perilesional, and non-lesional biopsies as well as serum) of HS patients stratified based on the severity of the diseases, type of treatment and response to drugs; the number of enrolled patients, with the same ethnic background, is a key issue, especially for the genetic studies, in this sense we do recommend the constitution of consortia to better address this key-point. A comparison and integration with the

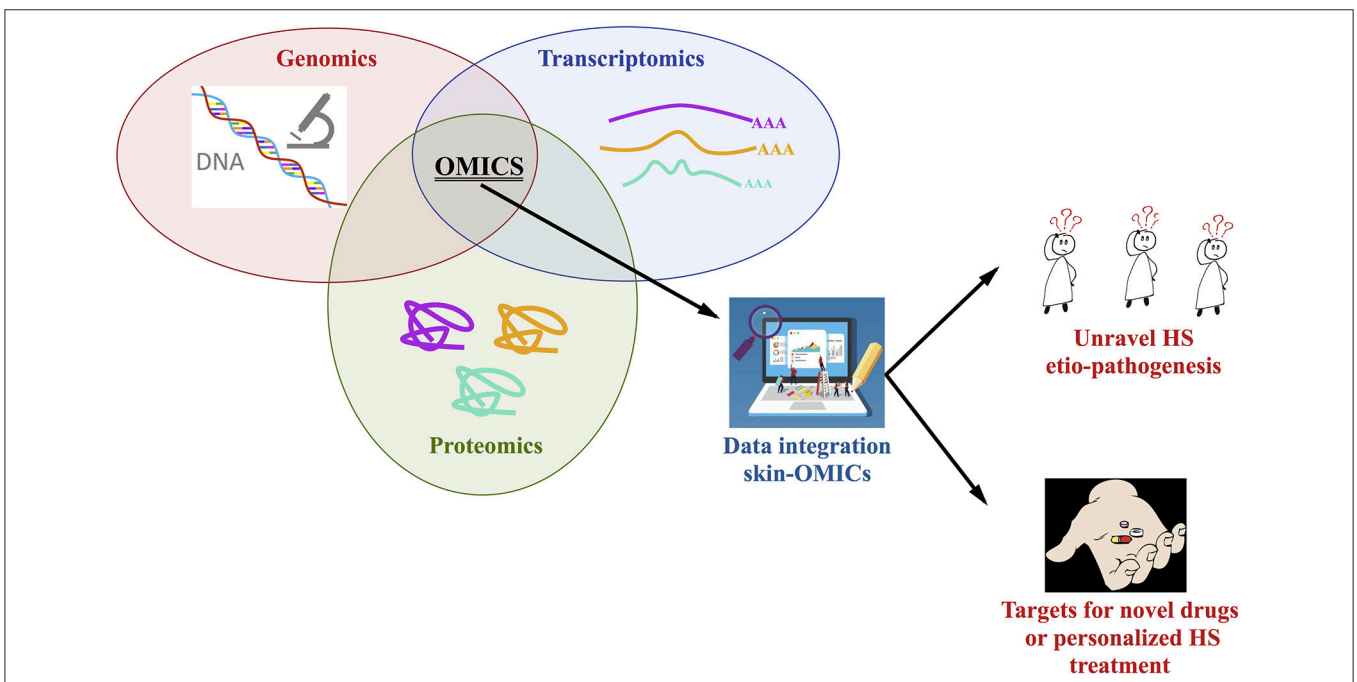

FIGURE 2 | Integrated OMICs pipeline set up for disclosing the actors involved in hidradenitis suppurativa pathogenesis and proposing a personalized treatment for the patients.

findings present in the OMICs repositories is mandatory, so in a theoretic pipeline the Skin-OMICs profile obtained from each HS patient should be compared and integrated with repositories and literature data by using appropriate InterOMICs approach (i.e., see the interesting work performed on 16 types of cancer integrating pathways and biological network data by Cava et al. (102). **Figure 2** shows the possible integrated strategy to be adopted for tailored diagnosis and treatment of HS patients.

In our opinion, this is the more rapid and robust approach to study the contribution of genome, transcriptome, proteome in the constitution of integrated pathways and networks able to better unravel HS etiopathogenesis, possibly discovering targets for novel drugs design or to personalize HS treatment, in accordance with the new challenges of the precision medicine.

AUTHOR CONTRIBUTIONS

PT contributed to the genetics and transcriptomics paragraphs. MB contributed to the genetics of γ secretase paragraph and drew the figures. GG contributed to the genotype-phenotype correlation paragraph and genetic diagnosis. AM and CZ contributed to the design and review of the manuscript and to genotype-phenotype correlation paragraph. SC contributed to the proteome, data integration paragraphs and manuscript design.

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Mechanisms of Inflammation in Neutrophil-Mediated Skin Diseases

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Neutrophil-mediated skin diseases, originally named neutrophilic dermatoses (NDs), are a group of conditions due to an altered neutrophil recruitment and activation, characterized by polymorphic cutaneous manifestations with possible internal organ involvement. Although a number of diseases are included in this setting, the two prototypic forms are pyoderma gangrenosum (PG) and Sweet's syndrome (SS) which usually present with skin ulcers and plaque-type lesions, respectively. They have central features significantly overlapping with autoinflammatory conditions which manifest as repeated episodes of tissue inflammation. However, in contrast to appropriate inflammatory responses to insults or to autoimmune disease, there is an absence of identifiable pathogens, autoantibodies, or autoreactive lymphocytes. The recognition of monogenic autoinflammatory diseases which can present with NDs has led to study several genes involved in autoinflammation in NDs. Based on discovering of a number of mutations involving different autoinflammatory genes, neutrophil-mediated skin diseases are nowadays regarded as a spectrum of polygenic autoinflammatory conditions. Although disease mechanisms have not yet been completely elucidated, NDs are recognized as diseases involving dysfunctional cellular signaling mediated by pathways mainly related to inflammasome and IL-1 with the contributory role of IL-17 and other effector molecules. The precise elucidation of the above-mentioned pathologic mechanisms may pave the way to tailored treatments for patients with different neutrophil-mediated skin diseases.

Keywords: neutrophil-mediated skin diseases, autoinflammation, inflammasome, cytokines, pyoderma gangrenosum

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INTRODUCTION

Neutrophilic dermatoses (NDs) are a heterogenous subset of conditions with common features and overlapping pathophysiology. They primarily present with cutaneous manifestations due to accumulation of neutrophils but may affect additional tissues. The most well-defined NDs include pyoderma gangrenosum (PG), Sweet's syndrome (SS), subcorneal pustular dermatosis, neutrophilic eccrine hidradenitis (NEH), bowel-associated dermatosis-arthritis syndrome (BADAS), rheumatoid neutrophilic dermatitis, Behçet's disease (BD), amicrobial pustulosis of the folds (APF) and generalized pustular psoriasis. The presentations by pathology

of the neutrophil-mediated autoinflammatory skin diseases for which there is genetic and immunological evidence are reported in **Table 1**. As PG with its syndromic forms is the prototypical ND and has a large body of research available, it will be the focal point of the discussion. Concerning the other entities, since there are no extensive research data available in the literature, their links with suggested mechanisms would be speculative. Each disease may present within an overlapping spectrum both clinically and histopathologically which can make diagnosis difficult and management challenging. The central features of NDs have significant overlap with disorders included within the spectrum of autoinflammatory conditions which manifest as reoccurring periods of tissue inflammation (18). However, in contrast to appropriate inflammatory responses to insults or to autoimmune disease, there is an absence of identifiable pathogens, autoantibodies, or autoreactive lymphocytes (16). The concept of autoinflammation arose out of the discovery of conditions resulting from specific genetic mutations leading to chronic inflammation devoid of autoreactive T cells (or antigen specific T cells) or autoantibodies. The first autoinflammatory disease identified, TNF (tumor necrosis factor) receptor-associated periodic syndrome (TRAPS), was described in 1999 upon identification of TNF receptor mutations in the autosomal dominant condition (19). Dysregulation of innate immunity signaling pathways particularly the overexpression of the proinflammatory cytokine interleukin (IL)-1, is considered to be the prominent mechanism behind the pathophysiology of these disorders (20).

TABLE 1 | Clinical, genetic, and immunological features of the main autoinflammatory neutrophil-mediated skin diseases.

Disease	Cutaneous presentation	Genetics	Immunology	References
PG	Ulcers with undermined, erythematous-violaceous borders	MEFV, NLRP3, NLRP12, NOD2, and LPIN2 mutations	Increased skin IL1β, IL1RI, IL1RII, TNFβ, TNFRI, TNFRII, IL17, IL17R, L-selectin, IL8, CXCL 1/2/3, CXCL16, RANTES, MMP-2, MMP-9, TIMP-1, TIMP-2, Siglec 5, Siglec 9, Fas, FasL, CD40, and CD40L	(1, 2)
		R52Q mutation in the PSTPIP1 gene	Not evaluated	(3)
		G258A and R52Q mutations in the PSTPIP1 gene	Not evaluated	(4)
		Not evaluated	Increased skin IL23	(5)
		Ptpn6 mutations	Increased serum IL1α	(6–8)
PAPA	Ulcers with undermined, erythematous-violaceous borders; inflammatory acne	E250 K in the PSTPIP1 gene	Increased serum IL1β	(9)
		E250Q and A230T mutations in the PSTPIP1 gene	Not evaluated	(10)
PASH	Ulcers with undermined, erythematous-violaceous borders; nodules, abscesses, fistulae	p.I591T, p.M694 V, p.V726A mutations in the MEFV gene; p.R702 W and p.G908R in the NOD2 gene; p.Q703 K in the NLRP3 gene; p.A106T in the IL1RN gene; p.E277D in the PSTPIP1 gene; and p.G8R in the PSMB8 gene	Increased skin IL1β, IL1RI, IL1RII, TNFα, TNFRI, TNFRII, IL-17, IL17R, L-selectin, IL-8, CXCL1/2/3, CXCL16, RANTES, MMP-2, MMP-9, TIMP-1, TIMP-2, Siglec 5, Siglec 9, Fas, FasL, CD40, and CD40L	(1)
		Increased CCTG microsatellite repeats in the PSTPIP1 gene	Not evaluated	(11)
		MEFV, NLRP3, NLRP12, NOD2, and LPIN2 mutations	IL-1-b, IL-17, TNFα, IL-8, CXCL1/2/3, and CXCL16	(2)
PAPASH	Ulcers with undermined, erythematous-violaceous borders; nodules, abscesses, fistulae	p.E277D missense mutation in the PSTPIP1 gene	Not evaluated	(12)
DIRA	Generalized pustular psoriasis	Monogenic (IL1RN mutations)	Increased serum IL1α, macrophage inflammatory protein 1α, TNFα, IL8, and IL6	(13)
DITRA	Generalized pustular psoriasis	Monogenic (IL36RN mutations)	Increased keratinocyte production of IL8 in response to proinflammatory cytokines (IL36α, IL36β, and IL36γ) as well as to IL1β and polyinosinic–polycytidylic acid	(14, 15)
CAPS	Urticaria-like lesions	Monogenic (NLRP3 mutations)	Increased serum IL1β and IL18	(16, 17)

CAPS, Cryopyrin-Associated Periodic Syndromes; CD40, cluster of differentiation 40; CD40, CD40 ligand; CXCL, chemokine (C-X-C motif) ligand; DIRA, Deficiency of IL-1 receptor antagonist; DITRA, Deficiency of IL-36 receptor antagonist; E-selectin, endothelial selectin; IL, interleukin; IL1RN, interleukin-1 receptor antagonist; IL-xR, interleukin-x receptor; LPIN2, Lipin 2; L-selectin, leukocyte selectin; MEFV, Mediterranean fever; MMP, matrix metalloproteinase; NLRP, nucleotide-binding domain, leucine-rich repeat containing gene family, pyrin domain-containing protein; NOD2, Nucleotide-binding oligomerization domain-containing protein 2; PAPA, Pyogenic sterile arthritis, Pyoderma Gangrenosum, and acne; PAPASH, Pyogenic sterile arthritis, PG, and acne; PASH, Pyoderma Gangrenosum, Acne and Suppurative Hidradenitis; PG, Pyoderma Gangrenosum; PSMB8, proteasome subunit beta 8; PSTPIP1, proline–serine–threonine phosphatase interactive protein 1; RANTES, Regulated upon Activation, Normal T cell Expressed, and Secreted; Siglec, Sialic acid-binding immunoglobulin-type lectins; TIMP, Tissue inhibitor of metalloproteinases; TNF, tumor necrosis factor; TNFR, tumor necrosis factor receptor; VEGF, vascular endothelial growth factor.

MECHANISMS OF INFLAMMATION IN NEUTROPHILIC DERMATOSES

Insights From Inherited Autoinflammatory Syndromes

The recognition of several monogenic diseases which can present with ND has led to an improved understanding of the possible mechanisms of polygenic non-mendelian inherited ND. These monogenic syndromes include CAPS (Cryopyrin-Associated Periodic Syndromes), DIRA [Deficiency of IL-1 receptor antagonist (IL-1RA)], DITRA [Deficiency of IL-36 receptor antagonist (IL-36RA)], PAPA (Pyogenic sterile arthritis, PG, and acne), and chronic recurrent multifocal osteomyelitis (CRMO) (21, 22).

CAPS are a group of rare inherited inflammatory disorders associated with dominant mutations in the cryopyrin-coding gene NLRP3 (nucleotide-binding domain, leucine-rich repeat containing gene family, pyrin domain-containing protein 3) on chromosome 1q44 which is also known as CIAS1, PYPAF1, or NALP3. Currently, more than 90 mutations involving NLRP3 and associated with CAPS phenotypes have been reported.

CAPS contain a spectrum of hereditary periodic fever syndromes including familial cold autoinflammatory syndrome (FCAS), Muckle-Wells Syndrome (MWS), and chronic infantile neurological cutaneous and articular syndrome (CINCA), also known as neonatal-onset multisystem inflammatory disease (NOMID). Characteristic symptoms are periodic fever and urticarial lesions. Dependent on severity, they can be associated with several clinical manifestations, including arthritis, conjunctivitis, amyloidosis, sensorineural hearing loss, aseptic meningitis, and/or cerebral atrophy (17).

DIRA is an autosomal recessive mutation in IL1RN (IL-1RA gene) on chromosome 2 leading to the absence of IL-1RA, resulting in an IL-1 signaling hyperactivity (13, 23). DIRA manifest as perinatal-onset pustular dermatitis, joint swelling, painful osteolytic lesions, and periostitis.

DITRA is caused by homozygous or compound heterozygous damaging mutations in IL36RN and is characterized by generalized pustular rashes and systemic inflammation (14, 15). IL36RN encodes for IL-36RA which inhibits binding of IL-36 to its receptor. When IL36RA is functionally impaired there is an enhanced IL-36R signaling which directly and indirectly attracts immune cells, especially neutrophils, giving rise to the pustular rashes (14, 15). IL-36 has been reported to be upregulated in a psoriasis-like inflammatory mouse model (24), confirming the role of this cytokine family in the pathogenesis of pustular psoriasis (25). Moreover, a strong correlation has been demonstrated in human psoriatic skin between the expression of IL-36 and that of other cytokines, such as IL-17, IL-23, TNF-α, and IFN-γ (26), suggesting that a positive gene expression loop might occur in psoriasis. Moreover, IL-36 also enhances IL-1α levels, further amplifying the inflammatory network (27). Thus, the IL-1/IL-36 inflammatory axis appears to be a key player of disease pathology in generalized pustular psoriasis (28) and its role may be intriguingly hypothesized also in other NDs, although it needs to be confirmed by dedicated studies.

PAPA syndrome is due to two primary mutations (A230T and E250Q) in the gene encoding proline–serine–threonine phosphatase interactive protein 1 (PSTPIP1) (10, 29). Hyperphosphorylation of the mutated PSTPIP1 protein results in increased pyrin mediated activation of the inflammasome, dysregulation of caspase 1, and overexpression of IL-1β (29). The presence of a prototypical ND such as PG in the context of PAPA syndrome, caused by a single inflammasome regulator gene mutation, suggests an autoinflammatory component in the pathophysiology of NDs (30). The occurrence of mutations in a single gene encoding an inflammasome regulating protein, as seen in PAPA, is similar to what happens in the first monogenic autoinflammatory conditions identified, i.e., familial Mediterranean fever and CAPS. Yet further studies have identified autoinflammatory syndromes with features of ND that are linked to mutations in multiple genes. In addition to PAPA, PG can present with other autoinflammatory syndromes including PG, acne, and suppurative hidradenitis (PASH) and pyogenic arthritis, PG, acne, and suppurative hidradenitis (PAPASH). The findings of these syndromic forms of PG along with reports of familial presentations of PG suggest the shared genetic basis of this ND (1, 11, 12, 31, 32).

Investigation of 7 cases of PASH and 13 cases of isolated PG revealed multiple mutations in a variety of autoinflammatory genes, including PSTPIP1, Mediterranean fever (MEFV), Nucleotide-binding oligomerization domain-containing protein 2 (NOD2), NLRP3, NLRP12, Lipin 2 (LPIN2) (2). The MEFV gene encodes for the protein pyrin, which is an innate immune system sensor which plays a central role in inflammasome activation. Different mutations in the genetic sequence can lead to variable clinical presentations with overlapping features.

In NDs, MEFV (S242R) mutations have been identified and lead to a chronically active pathogen-related response with inflammasome activation and IL-1β secretion (33). NOD2 is an intracellular pattern recognition receptors (PRRs) that plays a key role in orchestrating the proper assembly of autophagy-related proteins. Autophagy involves a cellular response resulting in the degradation of cytoplasmic components and is important in the transfer of microbial components to intracellular PRRs. The catabolic autophagy pathway responds to a wide variety of cellular stressors including nutrient deprivation, hypoxia, DNA damage, mechanical injury, reactive oxygen species (ROS), and the presence of microbial ligands (34). Loss of function mutations in NOD2 result in impaired autophagy and have been associated with inflammatory bowel disease (IBD), a condition characterized by heightened production of proinflammatory cytokines and often associated with ND comorbidity (35). Autophagy has been shown to be important in mitochondrial homeostasis (36) and its deficiency is associated with increased mitochondrial membrane permeability and ROS production, and the release of mitochondrial DNA into the cytosol (36–38). Mitochondrial DNA and ROS are both activators of the NLRP3 inflammasome (36, 37). Studies have revealed that macrophages with defective autophagy have decreased NLRP3 inflammasome activation (38). We speculate that mutations in NOD2 lead to defective autophagy causing increased production of ROS and the release of mitochondrial DNA resulting in NLRP3 activation

and subsequent ND. These findings point to the polygenic basis of NDs and helped establish the categorization of NDs among the autoinflammatory disorders. The polygenic nature suggests that NDs arise from a multifactorial response in genetically predisposed patients.

Neutrophil Production and Recruitment

In normal physiology, neutrophils are heralded as major effector cells in acute inflammation. As the most abundant leukocytes in circulation, neutrophils have been extensively described as protagonists against infection and innate immune system responders to insults. Within the bone marrow, the production and differentiation of neutrophils is regulated primarily by granulocyte colony-stimulating factor (G-CSF) (39). G-CSF also acts to promote release of mature neutrophils from the bone marrow into circulation. This is accomplished by the uncoupling of the CXC-chemokine receptor 4 (CXCR4) and CXC-chemokine ligand 12 (CXCL12) (39).

Within tissues, the "neutrostat" loop is a feedback pathway which normally suppresses neutrophilic response by suppressing production of G-CSF. This loop is initiated after phagocytosis of infiltrative neutrophils, resulting in suppression of IL-23 production by resident macrophages and dendritic cells and subsequent decreased secretion of IL-17, an important promoter of G-CSF production (40–42). Evaluation of patients with NDs has shown significantly elevated serum G-CSF, and therapeutic G-CSF is implicated in a majority of drug-induced SS cases, indicating a plausible but undiscovered contributing mechanism underlying ND (43–47). In addition, elevated IL-17 levels have been found in tissue samples from patients with PG, SS, and APF, a rare ND presenting with pustular lesions typically involving the skin folds and anogenital area (48–52). IL-17 is a key cytokine in both activation and induction of neutrophils to produce IL-8, a potent chemokine that is the principle chemoattractant of neutrophils. Increased levels of IL-8 have been found in ND lesional skin, and the chemokine works synergistically with TNF-α to potentiate and maintain a proinflammatory state (1, 2, 16, 18–21, 49, 50, 53). In mice models, experiments have revealed that protein kinase C α (PKCα) within keratinocytes promotes neutrophil infiltration of the epidermis, and may also play a central role in upregulation of G-CSF and IL-6 gene expression independent of TNF-α signaling, giving the possibility of a peripheral mechanism of ND (54).

Innate Immune System Activation

The innate immune system uses germline encoded PRRs to recognize pathogen-associated molecular patterns (PAMPs), including (foreign) PAMPs and (endogenous) damage-associated molecular patterns (DAMPs), to initiate the production of proinflammatory cytokines (55). Changes in local cellular homeostasis including temperature, pH, oxygen, and osmolarity are recognized as DAMPs by resident tissue macrophages (55). Recognition of DAMPs initiates an inflammatory cascade involving the inflammasome which consists of a central scaffold of proteins, a sensor (including Nod-like Receptors), the adaptor protein ASC [apoptosis associated speck-like protein containing a caspase-associated recruitment domain (CARD)] and the effector protein caspase-1 (22). Members of the NLRP (Nucleotide-binding oligomerization domain, Leucine-rich Repeat and Pyrin domain-containing) family, are the primary cytoplasmic PRRs that mediate inflammasome activation (55). Oligomerization of the inflammasome results in caspase-1 activation leading to the cleavage of pro-IL-1β and pro-IL-18 to IL-1β and IL-18 (22, 55). Gain-of-function mutations in NLRP3 gene are responsible for the development of autosomal dominant inflammatory disorders which typically presents with episodic urticarial neutrophil-rich cutaneous lesions, known as CAPS (see above) (56). Although NLRP3 mutations are prototypically responsible for inflammasome activation, other mutations such as those involving IL-1 and IL-36 pathway genes may also induce inflammasome activation (13, 14). The symptoms of CAPS are the result of overexpression of IL-1β secondary to constitutive activation of the cytoplasmic macromolecular complex. Evaluation of patients with ND have also shown elevated serum and lesional tissues IL-β levels (50, 57, 58). In addition, keratinocytes exposed to ultraviolet B irradiation, contact allergens or in the setting of psoriasis activate similar inflammasome pathways resulting in IL-1β production and subsequent neutrophil localization and activation (59–61), although there is support from mouse models that bone marrow-derived cells with isolated NLRP3 mutations are sufficient to induce IL-1β associated cutaneous autoinflammation (62).

Immune Signal Transduction

Immune cell proinflammatory signal transduction is inhibited by the tyrosine phosphatase known as Src homology region 2 (SH2) domain–containing phosphatase-1 (SHP-1) (63, 64). Dysfunctional activity of SHP-1 is associated with various diseases including multiple sclerosis, leukemia and psoriatic arthritis (65–69). SHP-1, also known as *PTPN6* (protein tyrosine phosphatase nonreceptor type 6), is encoded by the gene *Ptpn6*. Heterozygous mutations and splice variants of *Ptpn6* have been identified in patients with PG and SS (4).

Ptpn6^{spin} mice are the product of a Y208N (or Tyr208Asn) missense mutation leading to amino acid substitution in the carboxy-terminal SH2 domain of SHP-1. These mice develop severe cutaneous inflammation driven by overexpression of IL-1α (6). Histopathologically, the inflammatory cutaneous lesions resemble human NDs, with neutrophil-rich infiltrate and pustules within the epidermis, and are associated with neutrophilia. These data may support the role of mutations involving *Ptpn6* in triggering NDs in humans.

The Kanneganti group have utilized this murine model to characterize key regulatory components of neutrophil-mediated cutaneous autoinflammation. These authors have surprisingly shown that IL-1α signaling, but not IL-1β or caspase-1 associated inflammasome, plays a key role in orchestrating cutaneous autoinflammation in Ptpn6^{spin} mice (6). Their work has elucidated the complex regulation of the IL-1α pathway and identified a number of signaling components as pivotal in the development of cutaneous inflammation in Ptpn6^{spin} mice, such as IL-1 receptor (IL-1R), myeloid differentiation primary response gene 88 (MyD88) (7), spleen tyrosine kinase (Syk)

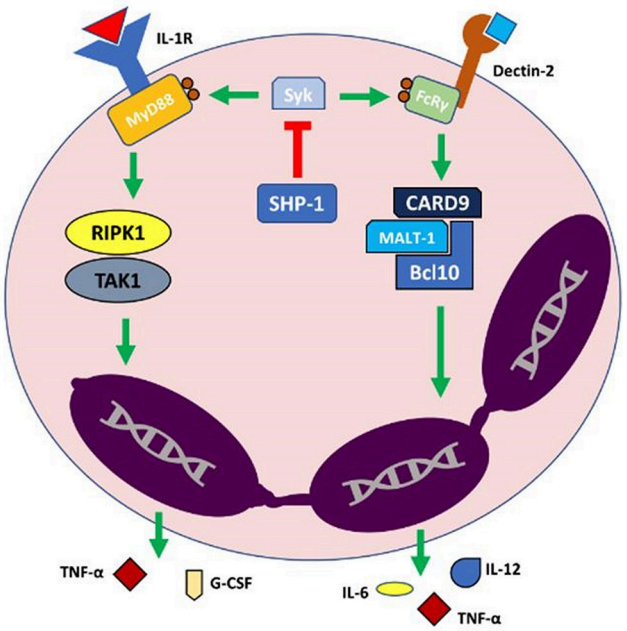

FIGURE 1 | Model of IL-1α (Interleukin 1α)-mediated autoinflammation in neutrophilic dermatoses. In normal physiologic conditions SHP-1 (Src homology region 2 domain-containing phosphatase-1) acts to inhibit Syk (spleen tyrosine kinase) which subsequently downregulates the activation of IL-1R and Dectin-2 proinflammatory pathways. Dysfunction in SHP-1 leads to uncoupled Syk activity, subsequently leading to a neutrophil-driven autoinflammatory state via an exaggerated release of proinflammatory cytokines and other effector molecules. Bcl10, B-cell lymphoma/leukemia 10; CARD9, Caspase recruitment domain-containing protein 9; FcRy, Fc receptor common γ chain; G-CSF, Granulocyte Colony Stimulating Factor; IL-1R, Interleukin 1 receptor; MALT-1, Mucosa-associated lymphoid tissue lymphoma translocation protein 1; MyD88, Myeloid differentiation primary response 88; RIPK1, Receptor-interacting serine/threonine-protein kinase 1; TAK1, Transforming growth factor beta-activated kinase 1; TNF-α, Tumor necrosis factor α.

(7), receptor interacting protein kinase 1 (RIPK1) (6), tumor growth factor-β activated kinase 1 (TAK1) (7) and apoptosis signal-regulating kinase 1 (ASK1) (8).

SHP-1 was also shown to regulate IL-1α signaling primarily through preventing phosphorylation of MyD88 by Syk (7). This pathway is illustrated in **Figure 1**. Of note, the Kanneganti group showed that inflammation in Ptpn6^{spin} mice is independent of toll-like receptors (TLRs), IFN-α/β receptor (IFNAR), integrin β-3 (ITGB3), NOD2–RIPK2 signaling, and independent of stimulator of IFN genes (STING) (7, 70). Support for unique role of IL-1α signaling in the development of cutaneous inflammation in Ptpn6^{spin} mice is strengthened by ruling out the influence of these additional innate immune signaling pathways (7, 70).

Recently, CARD9 signal transduction was identified as an essential mediator of cutaneous inflammation in Ptpn6^{spin} mice (71).

CARD9 is an adapter protein downstream of Syk and is central transducer for multiple innate signaling pathways including the C type lectin receptors, Dectin-1 and Mincle pathways (72). CARD9 forms a complex with MALT1 (mucosa-associated lymphoid tissue lymphoma translocation protein 1) and BCL10 (B-cell lymphoma/leukemia 10) leading to initiation of the NF-κB (nuclear factor kappa-light-chain-enhancer of activated B cells) signaling cascade and increased transcription of pro-inflammatory cytokines (TNF and IL-6) resulting in T helper (Th)17 polarization (73).

As previously discussed, IL-17 is a key signaling molecule in ND pathogenesis. CARD9 is thus linked to cytokine production, innate anti-fungal immunity, and myeloid cell activation (72). Identification of the key role of CARD9 in the autoinflammatory signaling pathway suggests the interconnection between NDs and other autoinflammatory conditions. Interestingly, CARD9 mutations are associated with IBD (74) and deletion in CARD9 significantly dampens the IL1-mediated cutaneous inflammatory disease in a mouse model knockout for CARD9 (70). Aberrant signaling involving SHP-1, Syk, and CARD9 can lead to neutrophil-mediated autoinflammation driven by overproduction of IL-1α. Thus, blocking these signals may provide a novel approach to design effective therapeutic strategies to treat NDs.

PATHOGENESIS-DRIVEN TREATMENT OF NEUTROPHILIC DERMATOSES

Several lines of evidence indicate that IL-1 blockers, namely anakinra, rilonacept, gevokizumab, and canakinumab, are effective in the treatment of skin manifestations of different autoinflammatory disorders. The introduction of these pharmacological agents represents a breakthrough not only in the management of monogenic inherited autoinflammatory diseases but also of skin diseases where neutrophils play a crucial pathogenic role. More specifically, Brenner et al. (75) reported complete healing of PG and acne in a patient affected by PAPA after 1 month-therapy with anakinra 100 mg/day. Similarly, canakinumab 150 mg every 8 weeks led to complete remission of PG and acne lesions in another PAPA patient. Two case reports are present in the literature on two patients with PASH treated with anakinra, who both experienced a partial remission of skin lesions (11, 76). In an open-label, proof of concept study conducted on six patients with active PG who were given three subcutaneous injections once every 4 weeks of the anti-IL-1β monoclonal antibody gevokizumab, complete remission of PG was observed in four patients, a partial response in one patient and another one did not respond. (ClinicalTrials.gov Identifier: NCT01882504) Conversely, a phase 3 trial demonstrated limited effectiveness of the same drug on PG patients. (ClinicalTrials.gov Identifier: NCT02326740 and NCT02315417) Complete PG clearance under canakinumab treatment has been described in two patients after 3 (77) and 12 months (78), respectively. Canakinumab effectiveness has also been assessed in an open-label study on five PG patients unresponsive to systemic steroids, with three out of five achieving complete remission at 16-week follow-up visit (79). A prompt and long-lasting response to anakinra in terms of both cutaneous and systemic manifestations has been described in a patient with SS (80). In another SS patient,

Kluger et al. observed that, despite a relatively rapid response to anakinra, both skin and systemic symptoms relapsed upon drug withdrawal (81). Amazan et al. reported on a woman with steroid- and anti-TNFα- refractory APF who experienced complete healing of her lesions on a regimen of 100 mg/day anakinra (82).

A rapid and robust clinical response to secukinumab, an anti-IL-17 monoclonal antibody, was reported in an adolescent with severe cutaneous manifestations due to DITRA (83). In addition to the latter clinical observation, a pathomechanistic link between IL-36 and Th17 differentiation may be postulated also based on the findings by Carrier et al. (26), who showed a direct correlation between IL-36 gene expression and IL-17 levels in the lesional skin of psoriatic patients.

Future perspectives in the management of PG involve the IL-1α blockade. IL-1α overproduction has been demonstrated in response to deregulated SHP-1 activity triggering a severe neutrophil-mediated inflammatory disease that develops independently of inflammasome. Based on these findings, there is an ongoing phase 2 open-label trial using bermekimab, an IL-1α inhibitor, in PG (ClinicalTrials.gov Identifier: NCT01965613).

CONCLUSIONS

NDs are a complex, variable and heterogenous group of diseases which have significant overlap in presentation and pathogenesis. Our understanding of the molecular mechanisms of NDs is based primarily on the discovery of familial variants, classification of autoinflammatory syndromes and development of mouse models. While understanding of disease mechanisms has not yet been completely elucidated, NDs are recognized as a polygenic multifactorial disease process which involves dysfunctional cellular signaling mediated by pathways mainly related to inflammasome and IL-1 with the contributory role of IL-17 and other effector molecules. The precise elucidation of the above-mentioned pathologic mechanisms will pave the way to tailored treatments for patients with different NDs.

AUTHOR CONTRIBUTIONS

AO-L, MC, and AM designed and reviewed the paper and contributed in drafting the manuscript. MH drafted the manuscript. GG and DM contributed in drafting and reviewing the manuscript. All the authors approved the final version of the manuscript.

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REVIEW
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Behçet's Disease: An Overview of Etiopathogenesis

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Behçet's disease (BD) is a systemic inflammatory disease with a chronic, relapsing-remitting course of unknown etiology hallmarked predominantly by mucocutaneous lesions and ocular involvement. BD shares some common features with autoimmune and autoinflammatory diseases and spondyloarthropathies (MHC-I-opathies). It is related to more than one pathogenic pathway triggered by environmental factors such as infectious agents in genetically predisposed subjects. The interplay between genetic background and immune system is linked to the BD presentation. Genetic factors have been investigated extensively, and several recent genome-wide association studies have confirmed *HLA-B*51* to be the strongest genetic susceptibility factor. However, new non-HLA susceptibility genes have been identified. Genetic variations in the genes encoding the cytokines could affect their function and be associated with disease susceptibility. Infectious agents such as *Streptococcus sanguinis* or the differences in salivary or gut microbiome composition can be considered to trigger the innate-derived inflammation, which is, subsequently, sustained by adaptive immune responses. Altered trimming of microbial and/or endogenous peptides by endoplasmic reticulum aminopeptidase 1 (ERAP1), presented by *HLA-B*51*, may play a key role in BD pathogenesis causing an alteration in T cell balance with downregulation of Tregs and expansion of Th1 and Th17. The activity of neutrophils is increased and there is an intense neutrophil infiltration in the early stage of inflammation in organs affected by the disease. Association with *HLA-B*51* and increased IL-17 response seems to have an important role in neutrophil activity. In this paper, we provide an overview of the most recent advances on BD etiopathogenesis.

Keywords: Behçet's disease, etiology, genetics, immunology, infectious agents

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INTRODUCTION

Behçet's disease (BD) is a systemic inflammatory disease with a chronic, relapsing-remitting course, and its etiology is still unknown. The disease is characterized by a range of clinical manifestations including oral aphthae, genital ulcers, skin lesions, ocular, vascular, articular, gastrointestinal, urogenital, pulmonary, and neurologic involvement. BD is prevalent in regions along the "Silk Road," extending from Japan to Mediterranean countries. BD often begins between the ages of 20–40. The disease is equally distributed between men and women and the diagnosis can be made only on the basis of clinical symptoms and signs. The course of the disease is more severe in male patients with younger age at onset and an increased number of organs affected at diagnosis (1).

Disease can be recognized by clinical findings because of the absence of a universally accepted diagnostic laboratory test. BD diagnosis is largely based on mucocutaneous symptoms which are a common characteristic of various diagnostic criteria used in the diagnosis of the disease so far (2). The International Study Group for Behçet's disease criteria (requires the presence of oral ulcer plus any two of recurrent genital ulcer, typical eye lesions, typical cutaneous lesions, or a positive skin pathergy test) is the most commonly used and internationally recognized diagnostic criteria by the authors of this field (2, 3).

Besides considerable morbidity, BD has increased mortality because of the pulmonary artery and large vessel, neurological, and gastrointestinal involvements. Therefore, knowing the etiopathogenesis of BD is extremely important to better understand the disease and, more importantly, to develop targeted therapies. BD has been listed among autoimmune diseases by some authors because of positive response to classical immunosuppressive agents and involvement of autoantigens and antigen-specific T cells. Others claim the disease should be included in the group of autoinflammatory diseases because of unprovoked episodes of inflammation without evidence of antigen-specific T cells or autoantibodies, increased activity of neutrophils, elevated levels of interleukin (IL)-1β (4). Most authors evaluate the disease as a spondyloarthropathy (MHC-I-opathy) based on Human Leukocyte Antigen (HLA) class I association and epistatic endoplasmic reticulum aminopeptidase 1 (ERAP-1) interactions, increased T helper (Th) 17 type immune response, neutrophilic inflammation and barrier dysfunction in environmentally exposed organs (5). According to the current literature, BD cannot be definitely classified under any of these three groups and defining it as autoimmune, autoinflammatory or spondyloarthropathy appears to be a simplified approach (6). BD shares some common features with all the above-mentioned entities and involves more than one pathogenic pathway triggered by environmental factors such as infectious agents in genetically predisposed subjects. We will discuss the most recent evidences on the etiology of BD under the subtitles of infectious, genetic and immunological etiology sections of this review (1, 7, 8).

INFECTIONS

Infectious agents have long been proposed as triggering factors in BD development. Antigens from viruses such as herpes simplex virus (HSV)-1 or bacteria belonging to *Streptococcus* species such as *Streptococcus sanguinis* have been suspected to have high homology with human proteins such as heat-shock proteins (HSP) and the cross-reaction leads to an immune response in genetically predisposed individuals (1, 9). Professor Hulusi Behçet was indeed one of the first authors who regarded the disease as possibly related to an infectious agent (10). Several studies have investigated the association between HSV-1 and BD. Studd et al. in an situ DNA-RNA hybridization method, detected a higher frequency of hybridization between HSV-1 DNA and complementary RNA in mononuclear cells of BD patients compared with healthy controls. The results show the presence of at least a portion of the HSV-1 genome in mononuclear cells of BD patients (11).

Several *Streptococcus* strains have become increasingly important in infectious etiology. The development of some clinical manifestations of the disease in hypersensitivity tests against streptococcal antigens is one of the most relevant evidences (12). In addition, antibodies against *S. sanguinis* and *S. pyogenes* were obtained more frequent in BD patients than in controls (13). Streptococcal 65-kDa HSP from an uncommon serotype (KTH-1, strain BD113-20) of oral *S. sanguinis* has been reported to be an important trigger in the pathogenesis (14). Neurofilament medium (Nf-M) was recently suggested as possible antigen able to trigger an immune response via molecular mimicry with bacterial HSP-65 (15). Immunoglobulin M in BD patients has been reported able to react with some streptococcal proteins such as streptococcal α-enolase and glyceraldehyde 3-phosphate dehydrogenase (16).

Cho et al. demonstrated that the *S. sanguinis* GroEL protein is a target of the serum anti-*S. sanguinis* IgA antibody. In addition, serum IgA reactivity against recombinant *S. sanguinis* GroEL has been correlated to reactivity against recombinant human hnRNP A2/B1 suggesting how autoreactive lymphocytes may be activated by infectious triggering (17).

As BD usually starts from the oral mucosa, it has been speculated that oral microbial flora may be implicated in the pathogenesis of the disease (18). BD patients can develop new-onset oral ulceration or experience both cutaneous and systemic flare-ups following dental procedures or surgical treatments for chronic tonsillitis (19, 20). Antimicrobial agents have been used successfully for treating various disease symptoms (21). Several previous studies and our experience showed oral health impairment in BD patients compared with healthy subjects (18, 22, 23). Oral health improvement in BD patients may positively modify their disease course. Dental treatments in BD patients could be associated with a relapse of oral aphthae in the short time but could decrease their number in longer follow-up (∼6 months) (24), also leading to better oral health in the long-term follow-up. Higher levels of various *Streptococci* were found in the oral mucosa of BD patients. In addition, *S. sanguinis* strain resulted able to induce the secretion of inflammatory cytokines by the KTH-1 cells. It is plausible that an inflammation process induced by infectious agents in subjects with predisposing genetic background leads to the development of BD (25, 26).

No association between BD and other bacterial species such as *Borrelia burgdorferi* and or *Helicobacter pylori* have been found (27, 28). Cytomegalovirus, Epstein-Barr virus, Parvovirus B19, Varicella zoster virus, Hepatitis virus have also been investigated as possible triggering factors but these studies were characterized by low-level evidences (29, 30).

Recent studies have shown that the differences in salivary or gut microbiome composition may have a role in the pathogenesis. In a study of the salivary microbiome using high- throughput sequencing of the 16S rRNA V4 region, Coit et al. reported that BD patients have a significantly less diverse microbial community structure than healthy controls (31). In another study, Consolandi et al. compared the fecal microbiota of BD

patients to healthy controls. They reported both a peculiar dysbiosis of the gut microbiota and a significant decrease of butyrate production in BD patients. Authors speculated that a defect of butyrate production might lead to both reduced T regulatory cells (Tregs) responses and activation of immunopathological T-effector responses (32).

In summary, until now, no infectious agent has been isolated as the specific etiologic agent. Additionally, results of antibacterial and antiviral treatments are controversial. However, there is a general agreement that infectious agents or microbiome is not directly responsible for the emergence of BD, but they play a triggering role in the development of the disease by causing dysfunction of the immune system.

GENETICS

Increased prevalence of the disease along the ancient "Silk Road," familial aggregation, association with the genes inside the major histocompatibility complex (MHC) region and outside the MHC region are the main evidence of genetic influence and a complex inheritance model of disease (33, 34). The strongest genetic susceptibility factor for BD is located inside the MHC class I region including the Human Leukocyte Antigen-B51 (*HLA-B*51*). The odds ratio for individuals carrying *HLA-B*51/B5* allele to develop BD compared with no-carriers was found to be 5.78 (33).

Genome-wide association studies (GWAS) have clearly shown the role of several single nucleotide polymorphisms (SNPs) in the etiopathogenesis of various diseases, including BD (35–41). In a multicenter study, Hughes et al. studied the association between *HLA-B*51* and BD as well as other risk loci within the HLA region: 8572 variants were screened, and imputation and meta-analysis of 24834 variants were performed in two independent groups of BD patients. The most significant association was with rs116799036, which is located between *HLA-B* and MHC Class I Polypeptide-Related Sequence A *(MICA)* (42). Recently, Takeuchi et al. genotyped 1900 Turkish BD and 1779 controls with the *Immunochip* and demonstrated that the major BD-related polymorphism was known as rs1050502, an HLA-B*51 gene variant (43). However, the presence of *HLA-B*51* alone only partially explains the genetic disease risk and all clinical manifestations of BD. Several recent GWAS have confirmed the association between BD and *HLA-B*51*, except for Fei et al's investigation. These studies also revealed new susceptibility loci both on other HLA Class I regions and on non-HLA genes (35–41). These genes provide a significant role in understanding disease pathogenesis and offer novel treatment strategies.

In general, BD-associated gene polymorphisms were localized in molecules responding to microorganisms, as well as in genes encoding cytokines and adhesion molecules. Polymorphisms within genes encoding the cytokines may affect their function and may be associated with disease predisposition (44). Researchers identified several non-HLA genetic associations by GWAS including *ERAP1, IL23 receptor (IL23R)*, *IL-23R/IL-12RB2, IL-10*, and *STAT* genes (38, 45).

ERAP1 variations have been identified as significant predictive loci of BD susceptibility. The gene encodes an amino-peptidase having the critical role to trim N-terminal of peptides. This mechanism was affected by the amino acids sequence of the corresponding protein (46–51). *ERAP1* is characterized by several common polymorphisms encoding variant amino acids related not only to BD, but also to ankylosing spondylitis (AS) and psoriasis (47–51). The same SNPs associated to BD risk resulted protective against AS and psoriasis: this effect depends on the different HLA interacting with *ERAP1* (46, 49). *ERAP1* polymorphisms was a risk factor preferentially in BD patients with HLA-B*51-positivity; *ERAP1* rs17482078 (p.Arg725Gln) might influence the peptide repertoire binding to *HLA-B*51* (47). A recent paper suggested the critical role of the altered peptide presentation by *HLA-B*51* in influencing disease pathogenesis (52). Based on these alterations, T-cell and natural killer (NK) cell recognition were probably affected, providing the basis for the association of *ERAP1* and *HLA-B*51* with BD (53). A very recent alternative pathogenic hypothesis linking *HLA-B*51* with BD involves the gut microbiome and the *HLA-B*51* misfolding. Both ER stress and unfolded proteins were consequences of the misfolding and also the inflammation trigger. Some combination of the misfolded proteins probably influences BD pathogenesis, but this point has not yet been addressed in BD patients and several small studies reported a role in AS pathogenesis with HLA-B-27 (52).

The association between SNPs of *IL-10* and *IL-23R/IL-12RB2* genes and BD was demonstrated in Turkish (35, 40) and Japanese population (35, 39). A reduced mRNA expression in BD patients monocytes was recognized in the presence of the A-allele of rs1518111 *IL-10* compared with wild-type G-allele. PBMCs or monocytes produced significantly less IL-10 following stimulation with Toll-like receptor (TLR) ligands in individuals homozygous for A-allele of rs1518111 (35). Afkari et al. showed that *IL-10* rs1800872 A allele contributes to BD genetic risk by modulating IL-10 expression: BD patient group showed lower gene expression levels compared to the controls (54). Most disease-associated GWAS variants were found to be localized on the IL-23R side of the hotspot. These results indicate the association of BD with IL-23R rather than IL-12RB2. The association of *IL23R* rs17375018 and a haplotype of four gene variations and BD was reported, but no functional data were available for this variation. Targeted resequencing of *IL23R* in BD Japanese and Turkish patients showed novel association pieces of evidence including the reduced frequency of those rare missense variations with a protective role by reduced IL-23-dependent IL-17 production, as demonstrated in Crohn's disease (35). IL23 induces T cell activation for IL17 production and is one of the most significant activators of Th17 pathway (1). The association between BD susceptibility and *IL23R-IL12RB2* locus was confirmed in a Korean population: the intergenic rs1495965 SNP was significantly related with BD risk both in discovery and replication phases (55).

Association between STAT4 rs7574070 and BD was underlined in different studies (35, 37, 38). In addition, the disease-associated A allele was related to increased gene expression, greater severity of disease course and higher IL-17 production (35). *IL1A-IL1B*, *IRF8*, and *CEBPB-PTPN1* were three novel disease markers recently identified by direct

genotyping in GWAS besides *ADO-EGR2* discovered by imputation (43).

The variation of the promoter region of *TNF* has also been reported as a risk marker for BD. Alterations of TNF expression related to gene polymorphisms may be responsible for the higher cytokine activity (56, 57). Polymorphic alleles were more frequent in BD patients and were related to higher TNF production by monocytes or mononuclear cells (45, 57). Mutations in the Mediterranean fever gene were also considered additional BD susceptibility factors (45).

The role of other genes located outside the HLA region, encoding chemokines (e.g., CCR1-CCR3, CCR5), cytokines (such as for IL-1β, IL-6, IL-8, IL-12, IL-17, IL-18, IL-23), oxidative stress-related proteins (glutathione transferase and myeloperoxidase), cell membrane receptors (TNFRSF1A, TLR2, 4, 7, 9), immunoregulatory proteins (e.g., IRF1, IRF5, CTLA-4, NF-jB), extracellular proteins (like ICAM- 1, MMP-9), and others including those for KLRC4, TNFAIP3, DEFA1, NEMO, NOD2, TLR4, and FUT2 were analyzed in several investigations with conflicting findings (45, 58–62).

Besides genetic contribution, also epigenetic processes, such as DNA methylation, histone modification, and non-coding RNAs, microRNA (miRNA) in particular, have been suggested as involved in BD pathogenesis (45, 63). The epigenetic aspects were also investigated by analyzing miRNA signatures associated with BD patients with active disease and showed that miRNAs target pathways relevant in BD, such as TNF, IFN-γ, and vascular endothelial growth factor receptor signaling cascades (64, 65). Alipour et al. reported that disease pathogenesis could be affected by altered methylation levels of interspersed repetitive sequences (IRs) elements, as well as by histone modifications and miRNA regulation, in particular, higher levels of miR-182 and miR-3591-3p and lower levels of miR-155, miR-638 and miR-4488 (63).

Recently, Zhou et al. screened a Caucasian family formed by an affected mother and two affected daughters presenting with oral and genital ulcers, uveitis, and arthralgia/arthritis clinical signs. Exome sequencing revealed two strong candidate variants, p.C78W of *TNFRSF9* and p.L227X of *TNFAIP3* genes. These mutations affect immune cell survival and proinflammatory cytokine production. Therefore, one or two of this mutation may contribute to this dominantly-inherited condition and can help us to understand how BD symptoms develop (66).

IMMUNITY

Activated innate immunity plays an important role in the pathogenesis of BD. Microbial triggers are sensed and processed by the innate immune system via pathogen-related and/or danger-associated molecular patterns. Overproduction of inflammatory cytokines by innate immune cells such as macrophages and dendritic cells may cause a higher production of adaptive Th1- and Th17-related cytokines. BD lesions in their early stages are predominated by neutrophils which are major immunoregulatory cell group of the innate immune system. Another member of innate immunity, natural killer (NK) cells are also found in BD lesions (67).

BD is considered as a neutrophilic vasculitis and the role of neutrophils in BD pathogenesis has long been known (7).

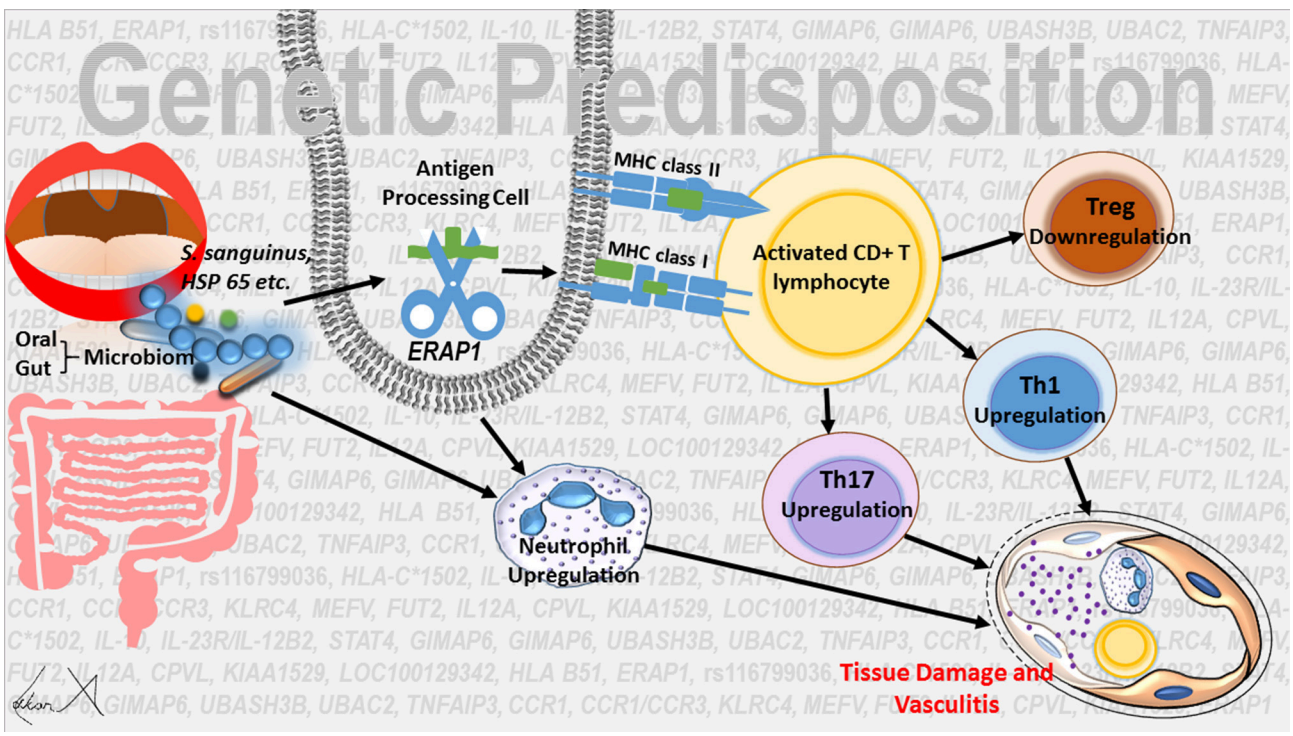

FIGURE 1 | Possible regulation mechanisms in the etiopathogenesis of Behçet's disease.

Surface molecules, indicating neutrophil activation status (CD10, CD14, and CD16), oxidative burst and phagocytic function of neutrophils have been explored and the presence of proactive neutrophils in BD patients was reported (68). Tissue injury in BD can be modulated by neutrophils in several manners: neutrophils were hyperactivated, probably *HLA B*51*-associated, and usually were involved in perivascular infiltration (68, 69). No significant differences were observed in the oxidative burst, phagocytic microbicide activities or cytokine pattern when BD patients and controls were compared in Perazzio and colleagues' study. However, significant differences in phagocytic dysfunction were found in patients with severe active disease compared with subjects with mild disease (45, 70). In addition, the structural and functional modification of fibrinogen resulted related to reactive oxygen species and neutrophil activation via neutrophil NADPH oxidase (69). Therefore, neutrophil activation was considered as the main source of oxidative stress through the oxidation of proteins. Hyper-activated neutrophils secrete some cytokines that are both autocrine and also stimulate Th1 cells (45). Recently, Yavuz et al. reported that testosterone causes a significant neutrophil activation together with Th-1 type immune alterations which may explain a more aggressive disease with a higher mortality rate in male BD patients (71).

NK cells were also identified in BD lesions where seems they have a role in driving the CD4+ Th1 response which is the main feature of BD lesions (72, 73). However, several studies underlined increased NK cells in the peripheral blood, in particular during the active phases of the disease (72, 74, 75).

Dysregulation of the immune system contributes to BD etiopathogenesis, with increased systemic levels of inflammatory cytokines (45, 72). It's well known that CD4+T cells can differentiate into two types: Th1 cells subset, which secretes IFN-γ, IL-2, and TNF and promotes cell-mediated immunity, and Th2 cells, which produce IL-4, IL-5, IL-10, and IL-13 and promote antibody-mediated immunity (76).

The alteration of T cell balance, especially Th1/Th17 expansion and decreased regulation by Tregs, are supposed to have a significant role in BD pathogenesis (7, 43). In particular, increased frequencies of Th17 cells were reported in the BD cutaneous lesions (77). Th17 and IL-17 pathways might have a part in the development and/or activity of BD (1). Increased production of IL-17, IL-23, and IFN-γ by PBMCs besides increased frequencies of IL-17 and IFN-γ producing T cells in BD patients with active uveitis was reported (78). IL-17 levels of BD patients with active stages of uveitis, oral and genital ulcers and articular symptoms were significantly higher compared with patients with inactive stages of the same symptoms. Hamzaoui et al. demonstrated that the percentage of circulating Th17 cells and plasma interleukin IL-17 levels were increased in active BD (52, 79). Increased neutrophil activity and neutrophil infiltration in the affected organs of BD might be caused by the increased IL-17 response (80). A recent study reported that, under Th17-stimulating conditions, T cells express both IL-17 and IFN-γ. Production of large amounts of IL-17 and IFN-γ by all lymphocyte subsets in BD patients were associated with increased innate responses, early tissue neutrophil infiltrations and late adaptive immunity (67). Moreover, in experimental autoimmune uveitis (EAU) the role of Herpesvirus entry mediator (HVEM), a member of the Tumor Necrosis Factor Receptor family, has been evaluated. The HVEM seemed to be involved as a co-signaling molecule inducing both Th1 and Th17 responses in EAU. In addition, in the same mouse model, the use of anti-HVEM antibodies blocking HVEM co-signal ameliorated EAU (81).

Takeuchi et al. compared the proinflammatory and Th1-, Th2-, and Th17-related cytokines frequency in a group of BD patients with recurrent uveitis and a group of remitted uveitis before and after infliximab treatment. They found higher levels of IL-1β, IL-4, IL-17A, IL-17F, IL-21, IL-22, IL-31, IFN-γ, sCD40L, and TNF-α, with a significant difference for IL-17F, in BD-recurrent uveitis patients respect to the BD-remitted uveitis group, before drug infusion. In addition, only IL-10 levels were found higher in the remission group than in the other group (82). Emmi et al. showed that cytotoxic Th1 and Th 17 cells can play a role in inducing mucosal damage during the early stages in BD patient with active intestinal involvement (83). These results confirm that Th17 and IL-17 pathway are active and play an important role, particularly in acute attacks of the disease. Conversely, a reduction in Tregs and cytokine IL-10 were notified in the disease (72, 84).

Due to recent progress in molecular methods and basic scientific researches, our knowledge about the disease has considerably increased. GWAS have become a very important step in understanding BD pathogenesis. New genes such as ERAP1 have been introduced which help to understand the possible pathogenic mechanism of *HLA-B*51*. In the future, similar studies in different populations with a higher number of patients will provide significant advances in the etiopathology of BD. Despite all these advances, clinical expression of the disease is quite heterogeneous and show regional differences. The underlying environmental and genetic factors of this situation are not fully elucidated. Being a complex disease, BD is related more than one pathogenic pathway. Although, management of the disease has evolved noticeably because of more effective and targeted therapies we still need new treatment options for severe and non-responsive cases such as biological treatments developed for the underlying etiopathological mechanism (85).

In conclusion, environmental factors (*S. sanguinis* etc.) or the differences in salivary or gut microbiome composition can trigger the innate-derived inflammation, which may be subsequently sustained by adaptive immune responses. Epistatic interactions between *HLA-B*51* and *ERAP1* variants seems to cause T cell homestasis perturbation, especially Th1 and Th 17 activation and Tregs response suppression. The activity of neutrophils is increased and there is an intense neutrophil infiltration in the early stage of inflammation in organs affected by the disease. Association with *HLA-B*51* and increased IL-17 response have a key role in the neutrophil activity (**Figure 1**).

AUTHOR CONTRIBUTIONS

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

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