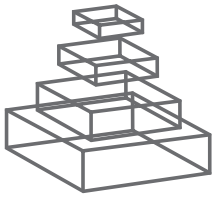
frontiers
RESEARCH TOPICS

INTERLEUKIN-1 LIGANDS AND RECEPTORS IN INFLAMMATION AND IMMUNITY

Topic Editors
Cecilia Garlanda and Alberto Mantovani

frontiers in
IMMUNOLOGY



frontiers

Cover image provided by Ibbl sarl, Lausanne CH

ISSN 1664-8714
ISBN 978-2-88919-197-0
DOI 10.3389/978-2-88919-197-0

ABOUT FRONTIERS

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

FRONTIERS JOURNAL SERIES

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

DEDICATION TO QUALITY

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

WHAT ARE FRONTIERS RESEARCH TOPICS?

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

INTERLEUKIN-1 LIGANDS AND RECEPTORS IN INFLAMMATION AND IMMUNITY

Topic Editors:
Cecilia Garlanda, Humanitas Clinical and Research Center, Italy
Alberto Mantovani, Humanitas Clinical and Research Center and University of Milan, Italy

IL-1R like receptors (ILRs), as well as Toll Like Receptors (TLRs), are members of a superfamily of phylogenetically conserved proteins of innate immunity and inflammation, characterized by the presence of a conserved intracellular domain, the Toll/IL-1R (TIR) domain. Most members of both subfamilies are responsible of the activation of an evolutionarily conserved signaling pathway leading to inflammation and innate responses: upon ligand binding, dimerization of receptor TIR domains, recruitment of TIR domain containing adapter proteins and activation of signaling occur. This pathway involves Myeloid differentiation factor 88 (MyD88), IL-1R associated kinases (IRAKs), and tumor necrosis factor receptor-associated factor 6 (TRAF6) and leads to activation of nuclear factor kappa B (NF-kB), activator protein-1 (AP-1), c-Jun N-terminal kinase (JNK), p38 mitogen-associated protein kinase and members of the interferon regulatory factor family.

Depending on the structure of the extracellular region, the family is subdivided in TLRs bearing leucine-rich repeats and ILRs bearing Ig-like domains. Whereas TLRs are receptors for specific pathogen associated molecular patterns and of necrotic cell-derived danger signals and act as sensors for microorganisms and tissue damage, the ILR subfamily includes the receptors and the accessory proteins (AcP) for pro- and anti-inflammatory molecules of the IL-1 family. ILRs are involved in the initiation of an amplification cascade of innate resistance, contribute to the activation and orientation of adaptive immunity and play a key role in inflammatory conditions. In particular, ILRs ligand family includes pro-inflammatory molecules such as IL-1α and IL-1β (IL-1F1), IL-18/IL-1F4, IL-36α/IL-1F6, IL-36β/IL-1F8 and IL-36γ/IL-1F9. Other members of the IL-1 family show anti-inflammatory activity: IL-1Ra/IL-1F3 binds to IL-1R1 inhibiting the recruitment of IL-1RAcP and competes with IL-1α and IL-1β for receptor binding; IL-36Ra binds IL-1Rrp2 antagonizing IL-36α, IL-36β and IL-36γ; IL-37 produces anti-inflammatory effects; and finally IL-33 binds to T1/ST2, recruits IL-1RAcP and induces the expression of anti-inflammatory cytokines.

Uncontrolled or deregulated activation of ILRs- or TLRs-dependent inflammatory and immune responses can be detrimental for the host and potentially causes tissue damage and acute or chronic inflammatory disorders. For the IL-1 system, which has served as a paradigm for the definition of signaling and regulatory mechanisms, the control is exerted at different levels, both extracellularly and intracellularly, for instance by polypeptide antagonists, decoy receptors, signaling molecules and miRNA. In particular, decoy receptors, such as IL-1R2, bind ligands that are no longer available for the transducing receptors or form dominant negative non-signaling complexes with AcPs. TIR8/SIGIRR inhibits the activation of the signaling pathway by TLRs and IL-1R by interfering with the association of adaptor molecules to the receptor complex.

Thus, the family of IL-1 ligands and receptors evolved with the dual role of activating as well as reducing and modulating inflammation and innate and adaptive immune responses. This Research Topic is focused on different members of the IL-1 ligand and receptor family, on their function as activators or suppressors of inflammation, activation and polarization of adaptive responses, on their involvement in pathology and potential therapeutic targeting.

Table of Contents

frontiers in
IMMUNOLOGY

EDITORIAL
published: 20 November 2013
doi: 10.3389/fimmu.2013.00396

Ligands and receptors of the interleukin-1 family in immunity and disease

Cecilia Garlanda*[1] *and Alberto Mantovani*[1,2]

[1] *Department of Inflammation and Immunology, Humanitas Clinical and Research Center, Rozzano, Italy*
[2] *Department of Biotechnology and Translational Medicine, University of Milan, Rozzano, Italy*
**Correspondence: alberto.mantovani@humanitasresearch.it*

Edited by:
Kendall A. Smith, Cornell University, USA

Keywords: interleukin-1, decoy receptor, inflammation, innate immunity, adaptive immunity, autoinflammatory diseases

IL-1 has served as a ground breaking molecule in immunology and it is now experiencing a renaissance. Originally the description of a cytokine acting at vanishingly low concentration on cells and organs as diverse as the hypothalamus (fever) and T cells (1) was without precedent in biology and paved the way to the whole field of cytokines and their pleiotropic mode of action.

The discovery of the importance of IL-1 in defense against bacteria and of the Toll-IL-1 resistance (TIR, as originally defined) domain was upstream of the discovery of Toll-like receptors (2). Along the same line, the identification of MyD88 as the key adaptor in the IL-1 receptor signaling cascade (3) prompted its identification in Toll/TLR4 signaling (4, 5). The type II IL-1 receptor was identified as a decoy for IL-1, thus providing a new paradigm in receptor biology (6), subsequently extended to other cytokines and growth factors (7). Stunning from this strong roots, IL-1 has in recent years seen a renaissance. New relatives of IL-1 and IL-1R have been identified and their function has been defined in innate and adaptive immune responses. IL-1 family members have emerged as key players in the differentiation of the main T helper subsets, Th1, Th2, and Th17. Finally, anti-IL-1 strategies have had a tremendous impact in autoinflammatory diseases and are being tested in a variety of clinical conditions.

This volume brings together eight articles that are intended to provide a summary about IL-1 family ligand and receptors in inflammation and immunity. The eight articles are briefly described below.

van de Veerdonk et al. focus their review on the IL-1 family of ligands, describe their biological functions and provide new insights in their biology (8). In particular they focus on the new IL-1 family members, IL-37 and the cytokines belonging to the IL-36 subfamily and on the potency of blocking IL-1 in disease. Among the ligands, a special focus on the biology of IL-18 as well as its role in human disease is provided by the review by Dinarello et al. (9). IL-18 is synthesized as an inactive precursor requiring processing by caspase-1 into an active cytokine, similarly to IL-1β, and is constitutively present in nearly all cell types. The activity of IL-18 is balanced by the presence of a high affinity naturally occurring IL-18 binding protein (IL-18BP), which is now in clinical trials.

Most members of the IL-1 family, including the master pro-inflammatory cytokine IL-1β, are leaderless proteins and are released from the cell though a "non-classical" pathway of secretion. Rubartelli et al. review current hypotheses on the mechanisms of externalization of IL-1 family members and discuss their relevance with respect to the different functions, as cytokines or as DAMPs, played by IL-1 family members (10).

Members of IL-1R like receptor family include signaling molecules and negative regulators. In our review, we present the latter, which include the prototypic decoy receptor type 2 IL-1R and "receptors" with regulatory function, such as TIR8/SIGIRR (11). We suggest that the presence of multiple pathways of negative regulation of members of the IL-1/IL-1R family emphasizes the need for a tight control of members of this fundamental system, which mediates potentially devastating local and systemic inflammatory reactions.

Voronov et al. present the role of IL-1 as a pleiotropic cytokine in the context of cancer (12). In their secreted form, IL-1α and IL-1β are involved in tumorigenesis and tumor invasiveness, whereas IL-1α, when expressed on the cell membrane, stimulates anti-tumor cell immunity. Differential patterns of IL-1α and IL-1β expression and function have been observed in different tumors, thus the authors suggest that better understanding of the role of IL-1α and IL-1β in distinct malignancies will enable the application of novel IL-1 modulation approaches in cancer patients as an adjunct to conventional approaches.

Lopetuso et al. discuss the dichotomous functions of IL-1 family members, such as IL-1, IL-1Ra, IL-18, and IL-33, in gastrointestinal-related inflammatory disorders, depending on the phase of disease or homeostasis and show that IL-37 is emerging as a potent anti-inflammatory cytokine which downregulates colitis (13). In addition, they present data on IL-1 family members suggesting novel pathogenic hypotheses and translational implications for inflammatory bowel disease (IBD) and inflammation-associated colorectal cancer.

The review by Federici et al. presents inherited autoinflammatory diseases secondary to mutations of proteins of the intracellular pathways deputed to the activation and secretion of IL-1β (14). The authors show that the understanding of the molecular pathways involved in these disorders has clarified that similar pathogenic mechanisms play also a crucial role in sustaining inflammation in several multi-factorial inflammatory disorders and opened new perspectives for the treatment of these autoinflammatory disorders based on IL-1 blockers.

Finally, Santarlasci et al. discuss the involvement of IL-1α and IL-1β in the differentiation, activation, and maintenance or survival of the different Th cell subsets (15). Indeed, the differential

expression of IL-1R1 on human $CD4^+$ T cell subsets confers distinct capacities to acquire specific effector functions. In particular, IL-1β is a key cytokine in Th17 development, acting through IL-1R1 expressed already by the naïve $CD4^+$ Th17 precursor, and interestingly by a sub-set of Th1 cells possibly derived by plasticity of Th17 cells.

The reviews collected in this issue of Frontiers will hopefully provide the reader with the sense of diversity and impact of IL-1 family members in the activation and regulation of innate and adaptive immune responses and in immunopathology.

REFERENCES

1. Dinarello CA. Anti-inflammatory agents: present and future. *Cell* (2010) **140**:935–50. doi:10.1016/j.cell.2010.02.043
2. O'Neill LA. Toll-like receptor signal transduction and the tailoring of innate immunity: a role for Mal? *Trends Immunol* (2002) **23**:296–300. doi:10.1016/S1471-4906(02)02222-6
3. Muzio M, Ni J, Feng P, Dixit VM. IRAK (Pelle) family member IRAK-2 and MyD88 as proximal mediators of IL-1 signaling. *Science* (1997) **278**:1612–5. doi:10.1126/science.278.5343.1612
4. Medzhitov R, Preston-Hurlburt P, Kopp E, Stadlen A, Chen C, Ghosh S, et al. MyD88 is an adaptor protein in the hToll/IL-1 receptor family signaling pathways. *Mol Cell* (1998) **2**:253–8. doi:10.1016/S1097-2765(00)80136-7
5. Muzio M, Natoli G, Saccani S, Levrero M, Mantovani A. The human toll signaling pathway: divergence of nuclear factor kappaB and JNK/SAPK activation upstream of tumor necrosis factor receptor-associated factor 6 (TRAF6). *J Exp Med* (1998) **187**:2097–101. doi:10.1084/jem.187.12.2097
6. Colotta F, Re F, Muzio M, Bertini R, Polentarutti N, Sironi M, et al. Interleukin-1 type II receptor: a decoy target for IL-1 that is regulated by IL-4. *Science* (1993) **261**:472–5. doi:10.1126/science.8332913
7. Mantovani A, Locati M, Vecchi A, Sozzani S, Allavena P. Decoy receptors: a strategy to regulate inflammatory cytokines and chemokines. *Trends Immunol* (2001) **22**:328–36. doi:10.1016/S1471-4906(01)01941-X
8. van de Veerdonk FL, Netea MG. New insights in the immunobiology of IL-1 family members. *Front Immunol* (2013) **4**:167. doi:10.3389/fimmu.2013.00167
9. Dinarello CA, Novick D, Kim S, Kaplanski G. Interleukin-18 and IL-18 binding protein. *Front Immunol* (2013) **4**:289. doi:10.3389/fimmu.2013.00289
10. Carta S, Lavieri R, Rubartelli A. Different members of the IL-1 family come out in different ways: DAMPs vs. cytokines? *Front Immunol* (2013) **4**:123. doi:10.3389/fimmu.2013.00123
11. Garlanda C, Riva F, Bonavita E, Gentile S, Mantovani A. Decoys and regulatory "receptors" of the IL-1/toll-like receptor superfamily. *Front Immunol* (2013) **4**:180. doi:10.3389/fimmu.2013.00180
12. Voronov E, Dotan S, Krelin Y, Song X, Elkabets M, Carmi Y, et al. Unique versus redundant functions of IL-1alpha and IL-1beta in the tumor microenvironment. *Front Immunol* (2013) **4**:177. doi:10.3389/fimmu.2013.00177
13. Lopetuso LR, Chowdhry S, Pizarro TT. Opposing functions of classic and novel IL-1 family members in gut health and disease. *Front Immunol* (2013) **4**:181. doi:10.3389/fimmu.2013.00181
14. Federici S, Martini A, Gattorno M. The central role of anti-IL-1 blockade in the treatment of monogenic and multi-factorial autoinflammatory diseases. *Front Immunol* (2013) **4**:351. doi:10.3389/fimmu.2013.00351
15. Santarlasci V, Cosmi L, Maggi L, Liotta F, Annunziato F. IL-1 and T helper immune responses. *Front Immunol* (2013) **4**:182. doi:10.3389/fimmu.2013.00182

Received: 05 November 2013; accepted: 07 November 2013; published online: 20 November 2013.

*Citation: Garlanda C and Mantovani A (2013) Ligands and receptors of the interleukin-1 family in immunity and disease. Front. Immunol. **4**:396. doi: 10.3389/fimmu.2013.00396*

This article was submitted to Inflammation, a section of the journal Frontiers in Immunology.

frontiers in
IMMUNOLOGY

REVIEW ARTICLE
published: 08 July 2013
doi: 10.3389/fimmu.2013.00167

New insights in the immunobiology of IL-1 family members

Frank L. van de Veerdonk* and Mihai G. Netea

Department of Medicine, Radboud University Nijmegen Medical Center, Nijmegen Institute for Infection, Inflammation and Immunity (N4i), Nijmegen, Netherlands

Edited by:
Cecilia Garlanda, Istituto Clinico Humanitas, Italy

Reviewed by:
Massimo Gadina, National Institute of Arthritis Musculoskeletal and Skin diseases-National Institutes of Health, USA
Barbara Viviani, University of Milan, Italy

****Correspondence:***
Frank L. van de Veerdonk, Department of Medicine, Radboud University Nijmegen Medical Center, P.O. Box 9101, 6500HB Nijmegen, Netherlands
e-mail: f.veerdonk@aig.umcn.nl

The interleukin-1 (IL 1) family of ligands is associated with acute and chronic inflammation, and plays an essential role in the non-specific innate response to infection. The biological properties of IL 1 family ligands are typically pro-inflammatory. The IL 1 family has 11 family members and can be categorized into subfamilies according to the length of their precursor and the length of the propiece for each precursor (**Figure 1**). The IL 1 subfamily consists of IL 1α, IL 1β, and IL 33, with the longest propieces of the IL 1 family. IL 18 and IL 37 belong to the IL 18 subfamily and contain smaller propieces than IL 1 and IL-33. Since IL 37 binds to the IL 18Rα chain it is part of the IL 18 subfamily, however it remains to be elucidated how the propiece of IL 37 is removed. IL 36α, β, and γ as well as IL 36 Ra belong to the IL 36 subfamily. In addition, IL 38 likely belongs to this family since it has the ability to bind to the IL 36R. The IL 36 subfamily has the shortest propiece. The one member of the IL 1 family that cannot be categorized in these subfamilies is IL 1 receptor antagonist (IL 1Ra), which has a signal peptide and is readily secreted. In the present review we will describe the biological functions of the IL-1F members and new insights in their biology.

Keywords: interleukin-1, cytokines, ligands, review, biology

INTRODUCTION

The interleukin-1 (IL-1) family of ligands is associated with acute and chronic inflammation, and plays an essential role in the non-specific innate response to infection. The biological properties of IL-1 family ligands are typically pro-inflammatory. The IL-1 family has 11 family members and can be categorized into subfamilies according to the length of their precursor and the length of the propiece for each precursor (**Figure 1**). The IL-1 subfamily consists of IL-1α, IL-1β, and IL-33, with the longest propieces of the IL-1 family. IL-18 and IL-37 belong to the IL-18 subfamily and contain smaller propieces then IL-1 and IL-33. Since IL-37 binds to the IL-18Rα chain it is part of the IL-18 subfamily, however it remains to be elucidated how the propiece of IL-37 is removed. IL-36α, β, and γ as well as IL-36 Ra belong to the IL-36 subfamily. In addition, IL-38 likely belongs to this family since it has the ability to bind to the IL-36R. The IL-36 subfamily has the shortest propiece. The one member of the IL-1 family that cannot be categorized in these subfamilies is IL-1 receptor antagonist (IL-1Ra), which has a signal peptide and is readily secreted. In the present review we will describe the biological functions of the IL-1F members and new insights in their biology.

THE IL-1 SUBFAMILY

INTERLEUKIN-1α

IL-1α does not have a signal peptide, binds to nuclear DNA, and is released from the cell upon death after which it can bind to the IL-1R1 receptor as either an unprocessed precursor or a processed protein. Primary cells such as keratinocytes, thymic epithelium, hepatocytes, endothelial cells, fibroblasts, and the epithelial cells of mucus membranes contain constitutive levels of intracellular IL-1α precursor (Hacham et al., 2002). Furthermore, precursor IL-1α can be found on the surface of several cells, particularly on monocytes and B-lymphocytes, referred to as membrane bound IL-1α (Kurt-Jones et al., 1985). Membrane bound IL-1α is biologically active (Kaplanski et al., 1994), and its biological activities are neutralized by antibodies specific to IL-1α. Endothelial cells undergoing stress-induced apoptosis release membrane apoptotic body-like particles containing full-length IL-1α precursor and the processed mature form (Berda-Haddad et al., 2011). When injected into mice, apoptotic body-like particles containing the IL-1α precursor induce neutrophilic infiltration that can be prevented by neutralization of IL-1α (Berda-Haddad et al., 2011). Although the IL-1α precursor is biologically active, the processed form is more active. The processing of the IL-1α precursor is accomplished by calpain II, a membrane-associated, calcium-dependent cysteine protease (Miller et al., 1994), and calcium influx induces IL-1α secretion of the processed form (Gross et al., 2012).

It has been proposed that IL-1α acts as an autocrine growth factor since the intracellular regulating normal cellular differentiation, particularly in epithelial and ectodermal cells. In support of this concept, neutralizing intracellular IL-1α reduces senescence in endothelial cells (Maier et al., 1990), and constitutive IL-1α precursor can bind to HAX-1 in fibroblasts that subsequently translocates as a complex to the nucleus (Kawaguchi et al., 2006). Although these data support the concept that IL-1α can act as an autocrine growth factor, it should be noted that mice deficient in IL-1α show no defects in growth and development, including skin, fur, epithelium, and gastrointestinal function (Horai et al., 1998). However, since mice deficient in IL-1α still retain the N-terminal propiece (Werman et al., 2004) and this N-terminal propiece of IL-1α has been shown to bind HAX-1 (Yin et al., 2001) it could still be that the propiece of IL-1α is responsible for the proposed autocrine growth factor function of IL-1α.

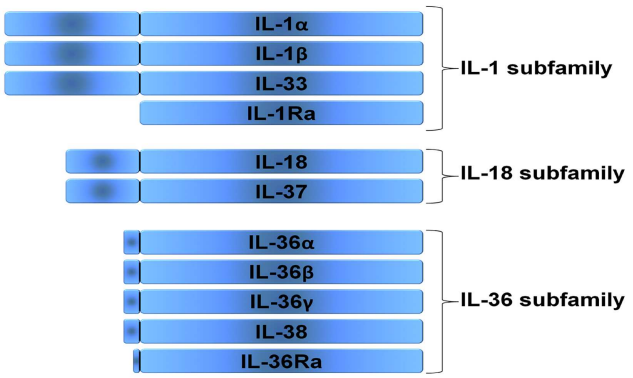


FIGURE 1 | Subfamilies according to the length of their precursor. Three families can be distinguished in the IL-1 family, the IL-1 subfamily, the IL-18 subfamily, and the IL-36 subfamily. The IL-1 receptor antagonist (IL-1Ra) cannot be categorized in these subfamilies, since it has a signal peptide and is readily secreted.

IL-1α plays an important role in sterile inflammation. Upon necrotic cell death the IL-1α precursor is released (Carmi et al., 2009; Cohen et al., 2010) and binds to the IL-1 receptor on nearby tissue macrophages and epithelial cells (Luheshi et al., 2011; Rider et al., 2011). This will trigger a pro-inflammatory response characterized by neutrophilic influx that is followed by influx of monocytes (Rider et al., 2011). This is underlined by the observation that extracts of tumor cells induce neutrophilic inflammation, which does not occur in mice deficient in IL-1RI and that can be prevented by neutralization of IL-1α (Chen et al., 2007). Thus, IL-1α, either the unprocessed precursor or the cleaved form can be seen as an alarmin (Chan et al., 2012). Furthermore, platelets also contain IL-1α (Hawrylowicz et al., 1989), and platelet-derived IL-1α has been described to be important in brain injury in stroke models (Thornton et al., 2010) and in atherosclerosis (Gawaz et al., 2000).

In mice fed a high-fat diet, serum amyloid A protein, a marker of inflammation in atherogenesis, was markedly lower in IL-1α-deficient mice compared to wild type or IL-1β-deficient mice (Kamari et al., 2007). IL-1α-deficient mice had significantly higher levels of non-high density lipoprotein cholesterol. The beneficial effect of IL-1α deficiency was due to hematopoietic cells transferred from the bone marrow of IL-1α-deficient mice resulting in a reduction in aortic lesion size twice that observed in mice transplanted with IL-1β-deficient bone marrow cells. Therefore, IL-1α appears to play an important role in the pathogenesis of lipid-mediated atherogenesis and this may be due to an effect of membrane IL-1α.

INTERLEUKIN-1β

IL-1β is a highly inflammatory cytokine as reviewed in Dinarello (2011a), and is primarily a product of monocytes, macrophages, and dendritic cells (DC) as well as B-lymphocytes and NK cells. Caspase-1, an intracellular cysteine protease, is responsible for the conversion of inactive IL-1β precursor into the active cytokine (**Figure 2**). Caspase-1 likewise needs to be processed in order to become active. This activation of caspase-1 is dependent on a complex of intracellular proteins termed the inflammasome (Agostini et al., 2004; Martinon et al., 2009). One critical component of the inflammasome is NLRP3, also termed cryopyrin since the gene was initially discovered in patients with "familial cold auto-inflammatory syndrome," a genetic disease characterized by fevers and elevated acute phase proteins following exposure to cold (Hoffman et al., 2001). Human blood monocytes contain constitutively active caspase-1, which is dependent on the presence of the key components of the inflammasome, namely ASC and NLRP3 (Netea et al., 2009). By contrast, other cells, such as macrophages and DC, need an additional trigger to activate caspase-1 (Netea et al., 2009). Non-caspase-1 mechanisms also exist to generate active forms of IL-1β. Sterile inflammation induces fever and increased production of hepatic acute phase proteins, which are absent in mice deficient in IL-1β, but present in mice deficient in caspase-1 (Fantuzzi et al., 1997a; Joosten et al., 2009). This observation can be explained by the fact that proteinase 3 from neutrophils can also process the IL-1β precursor extracellularly into an active cytokine (Coeshott et al., 1999; Joosten et al., 2009), as well as other proteases including elastase, matrix metalloprotease 9, and granzyme A (**Figure 2**).

Recently, autophagy has been reported to regulate IL-1β production (Saitoh et al., 2008). Autophagy is an ancient process of recycling cellular components, such as cytosolic organelles and protein aggregates, through degradation mediated by lysosomes. Autophagy is activated in conditions of cell stress, hypoxia, starvation, or growth factor deprivation, and it promotes cell survival by generating free metabolites and energy through degradation of the endogenous cellular components (Klionsky, 2007). In addition to its role in the pathophysiology of aging, cancer, and neurodegenerative diseases, autophagy can also modulate inflammatory responses (Schmid and Munz, 2007). A role for autophagy in production of pro-inflammatory cytokines, particularly IL-1β, has emerged with the reported link between ATG16L1 (an autophagy gene) function and IL-1β production. Macrophages from *ATG16L1*-deficient mice produce higher levels of IL-1β and IL-18 after stimulation with lipopolysaccharide (LPS) (Saitoh et al., 2008). These data suggest that higher activation of caspase-1 in *ATG16L1*-deficient mice accounts for the higher production of these caspase-1 dependent cytokines. Indeed, additional studies in *ATG16L1*-deficient mice point toward a regulatory effect of autophagy on caspase-1 activation through modulating the NLRP3 inflammasome (Saitoh et al., 2008; Tschopp and Schroder, 2010; Nakahira et al., 2011; Zhou et al., 2011). Autophagy can thus regulate IL-1β production by influencing caspase-1 activity (**Figure 2**). Furthermore, autophagosomes present in mouse macrophages can specifically degradate IL-1β precursor (Harris et al., 2011). Moreover, inhibition of autophagy in human primary monocytes leads to increased production of IL-1β (Crisan et al., 2011) (**Figure 2**). However, in the same cells TNFα production was decreased by autophagy inhibition, suggesting that there are divergent effects of autophagy on the production of these two important pro-inflammatory cytokines. Interestingly, in human cells IL-1β mRNA transcription is elevated when autophagy is inhibited, whereas no effects

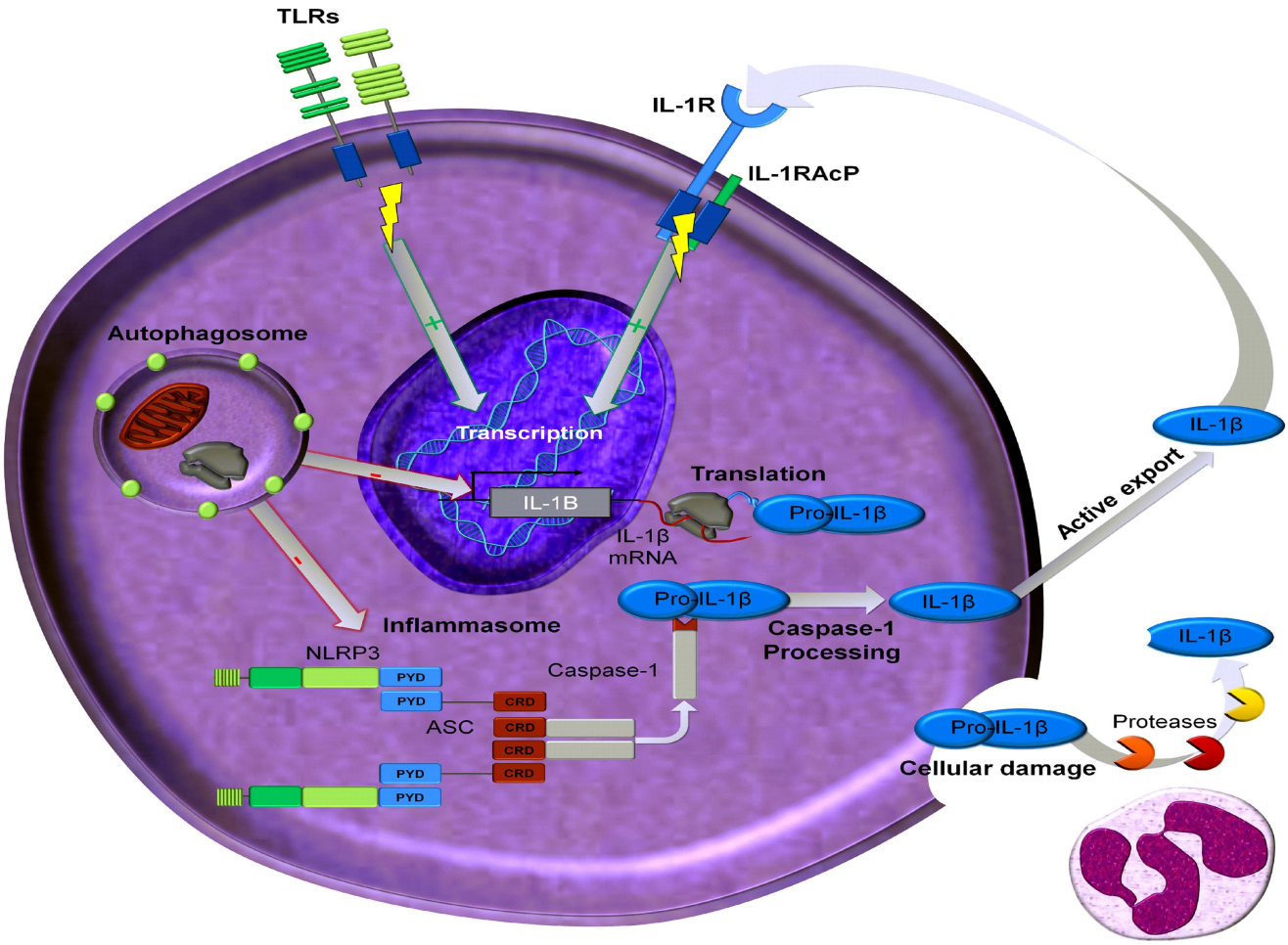


FIGURE 2 | Processing and regulation of IL-1β. Transcription of IL-1β mRNA can be induced by ligands that activate Toll like receptors (TLR) or by IL-1 ligands (IL-1α and IL-1β). This transcription can be regulated by autophagy. The precursor of IL-1β, namely pro-IL-1β, can be processed by active caspase-1 which is part of the inflammsome. In addition proteases, predominantly derived from neutrophils, can cleave extracellular pro-IL-1β, which will be present extracellularly in the setting of damaged inflammatory cells. This processed mature and bioactive IL-1β can then induce its own transcription.

can be observed on caspase-1 activation (Saitoh et al., 2008; Crisan et al., 2011; Harris et al., 2011). Despite these differences between mouse and human cells, autophagy modulates IL-1β production and inhibition of autophagy increases the production of IL-1β.

The potent inflammatory function of IL-1β is underlined by diseases that are specifically associated with IL-1β production. Although it has been described that IL-1 can play an important role in neurodegenerative diseases such as Alzheimer's disease, developmental diseases such as schizophrenia (Meyer, 2011) or even stress (Goshen and Yirmiya, 2009) most disease states that have been clearly linked with IL-1β mediated inflammation fall into the category of auto-inflammatory diseases, which are to be distinguished from the classic autoimmune diseases. Although inflammation is common to both auto-inflammatory and autoimmune diseases, in the case of IL-1-mediated disease, there is no evidence for a role of the adaptive immune system in its induction. Persons with activating mutations in one of the key genes that control the activation of caspase-1, namely NLRP3 (cryopyrin), can develop life-threatening systemic inflammation, which can be reversed by blocking IL-1β. Interestingly, in patients with these mutations in NLRP3, there is a decrease in steady state levels of pro-caspase-1 mRNA with IL-1Ra treatment (Goldbach-Mansky et al., 2006), suggesting that IL-1β stimulates its own production and processing. Other studies supporting this concept of IL-1-induced IL-1 have been reported (Goldbach-Mansky et al., 2006; Gattorno et al., 2007; Greten et al., 2007; Boni-Schnetzler et al., 2008). This explains the auto-inflammatory nature of these IL-1 mediated diseases, namely an initial trigger induces the production of IL-1β, which thereafter can induce itself. Type 2 diabetes appears to be an example of an auto-inflammatory disease where glucose induces IL-1β production from the insulin-producing beta cell and IL-1β induces the beta cell to produce its own IL-1β (Maedler et al., 2002).

No spontaneous disease has been reported in mice deficient in IL-1β. However, upon challenge, IL-1β-deficient mice differ significantly in their responses from wild-type mice. The most dramatic is the response to local inflammation induced by a local irritant. IL-1β-deficient mice will not have an acute phase response, develop anorexia, and have no fever within the first 24 h (Zheng et al., 1995; Fantuzzi et al., 1997b). These effects have also been observed in the same model using anti-IL-1R type I antibodies in wild-type mice (Zheng et al., 1995; Fantuzzi et al., 1997b). Reduced inflammation has also been observed in IL-1β-deficient mice that are exposed to zymosan-induced peritonitis (Fantuzzi et al., 1997b). IL-1β-deficient mice injected with LPS have little or no expression of leptin mRNA or leptin protein (Faggioni et al., 1998), however they do have elevated febrile responses to LPS, IL-1β, or IL-1α when compared to wild-type mice (Fantuzzi et al., 1996).

Interleukin-1β enhances T-cell activation and recognition of antigen. The specificity of this response was however not known initially. IL-1β, together with IL-6, and TGFβ have been reported to induce the development of Th17 cells, while IL-23 has been reported to be important for the maintenance of Th17 cells (Weaver et al., 2006; Dong, 2008; van de Veerdonk et al., 2009). The combination of IL-23 and IL-1β induces the development of human Th17 cells (Wilson et al., 2007). Interestingly, these cells also released IFNγ, displaying a phenotype common to both Th17 and Th1 cells (Wilson et al., 2007). The strong capacity of IL-1 to induce Th17 differentiation has also been linked to the induction and release of prostaglandins (Dinarello, 2011b). PGE2 are inducers of Th17 induction and inhibitors of cyclooxygenase decrease IL-17 production (Chizzolini et al., 2008; Smeekens et al., 2010). Furthermore, engagement of the aryl hydrocarbon receptor, a pathway demonstrated to be crucial for the generation of Th17 cells, has been shown to strongly induce IL-1β (Henley et al., 2004). IL-1β is also required for the production of IL-17 by NKT cells (Moreira et al., 2011) and of IL-22 from NK cells (Hughes et al., 2010).

Cytokines belonging to the IL-1 family have also been described to modulate neurons and functions of the central nervous system (CNS). For example IL-1β and its antagonist IL-1Ra have been extensively described for their ability to act within the CNS as modulators of hippocampal memory, as well as involvement in neuronal death (Yirmiya and Goshen, 2011; Hanamsagar et al., 2012). From these studies it is clear that in some context these cytokines that belong to the IL-1 family not only exert a pathological role but also play a role in homeostasis. These emerging observations underscore that the functions of inflammatory cytokines such a IL-1β are not only confined to the classical inflammatory response.

INTERLEUKIN-33

IL-33 belongs to the IL-1 subfamily, and was formerly termed IL-1F11. IL-33Rα is the ligand binding chain for IL-33 (Schmitz et al., 2005), and the co-receptor for IL-33 is the IL-1RAcP, which is also the co-receptor for IL-1α and IL-1β. The IL-33Rα chain is similar to IL-1R1, since it can bind the ligand but requires the IL-1RAcP to signal (Ali et al., 2007; Chackerian et al., 2007). The structure of IL-33 is closer to IL-18 than to IL-1β. Initially, IL-33 was considered closely related to IL-1β and IL-18 because the IL-33 precursor contains a caspase-1 site (Schmitz et al., 2005). However studies have revealed that caspase-1 cleaving of IL-33 actually results in loss of IL-33 activity and that the full-length IL-33 precursor can bind to IL-33Rα and is active (Cayrol and Girard, 2009). In addition, it has been reported that the caspase-1 cleavage site is similar to the consensus sequence for caspase-3 and that intracellular IL-33 precursor is a substrate for caspase-3 (Cayrol and Girard, 2009). Precursor IL-33 can also be processed by neutrophil proteinase 3 (PR3) into a biological active form of IL-33, however increasing the incubation time of PR3 will decrease the biological activity of IL-33 (Bae et al., 2012). Next to PR3 cleavage, neutrophil elastase and cathepsin G can cleave the IL-33 precursor, which results in the generation of IL-33 with different N-termini and varying levels of activity (Lefrancais et al., 2012). Thus, extracellular IL-33 is released as a precursor and can be processed by neutrophil enzymes which will generates active forms with varying levels of activity.

The dominant biological activity of IL-33 is the induction of Th2 cytokines, IL-4, IL-5, and IL-13 as well as other properties anticipated for a Th2 type cytokine. Therefore, the role of IL-33 in lung inflammation such as allergic type asthma has been studied extensively. Administration of IL-33 into the airways triggers an immediate allergic response in the lung of naïve mice and worsens the response in mice sensitized to antigen (Louten et al., 2011). When human IL-33 is administered to mice eosinophilic infiltration is a prominent finding in the lung and in allergic rhinitis as well as allergic conjunctivitis (Matsuba-Kitamura et al., 2010). Interestingly, it was recently described that interleukin-1α can control allergic sensitization to inhaled house dust mite (HDM) via the epithelial release of IL-33 (Willart et al., 2012). Mice deficient in IL-33Rα do not develop a Th2 response to Schistosoma egg antigen, and mice deficient in IL-33, are highly susceptible to *Strongyloides venezuelensis* (Yasuda et al., 2012). This infection induces a unique class of cells called natural helper cells or nuocytes, which produce IL-5 and IL-13 upon activation by IL-33, which results in eosinophilic infiltration into the lungs. This pulmonary eosinophilic inflammation causes damage that is IL-33 and IL-5 dependent (Yasuda et al., 2012).

Other impressive pathological findings such as changes in the arterial walls and intestinal tissues have also been observed when human IL-33 is injected in mice (Schmitz et al., 2005; Kim et al., 2012). In mice deficient in IL-33Rα, there is myocardial hypertrophy, ventricle dilation, and fibrosis of the heart suggesting that IL-33 plays a protective role in the heart (Sanada et al., 2007). Moreover, elevated levels of the extracellular domain of IL-33Rα predict outcomes in patients that have had a myocardial infarction (Sanada et al., 2007). Furthermore, administration of recombinant IL-33 inhibits the phosphorylation of IκB and reduces hypertrophy and fibrosis in a model of cardiomyocyte hypertrophy (Sanada et al., 2007). One of the more challenging aspects is the role of the IL-33 signaling pathway in the ApoE deficient mouse model of atherosclerosis. Arterial wall plaques of ApoE deficient mice on a

high-fat diet contain IL-33 and IL-33Rα. Atherosclerotic plaques were markedly reduced when these mice were treated with IL-33, however when Insoluble IL-33Rα was administered to neutralize IL-33 signaling the disease worsened (Miller et al., 2008).

Clearly IL-33 has properties that go beyond its role of inducing Th2 responses. For example, IL-33 can induce potent CD8(+) T-cell (CTL) responses to replicating, prototypic RNA, and DNA viruses in mice (Bonilla et al., 2012). Moreover, IL-33 is identical to a nuclear factor which is dominantly expressed in high endothelial venules (HEV) called NF-HEV (Carriere et al., 2007). Constitutive nuclear localization of IL-33 has also been reported in several other cell types such as type II lung epithelial cells (Yasuda et al., 2012), epithelial cells (Moussion et al., 2008), and pancreatic stellate cells (Masamune et al., 2010). IL-33 binding to DNA and acting as a nuclear factor resembles closely the IL-1α binding to chromatin and IL-1α functioning as a nuclear factor (Stevenson et al., 1997; Werman et al., 2004; Cohen et al., 2010). IL-33 precursor can bind NF-κB p65 and IL-1β-induced TNFα is reduced in cells overexpressing the IL-33 precursor (Ali et al., 2011). These data suggest that next to the ability of IL-33 to induce T-cell responses, IL-33 possesses anti-inflammatory activity which appears to be dependent on nuclear sequestration (Cohen et al., 2010).

THE IL-18 AND IL-37 SUBFAMILY

INTERLEUKIN-18

Interleukin-18 is extensively reviewed in this issue of Frontiers in Immunology by Dr. C. Dinarello.

INTERLEUKIN-37

IL-37, formerly termed IL-1F7, lacks a signal peptide and has a caspase-1 site. IL-37 can translocate to the nucleus following stimulation, similar to IL-1α and IL-33 (Sharma et al., 2008). Inhibition of caspase-1 markedly reduces nuclear entry of IL-37 (Sharma et al., 2008), suggesting that IL-37 translocates to the nucleus after caspase-1 processing, and acts as a transcriptional modulator reducing the production of LPS-stimulated pro-inflammatory cytokines. It must be noted that the secretion of IL-37 has not been documented with any certainty. It is likely that the IL-37 precursor exits the cell during cell death and that this precursor suppresses LPS-induced IL-1β, IL-6, and TNFα (Nold et al., 2010). It was from the first reports on IL-37 that recombinant IL-37 bound to the IL-18Rα (Pan et al., 2001; Kumar et al., 2002). In IL-37 transgenic mice this binding of IL-37 to IL-18Rα has also been observed (Nold et al., 2011), and it has been reported that IL-37 specifically binds to the third domain of the IL-18Rα (Bufler et al., 2002). However, IL-37 does not act as a classical receptor antagonist for IL-18, despite these studies showing binding of IL-37 to the IL-18Rα chain. High concentrations of IL-37 do not inhibit recombinant IL-18-induced IFNγ, and recombinant IL-37 modestly reduces IL-18-induced IFNγ in the presence of low concentrations of IL-18BP (Bufler et al., 2002).

A mouse homolog for human IL-37 has not been identified, therefore a strain of transgenic mice has been generated to study the *in vivo* biological function of IL-37 (Nold et al., 2010). No obvious phenotype in homozygous IL-37 transgenic mice (IL-37 tg) mice has been observed and breed normally. Importantly, these mice do not constitutively express mRNA levels of IL-37, which is most likely due to a functional instability sequence found in IL-37 that limits the half-life of IL-37 mRNA (Bufler et al., 2004). Upon stimulation with IL-1β or LPS, expression of IL-37 increases after 4–24 h and the IL-37 precursor can be found in peripheral blood cells isolated from the transgenic mice (Nold et al., 2003). Compared to wild-type mice, IL-37 transgenic mice are protected against LPS challenge (Nold et al., 2010). They display significantly less hypothermia, acidosis, hyperkalemia, hepatitis, and dehydration during LPS challenge. In addition, IL-6 and TNFα production is significantly less in whole blood cultures from IL-37 transgenic mice when stimulated by IL-1β or the combination of IL-12 plus IL-18. This anti-inflammatory activity of IL-37 is not limited to a reduction of the cytokines and chemokines, also DC isolated from the spleen of IL-37 transgenic mice have a marked reduction in their expression of CD86 and MHC II after LPS challenge (Nold et al., 2010). IL-37 transgenic mice subjected to dextran sulfate sodium (DSS)-induced colitis have significantly less clinical disease compared to wild-type mice (McNamee et al., 2011). A decreased leukocyte recruitment into the colonic lamina propria was observed in IL-37Tg mice which was associated with decreased proinflammatory cytokine production. Wild-type mice reconstituted with bone marrow from IL-37 transgenic mice were protected from colitis, suggesting that IL-37 originating from hematopoietic cells is sufficient to exert protective anti-inflammatory effects.

THE IL-36 SUBFAMILY

The IL-1 family members IL-1F5, IL-1F6, IL-1F8, IL-1F9, and IL-1F10 are now termed IL-36Ra, IL-36α, IL-36β, IL-36γ, and IL-38 respectively (Dinarello et al., 2010). Each member of the IL-36 subfamily binds to the IL-1Rpr2, now termed IL-36R (Towne et al., 2004). The IL-36 subfamily is closely related to the IL-1 subfamily because similar to the IL-1α and IL-1β and IL-33, the IL-36R forms a signaling complex with the IL-1RAcP (Towne et al., 2004; Ali et al., 2007).

INTERLEUKIN-36α, β, γ (IL-36)

IL-36α, IL-36β, and IL-36γ all have agonistic characteristics and signal through the IL-36R (Towne et al., 2004; Magne et al., 2006; Chustz et al., 2011). These IL-36 cytokines are mainly expressed in keratinocytes, bronchial epithelium, brain tissue, and monocytes/macrophages (Smith et al., 2000; Barksby et al., 2009). LPS derived from *E. coli* or *P. gingivalis* specifically induces expression of IL-36γ, but not IL-36α or IL-36β in THP-1 cells (Barksby et al., 2009). Peripheral blood lymphocytes are able to express IL-36γ in response to α-particles, which can be used for targeted cancer therapy (Turtoi et al., 2010), and T-lymphocytes have been reported to express IL-36α and IL-36β (Smith et al., 2000; Li et al., 2010; Vigne et al., 2011). Interestingly, it has recently been shown that γδ T-cells can express IL-36β under specific conditions (Yang et al., 2010). IL-36 cytokines like IL-1 and IL-18 also need to be processed in order to gain full bioactivity, although the enzyme responsible still remains to be determined (Towne et al., 2011).

IL-36β can induce expression of itself, and thus an autocrine/paracrine loop similar to IL-1 also seems to be present in the IL-36 subfamily of cytokines (Dinarello et al., 1987; Carrier et al., 2011). IL-36α, IL-36β, and IL-36γ can induce IL-17 and TNF expression in keratinocytes, which can be synergized by the cytokine IL-22 (Carrier et al., 2011). Furthermore, several reports indicate that epidermal growth factor signaling regulates the expression of IL-36α and IL-36β in the skin (Yang et al., 2010; Franzke et al., 2012) suggesting an important role of the agonists IL-36α and IL-36β in skin homeostasis. In line with this is the observation that transgenic mice which overexpress the IL-36α gene in basal keratinocytes display acanthosis and hyperkeratosis of the skin, which are characteristics of psoriatic skin lesions (Blumberg et al., 2007). IL-36 cytokine expression in bronchial epithelial cells can be induced by several pro-inflammatory stimuli (Vos et al., 2005; Chustz et al., 2011). In human lung fibroblasts, IL-36γ induces the chemokine IL-8 and the Th17 chemokine CCL20 (Chustz et al., 2011), suggesting that IL-36 cytokines can contribute to pro-inflammatory responses and in particular neutrophilic airway inflammation.

Furthermore, the IL-36R is highly expressed on microglial cells and astrocytes (Lovenberg et al., 1996; Berglöf et al., 2003; Wang et al., 2005). Murine IL-36β is expressed in neuron cells and in glial cells, but cannot be upregulated by LPS or IL-1β stimulation (Berglöf et al., 2003; Wang et al., 2005). Intraventricular injection of recombinant non-processed mouse IL-36β does not induce any of the classical IL-1 like responses such as fever or modification of food intake and body weight in mice (Berglöf et al., 2003). However, it must be noted that these studies have been performed with non-processed IL-36 agonists, which is shown to have significantly less bioactivity compared to its processed form (Towne et al., 2011).

IL-36 RECEPTOR ANTAGONIST

IL-36Ra shares homology with IL-1Ra but is unable to bind to the IL-1R1 since it significantly differs in loop conformations from IL-1Ra (Dunn et al., 2003). IL-36Ra inhibits IL-36γ-induced NFκB activation (Debets et al., 2001; Towne et al., 2004) in a way similar to IL-1Ra (Towne et al., 2011). However unlike IL-1Ra, IL-36Ra needs to be processed in order to gain antagonistic properties (Towne et al., 2011). Interestingly, IL-36Ra itself can induce mRNA of IL-4 and protein expression in glia cells, which can be attenuated by anti-SIGIRR antibodies. Moreover, the anti-inflammatory action IL-36Ra *in vivo* in the brain is dependent on IL-4 and SIGIRR (Costelloe et al., 2008). IL-36Ra reduces fungal-induced Th17 responses, however not in a classical dose-dependent manner (van de Veerdonk et al., 2012; Gresnigt et al., 2013). These reports suggest that IL-36Ra might be able to recruit the anti-inflammatory IL-1 orphan receptor SIGIRR and activate an anti-inflammatory signaling pathway, and thus does not act as a classical receptor antagonist such as IL-1Ra.

The importance of the biological activity of IL-36Ra in regulating skin inflammation has been demonstrated by several reports. IL-36Ra deficiency exacerbates skin lesions in IL-36α transgenic mice (Blumberg et al., 2007). Phorbol ester treatment of mouse skin overexpressing IL-36α results in an inflammatory condition with macroscopic and histological similarities to human psoriasis (Blumberg et al., 2010), and characteristic inflammation of human psoriatic skin transplanted into immunodeficient mice is dependent on the IL-36R (Blumberg et al., 2010). In patients with psoriasis anti-TNF treatment results in decreased expression of the IL-36 agonists and IL-36Ra, which was associated with improved clinical outcome (Johnston et al., 2011). This increased expression of *IL-36* agonists correlates with Th17 cytokines in human psoriatic skin lesions, although the expression of IL-36Ra by IL-17-stimulated keratinocytes derived from patients with psoriasis does not differ from healthy controls (Carrier et al., 2011; Muhr et al., 2011). Moreover it has recently been shown that mutations in *IL-36RN* can cause a rare life-threatening disease called general pustular psoriasis (GPP) (Marrakchi et al., 2011; Onoufriadis et al., 2011; Sugiura et al., 2012; Farooq et al., 2013). The currently found mutations in *IL-36RN* lead to introduction of a premature stop-codon, frameshift mutation, or an amino acid substitution which were found to result in a misfolded IL-36Ra protein that is less stable and poorly expressed (Marrakchi et al., 2011; Sugiura et al., 2012; Farooq et al., 2013). The misfolded IL-36Ra has less affinity with the IL-36R compared to the wild-type IL-36Ra protein, and therefore is not able to dampen IL-36R-mediated inflammation (Marrakchi et al., 2011; Sugiura et al., 2012; Farooq et al., 2013). These data indicate that IL-36Ra is a receptor antagonist, and that IL-36 signaling plays a significant role in regulating skin inflammation.

IL-36 cytokines might play a significant role in joint disease. Remarkably, only IL-36β is expressed joints of mice and humans (Magne et al., 2006). Interestingly, IL-36β can be measured in the serum of healthy human volunteers, but when serum IL-36β concentrations of healthy volunteers are compared to serum concentrations in rheumatoid arthritis there were no significant differences observed (Magne et al., 2006). However, a recent study showed that the IL-36R was not involved in the inflammatory response in a mouse model of collagen induced arthritis (Lamacchia et al., 2013). In a Caucasian cohort polymorphisms in IL-36β have been associated with spondylitis ankylopoetica, but not this association was not observed in Asian cohorts (Wu and Gu, 2007; Kim et al., 2008).

IL-36γ expression in the lung is significantly increased compared to non-challenged mice in a murine model of HDM-induced allergic inflammation. When recombinant IL-36γ is given intratracheally, this will result in neutrophil influx, but not eosinophilic influx in the lungs of mice, suggesting that IL-36γ is more involved in the regulation of neutrophilic airway inflammation (Chustz et al., 2011; Ramadas et al., 2011, 2012). Bronchial epithelial cells from patients with asthma that were infected with rhinovirus show a higher expression of IL-36γ compared to infected cells from healthy controls (Bochkov et al., 2010). These data support the concept that IL-36 cytokines might also play a significant role in regulating airway inflammation.

INTERLEUKIN-38

IL-1F10 has recently been renamed IL-38 (Dinarello et al., 2010). IL-38 shares 43% homology with IL-36Ra and 41% homology with IL-1Ra (Bensen et al., 2001). The IL-38 precursor lacks a

signal peptide and is 152 amino acids in length, and the natural N-terminus is unknown (Bensen et al., 2001). There is no caspase-1 consensus cleavage site present in the precursor of IL-38. IL-38 is predominantly expressed in the skin and in proliferating B-cells of the tonsil (Lin et al., 2001). The allele combinations that include IL-38 polymorphisms are associated with psoriatic arthritis and ankylosing spondylitis (Chou et al., 2006; Rahman et al., 2006; Guo et al., 2010), suggesting that IL-38 plays a role in the pathogenesis of these inflammatory diseases. Moreover, and suggesting an important role for this cytokine in human cardiovascular disease, polymorphisms in IL-38 were associated with CRP concentrations in humans in addition to polymorphisms in CRP, IL-6 receptor, and NLRP3 that were also associated with CRP concentrations (Dehghan et al., 2011).

Although it has been reported earlier that IL-38 binds to the IL-1 receptor type I this binding affinity of recombinant IL-38 was low (Lin et al., 2001), and more recently it was demonstrated that IL-38 can bind to the IL-36R similar to IL-36Ra (van de Veerdonk et al., 2012). The only biological activity reported so far is that IL-38 can reduce *Candida*-induced T helper 17 responses (van de Veerdonk et al., 2012). Notably, the dose-response suppression of IL-38 as well as that of IL-36Ra of *Candida*-induced IL-22 and IL-17 was not similar to the classic dose-response of IL-1 receptor antagonist, because low concentrations were optimal for inhibiting IL-22 production (van de Veerdonk et al., 2012). A non-classical dose-response has now been observed for IL-36Ra, IL-37, and IL-38 activity and it remains to be determined what the underlying mechanism and biological significance is of these findings.

INTERLEUKIN-1 RECEPTOR ANTAGONIST

The IL-1 receptor is expressed in nearly all tissues and its antagonism prevents receptor binding of either IL-1α or IL-1β, therefore its biological function is as diverse as the roles of IL-1α and IL-1β apart and combined. IL-1Ra can inhibit these responses by binding to the IL-1R1 and preventing the recruitment of IL-1RAcP, which will block IL-1 signaling (Dinarello, 1996). The potent inhibitory effect of IL-1Ra and its importance as a regulating protein in IL-1-mediated inflammation is underlined by a disease called deficiency in interleukin-1 receptor antagonist (DIRA) (Aksentijevich et al., 2009). This disease is characterized by severe sterile multifocal osteomyelitis, periostitis, and pustulosis (Aksentijevich et al., 2009). The life-threatening overwhelming inflammation of skin and bones in these patients can be resolved by treatment with recombinant IL-1Ra. Next to treating this rare disease it should be highlighted that IL-1Ra as a recombinant molecule is successful and on the rise as a new therapeutic agent for many diseases. The use of blocking IL-1 is extensively reviewed in Dinarello et al. (2012), and treating auto-inflammatory diseases with IL-1Ra such as Muckle-wells or gout is highly effective, and a growing list of diseases in which blocking IL-1 signaling with IL-1Ra is growing (Dinarello et al., 2012).

CONCLUSION

It is becoming clear that most members of the IL-1 family primarily promote inflammation and enhance specific acquired immune responses, while some members can provide a brake on inflammation, such as IL-1Ra and IL-36Ra. We are just beginning to understand the biological function of the new IL-1 family members, IL-37 and the cytokines belonging to the IL-36 subfamily, and we are increasingly appreciating the potency of blocking IL-1 in disease. This underscores that long after the initial discovery of IL-1, the cytokine biology of the IL-1 family is still contributing to understanding pathology of disease and remains an exciting field to study.

ACKNOWLEDGMENTS

We thank Prof. Charles Dinarello for his helpful comments. We thank Mark Gresnigt for drawing the figures. Frank L. van de Veerdonk was supported by a Veni grant of the Netherlands Organization for Scientific Research. Mihai G. Netea was supported by a Vici grant of the Netherlands Organization for Scientific Research.

REFERENCES

Agostini, L., Martinon, F., Burns, K., McDermott, M. F., Hawkins, P. N., and Tschopp, J. (2004). NALP3 forms an IL-1beta-processing inflammasome with increased activity in Muckle-Wells autoinflammatory disorder. *Immunity* 20, 319–325. doi:10.1016/S1074-7613(04)00046-9

Aksentijevich, I., Masters, S. L., Ferguson, P. J., Dancey, P., Frenkel, J., van Royen-Kerkhoff, A., et al. (2009). An autoinflammatory disease with deficiency of the interleukin-1-receptor antagonist. *N. Engl. J. Med.* 360, 2426–2437. doi:10.1056/NEJMoa0807865

Ali, S., Huber, M., Kollewe, C., Bischoff, S. C., Falk, W., and Martin, M. U. (2007). IL-1 receptor accessory protein is essential for IL-33-induced activation of T lymphocytes and mast cells. *Proc. Natl. Acad. Sci. U.S.A.* 104, 18660–18665. doi:10.1073/pnas.0705939104

Ali, S., Mohs, A., Thomas, M., Klare, J., Ross, R., Schmitz, M. L., et al. (2011). The dual function cytokine IL-33 interacts with the transcription factor NF-kappaB to dampen NF-kappaB-stimulated gene transcription. *J. Immunol.* 187, 1609–1616. doi:10.4049/jimmunol.1003080

Bae, S., Kang, T., Hong, J., Lee, S., Choi, J., Jhun, H., et al. (2012). Contradictory functions (activation/termination) of neutrophil proteinase 3 enzyme (PR3) in interleukin-33 biological activity. *J. Biol. Chem.* 287, 8205–8213. doi:10.1074/jbc.M111.295055

Barksby, H. E., Nile, C. J., Jaedicke, K. M., Taylor, J. J., and Preshaw, P. M. (2009). Differential expression of immunoregulatory genes in monocytes in response to *Porphyromonas gingivalis* and *Escherichia coli* lipopolysaccharide. *Clin. Exp. Immunol.* 156, 479–487. doi:10.1111/j.1365-2249.2009.03920.x

Bensen, J. T., Dawson, P. A., Mychaleckyj, J. C., and Bowden, D. W. (2001). Identification of a novel human cytokine gene in the interleukin gene cluster on chromosome 2q12-14. *J. Interferon Cytokine Res.* 21, 899–904. doi:10.1089/10799900175 3289505

Berda-Haddad, Y., Robert, S., Salers, P., Zekraoui, L., Farnarier, C., Dinarello, C. A., et al. (2011). Sterile inflammation of endothelial cell-derived apoptotic bodies is mediated by interleukin-1alpha. *Proc. Natl. Acad. Sci. U.S.A.* 108, 20684–20689. doi:10.1073/pnas.1116848108

Berglöf, E., Andre, R., Renshaw, B. R., Allan, S. M., Lawrence, C. B., Rothwell, N. J., et al. (2003). IL-1Rrp2 expression and IL-1F9 (IL-1H1) actions in brain cells. *J. Neuroimmunol.* 139, 36–43. doi:10.1016/S0165-5728(03)00130-9

Blumberg, H., Dinh, H., Dean, C., Trueblood, E. S., Bailey, K., Shows, D., et al. (2010). IL-1RL2 and its ligands contribute to the cytokine network in psoriasis. *J. Immunol.* 185, 4354–4362. doi:10.4049/jimmunol.1000313

Blumberg, H., Dinh, H., Trueblood, E. S., Pretorius, J., Kugler, D., Weng, N., et al. (2007). Opposing activities of two novel members of the IL-1 ligand family regulate skin inflammation. *J. Exp. Med.* 204, 2603–2614. doi:10.1084/jem.20070157

Bochkov, Y., Hanson, K. M., Keles, S., Brockman-Schneider, R., Jarjour, N. N., and Gern, J. E. (2010). Rhinovirus-induced modulation of gene expression in bronchial epithelial cells from subjects with asthma. *Mucosal Immunol.* 3, 69–80. doi:10.1038/mi.2009.109

Bonilla, W. V., Frohlich, A., Senn, K., Kallert, S., Fernandez, M., Johnson, S., et al. (2012). The alarmin interleukin-33 drives protective antiviral CD8(+) T cell responses. *Science* 335, 984–989. doi:10.1126/science.1215418

Boni-Schnetzler, M., Thorne, J., Parnaud, G., Marselli, L., Ehses, J. A., Kerr-Conte, J., et al. (2008). Increased interleukin (IL)-1beta messenger ribonucleic acid expression in beta-cells of individuals with type 2 diabetes and regulation of IL-1beta in human islets by glucose and autostimulation. *J. Clin. Endocrinol. Metab.* 93, 4065–4074. doi:10.1210/jc.2008-0396

Bufler, P., Azam, T., Gamboni-Robertson, F., Reznikov, L. L., Kumar, S., Dinarello, C. A., et al. (2002). A complex of the IL-1 homologue IL-1F7b and IL-18-binding protein reduces IL-18 activity. *Proc. Natl. Acad. Sci. U.S.A.* 99, 13723–13728. doi:10.1073/pnas.212519099

Bufler, P., Gamboni-Robertson, F., Azam, T., Kim, S. H., and Dinarello, C. A. (2004). Interleukin-1 homologues IL-1F7b and IL-18 contain functional mRNA instability elements within the coding region responsive to lipopolysaccharide. *Biochem. J.* 381, 503–510. doi:10.1042/BJ20040217

Carmi, Y., Voronov, E., Dotan, S., Lahat, N., Rahat, M. A., Fogel, M., et al. (2009). The role of macrophage-derived IL-1 in induction and maintenance of angiogenesis. *J. Immunol.* 183, 4705–4714. doi:10.4049/jimmunol.0901511

Carrier, Y., Ma, H. L., Ramon, H. E., Napierata, L., Small, C., O'Toole, M., et al. (2011). Inter-regulation of Th17 cytokines and the IL-36 cytokines in vitro and in vivo: implications in psoriasis pathogenesis. *J. Invest. Dermatol.* 131, 2428–2437. doi:10.1038/jid.2011.234

Carriere, V., Roussel, L., Ortega, N., Lacorre, D. A., Americh, L., Aguilar, L., et al. (2007). IL-33, the IL-1-like cytokine ligand for ST2 receptor, is a chromatin-associated nuclear factor in vivo. *Proc. Natl. Acad. Sci. U.S.A.* 104, 282–287. doi:10.1073/pnas.0606854104

Cayrol, C., and Girard, J. P. (2009). The IL-1-like cytokine IL-33 is inactivated after maturation by caspase-1. *Proc. Natl. Acad. Sci. U.S.A.* 106, 9021–9026. doi:10.1073/pnas.0812690106

Chackerian, A. A., Oldham, E. R., Murphy, E. E., Schmitz, J., Pflanz, S., and Kastelein, R. A. (2007). IL-1 receptor accessory protein and ST2 comprise the IL-33 receptor complex. *J. Immunol.* 179, 2551–2555.

Chan, J. K., Roth, J., Oppenheim, J. J., Tracey, K. J., Vogl, T., Feldmann, M., et al. (2012). Alarmins: awaiting a clinical response. *J. Clin. Invest.* 122, 2711–2719. doi:10.1172/JCI62423

Chen, C. J., Kono, H., Golenbock, D., Reed, G., Akira, S., and Rock, K. L. (2007). Identification of a key pathway required for the sterile inflammatory response triggered by dying cells. *Nat. Med.* 13, 851–856. doi:10.1038/nm1603

Chizzolini, C., Chicheportiche, R., Alvarez, M., de Rham, C., Roux-Lombard, P., Ferrari-Lacraz, S., et al. (2008). Prostaglandin E2 synergistically with interleukin-23 favors human Th17 expansion. *Blood* 112, 3696–3703. doi:10.1182/blood-2008-05-155408

Chou, C. T., Timms, A. E., Wei, J. C., Tsai, W. C., Wordsworth, B. P., and Brown, M. A. (2006). Replication of association of IL1 gene complex members with ankylosing spondylitis in Taiwanese Chinese. *Ann. Rheum. Dis.* 65, 1106–1109. doi:10.1136/ard.2005.046847

Chustz, R. T., Nagarkar, D. R., Poposki, J. A., Favoreto, S. Jr., Avila, P. C., Schleimer, R. P., et al. (2011). Regulation and function of the IL-1 family cytokine IL-1F9 in human bronchial epithelial cells. *Am. J. Respir. Cell Mol. Biol.* 45, 145–153. doi:10.1165/rcmb.2010-0075OC

Coeshott, C., Ohnemus, C., Pilyavskaya, A., Ross, S., Wieczorek, M., Kroona, H., et al. (1999). Converting enzyme-independent release of tumor necrosis factor alpha and IL-1beta from a stimulated human monocytic cell line in the presence of activated neutrophils or purified proteinase 3. *Proc. Natl. Acad. Sci. U.S.A.* 96, 6261–6266. doi:10.1073/pnas.96.11.6261

Cohen, I., Rider, P., Carmi, Y., Braiman, A., Dotan, S., White, M. R., et al. (2010). Differential release of chromatin-bound IL-1alpha discriminates between necrotic and apoptotic cell death by the ability to induce sterile inflammation. *Proc. Natl. Acad. Sci. U.S.A.* 107, 2574–2579. doi:10.1073/pnas.0915018107

Costelloe, C., Watson, M., Murphy, A., McQuillan, K., Loscher, C., Armstrong, M. E., et al. (2008). IL-1F5 mediates anti-inflammatory activity in the brain through induction of IL-4 following interaction with SIGIRR/TIR8. *J. Neurochem.* 105, 1960–1969. doi:10.1111/j.1471-4159.2008.05304.x

Crisan, T. O., Plantinga, T. S., van de Veerdonk, F. L., Farcas, M. F., Stoffels, M., Kullberg, B. J., et al. (2011). Inflammasome-independent modulation of cytokine response by autophagy in human cells. *PLoS ONE* 6:e18666. doi:10.1371/journal.pone.0018666

Debets, R., Timans, J. C., Homey, B., Zurawski, S., Sana, T. R., Lo, S., et al. (2001). Two novel IL-1 family members, IL-1 delta and IL-1 epsilon, function as an antagonist and agonist of NF-kappa B activation through the orphan IL-1 receptor-related protein 2. *J. Immunol.* 167, 1440–1446.

Dehghan, A., Dupuis, J., Barbalic, M., Bis, J. C., Eiriksdottir, G., Lu, C., et al. (2011). Meta-analysis of genome-wide association studies in >80 000 subjects identifies multiple loci for C-reactive protein levels. *Circulation* 123, 731–738. doi:10.1161/CIRCULATIONAHA.110.948570

Dinarello, C., Arend, W., Sims, J., Smith, D., Blumberg, H., O'Neill, L., et al. (2010). IL-1 family nomenclature. *Nat. Immunol.* 11, 973. doi:10.1038/ni1110-973

Dinarello, C. A. (1996). Biologic basis for interleukin-1 in disease. *Blood* 87, 2095–2147.

Dinarello, C. A. (2011a). A clinical perspective of IL-1beta as the gatekeeper of inflammation. *Eur. J. Immunol.* 41, 1203–1217. doi:10.1002/eji.201141550

Dinarello, C. A. (2011b). Interleukin-1 in the pathogenesis and treatment of inflammatory diseases. *Blood* 117, 3720–3732. doi:10.1182/blood-2010-07-273417

Dinarello, C. A., Ikejima, T., Warner, S. J., Orencole, S. F., Lonnemann, G., Cannon, J. G., et al. (1987). Interleukin 1 induces interleukin 1. I. Induction of circulating interleukin 1 in rabbits in vivo and in human mononuclear cells in vitro. *J. Immunol.* 139, 1902–1910.

Dinarello, C. A., Simon, A., and van der Meer, J. W. (2012). Treating inflammation by blocking interleukin-1 in a broad spectrum of diseases. *Nat. Rev. Drug Discov.* 11, 633–652. doi:10.1038/nrd3800

Dong, C. (2008). TH17 cells in development: an updated view of their molecular identity and genetic programming. *Nat. Rev. Immunol.* 8, 337–348. doi:10.1038/nri2295

Dunn, E. F., Gay, N. J., Bristow, A. F., Gearing, D. P., O'Neill, L. J., and Pei, X. Y. (2003). High-resolution structure of murine interleukin 1 homologue IL-1F5 reveals unique loop conformations for receptor binding specificity. *Biochemistry* 42, 10938–10944. doi:10.1021/bi0341197

Faggioni, R., Fantuzzi, G., Fuller, J., Dinarello, C. A., Feingold, K. R., and Grunfeld, C. (1998). IL-1 beta mediates leptin induction during inflammation. *Am. J. Physiol.* 274, R204–R208.

Fantuzzi, G., Ku, G., Harding, M. W., Livingston, D. J., Sipe, J. D., Kuida, K., et al. (1997a). Response to local inflammation of IL-1 beta-converting enzyme-deficient mice. *J. Immunol.* 158, 1818–1824.

Fantuzzi, G., Sacco, S., Ghezzi, P., and Dinarello, C. A. (1997b). Physiological and cytokine responses in interleukin-1β-deficient mice after zymosan-induced inflammation. *Am. J. Physiol.* 273, R400–R406.

Fantuzzi, G., Zheng, H., Faggioni, R., Benigni, F., Ghezzi, P., Sipe, J. D., et al. (1996). Effect of endotoxin in IL-1β-deficient mice. *J. Immunol.* 157, 291–296.

Farooq, M., Nakai, H., Fujimoto, A., Fujikawa, H., Matsuyama, A., Kariya, N., et al. (2013). Mutation analysis of the IL36RN gene in 14 Japanese patients with generalized pustular psoriasis. *Hum. Mutat.* 34, 176–183. doi:10.1002/humu.22203

Franzke, C.-W., Cobzaru, C., Triantafyllopoulou, A., Löffek, S., Horiuchi, K., Threadgill, D. W., et al. (2012). Epidermal ADAM17 maintains the skin barrier by regulating EGFR ligand-dependent terminal keratinocyte differentiation. *J. Exp. Med.* 209, 1105–1119. doi:10.1084/jem.2011225820913c

Gattorno, M., Tassi, S., Carta, S., Delfino, L., Ferlito, F., Pelagatti, M. A., et al. (2007). Pattern of interleukin-1beta secretion in response to lipopolysaccharide and ATP before and after interleukin-1 blockade in patients with CIAS1 mutations. *Arthritis Rheum.* 56, 3138–3148. doi:10.1002/art.22842

Gawaz, M., Brand, K., Dickfeld, T., Pogatsa-Murray, G., Page, S., Bogner, C., et al. (2000). Platelets induce alterations of

chemotactic and adhesive properties of endothelial cells mediated through an interleukin-1-dependent mechanism. Implications for atherogenesis. *Atherosclerosis* 148, 75–85. doi:10.1016/S0021-9150(99)00241-5

Goldbach-Mansky, R., Dailey, N. J., Canna, S. W., Gelabert, A., Jones, J., Rubin, B. I., et al. (2006). Neonatal-onset multisystem inflammatory disease responsive to interleukin-1beta inhibition. *N. Engl. J. Med.* 355, 581–592. doi:10.1056/NEJMoa055137

Goshen, I., and Yirmiya, R. (2009). Interleukin-1 (IL-1): a central regulator of stress responses. *Front. Neuroendocrinol.* 30:30–45. doi:10.1016/j.yfrne.2008.10.001

Gresnigt, M. S., Rosler, B., Jacobs, C. W., Becker, K. L., Joosten, L. A., van der Meer, J. W., et al. (2013). The IL-36 receptor pathway regulates *Aspergillus fumigatus*-induced Th1 and Th17 responses. *Eur. J. Immunol.* 43, 416–426. doi:10.1002/eji.201242711

Greten, F. R., Arkan, M. C., Bollrath, J., Hsu, L. C., Goode, J., Miething, C., et al. (2007). NF-kappaB is a negative regulator of IL-1beta secretion as revealed by genetic and pharmacological inhibition of IKKbeta. *Cell* 130, 918–931. doi:10.1016/j.cell.2007.07.009

Gross, O., Yazdi, A. S., Thomas, C. J., Masin, M., Heinz, L. X., Guarda, G., et al. (2012). Inflammasome activators induce interleukin-1alpha secretion via distinct pathways with differential requirement for the protease function of caspase-1. *Immunity* 36, 388–400. doi:10.1016/j.immuni.2012.01.018

Guo, Z. S., Li, C., Lin, Z. M., Huang, J. X., Wei, Q. J., Wang, X. W., et al. (2010). Association of IL-1 gene complex members with ankylosing spondylitis in Chinese Han population. *Int. J. Immunogenet.* 37, 33–37. doi:10.1111/j.1744-313X.2009.00889.x

Hacham, M., Argov, S., White, R. M., Segal, S., and Apte, R. N. (2002). Different patterns of interleukin-1alpha and interleukin-1beta expression in organs of normal young and old mice. *Eur. Cytokine Netw.* 13, 55–65.

Hanamsagar, R., Hanke, M. L., and Kielian, T. (2012). Toll-like receptor (TLR) and inflammasome actions in the central nervous system. *Trends Immunol.* 33, 333–342. doi:10.1016/j.it.2012.03.001

Harris, J., Hartman, M., Roche, C., Zeng, S. G., O'Shea, A., Sharp, F. A., et al. (2011). Autophagy controls IL-1beta secretion by targeting pro-IL-1beta for degradation. *J. Biol. Chem.* 286, 9587–9597. doi:10.1074/jbc.M110.202911

Hawrylowicz, C. M., Santoro, S. A., Platt, F. M., and Unanue, E. R. (1989). Activated platelets express IL-1 activity. *J. Immunol.* 143, 4015–4018.

Henley, D. V., Bellone, C. J., Williams, D. A., Ruh, T. S., and Ruh, M. F. (2004). Aryl hydrocarbon receptor-mediated posttranscriptional regulation of IL-1beta. *Arch. Biochem. Biophys.* 422, 42–51. doi:10.1016/j.abb.2003.11.022

Hoffman, H. M., Mueller, J. L., Broide, D. H., Wanderer, A. A., and Kolodner, R. D. (2001). Mutation of a new gene encoding a putative pyrin-like protein causes familial cold autoinflammatory syndrome and Muckle-Wells syndrome. *Nat. Genet.* 29, 301–305. doi:10.1038/ng756

Horai, R., Asano, M., Sudo, K., Kanuka, H., Suzuki, M., Nishihara, M., et al. (1998). Production of mice deficient in genes for interleukin (IL)-1alpha, IL-1beta, IL-1alpha/beta, and IL-1 receptor antagonist shows that IL-1beta is crucial in turpentine-induced fever development and glucocorticoid secretion. *J. Exp. Med.* 187, 1463–1475. doi:10.1084/jem.187.9.1463

Hughes, T., Becknell, B., Freud, A. G., McClory, S., Briercheck, E., Yu, J., et al. (2010). Interleukin-1beta selectively expands and sustains interleukin-22+ immature human natural killer cells in secondary lymphoid tissue. *Immunity* 32, 803–814. doi:10.1016/j.immuni.2010.06.007

Johnston, A., Xing, X., Guzman, A. M., Riblett, M., Loyd, C. M., Ward, N. L., et al. (2011). IL-1F5, -F6, -F8, and -F9: a novel IL-1 family signaling system that is active in psoriasis and promotes keratinocyte antimicrobial peptide expression. *J. Immunol.* 186, 2613–2622. doi:10.4049/jimmunol.1003162

Joosten, L. A., Netea, M. G., Fantuzzi, G., Koenders, M. I., Helsen, M. M., Sparrer, H., et al. (2009). Inflammatory arthritis in caspase 1 gene-deficient mice: contribution of proteinase 3 to caspase 1-independent production of bioactive interleukin-1beta. *Arthritis Rheum.* 60, 3651–3662. doi:10.1002/art.25006

Kamari, Y., Werman-Venkert, R., Shaish, A., Werman, A., Harari, A., Gonen, A., et al. (2007). Differential role and tissue specificity of interleukin-1alpha gene expression in atherogenesis and lipid metabolism. *Atherosclerosis* 195, 31–38. doi:10.1016/j.atherosclerosis.2006.11.026

Kaplanski, G., Farnarier, C., Kaplanski, S., Porat, R., Shapiro, L., Bongrand, P., et al. (1994). Interleukin-1 induces interleukin-8 secretion from endothelial cells by a juxtacrine mechanism. *Blood* 84, 4242–4248.

Kawaguchi, Y., Nishimagi, E., Tochimoto, A., Kawamoto, M., Katsumata, Y., Soejima, M., et al. (2006). Intracellular IL-1alpha-binding proteins contribute to biological functions of endogenous IL-1alpha in systemic sclerosis fibroblasts. *Proc. Natl. Acad. Sci. U.S.A.* 103, 14501–14506. doi:10.1073/pnas.0603545103

Kim, T.-J., Kim, T.-H., Lee, H.-J., Peddle, L., Rahman, P., Hu, P., et al. (2008). Interleukin 1 polymorphisms in patients with ankylosing spondylitis in Korea. *J. Rheumatol.* 35, 1603–1608.

Kim, Y. H., Yang, T. Y., Park, C. S., Ahn, S. H., Son, B. K., Kim, J. H., et al. (2012). Anti-IL-33 antibody has a therapeutic effect in a murine model of allergic rhinitis. *Allergy* 67, 183–190. doi:10.1111/j.1398-9995.2011.02735.x

Klionsky, D. J. (2007). Autophagy: from phenomenology to molecular understanding in less than a decade. *Nat. Rev. Mol. Cell Biol.* 8, 931–937. doi:10.1038/nrm2245

Kumar, S., Hanning, C. R., Brigham-Burke, M. R., Rieman, D. J., Lehr, R., Khandekar, S., et al. (2002). Interleukin-1F7B (IL-1H4/IL-1F7) is processed by caspase-1 and mature IL-1F7B binds to the IL-18 receptor but does not induce IFN-gamma production. *Cytokine* 18, 61–71. doi:10.1006/cyto.2002.0873

Kurt-Jones, E. A., Beller, D. I., Mizel, S. B., and Unanue, E. R. (1985). Identification of a membrane-associated interleukin-1 in macrophages. *Proc. Natl. Acad. Sci. U.S.A.* 82, 1204–1208. doi:10.1073/pnas.82.4.1204

Lamacchia, C., Palmer, G., Rodriguez, E., Martin, P., Vigne, S., Seemayer, C. A., et al. (2013). The severity of experimental arthritis is independent of IL-36 receptor signaling. *Arthritis Res. Ther.* 15, R38. doi:10.1186/ar4192

Lefrancais, E., Roga, S., Gautier, V., Gonzalez-de-Peredo, A., Monsarrat, B., Girard, J. P., et al. (2012). IL-33 is processed into mature bioactive forms by neutrophil elastase and cathepsin G. *Proc. Natl. Acad. Sci. U.S.A.* 109, 1673–1678. doi:10.1073/pnas.1115884109

Li, Y., Messina, C., Bendaoud, M., Fine, D. H., Schreiner, H., and Tsiagbe, V. K. (2010). Adaptive immune response in osteoclastic bone resorption induced by orally administered *Aggregatibacter actinomycetemcomitans* in a rat model of periodontal disease. *Mol. Oral Microbiol.* 25, 275–292. doi:10.1111/j.2041-1014.2010.00576.x

Lin, H., Ho, A. S., Haley-Vicente, D., Zhang, J., Bernal-Fussell, J., Pace, A. M., et al. (2001). Cloning and characterization of IL-1HY2, a novel interleukin-1 family member. *J. Biol. Chem.* 276, 20597–20602. doi:10.1074/jbc.M010095200

Louten, J., Rankin, A. L., Li, Y., Murphy, E. E., Beaumont, M., Moon, C., et al. (2011). Endogenous IL-33 enhances Th2 cytokine production and T-cell responses during allergic airway inflammation. *Int. Immunol.* 23, 307–315. doi:10.1093/intimm/dxr006

Lovenberg, T. W., Crowe, P. D., Liu, C., Chalmers, D. T., Liu, X. J., Liaw, C., et al. (1996). Cloning of a cDNA encoding a novel interleukin-1 receptor related protein (IL 1R-rp2). *J. Neuroimmunol.* 70, 113–122. doi:10.1016/S0165-5728(96)00047-1

Luheshi, N. M., Kovacs, K. J., Lopez-Castejon, G., Brough, D., and Denes, A. (2011). Interleukin-1alpha expression precedes IL-1beta after ischemic brain injury and is localised to areas of focal neuronal loss and penumbral tissues. *J. Neuroinflammation* 8, 186. doi:10.1186/1742-2094-8-186

Maedler, K., Sergeev, P., Ris, F., Oberholzer, J., Joller-Jemelka, H. I., Spinas, G. A., et al. (2002). Glucose-induced beta cell production of IL-1beta contributes to glucotoxicity in human pancreatic islets. *J. Clin. Invest.* 110, 851–860. doi:10.1172/JCI15318

Magne, D., Palmer, G., Barton, J. L., Mézin, F., Talabot-Ayer, D., Bas, S., et al. (2006). The new IL-1 family member IL-1F8 stimulates production of inflammatory mediators by synovial fibroblasts and articular chondrocytes. *Arthritis Res. Ther.* 8, R80. doi:10.1186/ar1946

Maier, J. A. M., Voulalas, P., Roeder, D., and Maciag, T. (1990). Extension of the life span of human endothelial cells by an interleukin-1α antisense oligomer. *Science* 249, 1570–1574. doi:10.1126/science.2218499

Marrakchi, S., Guigue, P., Renshaw, B. R., Puel, A., Pei, X. Y., Fraitag, S., et al. (2011). Interleukin-36-receptor antagonist deficiency and generalized pustular psoriasis.

N. Engl. J. Med. 365, 620–628. doi:10.1056/NEJMoa1013068

Martinon, F., Mayor, A., and Tschopp, J. (2009). The inflammasomes: guardians of the body. *Annu. Rev. Immunol.* 27, 229–265. doi:10.1146/annurev.immunol.021908.132715

Masamune, A., Watanabe, T., Kikuta, K., Satoh, K., Kanno, A., and Shimosegawa, T. (2010). Nuclear expression of interleukin-33 in pancreatic stellate cells. *Am. J. Physiol. Gastrointest. Liver Physiol.* 299, G821–G832. doi:10.1152/ajpgi.00178.2010

Matsuba-Kitamura, S., Yoshimoto, T., Yasuda, K., Futatsugi-Yumikura, S., Taki, Y., Muto, T., et al. (2010). Contribution of IL-33 to induction and augmentation of experimental allergic conjunctivitis. *Int. Immunol.* 22, 479–489. doi:10.1093/intimm/dxq035

McNamee, E. N., Masterson, J. C., Jedlicka, P., McManus, M., Grenz, A., Collins, C. B., et al. (2011). Interleukin 37 expression protects mice from colitis. *Proc. Natl. Acad. Sci. U.S.A.* 108, 16711–16716. doi:10.1073/pnas.1111982108

Meyer, U. (2011). Anti-inflammatory signaling in schizophrenia. *Brain Behav. Immun.* 25, 1507–1518. doi:10.1016/j.bbi.2011.05.014

Miller, A. C., Schattenberg, D. G., Malkinson, A. M., and Ross, D. (1994). Decreased content of the IL1 alpha processing enzyme calpain in murine bone marrow-derived macrophages after treatment with the benzene metabolite hydroquinone. *Toxicol. Lett.* 74, 177–184. doi:10.1016/0378-4274(94)90096-5

Miller, A. M., Xu, D., Asquith, D. L., Denby, L., Li, Y., Sattar, N., et al. (2008). IL-33 reduces the development of atherosclerosis. *J. Exp. Med.* 205, 339–346. doi:10.1084/jem.20071868

Moreira, A. P., Cavassani, K. A., Ismailoglu, U. B., Hullinger, R., Dunleavy, M. P., Knight, D. A., et al. (2011). The protective role of TLR6 in a mouse model of asthma is mediated by IL-23 and IL-17A. *J. Clin. Invest.* 121, 4420–4432. doi:10.1172/JCI44999

Moussion, C., Ortega, N., and Girard, J. P. (2008). The IL-1-like cytokine IL-33 is constitutively expressed in the nucleus of endothelial cells and epithelial cells in vivo: a novel 'alarmin'? *PLoS ONE* 3:e3331. doi:10.1371/journal.pone.0003331

Muhr, P., Zeitvogel, J., Heitland, I., Werfel, T., and Wittmann, M. (2011). Expression of interleukin (IL)-1 family members upon stimulation with IL-17 differs in keratinocytes derived from patients with psoriasis and healthy donors. *Br. J. Dermatol.* 165, 189–193. doi:10.1111/j.1365-2133.2011.10302.x

Nakahira, K., Haspel, J. A., Rathinam, V. A., Lee, S. J., Dolinay, T., Lam, H. C., et al. (2011). Autophagy proteins regulate innate immune responses by inhibiting the release of mitochondrial DNA mediated by the NALP3 inflammasome. *Nat. Immunol.* 12, 222–230. doi:10.1038/ni.1980

Netea, M. G., Nold-Petry, C. A., Nold, M. F., Joosten, L. A., Opitz, B., van der Meer, J. H., et al. (2009). Differential requirement for the activation of the inflammasome for processing and release of IL-1beta in monocytes and macrophages. *Blood* 113, 2324–2335. doi:10.1182/blood-2008-03-146720

Nold, M., Hauser, I. A., Hofler, S., Goede, A., Eberhardt, W., Ditting, T., et al. (2003). IL-18BPa:Fc cooperates with immunosuppressive drugs in human whole blood. *Biochem. Pharmacol.* 66, 505–510. doi:10.1016/S0006-2952(03)00294-6

Nold, M. F., Nold-Petry, C. A., Zepp, J. A., Lo, C., Garlanda, C., Mantovani, A., et al. (2011). Interleukin 37 exerts its anti-inflammatory functions by associating with IL-18R alpha and SIGIRR. *Cytokine* 56, 12. doi:10.1016/j.cyto.2011.07.333 (ABSTRACT).

Nold, M. F., Nold-Petry, C. A., Zepp, J. A., Palmer, B. E., Bufler, P., and Dinarello, C. A. (2010). IL-37 is a fundamental inhibitor of innate immunity. *Nat. Immunol.* 11, 1014–1022. doi:10.1038/ni.1944

Onoufriadis, A., Simpson, M. A., Pink, A. E., Di Meglio, P., Smith, C. H., Pullabhatla, V., et al. (2011). Mutations in IL36RN/IL1F5 are associated with the severe episodic inflammatory skin disease known as generalized pustular psoriasis. *Am. J. Hum. Genet.* 89, 432–437. doi:10.1016/j.ajhg.2011.07.022

Pan, G., Risser, P., Mao, W., Baldwin, D. T., Zhong, A. W., Filvaroff, E., et al. (2001). IL-1H, an interleukin 1-related protein that binds IL-18 receptor/IL-1Rrp. *Cytokine* 13, 1–7. doi:10.1006/cyto.2000.0799

Rahman, P., Sun, S., Peddle, L., Snelgrove, T., Melay, W., Greenwood, C., et al. (2006). Association between the interleukin-1 family gene cluster and psoriatic arthritis. *Arthritis Rheum.* 54, 2321–2325. doi:10.1002/art.21928

Ramadas, R. A., Ewart, S. L., Iwakura, Y., Medoff, B. D., and LeVine, A. M. (2012). IL-36alpha exerts pro-inflammatory effects in the lungs of mice. *PLoS ONE* 7:e45784. doi:10.1371/journal.pone.0045784

Ramadas, R. A., Ewart, S. L., Medoff, B. D., and LeVine, A. M. (2011). Interleukin-1 family member 9 stimulates chemokine production and neutrophil influx in mouse lungs. *Am. J. Respir. Cell Mol. Biol.* 44, 134–145. doi:10.1165/rcmb.2009-0315OC

Rider, P., Carmi, Y., Guttman, O., Braiman, A., Cohen, I., Voronov, E., et al. (2011). IL-1alpha and IL-1beta recruit different myeloid cells and promote different stages of sterile inflammation. *J. Immunol.* 187, 4835–4843. doi:10.4049/jimmunol.1102048

Saitoh, T., Fujita, N., Jang, M. H., Uematsu, S., Yang, B. G., Satoh, T., et al. (2008). Loss of the autophagy protein Atg16L1 enhances endotoxin-induced IL-1beta production. *Nature* 456, 264–268. doi:10.1038/nature07383

Sanada, S., Hakuno, D., Higgins, L. J., Schreiter, E. R., McKenzie, A. N., and Lee, R. T. (2007). IL-33 and ST2 comprise a critical biomechanically induced and cardioprotective signaling system. *J. Clin. Invest.* 117, 1538–1549. doi:10.1172/JCI30634

Schmid, D., and Munz, C. (2007). Innate and adaptive immunity through autophagy. *Immunity* 27, 11–21. doi:10.1016/j.immuni.2007.07.004

Schmitz, J., Owyang, A., Oldham, E., Song, Y., Murphy, E., McClanahan, T. K., et al. (2005). IL-33, an interleukin-1-like cytokine that signals via the IL-1 receptor-related protein ST2 and induces T helper type 2-associated cytokines. *Immunity* 23, 479–490. doi:10.1016/j.immuni.2005.09.015

Sharma, S., Kulk, N., Nold, M. F., Graf, R., Kim, S. H., Reinhardt, D., et al. (2008). The IL-1 family member 7b translocates to the nucleus and down-regulates proinflammatory cytokines. *J. Immunol.* 180, 5477–5482.

Smeekens, S. P., van de Veerdonk, F. L., van der Meer, J. W., Kullberg, B. J., Joosten, L. A., and Netea, M. G. (2010). The Candida Th17 response is dependent on mannan- and beta-glucan-induced prostaglandin E2. *Int. Immunol.* 22, 889–895. doi:10.1093/intimm/dxq442

Smith, D. E., Renshaw, B. R., Ketchem, R. R., Kubin, M., Garka, K. E., and Sims, J. E. (2000). Four new members expand the interleukin-1 superfamily. *J. Biol. Chem.* 275, 1169–1175. doi:10.1074/jbc.275.2.1169

Stevenson, F. T., Turck, J., Locksley, R. M., and Lovett, D. H. (1997). The N-terminal propiece of interleukin 1 alpha is a transforming nuclear oncoprotein. *Proc. Natl. Acad. Sci. U.S.A.* 94, 508–513. doi:10.1073/pnas.94.2.508

Sugiura, K., Takeichi, T., Kono, M., Ogawa, Y., Shimoyama, Y., Muro, Y., et al. (2012). A novel IL36RN/IL1F5 homozygous nonsense mutation, p.Arg10X, in a Japanese patient with adult-onset generalized pustular psoriasis. *Br. J. Dermatol.* 167, 699–701. doi:10.1111/j.1365-2133.2012.10953.x

Thornton, P., McColl, B. W., Greenhalgh, A., Denes, A., Allan, S. M., and Rothwell, N. J. (2010). Platelet interleukin-1alpha drives cerebrovascular inflammation. *Blood* 115, 3632–3639. doi:10.1182/blood-2009-11-252643

Towne, J. E., Garka, K. E., Renshaw, B. R., Virca, G. D., and Sims, J. E. (2004). Interleukin (IL)-1F6, IL-1F8, and IL-1F9 signal through IL-1Rrp2 and IL-1RAcP to activate the pathway leading to NF-kappaB and MAPKs. *J. Biol. Chem.* 279, 13677–13688. doi:10.1074/jbc.M400117200

Towne, J. E., Renshaw, B. R., Douangpanya, J., Lipsky, B. P., Shen, M., Gabel, C. A., et al. (2011). Interleukin-36 (IL-36) ligands require processing for full agonist (IL-36alpha, IL-36beta, and IL-36gamma) or antagonist (IL-36Ra) activity. *J. Biol. Chem.* 286, 42594–42602. doi:10.1074/jbc.M111.267922

Tschopp, J., and Schroder, K. (2010). NLRP3 inflammasome activation: the convergence of multiple signalling pathways on ROS production? *Nat. Rev. Immunol.* 10, 210–215. doi:10.1038/nri2725

Turtoi, A., Brown, I., Schläger, M., and Schneeweiss, F. H. (2010). Gene expression profile of human lymphocytes exposed to (211) At alpha particles. *Radiat. Res.* 174, 125–136. doi:10.1667/RR1659.1

van de Veerdonk, F. L., Gresnigt, M. S., Kullberg, B. J., van der Meer, J. W., Joosten, L. A., and Netea, M. G. (2009). Th17 responses and host defense against microorganisms: an overview. *BMB Rep.* 42, 776–787. doi:10.5483/BMBRep.2009.42.12.776

van de Veerdonk, F. L., Stoeckman, A. K., Wu, G., Boeckermann, A. N., Azam, T., Netea, M. G., et al. (2012). IL-38 binds to the IL-36 receptor and has biological effects

on immune cells similar to IL-36 receptor antagonist. *Proc. Natl. Acad. Sci. U.S.A.* 109, 3001–3005. doi:10.1073/pnas.1121534109

Vigne, S., Palmer, G., Lamacchia, C., Martin, P., Talabot-Ayer, D., Rodriguez, E., et al. (2011). IL-36R ligands are potent regulators of dendritic and T cells. *Blood* 118, 5813–5823. doi:10.1182/blood-2011-05-356873

Vos, J. B., van Sterkenburg, M., Rabe, K. F., Schalkwijk, J., Hiemstra, P. S., and Datson, N. (2005). Transcriptional response of bronchial epithelial cells to *Pseudomonas aeruginosa*: identification of early mediators of host defense. *Physiol. Genomics* 21, 324–336. doi:10.1152/physiolgenomics.00289.2004

Wang, P., Meinhardt, B., Andre, R., Renshaw, B. R., Kimber, I., Rothwell, N. J., et al. (2005). The interleukin-1-related cytokine IL-1F8 is expressed in glial cells, but fails to induce IL-1beta signalling responses. *Cytokine* 29, 245–250.

Weaver, C. T., Harrington, L. E., Mangan, P. R., Gavrieli, M., and Murphy, K. M. (2006). Th17: an effector CD4 T cell lineage with regulatory T cell ties. *Immunity* 24, 677–688. doi:10.1016/j.immuni.2006.06.002

Werman, A., Werman-Venkert, R., White, R., Lee, J. K., Werman, B., Krelin, Y., et al. (2004). The precursor form of IL-1alpha is an intracrine proinflammatory activator of transcription. *Proc. Natl. Acad. Sci. U.S.A.* 101, 2434–2439. doi:10.1073/pnas.0308705101

Willart, M. A., Deswarte, K., Pouliot, P., Braun, H., Beyaert, R., Lambrecht, B. N., et al. (2012). Interleukin-1alpha controls allergic sensitization to inhaled house dust mite via the epithelial release of GM-CSF and IL-33. *J. Exp. Med.* 209, 1505–1517. doi:10.1084/jem.20112691

Wilson, N. J., Boniface, K., Chan, J. R., McKenzie, B. S., Blumenschein, W. M., Mattson, J. D., et al. (2007). Development, cytokine profile and function of human interleukin 17-producing helper T cells. *Nat. Immunol.* 8, 950–957. doi:10.1038/ni1497

Wu, Z., and Gu, J. (2007). A meta-analysis on interleukin-1 gene cluster polymorphism and genetic susceptbility for ankylosing spondylitis. *Zhonghua Yi Xue Za Zhi* 87, 433–437.

Yang, J., Meyer, M., Müller, A.-K., Böhm, F., Grose, R., Dauwalder, T., et al. (2010). Fibroblast growth factor receptors 1 and 2 in keratinocytes control the epidermal barrier and cutaneous homeostasis. *J. Cell Biol.* 188, 935–952. doi:10.1083/jcb.200910126

Yasuda, K., Muto, T., Kawagoe, T., Matsumoto, M., Sasaki, Y., Matsushita, K., et al. (2012). Contribution of IL-33-activated type II innate lymphoid cells to pulmonary eosinophilia in intestinal nematode-infected mice. *Proc. Natl. Acad. Sci. U.S.A.* 109, 3451–3456. doi:10.1073/pnas.1201042109

Yin, H., Morioka, H., Towle, C. A., Vidal, M., Watanabe, T., and Weissbach, L. (2001). Evidence that HAX-1 is an interleukin-1 alpha N-terminal binding protein. *Cytokine* 15, 122–137. doi:10.1006/cyto.2001.0891

Yirmiya, R., and Goshen, I. (2011). Immune modulation of learning, memory, neural plasticity and neurogenesis. *Brain Behav. Immun.* 25, 181–213. doi:10.1016/j.bbi.2010.10.015

Zheng, H., Fletcher, D., Kozak, W., Jiang, M., Hofmann, K., Conn, C. C., et al. (1995). Resistance to fever induction and impaired acute-phase response in interleukin-1β deficient mice. *Immunity* 3, 9–19. doi:10.1016/1074-7613(95)90154-X

Zhou, R., Yazdi, A. S., Menu, P., and Tschopp, J. (2011). A role for mitochondria in NLRP3 inflammasome activation. *Nature* 469, 221–225. doi:10.1038/nature09663

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

*Received: 29 March 2013; accepted: 12 June 2013; published online: 08 July 2013. Citation: van de Veerdonk FL and Netea MG (2013) New insights in the immunobiology of IL-1 family members. Front. Immunol. **4**:167. doi: 10.3389/fimmu.2013.00167*

This article was submitted to Frontiers in Inflammation, a specialty of Frontiers in Immunology.

frontiers in
IMMUNOLOGY

REVIEW ARTICLE
published: 08 October 2013
doi: 10.3389/fimmu.2013.00289

Interleukin-18 and IL-18 binding protein

Charles A. Dinarello[1,2]*, Daniela Novick[3], Soohyun Kim[4] and Gilles Kaplanski[5,6]

[1] *Department of Medicine, University of Colorado Denver, Aurora, CO, USA*
[2] *Department of Medicine, University Medical Center Nijmegen, Nijmegen, Netherlands*
[3] *Department of Molecular Genetics, Weizmann Institute of Science, Rehovot, Israel*
[4] *Department of Biomedical Science and Technology, Konkuk University, Seoul, Republic of Korea*
[5] *UMR-S 1076, Aix Marseille Université, Marseille, France*
[6] *Service de Médecine Interne, Hôpital de la Conception, Assistance Publique Hôpitaux de Marseille, Marseille, France*

Edited by:
Cecilia Garlanda, Istituto Clinico Humanitas, Italy

Reviewed by:
Kingston H. Mills, Trinity College Dublin, Ireland
Jean-Philippe Girard, Centre national de la recherche scientifique, France

***Correspondence:**
Charles A. Dinarello, Department of Medicine, University of Colorado Denver, Denver, CO 80262, USA
e-mail: cdinare333@aol.com

Interleukin-18 (IL-18) is a member of the IL-1 family of cytokines. Similar to IL-1β, IL-18 is synthesized as an inactive precursor requiring processing by caspase-1 into an active cytokine but unlike IL-1β, the IL-18 precursor is constitutively present in nearly all cells in healthy humans and animals. The activity of IL-18 is balanced by the presence of a high affinity, naturally occurring IL-18 binding protein (IL-18BP). In humans, increased disease severity can be associated with an imbalance of IL-18 to IL-18BP such that the levels of free IL-18 are elevated in the circulation. Increasing number of studies have expanded the role of IL-18 in mediating inflammation in animal models of disease using the IL-18BP, IL-18-deficient mice, neutralization of IL-18, or deficiency in the IL-18 receptor alpha chain. A role for IL-18 has been implicated in several autoimmune diseases, myocardial function, emphysema, metabolic syndromes, psoriasis, inflammatory bowel disease, hemophagocytic syndromes, macrophage activation syndrome, sepsis, and acute kidney injury, although in some models of disease, IL-18 is protective. IL-18 plays a major role in the production of interferon-γ from T-cells and natural killer cells. The IL-18BP has been used safely in humans and clinical trials of IL-18BP as well as neutralizing anti-IL-18 antibodies are in clinical trials. This review updates the biology of IL-18 as well as its role in human disease.

Keywords: inflammation, autoimmune diseases, inflammasomes, interleukin-1, macrophages

INTRODUCTION TO IL-18

Interleukin-18 (IL-18) was first described in 1989 as "IFNγ-inducing factor" isolated in the serum of mice following an intraperitoneal injection of endotoxin. Days before, the mice had been pretreated with *Propionibacterium acnes*, which stimulates the reticuloendothelial system, particularly the Kupffer cells of the liver. Many investigators concluded that the serum factor was IL-12. With purification from mouse livers and molecular cloning of "IFNγ-inducing factor" in 1995 (1), the name was changed to IL-18. Surprisingly, the new cytokine was related to IL-1 and particularly to IL-1β. Similar to IL-1β, IL-18 is first synthesized as an inactive precursor and without a signal peptide, remains as an intracellular cytokine. The tertiary structure of the IL-18 precursor is closely related to the IL-37 precursor and the intron-exon borders of the IL-18 and IL-37 genes suggest a close association. Since 1995, many studies have used neutralization of endogenous IL-18 or IL-18-deficient mice to demonstrate the role for this cytokine in promoting inflammation and immune responses [reviewed in Ref. (2–4)]. However, the biology of IL-18 is hardly the recapitulation of IL-1β. There are several unique and specific differences between IL-18 and IL-1β. For example, in healthy human subjects and also in healthy mice, gene expression for IL-1β in blood mononuclear cells and hematopoietic cells is absent and there is no evidence that the IL-1β precursor is constitutively present in epithelial cells (5). In contrast, the IL-18 precursor is present in blood monocytes from healthy subjects and in the epithelial cells of the entire gastrointestinal tract. Peritoneal macrophages and mouse spleen also contain the IL-18 precursor in the absence of disease (5). The IL-18 precursor is also present in keratinocytes and nearly all epithelial cells. In this regard, IL-18 is similar to IL-1α and IL-33.

PRODUCTION AND ACTIVITY OF IL-18

PROCESSING OF THE IL-18 PRECURSOR BY CASPASE-1

The IL-18 precursor has a molecular weight of 24,000 and is processed by the intracellular cysteine protease caspase-1, which cleaves the precursor into an active mature molecule of 17,200. As with the processing of IL-1β, inactive pro-caspase-1 is first activated into active caspase-1 by the nucleotide-binding domain and leucine-rich repeat pyrin containing protein-3 (NLRP3) inflammasome. Following cleavage by active caspase-1, mature IL-18 is secreted from the monocyte/macrophage, although over 80% of the IL-18 precursor remains unprocessed inside the cell. Compared to wild-type mice, mice deficient in caspase-1 do not release circulating IFNγ following endotoxin. IL-12-induced IFNγ is also absent in caspase-1-deficient mice (6). Importantly, any phenotypic characteristic of caspase-1-deficient mice must be studied as whether the deficiency is due to reduced IL-1β or IL-18 activity. For example, the caspase-1-deficient mouse is resistant to colitis (7) but the IL-1β-deficient mouse is susceptible in the same disease model (8). Since neutralizing antibodies to IL-18 are protective in the dextran sodium sulfate (DSS) colitis model, caspase-1

deficiency appears to prevent processing of IL-18 (7, 9). On the other hand, there are examples where caspase-1 processing of IL-18 is not required. For example, Fas ligand (FasL) stimulation results in release of biologically active IL-18 in caspase-1-deficient murine macrophages (10).

Similar to IL-1α and IL-33, the IL-18 precursor is constitutively expressed in endothelial cells, keratinocytes, and intestinal epithelial cells throughout the gastrointestinal tract. Macrophages and dendritic cells are the primary sources for the release of active IL-18, whereas the inactive precursor remains in the intracellular compartment of mesenchymal cells. Also, similar to IL-1α and IL-33, the IL-18 precursor is released from dying cells and processed extracellularly, most likely by neutrophil proteases such as proteinase-3 (11).

Although Fas signaling triggers apoptosis, Fas signaling induces inflammatory cytokine production, including IL-18. In addition to inducing IL-18, Fas signaling activates caspase-8 in macrophages and dendritic cells, which results in processing and release of mature IL-1β and IL-18 (12). It was also reported that the processing of IL-1β and IL-18 takes place independently of NLRP3 or RIP3 (12).

PROCESSING AND SECRETION OF THE IL-18 PRECURSOR BY ADAM 33-MEDIATED VEGF-DEPENDENT MECHANISM

Because IL-18 stimulates vascular endothelial cells and promotes metastatic tumor cell invasion, studies had examined the mechanisms of IL-18 secretion from gastric cancer cell line. Vascular endothelial cell growth factor-D (VEGF-D) increased the expression as well as the secretion of IL-18 from the gastric cancer cell line (13). Since VEGF-D has a metalloprotease domain, knockdown of ADAM33 was examined and prevented the secretion of IL-18. Moreover, cell proliferation was reduced using ADAM33 small interfering RNA transfectants (13).

SIGNAL TRANSDUCTION BY IL-18

As shown in **Figure 1**, IL-18 forms a signaling complex by binding to the IL-18 alpha chain (IL-18Rα), which is the ligand binding chain for mature IL-18; however, this binding is of low affinity. In cells that express the co-receptor, termed IL-18 receptor beta chain (IL-18Rβ), a high affinity complex is formed, which then signals. The complex of IL-18 with the IL-18Rα and IL-18Rβ chains is similar to that formed by other members of the IL-1 family with the co-receptor, the IL-1R accessory chain IL-1RAcP. Following the formation of the heterodimer, the Toll-IL-1 receptor (TIR) domains approximate and it appears that the cascade of sequential recruitment of MyD88, the four IRAKs and TRAF-6 followed by the degradation of IκB and release of NFκB are nearly identical as that for IL-1 (14). However, there are differences between IL-1 and IL-18 signaling. With few exceptions, IL-1α or IL-1β are active on cells in the low nanograms per milliliter range and often in the picograms per milliliter range. In contrast, IL-18 activation of cells expressing the two IL-18 receptor chains requires 10–20 ng/mL and sometime higher levels (15, 16).

Although nearly all cells express the IL-1RI, not all cells express IL-1RAcP. Similarly, most cells express the IL-18Rα but not all cell express the IL-18Rβ. IL-18Rβ is expressed on T-cells and dendritic cells but not commonly expressed in mesenchymal cells.

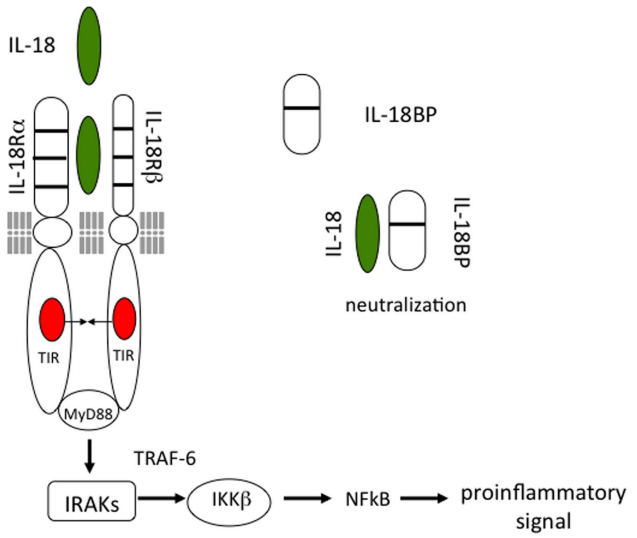


FIGURE 1 | Interleukin-18 signal transduction. IL-18 forms a signaling complex by binding to the IL-18 alpha chain (IL-18Rα). The co-receptor, termed IL-18 receptor beta chain (IL-18Rβ), is recruited to form a high affinity complex. Following the formation of the heterodimer, the Toll-IL-1 receptor (TIR) domains approximate triggering the binding of MyD88, phosphorylations of the four IRAKs, TRAF-6, and activation of NFκB. The IL-18BP is present in the extracellular compartment where it binds mature IL-18 and prevents binding to the IL-18 receptor.

The human lung epithelial cells line A549, derived from a lung carcinoma epithelial cell, does not express IL-18Rβ (17) and there is no signal unless IL-12 is present to induce IL-18Rβ (18). In the absence of IL-18Rβ, IL-18 binds to IL-18Rα without a pro-inflammatory signal. In A549 cells transfected with IL-18Rβ, IL-18 induces IL-8 and a large number of genes. One of these genes is the former IL-2-induced gene termed NK4 (19) now termed IL-32 (17). IL-32 is not a member of the IL-1 family but plays an important role in the regulation of cytokines such as IL-1β and TNFα. Importantly, IL-32 is an IL-18-inducible gene.

IL-18 AS AN IMMUNOREGULATORY CYTOKINE

ROLE OF IL-18 IN THE PRODUCTION OF IFNγ

Together with IL-12, IL-18 participates in the Th1 paradigm. This property of IL-18 is due to its ability to induce IFNγ either with IL-12 or IL-15. Without IL-12 or IL-15, IL-18 does not induce IFNγ. IL-12 or IL-15 increases the expression of IL-18Rβ, which is essential for IL-18 signal transduction. Importantly, without IL-12 or IL-15, IL-18 plays a role in Th2 diseases (20). The importance of IL-18 as an immunoregulatory cytokine is derived from its prominent biological property of inducing IFNγ from NK cells. Macrophage colony stimulating factor (M-CSF) induces human blood monocytes to differentiate into a subset of macrophages; these cells express a membrane-bound form of IL-18 (21). Membrane IL-18 is expressed in 30–40% of M-CSF-primed macrophages. In contrast, monocytes, dendritic cells, and monocytes differentiated into M1 macrophages did not express membrane IL-18. Although the expression of membrane IL-18 is caspase-1 dependent (21), LPS treatment was necessary for the release of membrane IL-18

(21). A major immunoregulating role for IL-18 is on the NK cell. Upon shedding of membrane IL-18 into a soluble form, NK cells expressed CCR7 and produced high levels of IFNγ. As expected, IFNγ production was prevented by neutralization of IL-18. This mechanism may account for the role of IL-18 as major IFNγ inducing factor from NK cells and the role of NK cells in the pathogenesis of autoimmune diseases.

The induction of IFNγ by IL-18 has been studied with co-inducer IL-12. For example, mice injected with the combination of IL-18 plus IL-12 develop high levels of IFNγ and die with hypoglycemia, intestinal inflammation, and inanition (22). In leptin-deficient mice, IL-18 plus IL-12 induce acute pancreatitis (23). Several human autoimmune diseases are associated with elevated production of IFNγ and IL-18. Diseases such as systemic lupus erythematosus, rheumatoid arthritis, Type-1 diabetes, Crohn's disease, psoriasis, and graft versus host disease are thought to be mediated, in part, by IL-18.

IL-18, IL-17, AND GAMMA/DELTA T-CELL ACTIVATION

The role for IL-17 in the pathogenesis of autoimmune diseases has been studied in animal models but also validated in humans treated with either neutralizing antibodies to IL-17 or the IL-17 receptor. However, blockade of IL-1 often prevents or markedly reduces the production of IL-17 *in vitro* as well as the development of autoimmunity in animal models (24–27). Indeed, there is increased IL-1β as well as increased IL-17 in children born with mutations in the naturally occurring IL-1Ra resulting in a severe inflammatory disease due to excessive IL-1β activity (28, 29). The high production of IL-17 in these children is thought to contribute to the severity of the disease. Is there a role for IL-18 in the production of IL-17?

Attention has focused on a role for IL-18 in Th17 responses primarily because both IL-1β and IL-18 are processed into active cytokines via caspase-1. Using a model for multiple sclerosis termed experimental autoimmune encephalomyelitis (EAE) (26), a role for IL-18 was studied. As expected, using the adjuvant of *Mycobacterium tuberculosis* plus the myelin-derived immunogen for EAE, bone marrow derived mouse dendritic cells released IL-1β and IL-18, which was dependent on caspase-1 (30). The primed dendritic cells induced IL-17 from T-cells, which when transferred to non-immunized mice resulted in the encephalomyelitis. However, the disease did not develop when the dendritic cells were exposed to a caspase-1 inhibitor (30). Treating the mice with either IL-1β or IL-18 restored the ability of the T-cell transfer to induce the disease. Moreover, treating the recipient mice with the caspase-1 inhibitor reduced the disease as well as reduced the production of IL-17 from CD4 positive T-cells as well as from gamma-delta T-cells. Gamma-delta T-cells produce IL-17 when stimulated with IL-18 plus IL-23, as these T-cells express high levels of the IL-18 receptor alpha chain. Thus, similar to caspase-1 dependent IL-1β, IL-18 induces T-cells to produce IL-17 and promote autoimmune responses to specific antigens.

IL-18 AND INFLAMMATION

PRO-INFLAMMATORY PROPERTIES OF IL-18

Interleukin-18 exhibits characteristics of other pro-inflammatory cytokines, such as increases in cell adhesion molecules, nitric oxide synthesis, and chemokine production. Blocking IL-18 activity reduces metastasis in a mouse model of melanoma; this is due to a reduction in IL-18-induced expression of vascular call adhesion molecule-1 (31). A unique property of IL-18 is the induction of FasL, which may account for the hepatic damage that takes place in macrophage activation syndrome (MAS) (10, 32). The induction of fever, a well-studied property of IL-1α and IL-1β as well as acute phase proteins, TNFα and IL-6, is not a significant property of IL-18. Injection of IL-18 into mice or rabbits does not produce fever (33, 34). In a clinical study of intravenous IL-18 dosing in patients with cancer, chills, and fevers were not common and were Grade 1 (low fevers). Unlike IL-1 and TNFα, fever in humans is observed in all patients at doses of 10 ng/kg whereas IL-18 fevers were observed in 3 of 21 patients and only at doses of 100 and 200 μg/kg (35).

Unlike IL-1 and TNFα, IL-18 does not induce cyclooxygenase-2 and hence there is no production of prostaglandin E2 (16, 36). IL-18 has been administered to humans for the treatment of cancer in order to increase the activity and expansion of cytotoxic T-cells. Not unexpectedly and similar to several cytokines, the therapeutic focus on IL-18 has shifted from its use as an immune stimulant to inhibition of its activity (3, 37).

Because IL-18 can increase IFNγ production, blocking IL-18 activity in autoimmune diseases is an attractive therapeutic target since anti-IL-12/23 reduces the severity of Crohn's disease as well as psoriasis. As discussed below, there appears to be a role for blocking IL-18 in Crohn's disease. However, there are several activities of IL-18 that are independent of IFNγ. For example, IL-18 inhibits proteoglycan synthesis in chondrocytes (38) and proteoglycan synthesis is essential for maintaining healthy cartilage. IL-18 also increases vascular cell adhesion molecule-1 (VCAM-1) expression in endothelial cells independently of IFNγ. VCAM-1 plays a major role in multiple sclerosis, other autoimmune diseases as well as in the metastatic process (39).

ROLE OF IL-18 IN MODELS OF INFLAMMATORY BOWEL DISEASE

Inflammatory bowel disease such as Crohn's disease is a complex autoimmune disease. Treatment is initially based on immunosuppressive drugs. Not surprisingly, anti-cytokines such as neutralizing monoclonal antibodies to TNFα (40) or to IL-12/23 provide effective treatment for many patients (41, 42). The reduction of IFNγ in Crohn's disease is linked to the clinical response to these agents (42). IL-18 is found in affected intestinal lesions from Crohn's disease patients as a mature protein but the IL-18 precursor form is present in uninvolved intestinal tissues (43). This observation was confirmed in a similar assessment of mucosal biopsies from Crohn's disease patients (44). Antisense RNA to IL-18 decreased IFNγ production in lamina propria mononuclear cells (44).

A commonly used mouse model for colitis is DSS, which is added to the drinking water and which damages the intestinal wall. Thus in DSS-induced colitis, the epithelial barrier defenses against luminal bacterial products are breeched. In this model, reducing IL-18 with a neutralizing antibody is protective and linked to a reduction in IFNγ (9). Blocking IL-18 with the IL-18 binding protein (IL-18BP) (see **Figure 1**) also reduces colitis induced by antigen sensitization (45). Since generation of active

IL-18 requires caspase-1, studies have also been performed in mice deficient in caspase-1 and subjected to DSS colitis. Nevertheless, despite many studies, the role of caspase-1 in DSS colitis remains unclear. The first study showed that mice deficient in caspase-1 were protected (7, 46). In addition, treatment of mice with a specific caspase-1 inhibitor was also effective in protecting against the colitis (47–49). In both studies, the effect of caspase-1 deficiency was linked to reduced IL-18 activity, whereas reducing IL-1 activity with the IL-1Ra was ineffective (7). In support of the role of IL-18 in DSS colitis, inhibition of endogenous merprin β to reduce the generation of active IL-18 was protective in DSS colitis (50).

However, a conundrum has developed whether caspase-1 deficiency is protective or detrimental in DSS colitis. DSS colitis is not the optimal model for Crohn's disease as the model is one of rapid loss of the protective barrier of the intestinal epithelium exposing the lamina propria mononuclear cells to a large amount and variety of bacterial products. Using the same DSS model, mice deficient in the adapter protein inflammasome component ASC experienced increased disease, morbidity, and precancerous lesions compared to wild-type mice exposed to DSS (51). Similarly, mice deficient in caspase-1 died rapidly from DSS compared to wild-type mice (52) whereas mice deficient in caspase-12, in which caspase-1 is enhanced were protected (52). Administration of exogenous IL-18 restored mucosal healing in caspase-1-deficient mice (52). Also, mice deficient in NLRP3 were more susceptible to either DSS or TNBS-induced colitis and exhibited decreased IL-1β as well as decreased beta-defensins (53). Macrophages from NLRP3-deficient mice failed to respond to MDP (53). Mice deficient in NLRP6 are also more vulnerable to DSS (54, 55) and the susceptibility appears to be due to lack of sufficient IL-18.

How to reconcile these data in mouse models of colitis was addressed by Siegmund (56). It is likely that IL-18, being constitutive in the intestinal epithelium, has a protective role in that the cytokine contributes to maintaining the intestinal barrier. With loss of the barrier, the microbial products stimulate macrophages in the lamina propria and caspase-1 dependent processing of IL-18 results in inflammation. In this model, inhibition of IL-18 production in caspase-1-deficient mice or treatment of wild-type mice with anti-IL-18 antibodies or caspase-1 inhibitors is protective. Worsening of disease in mice deficient in caspase-1 or NLRP3 or NLRP6 may lower the levels of active endogenous IL-18 needed to protect the epithelial barrier. Similarly, active endogenous IL-1β may be needed to protect to maintain the epithelial barrier by inducing growth factors.

Although it remains unclear why caspase-1 deficiency worsens DSS colitis, in humans with Crohn's disease, natalizumab, the antibody that blocks the very late antigen-4 (VLA-4), is highly effective in treating the disease. VLA-4 is the α4 subunit of the β-1 integrin. Anti-VLA-4 binds to the surface of macrophages and other myeloid cells and prevents the binding of these cells to the VLA-4 receptor on endothelial cells known as VCAM-1. Thus, the antibody disables the function of VCAM-1 and prevents the passage of macrophages and other myeloid cells into tissues such as the intestine in Crohn's disease and the brain in multiple sclerosis. Since IL-18 induces VCAM-1, blocking IL-18 would also reduce the passage of cells through the endothelium into to intestine.

IL-18, HYPERPHAGIA, AND THE METABOLIC SYNDROME

Whereas there is no constitutive gene expression for IL-1β in freshly obtained human PBMC, the same cells express constitutive mRNA for IL-18 (5). In western blot analysis from the same cells, the IL-18 precursor was present but not the IL-1β precursor. Similar observations were also made in mice (5). These findings suggest that IL-18 may act as regulator of homeostasis. Starting at age 16 weeks of age, IL-18-deficient mice start to overeat, become obese, and exhibit lipid abnormalities; there is increased atherosclerosis, insulin resistance, and diabetes mellitus reminiscent of the metabolic syndrome (57). IL-18Rα deficient mice also develop a similar phenotype. The higher body weight is attributed to enhanced food intake, in which the IL-18-deficient mice begin to diverge from wild-type animals at a relatively early age, and to reach values 30–40% higher than that of wild-type mice. Others have observed similar findings (58). A striking finding was an increase of more than 100% in the percent of adipose tissue in the IL-18-deficient animals that was accompanied by fat deposition in the arterial walls. The insulin resistance in these mice is corrected by exogenous recombinant IL-18. Mice deficient in IL-18 respond normally to a challenge with exogenous leptin suggesting that expression of the leptin receptor is unaffected. The unexpected and unique mechanism is responsible for the higher food intake in the IL-18-deficient animals appears to be due a central nervous system loss of appetite control. IL-18-deficient mice eat throughout the day whereas wild-type mice eat once, nocturnally.

IL-18 IN HEART DISEASE

Heart disease includes coronary vessel disease with associated myocardial infarction, post viral myocardiopathies, autoimmune heat disease, and chronic heart failure. Although survival from an acute myocardial infarction has decreased dramatically due to improved acute care, patients often progress to heart failure due to post infarction remodeling of the ventricles. Treatment options for heart failure vary but reducing cytokines is now being tested as a possible therapy. Based on pre-clinical as well as pilot clinical trials, blocking TNFα was tested in large trials but failed; using a higher dose of an antibody to TNFα (infliximab), there were more deaths compared to the placebo-treated patients. There are also pre-clinical studies demonstrating that blockade of IL-1β is effective (59, 60) and clinical trials using anakinra have revealed that blockade of IL-1 is effective in reducing post infarction remodeling (61, 62) as well as increased exercise tolerance (63). In fact, the largest trial in 17,200 patients using a neutralizing antibody to IL-1β aims to reduce cardiovascular events in high risk patients (64).

Increasing numbers of animal and clinical studies indicate a role for IL-18 in heart disease. The myocardium of patients with ischemic heart failure express the alpha chain of the IL-18 receptor and have elevated levels of circulating IL-18 and associated with death (65). Daily administration of IL-18 results in ventricular hypertrophy, increased collagen (66), and elevated left ventricular diastolic pressure in mice (67). As with all cytokine studies, validation of the role of a cytokine in a disease process is best assessed by specific blockade. In a model of myocardial suppression associated

with septic shock, mice were injected with LPS and a neutralizing antibody to murine IL-18 was administered (68). The rationale for the experiment was that IL-18 mediates the production of TNFα and IL-1β and to induce the expression of intercellular adhesion molecule-1 (ICAM-1) and VCAM-1. Mice were injected with LPS and left ventricular developed pressure was determined. Left ventricular developed pressure was depressed by 38% 6 h after LPS but pretreatment with anti-mouse IL-18 antibody attenuated LPS-induced myocardial dysfunction (by 92%) and ICAM-1/VCAM-1 expression (50 and 35% reduction, respectively).

In another study, human atrial muscle strips were obtained from patients undergoing by-pass surgery and the tissue was exposed to ischemia while contractile strength was measured. The addition of IL-18BP to the perfusate during and after the ischemic event resulted in improved contractile function from 35% of control to 76% with IL-18BP (69). IL-18BP treatment also preserved intracellular tissue creatine kinase levels (by 420%). Steady-state mRNA levels for IL-18 were elevated after ischemic and the concentration of IL-18 in myocardial homogenates was increased (control, 5.8 pg/mg versus I/R, 26 pg/mg). Active IL-18 requires cleavage of its precursor form by caspase-1; inhibition of caspase-1 also attenuated the depression in contractile force after ischemia (from 35% of control to 75.8% in treated atrial muscle). Because caspase-1 also cleaves the IL-1β precursor, IL-1 receptor blockade was accomplished by using the IL-1 receptor antagonist. IL-1 receptor antagonist added to the perfusate also resulted in a reduction of ischemia-induced contractile dysfunction.

In summary, these studies demonstrate a role for IL-18 in heart disease. Moreover, endogenous IL-18 is induced by IL-1β via caspase-1 under ischemic conditions in human myocardial tissue and that inhibition of caspase-1 reduces the processing of endogenous precursors of IL-18 and IL-1β and thereby prevents ischemia-induced myocardial dysfunction.

IL-18 AS A PROTECTIVE CYTOKINE

As stated above, mice deficient in caspase-1 experience increased disease severity when subjected to DSS colitis and that administration of exogenous IL-18 restored mucosal healing in these mice (52). In addition, IL-18 deficiency or IL-18 receptor deficiency results in the development of a metabolic syndrome in mice. Mice deficient in NLRP3 are more susceptible to DSS colitis, which is thought to be due to decreased IL-18 (53). Mice deficient in NLRP6 are also more vulnerable to DSS (54, 55) and the susceptibility appears to be due to lack of sufficient IL-18. As discussed below, a protective role for IL-18 is not limited to the gastrointestinal track. In the eye, a condition resembling "wet macula degeneration" worsens with antibodies to IL-18 (70).

Thus, there are a growing number of studies, which support a protective role for IL-18. The fact that mice deficient in IL-18 develop a metabolic syndrome-like phenotype is consistent with a role for IL-18 in homeostasis. A study in age related macular degeneration is also consistent with a protective role for IL-18. In that study, drusen, which is mixture of complement-derived and apolipoproteins and lipids, were shown to activate NLRP3 and induce the production of mature IL-1β and IL-18 (70). In a mouse model of "wet" age related macular degeneration, the disease was worse in mice deficient in NLRP3 but not in IL-1RI deficient mice (70). Therefore, IL-18 rather than IL-1α or IL-1β were protective and upon administration of IL-IL-18, the disease severity improved. Taken together, there is a case for IL-18 being a protective rather than inflammatory cytokine.

IL-18 BINDING PROTEIN

THE DISCOVERY OF THE IL-18BP

The discovery of the IL-18BP took place during the search for the soluble receptors for IL-18 (71). IL-18BP is a constitutively secreted protein, with an exceptionally high affinity for IL-18 (400 pM) (72) (**Figure 1**). Present in the serum of healthy humans at a 20-fold molar excess compared to IL-18 (73), IL-18BP may contribute to a default mechanism by which a Th1 response to foreign organisms is blunted in order to reduce triggering an autoimmune responses to a routine infection. IL-18BP deviates from the classical definition of soluble receptors since it does not correspond to the extracellular ligand binding domain of the IL-18 receptor, but is rather encoded by a separate gene. Thus IL-18BP belongs to a separate family of secreted proteins. As shown in **Figure 1**, IL-18BP contains only one IgG domain whereas the Type II IL-1 receptor contains three domains. In this regard, the single IgG domain of IL-18BP is similar to SIGIRR, which also has a single IgG domain and also functions as a decoy receptor. The salient property of IL-18BP in immune responses is in down-regulating Th1 responses by binding to IL-18 and thus reducing the induction of IFNγ (20). Since IL-18 also affects Th2 responses, IL-18BP also has properties controlling a Th2 cytokine response (20).

BALANCE OF IL-18 AND IL-18BP IN HUMAN DISEASE

IL-18 binding protein has a classic signal peptide, and therefore is readily secreted. Serum levels in healthy subjects are in the range of 2,000–3,000 pg/mL compared to the levels of IL-18 in the same sera of 80–120 pg/mL (73). Moreover, IL-18BP binds IL-18 with an affinity of 400 pM, an affinity significantly higher than that of IL-18Rα. Because a single IL-18BP molecule binds a single IL-18 molecule, one can calculate bound versus free IL-18 in a mixture of both molecules (73).

If one examines immunologically mediated diseases where IFNγ plays a pathological role such as Wegener's granulomatosis and systemic lupus erythematosus, one must consider the level of free IL-18 compared to IL-18 bound to IL-18BP. In fact, in these diseases both IL-18BP and IL-18 are high (74, 75) but the level of IL-18BP is not sufficiently high enough to neutralize IL-18 and therefore, the level of free IL-18 is higher than in healthy subjects. In MAS where IFNγ plays a pathological role, both IL-18BP and IL-18 are also high but the clinical and hematological abnormalities correlate with elevated free IL-18 (32).

A unique property of IL-18BP is that the molecule also binds IL-37 (76) and in doing so, enhances the ability of IL-18BP to inhibit the induction of IFNγ by IL-18. IL-37 binds to the IL-18Rα with a very low affinity but in mice expressing human IL-37, a profound anti-inflammatory effect is observed (77), particularly of LPS-induced cytokines and dendritic cell maturation (77). Human IL-37-expressing mice are also resistant to colitis (78). Thus, the anti-inflammatory property of IL-37 can be affected by the concentration of IL-18BP. As the concentration of IL-18BP increases and binds IL-37, there is the possibility that IL-37 becomes less

available as an anti-inflammatory cytokine. Indeed this has been observed in mice injected with IL-18BP. At low dosing of IL-18BP, there is reduced inflammation in a model of rheumatoid arthritis but as the doing of IL-18BP increases, the anti-inflammatory properties of IL-18BP are lost (79). **Table 1** summarizes several disease states in which IL-18 as well as IL-18BP are measured and in some studies, the level of free IL-18 has been reported.

REGULATION OF IL-18BP

IL-18 binding protein is highly regulated at the level of gene expression and unexpectedly, IFNγ increases gene expression and synthesis of IL-18BP (80, 81). Therefore, IFNγ driving an increase in the natural and potent inhibitor of IL-18 falls into the category of a negative feed-back loop. The concept is supported by clinical data showing that patients being treated with IFNα for hepatitis have elevated levels of IL-18BP (82, 83). IL-27, like IFNγ, functions as both a pro- as well as an anti-inflammatory cytokine and both may accomplish their roles as anti-inflammatory cytokines at the level of increased production of IL-18BP. In the skin, IL-27 also acts through a negative feed-back loop for inflammation. IL-27 is acting, as is IFNγ, by induction of IL-18BP gene expression and synthesis (84).

VIRAL IL-18BP

Natural neutralization of human IL-18 by IL-18BP takes place during a common viral infection. In *Molluscum contagiosum* infection, characterized by raised but bland eruptions, there are large numbers of viral particles in the epithelial cells of the skin but histologically there are few inflammatory or immunologically active cells in or near the lesions. Clearly, the virus fails to elicit an inflammatory or immunological response. Amino acid similarity exists between human IL-18BP and a gene found in various members of the poxviruses; the greatest degree of homology is found to be expressed by *M. contagiosum* gene (85). The ability of viral IL-18BP to reduce the activity of mammalian IL-18 likely explains the lack of inflammatory and immune cells in the virally infected tissues and provides further evidence for the natural ability of IL-18BP to interfere with IL-18 activity.

HEMOPHAGOCYTIC LYMPHO HISTIOCYTOSIS AND MACROPHAGE ACTIVATION SYNDROME

Hemophagocytic lympho histiocytosis (HLH) syndrome is a rare life-threatening condition characterized by a severe hyper-inflammatory state. There is a genetic form of HLH called familial hemophagocytic lympho histiocytosis (fHLH). However, HLH can

Table 1 | Levels of IL-18 and IL-18BP in human disease.

Disease	IL-18[a]	IL-18BP[b]	Free IL-18[a]	Reference
Sepsis	500–2,000	ND	ND	Emmanuilidis et al. (100)
Sepsis	250–10,000	22.5	250–3,000	Novick et al. (73)
Trauma	300–600	ND	ND	Mommsen et al. (101)
Schizophrenia	518	10	253	Palladino et al. (102)
Ulcerative colitis	274	ND	ND	Haas et al. (103)
Ulcerative colitis	393	4.7	250	Ludwiczek et al. (104)
Crohn's disease	387	ND	ND	Haas et al. (103)
Crohn's disease	546	5	340	Ludwiczek et al. (104)
Wegener's disease	240	14.5	84	Novick et al. (74)
Rheumatoid arthritis	230–400	ND	ND	Bokarewa and Hultgren (105)
SLE[c]	700	7.5	408	Favilli et al. (99)
SLE[c]	400	15	167	Novick et al. (75)
MAS[d]	2,200	35	660	Mazodier et al. (32)
Systemic JIA[e]	1,600–78,000	ND	ND	Jelusic et al. (106)
Adult Still's disease	1,000–6,000	ND	ND	Kawashima et al. (107)
Myocardial infarction	238	ND	ND	Blankenberg et al. (108)
Myocardial infarction	355	ND	ND	Narins et al. (109)
Coronary artery disease	356	13.7	125	Thompson et al. (110)
Metabolic syndrome	380	ND	ND	Troseid et al. (111)
Acute kidney injury[f]	500	ND	ND	Parikh et al. (112)
Acute kidney injury[f]	2,000	ND	ND	Vaidya et al. (113)
Acute kidney injury[f]	>360	ND	ND	Parikh et al. (114)
Acute kidney injury[f]	884	ND	ND	Sirota et al. (115)

[a] *Levels in picograms per milliliter, range, or mean.*

[b] *Levels in nanograms per milliliter, range, or mean.*

[c] *Systemic lupus erythematosus.*

[d] *Macrophage activation syndrome.*

[e] *Systemic juvenile idiopathic arthritis.*

[f] *Urine levels (mean in picograms per milliliter).*

be secondary to infections and lymphoma, and is called secondary MAS. The development of MAS is associated with several infectious diseases, notably due to Epstein–Barr virus, cytomegalovirus, herpes virus, or intracellular bacteria and parasites and also of various lymphomas, especially of T-cell lineage. In addition, patients with rheumatological conditions, particularly systemic onset juvenile arthritis (sJIA), but also systemic lupus erythematosus, Kawasaki disease, or systemic vasculitis can develop MAS (86–89). One of the most prominent hematologic and metabolic characteristics of MAS is thrombocytopenia and hepatic injury, respectively. Indeed, IFNγ may be responsible for the thrombocytopenia as well as several of the immunological abnormalities of the disorder.

IL-18 IN THE HEMOPHAGOCYTIC SYNDROMES

In the case of fHLH or MAS, gene expression for IL-18 is upregulated in peripheral mononuclear cells (90, 91) and serum IL-18 is unusually elevated (32, 92–95). Although levels of IL-18 in the circulation are below 1 ng/mL in inflammatory diseases such as severe sepsis, in active phase of fHLH or EBV-HLH, serum IL-18 is usually in the range of 5–7 ng/mL, and in fHLH complicating XIAP gene mutations as well as in MAS complicating sJIA, levels of circulating IL-18 can be in 20–30 ng/mL range (32, 96–98). However, it is necessary to calculate the level of free IL-18 since IL-18BP is present in the circulation in health and disease (73) (see **Table 1**) in lupus (75, 99), Wegener's granulomatosis (74). In patients with MAS, free IL-18 but not IL-12 concentrations significantly correlated with clinical status and the biologic markers of MAS such as anemia ($p < 0.001$), hypertriglyceridemia, and hyperferritinemia ($p < 0.01$) and also with markers of Th1 lymphocyte or macrophage activation, such as elevated concentrations of IFNγ and soluble IL-2 and TNFα receptor concentrations (32).

CONCLUDING REMARKS

Although clinical trials of IL-1 blocking therapies have focused attention on the biology IL-1, the role of IL-18 in health and disease is derived from animal models and measurements of IL-18 in various disease conditions. Nevertheless, with clinical trials of IL-18BP as well as neutralizing antibodies to IL-18 now underway, the role for this cytokine in treating human disease will become apparent. Certainly validated animal models support a role for IL-18 in acute renal injury, psoriasis, heart failure, MAS, and inflammatory bowel disease. Whether suppression of IL-18 will affect IL-17-mediated diseases such as multiple sclerosis or reduce metastatic melanoma will also be determined in clinical trials.

ACKNOWLEDGMENTS

The authors thank Tania Azam, Karin Mazodier, Laura Chiossonne, Catherine Farnarier, and Eric Vivier. These studies are supported by NIH AI-15614, AR-45584, and CA-04 6934 (to Charles A. Dinarello) and Agence Nationale de la Recherche-Maladies Rares 2007 et Projet National de Recherche Clinique 2007 (to Gilles Kaplanski).

REFERENCES

1. Okamura H, Nagata K, Komatsu T, Tanimoto T, Nukata Y, Tanabe F, et al. A novel costimulatory factor for gamma interferon induction found in the livers of mice causes endotoxic shock. *Infect Immun* (1995) **63**:3966–72.
2. Boraschi D, Dinarello CA. IL-18 in autoimmunity: review. *Eur Cytokine Netw* (2006) **17**(4):224–52.
3. Dinarello CA. Interleukin-18 and the pathogenesis of inflammatory diseases. *Semin Nephrol* (2007) **27**(1):98–114. doi:10.1016/j.semnephrol.2006.09.013
4. Tsutsui H, Nakanishi K. Immunotherapeutic applications of IL-18. *Immunotherapy* (2012) **4**(12):1883–94. doi:10.2217/imt.12.137
5. Puren AJ, Fantuzzi G, Dinarello CA. Gene expression, synthesis and secretion of IL-1β and IL-18 are differentially regulated in human blood mononuclear cells and mouse spleen cells. *Proc Natl Acad Sci U S A* (1999) **96**:2256–61. doi:10.1073/pnas.96.5.2256
6. Fantuzzi G, Reed DA, Dinarello CA. IL-12-induced IFNγ is dependent on caspase-1 processing of the IL-18 precursor. *J Clin Invest* (1999) **104**(6):761–7. doi:10.1172/JCI7501
7. Siegmund B, Lehr HA, Fantuzzi G, Dinarello CA. IL-1beta-converting enzyme (caspase-1) in intestinal inflammation. *Proc Natl Acad Sci U S A* (2001) **98**(23):13249–54. doi:10.1073/pnas.231473998
8. Besedovsky H, del Rey A, Sorkin E, Dinarello CA. Immunoregulatory feedback between interleukin-1 and glucocorticoid hormones. *Science* (1986) **233**(4764):652–4. doi:10.1126/science.3014662
9. Siegmund B, Fantuzzi G, Rieder F, Gamboni-Robertson F, Lehr HA, Hartmann G, et al. Neutralization of interleukin-18 reduces severity in murine colitis and intestinal IFN-γ and TNF-α production. *Am J Physiol Regul Integr Comp Physiol* (2001) **281**(4):R1264–73.
10. Tsutsui H, Matsui K, Okamura H, Nakanishi K. Pathophysiological roles of interleukin-18 in inflammatory liver diseases. *Immunol Rev* (2000) **174**:192–209. doi:10.1034/j.1600-0528.2002.017418.x
11. Sugawara S, Uehara A, Nochi T, Yamaguchi T, Ueda H, Sugiyama A, et al. Neutrophil proteinase 3-mediated induction of bioactive IL-18 secretion by human oral epithelial cells. *J Immunol* (2001) **167**(11):6568–75.
12. Bossaller L, Chiang PI, Schmidt-Lauber C, Ganesan S, Kaiser WJ, Rathinam VA, et al. Cutting edge: FAS (CD95) mediates noncanonical IL-1beta and IL-18 maturation via caspase-8 in an RIP3-independent manner. *J Immunol* (2012) **189**(12):5508–12. doi:10.4049/jimmunol.1202121
13. Kim KE, Song H, Hahm C, Yoon SY, Park S, Lee HR, et al. Expression of ADAM33 is a novel regulatory mechanism in IL-18-secreted process in gastric cancer. *J Immunol* (2009) **182**(6):3548–55. doi:10.4049/jimmunol.0801695
14. Weber A, Wasiliew P, Kracht M. Interleukin-1 (IL-1) pathway. *Sci Signal* (2010) **3**(105):cm1. doi:10.1126/scisignal.3105cm1
15. Morel JC, Park CC, Woods JM, Koch AE. A novel role for interleukin-18 in adhesion molecule induction through NFkappa B and phosphatidylinositol (PI) 3-kinase-dependent signal transduction pathways. *J Biol Chem* (2001) **276**(40):37069–75. doi:10.1074/jbc.M103574200
16. Lee JK, Kim SH, Lewis EC, Azam T, Reznikov LL, Dinarello CA. Differences in signaling pathways by IL-1beta and IL-18. *Proc Natl Acad Sci U S A* (2004) **101**(23):8815–20. doi:10.1073/pnas.0402800101
17. Kim SH, Han SY, Azam T, Yoon DY, Dinarello CA. Interleukin-32: a cytokine and inducer of TNFalpha. *Immunity* (2005) **22**(1):131–42. doi:10.1016/S1074-7613(04)00380-2
18. Nakanishi K, Yoshimoto T, Tsutsui H, Okamura H. Interleukin-18 regulates both Th1 and Th2 responses. *Ann Rev Immunol* (2001) **19**:423–74. doi:10.1146/annurev.immunol.19.1.423
19. Dahl CA, Schall RP, He HL, Cairns JS. Identification of a novel gene expressed in activated natural killer cells and T cells. *J Immunol* (1992) **148**(2):597–603.
20. Nakanishi K, Yoshimoto T, Tsutsui H, Okamura H. Interleukin-18 is a unique cytokine that stimulates both Th1 and Th2 responses depending on its cytokine milieu. *Cytokine Growth Factor Rev* (2001) **12**(1):53–72. doi:10.1016/S1359-6101(00)00015-0
21. Bellora F, Castriconi R, Doni A, Cantoni C, Moretta L, Mantovani A, et al. M-CSF induces the expression of a membrane-bound form of IL-18 in a subset of human monocytes differentiating in vitro toward macrophages. *Eur J Immunol* (2012) **42**(6):1618–26. doi:10.1002/eji.201142173

22. Nakamura S, Otani T, Ijiri Y, Motoda R, Kurimoto M, Orita K. IFN-gamma-dependent and -independent mechanisms in adverse effects caused by concomitant administration of IL-18 and IL-12. *J Immunol* (2000) **164**(6):3330–6.
23. Sennello JA, Fayad R, Pini M, Gove ME, Ponemone V, Cabay RJ, et al. Interleukin-18, together with interleukin-12, induces severe acute pancreatitis in obese but not in nonobese leptin-deficient mice. *Proc Natl Acad Sci U S A* (2008) **105**(23):8085–90. doi:10.1073/pnas.0804091105
24. Coccia M, Harrison OJ, Schiering C, Asquith MJ, Becher B, Powrie F, et al. IL-1beta mediates chronic intestinal inflammation by promoting the accumulation of IL-17A secreting innate lymphoid cells and CD4+ Th17 cells. *J Exp Med* (2012) **209**(9):1595–609. doi:10.1084/jem.20111453
25. Joosten LA. Excessive interleukin-1 signaling determines the development of Th1 and Th17 responses in chronic inflammation. *Arthritis Rheum* (2010) **62**(2):320–2. doi:10.1002/art.27242
26. Sutton C, Brereton C, Keogh B, Mills KH, Lavelle EC. A crucial role for interleukin (IL)-1 in the induction of IL-17-producing T cells that mediate autoimmune encephalomyelitis. *J Exp Med* (2006) **203**(7):1685–91. doi:10.1084/jem.20060285
27. Sutton CE, Lalor SJ, Sweeney CM, Brereton CF, Lavelle EC, Mills KH. Interleukin-1 and IL-23 induce innate IL-17 production from gammadelta T cells, amplifying Th17 responses and autoimmunity. *Immunity* (2009) **31**(2):331–41. doi:10.1016/j.immuni.2009.08.001
28. Aksentijevich I, Masters SL, Ferguson PJ, Dancey P, Frenkel J, van Royen-Kerkhoff A, et al. An autoinflammatory disease with deficiency of the interleukin-1-receptor antagonist. *N Engl J Med* (2009) **360**(23):2426–37. doi:10.1056/NEJMoa0807865
29. Reddy S, Jia S, Geoffrey R, Lorier R, Suchi M, Broeckel U, et al. An autoinflammatory disease due to homozygous deletion of the IL1RN locus. *N Engl J Med* (2009) **360**(23):2438–44. doi:10.1056/NEJMoa0809568
30. Lalor SJ, Dungan LS, Sutton CE, Basdeo SA, Fletcher JM, Mills KH. Caspase-1-processed cytokines IL-1beta and IL-18 promote IL-17 production by gammadelta and CD4 T cells that mediate autoimmunity. *J Immunol* (2011) **186**(10):5738–48. doi:10.4049/jimmunol.1003597
31. Vidal-Vanaclocha F, Fantuzzi G, Mendoza L, Fuentes AM, Anasagasti MJ, Martin J, et al. IL-18 regulates IL-1beta-dependent hepatic melanoma metastasis via vascular cell adhesion molecule-1. *Proc Natl Acad Sci U S A* (2000) **97**(2):734–9. doi:10.1073/pnas.97.2.734
32. Mazodier K, Marin V, Novick D, Farnarier C, Robitail S, Schleinitz N, et al. Severe imbalance of IL-18/IL-18BP in patients with secondary hemophagocytic syndrome. *Blood* (2005) **106**(10):3483–9. doi:10.1182/blood-2005-05-1980
33. Gatti S, Beck J, Fantuzzi G, Bartfai T, Dinarello CA. Effect of interleukin-18 on mouse core body temperature. *Am J Physiol Regul Integr Comp Physiol* (2002) **282**(3):R702–9.
34. Li S, Goorha S, Ballou LR, Blatteis CM. Intracerebroventricular interleukin-6, macrophage inflammatory protein-1 beta and IL-18: pyrogenic and PGE(2)-mediated? *Brain Res* (2003) **992**(1):76–84. doi:10.1016/j.brainres.2003.08.033
35. Robertson MJ, Mier JW, Logan T, Atkins M, Koon H, Koch KM, et al. Clinical and biological effects of recombinant human interleukin-18 administered by intravenous infusion to patients with advanced cancer. *Clin Cancer Res* (2006) **12**(14 Pt 1):4265–73. doi:10.1158/1078-0432.CCR-06-0121
36. Reznikov LL, Kim SH, Westcott JY, Frishman J, Fantuzzi G, Novick D, et al. IL-18 binding protein increases spontaneous and IL-1-induced prostaglandin production via inhibition of IFN-gamma. *Proc Natl Acad Sci U S A* (2000) **97**(5):2174–9. doi:10.1073/pnas.040582597
37. Tak PP, Bacchi M, Bertolino M. Pharmacokinetics of IL-18 binding protein in healthy volunteers and subjects with rheumatoid arthritis or plaque psoriasis. *Eur J Drug Metab Pharmacokinetics* (2006) **31**(2):109–16. doi:10.1007/BF03191127
38. Joosten LA, van De Loo FA, Lubberts E, Helsen MM, Netea MG, van der Meer JW, et al. An IFN-gamma-independent proinflammatory role of IL-18 in murine streptococcal cell wall arthritis. *J Immunol* (2000) **165**(11):6553–8.
39. Carrascal MT, Mendoza L, Valcarcel M, Salado C, Egilegor E, Telleria N, et al. Interleukin-18 binding protein reduces b16 melanoma hepatic metastasis by neutralizing adhesiveness and growth factors of sinusoidal endothelium. *Cancer Res* (2003) **63**(2):491–7.
40. ten Hove T, van Montfrans C, Peppelenbosch MP, van Deventer SJ. Infliximab treatment induces apoptosis of lamina propria T lymphocytes in Crohn's disease. *Gut* (2002) **50**(2):206–11. doi:10.1136/gut.50.2.206
41. Sandborn WJ, Feagan BG, Fedorak RN, Scherl E, Fleisher MR, Katz S, et al. A randomized trial of Ustekinumab, a human interleukin-12/23 monoclonal antibody, in patients with moderate-to-severe Crohn's disease. *Gastroenterology* (2008) **135**(4):1130–41. doi:10.1053/j.gastro.2008.07.014
42. Mannon PJ, Fuss IJ, Mayer L, Elson CO, Sandborn WJ, Present D, et al. Anti-interleukin-12 antibody for active Crohn's disease. *N Engl J Med* (2004) **351**(20):2069–79. doi:10.1056/NEJMoa033402
43. Pizarro TT, Michie MH, Bentz M, Woraratanadharm J, Smith MF Jr, Foley E, et al. IL-18, a novel immunoregulatory cytokine, is up-regulated in Crohn's disease: expression and localization in intestinal mucosal cells. *J Immunol* (1999) **162**(11):6829–35.
44. Monteleone G, Trapasso F, Parrello T, Biancone L, Stella A, Iuliano R, et al. Bioactive IL-18 expression is up-regulated in Crohn's disease. *J Immunol* (1999) **163**(1):143–7.
45. Ten Hove T, Corbaz A, Amitai H, Aloni S, Belzer I, Graber P, et al. Blockade of endogenous IL-18 ameliorates TNBS-induced colitis by decreasing local TNF-alpha production in mice. *Gastroenterology* (2001) **121**(6):1372–9. doi:10.1053/gast.2001.29579
46. Siegmund B. Interleukin-1b converting enzyme and intestinal inflammation. *Biochem Pharmacol* (2002) **7273**:1–8. doi:10.1016/S0006-2952(02)01064-X
47. Bauer C, Duewell P, Mayer C, Lehr HA, Fitzgerald KA, Dauer M, et al. Colitis induced in mice with dextran sulfate sodium (DSS) is mediated by the NLRP3 inflammasome. *Gut* (2010) **59**(9):1192–9. doi:10.1136/gut.2009.197822
48. Bauer C, Loher F, Dauer M, Mayer C, Lehr HA, Schonharting M, et al. The ICE inhibitor pralnacasan prevents DSS-induced colitis in C57BL/6 mice and suppresses IP-10 mRNA but not TNF-alpha mRNA expression. *Dig Dis Sci* (2007) **52**(7):1642–52. doi:10.1007/s10620-007-9802-8
49. Loher F, Bauer C, Landauer N, Schmall K, Siegmund B, Lehr HA, et al. The interleukin-1 beta-converting enzyme inhibitor pralnacasan reduces dextran sulfate sodium-induced murine colitis and T helper 1 T-cell activation. *J Pharmacol Exp Ther* (2004) **308**(2):583–90. doi:10.1124/jpet.103.057059
50. Banerjee S, Bond JS. Prointerleukin-18 is activated by meprin beta in vitro and in vivo in intestinal inflammation. *J Biol Chem* (2008) **283**(46):31371–7. doi:10.1074/jbc.M802814200
51. Allen IC, TeKippe EM, Woodford RM, Uronis JM, Holl EK, Rogers AB, et al. The NLRP3 inflammasome functions as a negative regulator of tumorigenesis during colitis-associated cancer. *J Exp Med* (2010) **207**(5):1045–56. doi:10.1084/jem.20100050
52. Dupaul-Chicoine J, Yeretssian G, Doiron K, Bergstrom KS, McIntire CR, LeBlanc PM, et al. Control of intestinal homeostasis, colitis, and colitis-associated colorectal cancer by the inflammatory caspases. *Immunity* (2010) **32**(3):367–78. doi:10.1016/j.immuni.2010.02.012
53. Hirota SA, Ng J, Lueng A, Khajah M, Parhar K, Li Y, et al. NLRP3 inflammasome plays a key role in the regulation of intestinal homeostasis. *Inflamm Bowel Dis* (2011) **17**(6):1359–72. doi:10.1002/ibd.21478
54. Elinav E, Strowig T, Kau AL, Henao-Mejia J, Thaiss CA, Booth CJ, et al. NLRP6 inflammasome regulates colonic microbial ecology and risk for colitis. *Cell* (2011) **145**(5):745–57. doi:10.1016/j.cell.2011.04.022
55. Chen CJ, Kono H, Golenbock D, Reed G, Akira S, Rock KL. Identification of a key pathway required for the sterile inflammatory response triggered by dying cells. *Nat Med* (2007) **13**(7):851–6. doi:10.1038/nm1603
56. Siegmund B. Interleukin-18 in intestinal inflammation: friend and foe? *Immunity* (2010) **32**(3):300–2. doi:10.1016/j.immuni.2010.03.010
57. Netea MG, Joosten LA, Lewis E, Jensen DR, Voshol PJ, Kullberg BJ, et al. Deficiency of interleukin-18

in mice leads to hyperphagia, obesity and insulin resistance. *Nat Med* (2006) **12**(6):650–6. doi:10.1038/nm1415

58. Zorrilla EP, Sanchez-Alavez M, Sugama S, Brennan M, Fernandez R, Bartfai T, et al. Interleukin-18 controls energy homeostasis by suppressing appetite and feed efficiency. *Proc Natl Acad Sci U S A* (2007) **104**(26):11097–102. doi:10.1073/pnas.0611523104
59. Abbate A, Van Tassell BW, Seropian IM, Toldo S, Robati R, Varma A, et al. Interleukin-1beta modulation using a genetically engineered antibody prevents adverse cardiac remodelling following acute myocardial infarction in the mouse. *Eur J Heart Fail* (2010) **12**(4):319–22. doi:10.1093/eurjhf/hfq017
60. Abbate A, Salloum FN, Vecile E, Das A, Hoke NN, Straino S, et al. Anakinra, a recombinant human interleukin-1 receptor antagonist, inhibits apoptosis in experimental acute myocardial infarction. *Circulation* (2008) **117**(20):2670–83. doi:10.1161/CIRCULATIONAHA.107.740233
61. Abbate A, Kontos MC, Grizzard JD, Biondi-Zoccai GG, Van Tassell BW, Robati R, et al. Interleukin-1 blockade with anakinra to prevent adverse cardiac remodeling after acute myocardial infarction (Virginia Commonwealth University Anakinra Remodeling Trial [VCU-ART] Pilot study). *Am J Cardiol* (2010) **105**(10):1371–77.e1. doi:10.1016/j.amjcard.2009.12.059
62. Abbate A, Van Tassell BW, Biondi-Zoccai G, Kontos MC, Grizzard JD, Spillman DW, et al. Effects of interleukin-1 blockade with anakinra on adverse cardiac remodeling and heart failure after acute myocardial infarction [from the Virginia Commonwealth University-Anakinra Remodeling Trial (2) (VCU-ART2) pilot study]. *Am J Cardiol* (2013) **111**(10):1394–400. doi:10.1016/j.amjcard.2013.01.287
63. Van Tassell BW, Arena R, Toldo S, Mezzaroma E, Azam T, Seropian IM, et al. Enhanced interleukin-1 activity contributes to exercise intolerance in patients with systolic heart failure. *PLoS One* (2012) **7**(3):e33438. doi:10.1371/journal.pone.0033438
64. Ridker PM, Thuren T, Zalewski A, Libby P. Interleukin-1beta inhibition and the prevention of recurrent cardiovascular events: rationale and design of the Canakinumab Anti-inflammatory Thrombosis Outcomes Study (CANTOS). *Am Heart J* (2011) **162**(4):597–605. doi:10.1016/j.ahj.2011.06.012
65. Mallat Z, Heymes C, Corbaz A, Logeart D, Alouani S, Cohen-Solal A, et al. Evidence for altered interleukin 18 (IL)-18 pathway in human heart failure. *FASEB J* (2004) **18**(14):1752–4.
66. Platis A, Yu Q, Moore D, Khojeini E, Tsau P, Larson D. The effect of daily administration of IL-18 on cardiac structure and function. *Perfusion* (2008) **23**(4):237–42.
67. Woldbaek PR, Sande JB, Stromme TA, Lunde PK, Djurovic S, Lyberg T, et al. Daily administration of interleukin-18 causes myocardial dysfunction in healthy mice. *Am J Physiol Heart Circ Physiol* (2005) **289**(2):H708–14. doi:10.1152/ajpheart.01179.2004
68. Raeburn CD, Dinarello CA, Zimmerman MA, Calkins CM, Pomerantz BJ, McIntyre RC Jr, et al. Neutralization of IL-18 attenuates lipopolysaccharide-induced myocardial dysfunction. *Am J Physiol* (2002) **283**(2):H650–7.
69. Pomerantz BJ, Reznikov LL, Harken AH, Dinarello CA. Inhibition of caspase 1 reduces human myocardial ischemic dysfunction via inhibition of IL-18 and IL-1beta. *Proc Natl Acad Sci U S A* (2001) **98**(5):2871–6. doi:10.1073/pnas.041611398
70. Doyle SL, Campbell M, Ozaki E, Salomon RG, Mori A, Kenna PF, et al. NLRP3 has a protective role in age-related macular degeneration through the induction of IL-18 by drusen components. *Nat Med* (2012) **18**:791–8. doi:10.1038/nm.2717
71. Novick D, Kim SH, Fantuzzi G, Reznikov L, Dinarello CA, Rubinstein M. Interleukin-18 binding protein: a novel modulator of the Th1 cytokine response. *Immunity* (1999) **10**:127–36. doi:10.1016/S1074-7613(00)80013-8
72. Kim SH, Eisenstein M, Reznikov L, Fantuzzi G, Novick D, Rubinstein M, et al. Structural requirements of six naturally occurring isoforms of the IL-18 binding protein to inhibit IL-18. *Proc Natl Acad Sci U S A* (2000) **97**:1190–5.
73. Novick D, Schwartsburd B, Pinkus R, Suissa D, Belzer I, Sthoeger Z, et al. A novel IL-18BP ELISA shows elevated serum il-18BP in sepsis and extensive decrease of free IL-18. *Cytokine* (2001) **14**(6):334–42. doi:10.1006/cyto.2001.0914
74. Novick D, Elbirt D, Dinarello CA, Rubinstein M, Sthoeger ZM. Interleukin-18 binding protein in the sera of patients with Wegener's granulomatosis. *J Clin Immunol* (2009) **29**(1):38–45. doi:10.1007/s10875-008-9217-0
75. Novick D, Elbirt D, Miller G, Dinarello CA, Rubinstein M, Sthoeger ZM. High circulating levels of free interleukin-18 in patients with active SLE in the presence of elevated levels of interleukin-18 binding protein. *J Autoimmun* (2011) **34**(2):121–6. doi:10.1016/j.jaut.2009.08.002
76. Bufler P, Azam T, Gamboni-Robertson F, Reznikov LL, Kumar S, Dinarello CA, et al. A complex of the IL-1 homologue IL-1F7b and IL-18-binding protein reduces IL-18 activity. *Proc Natl Acad Sci U S A* (2002) **99**(21):13723–8. doi:10.1073/pnas.212519099
77. Dinarello C, Arend W, Sims J, Smith D, Blumberg H, O'Neill L, et al. IL-1 family nomenclature. *Nat Immunol* (2010) **11**(11):973. doi:10.1038/ni1110-973
78. McNamee EN, Masterson JC, Jedlicka P, McManus M, Grenz A, Collins CB, et al. Interleukin 37 expression protects mice from colitis. *Proc Natl Acad Sci U S A* (2011) **108**(40):16711–6. doi:10.1073/pnas.1111982108
79. Banda NK, Vondracek A, Kraus D, Dinarello CA, Kim SH, Bendele A, et al. Mechanisms of inhibition of collagen-induced arthritis by murine IL-18 binding protein. *J Immunol* (2003) **170**(4):2100–5.
80. Muhl H, Kampfer H, Bosmann M, Frank S, Radeke H, Pfeilschifter J. Interferon-gamma mediates gene expression of IL-18 binding protein in nonleukocytic cells. *Biochem Biophys Res Commun* (2000) **267**(3):960–3. doi:10.1006/bbrc.1999.2064
81. Hurgin V, Novick D, Rubinstein M. The promoter of IL-18 binding protein: activation by an IFN-gamma-induced complex of IFN regulatory factor 1 and CCAAT/enhancer binding protein beta. *Proc Natl Acad Sci U S A* (2002) **99**(26):16957–62. doi:10.1073/pnas.262663399
82. Kaser A, Novick D, Rubinstein M, Siegmund B, Enrich B, Koch RO, et al. Interferon-alpha induces interleukin-18 binding protein in chronic hepatitis C patients. *Clin Exp Immunol* (2002) **129**(2):332–8. doi:10.1046/j.1365-2249.2002.01911.x
83. Ludwiczek O, Kaser A, Novick D, Dinarello CA, Rubinstein M, Vogel W, et al. Plasma levels of interleukin-18 and interleukin-18 binding protein are elevated in patients with chronic liver disease. *J Clin Immunol* (2002) **22**(6):331–7. doi:10.1023/A:1020600230977
84. Wittmann M, Bachmann M, Doble R, Pfeilschifter J, Werfel T, Mühl H. IL-27 regulates IL-18 binding protein in skin resident cells. *PLoS One* (2012) **7**(6):e38751. doi:10.1371/journal.pone.0038751
85. Xiang Y, Moss B. Correspondence of the functional epitopes of poxvirus and human interleukin-18-binding proteins. *J Virol* (2001) **75**(20):9947–54. doi:10.1128/JVI.75.20.9947-9954.2001
86. Grom AA. Macrophage activation syndrome and reactive hemophagocytic lymphohistiocytosis: the same entities? *Curr Opin Rheumatol* (2003) **15**(5):587–90. doi:10.1097/00002281-200309000-00011
87. Grom AA, Mellins ED. Macrophage activation syndrome: advances towards understanding pathogenesis. *Curr Opin Rheumatol* (2011) **22**(5):561–6. doi:10.1097/01.bor.0000381996.69261.71
88. Grom AA, Villanueva J, Lee S, Goldmuntz EA, Passo MH, Filipovich A. Natural killer cell dysfunction in patients with systemic-onset juvenile rheumatoid arthritis and macrophage activation syndrome. *J Pediatr* (2003) **142**(3):292–6. doi:10.1067/mpd.2003.110
89. Janka GE. Familial and acquired hemophagocytic lymphohistiocytosis. *Annu Rev Med* (2012) **63**:233–46. doi:10.1146/annurev-med-041610-134208
90. Ogilvie EM, Khan A, Hubank M, Kellam P, Woo P. Specific gene expression profiles in systemic juvenile idiopathic arthritis. *Arthritis Rheum* (2007) **56**(6):1954–65. doi:10.1002/art.22644
91. Sumegi J, Barnes MG, Nestheide SV, Molleran-Lee S, Villanueva J, Zhang K, et al. Gene expression profiling of peripheral blood mononuclear cells from children with active hemophagocytic lymphohistiocytosis. *Blood* (2011) **117**(15):e151–60. doi:10.1182/blood-2010-08-300046
92. Maeno N, Takei S, Imanaka H, Yamamoto K, Kuriwaki K, Kawano

Y, et al. Increased interleukin-18 expression in bone marrow of a patient with systemic juvenile idiopathic arthritis and unrecognized macrophage-activation syndrome. *Arthritis Rheum* (2004) **50**(6):1935–8. doi:10.1002/art.20268

93. Emmenegger U, Reimers A, Frey U, Fux C, Bihl F, Semela D, et al. Reactive macrophage activation syndrome: a simple screening strategy and its potential in early treatment initiation. *Swiss Med Wkly* (2002) **132**(17–18):230–6.
94. Nold-Petry CA, Lehrnbecher T, Jarisch A, Schwabe D, Pfeilschifter JM, Muhl H, et al. Failure of interferon gamma to induce the anti-inflammatory interleukin 18 binding protein in familial hemophagocytosis. *PLoS One* (2010) **5**(1):e8663. doi:10.1371/journal.pone.0008663
95. Honda K, Ohga S, Takada H, Nomura A, Ohshima K, Kinukawa N, et al. Neuron-specific enolase in hemophagocytic lymphohistiocytosis: a potential indicator for macrophage activation? *Int J Hematol* (2000) **72**(1):55–60.
96. Wada T, Muraoka M, Yokoyama T, Toma T, Kanegane H, Yachie A. Cytokine profiles in children with primary Epstein-Barr virus infection. *Pediatr Blood Cancer* (2013) **60**(7):E46–8. doi:10.1002/pbc.24480
97. Fitzgerald AA, Leclercq SA, Yan A, Homik JE, Dinarello CA. Rapid responses to anakinra in patients with refractory adult-onset Still's disease. *Arthritis Rheum* (2005) **52**(6):1794–803. doi:10.1002/art.21061
98. Larroche C, Mouthon L. Pathogenesis of hemophagocytic syndrome (HPS). *Autoimmunity Rev* (2004) **3**:69–75. doi:10.1016/S1568-9972(03)00091-0
99. Favilli F, Anzilotti C, Martinelli L, Quattroni P, De Martino S, Pratesi F, et al. IL-18 activity in systemic lupus erythematosus. *Ann N Y Acad Sci* (2009) **1173**:301–9. doi:10.1111/j.1749-6632.2009.04742.x
100. Emmanuilidis K, Weighardt H, Matevossian E, Heidecke CD, Ulm K, Bartels H, et al. Differential regulation of systemic IL-18 and IL-12 release during postoperative sepsis: high serum IL-18 as an early predictive indicator of lethal outcome. *Shock* (2002) **18**(4):301–5. doi:10.1097/00024382-200210000-00002
101. Mommsen P, Frink M, Pape HC, van Griensven M, Probst C, Gaulke R, et al. Elevated systemic IL-18 and neopterin levels are associated with posttraumatic complications among patients with multiple injuries: a prospective cohort study. *Injury* (2009) **40**(5):528–34. doi:10.1016/j.injury.2008.08.007
102. Palladino I, Salani F, Ciaramella A, Rubino IA, Caltagirone C, Fagioli S, et al. Elevated levels of circulating IL-18BP and perturbed regulation of IL-18 in schizophrenia. *J Neuroinflammation* (2012) **9**:206. doi:10.1186/1742-2094-9-206
103. Haas SL, Abbatista M, Brade J, Singer MV, Bocker U. Interleukin-18 serum levels in inflammatory bowel diseases: correlation with disease activity and inflammatory markers. *Swiss Med Wkly* (2009) **139**(9–10):140–5.
104. Ludwiczek O, Kaser A, Novick D, Dinarello CA, Rubinstein M, Tilg H. Elevated systemic levels of free interleukin-18 (IL-18) in patients with Crohn's disease. *Eur Cytokine Netw* (2005) **16**(1):27–33.
105. Bokarewa M, Hultgren O. Is interleukin-18 useful for monitoring rheumatoid arthritis? *Scand J Rheumatol* (2005) **34**(6):433–6. doi:10.1080/03009740510026724
106. Jelusic M, Lukic IK, Tambic-Bukovac L, Dubravcic K, Malcic I, Rudan I, et al. Interleukin-18 as a mediator of systemic juvenile idiopathic arthritis. *Clin Rheumatol* (2007) **26**(8):1332–4. doi:10.1007/s10067-006-0474-0
107. Kawashima M, Yamamura M, Taniai M, Yamauchi H, Tanimoto T, Kurimoto M, et al. Levels of interleukin-18 and its binding inhibitors in the blood circulation of patients with adult-onset Still's disease. *Arthritis Rheum* (2001) **44**(3):550–60. doi:10.1002/1529-0131(200103)44:3<550::AID-ANR103>3.0.CO;2-5
108. Blankenberg S, Luc G, Ducimetiere P, Arveiler D, Ferrieres J, Amouyel P, et al. Interleukin-18 and the risk of coronary heart disease in European men: the Prospective Epidemiological Study of Myocardial Infarction (PRIME). *Circulation* (2003) **108**(20):2453–9. doi:10.1161/01.CIR.0000099509.76044.A2
109. Narins CR, Lin DA, Burton PB, Jin ZG, Berk BC. Interleukin-18 and interleukin-18 binding protein levels before and after percutaneous coronary intervention in patients with and without recent myocardial infarction. *Am J Cardiol* (2004) **94**(10):1285–7. doi:10.1016/j.amjcard.2004.07.114
110. Thompson SR, Novick D, Stock CJ, Sanders J, Brull D, Cooper J, et al. Free Interleukin (IL)-18 levels, and the impact of IL18 and IL18BP genetic variation, in CHD patients and healthy men. *Arterioscler Thromb Vasc Biol* (2007) **27**(12):2743–9. doi:10.1161/ATVBAHA.107.149245
111. Troseid M, Seljeflot I, Arnesen H. The role of interleukin-18 in the metabolic syndrome. *Cardiovasc Diabetol* (2010) **9**:11. doi:10.1186/1475-2840-9-11
112. Parikh CR, Abraham E, Ancukiewicz M, Edelstein CL. Urine IL-18 is an early diagnostic marker for acute kidney injury and predicts mortality in the ICU. *J Am Soc Nephrol* (2005) **16**(10):3046–52. doi:10.1681/ASN.2005030236
113. Vaidya VS, Waikar SS, Ferguson MA, Collings FB, Sunderland K, Gioules C, et al. Urinary biomarkers for sensitive and specific detection of acute kidney injury in humans. *Clin Transl Sci* (2008) **1**(3):200–8. doi:10.1111/j.1752-8062.2008.00053.x
114. Parikh CR, Mishra J, Thiessen-Philbrook H, Dursun B, Ma Q, Kelly C, et al. Urinary IL-18 is an early predictive biomarker of acute kidney injury after cardiac surgery. *Kidney Int* (2006) **70**(1):199–203. doi:10.1038/sj.ki.5001527
115. Sirota JC, Walcher A, Faubel S, Jani A, McFann K, Devarajan P, et al. Urine IL-18, NGAL, IL-8 and serum IL-8 are biomarkers of acute kidney injury following liver transplantation. *BMC Nephrol* (2013) **14**:17. doi:10.1186/1471-2369-14-17

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

Received: 31 May 2013; accepted: 04 September 2013; published online: 08 October 2013.
*Citation: Dinarello CA, Novick D, Kim S and Kaplanski G (2013) Interleukin-18 and IL-18 binding protein. Front. Immunol. **4**:289. doi: 10.3389/fimmu.2013.00289*
This article was submitted to Inflammation, a section of the journal Frontiers in Immunology.

frontiers in
IMMUNOLOGY

REVIEW ARTICLE
published: 24 May 2013
doi: 10.3389/fimmu.2013.00123

Different members of the IL-1 family come out in different ways: DAMPs vs. cytokines?

Sonia Carta, Rosa Lavieri and Anna Rubartelli*

Cell Biology Unit, IRCSS Azienda Ospedale Università San Martino-IST, Genoa, Italy

Edited by:
Cecilia Garlanda, Istituto Clinico Humanitas, Italy

Reviewed by:
David Brough, University of Manchester, UK
Greta Guarda, University of Lausanne, Switzerland

****Correspondence:***
Anna Rubartelli, Cell Biology Unit, IRCSS Azienda Ospedale Università San Martino-IST, Largo Rosanna Benzi, 10, 16132 Genoa, Italy.
e-mail: anna.rubartelli@istge.it

Intercellular communications control fundamental biological processes required for the survival of multicellular organisms. Secretory proteins are among the most important messengers in this network of information. Proteins destined to the extracellular environment contain a signal sequence with the necessary information to target them to the Endoplasmic Reticulum, and are released by a "classical" pathway of secretion. However, in the early 1990s it became evident that non-classical mechanisms must exist for the secretion of some proteins, which in spite of their extracellular localization and function, lack a signal peptide. Indeed, the group of leaderless secretory proteins rapidly grew and is still growing. Many of them are implicated in the regulation of the inflammatory response. Interestingly, most members of the IL-1 family (IL-1F), including the master pro-inflammatory cytokine IL-1β, are leaderless proteins and find their way out of the cells in different manners. In this article, we will review current hypotheses on the mechanisms of externalization of IL-1F members and discuss their relevance with respect to the different functions (as cytokines or as DAMPs) played by the different IL-1 proteins.

Keywords: IL-1α, IL-1β, IL-18, IL-33, secretion, damage associated molecular pattern, TLR, IL-1F receptors

THE IL-1 FAMILY

IL-1 family (IL-1F) is evolutionary ancient. Eleven IL-1 members have been identified (**Table 1**) based on conservation of amino acid sequence, identity of gene structure, and three-dimensional structure (Dunn et al., 2001). Most of them (with the exception of IL-18 and IL-33, Nolan et al., 1998; Schmitz et al., 2005) map to chromosome 2 between the IL-1α and IL-1 Receptor antagonist (IL-1ra) loci (Nicklin et al., 2002), suggesting that each IL-1F member derives from the duplication of a common ancestral gene. Each IL-1 gene codes for a protein that contains a single structural domain formed from 12 beta strands connected by loop regions arranged in a beta-trefoil structure. IL-1F members differ most from each other within these loop regions (Dunn et al., 2001).

The various members of the IL-1F play different biologic activities all involved in innate immunity (Dinarello, 2009). Interestingly, although most IL-1F proteins are proinflammatory, also members endowed with anti-inflammatory properties exist, the most important being IL-1ra (Arend et al., 1998).

Most IL-1 family members share features which make them different from the other cytokines. First of all, they are synthesized as precursor proteins that subsequently undergo proteolytic maturation by converting enzymes. Proteolytic maturation is strictly required for activation of some members of IL-1 family, such as IL-1β, IL-18 (Dinarello, 1998a), and IL-37 (Boraschi et al., 2011). In the case of other IL-1F members, the precursor is able to engage its receptor and trigger a response on target cells. This is the case of IL-1α (Dinarello, 1998b) and IL-33 (Moussion et al., 2008). The major converting enzyme responsible for processing of IL-1β, IL-18, and IL-37 is caspase-1 (Black et al., 1988; Ghayur et al., 1997; Kumar et al., 2002). This convertase is produced as a zymogen (pro-caspase-1) and undergoes activation upon the assembly of intracellular multiprotein complexes named inflammasomes (Bauernfeind et al., 2011). Different types of Inflammasomes exist, each composed by a member of the nucleotide-binding domain leucine-rich repeat containing (NLR) gene family, adaptor proteins, and pro-caspase-1 molecules.

A second, important feature of IL-1F proteins is that only IL-1ra is a classical secretory protein endowed with a signal peptide: all the other members are leaderless (Dinarello, 2009).

LEADERLESS IL-1 CYTOKINES: HOW DO THEY GET OUT OF CELLS?

In principle, leaderless proteins, synthesized in the soluble cytosol, should stay there: a quite unlikely behavior for soluble mediators of inflammation. Alternatively, they must find a way out different from the classical secretory pathway. When the gene of IL-1β was cloned revealing the absence of a signal sequence (Auron et al., 1984) the first explanation for IL-1β externalization was that it was simply released by cells dying at the site of inflammation. This hypothesis was ruled out many years ago by two major evidences (Muesch et al., 1990; Rubartelli et al., 1990): (i) IL-1β is selectively released by LPS activated human monocytes: only the mature form of IL-1β, but neither the IL-1β precursor (pro-IL-1β) nor other cytosolic proteins are detectable in culture supernatants; (ii) viable cells are required for secretion of mature IL-1β: when activated monocytes were killed by freezing and thawing before incubating at 37°C, only pro-IL-1β accumulated in the culture supernatant.

Further studies confirmed that an active secretory pathway, different from the ER-Golgi one, exists for IL-1β and also for IL-18 (Andrei et al., 1999; MacKenzie et al., 2001; Qu et al., 2007).

Death as a mechanism of secretion was instead proposed for other members of the IL-1F, such as IL-1α and confirmed by various studies Sakurai et al. (2008), Luheshi et al. (2009), and Cohen et al. (2010).

SECRETION OF IL-1β AND IL-18

Secretion of IL-1β and IL-18 needs two signals (**Figure 1A**). A first signal, supplied by bacterial products that bind and activate Toll-like Receptors (TLR), triggers IL-1β expression and synthesis; this

Table 1 | IL-1 family members.

Family name	Name	Receptor/coreceptor	Property	Synthesized as precursor	Processing required for activity
IL-1F1	IL-1α	IL-1RI/IL-1RacP	Proinflammatory	Yes	No
IL-1F2	IL-1β	IL-1RI/IL-1RacP	Proinflammatory	Yes	Yes
IL-1F3	IL-1Ra	IL-1RI	Antagonist for IL-1α,β	No	No
IL-1F4	IL-18	IL-18Rα/IL-18Rβ	Proinflammatory	Yes	Yes
IL-1F5	IL-36Ra	IL-1Rrp2	Antagonist for IL-36	Yes	Yes
IL-1F6	IL-36α	IL-1Rrp2/IL-1RAcP	Proinflammatory	Yes	Yes
IL-1F7	IL-37	IL-18Rα, IL18BP	Anti-inflammatory	Yes	Yes
IL-1F8	IL-36β	IL-1Rrp2/IL-1RAcP	Proinflammatory	Yes	Yes
IL1-F9	IL-36γ	IL-1Rrp2/IL-1RAcP	Proinflammatory	Yes	Yes
IL-1F10	IL-38	IL-1Rrp2	? Antagonist	Yes	? No
IL-1F11	IL-33	ST2/IL-1RAcP	Proinflammatory	Yes	No

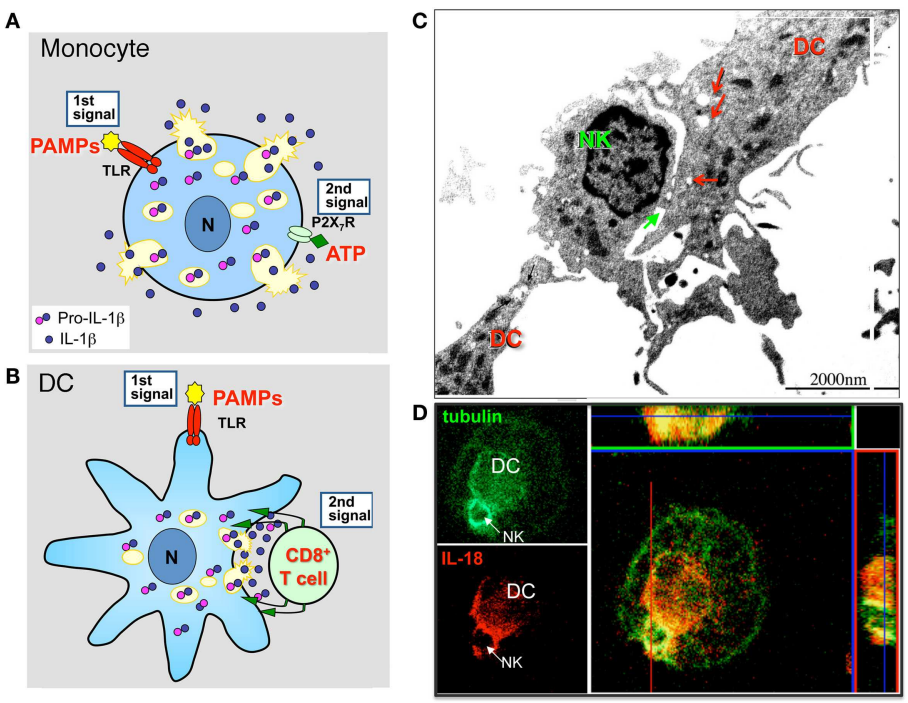


FIGURE 1 | Lysosome exocytosis allows diffuse and polarized secretion of IL-1β and IL-18. **(A)** Models of non-polarized secretion of IL-1β. TLR agonists (e.g., PAMPs) induce monocytes/macrophages to actively synthesize pro-IL-1β that accumulates into the cytosol and in part into secretory lysosomes. A second extracellular soluble signal (e.g., ATP) triggers generalized lysosome exocytosis (Andrei et al., 1999). A similar mechanism accounts for IL-18 secretion (Perregaux et al., 2000). **(B)** Models of polarized (lower panel) secretion of IL-1β. In DCs, a first maturative stimulus (e.g., TLR triggering), induces pro-IL-1β synthesis. The second signal is provided by antigen specific T cells that induces a $[Ca^{2+}]_i$ rise, followed by recruitment of IL-1β-containing secretory lysosomes toward the interacting T cell, and by exocytosis restricted to the intercellular space (immunological synapse) (Gardella et al., 2000b, 2001). A similar mechanism mediates IL-18 secretion in DCs interacting with autologous NK cells (Semino et al., 2005). **(C)**. Electron microscopy analysis of a DC interacting with a NK cell. Interaction between the two cells occurs primarily in correspondence with DC areas enriched by mitochondria and vesicles (red arrows). The immunological synapse is indicated by the green arrow. **(D)** Confocal microscopy analysis of tubulin (green) and IL-18 (red) in a DC/NK conjugate after 3 h of interaction. The strong co-staining of IL-18 and tubulin in both transversal and sagittal sections indicates that IL-18 from DC polarizes toward the NK/DC synapse and is transported along tubulin filaments. **(C,D)**: modified from Semino and Rubartelli (2010).

signal is needed also for IL-18 secretion even though IL-18 is constitutively expressed by myeloid cells (Perregaux et al., 2000). The second signal is provided by a variety of diverse stimuli: endogenous, such as extracellular ATP, or exogenous, such as microbial products or pathogenic crystals (Bauernfeind et al., 2011). However, while in murine macrophages a second signal is strictly required, in primary human monocytes the addition of second signals such as ATP strongly enhances secretion but is dispensable as bacterial products alone are sufficient to induce secretion of IL-1β, although at a lower extent and with slow kinetics (Piccini et al., 2008). In fact upon TLR-triggering, human monocytes externalize functionally effective amounts of their ATP that in turn stimulates autocrinally the monocyte purinergic P2 × 7 receptor, triggering the cascade of events that lead to inflammasome activation and IL-1β secretion (Piccini et al., 2008).

Several models of IL-1β and IL-18 secretion have been proposed but the precise mechanism remains elusive. In particular, where and how inflammasome activation and processing of the two pro-cytokines occur, as well as the link between processing and secretion are still unknown (Rubartelli, 2012). Most of the described IL-1β secretory pathways involve the externalization of the cytokine via vesicles (Andrei et al., 1999; MacKenzie et al., 2001; Qu et al., 2007). Secretory lysosomes, microvesicles shed from the plasma membrane, and exosomes have been identified as vesicles able to carry IL-1β out of the cell in different cell types (primary monocytes, monocyte continuous cell lines, mouse macrophages).

An additional, non-vesicular pathway of IL-1β secretion may take place in monocyte/macrophages where sustained activation of the NLRP3 or NLRC4 inflammasome cascade induces caspase-1-mediated pyroptotic death (Le Feuvre et al., 2002; Brough and Rothwell, 2007; Bergsbaken et al., 2009). In these cells, a direct efflux of cytosolic mature IL-1β occurs across hyper-permeable plasma membranes.

It is possible that differences in the cell type, in the functional state of the cells or in the culture conditions, as well as strength and duration of the stimulus (Carta et al., 2011; Lopez-Castejon and Brough, 2011) may account for the different vesicular or non-vesicular IL-1β secretory pathways used.

The first suggested mechanism is the secretory lysosome-dependent pathway, characterized in our lab on primary human monocytes (Andrei et al., 1999, 2004). In this model, pro-IL-1β is translocated together with caspase-1 into vesicles belonging to the endolysosomal compartment. The mature form of IL-1β is produced within the vesicles by caspase-1 cleavage, after which the endolysosomes fuse with the plasma membrane and the content is released into the extracellular space. The capacity to fuse with plasma membrane and to externalize the soluble content is a peculiarity of a subset of endolysosomes, called secretory lysosomes (Blott and Griffiths, 2002). These are Ca^{2+}-regulated secretory organelles displaying features of both classical endolysosomes and secretory granules responsible for regulated secretion in specialized cells (Blott and Griffiths, 2002). Particularly abundant in hemopoietic cells they participate in inflammatory and immune response by mobilizing their content into the external milieu in response to triggering signals. For instance, CTL and NK cells destroy their infected or tumor target cells by secreting cytolytic proteins, which are stored in secretory lysosomes.

Other leaderless proteins may be imported into cytoplasmic organelles related to the lysosomal compartment in myelomonocytic cells. Among IL-1F members, IL-18 seems to follow the same route as IL-1β (Semino et al., 2005). Also HMGB1, another inflammatory mediator, is present into endolysosomal related organelles of activated monocytes (Gardella et al., 2002). Given the implication of secretory lysosomes in many immune-inflammatory processes (Blott and Griffiths, 2002), lysosome-mediated secretion of IL-1β, IL-18, HMGB1 is consistent with the role played by these proteins in the modulation of innate immunity. Interestingly, the involvement of acidic vesicles in the export of leaderless proteins is evolutionary conserved as in *Dictyostelium discoideum*, translocation into exocytic contractile vacuoles of DdCAD-1, a leaderless adhesion protein, is necessary for its externalization (Sesaki et al., 1997).

LYSOSOME-MEDIATED POLARIZED SECRETION

In general, lysosome-mediated secretion is a regulated process in that a triggering signal is required to induce exocytosis (Blott and Griffiths, 2002). In the case of IL-1β, we have shown that LPS induce synthesis of pro-IL-1β with cytosolic accumulation and lysosomal translocation, then exogenous ATP triggers with IL-1β release (Andrei et al., 2004). A similar two step mechanism seems to account for the regulated secretion of IL-18 (Perregaux et al., 2000) and HMGB1 (Gardella et al., 2002). In all these cases, the signal triggering secretion is generated during the process of inflammation: ATP, promoting IL-1β and IL-18 secretion (Laliberte et al., 1997; Perregaux et al., 2000), is released by monocytes themselves after TLR stimulation and by other cells involved in inflammation (i.e., platelets) (Ferrari et al., 1997; Piccini et al., 2008); differently, active phospholipids such as phosphatidylcholine, possible responsible for secretion of HMGB1, appear later in the inflammatory microenvironment (Gardella et al., 2002).

Interestingly, not only inflammatory cells such as monocytes but also mature dendritic cells (DCs), the professional antigen presenting cells, express inflammatory leaderless cytokines such as IL-1β and IL-18. In these cells, secretion may be induced by antigen specific T cells (Gardella et al., 1999, 2000a,b, 2001) or NK cells (Semino et al., 2005). Morphological approaches allowed to demonstrated that interaction between DCs and $CD8^+$ T cells (Gardella et al., 2001) or NK cells (Semino et al., 2005) is associated with recruitment of IL-1β or IL-18-containing secretory lysosomes in the area of contact among the cells followed by polarization of these organelles, with evidence of lysosome exocytosis at the intercellular space, the so called "immunological synapse" (**Figures 1B–D**). These findings deserve two considerations. On the one hand, they underline the existence of a bidirectional cross talk between DCs and T lymphocytes or NK cell specifically interacting with them, in which the T or the NK cell induce the functional polarization of the DC and the DC responds by degranulation oriented toward the same interacting T or NK cell, with obvious relevance for the control of the immune response. On the other hand, the different way of regulating secretion by monocytes and DCs may account for

the different function of IL-1β and IL-18 in inflammation and immune response (**Figure 1**). Monocytes respond to soluble signals with generalized exocytosis, thus allowing the spreading of inflammatory cytokines in the microenvironment (**Figure 1A**). DCs respond to the localized signal provided by the interacting T or NK cell (**Figures 1B–D**). This restricts the area of release to the immunological synapse and allows activation of target cells without spreading of the cytokine, thus controlling inflammation. Thus, lysosome-mediated secretion of inflammatory leaderless proteins allows polarized secretion in non-polarized cells (Chimini and Rubartelli, 2005).

AUTOPHAGY AND IL-1β AND IL-18 SECRETION: LYSOSOME EXOCYTOSIS REVISITED?

Autophagy preserves the correct quality and quantity of the eukaryotic cytoplasm through two main highly conserved mechanisms: (i) cytosol autodigestion during starvation, which ensures cell-autonomous provision of energy and nutrients; (ii) removal of old organelles and aggregates exceeding the capacity of other cellular degradative systems (Levine and Kroemer, 2008). Recently, an involvement of autophagy in the process of leaderless secretion has been proposed. In fact, the secretion of the yeast leaderless secretory protein Acb1 was strongly enhanced by treatments that induce autophagy (nitrogen starvation or rapamycin). Accordingly, strains mutant for different key factors of autophagy are deficient in Acb1 secretion (Duran et al., 2010; Manjithaya et al., 2010). These results suggest that Acb1 is sequestered in autophagosomes that do not fuse with the vacuole but with endosomes to form amphisomes. Amphisomes might, in turn, fuse with the plasma membrane leading to Acb1 release in the extracellular medium (Giuliani et al., 2011). In agreement with the data on yeast, a link between autophagy and secretion of IL-1β is being emerging. In principle, the "secretory lysosomes" that were formerly found to containing IL-1β and caspase-1 may well represent autophagosomes, and like autophagosomes, may be destined either to fusion with lysosomes, and thereby to autophagic degradation of their cargoes, or to fusion with the plasma membrane, with externalization of their cargoes (**Figure 2**).

However, how autophagy regulates IL-1β secretion is highly debated. Both articles suggesting that autophagy inhibits secretion (Saitoh et al., 2008; Crisan et al., 2011; Harris et al., 2011; Zhou et al., 2011; Shi et al., 2012) and viceversa, that autophagy is required for IL-1β secretion (Dupont et al., 2011) have been published.

The hypothesis of autophagy as a positive mediator of IL-1β secretion is more appealing, according to the following considerations: (i) TLR triggering, a crucial step in IL-1β secretion, which is both necessary and sufficient to drive IL-1β synthesis, maturation, and secretion in human monocytes (Piccini et al., 2008), induces autophagy in myelomonocytic cells (Xu et al., 2007; Delgado et al., 2008); (ii) the hypothesis is consistent with the vesicular models of IL-1β secretion (Andrei et al., 1999; Qu et al., 2007). Besides, autophagy as a blocking mechanism for IL-1β secretion is supported by clear *in vitro* data, such as the strong inhibition of IL-1β secretion induced by substances that promote autophagy and, conversely, the enhancement observed with compounds that

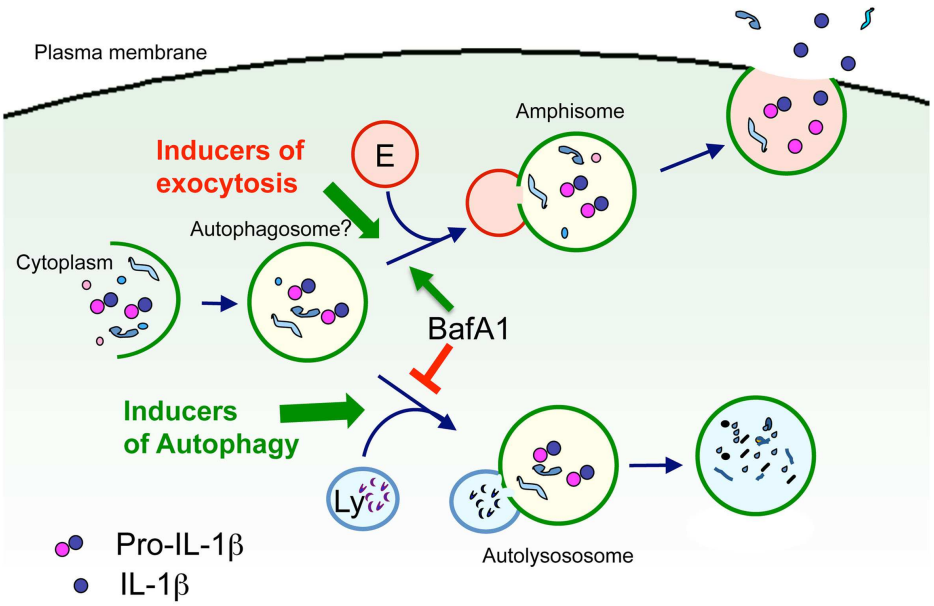


FIGURE 2 | Model of autophagosome involvement in IL-1β secretion. Cytosolic pro-IL-1β is sequestered in part into vesicles belonging to the autophagosomal-lysosomal compartment (autophagosomes?). Autophagosomes may then fuse either with lysosomes (Ly), resulting in degradation of pro-IL-1β, or with endosomes (E) to form amphisomes, were pro-IL-1β may undergo processing. Amphisomes then fuse with the plasma membrane leading to exocytosis of IL-1β. Type and strength of the stimuli may determine the fusion with either endosomes or lysosomes, and thus dictate the fate of pro-IL-1β toward degradation or secretion. Support to this hypothesis comes from the observation that the vacuolar H+ ATPase inhibitor bafilomycin A1 (BafA1) that prevents the fusion between autophagosomes and lysosomes, promotes IL-1β secretion (Sonia Carta and Anna Rubartelli, unpublished results).

block autophagy (Crisan et al., 2011; Harris et al., 2011; Shi et al., 2012) and by the results obtained in mice deficient of autophagy genes (Saitoh et al., 2008; Nakahira et al., 2011).

A possible explanation for these contrasting findings is that formation of pro-IL-1β containing autophagosomes is a prerequisite for IL-1β secretion, and it is induced by TLR activation. Autophagosomes may then undergo exocytosis, with secretion of IL-1β, or fuse to lysosomes, with degradation of pro-IL-1β, depending on the type and strength of the stimuli that trigger IL-1β producing cells. Thus, strong stimuli (e.g., high doses of TLR agonists) or exposure to inducer of autophagy such as rapamycin, would privilege maturation of autophagosome to autolysosome, and degradation of its content by the hydrolases provided by lysosomes. Weaker stimuli, such as continuous triggering by lower doses of TLR agonists would instead favor amphisome fusion with the plasma membrane, resulting in secretion (**Figure 2**).

THE TRAVEL OF CYTOKINES FROM INSIDE TO OUTSIDE DURING EVOLUTION. BOTH PASSIVE RELEASE AND ACTIVE SECRETION ACCOUNT FOR IL-1α AND IL-33 EXTERNALIZATION

The founding member of IL-1 family is probably IL-1α because of its close homology to acidic FGF, in turn one of the most ancient cytokines. It is conceivable that, at the beginning, IL-1F cytokines were, like FGF, intracellular growth factors, and repair molecules interacting with DNA as transcription factors (Dinarello, 2010) and were passively externalized by dying cells. When later in evolution Immunoglobulins appeared, they were used by extracellular cytokines/growth factors as cell surface receptors, and cytokine-mediated signaling evolved. Some IL-1F members including IL-1α and IL-33 retain intracellular (nuclear) function (Carriere et al., 2007; Cayrol and Girard, 2009). Interestingly, unlike IL-1β and IL-18, IL-1α, and IL-33 can activate their receptors on target cells as full-length molecules: thus when released from injured cells they can exert their biological activity in the absence of a proteolytic processing (Chen et al., 2007; Eigenbrod et al., 2008; Cayrol and Girard, 2009).

Based on the above observations, IL-1 family members can be divided into a group of cytokines that retain some intracellular function and are passively externalized upon cell lysis (the prototype being IL-1α), and a second group including cytokines that are stored into the cell cytosol before secretion, but do not play intracellular function and undergo regulated processing and secretion (the prototype being IL-1β).

Still, the situation is more complex. In fact, a number of reports indicate the possibility that also IL-1α and IL-33 are actively released by cells that maintain their integrity. IL-1α was reported to be secreted in response to heat shock (Mandinova et al., 2003) and through an unknown mechanism requiring caspase-1 (Gross et al., 2012). In the case of IL-33, intracellular calcium increase, regulated autocrinally by ATP and purinergic receptor stimulation, induces translocation from nucleus to cytoplasm and release of full-length IL-33 (Kouzaki et al., 2011). Extracellular ATP is a well-known inducer of inflammasome activation and IL-1β/IL-18 processing. The mechanism through which ATP induces IL-33 secretion seems to be different, since the unprocessed, full-length molecular form of IL-33 is secreted. However, we have previously observed that in human monocytes ATP drives exocytosis of pro-IL-1β containing vesicles also if caspase-1 is inhibited, resulting in secretion of the precursor form of the cytokine (Andrei et al., 2004). Moreover, both in monocytes and in DCs, calcium influx induces secretion of pro-IL-1β and pro-IL-18 (Gardella et al., 2000a, 2001; Andrei et al., 2004). Thus it is conceivable that the pathway described for IL-33 makes use of mechanisms (purinergic receptor stimulation and calcium influx) which are old and conserved during evolution. The ATP-mediated signaling may then have further specialized adding to the older function of inducing exocytosis the newer function of controlling inflammasome activation and hence bioactivity of cytokines such as IL-1β and IL-18.

Although the study by Kouzaki et al. (2011) did not investigate the subcellular localization of IL-33 after its nucleus-cytoplasmic translocation in airway epithelial cells, a recent report (Kakkar et al., 2012) indicates that, in fibroblasts, newly synthesized IL-33 first moves to the nucleus and then is translocated to cytoplasmic vesicles, a pathway reminiscent of that followed by HMGB1 (Gardella et al., 2002). Secretion of uncleaved IL-33 is induced by mechanical strain (i.e., application of a physical deformation) in the absence of cellular necrosis (Kakkar et al., 2012). Extracellular release of IL-33 is also observed in mice subjected to acute transaortic constriction, which causes mechanical stress in the left ventricle (Kakkar et al., 2012). Together, these data suggest that IL-1α and IL-33 may be secreted by cells that are subjected to non-lethal stress in addition of being released by necrotic cells.

EVOLUTION OF LEADERLESS SECRETION

Mechanisms of secretion of leaderless proteins were probably exploited by cells to get rid of proteins which can be harmful, either because they are misfolded, or too abundant, or mislocated. Such mechanisms exists in yeast, where toxic proteins are removed from the cytoplasm through a non-classical mechanism of secretion (Cleves et al., 1996). A similar mechanism operates also in mammals: for instance, the sulfotransferase rhodanese, which in physiological conditions accumulates in mitochondria, when overexpressed is rapidly externalized by transfected cells without any sign of cell lysis (Sloan et al., 1994). Similarly, expression of Green Fluorescent Protein (GFP) results in cytosolic accumulation of properly folded protein but also activates the secretion of misfolded GFP molecules (Tanudji et al., 2002). Leaderless secretion may thus act as a safety valve, maintaining cellular homeostasis when the cytoplasmic degradative pathways are overloaded.

STRESS AS A COMMON INDUCER OF IL-1F MEMBER LEADERLESS SECRETION

As discussed above the various members of the IL-1 family have exploited many different ways to get out of cells. However, all of them seem to be switched on by cellular stress due to changes in environmental conditions (Giuliani et al., 2011). This is true both for the passive release by dying cells exploited by IL-1α and IL-33, since death represents the last step of a cell subjected to stress, and for the more complex secretion of IL-1β and IL-18 that are regulated by redox stress (Cruz et al., 2007; Dostert et al., 2008; Hewinson et al., 2008; Meissner et al., 2008; Tassi

et al., 2009). Interestingly, both purinergic receptor stimulation and mechanical stress, cause a disturbance of redox homeostasis resulting in redox response (Wu et al., 2013); redox response is also required for inflammasome activation (Rubartelli, 2012). Interestingly, a recent study shows that mechanical stress, which as stated above induces IL-33 secretion, is sensed by NLRP3 inflammasome and leads to IL-1β processing and secretion (Wu et al., 2013). Mechanical stretch induces production of Reactive Oxygen Species (ROS) which are well-known players in the mechanism of NLRP3 inflammasome activation (Rubartelli, 2012). ROS production is also a very ancient cell defense mechanism (Naviaux, 2012) and induce redox signaling (Carta et al., 2009). It is possible that, depending on the cell type, the redox signaling is different, resulting in different effects on cytokine processing/release. For instance, while in professional inflammatory cells the evolution of the inflammasome complex favors redox-mediated processing of IL-1β and IL-18, in airway epithelial cells or fibroblasts the redox response could only induce cytokine externalization.

Stress as an inducer of secretion of many IL-1 family members is in agreement with the fact that the interleukin (IL)-1 family more than any other cytokine family is closely linked to the innate immune response, that is, to the first line of host defense against stressful noxia (Dinarello, 2009). This link became evident upon the discovery that the cytoplasmic domain of the IL-1 receptor type I is highly homologous to the cytoplasmic domains of all TLRs (Medzhitov et al., 1997). Fundamental inflammatory responses such as the induction of cyclooxygenase type 2, increased expression of adhesion molecules, or synthesis of nitric oxide are indistinguishable responses of both IL-1 and TLR ligands (Dinarello, 2009). Thus, IL-1F members are a "frontline" emergency cytokines produced very early in response to multiple stresses.

IL-1F MEMBERS: BORDERLINE BETWEEN DAMPs AND CYTOKINES

Another group of early inducers of inflammation are DAMPs. These are usually nuclear or cytosolic proteins, with a defined intracellular function that, when released by stressed cells undergoing necrosis, act as endogenous danger signals and initiate and perpetuate inflammation (Lotze et al., 2007). This is possible because DAMPs binds to specific receptors, unrelated to their intracellular function, whose engagement triggers inflammatory responses on target cells. The DAMPs features listed above are very similar to those associated to some IL-1F members, particularly IL-1α and IL-33. In fact, unlike IL-1β which is induced in a restricted number of inflammatory cells by inflammatory stimuli and undergoes regulated secretion, IL-1α and IL-33 are constitutively expressed and accumulate in large amounts in "barrier tissues" (epithelial and endothelial), the first ones entering in contact with external noxia (Dinarello, 2009; Liew et al., 2010). Both IL-1α and IL-33, although constitutively expressed, are further induced by stress conditions, thus increasing the amount of inflammatory mediator ready to be released in case of need. Interestingly, also certain DAMPs, such as HMGB1 are further increased in cells exposed to stress (Lotze et al., 2007).

The difference between cytokines such as IL-1α and IL-33 and DAMPs is therefore a moot point. The argument that DAMPs are only released by dying cells, whereas IL-1α and IL-33 can also be actively secreted has been discarded by the demonstration that the DAMP HMGB1 undergoes regulated secretion by certain cell types (Gardella et al., 2002). Actually, more recent data indicate that secretion of HMGB1, as well as of other leaderless secretory proteins not belonging to IL-1F, is also dependent on caspase-1 and inflammasome (Keller et al., 2008; Willingham et al., 2009; Lamkanfi et al., 2010). However, the underlying mechanism is unknown.

Rather, an important difference between DAMPs and IL-1F cytokines are the receptors. DAMPs bind to Pattern Recognition Receptors (PRR) such as TLRs (Leadbetter et al., 2002; Park et al., 2006; Yu et al., 2006), the most ancient membrane bound, and intracellular receptors that detect microbial invasion and initiate innate immune defenses (Kawai and Akira, 2010). These receptors are promiscuous because of their tendency to associate with different domains (Kawai and Akira, 2010), including domains present in DAMPs. During evolution, the appearance of PRR and their capacity to bind and be activated by some proteins released by dying cells, have provided a second life to these proteins, which became DAMPs.

IL-1F members have specific receptors belonging to the Toll-IL-1 receptor (TIR) superfamily by virtue of their intracellular signaling domain, shared with TLRs (O'Neill, 2008). These receptors are endowed with three extracellular immunoglobulin (Ig)-like repeats that bind IL-1F cytokines with high affinity. This implies that, at variance with DAMPs, a relatively low concentration of a given IL-1F cytokine is adequate to trigger a physiological response. The presence of specific receptor antagonists (i.e., IL-1ra) and binding proteins (i.e., IL-18 binding protein), which prevent cytokine-receptor interaction ensures a better control of the inflammatory response than in the case of DAMPs (Dinarello, 2009).

IL-1F receptors evolved after PRR. Before, cytokines worked as intracellular growth and repair molecules. Thus, in organisms (such as starfish) expressing IL-1 like molecules (Beck and Habicht, 1986) and TLRs but not cytokine receptors, an IL-1 like molecule, externalized by injured or stressed cells, was just diluted in the water and lost. Only when Ig appeared and cytokine receptors evolved, IL-1F members became able to work extracellularly as soluble mediators.

The independent evolution of leaderless secretion aimed at eliminating harmful intracellular proteins while maintaining cell integrity and of Ig superfamily members that specifically bind some leaderless secretory proteins, converged to assist in host defense: proteins fought off cells because no longer useful, became cytokines.

In conclusion, since TLR receptors are evolutionary more ancient than IL-1F receptors, it is conceivable that DAMPs are the first inflammatory mediators appeared along evolution. However, at variance with TLRs, IL-1F receptors are high affinity and non-promiscuous receptors. Although some IL-1F cytokines are passively released by dying cells, the production and activity of other IL-1F members (IL-18, IL-1β) is strictly regulated. Moreover, increasing evidence indicates that also IL-1α and IL-33,

classically considered as passively released, can be actively secreted by stressed cells. Lack of promiscuity and control of cytokine activity at several levels allows the generation of a complex network of cytokines that ensures the correct development and the positive outcome of inflammatory responses. Thus, although DAMPs are indeed able to trigger and perpetrate inflammation, the evolution of cytokines provided a strong impulse to innate immunity.

ACKNOWLEDGMENTS

We apologize to authors whose work could not be cited in this assay due to space limitations. We like to thank Dr. Patrizia Piccioli for critically reading the Ms. Anna Rubartelli is supported in part by grants from Compagnia San Paolo and Ricerca Corrente by Italian Health Ministry. Sonia Carta is the recipient of the "Young Investigators" grant GR-2010-2309622 from the Italian Ministry of Health.

REFERENCES

Andrei, C., Dazzi, C., Lotti, L., Torrisi, M. R., Chimini, G., and Rubartelli, A. (1999). The secretory route of the leaderless protein interleukin 1beta involves exocytosis of endolysosome-related vesicles. *Mol. Biol. Cell* 10, 1463–1475.

Andrei, C., Margiocco, P., Poggi, A., Lotti, L. V., Torrisi, M. R., and Rubartelli, A. (2004). Phospholipases C and A2 control lysosome-mediated IL-1 beta secretion: implications for inflammatory processes. *Proc. Natl. Acad. Sci. U.S.A.* 101, 9745–9750.

Arend, W. P., Malyak, M., Guthridge, C. J., and Gabay, C. (1998). Interleukin-1 receptor antagonist: role in biology. *Annu. Rev. Immunol.* 16, 27–55.

Auron, P. E., Webb, A. C., Rosenwasser, L. J., Mucci, S. F., Rich, A., Wolff, S. M., et al. (1984). Nucleotide sequence of human monocyte interleukin 1 precursor cDNA. *Proc. Natl. Acad. Sci. U.S.A.* 81, 7907–7911.

Bauernfeind, F., Ablasser, A., Bartok, E., Kim, S., Schmid-Burgk, J., Cavlar, T., et al. (2011). Inflammasomes: current understanding and open questions. *Cell. Mol. Life Sci.* 68, 765–783.

Beck, G., and Habicht, G. S. (1986). Isolation and characterization of a primitive interleukin-1-like protein from an invertebrate, *Asterias forbesi*. *Proc. Natl. Acad. Sci. U.S.A.* 83, 7429–7477.

Bergsbaken, T., Fink, S. L., and Cookson, B. T. (2009). Pyroptosis: host cell death and inflammation. *Nat. Rev. Microbiol.* 7, 99–109.

Black, R. A., Kronheim, S. R., Cantrell, M., Deeley, M. C., March, C. J., Prickett, K. S., et al. (1988). Generation of biologically active interleukin-1 beta by proteolytic cleavage of the inactive precursor. *J. Biol. Chem.* 263, 9437–9442.

Blott, E. J., and Griffiths, G. M. (2002). Secretory lysosomes. *Nat. Rev. Mol. Cell Biol.* 3, 122–131.

Boraschi, D., Lucchesi, D., Hainzl, S., Leitner, M., Maier, E., Mangelberger, D., et al. (2011). IL-37: a new anti-inflammatory cytokine of the IL-1 family. *Eur. Cytokine Netw.* 22, 127–147.

Brough, D., and Rothwell, N. J. (2007). Caspase-1-dependent processing of pro-interleukin-1beta is cytosolic and precedes cell death. *J. Cell. Sci.* 120, 772–781.

Carriere, V., Roussel, L., Ortega, N., Lacorre, D. A., Americh, L., Aguilar, L., et al. (2007). IL-33, the IL-1-like cytokine ligand for ST2 receptor, is a chromatin-associated nuclear factor in vivo. *Proc. Natl. Acad. Sci. U.S.A.* 104, 282–287.

Carta, S., Castellani, P., Delfino, L., Tassi, S., Venè, R., and Rubartelli, A. (2009). DAMPs and inflammatory processes: the role of redox in the different outcomes. *J. Leukoc. Biol.* 86, 549–555.

Carta, S., Tassi, S., Pettinati, I., Delfino, L., Dinarello, C. A., and Rubartelli, A. (2011). The rate of interleukin-1beta secretion in different myeloid cells varies with the extent of redox response to Toll-like receptor triggering. *J. Biol. Chem.* 286, 27069–27080.

Cayrol, C., and Girard, J. P. (2009). The IL-1-like cytokine IL-33 is inactivated after maturation by caspase-1. *Proc. Natl. Acad. Sci. U.S.A.* 2, 9021–9026.

Chen, C. J., Kono, H., Golenbock, D., Reed, G., Akira, S., and Rock, K. L. (2007). Identification of a key pathway required for the sterile inflammatory response triggered by dying cells. *Nat. Med.* 13, 851–856.

Chimini, G., and Rubartelli, A. (2005). "Novel pathways of protein secretion," in *Molecular Chaperones and Cell Signaling*, eds B. Henderson and A. G. Pockley (New York: Cambridge University Press), 45–60.

Cleves, A. E., Cooper, D. N., Barondes, S. H., and Kellym, R. B. (1996). A new pathway for protein export in *Saccharomyces cerevisiae*. *J. Cell Biol.* 133, 1017–1026.

Cohen, I., Rider, P., Carmi, Y., Braiman, A., Dotan, S., White, M. R., et al. (2010). Differential release of chromatin-bound IL-1alpha discriminates between necrotic and apoptotic cell death by the ability to induce sterile inflammation. *Proc. Natl. Acad. Sci. U.S.A.* 107, 2574–2579.

Crisan, T. O., Plantinga, T. S., van de Veerdonk, F. L., Farcas, M. F., Stoffels, M., Kullberg, B. J., et al. (2011). Inflammasome-independent modulation of cytokine response by autophagy in human cells. *PLoS ONE* 6:e18666. doi:10.1371/journal.pone.0018666

Cruz, C. M., Rinna, A., Forman, H. J., Ventura, A. L., Persechini, P. M., and Ojcius, D. M. (2007). ATP activates a reactive oxygen species-dependent oxidative stress response and secretion of proinflammatory cytokines in macrophages. *J. Biol. Chem.* 282, 2871–2879.

Delgado, M. A., Elmaoued, R. A., Davis, A. S., Kyei, G., and Deretic, V. (2008). Toll-like receptors control autophagy. *EMBO J.* 27, 1110–1121.

Dinarello, C. A. (1998a). Interleukin-1 beta, interleukin-18, and the interleukin-1 beta converting enzyme. *Ann. N. Y. Acad. Sci.* 856, 1–11.

Dinarello, C. A. (1998b). Interleukin-1, interleukin-1 receptors and interleukin-1 receptor antagonist. *Int. Rev. Immunol.* 16, 457–499.

Dinarello, C. A. (2009). Immunological and inflammatory functions of the interleukin-1 family. *Annu. Rev. Immunol.* 27, 519–550.

Dinarello, C. A. (2010). IL-1: discoveries, controversies and future directions. *Eur. J. Immunol.* 40, 599–606.

Dostert, C., Petrilli, V., Van Bruggen, R., Steele, C., Mossman, B. T., and Tschopp, J. (2008). Innate immune activation through Nalp3 inflammasome sensing of asbestos and silica. *Science* 320, 674–677.

Dunn, E., Sims, J. E., Nicklin, M. J., and O'Neill, L. A. (2001). Annotating genes with potential roles in the immune system: six new members of the IL-1 family. *Trends Immunol.* 22, 533–536.

Dupont, N., Jiang, S., Pilli, M., Ornatowski, W., Bhattacharya, D., and Deretic, V. (2011). Autophagy-based unconventional secretory pathway for extracellular delivery of IL-1β. *EMBO J.* 30, 4701–4711.

Duran, J. M., Anjard, C., Stefan, C., Loomis, W. F., and Malhotra, V. (2010). Unconventional secretion of Acb1 is mediated by autophagosomes. *J. Cell Biol.* 188, 527–536.

Eigenbrod, T., Park, J. H., Harder, J., Iwakura, Y., and Núñez, G. (2008). Cutting edge: critical role for mesothelial cells in necrosis-induced inflammation through the recognition of IL-1 alpha released from dying cells. *J. Immunol.* 181, 8194–8198.

Ferrari, D., Chiozzi, P., Falzoni, S., Dal Susino, M., Melchiorri, L., Baricordi, O. R., et al. (1997). Extracellular ATP triggers IL-1 beta release by activating the purinergic P2Z receptor of human macrophages. *J. Immunol.* 159, 1451–1458.

Gardella, S., Andrei, C., Costigliolo, S., Poggi, A., Zocchi, M. R., and Rubartelli, A. (1999). Interleukin-18 synthesis and secretion by dendritic cells are modulated by interaction with antigen-specific T cells. *J. Leukoc. Biol.* 66, 237–241.

Gardella, S., Andrei, C., Ferrera, D., Lotti, L. V., Torrisi, M. R., Bianchi, M. E., et al. (2002). The nuclear protein HMGB1 is secreted by monocytes via a non-classical, vesicle-mediated secretory pathway. *EMBO Rep.* 3, 995–1001.

Gardella, S., Andrei, C., Lotti, L. V., Poggi, A., Torrisi, M. R., Zocchi, M. R., et al. (2001). CD8(+) T lymphocytes induce polarized exocytosis of secretory lysosomes by dendritic cells with release of interleukin-1beta and cathepsin D. *Blood* 98, 2152–2159.

Gardella, S., Andrei, C., Poggi, A., Zocchi, M. R., and Rubartelli, A. (2000a). Control of interleukin-18 secretion by dendritic cells: role of calcium influxes. *FEBS Lett.* 481, 245–248.

Gardella, S., Andrei, C., Costigliolo, S., Olcese, L., Zocchi, M. R., and Rubartelli, A. (2000b). Secretion of bioactive interleukin-1beta by dendritic cells is modulated by interaction with antigen specific T cells. *Blood* 95, 3809–3815.

Ghayur, T., Banerjee, S., Hugunin, M., Butler, D., Herzog, L., Carter, A., et al. (1997). Caspase-1 processes IFN-gamma-inducing factor and regulates LPS-induced IFN-gamma production. *Nature* 386, 619–623.

Giuliani, F., Greve, A., and Rabouille, C. (2011). Unconventional secretion: a stress on GRASP. *Curr. Opin. Cell Biol.* 23, 498–504.

Gross, O., Yazdi, A. S., Thomas, C. J., Masin, M., Heinz, L. X., Guarda, G., et al. (2012). Inflammasome activators induce interleukin-1alpha secretion via distinct pathways with differential requirement for the protease function of caspase-1. *Immunity* 36, 388–400.

Harris, J., Hartman, M., Roche, C., Zeng, S. G., O'Shea, A., Sharp, F. A., et al. (2011). Autophagy controls IL-1beta secretion by targeting pro-IL-1beta for degradation. *J. Biol. Chem.* 286, 9587–9597.

Hewinson, J., Moore, S. F., Glover, C., Watts, A. G., and MacKenzie, A. B. (2008). A key role for redox signaling in rapid P2×7 receptor-induced IL-1 processing in human monocytes. *J. Immunol.* 180, 8410–8420.

Kakkar, R., Hei, H., Dobner, S., and Lee, R. T. (2012). Interleukin 33 as a mechanically responsive cytokine secreted by living cells. *J. Biol. Chem.* 287, 6941–6948.

Kawai, T., and Akira, S. (2010). The role of pattern-recognition receptors in innate immunity: update on Toll-like receptors. *Nat. Immunol.* 11, 373–384.

Keller, M., Rüegg, A., Werner, S., and Beer, H. D. (2008). Active caspase-1 is a regulator of unconventional protein secretion. *Cell* 132, 818–831.

Kouzaki, H., Iijima, K., Kobayashi, T., O'Grady, S. M., and Kita, H. (2011). The danger signal, extracellular ATP, is a sensor for an airborne allergen and triggers IL-33 release and innate Th2-type responses. *J. Immunol.* 186, 4375–4387.

Kumar, S., Hanning, C. R., Brigham-Burke, M. R., Rieman, D. J., Lehr, R., Khandekar, S., et al. (2002). Interleukin-1F7B (IL-1H4/IL-1F7) is processed by caspase-1 and mature IL-1F7B binds to the IL-18 receptor but does not induce IFN-gamma production. *Cytokine* 18, 61–71.

Laliberte, R. E., Perregaux, D. G., McNiff, P., and Gabel, C. A. (1997). Human monocyte ATP-induced IL-1 beta posttranslational processing is a dynamic process dependent on in vitro growth conditions. *J. Leukoc. Biol.* 62, 227–239.

Lamkanfi, M., Sarkar, A., Vande Walle, L., Vitari, A. C., Amer, A. O., Wewers, M. D., et al. (2010). Inflammasome-dependent release of the alarmin HMGB1 in endotoxemia. *J. Immunol.* 185, 4385–4392.

Le Feuvre, R. A., Brough, D., Iwakura, Y., Takeda, K., and Rothwell, N. J. (2002). Priming of macrophages with lipopolysaccharide potentiates P2×7-mediated cell death via a caspase-1-dependent mechanism, independently of cytokine production. *J. Biol. Chem.* 277, 3210–3218.

Leadbetter, E. A., Rifkin, I. R., Hohlbaum, A. M., Beaudette, B. C., Shlomchik, M. J., and Marshak-Rothstein, A. (2002). Chromatin-IgG complexes activate B cells by dual engagement of IgM and Toll-like receptors. *Nature* 416, 603–607.

Levine, B., and Kroemer, G. (2008). Autophagy in the pathogenesis of disease. *Cell* 132, 27–42.

Liew, F. Y., Pitman, N. I., and McInnes, I. B. (2010). Disease-associated functions of IL-33: the new kid in the IL-1 family. *Nat. Rev. Immunol.* 10, 103–110.

Lopez-Castejon, G., and Brough, D. (2011). Understanding the mechanism of IL-1β secretion. *Cytokine Growth Factor Rev.* 22, 189–195.

Lotze, M. T., Zeh, H. J., Rubartelli, A., Sparvero, L. J., Amoscato, A. A., Washburn, N. R., et al. (2007). The grateful dead: damage-associated molecular pattern molecules and reduction/oxidation regulate immunity. *Immunol. Rev.* 220, 60–81.

Luheshi, N. M., McColl, B. W., and Brough, D. (2009). Nuclear retention of IL-1 alpha by necrotic cells: a mechanism to dampen sterile inflammation. *Eur. J. Immunol.* 39, 2973–2980.

MacKenzie, A., Wilson, H. L., Kiss-Toth, E., Dower, S. K., North, R. A., and Surprenant, A. (2001). Rapid secretion of interleukin-1beta by microvesicle shedding. *Immunity* 15, 825–835.

Mandinova, A., Soldi, R., Graziani, I., Bagala, C., Bellum, S., Landriscina, M., et al. (2003). S100A13 mediates the copper-dependent stress-induced release of IL-1alpha from both human U937 and murine NIH 3T3 cells. *J. Cell. Sci.* 116, 2687–2696.

Manjithaya, R., Anjard, C., Loomis, W. F., and Subramani, S. (2010). Unconventional secretion of *Pichia pastoris* Acb1 is dependent on GRASP protein, peroxisomal functions, and autophagosome formation. *J. Cell Biol.* 188, 537–546.

Medzhitov, R., Preston-Hurlburt, P., and Janeway, C. A. Jr. (1997). A human homologue of the Drosophila Toll protein signals activation of adaptive immunity. *Nature* 388, 394–397.

Meissner, F., Molawi, K., and Zychlinsky, A. (2008). Superoxide dismutase 1 regulates caspase-1 and endotoxic shock. *Nat. Immunol.* 9, 866–872.

Moussion, C., Ortega, N., and Girard, J. P. (2008). The IL-1-like cytokine IL-33 is constitutively expressed in the nucleus of endothelial cells and epithelial cells in vivo: a novel 'alarmin'? *PLoS ONE* 3:e3331. doi:10.1371/journal.pone.0003331

Muesch, A., Hartmann, E., Rohde, K., Rubartelli, A., Sitia, R., and Rapoport, T. A. (1990). A novel pathway for secretory proteins? *Trends Biochem. Sci.* 15, 86–88.

Nakahira, K., Haspel, J. A., Rathinam, V. A., Lee, S. J., Dolinay, T., Lam, H. C., et al. (2011). Autophagy proteins regulate innate immune responses by inhibiting the release of mitochondrial DNA mediated by the NALP3 inflammasome. *Nat. Immunol.* 12, 222–230.

Naviaux, R. K. (2012). Oxidative shielding or oxidative stress? *J. Pharmacol. Exp. Ther.* 342, 608–618.

Nicklin, M. J., Barton, J. L., Nguyen, M., FitzGerald, M. G., Duff, G. W., and Kornman, K. (2002). A sequence-based map of the nine genes of the human interleukin-1 cluster. *Genomics* 79, 718–725.

Nolan, K. F., Greaves, D. R., and Waldmann, H. (1998). The human interleukin 18 gene IL18 maps to 11q22.2-q22.3, closely linked to the DRD2 gene locus and distinct from mapped IDDM loci. *Genomics* 51, 161–163.

O'Neill, L. A. (2008). The interleukin-1 receptor/Toll-like receptor superfamily: 10 years of progress. *Immunol. Rev.* 226, 10–18.

Park, J. S., Gamboni-Robertson, F., He, Q., Svetkauskaite, D., Kim, J. Y., Strassheim, D., et al. (2006). High mobility group box 1 protein interacts with multiple Toll-like receptors. *Am. J. Physiol. Cell Physiol.* 290, C917–C924.

Perregaux, D. G., McNiff, P., Laliberte, R., Conklyn, M., and Gabel, C. A. (2000). ATP acts as an agonist to promote stimulus-induced secretion of IL-1 beta and IL-18 in human blood. *J. Immunol.* 165, 4615–4623.

Piccini, A., Carta, S., Tassi, S., Lasiglié, D., Fossati, G., and Rubartelli, A. (2008). ATP is released by monocytes stimulated with pathogen-sensing receptor ligands and induces IL-1beta and IL-18 secretion in an autocrine way. *Proc. Natl. Acad. Sci. U.S.A.* 105, 8067–8072.

Qu, Y., Franchi, L., Nunez, G., and Dubyak, G. R. (2007). Nonclassical IL-1 beta secretion stimulated by P2×7 receptors is dependent on inflammasome activation and correlated with exosome release in murine macrophages. *J. Immunol.* 179, 1913–1925.

Rubartelli, A. (2012). Redox control of NLRP3 inflammasome activation in health and disease. *J. Leukoc. Biol.* 92, 951–958.

Rubartelli, A., Cozzolino, F., Talio, M., and Sitia, R. (1990). A novel secretory pathway for interleukin-1 beta, a protein lacking a signal sequence. *EMBO J.* 9, 1503–1510.

Saitoh, T., Fujita, N., Jang, M. H., Uematsu, S., Yang, B. G., Satoh, T., et al. (2008). Loss of the autophagy protein Atg16L1 enhances endotoxin-induced IL-1beta production. *Nature* 456, 264–268.

Sakurai, T., He, G., Matsuzawa, A., Yu, G. Y., Maeda, S., Hardiman, G., et al. (2008). Hepatocyte necrosis induced by oxidative stress and IL-1 alpha release mediate carcinogen-induced compensatory proliferation and liver tumorigenesis. *Cancer Cell* 14, 156–165.

Schmitz, J., Owyang, A., Oldham, E., Song, Y., Murphy, E., McClanahan, T. K., et al. (2005). IL-33, an interleukin-1-like cytokine that signals via the IL-1 receptor-related protein ST2 and induces T helper type 2-associated cytokines. *Immunity* 23, 479–490.

Semino, C., Angelini, G., Poggi, A., and Rubartelli, A. (2005). NK/iDC interaction results in IL-18 secretion by DCs at the synaptic cleft followed by NK cell activation and release of the DC maturation factor HMGB1. *Blood* 106, 609–616.

Semino, C., and Rubartelli, A. (2010). "NK cell-derived cytokines and delivery: NK cell synapses," in *Natural Killer Cells: Basic Science and Clinical Application*, eds M. T. Lotze, and A. W. Thomson (London: Academic Press, Elsevier Ltd.), 177–188.

Sesaki, H., Wong, E. F., and Siu, C. H. (1997). The cell adhesion molecule DdCAD-1 in Dictyostelium is targeted to the cell surface by a non-classical transport pathway involving contractile vacuoles. *J. Cell Biol.* 138, 939–951.

Shi, C. S., Shenderov, K., Huang, N. N., Kabat, J., Abu-Asab, M., Fitzgerald, K. A., et al. (2012). Activation of autophagy by inflammatory signals limits IL-1β production by targeting ubiquitinated inflammasomes for destruction. *Nat. Immunol.* 13, 255–263.

Sloan, I. S., Horowitz, P. M., and Chirgwin, J. M. (1994). Rapid secretion by a nonclassical pathway of overexpressed mammalian mitochondrial rhodanese. *J. Biol. Chem.* 269, 27625–27630.

Tanudji, M., Hevi, S., and Chuck, S. L. (2002). Improperly folded green fluorescent protein is secreted via a non-classical pathway. *J. Cell. Sci.* 115, 3849–3857.

Tassi, S., Carta, S., Vené, R., Delfino, L., Ciriolo, M. R., and Rubartelli, A. (2009). Pathogen-induced interleukin-1beta processing and secretion is regulated by a biphasic redox response. *J. Immunol.* 183, 1456–1462.

Willingham, S. B., Allen, I. C., Bergstralh, D. T., Brickey, W. J., Huang, M. T., Taxman, D. J., et al. (2009). NLRP3 (NALP3, Cryopyrin) facilitates in vivo caspase-1 activation, necrosis, and HMGB1 release via inflammasome-dependent and -independent pathways. *J. Immunol.* 183, 2008–2015.

Wu, J., Yan, Z., Schwartz, D. E., Yu, J., Malik, A. B., and Hu, G. (2013). Activation of NLRP3 inflammasome in alveolar macrophages contributes to mechanical stretch-induced lung inflammation and injury. *J. Immunol.* 190, 3590–3599.

Xu, Y., Jagannath, C., Liu, X. D., Sharafkhaneh, A., Kolodziejska, K. E., and Eissa, N. T. (2007). Toll-like receptor 4 is a sensor for autophagy associated with innate immunity. *Immunity* 27, 135–144.

Yu, M., Wang, H., Ding, A., Golenbock, D. T., Latz, E., Czura, C. J., et al. (2006). HMGB1 signals through toll-like receptor (TLR) 4 and TLR2. *Shock* 26, 174–179.

Zhou, R., Yazdi, A. S., Menu, P., and Tschopp, J. (2011). A role for mitochondria in NLRP3 inflammasome activation. *Nature* 469, 221–225.

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

Received: 08 April 2013; accepted: 08 May 2013; published online: 24 May 2013.
*Citation: Carta S, Lavieri R and Rubartelli A (2013) Different members of the IL-1 family come out in different ways: DAMPs vs. cytokines? Front. Immunol. **4**:123. doi: 10.3389/fimmu.2013.00123*
This article was submitted to Frontiers in Inflammation, a specialty of Frontiers in Immunology.

frontiers in
IMMUNOLOGY

REVIEW ARTICLE
published: 09 July 2013
doi: 10.3389/fimmu.2013.00180

Decoys and regulatory "receptors" of the IL-1/Toll-like receptor superfamily

Cecilia Garlanda[1]*, Federica Riva[2], Eduardo Bonavita[1], Stefania Gentile[1] and Alberto Mantovani[1,3]

[1] *Department of Inflammation and Immunology, Humanitas Clinical and Research Center, Rozzano, Italy*
[2] *Department of Veterinary Science and Public Health, University of Milan, Milan, Italy*
[3] *Department of Biotechnology and Translational Medicine, University of Milan, Rozzano, Milan, Italy*

Edited by:
Pietro Ghezzi, Brighton and Sussex Medical School, UK

Reviewed by:
Nick Gay, University of Cambridge, UK
Cem Gabay, University Hospitals of Geneva, Switzerland

****Correspondence:***
Cecilia Garlanda, Laboratory of Experimental Immunopathology, Istituto Clinico Humanitas, Via Manzoni 113, 20089 Rozzano, Italy
e-mail: cecilia.garlanda@humanitasresearch.it

Members of the IL-1 family play a key role in innate and adaptive immunity and in the pathogenesis of diverse diseases. Members of IL-1R like receptor (ILR) family include signaling molecules and negative regulators. The latter include decoy receptors (IL-1RII; IL-18BP) and "receptors" with regulatory function (TIR8/SIGIRR; IL-1RAcPb; DIGIRR). Structural considerations suggest that also TIGIRR-1 and IL-1RAPL may have regulatory function. The presence of multiple pathways of negative regulation of members of the IL-1/IL-1R family emphasizes the need for a tight control of members of this fundamental system.

Keywords: cytokine, interleukin-1, inflammation, decoy receptor

INTRODUCTION

IL-1R like receptors (ILRs) belong, together with Toll-like receptors (TLRs), to a superfamily of phylogenetically conserved proteins involved in innate immunity and inflammation (1–5). The common characteristic of the members of this family is the presence of a conserved domain in the cytoplasmic region, called TIR domain, originally defined as the Toll/IL-1-resistance and now generally assumed as an acronym for Toll/IL-1R domain. The TIR domain is involved in the activation of an evolutionarily conserved signaling pathway leading to NF-kB translocation to the nucleus and activation of protein kinases such as p38, c-Jun N-terminal kinases (JNKs), extracellular signal-regulated kinases (ERKs), and mitogen-activated protein kinases (mAPKs) (6). The ILR subfamily includes the receptors and the accessory proteins (AcP) for IL-1α (IL-1F1) and IL-1β (IL-1F2), IL-18/IL-1F4, IL-33/IL-F11, and other IL-1 family members (IL-36α/IL-1F6, IL-36β/IL-1F8, and IL-36γ/IL-1F9), which are involved in the initiation of an amplification cascade of innate resistance and inflammation and contribute to the activation and orientation of adaptive immunity (7–9). Some members of the family remain orphan receptors with still unknown ligands and functions. For instance, in the IL-1R subfamily, TIR8/SIGIRR, TIGIRR-1, and IL-1RAPL have no characterized ligands so far (2, 10, 11).

The activation of the ILR-dependent signaling cascade is tightly regulated. Indeed, the deregulated activation of these receptors, which lead to the production of proteins related to inflammation and immunity, potentially mediates damaging local and systemic inflammatory reactions. Several pathological conditions depend, at least in part, on the inflammatory potential of the IL-1 family members mentioned above. For instance, the IL-1 system represents a relevant therapeutic target in arthritis, type 2 diabetes, psoriasis, sepsis, ischemia and reperfusion, atherosclerosis, graft rejection, cancer (12–15). The regulatory mechanisms identified so far in the IL-1 system (ligands, receptors, signaling pathway) act extracellularly or intracellularly (16, 17). IL-1R antagonists (IL-1Ra)/IL-1F3 and IL-36Ra/IL-1F5 are polypeptide antagonists competing with IL-1 and IL-36α/IL-1F6, IL-36β/IL-1F8, and IL-36γ/IL-1F9, respectively, for receptor binding (3, 7, 18–20). IL-1RII lacks a signaling domain and by binding IL-1 prevents its interaction with a signaling receptor complex and therefore acts as a decoy, dominant-negative molecule, and scavenger. The negative regulator of ILR and TLR signaling, TIR8 (also known as SIGIRR), acts intracellularly. IRAK-M and MyD88s are intracellular negative regulators of ILRs and TLRs signaling (21, 22). Finally, ILR or TLR signaling proteins or transcription factors are targets of miRNAs, such as miR-155, miR-21, miR-146a, miR-132, miR-9, and miR-147, whose transcription is induced by inflammatory mediators [lipopolysaccharide (LPS), TNFα, IL-1 β] through NF-kB (23–25).

Here, we summarize our current understanding of the structure and function of negative regulatory receptors of the ILR family, in particular IL-1RII, which has served to defining the decoy receptor paradigm, and TIR8/SIGIRR, focusing on their regulatory roles in different pathological disorders dependent on ILRs and TLRs activity, and finally describe other largely uncharacterized members of the family with a negative regulatory potential, TIGIRR-1, IL-1RAPL, IL-1RAPb.

THE DECOY RECEPTOR IL-1RII

GENE AND PROTEIN

The first IL-1R was cloned from murine and human T cells, whereas IL-1RII was identified soon after in B lymphocytes and myelomonocytic cells (26, 27). On the basis of their structures, IL-1RI and IL-1RII belong to the Ig-like superfamily of receptors, with the extracellular portion containing 3 Ig-like domains. The signaling IL-1R complex includes the type I IL-1 receptor

(IL-1RI) and IL-1R AcP, which both have a cytoplasmic TIR domain (**Figure 1**).

The gene encoding IL-1RII is located on chromosome 2 (q12–22) in humans and in the centromeric region of chromosome 1 in mice (28), in cluster with IL-1RI and other members of the family (IL-33R, IL-18R, IL-36R). The type II receptor is highly conserved in evolution and is found in bony fish, where it functions to inhibit IL-1-induced inflammation (29). The third Ig domain of IL-1RII is homologous to the Ig domain of IL-18BP (30) and indeed, it has been suggested that IL-1RII and IL-18BP have a common ancestral gene and diverged at the level of fish (31). The IL-1RII locus spans about 38 kb of genomic DNA, of which about 21 kb contains the coding region. The exon structures of the extracellular portion of IL-1RII and IL-1RI receptors are identical and amino acid sequences share 28% homology. A single exon encodes the transmembrane region and a short cytoplasmic tail (29 amino acids) of IL-1RII, which has no TIR domain and does not signal (**Figure 1**). The human transcript encodes for a 386 amino acid glycosylated protein of 68 kDa, in contrast with IL-1RI which is a 80–85 kDa glycosylated protein and has a 213 amino acid cytoplasmic tail containing a TIR domain responsible for signaling (32).

IL-1RII can be proteolytically processed and released in a soluble form, via the actions of a metalloproteinase, A Disintegrin and Metalloprotease 17 (ADAM17, also know as TACE) (33, 34). In addition, IL-1RII can be processed in a manner similar to Amyloid β protein precursor (APP), by alpha-, beta-, and gamma-secretase: the ectodomain is shed in an alpha-secretase-like manner, whereas the IL-1RII C-terminal fragment undergoes further intramembrane proteolysis by gamma-secretase (35). Finally, the aminopeptidase regulator of TNFR1 shedding (ARTS-1) has been implicated in IL-1RII shedding in basal condition and upon cell stimulation with phorbol myristate acetate (PMA) (36).

Wang et al. (37) solved the structure of IL-1 and IL-1RAcP in complex with the extracellular domain of IL-1RII and showed that the mode of interaction among IL-1β, IL-1RII, and IL-1RAcP and the overall structure are extremely similar to those of the signaling ligand-receptor complex (IL-1, IL-1R1, IL-1AcP).

MECHANISMS OF NEGATIVE REGULATION

Several lines of evidence are consistent with the view that the IL-1RII is a bonafide IL-1 decoy.

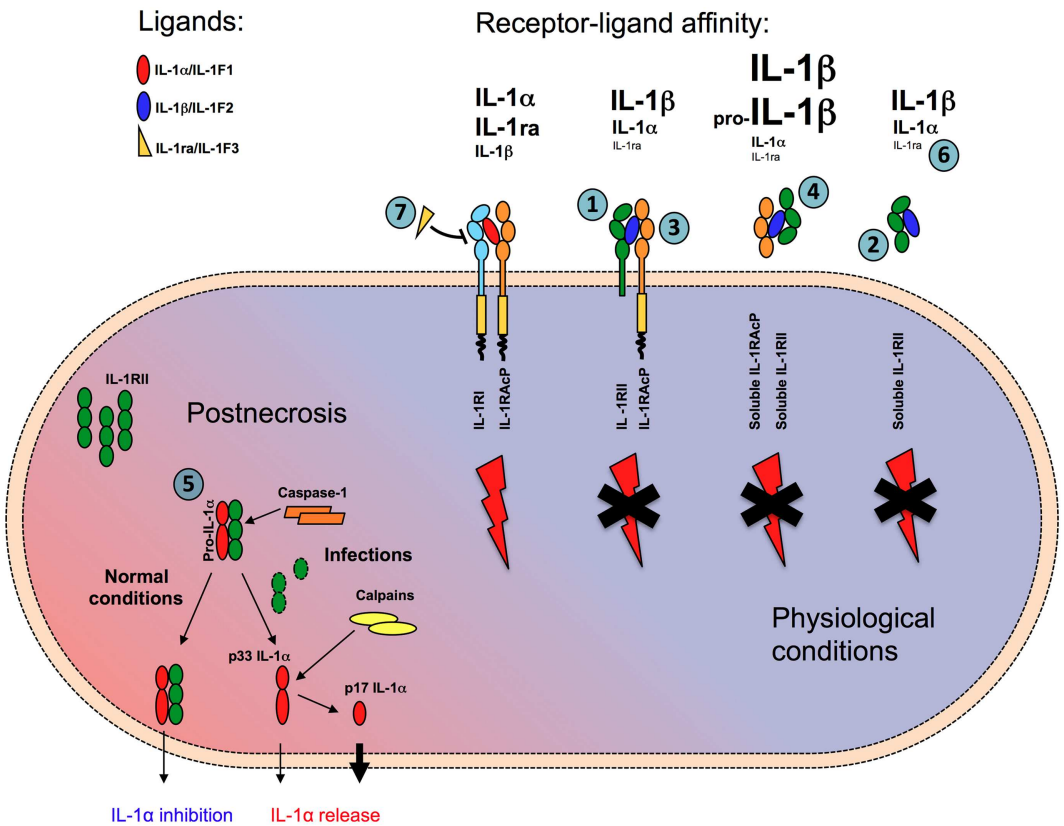


FIGURE 1 | Mechanisms of negative regulation mediated by IL-1RII. IL-1RII negatively regulates IL-1 responses in multiple ways. In membrane-bound (1) or soluble form (2) IL-1RII acts as a decoy, capturing with high-affinity IL-1β and IL-1α, but not IL-1ra, and preventing their interaction with IL-1RI. IL-1RII acts as a dominant-negative influencing IL-1RI-IL-1RAcp signaling receptor complex formation (3). The interaction of ligand-bound soluble IL-1RII with soluble IL-1RAcP increases the affinity for both IL-1α and IL-1β by about two orders of magnitude (4). Finally, in cytosol soluble form, IL-1RII interacts with pro-IL-1α preventing its pro-inflammatory activity during necrosis, until caspase-1 cleaves IL-1RII and allows the secretion of IL-1α (5). Because of low affinity for IL-1RII, IL-1ra does not compete with IL-1α and IL-1β for the interaction with the decoy receptor (6), whereas it competes with IL-1α and IL-1β antagonizing their interaction with the signaling receptor IL-1RI (7).

A first level of control is represented by the differential affinity of the signaling and decoy receptors for agonist or antagonist ligands of the IL-1 family (**Figure 1**). IL-1RI binds IL-1α with higher affinity than IL-1β ($K_d \approx 10^{-10}$ and 10^{-9} M, respectively) and IL-1ra with an affinity similar to that for IL-1α. By contrast, IL-1RII binds IL-1β and IL-1α with high affinity ($K_d \approx 10^{-9}$–10^{-10} and 10^{-8} M, respectively), but it binds IL-1ra at least 100 times less efficiently (38). Plasmon resonance analysis revealed that IL-1β has a slow off-rate from IL-1RII, whereas IL-1ra rapidly dissociates from IL-1RII but not from IL-1RI (39), in agreement with the need that the two regulators of IL-1 do not bind each other self defeating and frustrating their regulatory activity. By binding agonist ligands with high affinity without inducing signaling, IL-1RII acts as a molecular trap for IL-1 inhibiting its activity (27, 40) (**Figure 1**).

Second, IL-1RII also forms a complex with IL-1 and the IL-1RAcP. It therefore exerts a dominant-negative effect on the formation of a signaling receptor complex, by sequestering AcP, which is essential for signal transduction (41, 42) (**Figure 1**).

In addition, IL-1RII is also found in a soluble form, released from cells via the actions of a metalloproteinase (see above). Soluble IL-1RII is found in normal blood relatively at high concentrations, in the order of nanogram per milliliter. Cell-surface shedding is the major mechanism responsible of soluble IL-1RII generation, but in addition an alternatively spliced transcript encoding a soluble version of IL-1RII has been described (43). Soluble, but not cell-associated, IL-1RII binds pro-IL-1β and blocks its processing by IL-1-converting enzyme (ICE)/caspase-1 (38) (**Figure 1**). Soluble AcP, encoded by an alternatively spliced mRNA (44) and found at high levels in the circulation (300 ng/ml in humans), can interact with ligand-bound soluble IL-1RII, enhancing the latter's affinity for IL-1α and IL-1β by two orders of magnitude, while not affecting the very low affinity for IL-1Ra (40, 45) (**Figure 1**). In mouse and monkey, the interaction between AcP and IL-1RII is required for high-affinity binding of IL-1β and effective inhibition (45). Thus, the interaction with AcP renders IL-1RII a much more effective inhibitor of IL-1.

Finally, a further mechanism of negative control of IL-1α by IL-1RII during necrosis has recently been proposed (46). The soluble form of IL-1RII has been detected in the cytosol in large amounts, possibly because the IL-1RII signal peptide is short and relatively weak. In line with previous reports on systemic sclerosis fibroblasts (47), in this cytosolic form, soluble IL-1RII interacts with pro-IL-1α (**Figure 1**). This interaction protects pro-IL-1α from cleavage by different enzymes (calpain, granzyme B, chymase, and elastase) normally involved in the generation of the active form (48, 49) and prevents IL-1α activity (46). This blockade would be abrogated by active caspase-1 (for instance during infections), which specifically cleaves IL-1RII, causing dissociation from IL-1α, calpain processing, and complete restoration of IL-1α activity after necrosis or during regulated secretion (**Figure 1**). Since IL-1RII is expressed by a limited set of cell types, in contrast with IL-1RI, which is widely expressed, this mechanism of negative regulation would be cell type specific. Thus, the activity of IL-1α during necrosis and sterile inflammation would be somehow restricted to cell types which do not express IL-1RII. For instance, the high inflammatory profile of vascular smooth muscle cells to necrosis, which is IL-1α-dependent (50), would be in agreement with low levels of IL-1RII. These findings would explain the tissue specificity of inflammatory damage during necrosis.

The anti-inflammatory role of IL-1RII has been demonstrated in different pathological conditions in animal models. Gene-targeted mice overexpressing IL-1RII under the control of the human keratin gene promoter were resistant to PMA-induced chronic skin inflammation (51). Recombinant IL-1RII delivered via implanted human keratinocytes overexpressing soluble IL-1RII played a protective role in a mouse model of collagen-induced arthritis (52) and intravenous administration of soluble IL-1RII significantly reduced joint swelling and erosion in a model of arthritis in rabbit (53). Gene transfer of a soluble IL-1RII-Ig fusion protein reduced allograft rejection and prolonged graft survival in a rat model of heart transplantation, reduced infiltrating macrophages, and CD4+ T cells, and lowered levels of TNF-α and TGF-β (54). Similarly, IL-1RII ameliorated experimental autoimmune myocarditis by blocking IL-1 and inhibiting production of the cytokines [IL-6, transforming growth factor-β, retinoic acid-related orphan nuclear receptor (RORγt) and IL-17] involved in the polarization of Th17 cells (55). Finally, in a mouse model of endometriosis, consisting of human endometrial tissue implanted in nude mice, human soluble IL-1-RII administered intraperitoneally reduced the growth and dissemination of endometrial implants and the expression of IL-1β-dependent inflammatory, angiogenic, and cell growth mediators (56).

In support of the view that IL-1RII is a professional anti-IL-1 molecule, Pox viruses have acquired and retained a soluble version of type II IL-1R, that plays a key role in the regulation of pathogenicity (57).

Thus, IL-1RII negatively regulates IL-1 responses in multiple complementary ways. In membrane-bound or soluble form IL-1RII acts as a decoy, capturing with high affinity IL-1, and preventing it from interacting with IL-1RI. It acts as a dominant-negative influencing IL-1RI-IL-1RAcp signaling receptor complex formation. The interaction of ligand-bound soluble IL-1RII with soluble IL-1RAcP increases the affinity for both IL-1α and IL-1β by about two orders of magnitude and makes IL-1RII a powerful inhibitor for both agonists. Finally, in cytosol soluble form, IL-1RII interacts with pro-IL-1α preventing its pro-inflammatory activity during necrosis.

EXPRESSION

In contrast with IL-1RI, which is expressed by a large variety of cell types, IL-1RII is expressed by a limited set of cell types, which also often express IL-1RI: among leukocytes, IL-1RII is the predominant IL-1-binding protein found in monocytes, neutrophils, and B cells (26, 27, 40, 58). Monocyte differentiation to macrophages, in particular M2 or M2-like macrophages, is associated to increased expression of IL-1RII (58, 59). IL-1RII is also expressed by microglial cells, in particular upon stimulation with LPS and has been shown to regulate IL-1β actions by binding excess levels of this cytokine during brain inflammation (60). In addition, noradrenaline has been reported to upregulate IL-1RII in mixed microglia via β-adrenoceptor activation and downstream activation of protein kinase A and ERK, thus preventing IL-1β-induced neurotoxicity (61). Other stimuli involved in IL-1RII upregulation

in the CNS include cerebral ischemia, kainic acid administration, and central administration of IL-1β (62).

T regulatory cells (Tregs) have been shown to express surface and soluble functional IL-1RII, as well as IL-1Ra mRNA. This property has been exploited for the purification of activated human FOXP3+ regulatory T cells from expansion cultures (63). Activated human Tregs rapidly up-regulated IL-1RII and were able to neutralize IL-1β, which suggests a physiological significance for the expression of IL-1 decoy receptor on Tregs (64).

Differential levels of IL-1RII have been described in osteoclasts. In particular, lower expression of IL-1RII has been detected in large osteoclasts compared to small osteoclasts, and this is in line with increased resorptive activity of large osteoclasts in response to IL-1 (65). IL-1RII is also expressed by basal epithelial cells of the skin (66), epithelium of endometrium (67), vagina and urethra, and chondrocytes. Endothelial cells and fibroblasts generally express only IL-1RI and AcP.

Surface and soluble IL-1RII expression is strongly enhanced by anti-inflammatory signals. Glucocorticoid hormones (GCs), prostaglandins, the anti-inflammatory T helper 2 (Th2) cytokines (IL-4 and IL-13), and IL-27 induced augmented surface expression and release of IL-1RII *in vitro*, in particular in myelomonocytic cells, and *in vivo* (27, 58, 68–72). In particular, IL-4 and dexamethasone, by inducing IL-1RII, antagonized the prosurvival effect of IL-1 in neutrophils *in vitro* (27). IL-10 increased circulating soluble IL-1RII levels *in vivo* in mice. Aspirin increased IL-1RII release from mononuclear cell cultures *in vitro* and *in vivo* (73). IL-27 inhibited IL-1β-induced signaling in human macrophages by downregulating the expression of the signaling receptor IL-1RI, inducing expression of the receptor antagonist IL-1Ra, and by upregulating the expression of the decoy receptor IL-1RII (72). These data suggest that induction of IL-1RII contributes to the anti-inflammatory effect of these mediators.

In contrast, pro-inflammatory molecules inhibit IL-1RII expression. For instance, bacterial LPS caused a rapid shedding of surface IL-1RII in monocytes, followed by down-regulation of expression, whereas it stimulated the expression of IL-1RI, AcP and the adapter protein MyD88 (74). Interferon γ (IFN-γ) inhibited IL-1RII expression and release in myelomonocytic cells and counteracted IL-4-dependent upregulation of IL-1RII (71). In addition to LPS, chemoattractants such as formyl Meth-Leu-Phe (fMLP), reactive oxygen intermediates (ROI), TNF, and PMA caused rapid shedding of IL-1RII (33, 75, 76). PMA also induced alternatively spliced soluble IL-1RAcP (44). Thus, shedding of IL-1RII by circulating phagocytes and generation of alternatively spliced soluble IL-1RAcP induced by chemoattractants in the early steps of recruitment, could prepare cells to respond to IL-1 once they enter tissues.

Acetylated low density (ac-LDL) and very low density (VLDL) lipoprotein intracellular accumulation caused decreased IL-1RII mRNA and protein expression in macrophages *in vitro*. In agreement with these *in vitro* data, patients with familial combined hyperlipidemia showed decreased expression of IL-1RII in monocytes. Finally, IL-1RII expression in human atherosclerotic vessels was defective compared to non-atherosclerotic arteries (77).

Naturally circulating levels of soluble IL-1R type II are in the range of 5–10 ng/ml, although these can rise in certain chronic (78) or acute (79) inflammatory settings (see below), in part reflecting the activation of negative circuits of regulation of the cytokine action.

IL-1RII IN HUMAN PATHOLOGICAL CONDITIONS: DIAGNOSTIC AND THERAPEUTIC IMPLICATIONS

High levels of soluble IL-1RII are normally present in plasma of healthy individuals. Defective or increased expression of tissue or body fluid levels of soluble IL-1RII have been described in diverse pathological conditions, ranging from critical conditions to autoimmune diseases, neuroinflammatory diseases and tumors.

Increased blood levels of soluble IL-1RII have been detected in critically ill patients with infectious conditions such as sepsis, acute meningococcal infection, experimental endotoxemia, operative trauma, or necrotizing enterocolitis in preterm infants (73, 80, 81). In critically ill patients, IL-1RII levels were elevated especially in severe, systemic infection and culture-positive infections. In patients with a marked systemic inflammatory response syndrome, further pronounced increase of circulating IL-1RII levels was observed in patients developing sepsis (80). Treatment with glucocorticoids further increased IL-1RII levels, suggesting that it potentially behaves as a biomarker for the activation of anti-inflammatory pathways or for responsiveness to anti-inflammatory agents. In acute meningococcal infections, increased soluble IL-1RII levels correlated with disease severity, in particular with endotoxemia, complement-activation, and shock (82). Increased IL-1RII levels were also observed in patients upon treatment with aspirin (73).

IL-1Ra and/or IL-1RII increased levels were also detected in sera of multiple sclerosis patients after steroid treatment for relapse (83) and in the cerebrospinal fluid of patients with Alzheimer's disease, where it may be a marker of disease progression (84).

In psoriasis, IL-1ra and IL-1RII were both significantly overexpressed in the suprabasal and basal compartment, respectively, and inversely correlated with the expressions of IL-1α (66). Increased levels of soluble IL-1RII have been found in the synovial fluid (39) and plasma of individuals with RA (78), and these negatively correlated with severity of disease, suggesting IL-1RII acts as natural antagonist of IL-1-driven joint destruction. In contrast, plasma levels of IL-1Ra correlated positively with disease progression, possibly reflecting disease exacerbation (78). These data are in line with experimental *in vitro* and *in vivo* data showing that overexpression of IL-1RII in chondrocytes protected them from IL-1 stimulation (85), or that the transfer of cells overexpressing and releasing IL-1RII resulted in the inhibition of collagen-induced arthritis (52). These results, as well as the binding properties of IL-1RII (high affinity for IL-1, low affinity for IL-1ra), supported the development of IL-1RII as therapeutic molecule in rheumatoid arthritis (see below). The expression of both receptors for IL-1 was demonstrated by immunostaining and laser confocal microscopy in sarcolemma from human muscle tissue samples, at higher levels in patients with polymyositis and dermatomyositis as compared with healthy individuals, together with increased expression of IL-1α, IL-1β, and IL-1Ra (86).

IL-1RII is upregulated in some tumors, including pancreatic ductal adenocarcinoma (87), prostatic cancer and benign prostatic

hyperplasia (88), and ovarian cancer, where it provides a powerful distinction between primary and recurrent tumors (89).

In contrast to these conditions associated to upregulation of IL-1RII, in other contexts, defective expression of IL-1RII has been associated to the pathogenesis of the disease. For instance, gene-array analysis of osteoarthritic lesions indicated a lack of expression of IL-1RII and IL-1ra (85), suggesting that defective expression of negative regulators of the IL-1 system contributes to pathogenesis. Similarly, endometriosis and endometrioid ovarian cancer are associated with lower levels of serum and local IL-1RII and with IL-1RII polymorphisms (90–92). In the context of atherosclerosis, it has been proposed that, since macrophages from hyperlipidemic patients have decreased IL-1RII mRNA and protein expression, IL-1-dependent inflammation could be relatively unchecked during atheroma formation (77). Genome-wide association studies identified several candidate genes potentially involved in inflammatory bowel disease (IBD) pathogenesis, including IL-1RII (91).

Autoimmune inner ear disease is characterized by recurring episodes of sudden or progressive sensorineural hearing loss. Defective responsiveness to corticosteroid in this disease has been correlated to the low induction of IL-1RII in peripheral blood mononuclear cells (93).

Secretion of embryonic IL-1β is one of the first responses of the blastocyst to the receptive endometrium. IL-1β is involved in inducing molecular changes that are essential for attachment of the blastocyst, such as immunomodulation, angiogenesis, and endometrial tissue remodeling. In this context, it has been proposed that these IL-1 activities are regulated by chorionic gonadotropin, which acts directly on endometrial epithelial cells to down-regulate the synthesis and release of IL-1RII (94).

The IL-1 decoy receptor IL-1RII was originally tested as a therapeutic by Amgen in arthritis, based on the promising results in this context (45), but no clinical development of this agent has been reported. Recently, the soluble IL-1RI (Rilonacept) was introduced as therapeutic and approved by the FDA for selected autoinflammatory diseases, in particular cryopyrin-associated periodic syndromes (familial cold autoinflammatory syndrome and Muckle–Wells Syndrome) (95). The drug consists in a fusion protein containing the extracellular domains of IL-1R1 and IL-1RAcP coupled to the Fc region of human IgG1. Rilonacept acts similarly to soluble IL-1RII, as a decoy, by binding IL-1β and IL-1α with higher affinity than IL-1Ra (96).

THE NEGATIVE REGULATOR TIR8/SIGIRR

GENE AND PROTEIN

TIR8/SIGIRR gene is localized on human chromosome 11 and on murine chromosome 7 (97). The 410 amino acid-long protein is constituted by a single Ig extracellular domain, a transmembrane domain, an intracellular conserved TIR domain, and a 95 amino acid-long tail at the C-terminal, reminiscent of the intracellular tails of few ILR/TLR family members, in particular IL-1AcPb and TIGIRR (see below) (**Figure 2**). Both in human and mouse, TIR8/SIGIRR has several N- and O-glycosylation sites in the extracellular domain (97, 98). The sequence and pattern of expression of *TIR8/SIGIRR* is conserved among vertebrates, from chicken to humans (99). In particular, human and mouse protein sequences share 82% homology. TIR8/SIGIRR is expressed in several tissues, particularly in kidney, digestive tract, liver, lung, and lymphoid organs (97, 100).

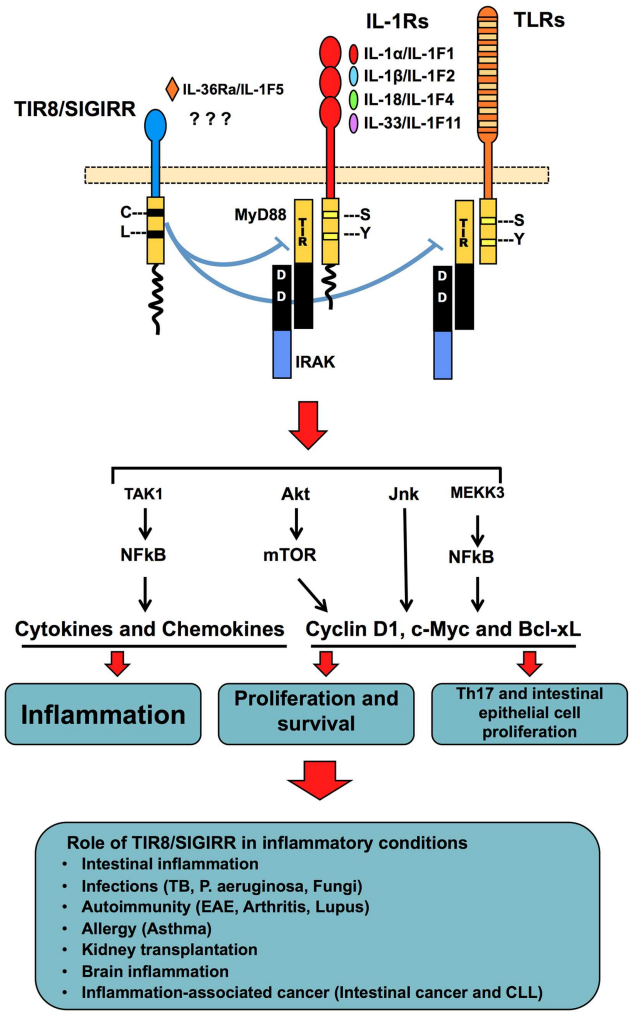


FIGURE 2 | TIR8/SIGIRR, a negative regulatory receptor of ILR and TLR signaling. TIR8/SIGIRR is composed by a single extracellular Ig domain, a transmembrane domain, an intracellular conserved TIR domain and a long intracellular tail. Two replaced amino acid in the TIR domain (Cys 222, Leu 305) are potentially involved in non-conventional activation. TIR8/SIGIRR is an orphan receptor, but IL-36Ra has been proposed as a TIR8/SIGIRR ligand in glial cells. TIR8/SIGIRR inhibits ILR and TLR signaling by competing with MyD88 and IRAK recruitment at the TIR domain thus dampening the signaling pathway leading to NF-kB activation. In T cells and epithelial cells, TIR8/SIGIRR inhibits IL-1-dependent activation of the Akt- mTOR pathway and of JNK, thus controlling cell proliferation and survival. *In vivo* studies in gene-targeted mice demonstrate that Tir8/Sigirr acts as a non-redundant negative regulator in different inflammatory conditions dependent on ILRs or TLRs.

TIR8/SIGIRR proximal promoter has a binding site for SP1, which enhances its transcription in basal conditions (101). LPS stimulation reduces SP1 binding to *TIR8/SIGIRR* promoter, possibly explaining the *TIR8/SIGIRR* down-regulation

in inflammatory conditions (LPS administration, ulcerative colitis, lung and urinary infections, infestations) (100–106). Recent studies demonstrated lower expression of *TIR8/SIGIRR* in fetal human enterocytes providing a reasonable explanation to the excessive inflammatory response in the immature intestine (107).

In contrast with these studies, *TIR8/SIGIRR* up-regulation was shown in human monocytes during sepsis and sterile systemic inflammation (108). Th2-lymphocytes expressed higher levels of *TIR8/SIGIRR* compared to Th1 polarized or non-differentiated lymphocytes (109). *Pseudomonas aeruginosa* infected mice showed up-regulation of *Tir8/Sigirr* in the cornea, macrophages, and Langerhans cells through the activity of vasoactive intestinal peptide (110). *Lactobacillus jensenii*, a probiotic microorganism, induced up-regulation of *TIR8/SIGIRR* in porcine Payer's patch antigen presenting cells through activation of TLR2 (111). Similarly, LPS-induced *Tir8/Sigirr* in murine Payer's patch DCs, but not in spleen DCs (112). These data suggest that Payer's patch DCs use Tir8/Sigirr to tune TLRs signaling.

MECHANISMS OF NEGATIVE REGULATION

The function of TIR8/SIGIRR consists in the specific inhibition of NF-kB and JNK activation following stimulation of ILR or TLR family members (102, 113). TIR8 can modulate the signal transduction activated by IL-1RI, IL-18R, T1/ST2, TLR1/2, TLR3, TLR4, TLR7, and TLR9 (98, 102, 109, 113–115) (**Figure 2**).

The extracellular Ig-like domain of TIR8/SIGIRR has been show to interfere with the dimerization of IL-1RI and IL-1RAcP. The cytoplasmic TIR domain binds TIR-containing adaptor molecules, which are no more available for signaling, whereas the cytoplasmic tail is not involved in the inhibitory activity (102, 114). A computational approach suggests a three-dimensional model for the interaction among the TIR domains of TLR4, TLR7, MyD88, and TIR8/SIGIRR. In this model, TIR8/SIGIRR binds TLR4 and TLR7 through its BB-loop region preventing their dimerization and MyD88 recruitment (116).

TIR8/SIGIRR can also regulate mTOR kinase activity in Th17 lymphocytes (117) and in intestinal epithelial cells (118) (**Figure 2**). These results are in agreement with the role of TIR8/SIGIRR in autoimmune diseases and in tumor suppression (see below).

ROLE OF TIR8/SIGIRR *IN VIVO*

Infection-associated inflammation

Tir8/Sigirr-deficient mice are more susceptible than wild type mice in several infections, such as tuberculosis, candidiasis, aspergillosis, *P. aeruginosa* infection, in terms of mortality and tissue damage due to an exaggerated inflammatory response (103, 106, 119, 120) (**Figure 2**). Results obtained with IL-1-blocking antibodies and IL-1RI-deficient mice indicated that in some of these infectious conditions (tuberculosis and *P. aeruginosa* lung infection), TIR8/SIGIRR played a major role in dampening inflammation induced by IL-1R activation.

Similarly, in a colitis mouse model, *Tir8/Sigirr*-deficient mice developed a more severe gut inflammation compared to wild type mice (113, 121). Commensal microflora activates enterocyte TLRs and consequently induces survival of epithelial cells and maintains gut homeostasis (122, 123). Lack of Tir8/Sigirr in colon epithelial cells was shown to be associated to constitutive NF-kB and JNK activation and up-regulated expression of Cyclin D1 and Bcl-xL in homeostatic conditions, which returned to the control level after depletion of commensal bacteria (121) (**Figure 2**).

Excessive systemic inflammation was observed in *Tir8/Sigirr*-deficient mice upon LPS challenge, and reduced inflammation and mortality were described in Tir8/Sigirr overexpressing mice in LPS-dependent acute lung injury model (102, 124). However, these phenotypes possibly depend on the genetic background since excessive systemic or local inflammatory reactions to LPS were not confirmed in other studies (102, 113).

In contrast with these data, in a urinary tract infection model, Tir8/Sigirr inhibited an effective host response against uropathogenic *E. coli*, as indicated by lower renal bacterial load and dysfunction in TIR8-deficient mice, associated to increased circulating and intrarenal neutrophils at the early phase of infection (125).

Sterile inflammatory conditions

Recent data suggest that TIR8/SIGIRR plays a direct role in inhibiting different IL-1-dependent signaling pathways, including IL-1R-mTOR, in Th17 lymphocytes, thus tuning initial Th17 differentiation and preventing Th17 cell-mediated pathogenic effects (117) (**Figure 2**). This effect was particularly evident in the control of CNS autoimmune inflammation in a model of experimental autoimmune encephalomyelitis (117). Tir8/Sigirr deficiency was also associated to increased susceptibility to develop autoimmunity in a model of systemic lupus erythematosus (B6lpr/lpr), as well as in a model of lupus nephritis induced by hydrocarbon oil (pristane) (115, 126). In the lpr/lpr model, Tir8/Sigirr deficiency was responsible for massive lymphoproliferation, peribronchial inflammation, and mesangio-proliferative glomerulonephritis, due to B and dendritic cell hyper-activation in TLR7- and TLR9-dependent response to autoantigens and nucleosomes (115). Tir8/Sigirr-deficient mice were also more susceptible than wild type mice to both zymosan-induced and collagen antibody-induced arthritis models, because of excessive inflammation at least in part dependent on IL-1 (127) (**Figure 2**).

In agreement with the results obtained in autoimmunity mouse models, *TIR8/SIGIRR* was down modulated together with other anti-inflammatory genes in psoriatic patients (128).

Studies on allergic inflammatory responses showed that Tir8/Sigirr plays an important role also in controlling the axis IL-33 – ST2 which is involved in Th2 cell polarization and Th2 cytokine production (109) (**Figure 2**).

DAMPs generated during renal ischemia/reperfusion are responsible of the activation of intrarenal DCs, macrophages, and neutrophils via TLRs and IL-1R, which are potentially involved in post ischemic renal failure. In models of renal ischemia/reperfusion or kidney transplantation, Tir8/Sigirr-deficient mice showed increased renal injury or severe graft rejection, respectively, associated to excessive cytokine and chemokine

production and consequently, leukocyte recruitment and amplified adaptive immune response against donor antigens (129, 130) (**Figure 2**).

Finally, in agreement with the expression in neurons, microglia, and astrocytes (131), TIR8/SIGIRR was shown to be a modulator of microglia activation by LPS, and of neuroinflammation (132). Furthermore, Tir8/Sigirr-deficient mice showed impaired cognitive and synaptic functions associated to up-regulated IL-1R1 and TLR4 signaling in hippocampal tissue in response to IL-1α and high mobility group box 1 (133). Studies on the anti-inflammatory activity of IL-36Ra in the brain demonstrated at least a partial involvement of TIR8/SIGIRR in down modulating glial cell inflammatory responses through the production of IL-4 (18).

Cancer-related inflammation

Chronic inflammation is associated with promotion of malignancy and tumor progression and several studies in animals have shown the protumoral role of IL-1 in this context (134, 135). Along the same line, in different murine models, TIR8/SIGIRR has been demonstrated to play a key protective role in the pathogenesis of cancer-related inflammation. In a model of colitis-associated cancer (CAC), a colorectal disease that arises in patients suffering from chronic IBD, Tir8/Sigirr-deficient mice were highly susceptible to both inflammation and carcinogenesis in terms of number, size, and severity of lesions (121, 136) (**Figure 2**). The mechanism proposed suggests that TIR8/SIGIRR plays a protective role probably by modulating the signaling activated by commensal bacteria through TLRs in the epithelial cells and consequently, downstream events, including production of inflammatory mediators and factors involved in cell survival and proliferation, leukocyte recruitment, and angiogenesis (137). Moreover, Tir8/Sigirr deficiency in the $Apc^{min/+}$ mouse model was associated to increased intestinal lesion development due to higher Akt-mTOR activity, a crucial tumorigenic pathway (118, 138). The data suggest that Tir8/Sigirr exerts a tumor suppressor activity by controlling IL-1- and TLR-induced mTOR-mediated cell cycle progression and consequent genetic instability (118).

In Chronic Lymphocytic Leukemia (CLL), human malignant B cells express lower levels of *TIR8/SIGIRR* mRNA than normal B cells (139, 140). Similar results were found in the mouse where CD19+ cells express lower levels of Tir8 messenger compared to CD19+ cells isolated from a transgenic mouse model of CLL (TCL1 mice) (141). In CLL, both genetic (e.g., MyD88 mutations) and micro environmental factors concur to the development, expansion, and progression of the disease (139, 142). In a murine CLL model, the absence of Tir8/Sigirr led to a more severe and earlier appearance of monoclonal B-cell expansions and to shortened life span. The disease mimicked the aggressive variant of human CLL, characterized by the appearance of prolymphocytes (141) (**Figure 2**), suggesting that TIR8/SIGIRR acts as an inhibitor of CLL progression through a still unclear molecular mechanism.

OTHER ILR WITH NEGATIVE REGULATORY PROPERTIES

IL-18 BINDING PROTEIN

IL-18 binding protein (IL-18BP) is a secreted high affinity IL-18 binding molecule, which acts as a potent inhibitor of IL-18 and a modulator of Th1 response. It is constituted by only one Ig-like domain and it is structurally and functionally similar to IL-1RII (30). Indeed, phylogenetic analysis suggests that IL-18BP and IL-1RII had a common ancestral gene and diverged at the level of fish (31). Recombinant IL-1F7 also binds to the IL-18BP, further increasing the ability of IL-18BP to neutralize IL-18 activity (143).

Proteins homolog to IL-18BP have been found in poxviruses (*Ectromelia*), which are responsible of neutralization of human IL-18 during the viral infection and of dampening the inflammatory response associated to the infection (144).

Further information about this molecule is available in the review by Dinarello et al. in this issue.

IL-1RAcPb

The IL-1RAcP is the receptor subunit of the IL-1RI complex, and it is also used by IL-36α/IL-1F6, IL-36β/IL-1F8 and IL-36γ/IL-1F9, and IL-33 receptors. It has been shown that an alternative form of AcP, called AcPb, can be generated by alternative splicing, in which the prototypical AcP C-terminal exon 12 is skipped and an alternative exon 12b is used (145, 146). Smith et al. (146) characterized this molecule and showed its regulatory properties in the brain. The C-terminus encoded by these two alternative exons has 35% amino acid identity, which includes conserved motifs of the TIR domain. Moreover, the exon 12b encodes a sequence of approximately 140 additional amino acids in the C-terminal of the TIR domain that has no homology to other protein sequences and is of unknown function. The general structure of AcPb is similar to that of AcP and suggests that the AcPb cytoplasmic domain is similar to AcP TIR domain. However, there is one area of substantial difference because of changed configuration in the DD loop and aD helix regions of the AcPb TIR domain, which remembers modification of TIR8/SIGIRR TIR domain, and altered charge distribution pattern on its surface. It has been proposed that these modifications could affect interaction with adapter and signaling molecules. Indeed, AcPb is capable of forming a ligand-dependent complex with IL-1R, but it does not lead to the recruitment of the adaptor molecules MyD88 and IRAK4 after stimulation with IL-1, and is unable to mediate specific IL-1 responses. In both human and mouse, the expression of the AcPb is restricted to the CNS (whole brain, fetal brain, cerebellum, and spinal cord). AcP and AcPb are coexpressed in the same cells, but AcPb is the more abundant isoform. It has been proposed that AcPb could also be recruited to other AcP-utilizing receptors, such as ST2 and IL-1Rrp2/IL-36R, which are expressed in the CNS, once they have bound their ligands (IL-33 and IL-36α,β,γ, respectively). In a model of LPS challenge in the CNS, AcPb-deficiency was associated to neuronal loss suggesting that AcPb may dampen the neurotoxic effects of IL-1 by modulating the intracellular signaling and gene expression response to LPS-induced IL-1, or possibly to other cytokines acting through AcP (146). The inhibitory effect of AcPb could depend on the failure to recruit MyD88 and IRAK4, on the competition with AcP in a IL-1 receptor complex containing multiple IL-1R and AcP molecules, or on unknown functions mediated by the C-terminal tail.

TIGIRR-1 AND IL-1RAPL

TIGIRR-1 and IL-1RAPL (also named TIGIRR-2) are localized on the X chromosome and share between 22 and 48%

overall identity to other ILR family members. IL-1RAPL and TIGIRR-1 exons are spread out over a very large segment of genomic DNA (more than 1500 kb for IL-1RAPL and 380 kb for TIGIRR-1). Both TIGIRR-1 and IL-1RAPL contain a signal peptide, three predicted extracellular Ig domains, a single transmembrane domain, and a highly conserved cytoplasmic region containing a C-terminal cytoplasmic extension reminiscent of the *Drosophila* Toll family, TIR8/SIGIRR, and AcPb cytoplasmic domains.

A negative regulatory role has not yet been reported for these two receptors. However, in *in vitro* studies performed with chimeric molecules, the cytoplasmic domains of TIGIRR-1 and IL-1RAPL fused to the extracellular and transmembrane domains of IL-1RI or AcP could not induce NF-κB, similarly to TIR8/SIGIRR, and in contrast with the cytoplasmic domains of other members of the ILR family (10). Other functional studies showed that IL-1RAPL can activate JNK but not the ERK or the p38 MAP kinases, whereas TIGIRR-1 cannot activate JNK. Deletion mutagenesis studies showed that the activation of JNK by IL-1RAPL does not depend on the integrity of its TIR domain, suggesting a distinct mechanism of signaling through this receptor (147).

TIGIRR-1 is highly conserved in human and mouse (94.5% identical at the amino acid level) and it is expressed in skin, liver, placenta, and fetal brain. IL-1RAPL, whose crystal structure has been determined (147), is expressed in heart, brain, ovary, skin, and to a lesser extent in tonsil, fetal liver, prostate, testis, small intestine, placenta, and colon. IL-1RAPL was identified as the gene responsible for hereditary non-syndromic mental retardation and autism linked to chromosome region Xp22.1–21.3 (148, 149). It is expressed in brain structures involved in the hippocampal memory system, and it has a role in brain development and function (150). No information are available about a potential role of IL-1RAPL in inflammation and defense, however, its C-terminal extension is reported to interact with neuronal calcium sensor-1 and regulate neurite outgrowth (150–152).

DIGIRR

Recently, a new member called DIGIRR was added to the ILR family (153). DIGIRR was discovered in teleost fish and showed high homology with TIR8/SIGIRR. DIGIRR is characterized by an extracellular portion comprising two Ig-like domains, a transmembrane domain and TIR domain carrying two amino acid substitutions (Arg419-Tyr420), which are responsible for the loss of signaling. The DIGIRR mRNA was found expressed in several tissues and in leukocytes and was upregulated by LPS, oppositely to TIR8/SIGIRR, suggesting a different mechanism of response to inflammatory stimuli between the two molecules. At the subcellular level, DIGIRR showed a peculiar distribution within the Golgi apparatus.

Different lines of evidence suggest that DIGIRR acts as negative regulator of LPS- and IL-1β-induced inflammation. Indeed, siRNA knock down of DIGIRR lead to increased production of IL-β-induced pro-inflammatory cytokines in liver, kidney, and leukocytes. Moreover, *in vitro* administration of DIGIRR to zebrafish embryos significantly inhibited LPS- and IL-1β-induced activation of NF-kB.

The discovery of DIGIRR could help to understand the evolution of ILR family members. Indeed, the authors suggest the hypothesis that DIGIRR and TIR8/SIGIRR derive from a common ancestral molecule that lost respectively one or two Ig-like extracellular domains, and Ser or Arg-Tyr- amino acids in the TIR domain. DIGIRR might represent an evolutionary intermediate molecule between IL-1R and TIR8/SIGIRR, demonstrating a shift from a potent receptor to a negative regulator.

CONCLUDING REMARKS

Studies conducted in the early 1990s suggesting that the non-signaling IL-1RII acts as a molecular trap for the agonist and the AcP, led to the formulation of the decoy paradigm, which has then been extended to other cytokine families and chemokines. Decoy receptors are now recognized as a general strategy to tune the actions of primary inflammatory cytokines and chemokines.

The list of ILR/TLR family receptors acting as negative regulators now includes TIR8/SIGIRR, which acts by modulating ILRs- or TLRs-dependent signaling. In addition to these molecules, soluble forms of signaling receptors or AcP act as decoys or negative regulators by trapping the ligands. For instance, T1/ST2 exists also as a soluble isoform obtained by differential mRNA processing, which acts as an antagonistic decoy receptor for IL-33 (154), and has been proposed in the therapy of arthritis (155). Similarly, soluble IL-1AcP, generated by alternative splicing, forms a complex with IL-1β and IL-1RII playing a protective role in arthritis (156) and has been pharmacologically exploited.

For several of these molecules further studies have to be performed to unequivocally define their role in disease and their potential as therapeutic targets. For instance, unfortunately there are no genetic evidence on the consequence of IL-1RII-gene deficiency or data supporting the relevance of TIR8/SIGIRR in human disease. In addition, the clinical development of IL-RII pharmacological targeting has not been reported. Finally, pharmacological approaches targeting TIR8/SIGIRR functions have not been developed yet and they will be necessary to assess whether TIR8/SIGIRR might be a therapeutic target in inflammatory conditions.

However, the existence of IL-1RII, together with IL-1Ra, TIR8/SIGIRR, brain AcPb, and other soluble receptors acting as molecular traps emphasizes the need for tight control of the IL-1 system, which mediates potentially devastating local and systemic inflammatory reactions.

ACKNOWLEDGMENTS

The contributions of the European Commission (European Research Council project HIIS, MUGEN LSHG-CT-2005-005203, MUVAPRED LSH-CT-2003-503240 and TIMER HEALTH F4-2011-281608), Ministero dell'Istruzione, dell'Università e della Ricerca [progetto FIRB RBLA039LSF (www.miur.it)], Associazione Italiana per la Ricerca sul Cancro (AIRC), Fondazione CARIPLO, and the Italian Cystic Fibrosis Research Foundation (http://www.fibrosicisticaricerca.it) are gratefully acknowledged.

REFERENCES

1. Takeda K, Kaisho T, Akira S. Toll-like receptors. *Annu Rev Immunol* (2003) **21**:335–76. doi:10.1146/annurev.immunol.21.120601.141126
2. O'Neill LA. The interleukin-1 receptor/Toll-like receptor superfamily: 10 years of progress. *Immunol Rev* (2008) **226**:10–8. doi:10.1111/j.1600-065X.2008.00701.x
3. Dinarello CA. Immunological and inflammatory functions of the interleukin-1 family. *Annu Rev Immunol* (2009) **27**:519–50. doi:10.1146/annurev.immunol.021908.132612
4. Dinarello CA. Anti-inflammatory agents: present and future. *Cell* (2010) **140**:935–50. doi:10.1016/j.cell.2010.02.043
5. van de Veerdonk FL, Netea MG, Dinarello CA, Joosten LA. Inflammasome activation and IL-1beta and IL-18 processing during infection. *Trends Immunol* (2011) **32**:110–6. doi:10.1016/j.it.2011.01.003
6. Barton GM, Medzhitov R. Toll-like receptor signaling pathways. *Science* (2003) **300**:1524–5. doi:10.1126/science.1085536
7. Towne JE, Garka KE, Renshaw BR, Virca GD, Sims JE. Interleukin (IL)-1F6, IL-1F8, and IL-1F9 signal through IL-1Rrp2 and IL-1RAcP to activate the pathway leading to NF-kappaB and MAPKs. *J Biol Chem* (2004) **279**:13677–88. doi:10.1074/jbc.M400117200
8. Arend WP, Palmer G, Gabay C. IL-1, IL-18, and IL-33 families of cytokines. *Immunol Rev* (2008) **223**:20–38. doi:10.1111/j.1600-065X.2008.00624.x
9. Dinarello C, Arend W, Sims J, Smith D, Blumberg H, O'Neill L, et al. IL-1 family nomenclature. *Nat Immunol* (2010) **11**:973. doi:10.1038/ni1110-973
10. Born TL, Smith DE, Garka KE, Renshaw BR, Bertles JS, Sims JE. Identification and characterization of two members of a novel class of the interleukin-1 receptor (IL-1R) family. Delineation of a new class of IL-1R-related proteins based on signaling. *J Biol Chem* (2000) **275**:29946–54. doi:10.1074/jbc.M004077200
11. Hasan U, Chaffois C, Gaillard C, Saulnier V, Merck E, Tancredi S, et al. Human TLR10 is a functional receptor, expressed by B cells and plasmacytoid dendritic cells, which activates gene transcription through MyD88. *J Immunol* (2005) **174**:2942–50.
12. Krelin Y, Voronov E, Dotan S, Elkabets M, Reich E, Fogel M, et al. Interleukin-1beta-driven inflammation promotes the development and invasiveness of chemical carcinogen-induced tumors. *Cancer Res* (2007) **67**:1062–71. doi:10.1158/0008-5472.CAN-06-2956
13. Liew FY, Pitman NI, McInnes IB. Disease-associated functions of IL-33: the new kid in the IL-1 family. *Nat Rev Immunol* (2010) **10**:103–10. doi:10.1038/nri2692
14. Dinarello CA. Interleukin-1 in the pathogenesis and treatment of inflammatory diseases. *Blood* (2011) **117**:3720–32. doi:10.1182/blood-2010-07-273417
15. Towne JE, Sims JE. IL-36 in psoriasis. *Curr Opin Pharmacol* (2012) **12**:486–90. doi:10.1016/j.coph.2012.02.009
16. Mantovani A, Locati M, Polentarutti N, Vecchi A, Garlanda C. Extracellular and intracellular decoys in the tuning of inflammatory cytokines and Toll-like receptors: the new entry TIR8/SIGIRR. *J Leukocyte Biol* (2004) **75**:738–42. doi:10.1189/jlb.1003473
17. Liew FY, Xu D, Brint EK, O'Neill LA. Negative regulation of toll-like receptor-mediated immune responses. *Nat Rev Immunol* (2005) **5**:446–58. doi:10.1038/nri1630
18. Costelloe C, Watson M, Murphy A, McQuillan K, Loscher C, Armstrong ME, et al. IL-1F5 mediates anti-inflammatory activity in the brain through induction of IL-4 following interaction with SIGIRR/TIR8. *J Neurochem* (2008) **105**:1960–9. doi:10.1111/j.1471-4159.2008.05304.x
19. Aksentijevich I, Masters SL, Ferguson PJ, Dancey P, Frenkel J, Van Royen-Kerkhoff A, et al. An autoinflammatory disease with deficiency of the interleukin-1-receptor antagonist. *N Engl J Med* (2009) **360**:2426–37. doi:10.1056/NEJMoa0807865
20. Reddy S, Jia S, Geoffrey R, Lorier R, Suchi M, Broeckel U, et al. An autoinflammatory disease due to homozygous deletion of the IL1RN locus. *N Engl J Med* (2009) **360**:2438–44. doi:10.1056/NEJMoa0809568
21. Kobayashi K, Hernandez LD, Galan JE, Janeway CA Jr., Medzhitov R, Flavell RA. IRAK-M is a negative regulator of Toll-like receptor signaling. *Cell* (2002) **110**:191–202. doi:10.1016/S0092-8674(02)00827-9
22. Janssens S, Burns K, Vercammen E, Tschopp J, Beyaert R. MyD88S, a splice variant of MyD88, differentially modulates NF-kappaB- and AP-1-dependent gene expression. *FEBS Lett* (2003) **548**:103–7. doi:10.1016/S0014-5793(03)00747-6
23. Bazzoni F, Rossato M, Fabbri M, Gaudiosi D, Mirolo M, Mori L, et al. Induction and regulatory function of miR-9 in human monocytes and neutrophils exposed to proinflammatory signals. *Proc Natl Acad Sci U S A* (2009) **106**:5282–7. doi:10.1073/pnas.0810909106
24. Nahid MA, Satoh M, Chan EK. MicroRNA in TLR signaling and endotoxin tolerance. *Cell Mol Immunol* (2011) **8**:388–403. doi:10.1038/cmi.2011.26
25. Quinn SR, O'Neill LA. A trio of microRNAs that control Toll-like receptor signalling. *Int Immunol* (2011) **23**:421–5. doi:10.1093/intimm/dxr034
26. McMahan CJ, Slack JL, Mosley B, Cosman D, Lupton SD, Brunton LL, et al. A novel IL-1 receptor, cloned from B cells by mammalian expression, is expressed in many cell types. *EMBO J* (1991) **10**:2821–32.
27. Colotta F, Re F, Muzio M, Bertini R, Polentarutti N, Sironi M, et al. Interleukin-1 type II receptor: a decoy target for IL-1 that is regulated by IL-4. *Science* (1993) **261**:472–5. doi:10.1126/science.8332913
28. Copeland NG, Silan CM, Kingsley DM, Jenkins NA, Cannizzaro LA, Croce CM, et al. Chromosomal location of murine and human IL-1 receptor genes. *Genomics* (1991) **9**:44–50. doi:10.1016/0888-7543(91)90219-5
29. Morrison RN, Young ND, Nowak BF. Description of an Atlantic salmon (*Salmo salar* L.) type II interleukin-1 receptor cDNA and analysis of interleukin-1 receptor expression in amoebic gill disease-affected fish. *Fish Shellfish Immunol* (2012) **32**:1185–90. doi:10.1016/j.fsi.2012.03.005
30. Novick D, Kim SH, Fantuzzi G, Reznikov LL, Dinarello CA, Rubinstein M. Interleukin-18 binding protein: a novel modulator of the Th1 cytokine response. *Immunity* (1999) **10**:127–36. doi:10.1016/S1074-7613(00)80013-8
31. Watanabe M, Goto N, Watanabe Y, Nishiguchi S, Shimada K, Yasunga T, et al. Evolution of interleukin-18 binding proteins and interleukin-1 receptor, type II proteins. *Int J Mol Med* (2005) **15**:561–6.
32. Mantovani A, Muzio M, Ghezzi P, Colotta C, Introna M. Regulation of inhibitory pathways of the interleukin-1 system. *Ann N Y Acad Sci* (1998) **840**:338–51. doi:10.1111/j.1749-6632.1998.tb09573.x
33. Orlando S, Sironi M, Bianchi G, Drummond AH, Boraschi D, Yabes D, et al. Role of metalloproteases in the release of the IL-1 type II decoy receptor. *J Biol Chem* (1997) **272**:31764–9. doi:10.1074/jbc.272.50.31764
34. Lorenzen I, Lokau J, Dusterhoft S, Trad A, Garbers C, Scheller J, et al. The membrane-proximal domain of A Disintegrin and Metalloprotease 17 (ADAM17) is responsible for recognition of the interleukin-6 receptor and interleukin-1 receptor II. *FEBS Lett* (2012) **586**:1093–100. doi:10.1016/j.febslet.2012.03.012
35. Kuhn PH, Marjaux E, Imhof A, De Strooper B, Haass C, Lichtenthaler SF. Regulated intramembrane proteolysis of the interleukin-1 receptor II by alpha-, beta-, and gamma-secretase. *J Biol Chem* (2007) **282**:11982–95. doi:10.1074/jbc.M700356200
36. Cui X, Rouhani FN, Hawari F, Levine SJ. Shedding of the type II IL-1 decoy receptor requires a multifunctional aminopeptidase, aminopeptidase regulator of TNF receptor type 1 shedding. *J Immunol* (2003) **171**:6814–9.
37. Wang D, Zhang S, Li L, Liu X, Mei K, Wang X. Structural insights into the assembly and activation of IL-1beta with its receptors. *Nat Immunol* (2010) **11**:905–11. doi:10.1038/ni.1925
38. Symons JA, Young PR, Duff GW. Soluble type II interleukin 1 (IL-1) receptor binds and blocks processing of IL-1 beta precursor and loses affinity for IL-1 receptor antagonist. *Proc Natl Acad Sci U S A* (1995) **92**:1714–8. doi:10.1073/pnas.92.5.1714

39. Arend WP, Malyak M, Smith MF Jr., Whisenand TD, Slack JL, Sims JE, et al. Binding of IL-1 alpha, IL-1 beta, and IL-1 receptor antagonist by soluble IL-1 receptors and levels of soluble IL-1 receptors in synovial fluids. *J Immunol* (1994) **153**: 4766–74.
40. Re F, Sironi M, Muzio M, Matteucci C, Introna M, Orlando S, et al. Inhibition of interleukin-1 responsiveness by type II receptor gene transfer: a surface "receptor" with anti-interleukin-1 function. *J Exp Med* (1996) **183**:1841–50. doi:10.1084/jem.183.4.1841
41. Lang D, Knop J, Wesche H, Raffetseder U, Kurrle R, Boraschi D, et al. The type II IL-1 receptor interacts with the IL-1 receptor accessory protein: a novel mechanism of regulation of IL-1 responsiveness. *J Immunol* (1998) **161**:6871–7.
42. Malinowsky D, Lundkvist J, Laye S, Bartfai T. Interleukin-1 receptor accessory protein interacts with the type II interleukin-1 receptor. *FEBS Lett* (1998) **429**:299–302. doi:10.1016/S0014-5793(98)00467-0
43. Liu C, Hart RP, Liu XJ, Clevenger W, Maki RA, De Souza EB. Cloning and characterization of an alternatively processed human type II interleukin-1 receptor mRNA. *J Biol Chem* (1996) **271**:20965–72. doi:10.1074/jbc.271.34.20965
44. Jensen LE, Muzio M, Mantovani A, Whitehead AS. IL-1 signaling cascade in liver cells and the involvement of a soluble form of the IL-1 receptor accessory protein. *J Immunol* (2000) **164**:5277–86.
45. Smith DE, Hanna R, Della F, Moore H, Chen H, Farese AM, et al. The soluble form of IL-1 receptor accessory protein enhances the ability of soluble type II IL-1 receptor to inhibit IL-1 action. *Immunity* (2003) **18**:87–96. doi:10.1016/S1074-7613(02)00514-9
46. Zheng Y, Humphry M, Maguire JJ, Bennett MR, Clarke MC. Intracellular interleukin-1 receptor 2 binding prevents cleavage and activity of interleukin-1alpha, controlling necrosis-induced sterile inflammation. *Immunity* (2013) **38**:285–95. doi:10.1016/j.immuni.2013.01.008
47. Kawaguchi Y, Nishimagi E, Tochimoto A, Kawamoto M, Katsumata Y, Soejima M, et al. Intracellular IL-1alpha-binding proteins contribute to biological functions of endogenous IL-1alpha in systemic sclerosis fibroblasts. *Proc Natl Acad Sci U S A* (2006) **103**:14501–6. doi:10.1073/pnas.0603545103
48. Kobayashi Y, Yamamoto K, Saido T, Kawasaki H, Oppenheim JJ, Matsushima K. Identification of calcium-activated neutral protease as a processing enzyme of human interleukin 1 alpha. *Proc Natl Acad Sci U S A* (1990) **87**:5548–52. doi:10.1073/pnas.87.14.5548
49. Afonina IS, Tynan GA, Logue SE, Cullen SP, Bots M, Luthi AU, et al. Granzyme B-dependent proteolysis acts as a switch to enhance the proinflammatory activity of IL-1alpha. *Mol Cell* (2011) **44**:265–78. doi:10.1016/j.molcel.2011.07.037
50. Clarke MC, Talib S, Figg NL, Bennett MR. Vascular smooth muscle cell apoptosis induces interleukin-1-directed inflammation: effects of hyperlipidemia-mediated inhibition of phagocytosis. *Circ Res* (2010) **106**:363–72. doi:10.1161/CIRCRESAHA.109.208389
51. Rauschmayr T, Groves RW, Kupper TS. Keratinocyte expression of the type 2 interleukin 1 receptor mediates local and specific inhibition of interleukin 1-mediated inflammation. *Proc Natl Acad Sci U S A* (1997) **94**:5814–9. doi:10.1073/pnas.94.11.5814
52. Bessis N, Guery L, Mantovani A, Vecchi A, Sims JE, Fradelizi D, et al. The type II decoy receptor of IL-1 inhibits murine collagen-induced arthritis. *Eur J Immunol* (2000) **30**:867–75. doi:10.1002/1521-4141(200003)30:3<867::AID-IMMU867>3.0.CO;2-M
53. Dawson J, Engelhardt P, Kastelic T, Cheneval D, MacKenzie A, Ramage P. Effects of soluble interleukin-1 type II receptor on rabbit antigen-induced arthritis: clinical, biochemical and histological assessment. *Rheumatology* (1999) **38**:401–6. doi:10.1093/rheumatology/38.5.401
54. Simeoni E, Dudler J, Fleury S, Li J, Pagnotta M, Pascual M, et al. Gene transfer of a soluble IL-1 type 2 receptor-Ig fusion protein improves cardiac allograft survival in rats. *Eur J Cardiothorac Surg* (2007) **31**:222–8. doi:10.1016/j.ejcts.2006.10.042
55. Chang H, Wang Y, Wu W, Li G, Hanawa H, Zou J. Hydrodynamics-based delivery of an interleukin-1 receptor II fusion gene ameliorates rat autoimmune myocarditis by inhibiting IL-1 and Th17 cell polarization. *Int J Mol Med* (2013) **31**:833–40. doi:10.3892/ijmm.2013.1276
56. Khoufache K, Bondza PK, Harir N, Daris M, Leboeuf M, Mailloux J, et al. Soluble human IL-1 receptor type 2 inhibits ectopic endometrial tissue implantation and growth: identification of a novel potential target for endometriosis treatment. *Am J Pathol* (2012) **181**:1197–205. doi:10.1016/j.ajpath.2012.06.022
57. Alcami A, Koszinowski UH. Viral mechanisms of immune evasion. *Immunol Today* (2000) **21**:447–55. doi:10.1016/S0167-5699(00)01699-6
58. Colotta F, Saccani S, Giri JG, Dower SK, Sims JE, Introna M, et al. Regulated expression and release of the IL-1 decoy receptor in human mononuclear phagocytes. *J Immunol* (1996) **156**:2534–41.
59. Martinez FO, Gordon S, Locati M, Mantovani A. Transcriptional profiling of the human monocyte-to-macrophage differentiation and polarization: new molecules and patterns of gene expression. *J Immunol* (2006) **177**:7303–11.
60. Pinteaux E, Parker LC, Rothwell NJ, Luheshi GN. Expression of interleukin-1 receptors and their role in interleukin-1 actions in murine microglial cells. *J Neurochem* (2002) **83**:754–63. doi:10.1046/j.1471-4159.2002.01184.x
61. McNamee EN, Ryan KM, Kilroy D, Connor TJ. Noradrenaline induces IL-1ra and IL-1 type II receptor expression in primary glial cells and protects against IL-1beta-induced neurotoxicity. *Eur J Pharmacol* (2010) **626**:219–28. doi:10.1016/j.ejphar.2009.09.054
62. Docagne F, Campbell SJ, Bristow AF, Poole S, Vigues S, Guaza C, et al. Differential regulation of type I and type II interleukin-1 receptors in focal brain inflammation. *Eur J Neurosci* (2005) **21**:1205–14. doi:10.1111/j.1460-9568.2005.03965.x
63. Tran DQ, Andersson J, Hardwick D, Bebris L, Illei GG, Shevach EM. Selective expression of latency-associated peptide (LAP) and IL-1 receptor type I/II (CD121a/CD121b) on activated human FOXP3+ regulatory T cells allows for their purification from expansion cultures. *Blood* (2009) **113**:5125–33. doi:10.1182/blood-2009-01-199950
64. Mercer F, Kozhaya L, Unutmaz D. Expression and function of TNF and IL-1 receptors on human regulatory T cells. *PLoS ONE* (2010) **5**:e8639. doi:10.1371/journal.pone.0008639
65. Trebec DP, Chandra D, Gramoun A, Li K, Heersche JN, Manolson MF. Increased expression of activating factors in large osteoclasts could explain their excessive activity in osteolytic diseases. *J Cell Biochem* (2007) **101**:205–20. doi:10.1002/jcb.21171
66. Debets R, Hegmans JP, Croughs P, Troost RJ, Prins JB, Benner R, et al. The IL-1 system in psoriatic skin: IL-1 antagonist sphere of influence in lesional psoriatic epidermis. *J Immunol* (1997) **158**:2955–63.
67. Akoum A, Jolicoeur C, Kharfi A, Aube M. Decreased expression of the decoy interleukin-1 receptor type II in human endometriosis. *Am J Pathol* (2001) **158**:481–9. doi:10.1016/S0002-9440(10)63990-9
68. Spriggs MK, Nevens PJ, Grabstein K, Dower SK, Cosman D, Armitage RJ, et al. Molecular characterization of the interleukin-1 receptor (IL-1R) on monocytes and polymorphonuclear cells. *Cytokine* (1992) **4**:90–5. doi:10.1016/1043-4666(92)90042-P
69. Colotta F, Re F, Muzio M, Polentarutti N, Minty A, Caput D, et al. Interleukin-13 induces expression and release of interleukin-1 decoy receptor in human polymorphonuclear cells. *J Biol Chem* (1994) **269**:12403–6.
70. Re F, Muzio M, De Rossi M, Polentarutti N, Giri JG, Mantovani A, et al. The type II "receptor" as a decoy target for interleukin 1 in polymorphonuclear leukocytes: characterization of induction by dexamethasone and ligand binding properties of the released decoy receptor. *J Exp Med* (1994) **179**:739–43. doi:10.1084/jem.179.2.739

71. Dickensheets HL, Donnelly RP. IFN-gamma and IL-10 inhibit induction of IL-1 receptor type I and type II gene expression by IL-4 and IL-13 in human monocytes. *J Immunol* (1997) **159**:6226–33.
72. Kalliolias GD, Gordon RA, Ivashkiv LB. Suppression of TNF-alpha and IL-1 signaling identifies a mechanism of homeostatic regulation of macrophages by IL-27. *J Immunol* (2010) **185**:7047–56. doi:10.4049/jimmunol.1001290
73. Daun JM, Ball RW, Burger HR, Cannon JG. Aspirin-induced increases in soluble IL-1 receptor type II concentrations in vitro and in vivo. *J Leukocyte Biol* (1999) **65**:863–6.
74. Penton-Rol G, Orlando S, Polentarutti N, Bernasconi S, Muzio M, Introna M, et al. Bacterial lipopolysaccharide causes rapid shedding, followed by inhibition of mRNA expression, of the IL-1 type II receptor, with concomitant up-regulation of the type I receptor and induction of incompletely spliced transcripts. *J Immunol* (1999) **162**:2931–8.
75. Colotta F, Orlando S, Fadlon EJ, Sozzani S, Matteucci C, Mantovani A. Chemoattractants induce rapid release of the interleukin 1 type II decoy receptor in human polymorphonuclear cells. *J Exp Med* (1995) **181**:2181–6. doi:10.1084/jem.181.6.2181
76. Orlando S, Matteucci C, Fadlon EJ, Buurman WA, Bardella MT, Colotta F, et al. TNF-alpha, unlike other pro- and anti-inflammatory cytokines, induces rapid release of the IL-1 type II decoy receptor in human myelomonocytic cells. *J Immunol* (1997) **158**:3861–8.
77. Pou J, Martinez-Gonzalez J, Rebollo A, Rodriguez C, Rodriguez-Calvo R, Martin-Fuentes P, et al. Type II interleukin-1 receptor expression is reduced in monocytes/macrophages and atherosclerotic lesions. *Biochim Biophys Acta* (2011) **1811**:556–63. doi:10.1016/j.bbalip.2011.05.014
78. Jouvenne P, Vannier E, Dinarello CA, Miossec P. Elevated levels of soluble interleukin-1 receptor type II and interleukin-1 receptor antagonist in patients with chronic arthritis: correlations with markers of inflammation and joint destruction. *Arthritis Rheum* (1998) **41**:1083–9. doi:10.1002/1529-0131(199806)41:6<1083::AID-ART15>3.0.CO;2-9
79. Giri JG, Wells J, Dower SK, McCall CE, Guzman RN, Slack J, et al. Elevated levels of shed type II IL-1 receptor in sepsis. Potential role for type II receptor in regulation of IL-1 responses. *J Immunol* (1994) **153**:5802–9.
80. Muller B, Peri G, Doni A, Perruchoud AP, Landmann R, Pasqualini F, et al. High circulating levels of the IL-1 type II decoy receptor in critically ill patients with sepsis: association of high decoy receptor levels with glucocorticoid administration. *J Leukoc Biol* (2002) **72**:643–9.
81. Chan KY, Leung FW, Lam HS, Tam YH, To KF, Cheung HM, et al. Immunoregulatory protein profiles of necrotizing enterocolitis versus spontaneous intestinal perforation in preterm infants. *PLoS ONE* (2012) **7**:e36977. doi:10.1371/journal.pone.0036977
82. van Deuren M, Van Der Ven-Jongekrijg J, Vannier E, Van Dalen R, Pesman G, Bartelink AK, et al. The pattern of interleukin-1beta (IL-1beta) and its modulating agents IL-1 receptor antagonist and IL-1 soluble receptor type II in acute meningococcal infections. *Blood* (1997) **90**:1101–8.
83. Dujmovic I, Mangano K, Pekmezovic T, Quattrocchi C, Mesaros S, Stojsavljevic N, et al. The analysis of IL-1 beta and its naturally occurring inhibitors in multiple sclerosis: the elevation of IL-1 receptor antagonist and IL-1 receptor type II after steroid therapy. *J Neuroimmunol* (2009) **207**:101–6. doi:10.1016/j.jneuroim.2008.11.004
84. Garlind A, Brauner A, Hojeberg B, Basun H, Schultzberg M. Soluble interleukin-1 receptor type II levels are elevated in cerebrospinal fluid in Alzheimer's disease patients. *Brain Res* (1999) **826**:112–6. doi:10.1016/S0006-8993(99)01092-6
85. Attur MG, Dave M, Cipolletta C, Kang P, Goldring MB, Patel IR, et al. Reversal of autocrine and paracrine effects of interleukin 1 (IL-1) in human arthritis by type II IL-1 decoy receptor. Potential for pharmacological intervention. *J Biol Chem* (2000) **275**:40307–15. doi:10.1074/jbc.M002721200
86. Grundtman C, Salomonsson S, Dorph C, Bruton J, Andersson U, Lundberg IE. Immunolocalization of interleukin-1 receptors in the sarcolemma and nuclei of skeletal muscle in patients with idiopathic inflammatory myopathies. *Arthritis Rheum* (2007) **56**:674–87. doi:10.1002/art.22388
87. Ruckert F, Dawelbait G, Winter C, Hartmann A, Denz A, Ammerpohl O, et al. Examination of apoptosis signaling in pancreatic cancer by computational signal transduction analysis. *PLoS ONE* (2010) **5**:e12243. doi:10.1371/journal.pone.0012243
88. Ricote M, Garcia-Tunon I, Bethencourt FR, Fraile B, Paniagua R, Royuela M. Interleukin-1 (IL-1alpha and IL-1beta) and its receptors (IL-1RI, IL-1RII, and IL-1Ra) in prostate carcinoma. *Cancer* (2004) **100**:1388–96. doi:10.1002/cncr.20142
89. Laios A, O'Toole SA, Flavin R, Martin C, Ring M, Gleeson N, et al. An integrative model for recurrence in ovarian cancer. *Mol Cancer* (2008) **7**:8. doi:10.1186/1476-4598-7-8
90. Akoum A, Al-Akoum M, Lemay A, Maheux R, Leboeuf M. Imbalance in the peritoneal levels of interleukin 1 and its decoy inhibitory receptor type II in endometriosis women with infertility and pelvic pain. *Fertil Steril* (2008) **89**:1618–24. doi:10.1016/j.fertnstert.2007.06.019
91. Keita M, Ainmelk Y, Pelmus M, Bessette P, Aris A. Endometrioid ovarian cancer and endometriotic cells exhibit the same alteration in the expression of interleukin-1 receptor II: to a link between endometriosis and endometrioid ovarian cancer. *J Obstet Gynaecol Res* (2011) **37**:99–107. doi:10.1111/j.1447-0756.2010.01320.x
92. Chun S, Kim H, Ku SY, Suh CS, Kim SH, Kim JG. The association between endometriosis and polymorphisms in the interleukin-1 family genes in Korean women. *Am J Repr Immunol* (2012) **68**:154–63. doi:10.1111/j.1600-0897.2012.01136.x
93. Vambutas A, Devoti J, Goldofsky E, Gordon M, Lesser M, Bonagura V. Alternate splicing of interleukin-1 receptor type II (IL1R2) in vitro correlates with clinical glucocorticoid responsiveness in patients with AIED. *PLoS ONE* (2009) **4**:e5293. doi:10.1371/journal.pone.0005293
94. Herrmann-Lavoie C, Rao CV, Akoum A. Chorionic gonadotropin down-regulates the expression of the decoy inhibitory interleukin 1 receptor type II in human endometrial epithelial cells. *Endocrinology* (2007) **148**:5377–84. doi:10.1210/en.2007-0368
95. Gillespie J, Mathews R, McDermott MF. Rilonacept in the management of cryopyrin-associated periodic syndromes (CAPS). *J Inflamm Res* (2010) **3**:1–8.
96. Dinarello CA, Simon A, Van Der Meer JW. Treating inflammation by blocking interleukin-1 in a broad spectrum of diseases. *Nat Rev Drug Discov* (2012) **11**:633–52. doi:10.1038/nrd3800
97. Thomassen E, Renshaw BR, Sims JE. Identification and characterization of SIGIRR, a molecule representing a novel subtype of the IL-1R superfamily. *Cytokine* (1999) **11**:389–99. doi:10.1006/cyto.1998.0452
98. Lech M, Garlanda C, Mantovani A, Kirschning CJ, Schlondorff D, Anders HJ. Different roles of TiR8/Sigirr on toll-like receptor signaling in intrarenal antigen-presenting cells and tubular epithelial cells. *Kidney Int* (2007) **72**:182–92. doi:10.1038/sj.ki.5002293
99. Riva F, Polentarutti N, Tribbioli G, Mantovani A, Garlanda C, Turin L. The expression pattern of TIR8 is conserved among vertebrates. *Vet Immunol Immunopathol* (2009) **131**:44–9. doi:10.1016/j.vetimm.2009.03.009
100. Polentarutti N, Rol GP, Muzio M, Bosisio D, Camnasio M, Riva F, et al. Unique pattern of expression and inhibition of IL-1 signaling by the IL-1 receptor family member TIR8/SIGIRR. *Eur Cytokine Netw* (2003) **14**:211–8.
101. Kadota C, Ishihara S, Aziz MM, Rumi MA, Oshima N, Mishima Y, et al. Down-regulation of single immunoglobulin interleukin-1R-related molecule (SIGIRR)/TIR8 expression in intestinal epithelial cells during inflammation. *Clin Exp Immunol* (2010) **162**:348–61. doi:10.1111/j.1365-2249.2010.04254.x
102. Wald D, Qin J, Zhao Z, Qian Y, Naramura M, Tian L, et al. SIGIRR, a negative regulator of Toll-like receptor-interleukin 1 receptor signaling. *Nat Immunol* (2003) **4**:920–7. doi:10.1038/ni968

103. Huang X, Hazlett LD, Du W, Barrett RP. SIGIRR promotes resistance against *Pseudomonas aeruginosa* keratitis by down-regulating type-1 immunity and IL-1R1 and TLR4 signaling. *J Immunol* (2006) **177**:548–56.
104. Ragnarsdottir B, Samuelsson M, Gustafsson MC, Leijonhufvud I, Karpman D, Svanborg C. Reduced toll-like receptor 4 expression in children with asymptomatic bacteriuria. *J Infect Dis* (2007) **196**:475–84. doi:10.1086/518893
105. Gopal R, Birdsell D, Monroy FP. Regulation of toll-like receptors in intestinal epithelial cells by stress and Toxoplasma gondii infection. *Parasite Immunol* (2008) **30**:563–76. doi:10.1111/j.1365-3024.2008.01055.x
106. Veliz Rodriguez T, Moalli F, Polentarutti N, Paroni M, Bonavita E, Anselmo A, et al. Role of Toll interleukin-1 receptor (IL-1R) 8, a negative regulator of IL-1R/Toll-like receptor signaling, in resistance to acute *Pseudomonas aeruginosa* lung infection. *Infect Immun* (2012) **80**:100–9. doi:10.1128/IAI.05695-11
107. Nanthakumar N, Meng D, Goldstein AM, Zhu W, Lu L, Uauy R, et al. The mechanism of excessive intestinal inflammation in necrotizing enterocolitis: an immature innate immune response. *PLoS ONE* (2011) **6**:e17776. doi:10.1371/journal.pone.0017776
108. Adib-Conquy M, Adrie C, Fitting C, Gattolliat O, Beyaert R, Cavaillon JM. Up-regulation of MyD88s and SIGIRR, molecules inhibiting Toll-like receptor signaling, in monocytes from septic patients. *Crit Care Med* (2006) **34**:2377–85. doi:10.1097/01.CCM.0000233875.93866.88
109. Bulek K, Swaidani S, Qin J, Lu Y, Gulen MF, Herjan T, et al. The essential role of single Ig IL-1 receptor-related molecule/Toll IL-1R8 in regulation of Th2 immune response. *J Immunol* (2009) **182**:2601–9. doi:10.4049/jimmunol.0802729
110. Jiang X, McClellan SA, Barrett RP, Zhang Y, Hazlett LD. Vasoactive intestinal peptide downregulates proinflammatory TLRs while upregulating anti-inflammatory TLRs in the infected cornea. *J Immunol* (2012) **189**:269–78. doi:10.4049/jimmunol.1200365
111. Villena J, Suzuki R, Fujie H, Chiba E, Takahashi T, Tomosada Y, et al. Immunobiotic *Lactobacillus jensenii* modulates the Toll-like receptor 4-induced inflammatory response via negative regulation in porcine antigen-presenting cells. *Clin Vaccine Immunol* (2012) **19**:1038–53. doi:10.1128/CVI.00199-12
112. Davies JM, MacSharry J, Shanahan F. Differential regulation of Toll-like receptor signalling in spleen and Peyer's patch dendritic cells. *Immunology* (2010) **131**:438–48. doi:10.1111/j.1365-2567.2010.03317.x
113. Garlanda C, Riva F, Polentarutti N, Buracchi C, Sironi M, De Bortoli M, et al. Intestinal inflammation in mice deficient in Tir8, an inhibitory member of the IL-1 receptor family. *Proc Natl Acad Sci U S A* (2004) **101**:3522–6. doi:10.1073/pnas.0308680101
114. Qin J, Qian Y, Yao J, Grace C, Li X. SIGIRR inhibits interleukin-1 receptor- and toll-like receptor 4-mediated signaling through different mechanisms. *J Biol Chem* (2005) **280**:25233–41. doi:10.1074/jbc.M501363200
115. Lech M, Kulkarni OP, Pfeiffer S, Savarese E, Krug A, Garlanda C, et al. Tir8/Sigirr prevents murine lupus by suppressing the immunostimulatory effects of lupus autoantigens. *J Exp Med* (2008) **205**:1879–88. doi:10.1084/jem.20072646
116. Gong J, Wei T, Stark RW, Jamitzky F, Heckl WM, Anders HJ, et al. Inhibition of Toll-like receptors TLR4 and 7 signaling pathways by SIGIRR: a computational approach. *J Struct Biol* (2010) **169**:323–30. doi:10.1016/j.jsb.2009.12.007
117. Gulen MF, Kang Z, Bulek K, Youzhong W, Kim TW, Chen Y, et al. The receptor SIGIRR suppresses Th17 cell proliferation via inhibition of the interleukin-1 receptor pathway and mTOR kinase activation. *Immunity* (2010) **32**:54–66. doi:10.1016/j.immuni.2009.12.003
118. Xiao H, Yin W, Khan MA, Gulen MF, Zhou H, Sham HP, et al. Loss of single immunoglobulin interlukin-1 receptor-related molecule leads to enhanced colonic polyposis in Apc(min) mice. *Gastroenterology* (2010) **139**:574–85. doi:10.1053/j.gastro
119. Garlanda C, Di Liberto D, Vecchi A, La Manna MP, Buracchi C, Caccamo N, et al. Damping excessive inflammation and tissue damage in *Mycobacterium tuberculosis* infection by Toll IL-1 receptor 8/single Ig IL-1-related receptor, a negative regulator of IL-1/TLR signaling. *J Immunol* (2007) **179**:3119–25.
120. Bozza S, Zelante T, Moretti S, Bonifazi P, Deluca A, D'Angelo C, et al. Lack of Toll IL-1R8 exacerbates Th17 cell responses in fungal infection. *J Immunol* (2008) **180**:4022–31.
121. Xiao H, Gulen MF, Qin J, Yao J, Bulek K, Kish D, et al. The Toll-interleukin-1 receptor member SIGIRR regulates colonic epithelial homeostasis, inflammation, and tumorigenesis. *Immunity* (2007) **26**:461–75. doi:10.1016/j.immuni.2007.02.012
122. Rakoff-Nahoum S, Paglino J, Eslami-Varzaneh F, Edberg S, Medzhitov R. Recognition of commensal microflora by toll-like receptors is required for intestinal homeostasis. *Cell* (2004) **118**:229–41. doi:10.1016/j.cell.2004.07.002
123. Karin M, Lawrence T, Nizet V. Innate immunity gone awry: linking microbial infections to chronic inflammation and cancer. *Cell* (2006) **124**:823–35. doi:10.1016/j.cell.2006.02.016
124. Chen X, Zhao Y, Wu X, Qian G. Enhanced expression of single immunoglobulin IL-1 receptor-related molecule ameliorates LPS-induced acute lung injury in mice. *Shock* (2011) **35**:198–204. doi:10.1097/SHK.0b013e3181f226f3
125. Leemans JC, Butter LM, Teske GJ, Stroo I, Pulskens WP, Florquin S. The toll interleukin-1 receptor (IL-1R) 8/single Ig domain IL-1R-related molecule modulates the renal response to bacterial infection. *Infect Immunity* (2012) **80**:3812–20. doi:10.1128/IAI.00422-12
126. Lech M, Skuginna V, Kulkarni OP, Gong J, Wei T, Stark RW, et al. Lack of SIGIRR/TIR8 aggravates hydrocarbon oil-induced lupus nephritis. *J Pathol* (2010) **220**:596–607. doi:10.1002/path.2678
127. Drexler SK, Kong P, Inglis J, Williams RO, Garlanda C, Mantovani A, et al. SIGIRR/TIR-8 is an inhibitor of Toll-like receptor signaling in primary human cells and regulates inflammation in models of rheumatoid arthritis. *Arthritis Rheum* (2010) **62**:2249–61. doi:10.1002/art.27517
128. Batliwalla FM, Li W, Ritchlin CT, Xiao X, Brenner M, Laragione T, et al. Microarray analyses of peripheral blood cells identifies unique gene expression signature in psoriatic arthritis. *Mol Med* (2005) **11**:21–9. doi:10.2119/2006-00003.Gulko
129. Lech M, Avila-Ferrufino A, Allam R, Segerer S, Khandoga A, Krombach F, et al. Resident dendritic cells prevent postischemic acute renal failure by help of single Ig IL-1 receptor-related protein. *J Immunol* (2009) **183**:4109–18. doi:10.4049/jimmunol.0900118
130. Noris M, Cassis P, Azzollini N, Cavinato R, Cugini D, Casiraghi F, et al. The Toll-IL-1R member Tir8/SIGIRR negatively regulates adaptive immunity against kidney grafts. *J Immunol* (2009) **183**:4249–60. doi:10.4049/jimmunol.0803549
131. Andre R, Lerouet D, Kimber I, Pinteaux E, Rothwell NJ. Regulation of expression of the novel IL-1 receptor family members in the mouse brain. *J Neurochem* (2005) **95**:324–30. doi:10.1111/j.1471-4159.2005.03364.x
132. Watson MB, Costello DA, Carney DG, McQuillan K, Lynch MA. SIGIRR modulates the inflammatory response in the brain. *Brain Behav Immun* (2010) **24**:985–95. doi:10.1016/j.bbi.2010.04.002
133. Costello DA, Watson MB, Cowley TR, Murphy N, Murphy Royal C, Garlanda C, et al. Interleukin-1alpha and HMGB1 mediate hippocampal dysfunction in SIGIRR-deficient mice. *J Neurosci* (2011) **31**:3871–9. doi:10.1523/JNEUROSCI.6676-10.2011
134. Voronov E, Shouval DS, Krelin Y, Cagnano E, Benharroch D, Iwakura Y, et al. IL-1 is required for tumor invasiveness and angiogenesis. *Proc Natl Acad Sci U S A* (2003) **100**:2645–50. doi:10.1073/pnas.0437939100
135. Colotta F, Allavena P, Sica A, Garlanda C, Mantovani A. Cancer-related inflammation, the seventh hallmark of cancer: links to genetic instability. *Carcinogenesis* (2009) **30**:1073–81. doi:10.1093/carcin/bgp127
136. Garlanda C, Riva F, Veliz T, Polentarutti N, Pasqualini F, Radaelli

E, et al. Increased susceptibility to colitis-associated cancer of mice lacking TIR8, an inhibitory member of the interleukin-1 receptor family. *Cancer Res* (2007) **67**:6017–21. doi:10.1158/0008-5472.CAN-07-0560

137. Karin M. Nuclear factor-kappaB in cancer development and progression. *Nature* (2006) **441**:431–6. doi:10.1038/nature04870
138. Aoki K, Tamai Y, Horiike S, Oshima M, Taketo MM. Colonic polyposis caused by mTOR-mediated chromosomal instability in Apc+/Delta716 Cdx2+ compound mutant mice. *Nat Genet* (2003) **35**:323–30. doi:10.1038/ng1265
139. Muzio M, Scielzo C, Bertilaccio MT, Frenquelli M, Ghia P, Caligaris-Cappio F. Expression and function of toll like receptors in chronic lymphocytic leukaemia cells. *Br J Haematol* (2009) **144**:507–16. doi:10.1111/j.1365-2141.2008.07475.x
140. Arvaniti E, Ntoufa S, Papakonstantinou N, Touloumenidou T, Laoutaris N, Anagnostopoulos A, et al. Toll-like receptor signaling pathway in chronic lymphocytic leukemia: distinct gene expression profiles of potential pathogenic significance in specific subsets of patients. *Haematologica* (2011) **96**:1644–52. doi:10.3324/haematol.2011.044792
141. Bertilaccio MT, Simonetti G, Dagklis A, Rocchi M, Rodriguez TV, Apollonio B, et al. Lack of TIR8/SIGIRR triggers progression of chronic lymphocytic leukemia in mouse models. *Blood* (2011) **118**:660–9. doi:10.1182/blood-2011-01-329870
142. Muzio M, Bertilaccio MT, Simonetti G, Frenquelli M, Caligaris-Cappio F. The role of toll-like receptors in chronic B-cell malignancies. *Leuk Lymphoma* (2009) **50**:1573–80. doi:10.1080/10428190903115410
143. Bufler P, Azam T, Gamboni-Robertson F, Reznikov LL, Kumar S, Dinarello CA, et al. A complex of the IL-1 homologue IL-1F7b and IL-18-binding protein reduces IL-18 activity. *Proc Natl Acad Sci U S A* (2002) **99**:13723–8. doi:10.1073/pnas.212519099
144. Xiang Y, Moss B. Correspondence of the functional epitopes of poxvirus and human interleukin-18-binding proteins. *J Virol* (2001) **75**:9947–54. doi:10.1128/JVI.75.20.9947-9954.2001
145. Lu HL, Yang CY, Chen HC, Hung CS, Chiang YC, Ting LP. A novel alternatively spliced interleukin-1 receptor accessory protein mIL-1RAcP687. *Mol Immunol* (2008) **45**:1374–84. doi:10.1016/j.molimm.2007.09.002
146. Smith DE, Lipsky BP, Russell C, Ketchem RR, Kirchner J, Hensley K, et al. A central nervous system-restricted isoform of the interleukin-1 receptor accessory protein modulates neuronal responses to interleukin-1. *Immunity* (2009) **30**:817–31. doi:10.1016/j.immuni.2009.03.020
147. Khan JA, Brint EK, O'Neill LA, Tong L. Crystal structure of the Toll/interleukin-1 receptor domain of human IL-1RAPL. *J Biol Chem* (2004) **279**:31664–70. doi:10.1074/jbc.M403434200
148. Ferrante MI, Ghiani M, Bulfone A, Franco B. IL1RAPL2 maps to Xq22 and is specifically expressed in the central nervous system. *Gene* (2001) **275**:217–21. doi:10.1016/S0378-1119(01)00659-X
149. Tabolacci E, Pomponi MG, Pietrobono R, Terracciano A, Chiurazzi P, Neri G. A truncating mutation in the IL1RAPL1 gene is responsible for X-linked mental retardation in the MRX21 family. *Am J Med Genet* (2006) **140**:482–7. doi:10.1002/ajmg.a.31107
150. Gao X, Xi G, Niu Y, Zhang S, Fu R, Zheng Z, et al. A study on the correlation between IL1RAPL1 and human cognitive ability. *Neurosci Lett* (2008) **438**:163–7. doi:10.1016/j.neulet.2008.03.084
151. Bahi N, Friocourt G, Carrie A, Graham ME, Weiss JL, Chafey P, et al. IL1 receptor accessory protein like, a protein involved in X-linked mental retardation, interacts with Neuronal Calcium Sensor-1 and regulates exocytosis. *Hum Mol Genet* (2003) **12**:1415–25. doi:10.1093/hmg/ddg147
152. Piton A, Michaud JL, Peng H, Aradhya S, Gauthier J, Mottron L, et al. Mutations in the calcium-related gene IL1RAPL1 are associated with autism. *Hum Mol Genet* (2008) **17**:3965–74. doi:10.1093/hmg/ddn300
153. Gu YF, Fang Y, Jin Y, Dong WR, Xiang LX, Shao JZ. Discovery of the DIGIRR gene from teleost fish: a novel Toll-IL-1 receptor family member serving as a negative regulator of IL-1 signaling. *J Immunol* (2011) **187**:2514–30. doi:10.4049/jimmunol.1003457
154. Hayakawa H, Hayakawa M, Kume A, Tominaga S. Soluble ST2 blocks interleukin-33 signaling in allergic airway inflammation. *J Biol Chem* (2007) **282**:26369–80. doi:10.1074/jbc.M704916200
155. Leung BP, Xu D, Culshaw S, McInnes IB, Liew FY. A novel therapy of murine collagen-induced arthritis with soluble T1/ST2. *J Immunol* (2004) **173**:145–50.
156. Smeets RL, Joosten LA, Arntz OJ, Bennink MB, Takahashi N, Carlsen H, et al. Soluble interleukin-1 receptor accessory protein ameliorates collagen-induced arthritis by a different mode of action from that of interleukin-1 receptor antagonist. *Arthritis Rheum* (2005) **52**:2202–11. doi:10.1002/art.21108

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

Received: 03 May 2013; accepted: 22 June 2013; published online: 09 July 2013.
*Citation: Garlanda C, Riva F, Bonavita E, Gentile S and Mantovani A (2013) Decoys and regulatory "receptors" of the IL-1/Toll-like receptor superfamily. Front. Immunol. **4**:180. doi: 10.3389/fimmu.2013.00180*
This article was submitted to Frontiers in Inflammation, a specialty of Frontiers in Immunology.

frontiers in
IMMUNOLOGY

REVIEW ARTICLE
published: 08 July 2013
doi: 10.3389/fimmu.2013.00177

Unique versus redundant functions of IL-1α and IL-1β in the tumor microenvironment

Elena Voronov, Shahar Dotan, Yakov Krelin, Xiaoping Song, Moshe Elkabets, Yaron Carmi, Peleg Rider, Idan Cohen, Marianna Romzova, Irena Kaplanov and Ron N. Apte*

The Shraga Segal Department of Microbiology, Immunology and Genetics, The Faculty of Health Sciences, Ben Gurion University of the Negev, Beer Sheva, Israel

Edited by:
Cecilia Garlanda, Istituto Clinico Humanitas, Italy

Reviewed by:
Silvano Sozzani, University of Brescia, Italy
Daisuke Kamimura, Osaka University, Japan

***Correspondence:**
Ron N. Apte, The Shraga Segal Department of Microbiology, Immunology and Genetics, The Faculty of Health Sciences, Ben Gurion University of the Negev, Beer Sheva 84105, Israel
e-mail: rapte@bgu.ac.il

Interleukin-1 (IL-1) is a major "alarm" upstream pro-inflammatory cytokine that also affects immunity and hematopoiesis by inducing cytokine cascades. In the tumor arena, IL-1 is produced by malignant or microenvironmental cells. As a pleiotropic cytokine, IL-1 is involved in tumorigenesis and tumor invasiveness but also in the control of anti-tumor immunity. IL-1α and IL-1β are the major agonists of IL-1, while IL-1Ra is a physiological inhibitor of preformed IL-1. In their secreted form, IL-1α and IL-1β bind to the same receptors and induce the same biological functions, but IL-1α and IL-1β differ in their compartmentalization within the producing cell or the microenvironment. IL-1β is only active in its processed, secreted form, and mediates inflammation, which promotes carcinogenesis, tumor invasiveness, and immunosuppression, whereas IL-1α is mainly cell-associated and in the tumor context, when expressed on the cell membrane, it stimulates anti-tumor cell immunity manifested by tumor regression. In the tumor milieu, extracellular levels of IL-1α are usually low and do not stimulate broad inflammation that promotes progression. Immunosuppression induced by IL-1β in the tumor microenvironment, mainly through MDSC induction, usually inhibits or masks anti-tumor cell immunity induced by cell-associated IL-1α. However, in different tumor systems, redundant or unique patterns of IL-1α and IL-1β expression and function have been observed. Recent breakthroughs in inflammasome biology and IL-1β processing/secretion have spurred the development of novel anti-IL-1 agents, which are being used in clinical trials in patients with diverse inflammatory diseases. Better understanding of the integrative role of IL-1α and IL-1β in distinct malignancies will facilitate the application of novel IL-1 modulation approaches at the bedside, in cancer patients with minimal residual disease (MRD), as an adjunct to conventional approaches to reduce the tumor burden.

Keywords: IL-1, carcinogenesis, tumor invasiveness, tumor-host interactions, immunogenicity, anti-tumor immunity, immunotherapy

EXPRESSION AND SECRETION OF IL-1α AND IL-1β – THE MAJOR IL-1 AGONISTIC MOLECULES

The IL-1 family consists of 11 agonist and antagonist molecules that are centrally involved in regulating inflammatory responses. These include IL-1α, IL-1β, IL-1Ra, IL-18, IL-33, IL-1Ra, IL-36α, IL-36β IL-36γ, and IL-38 [reviewed in Ref. (1–7)]. Here, we will mainly focus on the two major IL-1 agonistic molecules, i.e., IL-1α and IL-1β, and IL-1 receptor antagonist (IL-Ra), which is a physiological inhibitor of IL-1 signaling.

IL-1α and IL-1β are synthesized as precursors of 31 kDa that are further processed by proteases to their mature secreted 17 kDa forms. IL-1 differs from most other cytokines by lack of a signal sequence, thus not passing through the endoplasmic reticulum-Golgi pathway; its mechanisms of secretion are not yet completely understood. IL-1Ra, which has a signal peptide, is secreted in the ER-Golgi exocytic pathway. Generally, IL-1 is produced and secreted by various cell types upon inflammatory or stress conditions, predominantly by myeloid cells, which display the strongest capacity to produce and secrete IL-1. Stimulation of IL-1 production occurs through signaling of Toll-like receptors (TLRs), which recognize conserved microbial molecules of pathogens, i.e., pathogen-associated molecular patterns (PAMPs) [reviewed in Ref. (8–10)] as well as endogenous molecules, which are products of damaged cells, termed danger-associated molecular patterns (DAMPs) [reviewed in Ref. (11–13)]. The activation of TLR signaling via the NF-κB pathway leads to the generation of many cytokines; IL-1 is a central cytokine produced by this pathway. Signaling through surface IL-1Rs and most of the TLRs is common and converges from MyD88 to NF-κB activation and induction of an inflammatory response, including expression of IL-1.

PROCESSING OF IL-1β

The IL-1β-converting enzyme (ICE), or caspase-1, is a cysteine protease, that is activated in the cytosol on the inflammasome platform, and subsequently cleaves the inactive precursors of IL-1β, IL-18, and IL-33 into their mature secreted forms (2, 3, 14–18).

PROCESSING OF IL-1α

The precursor of IL-1α (ProIL-1α) is processed by the Ca^{2+}-dependent protease calpain into the mature 17 kDa form and the 16 kDa N-terminal cleavage product – the propiece of IL-1α, also termed IL-1α N-terminal peptide (IL-1NTP). The latent form of

calpain is activated in cells under inflammatory conditions and especially upon loss of plasma membrane integrity, which occurs during necrosis (19). However, intracellular ProIL-1α is present in many cells because they contain calpain inhibitors and are thus unable to process and secrete IL-1α. Other proteases, such as elastase, chymase, or Granzyme B can also process ProIL-1α into smaller molecules with high inflammatory potential (20–22). Recently some involvement of the inflammasome in IL-1α secretion has been demonstrated (23–25). A biologically active membrane-associated form of IL-1α (23 kDa), which is anchored to the membrane via a mannose-like receptor, has been demonstrated in activated cells that express the cytokine. However, it is not clear how IL-1α is inserted in the membrane.

IL-1 RECEPTORS

IL-1α and IL-1β signal through the same IL-1Rs, which belong to the immunoglobulin (Ig) supergene family and are abundantly expressed on many cell types. IL-1R of type I (IL-1R1) (80 kDa) is a signaling receptor, whereas the IL-1R of type II (IL-1R2) (68 kDa) serves as a decoy target, acting to reduce excessive amounts of IL-1 [reviewed in Ref. (1–7)]. Following the binding of IL-1 to IL-1R1, a second chain, i.e., the IL-1R acceptor protein (IL-1RAcP) is recruited. This heterodimeric complex triggers IL-1 signaling by activating the IL-1 receptor-associated kinase (IRAK) and ultimately leads to activation of NF-κB and its target genes. On the contrary, IL-1R2 and the IL-1Ra do not form this heterodimeric complex with the IL-1RAcP and therefore do not recruit IRAK. Signaling through surface IL-1R1 represents an evolutionary conserved mechanism homologous to the TLR pathway.

MAJOR BIOLOGICAL ACTIVITIES OF IL-1

EFFECTS OF IL-1 ON INFLAMMATORY RESPONSES

IL-1α and IL-1β are defined as "alarm cytokines" that are secreted by macrophages and initiate inflammatory responses, by inducing a cascade of other pro-inflammatory genes [reviewed in Ref. (1–7)]. Of major importance are cyclooxygenase type 2 (COX-2), inducible nitric oxide synthase (iNOS), chemokines/cytokines, and matrix metalloproteinases (MMPs). The IL-1 molecules stimulate their own and each other's production; this represents an important amplification loop of the inflammatory response. Also, IL-1 increases the expression of integrins on endothelial cells, stromal cells, and leukocytes and thereby promotes cell infiltration into inflamed tissues.

Recently, the unique alarmin function of ProIL-1α in sterile inflammation has been described by us and others (26–30). In tissue cells, such as epithelial cells, endothelial cells, and fibroblasts, ProIL-1α is located in the cytosol and nucleus. Upon stress induction, expression of ProIL-1α increases and it translocates into the nucleus, where it is bound to chromatin in a highly dynamic manner. In stressed cells, i.e., in hypoxic conditions, ProIL-1α expression is initially increased, with the involvement of the transcription factors HIF-1α and HIF-1β. Upon necrotic cell death, ProIL-1α is released and induces inflammation (31). However, following apoptotic death, the mobility of IL-1α is greatly reduced, it concentrates in dense nuclear foci and is not released into the environment (27). This represents a novel mechanism that explains why inflammatory responses are not generated upon apoptosis. Necrotic cells lacking IL-1α failed to induce this early inflammatory response. The early infiltrate found in Matrigel plugs containing lysates of necrotic cells consists mainly of neutrophils and myeloid progenitor cells; recruitment of cells is via IL-1R1 signaling (27, 29). Macrophages infiltrate such Matrigel plugs at later times and they actively secrete IL-1β, which terminates inflammation, resulting in wound healing and restoration of tissue homeostasis (29). These results indicate significant differences in the capacity of the major IL-1 agonistic molecules to alarm inflammatory cells, thereby controlling the inflammatory response. Recently, a novel mechanism to control IL-1α activity in necrotic cells has been described by Zheng et al. (21) and reviewed in Ref. (22). Zheng found that under normal conditions, IL-1α is synthesized as a p33 precursor that is sequestered in the cytosol by IL-1R2 where it cannot be cleaved by proteases or activate IL-1R1 signaling. However, after inflammasome activation, IL-1R2 can be cleaved by caspase-1 and ProIL-1α can be released and further processed by calpain to the highly active p17 mature IL-1α form. Previously, it was thought that ProIL-1α and mature IL-1α are active to the same extent. However, this study demonstrated that the affinity of mature IL-1α to IL-1R1 is about 50 times higher than that of ProIL-1α; in accordance, their biological activity significantly differs (22). Moreover, necrosis-induced IL-1α activity is tightly controlled in a cell type-specific manner (21). Thus, in cell types with a silent necrotic phenotype, IL-1R2 remains associated with ProIL-1α. In contrast, in cells with an inflammatory, necrotic phenotype, IL-1R2 is either absent or caspase-1 is activated before necrosis. Overall, the extent of inflammation in damaged tissues depends on the concentration of cleaved IL-1α, as well as the local expression of IL-1R1. This control mechanism evolved in order to prevent exacerbation of inflammation induced by necrotic cells in tissues with limited regenerative capacity, such as kidney, heart, and brain (21, 32). These findings suggested that sterile inflammation can occur even without activation of IL-1β. Other studies have also demonstrated inflammasome-dependent IL-1α release in sterile inflammation, which may further lead to ProIL-1β expression, caspase-1-dependent processing and release [reviewed in Ref. (2, 22)].

EFFECTS OF IL-1 ON IMMUNE RESPONSES

As a pleiotropic cytokine, IL-1 has diverse potentiating effects on the proliferation, differentiation, and function of various innate (NK cells, macrophages, granulocytes etc.), as well as specific immunocompetent cells (T and B cells) [reviewed in Ref. (1–7)]. Most pronounced are the effects of IL-1 on T cell activation. Initially, IL-1 was characterized as "the classical" co-stimulatory cytokine for T cell proliferation, inducing IL-2 secretion and expression of high affinity IL-2Rs by activated T cells (33). Recent studies by the Paul group demonstrated that IL-1β induces a robust and durable expansion of naïve and memory $CD4^+$ T cells (Th1, Th2, and Th17) in response to antigen stimulation and also enhances their function (34). The responding T cells must express IL-1R1 and different members of the IL-1 family were shown to activate particular STATs, which leads to the expression of relevant subset-specific transcription factors that reinforce the polarized phenotype; IL-33 and STAT5 induce Th2, IL-1β and

STAT3 induces Th17 and IL-18 and STAT4 induces Th1 cells (35). Stimulatory effects of IL-1 on activation of antigen-specific $CD8^+$ T cells, migration and killing were recently described (36). IL-1 can thus serve as an adjuvant in immunization, especially against weak immunogens. Indeed, in some studies, IL-1β has been characterized as an "endogenous adjuvant" that is generated following immunization with adjuvants, such as CFA and aluminum hydroxide (Alum) (37–39). The adjuvant properties of IL-1 in T cell activation possibly stem from its ability to serve as a danger signal, recruiting inflammatory cells to the site of antigen application and inducing maturation and activation of professional APCs.

EFFECTS OF IL-1 ON HEMATOPOIESIS

Multiple hemopoietic functions have been attributed to IL-1, especially to IL-1β [reviewed in Ref. (1–7)]. The *in vivo* importance of IL-1 in stimulating hematopoiesis is best demonstrated by its ability to rescue mice after lethal irradiation or chemotherapy, mainly via inducing recovery of the myeloid compartment (40). IL-1 was characterized as hemopoietin-1, a factor essential for hematopoiesis, acting by inducing the expression of receptors for colony-stimulating factors (CSFs) on primitive precursor cells (41). Of special relevance to the malignant process are the effects of IL-1 on immature $Gr\text{-}1^+CD11b^+$ myeloid cells, also termed myeloid-derived suppressor cells (MDSCs). MDSCs consist of cells committed to differentiate in the bone marrow (BM) into granulocytes, macrophages or dendritic cells [(reviewed in Ref. (42)]. In cancer or chronic inflammation, MDSCs expand in the BM in response to diverse systemic pro-inflammatory cytokines, including IL-1β; they subsequently exit the BM as immature cells and seed at sites of tumor/inflammation. They also accumulate in the spleen and lymph nodes. MDSCs remain immature and are further activated by inflammatory products to acquire immunosuppressive and pro-invasive characteristics, the latter mediated through secretion of VEGF and MMPs. MDSCs mainly consist of subpopulations of granulocyte MDSCs (G-MDSCs) and macrophage MDSCs (M-MDSCs). G-MDSCs have a limited lifespan and usually undergo apoptosis at tumor/chronic inflammation sites, while and M-MDSCs differentiate into M2 tumor-associated macrophages (TAMS). However, in some cases M-MDSCs mature into M1 anti-tumor macrophages.

SIMILARITIES AND DIFFERENCES BETWEEN IL-1α AND IL-1β

Mature secreted IL-1α and IL-1β as well as Pro IL-1α bind to the same receptors and exert the same biological activities, although changes in the affinity of binding of these ligands to IL-1R1 have been described. Generally, IL-1β, due to its secreted nature, has been considered to be the major IL-1 pro-inflammatory molecule and only few comparative studies on *in vivo* biological functions of both IL-1 agonistic molecules have been performed. However, some characteristics of IL-1α and IL-1β differ dramatically [reviewed in Ref. (1–7)]. IL-1β is not present in homeostatic conditions; it is induced and secreted only upon inflammatory signals and its secretion is tightly controlled at the levels of transcription, mRNA stability, translation, and processing. On the other hand, IL-1α is present in the cytosol, nucleus, or cell membrane in homeostatic states, as well as in inflammation, when its expression is upregulated. Importantly, IL-1α is only rarely secreted by living cells and in most cases is undetectable in body fluids. Previously, we demonstrated that *in vivo*, in steady-state homeostasis and in inflammation, IL-1α and IL-1β are differentially expressed in tissues, possibly pointing to their different physiological roles (32, 43).

IL-1α and IL-1β differ in the sub-cellular compartments in which they are active. IL-1β is solely active as an extracellular secreted product, while its precursor is inactive and there is no membrane-associated form of IL-1β. On the other hand, IL-1α is mainly present in its cell-associated forms (ProIL-1α, IL-1NTP and membrane-associated forms), but is only marginally secreted in its mature from, with the exception of activated myeloid cells [reviewed in Ref. (1–3)]. Very little is known about the biological activity of IL-1NTP. Intracellular forms of IL-1α were shown to translocate to the nucleus, due to a nuclear localization sequence (NLS) located within the structure of ProIL-1α and IL-1NTP, but lacking in the mature form of IL-1α. In cells that express ProIL-1α, but do not secrete it, the cytokine possibly acts in an intracrine manner from within the cell, without the need to be secreted, via signaling pathways that are not yet fully characterized. We have hypothesized that intracellular forms of IL-1α evolved as intracellular effector molecules undertaking important homeostatic regulatory functions beyond the realm of immunity and inflammation. These include effects on gene expression, cell growth, and differentiation, which were demonstrated in tissue-resident cells, such as endothelial cells, fibroblasts, smooth muscle cells, keratinocytes, epithelial cells, and brown fat cells [reviewed in Ref. (1–3)]. Thus, IL-1α belongs to a group of "dual function" cytokines (i.e., HMBG1 and IL-33) that are expressed in the cytosol and can enter the nucleus, where they perform homeostatic functions, but upon cell necrosis, they are released into the microenvironment and serve as alarmins by inducing inflammation [reviewed in Ref. (44, 45)].

We have hypothesized that the localization of the IL-1 molecules in the context of the producing cell and its microenvironment dictates their biological function in normal homeostasis and also in the malignant process [reviewed in Ref. (1, 46, 47)]. Thus, as will be shown below, membrane-associated IL-1α is immunostimulatory, while cytosolic ProIL-1α controls intracrine homeostatic functions (27, 48). However, when cytosolic ProIL-1α is released from damaged cells, it acts as an alarmin to initiate inflammation. Secreted IL-1 (mainly IL-1β), at low local doses, induces limited inflammatory responses followed by activation of specific immune mechanisms, while at high doses, broad inflammation accompanied by tissue-damage and tumor invasiveness are observed.

DIFFERENTIAL ACTIVITIES OF IL-1α AND IL-1β IN THE MALIGNANT PROCESS

In the tumor arena, IL-1 is an abundant cytokine that can be secreted by malignant or microenvironment cells and affect inflammation, hematopoiesis, and immunity. It is involved in all phases of the malignant process, such as tumorigenesis, tumor invasiveness and progression, as well as activation/suppression of anti-tumor immunity. In the malignant process, the target cells of IL-1 can include pre-malignant or malignant cells, as well as cells of the microenvironment that are activated by exogenous IL-1,

usually to produce inflammatory mediators that promote invasiveness. In tumorigenesis, IL-1 of microenvironment origin can propagate initial mutations by ROS or NO, produced by phagocytes, other microenvironment cells, or the mutated cells. It can then rescue initiated cells from apoptosis, enable their proliferation and further accumulation of mutations, ultimately leading to a malignant phenotype. IL-1 can then potentiate the invasiveness of malignant cells through stimulation of growth factors, angiogenesis, and tumor cell motility, leading to metastasis. In some cases, IL-1 can also enhance the immunogenicity of malignant cells and consequently reduce tumor invasiveness. As IL-1 is an upstream cytokine, its effects on the malignant process may be direct or indirect, being mediated by cytokines/mediators that it induces. Thus, at tumor sites, IL-1 induces a local cytokine network that is determined by the array of expressed cytokines, their relative concentrations, and the expression pattern of their receptors. This cytokine network dictates the dominant "net cytokine effect" and it fluctuates at various phases of tumor development. We have thoroughly studied the role of IL-1α and IL-1β in the malignant process and have shown that in many cases they perform distinct functions. The results of these studies are summarized below.

EFFECTS OF IL-1 ON TUMORIGENESIS

Tumorigenesis encompasses the *in vivo* induction of tumors cells by carcinogens or oncogenes, as well as *in vitro* transformation of normal or immortalized non-tumor forming cells into overt malignant cells that are capable of tumor formation in mice.

CONSTITUTIVE EXPRESSION OF IL-1β IN THE STOMACH CAN RESULT IN TUMORIGENESIS

The overexpression of human IL-1β fused to a signal peptide (ssIL-1β), in mouse stomach epithelial cells leads to development of spontaneous gastric inflammation, pre-neoplastic lesions, and in some cases, tumors. Thus, secreted IL-1β serves as both an initiator and a tumor promoter (49). This correlates with recruitment of MDSCs to the stomach and their *in situ* activation through the IL-1R1/NF-κB pathway. Gastric pre-neoplasia and MDSC mobilization were inhibited by the IL-1Ra. In this system, overexpressed levels of IL-1β in the stomach induced a strong local inflammatory response, driven by continuous NF-κB activation, which promoted extensive hyperplasia and subsequent tumorigenesis. The ssIL-1β construct, driven by the elastase promoter in the pancreas, resulted in severe chronic pancreatitis; the severity of lesions and local inflammation correlated to the extent of IL-1β expression (50). In this system, older mice developed acinar-ductal metaplasia, but no tumor development was observed.

LOCAL EXPRESSION OF IL-1β IS INVOLVED IN CHEMICAL CARCINOGENESIS

We have demonstrated the role of host-derived IL-1 molecules on susceptibility to chemical carcinogenesis induced by 3-methylcholanthrene (3-MCA), which acts both as an initiator and a tumor promoter, using a battery of IL-1 KO mice [IL-1α$^{-/-}$, IL-1β$^{-/-}$, IL-1α/β$^{-/-}$ (double KO mice), or IL-1Ra$^{-/-}$ mice] in comparison to wild-type (WT) mice (51). We found that deficiency of IL-1β leads to delayed 3-MCA-induced fibrosarcoma development. In mice deficient in IL-1β, tumors appeared only after a prolonged lag period and developed only in part of the treated mice. In WT and IL-1α$^{-/-}$ mice, carcinogenesis patterns were similar and all mice developed tumors. In IL-1Ra$^{-/-}$ mice, in which unattenuated levels of the IL-1 molecules are present, tumor development was more rapid than in WT mice. An early inflammatory response consisting of neutrophils was detected as early as 10 days after carcinogen injection. At later times, when tumor cells are already apparent, the local infiltrate consisted mainly of macrophages, which is consistent with the role of macrophages in tumor progression (52–54). Patterns of inflammation correlated with tumor development. Thus, in mice deficient in IL-1β, almost no inflammatory response was observed during tumor development, lack of IL-1α did not impair inflammation as compared to WT mice, while an heightened inflammatory response was evident in IL-1Ra KO mice. These results indicated for the first time that 3-MCA-induced carcinogenesis is inflammation-dependent, as previously it had been suggested that tumor development is controlled by immune surveillance mechanisms that eliminate the arising malignant cells [reviewed in Ref. (55)].

CONSTITUTIVE EXPRESSION OF IL-1α IN THE SKIN INITIATES BENIGN SKIN PAPILLOMAS

The role of wound healing in tumorigenesis has recently been reviewed (56, 57). In mice specifically over-expressing a transgene of MAPK kinase 1 in the suprabasal layer of the skin, where non-proliferating but differentiating keratinocytes reside, keratinocyte-derived IL-1α initiates wound-induced papilloma formation (58). In such mice, hyperproliferative epidermis and a chronic inflammatory infiltrate were observed. However, papillomas developed only upon skin wounding. In normal keratinocytes IL-1α is present constitutively in the cytosol and its expression increases in cells expressing the MAPK kinase 1 transgene, but it is not released into the microenvironment. Following a skin wound, IL-1α is released from dying cells and activates an inflammatory response in the suprabasal layer by infiltration of macrophages and γδT. Subsequently, immature keratinocytes are recruited into the suprabasal layer where they proliferate, leading to the development of benign tumors. Treatment with dexamethasone, which impairs cytokine production and cell infiltration, or with IL-1Ra, dramatically reduced the local inflammation and papilloma formation. IL-1β is not involved in wound-induced papilloma formation.

THE ALARMIN FUNCTION OF IL-1α CONTRIBUTES TO CARCINOGEN-INDUCED LIVER CARCINOGENESIS

In a model of diethylnitrosamine (DEN)-induced liver carcinogenesis in mice lacking p38α in hepatocytes, the activity of the carcinogen is enhanced as compared to that in WT mice. This is due to enhanced ROS accumulation in hepatocytes, hepatocyte cell death, and liver damage, which ultimately culminates in carcinogenesis. IL-1α is homeostatically expressed in hepatocytes and is released from dying hepatocytes; it stimulates local inflammatory responses, as well as a compensatory proliferative response that characterizes the regenerating liver. These events contribute to the development of hepatocellular carcinoma (HCC) (59). Inhibition of IL-1α or ablation of IL-1R1 prevents HCC development. In this model, IL-1α-induced IL-6 activates STAT3 and promotes liver regeneration and tumor outgrowth (60, 61). Similar effects

were observed during gastric neoplasia in mice with a conditional knockout of IKKβ (lack of NF-κB signaling) in gastric epithelial cells (GECs) after exposure to stress induced by Helicobacter felis infection or ionizing irradiation (62). This resulted in a local accentuated inflammatory response, manifested by increased ROS production, tissue damage, apoptosis followed by cell necrosis, and release of IL-1α from GECs. This inflammatory response ultimately resulted in rapid progression to gastric pre-neoplasia, which was inhibited by blocking IL-1 signaling.

ENDOGENOUS EXPRESSION OF IL-1α IN ONCOGENE-TRANSFORMED CELLS FACILITATES THEIR INVASIVE POTENTIAL

Several oncogenes, including Ras, Myc, and Ret not only mediate neoplastic transformation, but also activate intrinsically inflammatory cytokines that establish the pro-invasive tumor microenvironment [reviewed in Ref. (63)]. Thus, in a model of two-stage skin carcinogenesis (DMBA/TPA), mutated Ras appears early in initiated cells, whereas inflammation induces tumor promotion. Mice lacking IL-1R1 or MyD88 are less sensitive to topical skin carcinogenesis (64). The role of IL-1 in acquiring the malignant phenotype of Ras transformed primary keratinocytes was studied. It was shown that Ras-transduced keratinocytes concomitantly express IL-1α that acts in an autocrine loop together with IL-1R and MyD88. This loop controls defects in keratinocyte differentiation that are observed in papillomas and skin malignancies, as well as NF-κB activation. Treatment with IL-1Ra, reversed the differentiation defects and inhibited pro-inflammatory gene expression in keratinocytes, indicating that IL-1α is secreted from the cells and then activates them in an autocrine manner through IL-1R1 (64). Similar findings were found in a model of pancreatic ductal carcinoma (PDAC), in which constitutive K-ras and NF-κB activation are characteristic. In this model, early mutation activation of K-ras occurs, but the pathways leading to NF-κB activation are not clear. Mutated Ras induces an IL-1α-dependent mechanism that leads to constitutive NF-κB activation and tumor promotion (65). Thus, mutated K-ras induces AP-1 that subsequently activates IL-1α expression/secretion, which further leads to NF-κB activation in an autocrine manner, resulting in expression of its target genes, ultimately leading to PDAC development and invasiveness. Constitutive NF-κB activity is mediated by feed-forward loops activated by two NF-κB target genes: IL-1α and p62 – an adaptor protein that prolongs NF-κB activation by intervening in K63-polyubiquitination, which are both constitutively induced as a result of NF-κB activation and further fuel its constant activation. IL-1β is not involved in this regulatory circuit. These results substantiate the significance of the NF-κB/IKKβ pathway as a key link between inflammation and cancer, inducing pro-inflammatory cytokines in myeloid cells and anti-apoptotic pathways in epithelial cells [reviewed in Ref. (66, 67)]. The mechanisms of unique induction of either IL-1α or IL-1β in initiated cells are still unknown and are possibly dependent on the cell type.

TUMOR CELL- OR HOST-DERIVED IL-1α PREFERENTIALLY ACTIVATES ANTI-TUMOR CELL IMMUNITY

ANTI-TUMOR EFFECTS OF CELL-ASSOCIATED IL-1α

We demonstrated the anti-tumor effects of IL-1α expression by malignant cells in different experimental systems, using oncogene-transformed fibroblasts that constitutively express IL-1α, possibly due to alterations in the control of IL-1α expression induced by the oncogene. Fibrosarcoma cells were transfected with cDNA of ProIL-1α and lymphoma cells were induced to express IL-1α in a transient manner, following *in vitro* activation of the cells with immunomodulators/cytokines (68–73). In these cell lines, IL-1α is expressed in the cytosol or on the membrane, but is not secreted. In such cells, IL-1β is not expressed. IL-1α-expressing tumor cells usually fail to cause tumor development in intact mice but if tumors occur, they subsequently regress. Tumor regression in this instance is mainly mediated by $CD8^+$ T cells, with some contribution of NK cells and macrophages. Regression of tumors from IL-1α-positive fibrosarcoma cells does not require activation of $CD4^+$ T cells, which suggests that cell-associated IL-1α may act as a membrane-associated co-stimulatory molecule or focused adjuvant that directly activates $CD8^+$ T cells. Tumor cell-associated IL-1α also potentiates antigen presentation by the malignant cells themselves, possibly through IFNγ-induced MHC class II expression, and also via cross-presentation by professional APCs. Tumor regression induces a long-term specific immune memory that protects mice against a challenge with violent parental cells.

Tumor cell-associated IL-1α was shown to be effective in tumor cell vaccines used to intervene in the growth of tumor cells of the corresponding violent line (non-IL-1 expressing). Thus, the vaccine induced regression of violent fibrosarcomas when applied at a critical "therapeutic window" 5–10 days (single application of Mitomycin-C-treated tumor cells) after inoculation of the malignant cells (70).

The "natural" membrane-associated form of IL-1α is important for exerting anti-tumor effects, as it acts as an adhesion-molecule, allowing efficient cell-to-cell interactions between malignant and immune effector cells that bear IL-1Rs, which enables better killing. Membrane IL-1α is also effective as a focused adjuvant that efficiently acts at low levels of expression, below those which are toxic to the host. Other studies have also emphasized the effectiveness of membrane-associated cytokines expressed on engineered tumor cells (i.e., IFNγ, GM-CSF, M-CSF, TNFα, and IL-12) (74–76).

HOST-DERIVED IL-1α IS ESSENTIAL FOR IMMUNOEDITING DURING CARCINOGENESIS

We have shown that patterns of IL-1 expression in the microenvironment can affect the immunogenicity of the arising malignant cells during tumorigenesis. Immunoediting has mainly been studied in the process of experimental carcinogenesis induced by 3-MCA. Thus, we have shown that transplantable 3-MCA-induced fibrosarcoma cell lines obtained from $IL\text{-}1\alpha^{-/-}$ mice failed to induce tumors in immune intact mice, whereas in sublethally irradiated mice, tumors do develop (77). This is despite the fact that tumor incidence and the nature of the local inflammatory response were comparable in 3-MCA-treated $IL\text{-}1\alpha^{-/-}$ mice and WT mice. Impaired immunoediting occurs in 3-MCA-induced tumors in various immunodeficient mice that lack critical components essential for the development of anti-tumor cell immunity. These include mice lacking immunosurveillance cells, such as $Rag2^{-/-}$ mice, which lack T cells and B cells, nude mice, $CD1d^{-/-}$ mice, which lack CD1d-restricted T cells, and

Ja18$^{-/-}$ mice, lacking semi-invariant NKT cells or mice deficient in cytokines critical for anti-tumor immunity, such as IFNγ and IL-12 [reviewed in Ref. (55, 78)]. IL-1α can now be added to the list of cytokines of importance in immunoediting. It is not yet known which form of IL-1α (secreted or cell-associated) is involved in the process of immunoediting. The process of immunoediting in immunodeficient hosts allows the survival of malignant cell variants, which are "universally immunogenic," as they express surface adhesion or co-stimulatory molecules (i.e., ICAM-1 or 2, LFA-1 or 3, CD1d, VLA-4, B7 etc.) that are essential for the development of anti-tumor immunity. Thus, IL-1α$^{-/-}$ cell lines were shown to express more surface MHC class I molecules, co-stimulatory molecules (i.e., B7.1 and B7.2) as well as adhesion molecules, such as L-selectin and ICAM-1, as compared to fibrosarcoma cells from WT mice. IL-1α$^{-/-}$ immunogenic cells are rejected in intact mice by conventional innate and specific anti-tumor immune effector cells, including NK cells, as well as by CD4^{+} and CD8^{+} T cells. Immune impairments in IL-1α$^{-/-}$ mice, including in NK development and in the killing capacity of NK cells, LAK cells, and CTLs, were characterized. The role of IL-1α in activation of immunosurveillance cells was shown in transgenic mice over-expressing IL-1α in the skin (79). In such mice, DMBA/TPA treatment induced skin tumors at very low incidence compared to WT mice, due to the rapid eradication of arising malignant cells by innate effector cells activated by local IL-1α in the skin. We hypothesize that IL-1α in the tumor microenvironment is immunostimulatory, rather that inflammatory, due to its localization on the surface of cells and its limited secretion, as compared to IL-1β. However, when IL-1β levels in tissues are limited, it can also be immunostimulatory, as will be shown below.

TUMOR CELL- OR HOST-DERIVED IL-1β IS INVOLVED IN TUMOR INVASIVENESS

IL-1β SECRETED BY MALIGNANT CELLS INCREASES THEIR INVASIVE POTENTIAL AND INDUCES TUMOR-MEDIATED IMMUNE SUPPRESSION

To assess effects of tumor cell-associated IL-1β on tumorigenicity patterns, we transfected violent fibrosarcoma cells with constructs bearing the cDNAs of the mature form of IL-1β or the mature form of IL-1β ligated to a signal sequence (ssIL-1β), to induce potent secretion of IL-1β through the endoplasmic reticulum-Golgi pathway (80, 81). We found that IL-1β- and ssIL-1β-transfected fibrosarcoma tumors were more invasive than the violent parental cells or mock-transfected cells. The invasiveness of the malignant cells correlated with the amount of IL-1β that was secreted by them. In addition, only the ssIL-1β transfectants, which secrete relatively high levels of the cytokine, exhibited a metastatic potential. Enhanced angiogenesis patterns, as evidenced by high vessel density in tumors and increased secretion of VEGF by the malignant cells, were observed in tumors secreting IL-1β. Similar observations were described in other experimental systems using IL-1β-transfected tumor cells (82–84). No anti-tumor effector cells or cytokines that potentiate anti-tumor immunity (i.e., IFNγ and IL-2) could be detected in spleens of mice injected with fibrosarcoma cells transfected with IL-1β or ssIL-1β or with the violent parental cells. In contrast, effective anti-tumor cell immune responses were observed in mice injected with fibrosarcoma cells transfected with ProIL-1α, as indicated above.

Further studies have shown that general anergy develops in mice bearing tumors of IL-1β secreting cells, mediated by MDSCs (80, 82, 85). Resection of large tumors of IL-1β secreting cells completely restored immune reactivity and reversed the MDSC response within 7–10 days. Treatment of tumor-bearing mice with the IL-1Ra reduced tumor growth and attenuated the MDSC response.

In spite of tumor-mediated suppression, resection of large tumors of IL-1β secreting cells, followed by a challenge (2 months after tumor resection) with the violent parental cells induced resistance in mice; protection was not observed in mice bearing tumors of mock-transfected fibrosarcoma cells. Thus, in mice bearing tumors of IL-1β secreting cells, anti-tumor cell specific immunity is activated, due to the adjuvant-like effects of IL-1β; however, protective immunity is not manifested, due to suppression of immune effector mechanisms. It is notable that when tumor cells expressing membrane-associated IL-1α are injected into mice, anti-tumor immune responses occur without concomitant tumor-mediated suppression and thus the malignant cells are rejected.

TUMOR-MEDIATED ANGIOGENESIS IS LARGELY STIMULATED BY MICROENVIRONMENT IL-1β

We have studied in detail the role of IL-1β in tumor-mediated angiogenesis, which is almost non-existent upon injection of tumor cells into IL-1β KO mice or following neutralization of IL-1β in WT mice (86, 87). Inflammation usually accompanies tumor-mediated angiogenesis. We have used B16 melanoma cells encapsulated in Matrigel plugs, in order to characterize cell/cytokine interactions in the early angiogenic response (88). We have characterized a newly described auto-induction circuit in which IL-1β and VEGF interact and induce each other. Tumor-mediated angiogenesis is inhibited if either IL-1β or VEGF are neutralized and it does not occur in IL-1β KO mice. The IL-1β and VEGF circuit acts via interactions between BM-derived VEGFR1^{+}/IL-1R1^{+} immature myeloid cells (MDSCs) and tissue-resident endothelial cells. Myeloid cells do not directly stimulate endothelial cells for migration and subsequent blood vessel formation. However, myeloid cells produce IL-1β and a network of pro-inflammatory cytokines/molecules, which subsequently activate resting endothelial cells to produce VEGF, as well as other direct pro-angiogenic factors. Subsequently, VEGF activates endothelial cells for blood vessel formation. IL-1β thus provides the inflammatory microenvironment for angiogenesis and tumor progression. We have shown that IL-1β inhibition stably reduces tumor growth, by limiting inflammation and by inducing the maturation of MDSCs into M1 macrophages, which do not promote tumor invasiveness and can be cytotoxic/cytostatic for tumor cells and can also serve as APCs that induce anti-tumor immunity. Thus, this study has characterized IL-1β as a major mediator in the tumor microenvironment that recruits MDSCs and also controls their immature pro-invasive and immunosuppressive state; ablation of IL-1β alters the pro-tumor microenvironment into an anti-tumor one.

MICROENVIRONMENT IL-1 ACTIVATES CANCER STEM CELLS

In murine tumor models and in cancer patients, it was shown that IL-1β increases tumor invasiveness [reviewed in Ref. (1, 46, 47)].

Recently, direct effects of IL-1β on cancer stem cells (CSCs) or the niche that favors CSC formation were described. In *in vitro* studies, recombinant IL-1β increased the sphere forming capacity of CSCs and enhanced expression of stemness genes (i.e., Bmi1 and Nestin), as well as Zeb1 that is an important regulator of EMT and self-renewal (89). Furthermore, the Weinberg group has described a circuit in which carcinoma-derived IL-1 creates the niche for the transition of "regular tumor cells" into CSCs (90). Thus, in the tumor microenvironment, carcinoma-derived IL-1 (IL-1α and IL-1β) activates mesenchymal stem cells (MSCs) to produce PGE_2 and other cytokines, such as IL-6, IL-8, Gro-α, and RANTES that in turn act on the carcinoma cells and induce activation of β-catenin and transition into CSCs. These effects were largely neutralized by the IL-1Ra or siRNAs against IL-1α and IL-1β. Thus, IL-1 in the tumor microenvironment can support development and expansion of CSCs and thus amplify the malignant process and support metastasis formation.

ANTI-TUMOR EFFECTS OF MICROENVIRONMENT IL-1β IN MICE WITH MINIMAL RESIDUAL DISEASE

Elegant studies by the Zitvogel group demonstrated that tissue-damage following cancer treatment with some chemotherapeutical drugs activates DCs in the tumor microenvironment to present tumor antigens and further stimulate anti-tumor immunity that synergizes with the chemotherapy (91). In the milieu of anthracycline-treated tumors, the NLRP3 inflammasome is activated and stimulates IL-1β production that is essential for activating IFNγ producing $CD8^+$ T cells. In addition, patients with breast cancer with a loss-of-function allele of P2X7R, which is essential for activation of the NLRP3 inflammasome and IL-1β processing/secretion, develop a more rapid metastatic disease than individuals with the normal allele. This may represent a unique scenario in which a low tumor burden, possibly accompanied by relative low levels of IL-1β in the microenvironment could activate local immunity. These results will hopefully open new avenues for use of IL-1β, and possibly also IL-1α, in cancer immunotherapy in tumor-debulked patients.

INTERACTIONS BETWEEN TUMOR CELL- AND MICROENVIRONMENT-DERIVED IL-1 IN THE CONTROL OF TUMOR INVASIVENESS OF 3-MCA-INDUCED TUMOR CELL LINES

In the tumor microenvironment, interactions between IL-1 derived from the malignant cells or from inflammatory cells interact and determine the invasive potential of the tumor. Transplantation assays, in which 3-MCA-induced fibrosarcoma cell lines that were derived from WT or IL-1 KO mice were injected into the same strains of mice, enabled us to define the role of IL-1 expressed by the malignant cells or the microenvironment in tumor progression (51, 77, 92–94).

3-MCA-induced fibrosarcoma cell lines from WT mice manifested low invasiveness in IL-1 deficient mice, intermediate invasiveness in WT mice, and high invasiveness in $IL\text{-}1Ra^{-/-}$ mice, pointing to the importance of microenvironment-derived IL-1 in tumor progression. The contribution of tumor cell-derived IL-1 was demonstrated following injection of 3-MCA fibrosarcoma cell lines from IL-1 KO mice into WT mice. Thus, fibrosarcoma cells obtained from mice deficient in IL-1β failed to grow in WT mice, due to their inability to recruit a local inflammatory response that is essential for tumor invasiveness. Furthermore, invasive 3-MCA-induced fibrosarcoma cells from IL-1Ra KO mice were only weakly tumorigenic in IL-1 deficient mice (92–94). We suggest that initially, upon injection of tumor cells into mice, the malignant cells express relatively small amounts of IL-1 that subsequently induces broad inflammation mediated by infiltrating cells, ultimately leading to tumor invasiveness. In malignant cells, IL-1 can be constitutively expressed due to oncogene activation or it can be induced by danger signals in the microenvironment. IL-1 of the microenvironment is critical to induce tumor outgrowth and progression leading to invasiveness of 3-MCA fibrosarcomas.

In naturally occurring tumor cells, both IL-1 molecules can be expressed and interact. The expression of IL-1α in 3-MCA-induced tumor cells from IL-1β KO mice concomitantly activated a strong T helper and CTL response, which also contributed to the reduced *in vivo* growth of these cells in WT mice. When cell lines from IL-1α KO mice were injected into mice, progressive tumor growth occurred, due to the pro-invasive and immunosuppressive effects of tumor cell-derived IL-1β and the absence of immunostimulatory effects of tumor cell-associated IL-1α that is missing in these cells. Cell lines that originated in 3-MCA-treated $IL\text{-}1Ra^{-/-}$ mice were very invasive and metastatic, compared to cell lines originating in WT mice, due to high-unattenuated levels of IL-1 expression in the malignant cells, which facilitates their invasiveness and also promotes immunosuppressive mechanisms. Thus, fibrosarcoma cell-derived IL-1α and IL-1β do not act in concert and each IL-1 molecule has unique effects on tumor invasiveness or on anti-tumor cell immune responses. At tumor sites, immunosuppressive effector cells, induced by excessive expression of IL-1β inhibit or mask the induction/function of anti-tumor immunosurveillance induced by tumor cell-derived IL-1α. At tumor sites, effects of tumor-derived IL-1 and host-derived IL-1 interact and modulate tumor progression. Elucidation of these interaction patterns should enable better understanding of the overall role of IL-1 in the malignant process.

COMPLEX EFFECTS OF IL-1 ON THE INVASIVENESS OF HUMAN TUMORS

In experimental tumor models in WT mice and in cancer patients, increased local levels of IL-1 at tumor sites usually correlate with tumor invasiveness and a bad prognosis [reviewed in Ref. (1, 46, 47)]. Very little is known about interactions between IL-1α and IL-1β expressed at tumor sites by either the malignant or microenvironment cells. Most studies usually assess only one of the IL-1 agonistic molecules and do not discriminate between patterns of its expression in the malignant cells versus the microenvironment. The levels of IL-1 expression at tumor sites are also not compared to homeostatic levels in the given organ. Thus, insufficient characterization of IL-1 at tumor sites has led to some inconsistencies concerning the impact of the concerted action of IL-1α and IL-1β on the malignant process. For example, Okamoto et al. showed constitutively active NLRP3 inflammasome and IL-1β secretion in melanoma cell lines derived from late stage patients, where selection for cells expressing

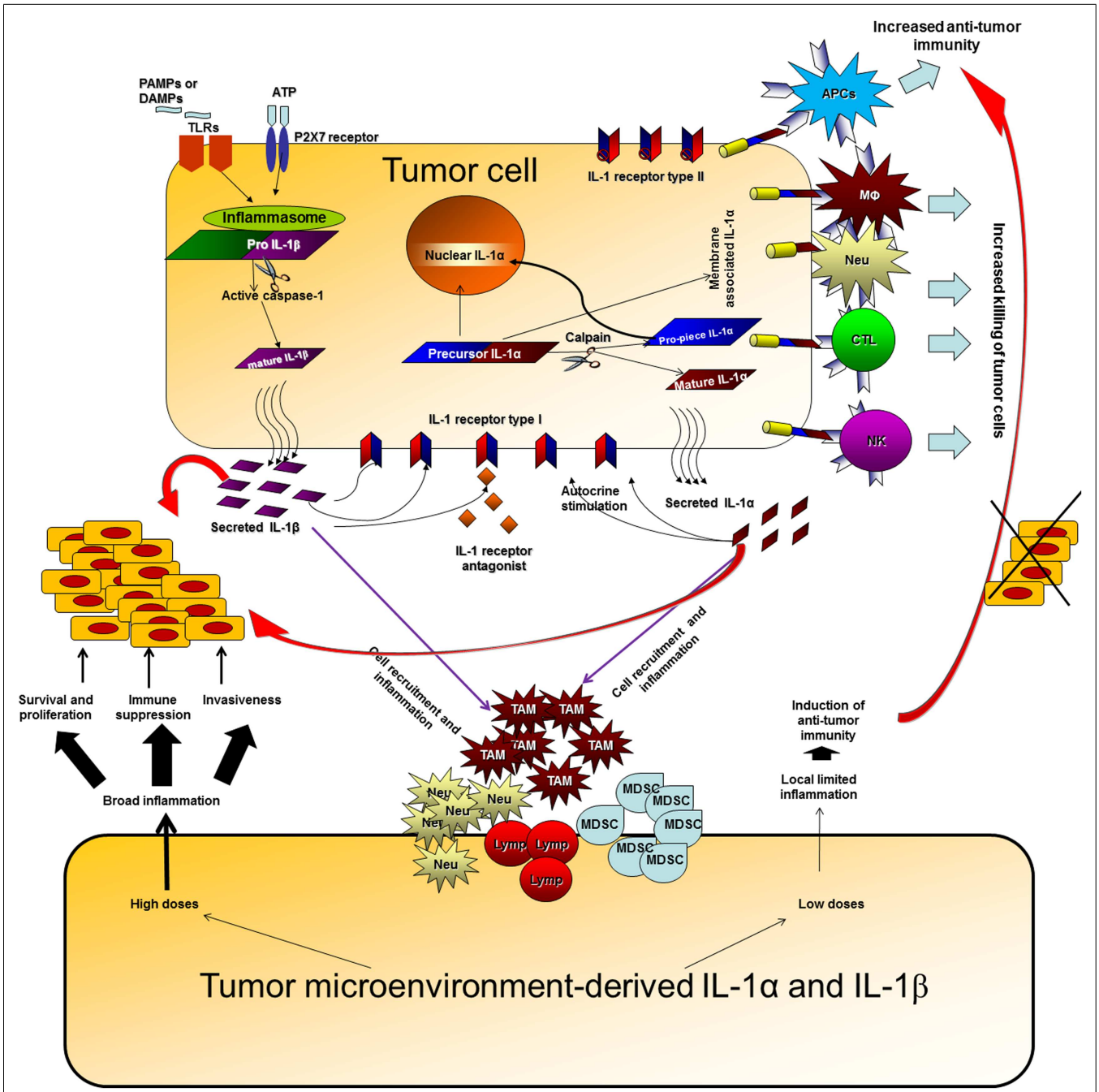


FIGURE 1 | Effects of IL-1 at tumor sites. IL-1 can be produced at tumor sites by the malignant cells or by diverse cells in the tumor microenvironment. IL-1 generated by tumor cells can affect the malignant cells in an autocrine or paracrine manner, enabling proliferation, and invasiveness. In parallel, IL-1 secreted by malignant cells activates microenvironment residing or infiltrating cells to produce additional IL-1, which then induces a cytokine network, which further activates tumor invasiveness. High doses of IL-1 at tumor sites usually result in an invasive potential and immunosuppression. Expression of IL-1α on the membrane of malignant cells increases their immunogenicity and leads to induction of efficient anti-tumor responses. Membrane IL-1α expressed on infiltrating cells possibly also promotes the development of anti-tumor cell immunity. Low levels of IL-1 at tumor sites at early stages of tumor development or upon IL-1 attenuation, usually result in efficient anti-tumor immunity, in the absence of immunosuppression mainly mediated by IL-1-induced MDSCs and also Tregs. When immunosuppression is evident at tumor sites, it hinders the development or masks the function of anti-tumor immunity and thus invasive growth results. Host- and tumor cell-derived IL-1 induce each other and together fuel the local, and sometimes systemic, inflammatory response. Intracellular ProIL-1α in tumor cells induces intracrine functions following translocation into the nucleus. These are related to survival, proliferation, or gene expression; however, they were not sufficiently characterized in the context of the malignant process.

invasiveness-promoting molecules had already occurred. This can also explain the increased invasive phenotype of progressive melanoma tumors (95). In a different study on melanoma patients performed by the Grimm group, IL-1α was expressed in most primary tumors (98%) and approximately half (55%) of metastases. IL-1α was also expressed in 73% of inspected nevi. IL-1β was expressed in approximately 10% of primary or metastatic melanoma samples, but its expression strongly correlated with IL-1α expression (96). The mechanisms of preferential IL-1α expression in human melanomas are not known. By using cell lines obtained from human melanoma patients, typical molecular pathways of inflammation, including secretion of ROS, NO, COX-2, as well as NF-κB and c-Jun activation, were observed in malignant melanoma cells upon activation by endogenous IL-1, which also promotes *in vitro* cell proliferation. These effects were most pronounced in cell lines producing significant amounts of IL-1 and were abrogated by antibodies against IL-1R1 or siRNA of IL-1α and IL-1β. Furthermore, blocking IL-1 signaling in melanoma cell lines induced autophagy, which might further lead to cell death. IL-1 secreted by melanoma cells also affects recruitment and activation of inflammatory cells at tumor sites, which contribute to invasiveness. The correlation between IL-1 expression in tumors and its secretion is still unknown and awaits further investigation.

FUTURE PROSPECTS FOR IL-1 MANIPULATION IN ANTI-TUMOR THERAPIES

The network of cytokines and immune/inflammatory cells in the tumor microenvironment controls the fate of the malignant process. In the tumor microenvironment, the balance between the "wound healing" type of inflammation, which promotes tumor progression and immune escape, and "favorable" limited inflammatory responses, in which professional APCs are activated and induce anti-tumor adaptive immunity, determines the direction of the malignant process [reviewed in Ref. (97–101)]. Due to the plethora of activities of IL-1 in the malignant process and its dominant role in determining local cytokine networks at tumor sites, neutralization of IL-1 as a single target molecule has potential to tilt the balance between destructive inflammation and protective anti-tumor immunity in the tumor microenvironment (**Figure 1**). For example, we have shown that 4T1 cells produce invasive and metastatic breast tumors which grow progressively in WT mice, whereas, they cause tumors that grow initially but later regress in IL-1 KO mice, due to the efficient induction of a CTL-mediated anti-tumor response in these mice.

Use of genetically engineered cells or mice with distinct patterns of IL-1 expression, have shown that IL-1α and IL-1β have distinct effects at tumor sites. Thus, IL-1β promotes invasiveness and immunosuppression, while IL-1α is mainly immunostimulatory. This is true for both tumor cell-derived or microenvironment-derived IL-1. Immunosuppression induced by IL-1β usually acts in a dominant manner and masks the immunostimulatory anti-tumor effects of IL-1α.

However, in "real-life" both IL-1 molecules are usually expressed and they induce each other. Further studies should establish the "IL-1 map" in individual tumors, taking into account patterns of expression, secretion, and levels of IL-1α and IL-1β in malignant or infiltrating cells. Having these data should facilitate the design of treatment protocols based on IL-1 manipulation.

IL-1 neutralization agents are available at the present time (102–104). Thus, the IL-1Ra, also called Anakinra (Kineret; Amgen/Biovitrum) is FDA-approved and has been shown to be safe and efficient in alleviating symptoms of rheumatoid arthritis and auto-immune diseases. Characterization of the inflammasome pathway of IL-1β processing and secretion encouraged the development of novel anti-IL-1 agents that are now being tested in different clinical trials in diverse diseases with inflammatory manifestations. Some of these trials have produced initial promising results. These agents now await testing in cancer patients, once protocols are established for their integration into first-line anti-tumor therapies. Optimally, IL-1 neutralization should be most effective in patients with minimal residual disease (MRD), to prevent tumor recurrence and metastasis. In such patients, tumor-mediated immunosuppression and inflammation should be reduced and enable induction of protective anti-tumor immune responses in a microenvironment that does not favor invasiveness. These treatments may be given for extended periods, to convert MRD to a chronic state, provided that resistance to anti-IL-1 therapy does not develop. Neutralization of tumor-associated IL-1, especially IL-1β, should not be complete, in order not to compromise the immune system of patients. In addition, due to the adjuvanticity of cell-associated IL-1α, tumor cell vaccines based on constitutive or transient IL-1α expression has the potential to induce anti-tumor cell immunity in patients with MRD. In such patients, one can envision initial systemic neutralization of IL-1β followed by application of IL-1α expressing tumor cell vaccines. In conclusion, better understanding the integrative role that IL-1α and IL-1β play in animal experimental models and in cancer patients, together with "IL-1 mapping" at tumor sites, as indicated above, should pave the way for safe and efficient application of anti-IL-1 therapies at the bedside for cancer patients.

ACKNOWLEDGMENTS

Ron N. Apte was supported by the Israel Ministry of Science (MOS) jointly with the Deutsches Krebsforschungscentrum (DKFZ), Heidelberg, Germany, the Israel Science Foundation funded by the Israel Academy of Sciences and Humanities, the Israel Cancer Association and the Israel Ministry of Health Chief Scientist's Office, FP7: "Cancer and Inflammation" (INFLACARE) and The Binational (Israel–USA) Science Foundation. Prof. Ron N. Apte is an incumbent of the Irving Isaac Sklar Chair in Endocrinology and Cancer. Elena Voronov was supported by the Israel Cancer Association, the Israel Ministry of Health Chief Scientist's Office, and the Concern Foundation, FP7: "Cancer and Inflammation" (INFLA-CARE) and ISF (the last two in cooperation with Prof. Ron N. Apte). The authors would like to thank their collaborators: Prof. Charles A. Dinarello, the University of Colorado, Denver, CO, USA; Profs. Margot Zoller and Margareta Mueller, the German Cancer Center (DKFZ), Heidelberg, Germany; Prof. Michael Martin, Justus Liebig University, Giessen, Germany; Prof. Yoichiro Iwakura, University of Tokyo, Tokyo, Japan. We would also like to thank Mrs. Rosalyn M. White for her devoted help in preparation of this MS.

REFERENCES

1. Apte RN, Voronov E. Is interleukin-1 a good or bad 'guy' in tumor immunobiology and immunotherapy? *Immunol Rev* (2008) **222**:222–41. doi:10.1111/j.1600-065X
2. Dinarello CA. Immunological and inflammatory functions of the interleukin-1 family. *Annu Rev Immunol* (2009) **27**:519–50. doi:10.1146/annurev.immunol.021908.132612
3. Dinarello CA. Interleukin-1 in the pathogenesis and treatment of inflammatory diseases. *Blood* (2011) **117**:3720–32. doi:10.1182/blood-2010-07-273417
4. Gabay C, Lamacchia C, Palmer G. IL-1 pathways in inflammation and human diseases. *Nat Rev Rheumatol* (2010) **6**:232–41. doi:10.1038/nrrheum.2010.4
5. Garlanda C, Anders HJ, Mantovani A. TIR8/SIGIRR: an IL-1R/TLR family member with regulatory functions in inflammation and T cell polarization. *Trends Immunol* (2009) **30**:439–46. doi:10.1016/j.it.2009.06.001
6. O'Neill LA. The interleukin-1 receptor/Toll-like receptor superfamily: 10 years of progress. *Immunol Rev* (2008) **226**:10–8. doi:10.1111/j.1600-065X.2008.00701.x
7. Sims JE, Smith DE. The IL-1 family: regulators of immunity. *Nat Rev Immunol* (2010) **10**:89–102. doi:10.1038/nri2691
8. Iwasaki A, Medzhitov R. Regulation of adaptive immunity by the innate immune system. *Science* (2010) **327**:291–5. doi:10.1126/science.1183021
9. Palm NW, Medzhitov R. Pattern recognition receptors and control of adaptive immunity. *Immunol Rev* (2009) **227**:221–33. doi:10.1111/j.1600-065X.2008.00731.x
10. Takeuchi O, Akira S. Pattern recognition receptors and inflammation. *Cell* (2010) **140**:805–20. doi:10.1016/j.cell.2010.01.022
11. Carta S, Castellani P, Delfino L, Tassi S, Vene R, Rubartelli A. DAMPs and inflammatory processes: the role of redox in the different outcomes. *J Leukoc Biol* (2009) **86**:549–55. doi:10.1189/jlb.1008598
12. Rock KL, Kono H. The inflammatory response to cell death. *Annu Rev Pathol* (2008) **3**:99–126. doi:10.1146/annurev.pathmechdis.3.121806.151456
13. Srikrishna G, Freeze HH. Endogenous damage-associated molecular pattern molecules at the crossroads of inflammation and cancer. *Neoplasia* (2009) **11**:615–28.
14. Eisenbarth SC, Flavell RA. Innate instruction of adaptive immunity revisited: the inflammasome. *EMBO Mol Med* (2009) **1**:92–8. doi:10.1002/emmm.200900014
15. Franchi L, Eigenbrod T, Munoz-Planillo R, Nunez G. The inflammasome: a caspase-1-activation platform that regulates immune responses and disease pathogenesis. *Nat Immunol* (2009) **10**:241–7. doi:10.1038/ni.1703
16. Latz E. The inflammasomes: mechanisms of activation and function. *Curr Opin Immunol* (2010) **22**:28–33. doi:10.1016/j.coi.2009.12.004
17. Martinon F, Mayor A, Tschopp J. The inflammasomes: guardians of the body. *Annu Rev Immunol* (2009) **27**:229–65. doi:10.1146/annurev.immunol.021908.132715
18. Schroder K, Tschopp J. The inflammasomes. *Cell* (2010) **140**:821–32. doi:10.1016/j.cell.2010.01.040
19. Wang KK. Calpain and caspase: can you tell the difference? *Trends Neurosci* (2000) **23**:20–6. doi:10.1016/S0166-2236(99)01536-2
20. Afonina IS, Tynan GA, Logue SE, Cullen SP, Bots M, Luthi AU, et al. Granzyme B-dependent proteolysis acts as a switch to enhance the proinflammatory activity of IL-1alpha. *Mol Cell* (2011) **44**:265–78. doi:10.1016/j.molcel.2011.07.037
21. Zheng Y, Humphry M, Maguire JJ, Bennett MR, Clarke MC. Intracellular interleukin-1 receptor 2 binding prevents cleavage and activity of interleukin-1alpha, controlling necrosis-induced sterile inflammation. *Immunity* (2013) **38**:285–95. doi:10.1016/j.immuni.2013.01.008
22. Di Paolo NC, Shayakhmetov DM. Interleukin-1 receptor 2 keeps the lid on interleukin-1alpha. *Immunity* (2013) **38**:203–5. doi:10.1016/j.immuni.2013.02.001
23. Fettelschoss A, Kistowska M, LeibundGut-Landmann S, Beer HD, Johansen P, Senti G, et al. Inflammasome activation and IL-1beta target IL-1alpha for secretion as opposed to surface expression. *Proc Natl Acad Sci U S A* (2011) **108**:18055–60. doi:10.1073/pnas.1109176108
24. Gross O, Yazdi AS, Thomas CJ, Masin M, Heinz LX, Guarda G, et al. Inflammasome activators induce interleukin-1alpha secretion via distinct pathways with differential requirement for the protease function of caspase-1. *Immunity* (2012) **36**:388–400. doi:10.1016/j.immuni.2012.01.018
25. Keller M, Ruegg A, Werner S, Beer HD. Active caspase-1 is a regulator of unconventional protein secretion. *Cell* (2008) **132**:818–31. doi:10.1016/j.cell.2007.12.040
26. Chen GY, Nunez G. Sterile inflammation: sensing and reacting to damage. *Nat Rev Immunol* (2010) **10**:826–37. doi:10.1038/nri2873
27. Cohen I, Rider P, Carmi Y, Braiman A, Dotan S, White MR, et al. Differential release of chromatin-bound IL-1alpha discriminates between necrotic and apoptotic cell death by the ability to induce sterile inflammation. *Proc Natl Acad Sci U S A* (2010) **107**:2574–9. doi:10.1073/pnas.0915018107
28. Lukens JR, Gross JM, Kanneganti TD. IL-1 family cytokines trigger sterile inflammatory disease. *Front Immunol* (2012) **3**:315. doi:10.3389/fimmu.2012.00315
29. Rider P, Carmi Y, Guttman O, Braiman A, Cohen I, Voronov E, et al. IL-1alpha and IL-1beta recruit different myeloid cells and promote different stages of sterile inflammation. *J Immunol* (2011) **187**:4835–43. doi:10.4049/jimmunol.1102048
30. Rock KL, Lai JJ, Kono H. Innate and adaptive immune responses to cell death. *Immunol Rev* (2011) **243**:191–205. doi:10.1111/j.1600-065X.2011.01040.x
31. Rider P, Kaplanov I, Romzova M, Bernardis L, Braiman A, Voronov E, et al. The transcription of the alarmin cytokine interleukin-1 alpha is controlled by hypoxia inducible factors 1 and 2 alpha in hypoxic cells. *Front Immunol* (2012) **3**:290. doi:10.3389/fimmu.2012.00290
32. Hacham M, Argov S, White RM, Segal S, Apte RN. Different patterns of interleukin-1alpha and interleukin-1beta expression in organs of normal young and old mice. *Eur Cytokine Netw* (2002) **13**:55–65.
33. Chambers CA, Allison JP. Costimulatory regulation of T cell function. *Curr Opin Cell Biol* (1999) **11**:203–10. doi:10.1016/S0955-0674(99)80027-1
34. Ben-Sasson SZ, Hu-Li J, Quiel J, Cauchetaux S, Ratner M, Shapira I, et al. IL-1 acts directly on CD4 T cells to enhance their antigen-driven expansion and differentiation. *Proc Natl Acad Sci U S A* (2009) **106**:7119–24. doi:10.1073/pnas.0902745106
35. Guo L, Wei G, Zhu J, Liao W, Leonard WJ, Zhao K, et al. IL-1 family members and STAT activators induce cytokine production by Th2, Th17, and Th1 cells. *Proc Natl Acad Sci U S A* (2009) **106**:13463–8. doi:10.1073/pnas.0906988106
36. Ben-Sasson SZ, Hogg A, Hu-Li J, Wingfield P, Chen X, Crank M, et al. IL-1 enhances expansion, effector function, tissue localization, and memory response of antigen-specific CD8 T cells. *J Exp Med* (2013) **210**:491–502. doi:10.1084/jem.20122006
37. Eisenbarth SC, Colegio OR, O'Connor W, Sutterwala FS, Flavell RA. Crucial role for the Nalp3 inflammasome in the immunostimulatory properties of aluminium adjuvants. *Nature* (2008) **453**:1122–6. doi:10.1038/nature06939
38. Franchi L, Nunez G. The Nlrp3 inflammasome is critical for aluminium hydroxide-mediated IL-1beta secretion but dispensable for adjuvant activity. *Eur J Immunol* (2008) **38**:2085–9. doi:10.1002/eji.200838549
39. Li H, Nookala S, Re F. Aluminum hydroxide adjuvants activate caspase-1 and induce IL-1beta and IL-18 release. *J Immunol* (2007) **178**:5271–6.
40. Neta R, Douches S, Oppenheim JJ. Interleukin 1 is a radioprotector. *J Immunol* (1986) **136**:2483–5.
41. Mochizuki DY, Eisenman JR, Conlon PJ, Larsen AD, Tushinski RJ. Interleukin 1 regulates hematopoietic activity, a role previously ascribed to hemopoietin 1. *Proc Natl Acad Sci U S A* (1987) **84**:5267–71. doi:10.1073/pnas.84.15.5267
42. Gabrilovich DI, Ostrand-Rosenberg S, Bronte V. Coordinated regulation of myeloid cells by tumours. *Nat*

Rev Immunol (2012) **12**:253–68. doi:10.1038/nri3175
43. Hacham M, Cristal N, White RM, Segal S, Apte RN. Complementary organ expression of IL-1 vs. IL-6 and CSF-1 activities in normal and LPS-injected mice. *Cytokine* (1996) **8**:21–31. doi:10.1006/cyto.1995.0004
44. Lotze MT, Deisseroth A, Rubartelli A. Damage associated molecular pattern molecules. *Clin Immunol* (2007) **124**:1–4. doi:10.1016/j.clim.2007.02.006
45. Raucci A, Palumbo R, Bianchi ME. HMGB1: a signal of necrosis. *Autoimmunity* (2007) **40**:285–9. doi:10.1080/08916930701356978
46. Apte RN, Dotan S, Elkabets M, White MR, Reich E, Carmi Y, et al. The involvement of IL-1 in tumorigenesis, tumor invasiveness, metastasis and tumor-host interactions. *Cancer Metastasis Rev* (2006) **25**:387–408. doi:10.1007/s10555-006-9004-4
47. Apte RN, Krelin Y, Song X, Dotan S, Recih E, Elkabets M, et al. Effects of microenvironment- and malignant cell-derived interleukin-1 in carcinogenesis, tumour invasiveness and tumour-host interactions. *Eur J Cancer* (2006) **42**:751–9. doi:10.1016/j.ejca.2006.01.010
48. Werman A, Werman-Venkert R, White R, Lee JK, Werman B, Krelin Y, et al. The precursor form of IL-1alpha is an intracrine proinflammatory activator of transcription. *Proc Natl Acad Sci U S A* (2004) **101**:2434–9. doi:10.1073/pnas.0308705101
49. Tu S, Bhagat G, Cui G, Takaishi S, Kurt-Jones EA, Rickman B, et al. Overexpression of interleukin-1beta induces gastric inflammation and cancer and mobilizes myeloid-derived suppressor cells in mice. *Cancer Cell* (2008) **14**:408–19. doi:10.1016/j.ccr.2008.10.011
50. Marrache F, Pendyala S, Bhagat G, Betz KS, Song Z, Wang TC. Role of bone marrow-derived cells in experimental chronic pancreatitis. *Gut* (2008) **57**:1113–20. doi:10.1136/gut.2007.143271
51. Krelin Y, Voronov E, Dotan S, Elkabets M, Reich E, Fogel M, et al. Interleukin-1beta-driven inflammation promotes the development and invasiveness of chemical carcinogen-induced tumors. *Cancer Res* (2007) **67**:1062–71. doi:10.1158/0008-5472.CAN-06-2956
52. Balkwill FR, Mantovani A. Cancer-related inflammation: common themes and therapeutic opportunities. *Semin Cancer Biol* (2012) **22**:33–40. doi:10.1016/j.semcancer.2011.12.005
53. Galdiero MR, Garlanda C, Jaillon S, Marone G, Mantovani A. Tumor associated macrophages and neutrophils in tumor progression. *J Cell Physiol* (2013) **228**:1404–12. doi:10.1002/jcp.24260
54. Sica A, Mantovani A. Macrophage plasticity and polarization: in vivo veritas. *J Clin Invest* (2012) **122**:787–95. doi:10.1172/JCI59643
55. Smyth MJ, Dunn GP, Schreiber RD. Cancer immunosurveillance and immunoediting: the roles of immunity in suppressing tumor development and shaping tumor immunogenicity. *Adv Immunol* (2006) **90**:1–50. doi:10.1016/S0065-2776(06)90001-7
56. Kuraishy A, Karin M, Grivennikov SI. Tumor promotion via injury- and death-induced inflammation. *Immunity* (2011) **35**:467–77. doi:10.1016/j.immuni.2011.09.006
57. Mantovani A, Biswas SK, Galdiero MR, Sica A, Locati M. Macrophage plasticity and polarization in tissue repair and remodelling. *J Pathol* (2013) **229**:176–85. doi:10.1002/path.4133
58. Arwert EN, Lal R, Quist S, Rosewell I, van Rooijen N, Watt FM. Tumor formation initiated by nondividing epidermal cells via an inflammatory infiltrate. *Proc Natl Acad Sci U S A* (2010) **107**:19903–8. doi:10.1073/pnas.1007404107
59. Sakurai T, He G, Matsuzawa A, Yu GY, Maeda S, Hardiman G, et al. Hepatocyte necrosis induced by oxidative stress and IL-1 alpha release mediate carcinogen-induced compensatory proliferation and liver tumorigenesis. *Cancer Cell* (2008) **14**:156–65. doi:10.1016/j.ccr.2008.06.016
60. Grivennikov SI, Karin M. Inflammatory cytokines in cancer: tumour necrosis factor and interleukin 6 take the stage. *Ann Rheum Dis* (2011) **70**(Suppl 1):i104–8. doi:10.1136/ard.2010.140145
61. He G, Yu GY, Temkin V, Ogata H, Kuntzen C, Sakurai T, et al. Hepatocyte IKKbeta/NF-kappaB inhibits tumor promotion and progression by preventing oxidative stress-driven STAT3 activation. *Cancer Cell* (2010) **17**:286–97. doi:10.1016/j.ccr.2009.12.048
62. Shibata W, Takaishi S, Muthupalani S, Pritchard DM, Whary MT, Rogers AB, et al. Conditional deletion of IkappaB-kinase-beta accelerates helicobacter-dependent gastric apoptosis, proliferation, and preneoplasia. *Gastroenterology* (2010) **138**(1022-34):e1–10. doi:10.1053/j.gastro.2009.11.054
63. Borrello MG, Degl'Innocenti D, Pierotti MA. Inflammation and cancer: the oncogene-driven connection. *Cancer Lett* (2008) **267**:262–70. doi:10.1016/j.canlet.2008.03.060
64. Cataisson C, Salcedo R, Hakim S, Moffitt BA, Wright L, Yi M, et al. IL-1R-MyD88 signaling in keratinocyte transformation and carcinogenesis. *J Exp Med* (2012) **209**:1689–702. doi:10.1084/jem.20101355
65. Ling J, Kang Y, Zhao R, Xia Q, Lee DF, Chang Z, et al. KrasG12D-induced IKK2/beta/NF-kappaB activation by IL-1alpha and p62 feedforward loops is required for development of pancreatic ductal adenocarcinoma. *Cancer Cell* (2012) **21**:105–20. doi:10.1016/j.ccr.2011.12.006
66. Grivennikov SI, Karin M. Dangerous liaisons: STAT3 and NF-kappaB collaboration and crosstalk in cancer. *Cytokine Growth Factor Rev* (2010) **21**:11–9. doi:10.1016/j.cytogfr.2009.11.005
67. Pikarsky E, Porat RM, Stein I, Abramovitch R, Amit S, Kasem S, et al. NF-kappaB functions as a tumour promoter in inflammation-associated cancer. *Nature* (2004) **431**:461–6. doi:10.1038/nature02924
68. Douvdevani A, Huleihel M, Segal S, Apte RN. Aberrations in interleukin-1 expression in oncogene-transformed fibrosarcoma lines: constitutive interleukin-1 alpha transcription and manifestation of biological activity. *Eur Cytokine Netw* (1991) **2**:257–64.
69. Douvdevani A, Huleihel M, Zoller M, Segal S, Apte RN. Reduced tumorigenicity of fibrosarcomas which constitutively generate IL-1 alpha either spontaneously or following IL-1 alpha gene transfer. *Int J Cancer* (1992) **51**:822–30. doi:10.1002/ijc.2910510526
70. Dvorkin T, Song X, Argov S, White RM, Zoller M, Segal S, et al. Immune phenomena involved in the in vivo regression of fibrosarcoma cells expressing cell-associated IL-1{alpha}. *J Leukoc Biol* (2006) **80**:96–106. doi:10.1189/jlb.0905509
71. Voronov E, Weinstein Y, Benharroch D, Cagnano E, Ofir R, Dobkin M, et al. Antitumor and immunotherapeutic effects of activated invasive T lymphoma cells that display short-term interleukin 1alpha expression. *Cancer Res* (1999) **59**:1029–35.
72. Zoller M, Douvdevani A, Segal S, Apte RN. Interleukin-1 production by transformed fibroblasts. II. Influence on antigen presentation and T-cell-mediated anti-tumor response. *Int J Cancer* (1992) **50**:450–7. doi:10.1002/ijc.2910500320
73. Zoller M, Douvdevani A, Segal S, Apte RN. Interleukin-1 produced by tumorigenic fibroblasts influences tumor rejection. *Int J Cancer* (1992) **50**:443–9. doi:10.1002/ijc.2910500320
74. el-Shami KM, Tzehoval E, Vadai E, Feldman M, Eisenbach L. Induction of antitumor immunity with modified autologous cells expressing membrane-bound murine cytokines. *J Interferon Cytokine Res* (1999) **19**:1391–401. doi:10.1089/107999099312858
75. Marr RA, Addison CL, Snider D, Muller WJ, Gauldie J, Graham FL. Tumour immunotherapy using an adenoviral vector expressing a membrane-bound mutant of murine TNF alpha. *Gene Ther* (1997) **4**:1181–8. doi:10.1038/sj.gt.3300528
76. Soo Hoo W, Lundeen KA, Kohrumel JR, Pham NL, Brostoff SW, Bartholomew RM, et al. Tumor cell surface expression of granulocyte-macrophage colony-stimulating factor elicits antitumor immunity and protects from tumor challenge in the P815 mouse mastocytoma tumor model. *J Immunol* (1999) **162**:7343–9.
77. Elkabets M, Krelin Y, Dotan S, Cerwenka A, Porgador A, Lichtenstein RG, et al. Host-derived interleukin-1alpha is important in determining

the immunogenicity of 3-methylcholanthrene tumor cells. *J Immunol* (2009) **182**: 4874–81. doi:10.4049/jimmunol.0803916

78. Schreiber RD, Old LJ, Smyth MJ. Cancer immunoediting: integrating immunity's roles in cancer suppression and promotion. *Science* (2011) **331**: 1565–70. doi:10.1126/science.1203486
79. Murphy JE, Morales RE, Scott J, Kupper TSIL-. 1 alpha, innate immunity, and skin carcinogenesis: the effect of constitutive expression of IL-1 alpha in epidermis on chemical carcinogenesis. *J Immunol* (2003) **170**: 5697–703.
80. Song X, Krelin Y, Dvorkin T, Bjorkdahl O, Segal S, Dinarello CA, et al. CD11b+/Gr-1+ immature myeloid cells mediate suppression of T cells in mice bearing tumors of IL-1{beta}-secreting cells. *J Immunol* (2005) **175**:8200–8.
81. Song X, Voronov E, Dvorkin T, Fima E, Cagnano E, Benharroch D, et al. Differential effects of IL-1 alpha and IL-1 beta on tumorigenicity patterns and invasiveness. *J Immunol* (2003) **171**:6448–56.
82. Bunt SK, Sinha P, Clements VK, Leips J, Ostrand-Rosenberg S. Inflammation induces myeloid-derived suppressor cells that facilitate tumor progression. *J Immunol* (2006) **176**: 284–90.
83. Nakao S, Kuwano T, Tsutsumi-Miyahara C, Ueda S, Kimura YN, Hamano S, et al. Infiltration of COX-2-expressing macrophages is a prerequisite for IL-1 beta-induced neovascularization and tumor growth. *J Clin Invest* (2005) **115**:2979–91. doi:10.1172/JCI23298
84. Saijo Y, Tanaka M, Miki M, Usui K, Suzuki T, Maemondo M, et al. Proinflammatory cytokine IL-1 beta promotes tumor growth of Lewis lung carcinoma by induction of angiogenic factors: in vivo analysis of tumor-stromal interaction. *J Immunol* (2002) **169**:469–75.
85. Elkabets M, Ribeiro VSG, Dinarello CA, Ostrand-Rosenberg S, Di Santo J, Apte RN, et al. IL-1β regulates a novel myeloid-derived suppressor cell subset that impairs NK cell development and function. *Eur J Immunol* (2010) **40**(12):3347–57. doi:10.1002/eji.201041037
86. Bar D, Apte RN, Voronov E, Dinarello CA, Cohen SA. continuous delivery system of IL-1 receptor antagonist reduces angiogenesis and inhibits tumor development. *FASEB J* (2004) **18**:161–3.
87. Voronov E, Shouval DS, Krelin Y, Cagnano E, Benharroch D, Iwakura Y, et al. IL-1 is required for tumor invasiveness and angiogenesis. *Proc Natl Acad Sci U S A* (2003) **100**:2645–50. doi:10.1073/pnas.0437939100
88. Carmi Y, Dotan S, Rider P, Kaplanov I, White MR, Baron R, et al. The role of IL-1beta in the early tumor cell-induced angiogenic response. *J Immunol* (2013) **190**:3500–9. doi:10.4049/jimmunol.1202769
89. Li Y, Wang L, Pappan L, Galliher-Beckley A, Shi J. IL-1 beta promotes stemness and invasiveness of colon cancer cells through Zeb1 activation. *Mol Cancer* (2012) **11**:87. doi:10.1186/1476-4598-11-87
90. Li HJ, Reinhardt F, Herschman HR, Weinberg RA. Cancer-stimulated mesenchymal stem cells create a carcinoma stem cell niche via prostaglandin E2 signaling. *Cancer Discov* (2012) **2**:840–55. doi:10.1158/2159-8290.CD-12-0101
91. Ghiringhelli F, Apetoh L, Tesniere A, Aymeric L, Ma Y, Ortiz C, et al. Activation of the NLRP3 inflammasome in dendritic cells induces IL-1beta-dependent adaptive immunity against tumors. *Nat Med* (2009) **15**:1170–8. doi:10.1038/nm.2028
92. Marhaba R, Nazarenko I, Knofler D, Reich E, Voronov E, Vitacolonna M, et al. Opposing effects of fibrosarcoma cell-derived IL-1alpha and IL-1beta on immune response induction. *Int J Cancer* (2008) **123**:134–45. doi:10.1002/ijc.23503
93. Nazarenko I, Marhaba R, Reich E, Voronov E, Vitacolonna M, Hildebrand D, et al. Tumorigenicity of IL-1alpha- and IL-1beta-deficient fibrosarcoma cells. *Neoplasia* (2008) **10**:549–62.
94. Voronov E, Reich E, Dotan S, Dransh P, Cohen I, Huszar M, et al. Effects of IL-1 molecules on growth patterns of 3-MCA-induced cell lines: an interplay between immunogenicity and invasive potential. *J Immunotoxicol* (2010) **7**:27–38. doi:10.3109/15476910903405528
95. Okamoto M, Liu W, Luo Y, Tanaka A, Cai X, Norris DA, et al. Constitutively active inflammasome in human melanoma cells mediating autoinflammation via caspase-1 processing and secretion of interleukin-1beta. *J Biol Chem* (2010) **285**:6477–88. doi:10.1074/jbc.M109.064907
96. Qin Y, Ekmekcioglu S, Liu P, Duncan LM, Lizee G, Poindexter N, et al. Constitutive aberrant endogenous interleukin-1 facilitates inflammation and growth in human melanoma. *Mol Cancer Res* (2011) **9**:1537–50. doi:10.1158/1541-7786.MCR-11-0279
97. Biswas SK, Mantovani A. Macrophage plasticity and interaction with lymphocyte subsets: cancer as a paradigm. *Nat Immunol* (2010) **11**:889–96. doi:10.1038/ni.1937
98. Bui JD, Schreiber RD. Cancer immunosurveillance, immunoediting and inflammation: independent or interdependent processes? *Curr Opin Immunol* (2007) **19**:203–8. doi:10.1016/j.coi.2007.02.001
99. DeNardo DG, Andreu P, Coussens LM. Interactions between lymphocytes and myeloid cells regulate pro- versus anti-tumor immunity. *Cancer Metastasis Rev* (2010) **29**:309–16. doi:10.1007/s10555-010-9223-6
100. Ghiringhelli F, Apetoh L, Housseau F, Kroemer G, Zitvogel L. Links between innate and cognate tumor immunity. *Curr Opin Immunol* (2007) **19**:224–31. doi:10.1016/j.coi.2007.02.003
101. Ostrand-Rosenberg S. Immune surveillance: a balance between protumor and antitumor immunity. *Curr Opin Genet Dev* (2008) **18**:11–8. doi:10.1016/j.gde.2007.12.007
102. Dinarello CA. Anti-inflammatory agents: present and future. *Cell* (2010) **140**:935–50. doi:10.1016/j.cell.2010.02.043
103. Dinarello CA. Why not treat human cancer with interleukin-1 blockade? *Cancer Metastasis Rev* (2010) **29**:317–29. doi:10.1007/s10555-010-9229-0
104. Dinarello CA, Simon A, van der Meer JW. Treating inflammation by blocking interleukin-1 in a broad spectrum of diseases. *Nat Rev Drug Discov* (2012) **11**(8):633–52. doi:10.1038/nrd3800

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

Received: 18 April 2013; accepted: 19 June 2013; published online: 08 July 2013.
*Citation: Voronov E, Dotan S, Krelin Y, Song X, Elkabets M, Carmi Y, Rider P, Cohen I, Romzova M, Kaplanov I and Apte RN (2013) Unique versus redundant functions of IL-1α and IL-1β in the tumor microenvironment. Front. Immunol. **4**:177. doi: 10.3389/fimmu.2013.00177*
This article was submitted to Frontiers in Inflammation, a specialty of Frontiers in Immunology.

frontiers in
IMMUNOLOGY

REVIEW ARTICLE
published: 09 July 2013
doi: 10.3389/fimmu.2013.00181

Opposing functions of classic and novel IL-1 family members in gut health and disease

Loris R. Lopetuso[1,2], Saleem Chowdhry[1,3] and Theresa T. Pizarro[1]*

[1] *Department of Pathology, Case Western Reserve University School of Medicine, Cleveland, OH, USA*
[2] *Internal Medicine, Gastroenterology Division, Catholic University of Rome, Rome, Italy*
[3] *Digestive Health Institute, University Hospitals, Cleveland, OH, USA*

Edited by:
Cecilia Garlanda, Istituto Clinico Humanitas, Italy

Reviewed by:
Miriam Wittmann, University of Leeds, UK
Jennifer E. Towne, Amgen Inc, USA

****Correspondence:***
Theresa T. Pizarro, Department of Pathology, Case Western Reserve University School of Medicine, 2103 Cornell Road, WRB 5534, Cleveland, OH 44106, USA
e-mail: theresa.pizarro@case.edu

In addition to their well-established role(s) in the pathogenesis of gastrointestinal (GI)-related inflammatory disorders, including inflammatory bowel disease (IBD) and inflammation-associated colorectal cancer (CRC), emerging evidence confirms the critical involvement of the interleukin-1 (IL-1) cytokine family and their ligands in the maintenance of normal gut homeostasis. In fact, the paradigm that IBD occurs in two distinct phases is substantiated by the observation that classic IL-1 family members, such as IL-1, the IL-1 receptor antagonist (IL-1Ra), and IL-18, possess dichotomous functions depending on the phase of disease, as well as on their role in initiating vs. sustaining chronic gut inflammation. Another recently characterized IL-1 family member, IL-33, also possesses dual functions in the gut. IL-33 is upregulated in IBD and potently induces Th2 immune responses, while also amplifying Th1-mediated inflammation. Neutralization studies in acute colitis models, however, have yielded controversial results and recent reports suggest a protective role of IL-33 in epithelial regeneration and mucosal wound healing. Finally, although little is currently known regarding the potential contribution of IL-36 family members in GI inflammation/homeostasis, another IL-1 family member, IL-37, is emerging as a potent anti-inflammatory cytokine with the ability to down-regulate colitis. This new body of information has important translational implications for both the prevention and treatment of patients suffering from IBD and inflammation-associated CRC.

Keywords: IL-1 family of cytokines, inflammatory bowel disease, colitis, inflammation-associated colon cancer, intestinal homeostasis, Toll/IL-1 receptor family, mucosal wound healing, intestinal fibrosis

BACKGROUND AND INTRODUCTION

STRUCTURE AND FUNCTION OF THE INTESTINAL GUT MUCOSA

The gastrointestinal (GI) tract, with its epithelial barrier consisting of a total surface area of approximately 200 m^2, is man's most widely exposed organ system to the external environment. The intestinal barrier represents a functional unit responsible for two main tasks crucial for survival of the individual: allowing nutrient absorption, and defending the body from penetration of unwanted, often dangerous, macromolecules. In fact, the gut mucosa is a multi-layered system consisting of an external "anatomical" barrier and an inner "functional" immunological barrier. Commensal gut microbiota, a mucous layer, and the intestinal epithelial monolayer constitute the anatomical barrier. The deeper, inner layer consists of a complex network of immune cells organized in a specialized and compartmentalized system known as gut-associated lymphoid tissue or GALT. GALT represents both isolated and aggregated lymphoid follicles and is one of the largest lymphoid organs, containing up to 70% of the body's total number of immunocytes, and is involved in responding to pathogenic microorganisms and providing immune tolerance to commensal bacteria. The ability of GALT to interact with luminal antigens rests on specific mucosal immune cells (i.e., dendritic cells and M-cells), primarily localized to Peyer's patches within the ileum, that are intimately positioned at the mucosal-environmental interface and internalize microorganisms and macromolecules. These specialized immune cells have the ability to present antigen to naïve T-lymphocytes, which subsequently produce cytokines and activate mucosal immune responses, when needed. From the intracellular point of view, inflammasomes are a group of protein complexes that assemble upon recognition of a diverse set of noxious stimuli and are now considered the cornerstone of the intracellular surveillance system. They are able to sense both microbial and damage-associated molecular patterns (DAMPs) and initiate a potent innate, anti-microbial immune response (1). The interaction of these components sustains the maintenance of the delicate equilibrium needed for intestinal homeostasis. Many factors can alter this balance, including alterations in the gut microflora, modifications of the mucus layer, and epithelial damage, leading to increased intestinal permeability and translocation of luminal contents to the underlying mucosa (2). The integrity of these structures is necessary for the maintenance of normal intestinal barrier function. Dysregulation of any of the aforementioned components have been implicated not only in the pathogenesis of inflammatory bowel disease (IBD), but many other GI disorders, including infectious enterocolitis, irritable bowel syndrome, small intestinal bowel overgrowth, and allergic food intolerance (3–5).

THE TOLL/IL-1 RECEPTOR SUPERFAMILY IN THE GI TRACT

The role of the Toll/IL-1 Receptor (TIR) superfamily and their respective ligands, of which interleukin-1 (IL-1)-like molecules belong, is well established in the pathogenesis of several autoinflammatory and chronic immune disorders (6). However, the emerging concept that Toll-like receptors (TLRs), as well as IL-1 and its related cytokine family members, also play a critical role in health and the maintenance of immune homeostasis is gaining increasing acceptance. The GI system, in fact, represents one of the best examples of where these opposing mechanisms simultaneously take place (7). A large body of literature exists that support the contribution of various TLRs and IL-1 family members, particularly IL-1 and IL-18, to the pathogenesis of IBD, such as Crohn's disease (CD) and ulcerative colitis (UC), as well as GI-related cancers. However, while selective blockade of pro-inflammatory cytokines is one of the most effective strategies to down-regulate mucosal inflammation in IBD (8), Phase I clinical trials using strategies to neutralize either IL-1 or IL-18 have failed to show significant efficacy in treating patients with UC and CD, respectively. One potential cause for this failure is the dichotomous functions of these IL-1 family members in inducing disease pathogenesis, while simultaneously promoting protection, within the intestinal gut mucosa.

In fact, new insights into the role of cytokine-driven pathways in mucosal immunity have been described based on several recent studies in animal models of acute intestinal injury, repair, and chronic inflammation. Information derived from these studies reveal that intestinal homeostasis and inflammation are driven by cellular elements and soluble mediators that mediate both processes, with several cytokines exhibiting opposing roles, depending upon the specific setting. This concept is most strongly supported by members of the IL-1 family of cytokines in the pathogenesis of IBD (**Table 1**) (9–22), where the same cytokine can possess both classic pro-inflammatory properties, as well as protective, anti-inflammatory functions, which is primarily dependent on the presence of receptor-bearing cells during the host's disease state. Related to this notion is the dogma that chronic intestinal inflammation characteristic of IBD develops through two distinct phases (21). Early disease refers to the initial events that take place when homeostatic mechanisms initially fail and acute inflammatory responses cannot be resolved. In contrast, late disease refers to the period when adaptive immunity has been irreversibly primed toward a specific effector phenotype. During these distinct stages of disease progression, innate cytokines play diverse, and often times, dichotomous roles (21).

As such, aside from the established pro-inflammatory properties of IL-1α, IL-1β, IL-18, and their downstream signaling molecules shared with TLR family members, such as nuclear factor kappa-light-chain-enhancer of activated B cells (NF-κB) and myeloid differentiation primary response 88 (MyD88), a growing body of evidence indicates that these mediators are necessary for the maintenance of mucosal homeostasis by effectively handling microbiota, as well as by protecting and restoring the integrity of the epithelial barrier (23–25). While little is known regarding the

Table 1 | Role of IL-1 cytokine family members in IBD and in GI-related cancers.

Common name	IL-1 family name	Ligand-binding chain	Disease association	Potential role in IBD	Potential role in GI-related cancers
IL-Iα	IL-1F1	IL-IR type I	CD, UC	Protective during early phase of inflammation	Induction of tumor growth, metastasis formation, and angiogenesis in gastric, liver, colon, and pancreatic cancer
IL-1β	IL-1F2	IL-IR type I	CD, UC	Protective during early phase of inflammation	Induction of tumor growth, metastasis formation, and angiogenesis in gastric, liver, colon, and pancreatic cancer
IL-IRa	IL-1F3	IL-IR type I	UC	Potential dual role	Protective
IL-18	IL-1F4	IL-18Rα	CD	Protective during early phase of inflammation	Protective in inflammation-associated colon cancer
IL-36Ra	IL-1F5	IL-IRrp2	Unknown	Unknown	Unknown
IL-36α	IL-1F6	IL-IRrp2	Unknown	Unknown	Unknown
IL-37	IL-1F7	IL-18Ra	Unknown for human IBD, antagonist for DSS colitis	Protective (correlates with breakdown of intestinal barrier)	Expressed in colon cancer cells
IL-36β	IL-1F8	IL-IRrp2	Unknown	Unknown	Unknown
IL-36γ	IL-1F9	IL-IRrp2	Unknown	Unknown	Unknown
IL-38	IL-1F10	IL-IRrp2	Unknown	Unknown	Unknown
IL-33	IL-1F11	ST2	UC	Protective	Possible support of tumor formation and progression

potential contributions of other IL-1 family members, such as IL-36, IL-36Ra, IL-37, and IL-38, in chronic intestinal inflammation and gut health, the evolving literature regarding the role of IL-33, the most recently described IL-1 family member is, at present, ambiguous and may reflect yet another example of an innate-type cytokine that possesses multiple functions depending on the immunological status and genetic susceptibility of the host. Although one of the first observations of IL-33-dependent functions in the gut was potent epithelial proliferation and mucus production (26), suggesting the promotion of mucosal repair and healing, dysregulated or uncontrolled IL-33 production may also lead to more pathogenic features characteristic of IBD, including epithelial barrier dysfunction, chronic, relapsing inflammation, and formation of fibrotic lesions (27, 28).

In the present review, we will comprehensively evaluate the role of IL-1 family members and their associated ligands in modulating mucosal homeostasis and chronic inflammation within the GI tract, as well as touch on the potential contribution of these important receptor-ligand pairings to GI tumorigenesis and cancer. Moreover, we speculate about the potential implications of the opposing functions of IL-1 family members for treating chronic intestinal inflammation and inflammation-associated colorectal cancer (CRC), as well as in designing more efficacious strategies for the prevention and treatment of these devastating GI pathologies.

PATHOGENIC ROLE OF CLASSIC IL-1 FAMILY MEMBERS IN CHRONIC INTESTINAL INFLAMMATION AND INFLAMMATION-ASSOCIATED CRC

PATHOGENIC EFFECTS OF IL-1α, IL-1β, AND IL-1RA DURING CHRONIC INTESTINAL INFLAMMATION

IL-1α and IL-1β (IL-1F1 and F2, respectively) are derived from different genes, but are functionally similar, and both bind to the IL-1R type I (IL-1RI) (**Figure 1**). This is followed by recruitment of the co-receptor chain, IL-1R accessory protein (IL-1RAcP), and

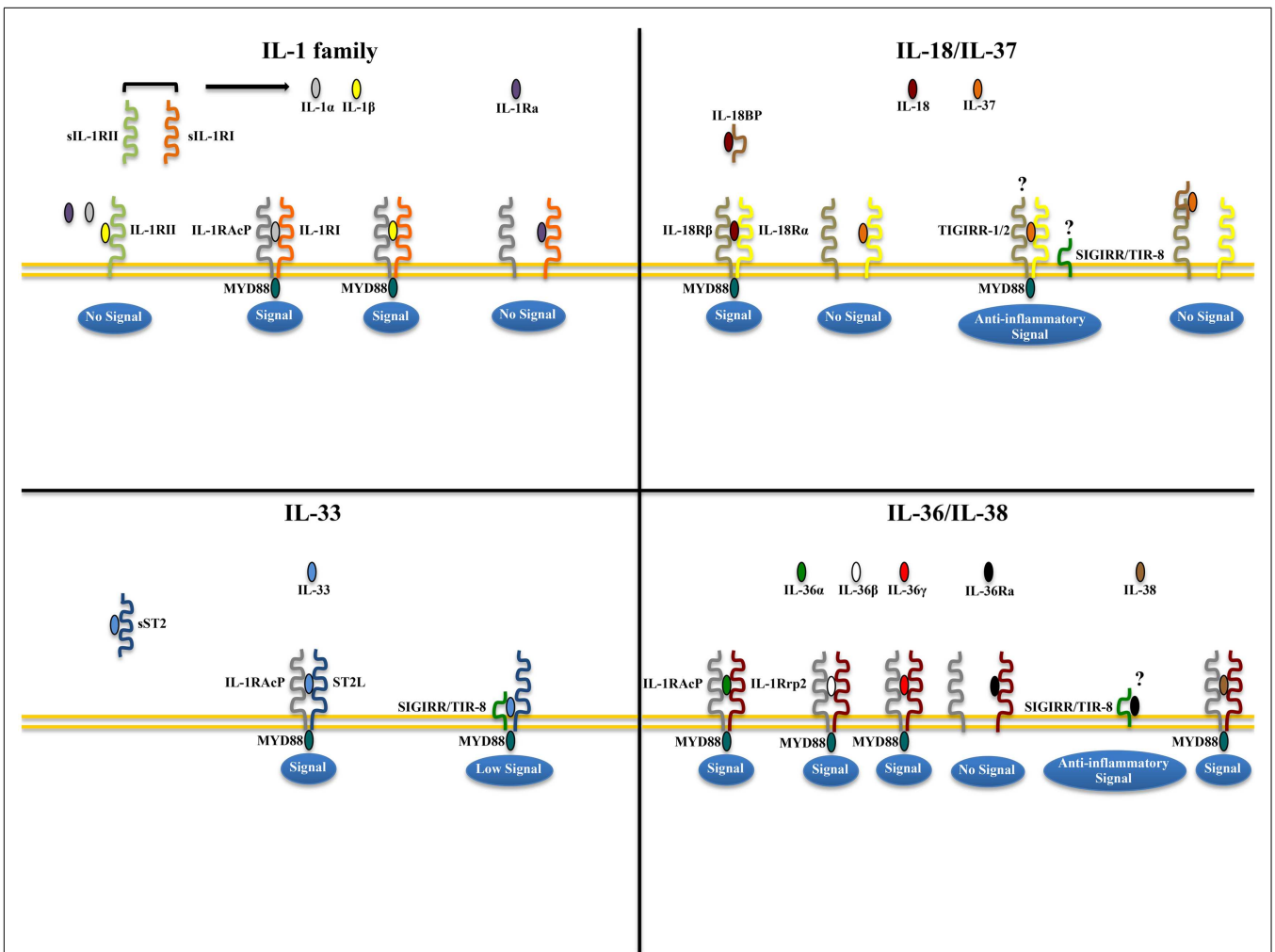


FIGURE 1 | Receptor-ligand pairing of IL-1 family members. Productive pairings of ligand, binding receptor, and accessory protein for the IL-1 (upper left), IL-18/IL-37 (upper right), IL-33 (lower left), and IL-36/IL-38 (lower right) systems. The overall bioactivity of IL-1 family agonists is dependent on the prevalent isoform and receptor binding domain/accessory protein present on effector cells. Promiscuous receptor/co-receptor binding of agonists and antagonists imply that IL-1 family members cannot be considered in isolation, but in the context of other IL-1 family members that can influence their overall integrative effects and impact on disease pathogenesis.

a receptor complex is formed. The IL-1R complex can then recruit the adaptor protein, MyD88, to the TIR domain, after which several kinases are phosphorylated, NF-κB translocates to the nucleus, and the transcription of several inflammatory genes takes place (**Figure 2**). Although they exhibit similar biological activities, IL-1α and IL-1β differ in the manner in which they are processed and secreted. IL-1α is localized in the cytosol or cell membrane and is believed to regulate the intracellular environment (29), but can also be secreted into the extracellular compartment and serve as a soluble mediator (30). In contrast, IL-1β is first cleaved to its mature active form and then secreted extracellularly. Patients with infectious or inflammatory conditions exhibit elevated plasma concentrations of IL-1β but not IL-1α, suggesting a systemic role for IL-1β (30). With the sole exception of IL-1 receptor antagonist (IL-1Ra), each member of the IL-1 family is first synthesized as a precursor molecule without a clear signal peptide for processing and secretion. IL-1α, similar to the newest member of the IL-1 family, IL-33, has the ability to bind its precursor form to IL-1Rs and trigger signal transduction. Moreover, both IL-1β and IL-33 are also considered "dual-function" cytokines in that, in addition to binding to their respective cell surface receptors, their intracellular precursor forms have the ability to translocate to the nucleus and can influence subsequent downstream transcription (31, 32). In general, the nuclear function of IL-1α or IL-33 is transcription of pro-inflammatory genes. In contrast, the precursor forms of IL-1β and IL-18 do not bind to their respective receptors, are not active, requiring cleavage by either intracellular caspase-1 or extracellular neutrophilic proteases (6).

The biologic effects of IL-1 are regulated by naturally produced inhibitors, including IL-1Ra (IL-1F3), that binds to the IL-1RI and is specific for preventing the activity of IL-1α and IL-1β, without possessing any agonist function (6, 33). In addition, binding to the IL-1 receptor type II (IL-1RII), expressed mostly on macrophages, neutrophils, and B cells, does not result in productive signaling due to the lack of a cytoplasmic domain, for which docking of MyD88 cannot take place. IL-1RII binds IL-1β with a greater affinity than IL-1RI and works as a decoy receptor by sequestering IL-1β, thereby operating as a functional IL-1 antagonist. Because IL-1RAcP is recruited to the IL-1RII-IL-1β complex, the decoy receptor also serves to sequester the accessory receptor from participating in IL-1 signaling through IL-1RI (6). Finally, an additional tactic that IL-1Rs use to regulate the activity of IL-1 is by proteolytic cleavage of their extracellular domains. Shedding of IL-1RII results is the soluble form of IL-1RII (sIL-1RII) that has an increased affinity for IL-1β compared to IL-1α and IL-1Ra (34–38), thereby contributing to the antagonism of IL-1 by preferentially neutralizing IL-1β's bioactivity. In addition, an alternate form of IL-1RAcP also exists that consists of only its extracellular

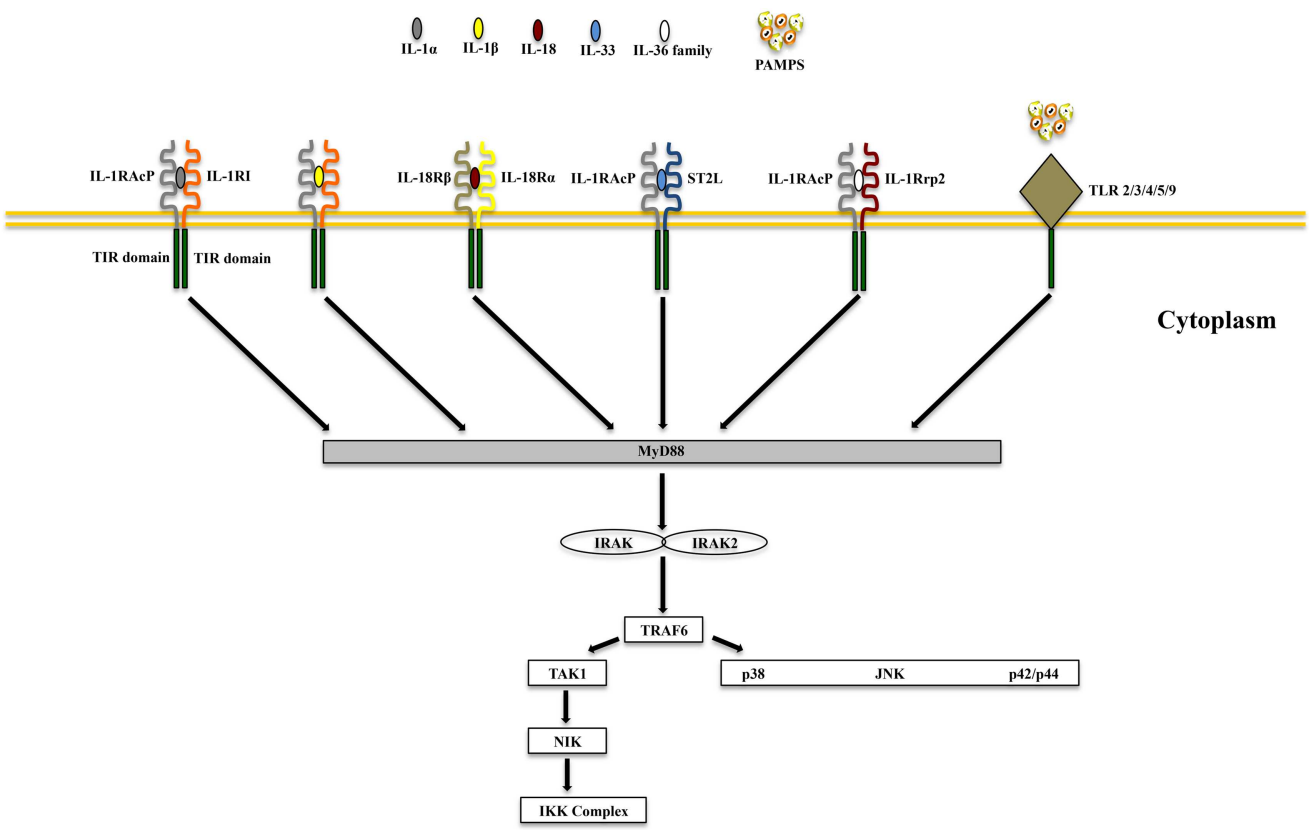


FIGURE 2 | Common signaling pathway for Toll/IL-1R (TIR) family members. TLRs possess significant homology to IL-1R family members and share a similar cytoplasmic TIR domain with receptors of IL-1, IL-18, IL-33, and IL-36. The TIR domain is essential for recruitment of cytoplasmic adapter proteins, which in turn initiates downstream signaling cascades. PAMPS, pathogen-associated molecular patterns.

domain; this soluble IL-1RAcP has the ability to associate with ligand-bound sIL-1RII, which results in an increased affinity of binding to both IL-1α and β, further establishing sIL-1RII as a potent inhibitor of IL-1 (39). Conversely, similar to its membrane bound form, sIL-1RI retains that ability to bind IL-1α and IL-1Ra with greater affinity than IL-1β, and can therefore be regarded as promoting a pro-inflammatory phenotype by sequestering IL-1Ra and limiting its anti-inflammatory effects on IL-1RI-bearing target cells, and by facilitating free IL-1β to bind to cell surface IL-1RI to promote pro-inflammatory immune responses (36, 38, 40, 41). Therefore, from a clinical perspective, the balance between IL-1 agonists, antagonists, and the amount of surface as well as soluble IL-1Rs affect the overall degree and severity of inflammation in several diseases, including IBD.

Gut mucosal inflammation is characterized by infiltration of neutrophils and mononuclear cells, which upon activation, are important sources of cytokines and other inflammatory mediators. IL-1α and IL-1β play key roles in intestinal inflammation, as they are produced early and induce the production of many other cytokines, amplifying their pro-inflammatory action (6). A marked increase in IL-1 production by isolated lamina propria mononuclear cells (LPMCs), most prominently from tissue histiocytes or macrophages, and by intestinal mucosal tissues has been reported in patients with active IBD by several groups (13, 42–44). Furthermore, tissue levels of IL-1 also closely correlate with the degree of observed mucosal inflammation and necrosis (9).

One of the earliest bodies of work dissecting the role of IL-1 in experimental colitis was performed using a rabbit immune complex-mediated model that possesses some features of UC (9, 12, 45, 46). Results from theses studies have provided insight into the bi-directional effects of an innate-type cytokine (i.e., IL-1). In this model, both IL-1α and IL-1β are increased in the inflamed intestinal tissues and display pro-inflammatory properties, as neutralization by either endogenous or exogenous IL-1Ra administration resulted in significant amelioration of colitis (12, 45, 46). Despite these findings, administration of recombinant IL-1β had a similar beneficial effect, indicating that IL-1β is necessary for mucosal protection and maintenance of homeostasis in this model (9). In fact, the currently accepted paradigm is that an imbalance of pro- and anti-inflammatory mediators, as exemplified by the IL-1/IL-1Ra system, is a key mechanism in the pathogenesis of IBD (47).

Interleukin-1 receptor antagonist, primarily produced by intestinal epithelial cells (IECs) and LPMCs within the gut mucosa (48), regulates the bioactivity of IL-1 and a marked decrease in the mucosal IL-1Ra/IL-1 ratio was found in both CD and UC patients when compared to control subjects (13). In this study, the IL-1Ra/IL-1 ratio correlated closely with the clinical severity of disease and was specific for IBD since this trend was not observed in patients with self-limiting colitis. Although the precise mechanism(s) as to why this imbalance occurs in IBD is not specifically known, several groups have reported an association between carriage of the IL-1RN allele 2 (IL-1RN*2) of the IL-1Ra variable number of tandem repeats (VNTR) polymorphism and low production of IL-1Ra, as well as increased severity of disease in UC patients of several ethnic backgrounds (49–51). Finally, as indicated earlier, the expression and presence of cell surface and soluble IL-1Rs can affect the severity and overall disease phenotype that manifests in patients with IBD. In a study that surveyed circulating plasma and colonic tissue levels of IL-1α, IL-1β, IL-1Ra, sIL-1RI, and sIL-1RII from IBD patients and controls, it was found that sIL-1RI served as a systemic biomarker of disease activity in CD patients, while local shedding of the functional antagonist, sIL-1RII, was associated with decreased colonic inflammation in CD, but not in UC, patients (52).

Taken together, the pathogenesis of chronic intestinal inflammation is characterized by a robust elevation of IL-1 family members promoting agonist activity, including IL-1α and IL-1β, whose primary source are LPMCs of myeloid lineage. A recent study, however, also provides evidence that during acute experimental colitis, IL-1α is potently produced by the intestinal epithelium (53). At the same time, production of IEC- and LP macrophage-derived IL-1Ra is not adequate to overcome the overwhelming pro-inflammatory effects of IL-1, resulting in perpetuation of chronic intestinal inflammation. This deficit in IL-1Ra can be due to carriage of a genetic polymorphism that infers low production, particularly in UC patients. Aside from IL-1 ligands, another facet of overall IL-1 biology to consider in a disease setting is the contribution of IL-1Rs. Within the gut mucosa, almost all cell types have the ability to respond to IL-1 ligands and express IL-1RI and II. The ability of these cells to shed soluble forms of IL-1Rs have indicated that sIL-1RI plasma levels may serve as a biomarker for disease activity and local sIL-1RII is associated with decreased colonic inflammation, specifically in CD patients. To date, however, a comprehensive study as to the precise distribution of IL-1Rs, including their co-receptors, their cellular sources, and potential trigger(s) to induce shedding during chronic intestinal inflammation, has not been performed. The results derived from these studies would provide critical information regarding the precise contribution of different IL-1R-bearing gut mucosal cell types during the course of disease, as well as aid in the design of more effective therapies to restore the IL-1/IL-1Ra imbalance (**Figure 3**).

PATHOGENIC EFFECTS OF IL-1α, IL-1β, AND IL-1RA IN GI-RELATED CANCERS

In addition to their contribution to chronic intestinal inflammation, IL-1 has also been implicated in tumorigenesis and tumor progression in the GI tract. Cancer cells can directly produce IL-1 or can induce cells within the tumor microenvironment to do so (54). IL-1β is upregulated in colon cancer, and patients with IL-1β-producing tumors generally possess a bad prognosis (55–57). The expression patterns of IL-1, in general, vary since it is expressed in either an autocrine or paracrine fashion (58). Co-culture studies on human melanocytic cells showed that IL-1α and IL-1 β exhibit autocrine behavior by stimulating tumor cells themselves to invade and proliferate, or exert paracrine effects on stromal cells in the microenvironment. The exact mechanism(s) by which IL-1 promotes tumor growth remain unclear, although it is believed to act primarily in an indirect fashion. In human colon cancer lines, IL-1 induces expression of metastatic genes, such as matrix metalloproteinases (MMPs), and stimulates nearby cells to produce angiogenic proteins and growth factors (59), including vascular endothelial growth factor (VEGF), IL-8, IL-6, tumor necrosis factor (TNF), and transforming growth factor (TGF)β (30, 60–62).

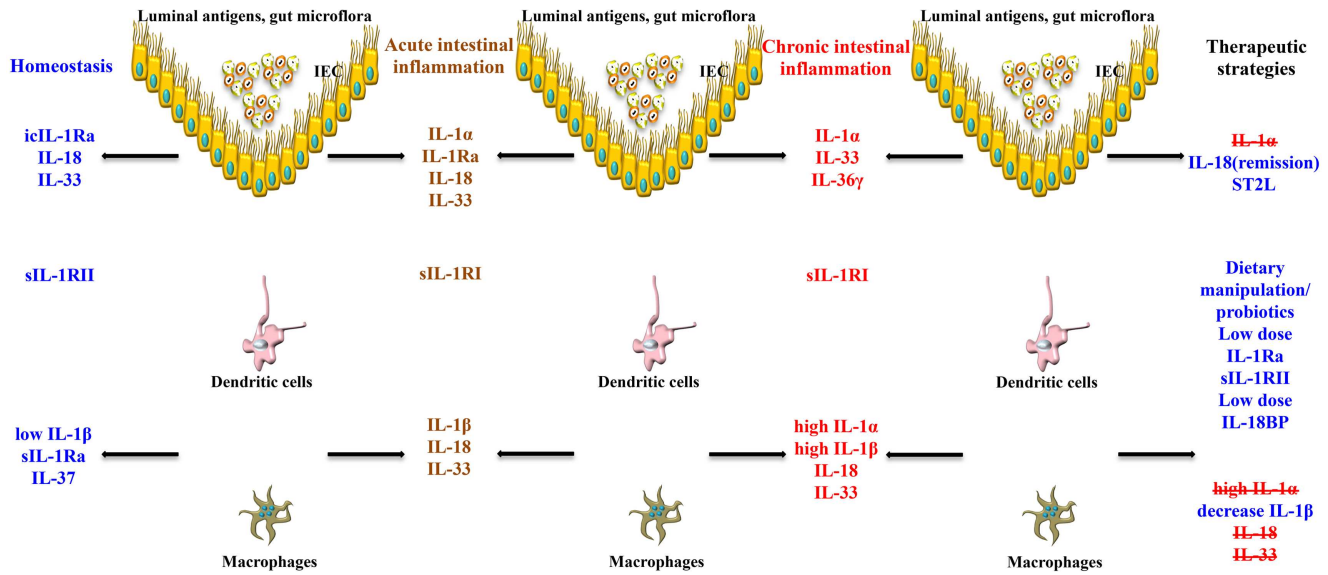


FIGURE 3 | Role of IL-1 family members in the gut mucosa and potential therapeutic strategies to restore intestinal homeostasis. The effects of the same IL-1 family member can vary, and are often dichotomous, depending on the cell source, phase of disease (early vs. late), and inflammatory state (acute vs. chronic) of the host. IECs and APCs are critically involved in maintaining gut homeostasis, but also in triggering gut inflammation. icIL-1Ra, IL-18, and IL-33 released by IECs, together with sIL-1Ra, IL-37, and low levels of IL-1β produced by APCs, and sIL-1RII shed by macrophages, promote physiological inflammation, and homeostasis. Chronic intestinal inflammation results when this balance is disrupted, often changing the cellular sources and absolute levels of IL-1 family members. Based on this imbalance, therapeutic strategies aimed at modulating levels of IL-1 family members can be designed to reestablish gut health.

Further studies in IL-1 transgenic mice demonstrate the necessity of IL-1 in tumor growth, metastasis, and angiogenesis (62, 63). Sawai et al. (64) evaluated the role of IL-1 in metastatic and non-metastatic human pancreatic cancer cell lines and showed that metastatic lines demonstrate increased IL-1RI expression compared to non-metastatic cell lines, and exposure to IL-1α results in increased α6- and β1-integrin subunit expression, whereas IL-1α exposure to non-metastatic lines has no effect. Additionally, IL-1α induces adhesion and invasion into laminin in human metastatic cell lines, but not in non-metastatic cell lines. This study highlights the importance of IL-1α for invasiveness and angiogenic properties *in vitro*, and confirms that only those cancer cell lines that show highly metastatic properties express IL-1α mRNA (65). These findings have also been confirmed for colon and gastric cancers. Human colon cancer-derived IL-1α induces angiogenesis by its action upon the microenvironment, and thereby contributes to metastasis (66). Along this same line, a significant correlation between IL-1α expression and metastasis in human gastric carcinomas has also been established (67, 68). Moreover, increased IL-1 production by gastric epithelial cells leads to gastric inflammation and the development of gastric dysplasia and cancer, as demonstrated in IL-1 transgenic mice (69). In fact, the administration of IL-1Ra has been proposed as a therapeutic regimen for different neoplasias (63).

Similar to IL-1, several lines of evidence point to the involvement of the another IL-1 family co-receptor member, single Ig IL-1R related molecule (SIGIRR), also known as Toll/IL-1R 8 (TIR8), in colitis-associated cancer in mice (70). SIGIRR/TIR8 is an orphan receptor that inhibits signaling from IL-1R/TLR complexes, possibly by trapping IL-1R-associated kinase (IRAK)-1 and TNFR-associated factor (TRAF)6 (71, 72), and is characterized by the presence of a single immunoglobulin domain in its extracellular region, a conserved TIR domain, and a 95-amino-acid long tail with inhibitory properties (73, 74) (**Figure 1**). SIGIRR/TIR8 is expressed in several tissues, especially in the digestive tract, and cell-type expression is particularly high in epithelial cells (74, 75). SIGIRR/TIR8 functions as a negative regulator for LPS and IL-1 signaling through its interaction with TLR4 and the IL-1R complex (76). Accordingly, there is evidence for a non-redundant regulatory role of this molecule in inflammation within the GI mucosa (75). *Tir8* deficient mice exhibit dramatic intestinal inflammation (colitis) in response to dextran sodium sulfate (DSS) administration in regard to weight loss, intestinal bleeding, and mortality, and show increased susceptibility to carcinogenesis in response to azoxymethane (AOM)/DSS administration (70). This increased susceptibility to colitis-associated cancer in *Tir8* deficient mice is linked to increased permeability and local production of prostaglandin E2 (PGE2), pro-inflammatory cytokines, and chemokines. In fact, colonic epithelial cells from *Tir8* deficient mice display commensal bacteria-dependent homeostatic defects, as shown by constitutive upregulation of pro-inflammatory genes, and increased inflammatory and tumorigenic responses to DSS and AOM/DSS challenge, respectively (77). As such, gut epithelial-specific expression of the *Tir8* transgene reduces colonic epithelial cell survival, abrogates the hypersensitivity of *Tir8* KO mice to DSS-induced colitis, and reduces

AOM/DSS-induced tumorigenesis (77). These findings have been confirmed in $Apc^{min/(}$ mice, a spontaneous ileal polyposis model. Introduction of *Tir8* deficiency into the $Apc^{min/(}$ mice leads to increased loss of heterozygosity of *Apc* and colonic microadenoma formation. Importantly, the increased tumorigenesis in $Apc^{min/(}/Tir8^{-/-}$ mice is dependent on the presence of the commensal flora, underscoring the role of dysregulated commensal bacteria-TLR signaling in colonic tumor initiation (78).

The impact of the relationship between the gut microbiota and IL-1 family members on colitis-driven CRC also involves the inflammasome. Inflammasomes comprise, in essence, a multiprotein platform for the activation of inflammatory caspases, of which caspase-1 appears to play a dominant role (79). They include a sensor protein, an adaptor protein [apoptosis-associated speck-like protein (ASC) containing a caspase activation and recruitment domain (CARD)], and an inflammatory caspase. Sensor proteins belong to two families of proteins: the nucleotide-binding oligomerization domain (NOD)-like receptor (NLR) family and the pyrin and hemopoietic expression, interferon-inducibility, nuclear localization (HIN) domain-containing protein (PYHIN) family. Tight control of caspase-1 activation by inflammasomes, in particular of NOD-like receptor family pyrin domain-containing 3 (NLRP3, also referred to as Nalp3, CIAS1, or Cryopyrin), is critical since the processing and release of IL-1β and IL-18, as well as a subset of leaderless proteins that facilitate tissue repair, are directly regulated by caspase-1 (80). Homotypic interactions between the pyrin domain in the N-terminus of NLRP3 and the bipartite adaptor protein ASC (encoded by *Pycard*) bridge the association of caspase-1 to NLRP3 in the inflammasome. Mice lacking the inflammasome adaptor protein ASC and caspase-1 demonstrate increased disease outcome, morbidity, histopathology, and polyp formation in the AOM/DSS model of CRC (81). The increased tumor burden correlates with attenuated levels of IL-1β and IL-18 at the tumor site. In particular, leucine-rich-repeat-containing $Nlrp3^{-/-}$ mice show an increase in acute and recurring colitis and colitis-associated cancer, although the disease outcome is less severe in $Nlrp3^{-/-}$ mice than in $Pycard^{-/-}$ or $Casp1^{-/-}$ animals. No significant differences have been found in disease progression or outcome in NLR family CARD domain-containing protein 4 $(Nlrc4)^{-/-}$ mice compared to similarly treated wild-type (WT) animals. Bone marrow reconstitution experiments show that *Nlrp3* gene expression and function in hematopoietic cells, rather than IECs or stromal cells, is responsible for protection against increased tumorigenesis (81). These data suggest that the inflammasome functions as an attenuator of colitis and colitis-driven CRC. Taken together, the imbalance of IL-1 agonists with IL-1 antagonists and their associated receptors/co-receptors within the GI tract may not be limited to promoting inflammatory processes, but may also be important in tumorigenesis and tumor progression (**Figure 4**). Re-establishing this balance may represent a new therapeutic target in the treatment of GI-related cancers.

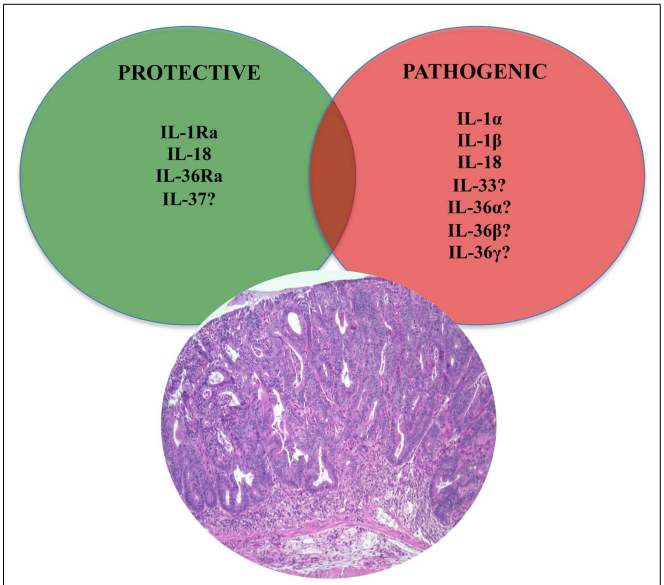


FIGURE 4 | Role of IL-1 family members in colon cancer. Similar to chronic intestinal inflammation characteristic of IBD, an imbalance between protective and pathogenic IL-1 family members is also an important mechanism leading to intestinal tumorigenesis and the development of GI-related cancers. In fact, IL-1 cytokines play an important role in sustaining tumor growth by stimulating growth factor production and modulating host immune responses against tumor cells. While the roles of classic IL-1 family members, such as IL-1 and IL-18, have been firmly established, only speculation can be made on other, newer IL-1 family members, such as IL-33, IL-36, and IL-37.

IL-18 IN CHRONIC INTESTINAL INFLAMMATION

IL-18 (IL-1F4) was initially characterized as a novel IFNγ-inducing factor in mice infected with *Propionibacterium acnes* and subsequently challenged with a sublethal dose of LPS; as such, this factor was originally coined IFNγ inducing factor or IGIF (82). After cloning, IL-18 was shown to induce IFNγ in the presence of a mitogen or IL-2, and these effects were shown to be independent of IL-12 (83). IL-18 is widely expressed throughout various organ systems in the body and in cells of both hematopoietic and non-hematopoietic cell lineages (e.g., macrophages, dendritic cells, Kupffer cells, keratinocytes, osteoblasts, adrenal cortex cells, IECs, microglial cells, and synovial fibroblasts) (14, 84–90). Within the gut mucosa, IL-18 is primarily produced by IECs, tissue histiocytes (or macrophages), and dendritic cells (14, 15, 91). IL-18 exerts its biological effects through binding to the IL-18R complex, which is a heterodimer consisting of an α chain (IL-18Rα or IL-1R related protein 1, IL-1Rrp1), that is responsible for extracellular binding of IL-18, and a non-binding, signal-transducing β chain (IL-18Rβ or Accessory Protein Like, AcPL) (**Figure 1**). Both chains are members of the IL-1R family and are required for functional IL-18 signaling, that similar to IL-1, occurs through MyD88/IRAK, leading to the downstream activation of NF-κB (92–95) (**Figure 2**). The IL-18R complex is expressed on a variety of cell types, including T- and B-lymphocytes, macrophages, neutrophils, natural killer cells, endothelial cells, and smooth muscle cells (96–99). It can be upregulated on naïve T cells, Th1 cells, and B cells by IL-12 (93, 100). In contrast, T cell receptor ligation in the presence of IL-4 results in downregulation of the IL-18R complex (101). Although initially described as a Th1 polarizing cytokine, IL-18 has been shown to be a pleiotropic cytokine that can mediate both Th1- and Th2-driven immune responses (102, 103).

An additional family member that affects the overall bioactivity of IL-18 is the IL-18 binding protein (IL-18BP), a naturally occurring, soluble protein that effectively inhibits IL-18 by preventing its interaction with the endogenous receptor (**Figure 1**) (104). The human IL-18BP gene encodes at least four distinct isoforms (IL-18BPa-d), which are derived by alternative splicing (105). IL-18BP isoforms a and c neutralize the biological activity of IL-18, whereas b and d do not (106). The high affinity of IL-18 to the IL-18BP has a significant impact on its overall bioactivity and clinical relevance during disease states as ligand passing of IL-18 from the IL-18BP to its cell-bound receptor does not occur due to this unusually tight binding (104, 106, 107). Thus, the IL-18/IL-18BP system possesses several biological activities that underscore the potential for IL-18 to serve as a key mediator in the pathogenesis of several chronic inflammatory disorders, including IBD.

Our group and others were the first to report that IL-18 is upregulated in patients with IBD, particularly in CD (14, 15). IL-18 is present in the serum of CD patients, and bioactive IL-18 expression along with IL-18-induced cytokines are increased in mucosal biopsies of patients with IBD compared to controls, in involved vs. non-involved lesions, and in chronic advanced compared to early asymptomatic disease (14, 15, 91). Interestingly, immunohistochemistry (IHC) studies of colonic tissues derived from IBD patients and controls reveal a distinct pattern of IL-18 expression that may uncover potential IL-18-dependent mechanisms involved in maintaining gut health and in the pathogenesis of chronic intestinal inflammation (14) (**Figure 3**). In these studies, a dramatic shift in IL-18 expression is observed within the gut mucosa of CD patients as inflammation became more severe. In non-involved areas, IL-18 is immunolocalized almost exclusively to the epithelium, similar to that found in uninflamed tissues from the resected healthy margins of colon cancer patients. As disease severity increases, IL-18 expression switches from the epithelium to the lamina propria, specifically in cells morphologically consistent with tissue histiocytes/macrophages, wherein the most severe cases lack epithelial-derived IL-18. This trend appears to be specific for CD as IL-18 expression in UC patients is limited to the epithelium, independent of disease severity (14).

The IL-18 BP is also differentially expressed in intestinal tissues from IBD patients. Intestinal endothelial cells and macrophages are the major source of IL-18BP in the submucosa, and in CD, an increased number of IL-18BP-expressing macrophages and endothelial cells, specifically isoforms a, c, and d, has been detected (105). The presence of IL-18BP in CD lesions suggests neutralization of IL-18 activity, unless patients with active CD preferentially undergo differential splicing to produce more of the inactive isoforms (b and d) than the a and c bioactive isoforms. These patients would then have a reduced ability to regulate IL-18 activity during the course of the disease. In fact, free IL-18 is still observed in specimens from active CD and highlights the complexity of regulating bioactivity of IL-18. The importance of the IL-18BP in regulating IL-18 has also been reported in pediatric IBD patients, particularly in CD (108). IL-18BP does not sequester all free IL-18, which is increased not only local gut tissues, but also in the serum of children with active CD.

Although the majority of studies characterizing IL-18 and IL-18BP in IBD have been mostly descriptive in nature, they have laid the foundation that underscores the importance of the balance between IL-18 and IL-18BP in gut health and the pathogenesis of chronic intestinal inflammation, particularly in patients with CD. Similar to assessing global IL-1 bioactivity, expression of the IL-18R/co-receptor system on effector target cells should also be considered when evaluating the overall biological effects of IL-18. To date, a comprehensive study has not been performed to measure IL-18Rs and/or co-receptors in either CD or UC patients. However, polymorphisms in the IL-18 accessory protein (IL-18RAcP/IL-18RAcPL/IL-18Rβ), as well as IL-18, have been linked to IBD susceptibility (109–111). In fact, IL-18 expression is reportedly altered by a number of polymorphisms including three single-nucleotide polymorphisms (SNPs) in the IL-18 promoter at positions -137, -607, and -656, relative to the transcriptional start site (112). Transcription analysis of the first two polymorphisms showed that they cause altered transcription factor binding and gene expression (112). Similarly, SNP rs917997 correlates with altered expression of IL-18Rβ and is strongly associated with IBD and celiac disease (111, 113).

Animal models of IBD have provided a critical tool to mechanistically determine the potential role of IL-18 during the pathogenesis of colitis (120). Initial studies, in fact, support a pathogenic role for IL-18. In mice with either 2,4,6-trinitrobenzene sulfonic acid (TNBS)- or DSS-induced colitis, intestinal IL-18 levels of both macrophage and epithelial cell origin were found to be markedly elevated (114, 115). IL-18 expression, in co-operation with IL-12, leads to the expansion of Th1 $CD4^+$ T cells (116) and production of the prototypic Th1 cytokines, IFN(, and TNF (117). Further evidence that IL-18 plays an important role in the chronic phase of intestinal inflammation was demonstrated using a T-cell dependent adoptive transfer model, wherein local administration of an adenovirus expressing anti-sense IL-18 mRNA had the ability to effectively treat colitis in recipient SCID mice (118). In fact, neutralization or targeted gene deletion of IL-18 results in amelioration of both chemically- and immunologically-mediated colitis (117–120), which may occur through a mechanism wherein local TNF production is dampened (114). Moreover, transgenic overexpression of IL-18 is associated with exacerbated colitis, which displays a marked infiltration of mucosal macrophages (121). The cellular re-distribution of IL-18, from IECs to gut mucosal macrophages, may be responsible for the pro-inflammatory role that IL-18 appears to play during chronic inflammatory responses within the gut mucosa. Using the SAMP1/YitFc (SAMP) model of spontaneous CD-like ileitis (122), our group previously reported that the mouse IL-18 gene is located within an interval on chromosome 9 that confers genetic susceptibility to disease in these mice (123). Similar to human CD, SAMP mice display a dramatic shift in the cellular source of IL-18 as disease becomes more severe, from IECs to LPMCs (124). The temporal and spatial expression of IL-18, in regard to the cellular source, as well as the presence or absence of specific IL-18R bearing cells, may explain the observed differential effects of IL-18 during the innate, early phases, vs. the later, chronic stages, of IBD. Together, these data indicate that IL-18 represents a central mediator in the pathogenesis of intestinal inflammation and is able to play very different roles during the inflammatory process depending on the host's inflammatory state. As such, therapeutic strategies to alter IL-18 bioactivity need

to be carefully addressed to determine the appropriate dose (low vs. high) and most beneficial time (early vs. late) to neutralize endogenously produced IL-18 during chronic inflammatory diseases, including IBD (**Figure 3**).

IL-18 IN INFLAMMATION-ASSOCIATED GI-RELATED CANCERS

Aside from its established role in mucosal innate and adaptive immunity within the GI tract, IL-18 has also been identified as a mediator that both promotes and suppresses the process of oncogenesis (**Figure 4**). Although discussed in detail later in this review, IL-18's protective effects include the ability to induce cell death and tumor regression through NK cell activation (125). In experimental cancer models, IL-18 expression in tumor cells has been shown to enhance both specific and non-specific anti-tumor immune responses (126, 127). On the other hand, IL-18 mRNA expression and serum levels correlate with the development and progression of gastric cancers (128), and may be associated with esophageal carcinoma. IL-18 upregulates expression of VEGF (129) and thrombospondin (130), suggesting its effectiveness in promoting angiogenesis. IL-18 also promotes metastasis by inducing cell adhesion molecules (131) and MMPs (132), while facilitating immune evasion by increasing the expression of Fas ligand on tumor cells (133). Similar to IBD, polymorphisms in the IL-18 promoter region are also strongly associated to GI-related cancers. In particular the rs917997 genotype appears to correlate with patient risk of reflux progressing to Barrett's and esophagus adenocarcinoma (134, 135). IL-18 promoter polymorphisms are also associated with an increased risk for the development of gastric and colorectal cancers (136, 137).

PROTECTIVE ROLE OF CLASSIC IL-1 FAMILY MEMBERS IN MAINTAINING INTESTINAL HOMEOSTASIS AND GUT HEALTH

PROTECTIVE EFFECTS OF IL-1α, IL-1β, AND IL-1RA

As previously mentioned, initial studies using a rabbit model of colitis revealed the potential dual role of IL-1 as a classic pro-inflammatory cytokine (12, 46, 138) as well as a mediator that has beneficial effects, particularly the IL-1β isoform, promoting gut mucosal protection (45). Interestingly, protection by IL-1β is only achieved with administration of low dose IL-1β, and only when given 24 h, but not 30 min, before the induction of colitis. Such protective effects of low dose IL-1 have also been shown in other disease models, such as arthritis (139) and sepsis (140). Similarly, in a mouse model of DSS-induced colitis, neutralization of IL-1 activity during the acute phase of disease was associated with exacerbated severity of inflammation and delayed recovery from injury (23). No effect was observed during the chronic stage of colitis, suggesting that IL-1 may have opposing effects during the progression of colitis by inferring protection during early, acute inflammation, but exerting more pro-inflammatory functions in later stages during the chronic phase of disease (**Figure 3**).

An alternative hypothesis to support the dichotomous role of IL-1 in IBD is that IL-1α and IL-1β possess opposing roles during the progression of chronic intestinal inflammation. In support of this concept, a recent study by Bersudsky et al. demonstrates that the precursor form of IL-1α, derived primarily from damaged IECs following DSS-induced colitis, can act as a classic alarmin by initiating and sustaining colitis, while IL-1α KO mice show little disease with increased recovery (53). Conversely, myeloid cell-derived IL-1β in the same colitic model induces the restitution and repair of IECs and improves gut barrier function during the recovery phase of acute inflammation. Furthermore, while specific blockade of IL-1α leads to amelioration of colitis, administration of IL-1Ra or anti-IL-1β antibodies do not effectively treat DSS colitis (53). Taken together, understanding the potential opposing roles of IL-1 agonists, such as IL-1α and IL-1β, during the initiation and progression of chronic intestinal inflammation, will shed further light on precise therapeutic modalities that will lead to more efficacious treatment of patients with IBD (**Figure 3**).

IL-18-DEPENDENT PROTECTION DURING INTESTINAL INFLAMMATION

Based on more recent studies, results point to the possibility of IL-18 possessing dichotomous roles during the progression of IBD, which may be related to phase of disease, as well as the cellular sources of both ligand and receptors/co-receptors (25). In fact, at the onset, or initiation of intestinal inflammation, IL-18 derived from IECs may exert a protective role, facilitating tissue repair and promoting mechanisms to induce homeostasis. In support of this concept is the observation that IL-18 and IL-18R KO mice are more susceptible to acute DSS colitis than their WT littermates (141). In addition, epithelial-derived IL-18 is critical for the protection from DSS colitis conferred by NLR-mediated signaling, as shown in studies utilizing mice deficient in *Nlrp3* (142).

In fact, similar to IL-1, emerging evidence highlights the control of IL-18 activation and the overall regulation of intestinal mucosal immune responses exerted by the inflammasome (80). As mentioned earlier, tight regulation of caspase-1 activation by inflammasomes is critical since the processing and release of IL-1β and IL-18 are directly regulated by caspase-1 (80). IL-18 is upregulated at the site of inflammation in DSS-exposed WT, but not in *Nlrp3*$^{-/-}$, *Pycard*$^{-/-}$, and *Casp1*$^{-/-}$, mice (142). Nlrp3, Asc and Caspase-1/11 KO mice are also hyper-sensitive to acute DSS colitis, with low colonic IL-18 levels associated with disease susceptibility, while administration of exogenous IL-18 ameliorates colitis severity (142). Nevertheless, Bauer et al. (143) demonstrated that *Nlrp3* KO mice are protected from DSS-induced colitis, suggesting that DSS itself may activate the NLRP3 inflammasome. These results support that concept that different inflammasomes may exert differential and redundant effects on the development and progression of inflammation that may be additive or divergent, resulting in a hierarchical combinatorial net effect on intestinal inflammation (144). Thus, activation of a particular inflammasome in hematopoietic cells, such as dendritic cells and macrophages, may result in local release of IL-1β and/or IL-18 that induces inflammatory changes, such as secretion of IFNγ, while IL-18 secretion from IECs, through a different inflammasome, may play a local role in tissue regeneration and wound mucosal healing in response to injury. Such differential and cell-specific contributions of inflammasome signaling remain to be demonstrated experimentally. Moreover, inflammasomes are able to induce pyroptosis in damaged or infected IECs, which may affect tissue regeneration and consequently, the level of microbial influx into the LP and its effects on the severity of colitis (80). These effects may be induced by different inflammasomes and introduce

a complex net effect based on temporal and microanatomical variations. However, mutations in the inflammasome pathway may also affect colitis differently, depending on the composition of the commensal microbiota that is present in the host since the inflammasome is a critical regulator of colonic microbial ecology (145). This observation also underscores the role of the commensal flora in intestinal immune homeostasis and further demonstrates the complexity of the gut mucosal immune system.

PROTECTIVE ROLE OF IL-18 IN GI TUMORIGENESIS

In contrast to its established, pathogenic role in tumorigenesis, IL-18 has been shown to represent a key protective cytokine in the development of inflammation-associated CRC using the AOM/DSS-induced model of colitis-associated cancer (146). An association between chronic inflammation and tumor development and progression is well established and as such, it is not surprising that a cytokine that has protective properties against inflammation can also reduce tumorigenesis associated with chronic inflammation. In fact, IL-18 and IL-18R KO mice are known to be highly susceptible to both DSS-induced colitis and colorectal tumorigenesis (147). In addition, MyD88 KO mice, which are defective in both IL-1β and IL-18 production, exhibit increased colonic epithelial proliferation, damage and colorectal tumorigenesis (147). Furthermore, administration of exogenous IL-18 can alleviate the severity of colitis and colitis-induced tumorigenesis in caspase-1/11 and Nlrp3 KO mice (148). In contrast, IL1R KO mice show equal numbers of colorectal tumors in the CRC AOM/DSS model, highlighting the unique and essential role of IL-18 during intestinal tumor progression (148). As such, and taking into consideration the previous discussion regarding IL-18's pathogenic role in CRC, the contribution of IL-18 in tumorigenesis and the development of intestinal-specific cancer is clearly dichotomous. However, based on the current data, it appears that, similar to the role of IL-1 family members in intestinal inflammation, IL-18 primarily infers protection during early events leading to the development of GI cancers, including epithelial repair processes (147) and anti-tumor immune responses (126, 127), while during later stages, IL-18 supports events sustaining tumor growth [e.g., angiogenesis (129) and metastasis (130)].

IL-37

IL-37 (IL-1F7) was first identified in 2000 and is one of the most recently characterized members of IL-1 family (149). In general, IL-37 has been shown to have potent anti-inflammatory properties and there is currently intense interest in elucidating its precise role in chronic intestinal inflammation and inflammation-associated CRC. Its relationship to IL-18 is that it binds to IL-18Rα, but unlike IL-18, it does not bind to the IL-18Rβ subunit or the accessory protein, IL-1RAcP (150–152) (**Figures 1** and **2**). Data, however, specifically investigating IL-37b, which is the most abundant form of IL-37 and the most studied, its binding to IL-18Rα, and whether IL-37 represents a competitive antagonist for IL-18 and its functions, remains unclear. An alternative hypothesis is that the IL-37b/IL-18Rα complex uses an accessory protein, such as SIGIRR/TIR8 (153), thereby activating a yet unknown anti-inflammatory pathway (**Figure 1**). It has also been suggested that IL-37 may bind weakly to the IL-18BP and render the IL-18Rβ useless for IL-18 by co-receptor competition (152) (**Figure 1**). In addition, recent studies have shown that the mature form of IL-37b may also translocate to the nucleus, similar to IL-1α and IL-33, and possess a regulatory role in gene transcription (154). At present, five splice variants (IL-37a-f) have been identified in humans; however, none of these variants are present in mice. Splice variant a, b, and c are expressed in lymph nodes, thymus, bone marrow, lung, testis, placenta uterus, skin, and colon; in addition, these variants are expressed in variety of immune cells, such as NK cells, monocytes, and stimulated B cells, while isoforms d and e are only expressed in testis and bone marrow (155). As mentioned earlier, IL-37b is the most abundant isoform and, relevant to the present review, is expressed in the cytoplasm of plasma cells in epithelial crypts, in the lamina propria of normal colon, and in the stroma of colon carcinomas. As with other IL-1 family members, IL-37 is synthesized as a precursor molecule that is cleaved by caspase-1 to its mature form (151).

In regard to its role in the pathogenesis of chronic intestinal inflammation and inflammation-associated CRC, very little has been reported at present (**Table 1**). *In vitro* studies on macrophages and epithelial cells overexpressing IL-37b, as well as *in vivo* experiments in transgenic mice overexpressing human IL-37b, show reduced DC activation and decreased production of pro-inflammatory and Th1/Th17 cytokines, including IL-1β, IL-6, IFNγ, and IL-17 following LPS stimulation. *In vivo* studies suggest that these effects may be mediated through the Smad3 pathway (156). In addition, IL-37b-tg mice exposed to DSS further upregulate IL-37b expression after epithelial injury and display a significant reduction in the severity of colitis compared to WT controls (16). IL-37 is also expressed in the colorectal carcinoma cell line, CCL-247, and in the stroma of colon cancer tumors, wherein IHC revealed intense staining in plasma cells of both normal and diseased colon, suggesting a potential role of IL-37 in antibody production, B-cell activation, and in colon tumorigenesis (151). Therefore, while initial reports indicate that IL-37 may play an anti-inflammatory role in acute colitis (**Figure 3**), further studies are warranted to elucidate the precise role in both chronic intestinal inflammation as well as inflammation-associated CRC (**Figure 4**).

DICHOTOMOUS ROLE OF IL-33, THE NEWEST MEMBER OF THE IL-1 FAMILY, IN INTESTINAL INFLAMMATION AND MUCOSAL WOUND HEALING

IL-33, also known as IL-1F11, is a protein with dual function that can act both as signaling cytokine as well as an intracellular nuclear factor (157) (**Table 1**). In the GI tract, IL-33 is primarily expressed in non-hematopoietic cells, including fibroblasts, adipocytes, smooth muscle cells, endothelial cells, and IECs (26, 158, 159), but is also present in cells of hematopoietic origin, particularly in restricted populations of professional antigen presenting cells, such as macrophages and DCs (26). IL-33 exerts its biological effects through binding to its receptor, IL-1 receptor-like 1 (IL1RL1), also known as ST2 (26, 28), and in the presence of IL-33, ST2 pairs with its co-receptor, IL-1RAcP, and signals through mitogen-activated protein kinase (MAPK)- and NF-κB-dependent pathways (26, 160) (**Figure 2**). Similar to IL-18Rα, the co-receptor SIGIRR/TIR8 can also dimerize with ST2 and likely acts as a negative regulator of the IL-33/ST2 signaling pathway, ultimately reducing IL-33's biological effects

(161) (**Figure 1**). To date, a very limited amount of information is available regarding the biologic and pathophysiologic relevance of IL-33 isoforms/splice variants, ST2 splice variants, and alternative ST2/SIGIRR signaling.

IL-33 IN MAINTAINING GUT HOMEOSTASIS

In regard to its role in the GI tract, emerging evidence suggests that IL-33 plays a critical role in maintaining normal gut homeostasis. IL-33 enhances mucosal defenses against intestinal parasites and bacteria, as described for *Toxoplasma gondii* (162), *Pseudomonas aeruginosa* (163), and *Leptospira* (164) infection, indicating a primary role in mucosal protection. In addition, one of the earliest observations regarding the biological activity of IL-33 was its ability to promote epithelial proliferation and mucus production (26), which are obvious functions involved in epithelial restitution and repair, as well as overall mucosal wound healing and protection. Similar to IL-1α, increasing evidence also indicates that IL-33 can function as a prototypic "alarmin," passively released upon cellular damage, stress, or necrosis, and able to serve as a danger signal/alarmin to alert the immune system of a local threat, such as trauma or infection (159, 165–167). In this setting, IL-33 has the ability to signal local, innate immune responses in an effort to mount an effective, physiological inflammatory reaction in order to restore normal gut homeostasis.

IL-33 has also been shown to activate mast cells, which are distributed throughout barrier tissues, such as the skin and mucosa, including the intraepithelial space of the intestine. Mast cells are classically considered important late-stage effector cells during Th2-associated immune responses, such as host responses against parasitic helminths in mucosal tissues (168). However, recent studies show that mast cells are able to initiate and orchestrate type 2 immunity against helminth infection through the regulation of tissue-derived cytokines. In fact, mast cell-deficient mouse strains and mice treated with the mast cell stabilizing agent, cromolyn sodium, show dramatically reduced Th2 priming and type 2 cytokine production and harbor an increased burden of parasites following infection with the GI helminthes, *Heligmosomoides polygyrus bakeri* and *Trichuris muris*. In addition, early production of the tissue-derived cytokines IL-25, IL-33, and thymic stromal lymphopoietin (TSLP), is significantly diminished in mast cell-deficient mice. Finally, repair of mast cell deficiency increases production of IL-25, IL-33, and TSLP, restores progenitor cell number and Th2 priming, and reduces intestinal parasite burden. These data reveal the important link between IL-33 and an innate IgE-independent role for mast cells in orchestrating type 2 immune responses. Mast cell degranulation, which is crucial for the activation of dendritic cells and recruitment of neutrophils and T cells to the site of infection (169–171), is also needed for the enhanced expression and production of the tissue-derived IL-25, IL-33, and TSLP, which are required for the optimal orchestration and priming of type 2 immunity (172, 173) and are obvious, apparent events important in intestinal mucosal protection against infection.

IL-33/ST2 AXIS IN IBD

In regard to chronic intestinal inflammation, it is now well established, and confirmed by several groups, that increased IL-33 expression is associated with IBD when compared to healthy controls, particularly in UC patients (17–20). In addition, a potential genetic predisposition to dysregulated IL-33/ST2 function may exist as a recent study describes the novel observation of association between the rs3939286 IL-33 polymorphism and IBD, and between the IL1RL1 rs13015714 and CD, in a well-characterized Italian cohort of adult and early onset IBD patients (155). The distribution of IL-33 expression in the gut mucosa is primarily localized to non-hematopoietic cells, particularly IECs (17, 18, 20) and myofibroblasts (19). In addition, *ex vivo* studies on isolated intestinal mucosal cell populations and immunolocalization on full-thickness intestinal tissues show that IL-33 is also expressed by a wide variety of cell types (17, 19, 22), such as fibroblasts, smooth muscle cells, endothelial cells (26, 174), and adipocytes (17, 158). In active UC, IL-33 is localized to, and potently expressed by, IECs, as well as infiltrating LPMCs, belonging to the monocyte/macrophage and B-cell lineages (17–19). It has also been originally reported by Kobori et al. (19), and later confirmed (22), that IL-33 is expressed in activated subepithelial myofibroblasts (SEMFs) situated below ulcerative lesions in UC, but not in CD, patients supporting a potential functional role for IL-33 in ulcer/wound healing, which may be different in UC compared to CD (**Figure 3**).

Similar to IL-33, its receptor, ST2, is also increased in the intestinal mucosa of IBD patients (17, 18). Importantly, the intestinal tissue expression pattern of ST2 is different in healthy mucosa compared to that found in chronically inflamed IBD patients, wherein ST2 is abundantly expressed in macroscopically non-inflamed colon epithelium, while during chronic inflammatory processes characterizing either UC or CD, its expression is lost/decreased and redistributed (28). This epithelial-derived tissue expression for ST2 appears to be IBD-specific since non-specific colitis (e.g., diverticulitis and infectious colitis) do not present with this same expression pattern (17). Taken together, considering the potential role of IL-33 in promoting mucosal protection, as well as its tissue distribution in IBD, it is tempting to speculate that the primary role for IL-33 is, in fact, to induce epithelial restitution and repair and mucosal healing (27). In addition, further analysis has shown that the ST2 variant for which expression is altered in the epithelium of IBD patients is ST2L, IL-33's signaling transmembrane receptor (18, 28). As such, it is possible that impaired epithelial ST2L expression, specifically in IBD patients, may represent an inherent epithelial defect or a negative feedback response to chronic exposure of elevated IL-33 concentrations. One cannot rule out, however, that IL-33 may have pathogenic, as opposed to protective, effects by indirectly damaging or disrupting epithelial barrier function through, for example, recruitment of neutrophils and eosinophils, as well as consider its effects in mounting potent Th2, Th17, and potentiate Th1, immune responses that can amplify and sustain chronic intestinal inflammation. In fact, the dichotomous role of IL-33 has been best characterized in the intestine, where it can possess both protective and pro-inflammatory functions, depending upon the immunological status of the host and/or the type and phase of the ongoing inflammatory process (21, 28).

ROLE OF IL-33 IN EXPERIMENTAL MODELS OF ACUTE COLITIS

Interestingly, investigation into the role of IL-33 in the development of intestinal inflammation using an acute model of DSS colitis has generated mixed results, and likely reflects the

dichotomous roles of IL-33 in both inducing inflammation as well as promoting epithelial restitution/repair and mucosal healing. In fact, DSS administration to IL-33 deficient mice results in less severe colitis than in WT controls, with decreased granulocyte infiltration (175), while exogenous administration of IL-33 to DSS-treated mice further aggravates colitis and induces the influx of neutrophils (176), suggesting a pathogenic role of IL-33, at least in an acute inflammatory setting. Although it is unclear as to what factor(s) precisely regulate IL-33 in the gut, it has recently been shown that severe colonic inflammation with a marked increase in IL-33-producing macrophages results after DSS administration to mice expressing a truncated form of the receptor for TGFβ, supporting a pathogenic function for IL-33 during acute colitis and indicate a direct effect of TGFβ on macrophages to limit IL-33 expression (177). Imaeda et al. also reported an exacerbation of DSS-induced colitis upon treatment with IL-33, hypothesized to occur by IL-33-dependent induction of pathogenic Th2 cytokines; although in the same mice, IL-33 restores goblet cells that were found to be depleted in IL-33-untreated mice (178). In addition, during the recovery phase of DSS-induced colitis, while weight recovery is markedly delayed in IL-33 deficient mice, no significant difference in colonic inflammation is observed between these mice and WT littermates (175). The authors propose that in this particular model, IL-33 plays an important role in driving acute, innate immune responses, but is dispensable in the maintenance of chronic intestinal inflammation. Alternatively, the possibility exists that the delayed weight recovery observed in IL-33 deficient mice, but not in WT littermates, is due to the lack of IL-33-driven epithelial regeneration and restoration of barrier function leading to a dampened ability for mucosal healing.

In fact, as opposed to their results obtained from IL-33 treatment in acute DSS colitis, Groβ et al. showed that IL-33 administration during repeated, chronic cycling of DSS causes a reduction of colitis, suppresses IFNγ, and decreases bacterial translocation (176), supporting a protective role of IL-33 that the authors suggest may occur by switching from Th1- to Th2-driven immune responses. These results are supported by a recent study using the TNBS-induced model of colitis (179). Although the aforementioned study utilized an acute, 4-day protocol, exogenous administration of IL-33 was shown to ameliorate TNBS-induced colitis and induce the production of Th2-type cytokines (179). In addition, the protective effect of IL-33 was diminished after depletion of T-regulatory cells (T_{regs}). The authors propose that, mechanistically, IL-33 has an indirect effect on the development of $Foxp3^+$ T_{regs} by increasing the expression of epithelial-derived TSLP and retinoic acid, which promotes the activation of $CD103^+$ DCs (180) and leads to the induction of $Foxp3^+$ T_{reg} development (181). The ultimate IL-33-induced expansion of $Foxp3^+$ T_{regs} facilitates the observed decrease in the severity of colitis.

ROLE OF IL-33 IN EXPERIMENTAL CHRONIC INTESTINAL INFLAMMATION

In SAMP mice, IL-33 expression patterns in the gut mucosa and within the systemic circulation of IBD patients were confirmed (17). IL-33 gut mucosal tissue levels in SAMP mice progressively increase over time and demonstrate a positive correlation with ileal inflammation, with epithelial cells exclusively expressing full-length IL-33 (17). Although the precise, mechanistic role of IL-33 has not yet been addressed in the SAMP model, preliminary studies blocking IL-33 signaling by administration of an antibody against ST2 indicate a pathogenic role during the chronic phase of disease development (182, 183). In fact, neutralization of IL-33 interferes with the massive influx of eosinophils into the gut mucosa (183) and potently decreases fibrosis and fibrogenic gene expression (182), characteristic of SAMP mice. Interestingly, although blockade of IL-33 has a significant effect on decreasing the overall severity of ileal inflammation in SAMP mice, the magnitude of this reduction is approximately 30%, which may reflect a need for optimizing treatment dosage or alternatively, represents an opposing effect of interfering with epithelial repair and mucosal healing. Investigation is further warranted to study the role of IL-33 during the early, acute phase of SAMP ileitis, as well as the specific role of epithelial-derived IL-33 and IL-33's direct effects on the intestinal epithelium.

IL-33 AND ST2 IN INTESTINAL FIBROSIS

Although the role of IL-33 has not yet been fully investigated in the pathogenesis of intestinal fibrosis, several lines of evidence indicate that the IL-33/ST2 axis may represent an important mediator in this process. Within the gut mucosa, SEMFs have been reported as a primary source of IL-33, specifically in UC patients where they are situated below ulcerative mucosal lesions (19, 22). In fact, Sponheim et al. observed that a prominent feature of IBD-associated IL-33 expression is the accumulation of both fibroblasts and myofibroblasts in ulcerations of UC lesions (22). Although, the association and localization of IL-33-producing SEMFs with mucosal ulcerations suggests an important role in wound healing, one cannot rule out its potential role in gut-associated fibrosis, particularly in the setting of cycling of chronic tissue damage and repair, characteristic of IBD. Taken together, there is clear evidence of the IL-33/ST2 axis in maintaining normal gut homeostasis, particularly in promoting mucosal wound healing and repair. When dysregulated, this important ligand-binding pair can also play a critical role in the progression of chronic inflammation and fibrosis, leading to such GI-related disorders as IBD.

EMERGING ROLE OF THE IL-33/ST2 AXIS IN GI-RELATED CANCERS

Finally, based on the established role of IL-1 family members in GI-related cancers, the possibility exists that IL-33 can likewise play an important role in GI-associated tumor formation. In fact, a recent study has reported elevated IL-33 levels in the serum of gastric cancer patients that correlates with several poor prognostic factors, including depth of invasion, distant metastasis, and advanced stage, but not with the classic tumor markers, CEA and CA 19-9 (184). Of note, however, no significant difference in IL-33 expression was found between four gastric cancer cell lines and the normal gastric cell line, GES-1, which may indicate that IL-33 expression can either be modulated by local environmental factors and/or produced by other cells responding to gastric cancer epithelial cells. As such, the initial observation of increased, circulating IL-33 levels in gastric cancer patients may be related to the progression of the cancer. In addition, based on IL-33's ability to shift host immune responses to a Th2 phenotype, downregulation of tumor-specific immune responses can occur by inhibiting tumor antigen presentation (185, 186). From this point of view, IL-33 may represent one of the effective weapons tumor cells utilize

in order to create an ideal environment to obtain, and maintain, optimal growth conditions, further supporting the role of the IL-33/ST2 axis in tumor formation and the progression of cancer (**Figure 4**).

POTENTIAL CONTRIBUTION(S) OF THE IL-36-RELATED CYTOKINES IN GUT HEALTH AND DISEASE

In the last two decades, human genome sequence analysis has helped to identify new members of the IL-1 family. Three new members IL-36α, β, and γ, previously known as IL-1F6, IL-1F8, and IL-1F9, respectively, have been shown to bind to a heterodimeric receptor, IL-36R, also known as IL-1 receptor-related protein 2 (IL-1Rrp2), in a manner similar to the binding of IL-1α and IL-1β to IL-1RI. Consistent with the promiscuous nature of IL-1 family members, the IL-36 complex then recruits IL-1RAcP, thereby activating downstream NF-κB and MAPK pathways (187, 188). Interestingly, IL-36 family members also includes a receptor antagonist, IL-36Ra, similar to IL-1Ra, suggesting significant homology between these two IL-1 subfamilies (**Figure 1**).

At present, there are no known reports regarding the association between IL-36 and chronic intestinal inflammation, including IBD, as well as inflammation-associated CRC. Most of the published studies concerning IL-36 and disease pathogenesis come from either the psoriatic or pulmonary literature. In skin, all three IL-36 agonist ligands are highly expressed in psoriatic skin lesions (189–191). Johnston et al. has shown that TNF and IL-17 stimulation of human keratinocytes can induce IL-36, and IL-36 can, in turn, stimulate production of anti-microbial peptides and MMPs in human epidermal cells (192). Muhr et al. confirmed these findings and demonstrated that IL-17 potently induces greater amounts of IL-36 in keratinocytes obtained from psoriatic patients compared to healthy controls (193). Others have described increased expression of TNF and IL-6 in

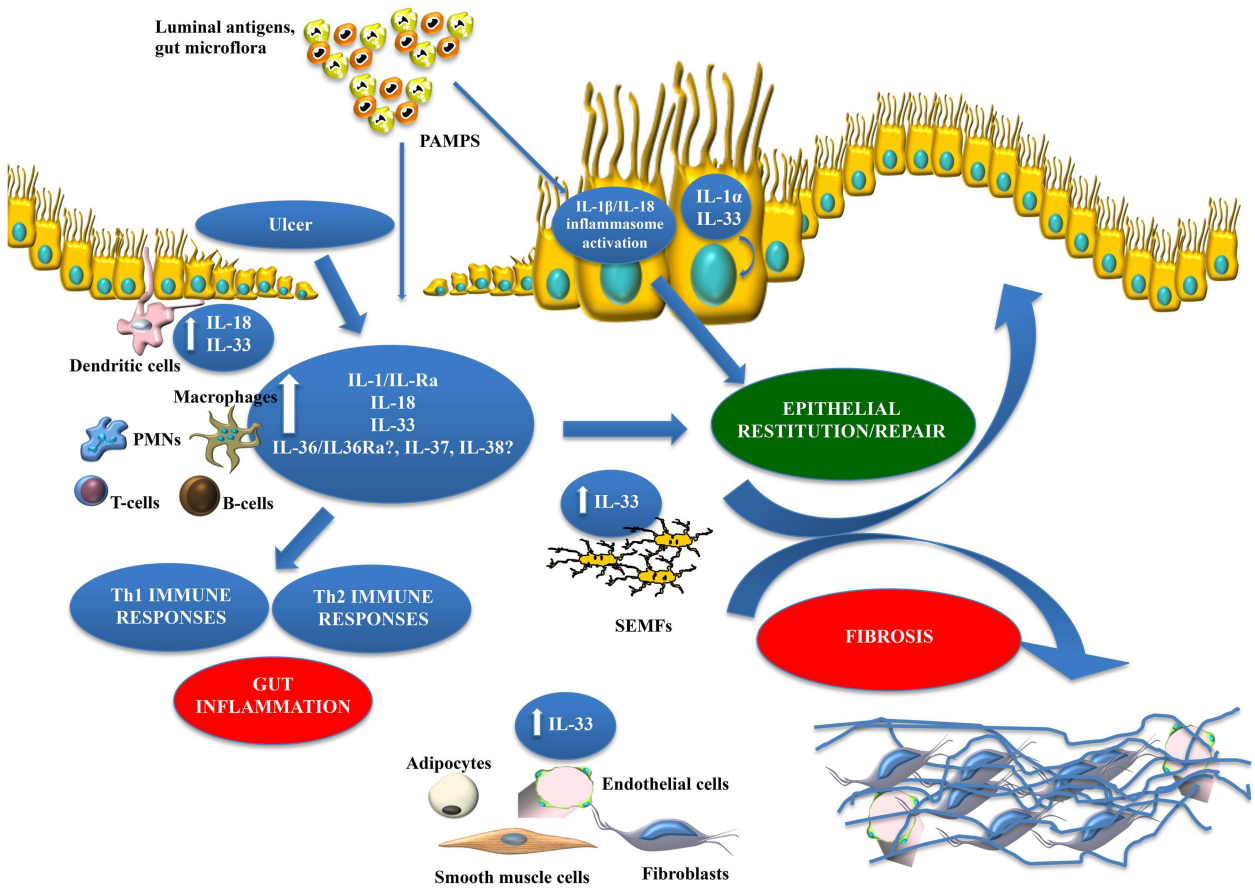


FIGURE 5 | Working hypothesis summarizing opposing functions of IL-1 family members within the gut mucosa. The balance between pro-inflammatory and protective cytokines is crucial for the maintenance of gut homeostasis. Damage to the epithelium (e.g., ulcer formation) and other pro-inflammatory stimuli, including PAMPs derived from luminal antigens and the local intestinal microflora, induce the expression of IL-1 family members that are subsequently released by necrotic IECs as potential alarmins (e.g., IL-33 and IL-1α). Depending on the cellular source and presence of receptor-bearing effector cells, IL-18 can possess very different functions within the gut mucosa. IL-33 may also act on various immune cell populations, including macrophages, and T- and B-cells, eliciting a pro-inflammatory response and promoting Th2 immunity. Concomitantly, IL-33 can also induce epithelial proliferation and repair, and overall wound healing by acting directly or indirectly on IECs and SEMFs. Alternatively, chronic mucosal damage, granulomatous inflammation, and dysregulated activation of mesenchymal cells, such as SEMFs and fibroblasts, can lead to fibrosis and the formation of intestinal fibrotic lesions. Therapeutic interventions should consider all of the aforementioned processes and whether targeting specific IL-1 family members may be more efficacious during active disease vs. maintaining remission.

IL-36-stimulated keratinocytes, suggesting a mutual regulation of these inflammatory mediators (194). Recent studies have also supported an important role of IL-36γ in various lung pathologies. IL-36γ expression is reported to be increased in mice after allergen challenge, and intratracheal administration of IL-36γ leads to airway hyper-responsiveness, neutrophil accumulation and pro-inflammatory cytokine production (195–197). In addition, IL-36 signaling promotes Th1 polarization of naïve $CD4^+$ T cells (198) and induction of Th17 immune response in lung disease (196, 199). Finally, increased IL-36α expression was reported in eosinophilic esophagitis, indicating a possible role of IL-36 in Th2-type immune responses (200). Taken together, these data imply an important pro-inflammatory role for IL-36 ligands in chronic immune disorders, although it is unclear at present whether IL-36 is prone to promoting Th1, Th2, and/or Th17 immunity and whether, like other IL-1 family members, IL-36 may possess dichotomous functions in the setting of health and disease states. In addition to the three described IL-36 agonists, the IL-36Ra and IL-38, previously known as IL-1F5 and IL-1F10, respectively, also bind to the IL-36R; however, differently from IL-36α, β, and γ, IL-36Ra and IL-38 both serve as antagonists for the biological activities of IL-36 (187, 188, 190, 201). Interestingly, IL-36Ra has been shown to possess an anti-inflammatory effect localized to the brain and mediated through a unique TIR8/SIGIRR-dependent pathway (202).

On the basis of the limited availability of published data and preliminary findings, IL-36 may potentially play an important role in chronic inflammatory disorders, including IBD. Investigation into the role of the IL-36 family of cytokines in chronic intestinal inflammation and inflammation-associated CRC, in fact, is an active area of research that may uncover further pathogenic mechanism(s) involved in GI-related pathologies and may provide the foundation for IL-36 to serve as a potential therapeutic target in the near future.

CONCLUSION

The present review provides evidence that members of the IL-1 family of cytokines possess dichotomous, often opposing functions in both the maintenance of normal gut homeostasis and in the pathogenesis of chronic intestinal inflammation and inflammation-associated CRC. We hypothesize that their effects vary, depending on the phase of disease (early vs. late), as well as the inflammatory state (acute vs. chronic) of the host. In general, early activation of the intestinal epithelium by pathogenic organisms and/or other noxious environmental antigens elicits the production of epithelial-derived IL-1 family members, including intracellular (ic)IL-1Ra, IL-1α, IL-18, and IL-33. Epithelial disruption often occurs, facilitating translocation of luminal bacterial products and the recruitment of innate immune cells, primarily neutrophils, and macrophages that are also a potent source of secreted (s)IL-1Ra, IL-1β, IL-18, and IL-33. Normally, early expression of these mediators function to dampen acute inflammation and promote epithelial repair and restitution, with the end goal of limiting gut mucosal damage and restoring intestinal homeostasis. Under conditions of either uncontrolled and/or persistent inflammation (e.g., as a result of innate immune dysfunction or host genetic predisposition), infiltration of adaptive immune cells bearing various IL-1R family members occurs during the later phases of inflammation, making available an effector population able to respond to IL-1-like ligands. For example, the presence of naïve $CD4^+$ T cells expressing the IL-18R have the ability to respond to IL-18, and in combination with IL-12, represents one of the most potent stimuli for IFNγ production and Th1 polarized effector responses, thereby promoting chronic Th1-mediated inflammation. Similar effects can occur upon IL-33 stimulation of naïve $CD4^+$ T cells, but in this case, a robust Th2 immune response results. Furthermore, several levels of regulation exist within each subfamily of IL-1 family members, often including the presence of several agonist isoforms (both precursor and mature, cleaved forms), receptor antagonists, as well as soluble and cell-bound decoy receptors. In addition, the promiscuity of IL-1 family ligands with both binding receptors as well as recruited accessory proteins, instills yet another level of regulation that should be considered when determining the overall biological effects of a specific IL-1 family member agonist. In fact, IL-1 family members cannot be considered in isolation, but with other IL-1-related proteins that can influence their overall interactive effects. An imbalance in the equilibrium between IL-1 family components, dependent on prevalent isoform and receptor binding domain/accessory protein present on effector cells, maybe responsible for either driving pathogenic events, including chronic intestinal inflammation, fibrosis, and CRC, or for promoting protection by inducing epithelial repair, mucosal wound healing, and restoration of gut homeostasis (summarized in **Figure 5**). Based on this new information and the emerging concept that IL-1 family members can possess opposing role in gut health and disease, novel pathogenic hypotheses can be formed that have important translational implications in regard to the prevention and treatment of chronic intestinal inflammation, including CD and UC, and CRC.

ACKNOWLEDGMENTS

The authors acknowledge continued support from the National Institutes of Health: DK056762, DK091222, and AI102269, and a Research Award from the DeGregorio Family Foundation (all to Theresa T. Pizarro).

REFERENCES

1. Strowig T, Henao-Mejia J, Elinav E, Flavell R. Inflammasomes in health and disease. *Nature* (2012) **481**(7381):278–86. doi:10.1038/nature10759
2. Scaldaferri F, Pizzoferrato M, Gerardi V, Lopetuso L, Gasbarrini A. The gut barrier: new acquisitions and therapeutic approaches. *J Clin Gastroenterol* (2012) **46**(Suppl):S12–7. doi:10.1097/MCG.0b013e31826ae849
3. Camilleri M, Madsen K, Spiller R, Van Meerveld BG, Verne GN. Intestinal barrier function in health and gastrointestinal disease. *Neurogastroenterol Motil* (2012) **24**(6):503–12. doi:10.1111/j.1365-2982.2012.01921.x
4. Fasano A. Leaky gut and autoimmune diseases. *Clin Rev Allergy Immunol* (2012) **42**(1):71–8. doi:10.1007/s12016-011-8291-x
5. Fasano A. Zonulin and its regulation of intestinal barrier function: the biological door to inflammation, autoimmunity, and cancer. *Physiol Rev* (2011) **91**(1):151–75. doi:10.1152/physrev.00003.2008
6. Dinarello CA. Interleukin-1 in the pathogenesis and treatment of inflammatory diseases. *Blood* (2011) **117**(14):3720–32. doi:10.1182/blood-2010-07-273417

7. Pizarro TT, Cominelli F. Cytokine therapy for Crohn's disease: advances in translational research. *Annu Rev Med* (2007) **58**:433–44. doi:10.1146/annurev.med.58.121205.100607
8. Rutgeerts P, Vermeire S, Van Assche G. Biological therapies for inflammatory bowel diseases. *Gastroenterology* (2009) **136**(4):1182–97. doi:10.1053/j.gastro.2009.02.001
9. Cominelli F, Nast CC, Clark BD, Schindler R, Lierena R, Eysselein VE, et al. Interleukin 1 (IL-1) gene expression, synthesis, and effect of specific IL-1 receptor blockade in rabbit immune complex colitis. *J Clin Invest* (1990) **86**(3):972–80. doi:10.1172/JCI114799
10. Andus T, Daig R, Vogl D, Aschenbrenner E, Lock G, Hollerbach S, et al. Imbalance of the interleukin 1 system in colonic mucosa – association with intestinal inflammation and interleukin 1 receptor antagonist [corrected] genotype 2. *Gut* (1997) **41**(5):651–7. doi:10.1136/gut.41.5.651
11. Nishiyama T, Mitsuyama K, Toyonaga A, Sasaki E, Tanikawa K. Colonic mucosal interleukin 1 receptor antagonist in inflammatory bowel disease. *Digestion* (1994) **55**(6):368–73. doi:10.1159/000201167
12. Ferretti M, Casini-Raggi V, Pizarro TT, Eisenberg SP, Nast CC, Cominelli F. Neutralization of endogenous IL-1 receptor antagonist exacerbates and prolongs inflammation in rabbit immune colitis. *J Clin Invest* (1994) **94**(1):449–53. doi:10.1172/JCI117345
13. Casini-Raggi V, Kam L, Chong YJ, Fiocchi C, Pizarro TT, Cominelli F. Mucosal imbalance of IL-1 and IL-1 receptor antagonist in inflammatory bowel disease. A novel mechanism of chronic intestinal inflammation. *J Immunol* (1995) **154**(5):2434–40.
14. Pizarro TT, Michie MH, Bentz M, Woraratanadharm J, Smith MF Jr, Foley E, et al. IL-18, a novel immunoregulatory cytokine, is up-regulated in Crohn's disease: expression and localization in intestinal mucosal cells. *J Immunol* (1999) **162**(11):6829–35.
15. Monteleone G, Trapasso F, Parrello T, Biancone L, Stella A, Iuliano R, et al. Bioactive IL-18 expression is up-regulated in Crohn's disease. *J Immunol* (1999) **163**(1):143–7.
16. McNamee EN, Masterson JC, Jedlicka P, McManus M, Grenz A, Collins CB, et al. Interleukin 37 expression protects mice from colitis. *Proc Natl Acad Sci U S A* (2011) **108**(40):16711–6. doi:10.1073/pnas.1111982108
17. Pastorelli L, Garg RR, Hoang SB, Spina L, Mattioli B, Scarpa M, et al. Epithelial-derived IL-33 and its receptor ST2 are dysregulated in ulcerative colitis and in experimental Th1/Th2 driven enteritis. *Proc Natl Acad Sci U S A* (2010) **107**(17):8017–22. doi:10.1073/pnas.0912678107
18. Beltran CJ, Nunez LE, Diaz-Jimenez D, Farfan N, Candia E, Heine C, et al. Characterization of the novel ST2/IL-33 system in patients with inflammatory bowel disease. *Inflamm Bowel Dis* (2010) **16**(7):1097–107. doi:10.1002/ibd.21175
19. Kobori A, Yagi Y, Imaeda H, Ban H, Bamba S, Tsujikawa T, et al. Interleukin-33 expression is specifically enhanced in inflamed mucosa of ulcerative colitis. *J Gastroenterol* (2010) **45**(10):999–1007. doi:10.1007/s00535-010-0245-1
20. Seidelin JB, Bjerrum JT, Coskun M, Widjaya B, Vainer B, Nielsen OH. IL-33 is upregulated in colonocytes of ulcerative colitis. *Immunol Lett* (2010) **128**(1):80–5. doi:10.1016/j.imlet.2009.11.001
21. Bamias G, Corridoni D, Pizarro TT, Cominelli F. New insights into the dichotomous role of innate cytokines in gut homeostasis and inflammation. *Cytokine* (2012) **59**(3):451–9. doi:10.1016/j.cyto.2012.06.014
22. Sponheim J, Pollheimer J, Olsen T, Balogh J, Hammarstrom C, Loos T, et al. Inflammatory bowel disease-associated interleukin-33 is preferentially expressed in ulceration-associated myofibroblasts. *Am J Pathol* (2010) **177**(6):2804–15. doi:10.2353/ajpath.2010.100378
23. Kojouharoff G, Hans W, Obermeier F, Mannel DN, Andus T, Scholmerich J, et al. Neutralization of tumour necrosis factor (TNF) but not of IL-1 reduces inflammation in chronic dextran sulphate sodium-induced colitis in mice. *Clin Exp Immunol* (1997) **107**(2):353–8. doi:10.1111/j.1365-2249.1997.291-ce1184.x
24. Tebbutt NC, Giraud AS, Inglese M, Jenkins B, Waring P, Clay FJ, et al. Reciprocal regulation of gastrointestinal homeostasis by SHP2 and STAT-mediated trefoil gene activation in gp130 mutant mice. *Nat Med* (2002) **8**(10):1089–97. doi:10.1038/nm763
25. Reuter BK, Pizarro TT. Commentary: the role of the IL-18 system and other members of the IL-1R/TLR superfamily in innate mucosal immunity and the pathogenesis of inflammatory bowel disease: friend or foe? *Eur J Immunol* (2004) **34**(9):2347–55. doi:10.1002/eji.200425351
26. Schmitz J, Owyang A, Oldham E, Song Y, Murphy E, McClanahan TK, et al. IL-33, an interleukin-1-like cytokine that signals via the IL-1 receptor-related protein ST2 and induces T helper type 2-associated cytokines. *Immunity* (2005) **23**(5):479–90. doi:10.1016/j.immuni.2005.09.015
27. Lopetuso LR, Scaldaferri F, Pizarro TT. Emerging role of the interleukin (IL)-33/ST2 axis in gut mucosal wound healing and fibrosis. *Fibrogenesis Tissue Repair* (2012) **5**(1):18. doi:10.1186/1755-1536-5-18
28. Pastorelli L, De Salvo C, Cominelli MA, Vecchi M, Pizarro TT. Novel cytokine signaling pathways in inflammatory bowel disease: insight into the dichotomous functions of IL-33 during chronic intestinal inflammation. *Therap Adv Gastroenterol* (2011) **4**(5):311–23. doi:10.1177/1756283X11410770
29. Dinarello CA. Biologic basis for interleukin-1 in disease. *Blood* (1996) **87**(6):2095–147.
30. Keller M, Ruegg A, Werner S, Beer HD. Active caspase-1 is a regulator of unconventional protein secretion. *Cell* (2008) **132**(5):818–31. doi:10.1016/j.cell.2007.12.040
31. Carriere V, Roussel L, Ortega N, Lacorre DA, Americh L, Aguilar L, et al. IL-33, the IL-1-like cytokine ligand for ST2 receptor, is a chromatin-associated nuclear factor in vivo. *Proc Natl Acad Sci U S A* (2007) **104**(1):282–7. doi:10.1073/pnas.0606854104
32. Werman A, Werman-Venkert R, White R, Lee JK, Werman B, Krelin Y, et al. The precursor form of IL-1alpha is an intracrine proinflammatory activator of transcription. *Proc Natl Acad Sci U S A* (2004) **101**(8):2434–9. doi:10.1073/pnas.0308705101
33. Arend WP. Interleukin-1 receptor antagonist. *Adv Immunol* (1993) **54**:167–227. doi:10.1016/S0065-2776(08)60535-0
34. Giri JG, Newton RC, Horuk R. Identification of soluble interleukin-1 binding protein in cell-free supernatants. Evidence for soluble interleukin-1 receptor. *J Biol Chem* (1990) **265**(29):17416–9.
35. Symons JA, Eastgate JA, Duff GW. Purification and characterization of a novel soluble receptor for interleukin 1. *J Exp Med* (1991) **174**(5):1251–4. doi:10.1084/jem.174.5.1251
36. Arend WP, Malyak M, Smith MF Jr, Whisenand TD, Slack JL, Sims JE, et al. Binding of IL-1 alpha, IL-1 beta, and IL-1 receptor antagonist by soluble IL-1 receptors and levels of soluble IL-1 receptors in synovial fluids. *J Immunol* (1994) **153**(10):4766–74.
37. Symons JA, Young PR, Duff GW. Soluble type II interleukin 1 (IL-1) receptor binds and blocks processing of IL-1 beta precursor and loses affinity for IL-1 receptor antagonist. *Proc Natl Acad Sci U S A* (1995) **92**(5):1714–8. doi:10.1073/pnas.92.5.1714
38. Burger D, Chicheportiche R, Giri JG, Dayer JM. The inhibitory activity of human interleukin-1 receptor antagonist is enhanced by type II interleukin-1 soluble receptor and hindered by type I interleukin-1 soluble receptor. *J Clin Invest* (1995) **96**(1):38–41. doi:10.1172/JCI118045
39. Smith DE, Hanna R, Della F, Moore H, Chen H, Farese AM, et al. The soluble form of IL-1 receptor accessory protein enhances the ability of soluble type II IL-1 receptor to inhibit IL-1 action. *Immunity* (2003) **18**(1):87–96. doi:10.1016/S1074-7613(02)00514-9
40. Giri JG, Wells J, Dower SK, McCall CE, Guzman RN, Slack J, et al. Elevated levels of shed type II IL-1 receptor in sepsis. Potential role for type II receptor in regulation of IL-1 responses. *J Immunol* (1994) **153**(12):5802–9.
41. Preas HL II, Reda D, Tropea M, Vandivier RW, Banks SM, Agosti

JM, et al. Effects of recombinant soluble type I interleukin-1 receptor on human inflammatory responses to endotoxin. *Blood* (1996) **88**(7):2465–72.
42. Mahida YR, Wu K, Jewell DP. Enhanced production of interleukin 1-beta by mononuclear cells isolated from mucosa with active ulcerative colitis of Crohn's disease. *Gut* (1989) **30**(6):835–8. doi:10.1136/gut.30.6.835
43. Youngman KR, Simon PL, West GA, Cominelli F, Rachmilewitz D, Klein JS, et al. Localization of intestinal interleukin 1 activity and protein and gene expression to lamina propria cells. *Gastroenterology* (1993) **104**(3):749–58.
44. Ligumsky M, Simon PL, Karmeli F, Rachmilewitz D. Role of interleukin 1 in inflammatory bowel disease – enhanced production during active disease. *Gut* (1990) **31**(6):686–9. doi:10.1136/gut.31.6.686
45. Cominelli F, Nast CC, Llerena R, Dinarello CA, Zipser RD. Interleukin 1 suppresses inflammation in rabbit colitis. Mediation by endogenous prostaglandins. *J Clin Invest* (1990) **85**(2):582–6. doi:10.1172/JCI114476
46. Cominelli F, Nast CC, Duchini A, Lee M. Recombinant interleukin-1 receptor antagonist blocks the proinflammatory activity of endogenous interleukin-1 in rabbit immune colitis. *Gastroenterology* (1992) **103**(1):65–71.
47. Cominelli F. Cytokine-based therapies for Crohn's disease – new paradigms. *N Engl J Med* (2004) **351**(20):2045–8. doi:10.1056/NEJMp048253
48. Daig R, Rogler G, Aschenbrenner E, Vogl D, Falk W, Gross V, et al. Human intestinal epithelial cells secrete interleukin-1 receptor antagonist and interleukin-8 but not interleukin-1 or interleukin-6. *Gut* (2000) **46**(3):350–8. doi:10.1136/gut.46.3.350
49. Mansfield JC, Holden H, Tarlow JK, Di Giovine FS, McDowell TL, Wilson AG, et al. Novel genetic association between ulcerative colitis and the anti-inflammatory cytokine interleukin-1 receptor antagonist. *Gastroenterology* (1994) **106**(3):637–42.
50. Tountas NA, Casini-Raggi V, Yang H, Di Giovine FS, Vecchi M, Kam L, et al. Functional and ethnic association of allele 2 of the interleukin-1 receptor antagonist gene in ulcerative colitis. *Gastroenterology* (1999) **117**(4):806–13. doi:10.1016/S0016-5085(99)70338-0
51. Carter MJ, di Giovine FS, Jones S, Mee J, Camp NJ, Lobo AJ, et al. Association of the interleukin 1 receptor antagonist gene with ulcerative colitis in Northern European Caucasians. *Gut* (2001) **48**(4):461–7. doi:10.1136/gut.48.4.461
52. Ludwiczek O, Vannier E, Borggraefe I, Kaser A, Siegmund B, Dinarello CA, et al. Imbalance between interleukin-1 agonists and antagonists: relationship to severity of inflammatory bowel disease. *Clin Exp Immunol* (2004) **138**(2):323–9. doi:10.1111/j.1365-2249.2004.02599.x
53. Bersudsky M, Luski L, Fishman D, White RM, Ziv-Sokolovskaya N, Dotan S, et al. Non-redundant properties of IL 1α and IL 1β during acute colon inflammation in mice. *Gut* (2013). doi:10.1136/gutjnl-2012-303329. [Epub ahead of print].
54. Portier M, Zhang XG, Ursule E, Lees D, Jourdan M, Bataille R, et al. Cytokine gene expression in human multiple myeloma. *Br J Haematol* (1993) **85**(3):514–20. doi:10.1111/j.1365-2141.1993.tb03341.x
55. Apte RN, Krelin Y, Song X, Dotan S, Recih E, Elkabets M, et al. Effects of micro-environment- and malignant cell-derived interleukin-1 in carcinogenesis, tumour invasiveness and tumour-host interactions. *Eur J Cancer* (2006) **42**(6):751–9. doi:10.1016/j.ejca.2006.01.010
56. Elaraj DM, Weinreich DM, Varghese S, Puhlmann M, Hewitt SM, Carroll NM, et al. The role of interleukin 1 in growth and metastasis of human cancer xenografts. *Clin Cancer Res* (2006) **12**(4):1088–96. doi:10.1158/1078-0432.CCR-05-1603
57. Gemma A, Takenaka K, Hosoya Y, Matuda K, Seike M, Kurimoto F, et al. Altered expression of several genes in highly metastatic subpopulations of a human pulmonary adenocarcinoma cell line. *Eur J Cancer* (2001) **37**(12):1554–61. doi:10.1016/S0959-8049(01)00154-X
58. Apte RN, Voronov E. Interleukin-1 – a major pleiotropic cytokine in tumor-host interactions. *Semin Cancer Biol* (2002) **12**(4):277–90. doi:10.1016/S1044-579X(02)00014-7
59. Konishi N, Miki C, Yoshida T, Tanaka K, Toiyama Y, Kusunoki M. Interleukin-1 receptor antagonist inhibits the expression of vascular endothelial growth factor in colorectal carcinoma. *Oncology* (2005) **68**(2-3):138–45. doi:10.1159/000086768
60. Barille S, Akhoundi C, Collette M, Mellerin MP, Rapp MJ, Harousseau JL, et al. Metalloproteinases in multiple myeloma: production of matrix metalloproteinase-9 (MMP-9), activation of proMMP-2, and induction of MMP-1 by myeloma cells. *Blood* (1997) **90**(4):1649–55.
61. Akagi Y, Liu W, Xie K, Zebrowski B, Shaheen RM, Ellis LM. Regulation of vascular endothelial growth factor expression in human colon cancer by interleukin-1beta. *Br J Cancer* (1999) **80**(10):1506–11. doi:10.1038/sj.bjc.6690553
62. Voronov E, Shouval DS, Krelin Y, Cagnano E, Benharroch D, Iwakura Y, et al. IL-1 is required for tumor invasiveness and angiogenesis. *Proc Natl Acad Sci U S A* (2003) **100**(5):2645–50. doi:10.1073/pnas.0437939100
63. Lewis AM, Varghese S, Xu H, Alexander HR. Interleukin-1 and cancer progression: the emerging role of interleukin-1 receptor antagonist as a novel therapeutic agent in cancer treatment. *J Transl Med* (2006) **4**:48. doi:10.1186/1479-5876-4-48
64. Sawai H, Funahashi H, Yamamoto M, Okada Y, Hayakawa T, Tanaka M, et al. Interleukin-1alpha enhances integrin alpha(6)beta(1) expression and metastatic capability of human pancreatic cancer. *Oncology* (2003) **65**(2):167–73. doi:10.1159/000072343
65. Sawai H, Okada Y, Funahashi H, Matsuo Y, Takahashi H, Takeyama H, et al. Interleukin-1alpha enhances the aggressive behavior of pancreatic cancer cells by regulating the alpha6beta1-integrin and urokinase plasminogen activator receptor expression. *BMC Cell Biol* (2006) **7**:8. doi:10.1186/1471-2121-7-8
66. Matsuo Y, Sawai H, Ma J, Xu D, Ochi N, Yasuda A, et al. IL-1alpha secreted by colon cancer cells enhances angiogenesis: the relationship between IL-1alpha release and tumor cells' potential for liver metastasis. *J Surg Oncol* (2009) **99**(6):361–7. doi:10.1002/jso.21245
67. Tomimatsu S, Ichikura T, Mochizuki H. Significant correlation between expression of interleukin-1alpha and liver metastasis in gastric carcinoma. *Cancer* (2001) **91**(7):1272–6. doi:10.1002/1097-0142(20010401)91:7<1272::AID-CNCR1128>3.0.CO;2-Z
68. Sakamoto K, Hikiba Y, Nakagawa H, Hayakawa Y, Yanai A, Akanuma M, et al. Inhibitor of kappaB kinase beta regulates gastric carcinogenesis via interleukin-1alpha expression. *Gastroenterology* (2010) **139**(1):226–38.e6. doi:10.1053/j.gastro.2010.03.047
69. Tu S, Bhagat G, Cui G, Takaishi S, Kurt-Jones EA, Rickman B, et al. Overexpression of interleukin-1beta induces gastric inflammation and cancer and mobilizes myeloid-derived suppressor cells in mice. *Cancer Cell* (2008) **14**(5):408–19. doi:10.1016/j.ccr.2008.11.004
70. Garlanda C, Riva F, Veliz T, Polentarutti N, Pasqualini F, Radaelli E, et al. Increased susceptibility to colitis-associated cancer of mice lacking TIR8, an inhibitory member of the interleukin-1 receptor family. *Cancer Res* (2007) **67**(13):6017–21. doi:10.1158/0008-5472.CAN-07-0560
71. Mantovani A, Locati M, Polentarutti N, Vecchi A, Garlanda C. Extracellular and intracellular decoys in the tuning of inflammatory cytokines and Toll-like receptors: the new entry TIR8/SIGIRR. *J Leukoc Biol* (2004) **75**(5):738–42. doi:10.1189/jlb.1003473
72. Wald D, Qin J, Zhao Z, Qian Y, Naramura M, Tian L, et al. SIGIRR, a negative regulator of Toll-like receptor-interleukin 1 receptor signaling. *Nat Immunol* (2003) **4**(9):920–7. doi:10.1038/ni968
73. Thomassen E, Renshaw BR, Sims JE. Identification and characterization of SIGIRR, a molecule representing a novel subtype of the IL-1R superfamily. *Cytokine* (1999) **11**(6):389–99. doi:10.1006/cyto.1998.0452
74. Polentarutti N, Rol GP, Muzio M, Bosisio D, Camnasio M, Riva F, et

al. Unique pattern of expression and inhibition of IL-1 signaling by the IL-1 receptor family member TIR8/SIGIRR. *Eur Cytokine Netw* (2003) **14**(4):211–8.

75. Garlanda C, Riva F, Polentarutti N, Buracchi C, Sironi M, De Bortoli M, et al. Intestinal inflammation in mice deficient in Tir8, an inhibitory member of the IL-1 receptor family. *Proc Natl Acad Sci U S A* (2004) **101**(10):3522–6. doi:10.1073/pnas.0308680101
76. Qin J, Qian Y, Yao J, Grace C, Li X. SIGIRR inhibits interleukin-1 receptor- and Toll-like receptor 4-mediated signaling through different mechanisms. *J Biol Chem* (2005) **280**(26):25233–41. doi:10.1074/jbc.M501363200
77. Xiao H, Gulen MF, Qin J, Yao J, Bulek K, Kish D, et al. The Toll-interleukin-1 receptor member SIGIRR regulates colonic epithelial homeostasis, inflammation, and tumorigenesis. *Immunity* (2007) **26**(4):461–75. doi:10.1016/j.immuni.2007.02.012
78. Xiao H, Yin W, Khan MA, Gulen MF, Zhou H, Sham HP, et al. Loss of single immunoglobulin interlukin-1 receptor-related molecule leads to enhanced colonic polyposis in Apc(min) mice. *Gastroenterology* (2010) **139**(2):574–85. doi:10.1053/j.gastro.2010.04.043
79. Martinon F, Burns K, Tschopp J. The inflammasome: a molecular platform triggering activation of inflammatory caspases and processing of proIL-beta. *Mol Cell* (2002) **10**(2):417–26. doi:10.1016/S1097-2765(02)00599-3
80. Elinav E, Henao-Mejia J, Flavell RA. Integrative inflammasome activity in the regulation of intestinal mucosal immune responses. *Mucosal Immunol* (2013) **6**(1):4–13. doi:10.1038/mi.2012.115
81. Allen IC, TeKippe EM, Woodford RM, Uronis JM, Holl EK, Rogers AB, et al. The NLRP3 inflammasome functions as a negative regulator of tumorigenesis during colitis-associated cancer. *J Exp Med* (2010) **207**(5):1045–56. doi:10.1084/jem.20100050
82. Okamura H, Nagata K, Komatsu T, Tanimoto T, Nukata Y, Tanabe F, et al. A novel costimulatory factor for gamma interferon induction found in the livers of mice causes endotoxic shock. *Infect Immun* (1995) **63**(10):3966–72.
83. Kohno K, Kataoka J, Ohtsuki T, Suemoto Y, Okamoto I, Usui M, et al. IFN-gamma-inducing factor (IGIF) is a costimulatory factor on the activation of Th1 but not Th2 cells and exerts its effect independently of IL-12. *J Immunol* (1997) **158**(4):1541–50.
84. Udagawa N, Horwood NJ, Elliott J, Mackay A, Owens J, Okamura H, et al. Interleukin-18 (interferon-gamma-inducing factor) is produced by osteoblasts and acts via granulocyte/macrophage colony-stimulating factor and not via interferon-gamma to inhibit osteoclast formation. *J Exp Med* (1997) **185**(6):1005–12. doi:10.1084/jem.185.6.1005
85. Stoll S, Muller G, Kurimoto M, Saloga J, Tanimoto T, Yamauchi H, et al. Production of IL-18 (IFN-gamma-inducing factor) messenger RNA and functional protein by murine keratinocytes. *J Immunol* (1997) **159**(1):298–302.
86. Conti B, Jahng JW, Tinti C, Son JH, Joh TH. Induction of interferon-gamma inducing factor in the adrenal cortex. *J Biol Chem* (1997) **272**(4):2035–7. doi:10.1074/jbc.272.4.2035
87. Matsui K, Yoshimoto T, Tsutsui H, Hyodo Y, Hayashi N, Hiroishi K, et al. *Propionibacterium acnes* treatment diminishes CD4+ NK1.1+ T cells but induces type I T cells in the liver by induction of IL-12 and IL-18 production from Kupffer cells. *J Immunol* (1997) **159**(1):97–106.
88. Stoll S, Jonuleit H, Schmitt E, Muller G, Yamauchi H, Kurimoto M, et al. Production of functional IL-18 by different subtypes of murine and human dendritic cells (DC): DC-derived IL-18 enhances IL-12-dependent Th1 development. *Eur J Immunol* (1998) **28**(10):3231–9. doi:10.1002/(SICI)1521-4141(199810)28:10<3231::AID-IMMU3231>3.3.CO;2-H
89. Prinz M, Hanisch UK. Murine microglial cells produce and respond to interleukin-18. *J Neurochem* (1999) **72**(5):2215–8. doi:10.1046/j.1471-4159.1999.0722215.x
90. Gracie JA, Robertson SE, McInnes IB. Interleukin-18. *J Leukoc Biol* (2003) **73**(2):213–24. doi:10.1189/jlb.0602313
91. Kanai T, Watanabe M, Okazawa A, Nakamaru K, Okamoto M, Naganuma M, et al. Interleukin 18 is a potent proliferative factor for intestinal mucosal lymphocytes in Crohn's disease. *Gastroenterology* (2000) **119**(6):1514–23. doi:10.1053/gast.2000.20260
92. Torigoe K, Ushio S, Okura T, Kobayashi S, Taniai M, Kunikata T, et al. Purification and characterization of the human interleukin-18 receptor. *J Biol Chem* (1997) **272**(41):25737–42. doi:10.1074/jbc.272.41.25737
93. Hoshino K, Tsutsui H, Kawai T, Takeda K, Nakanishi K, Takeda Y, et al. Cutting edge: generation of IL-18 receptor-deficient mice: evidence for IL-1 receptor-related protein as an essential IL-18 binding receptor. *J Immunol* (1999) **162**(9):5041–4.
94. Parnet P, Garka KE, Bonnert TP, Dower SK, Sims JE. IL-1Rrp is a novel receptor-like molecule similar to the type I interleukin-1 receptor and its homologues T1/ST2 and IL-1R AcP. *J Biol Chem* (1996) **271**(8):3967–70.
95. Born TL, Thomassen E, Bird TA, Sims JE. Cloning of a novel receptor subunit, AcPL, required for interleukin-18 signaling. *J Biol Chem* (1998) **273**(45):29445–50. doi:10.1074/jbc.273.45.29445
96. Leung BP, Culshaw S, Gracie JA, Hunter D, Canetti CA, Campbell C, et al. A role for IL-18 in neutrophil activation. *J Immunol* (2001) **167**(5):2879–86.
97. Hyodo Y, Matsui K, Hayashi N, Tsutsui H, Kashiwamura S, Yamauchi H, et al. IL-18 up-regulates perforin-mediated NK activity without increasing perforin messenger RNA expression by binding to constitutively expressed IL-18 receptor. *J Immunol* (1999) **162**(3):1662–8.
98. Gerdes N, Sukhova GK, Libby P, Reynolds RS, Young JL, Schonbeck U. Expression of interleukin (IL)-18 and functional IL-18 receptor on human vascular endothelial cells, smooth muscle cells, and macrophages: implications for atherogenesis. *J Exp Med* (2002) **195**(2):245–57. doi:10.1084/jem.20011022
99. Afkarian M, Sedy JR, Yang J, Jacobson NG, Cereb N, Yang SY, et al. T-bet is a STAT1-induced regulator of IL-12R expression in naive CD4+ T cells. *Nat Immunol* (2002) **3**(6):549–57. doi:10.1038/ni794
100. Yoshimoto T, Takeda K, Tanaka T, Ohkusu K, Kashiwamura S, Okamura H, et al. IL-12 up-regulates IL-18 receptor expression on T cells, Th1 cells, and B cells: synergism with IL-18 for IFN-gamma production. *J Immunol* (1998) **161**(7):3400–7.
101. Smeltz RB, Chen J, Hu-Li J, Shevach EM. Regulation of interleukin (IL)-18 receptor alpha chain expression on CD4(+) T cells during T helper (Th)1/Th2 differentiation. Critical downregulatory role of IL-4. *J Exp Med* (2001) **194**(2):143–53. doi:10.1084/jem.194.2.143
102. Nakanishi K, Yoshimoto T, Tsutsui H, Okamura H. Interleukin-18 is a unique cytokine that stimulates both Th1 and Th2 responses depending on its cytokine milieu. *Cytokine Growth Factor Rev* (2001) **12**(1):53–72. doi:10.1016/S1359-6101(00)00015-0
103. Nakanishi K, Yoshimoto T, Tsutsui H, Okamura H. Interleukin-18 regulates both Th1 and Th2 responses. *Annu Rev Immunol* (2001) **19**:423–74. doi:10.1146/annurev.immunol.19.1.423
104. Novick D, Kim SH, Fantuzzi G, Reznikov LL, Dinarello CA, Rubinstein M. Interleukin-18 binding protein: a novel modulator of the Th1 cytokine response. *Immunity* (1999) **10**(1):127–36. doi:10.1016/S1074-7613(00)80013-8
105. Corbaz A, Ten Hove T, Herren S, Graber P, Schwartsburd B, Belzer I, et al. IL-18-binding protein expression by endothelial cells and macrophages is up-regulated during active Crohn's disease. *J Immunol* (2002) **168**(7):3608–16.
106. Kim SH, Eisenstein M, Reznikov L, Fantuzzi G, Novick D, Rubinstein M, et al. Structural requirements of six naturally occurring isoforms of the IL-18 binding protein to inhibit IL-18. *Proc Natl Acad Sci U S A* (2000) **97**(3):1190–5. doi:10.1073/pnas.97.3.1190
107. Meng X, Leman M, Xiang Y. Variola virus IL-18 binding protein interacts with three human IL-18 residues that are part of a binding site for human IL-18 receptor alpha subunit. *Virology* (2007) **358**(1):211–20. doi:10.1016/j.virol.2006.08.019

108. Leach ST, Messina I, Lemberg DA, Novick D, Rubenstein M, Day AS. Local and systemic interleukin-18 and interleukin-18-binding protein in children with inflammatory bowel disease. *Inflamm Bowel Dis* (2008) **14**(1):68–74. doi:10.1002/ibd.20272
109. Takagawa T, Tamura K, Takeda N, Tomita T, Ohda Y, Fukunaga K, et al. Association between IL-18 gene promoter polymorphisms and inflammatory bowel disease in a Japanese population. *Inflamm Bowel Dis* (2005) **11**(12):1038–43. doi:10.1097/01.MIB.0000182868.67025.b9
110. Tamura K, Fukuda Y, Sashio H, Takeda N, Bamba H, Kosaka T, et al. IL18 polymorphism is associated with an increased risk of Crohn's disease. *J Gastroenterol* (2002) **37**(Suppl 14):111–6.
111. Zhernakova A, Festen EM, Franke L, Trynka G, van Diemen CC, Monsuur AJ, et al. Genetic analysis of innate immunity in Crohn's disease and ulcerative colitis identifies two susceptibility loci harboring CARD9 and IL18RAP. *Am J Hum Genet* (2008) **82**(5):1202–10. doi:10.1016/j.ajhg.2008.03.016
112. Giedraitis V, He B, Huang WX, Hillert J. Cloning and mutation analysis of the human IL-18 promoter: a possible role of polymorphisms in expression regulation. *J Neuroimmunol* (2001) **112**(1-2):146–52. doi:10.1016/S0165-5728(00)00407-0
113. Hunt KA, Zhernakova A, Turner G, Heap GA, Franke L, Bruinenberg M, et al. Newly identified genetic risk variants for celiac disease related to the immune response. *Nat Genet* (2008) **40**(4):395–402. doi:10.1038/ng.102
114. Ten Hove T, Corbaz A, Amitai H, Aloni S, Belzer I, Graber P, et al. Blockade of endogenous IL-18 ameliorates TNBS-induced colitis by decreasing local TNF-alpha production in mice. *Gastroenterology* (2001) **121**(6):1372–9. doi:10.1053/gast.2001.29579
115. Maerten P, Shen C, Colpaert S, Liu Z, Bullens DA, van Assche G, et al. Involvement of interleukin 18 in Crohn's disease: evidence from in vitro analysis of human gut inflammatory cells and from experimental colitis models. *Clin Exp Immunol* (2004) **135**(2):310–7. doi:10.1111/j.1365-2249.2004.02362.x
116. Kanai T, Watanabe M, Okazawa A, Sato T, Hibi T. Interleukin-18 and Crohn's disease. *Digestion* (2001) **63**(Suppl 1):37–42. doi:10.1159/000051909
117. Siegmund B, Fantuzzi G, Rieder F, Gamboni-Robertson F, Lehr HA, Hartmann G. et al. Neutralization of interleukin-18 reduces severity in murine colitis and intestinal IFN-gamma and TNF-alpha production. *Am J Physiol Regul Integr Comp Physiol* (2001) **281**(4):R1264–73.
118. Wirtz S, Becker C, Blumberg R, Galle PR, Neurath MF. Treatment of T cell-dependent experimental colitis in SCID mice by local administration of an adenovirus expressing IL-18 antisense mRNA. *J Immunol* (2002) **168**(1):411–20.
119. Kanai T, Watanabe M, Okazawa A, Sato T, Yamazaki M, Okamoto S, et al. Macrophage-derived IL-18-mediated intestinal inflammation in the murine model of Crohn's disease. *Gastroenterology* (2001) **121**(4):875–88. doi:10.1053/gast.2001.28021
120. Sivakumar PV, Westrich GM, Kanaly S, Garka K, Born TL, Derry JM, et al. Interleukin 18 is a primary mediator of the inflammation associated with dextran sulphate sodium induced colitis: blocking interleukin 18 attenuates intestinal damage. *Gut* (2002) **50**(6):812–20. doi:10.1136/gut.50.6.812
121. Ishikura T, Kanai T, Uraushihara K, Iiyama R, Makita S, Totsuka T, et al. Interleukin-18 overproduction exacerbates the development of colitis with markedly infiltrated macrophages in interleukin-18 transgenic mice. *J Gastroenterol Hepatol* (2003) **18**(8):960–9. doi:10.1046/j.1440-1746.2003.03097.x
122. Pizarro TT, Pastorelli L, Bamias G, Garg RR, Reuter BK, Mercado JR, et al. SAMP1/YitFc mouse strain: a spontaneous model of Crohn's disease-like ileitis. *Inflamm Bowel Dis* (2011) **17**(12):2566–84. doi:10.1002/ibd.21638
123. Kozaiwa K, Sugawara K, Smith MF Jr, Carl V, Yamschikov V, Belyea B, et al. Identification of a quantitative trait locus for ileitis in a spontaneous mouse model of Crohn's disease: SAMP1/YitFc. *Gastroenterology* (2003) **125**(2):477–90. doi:10.1016/S0016-5085(03)00876-X
124. Pizarro TT, Arseneau KO, Cominelli F. Lessons from genetically engineered animal models XI. Novel mouse models to study pathogenic mechanisms of Crohn's disease. *Am J Physiol Gastrointest Liver Physiol* (2000) **278**(5):G665–9.
125. Cho D, Kim TG, Lee W, Hwang YI, Cho HI, Han H, et al. Interleukin-18 and the costimulatory molecule B7-1 have a synergistic anti-tumor effect on murine melanoma; implication of combined immunotherapy for poorly immunogenic malignancy. *J Invest Dermatol* (2000) **114**(5):928–34. doi:10.1038/sj.jid.5600685
126. Han MY, Zheng S, Yu JM, Peng JP, Guo QS, Wang JL. Study on interleukin-18 gene transfer into human breast cancer cells to prevent tumorigenicity. *J Zhejiang Univ Sci* (2004) **5**(4):472–6. doi:10.1631/jzus.2004.0472
127. Xia D, Li F, Xiang J. Engineered fusion hybrid vaccine of IL-18 gene-modified tumor cells and dendritic cells induces enhanced antitumor immunity. *Cancer Biother Radiopharm* (2004) **19**(3):322–30. doi:10.1089/1084978041424990
128. Ye ZB, Ma T, Li H, Jin XL, Xu HM. Expression and significance of intratumoral interleukin-12 and interleukin-18 in human gastric carcinoma. *World J Gastroenterol* (2007) **13**(11):1747–51.
129. Kim KE, Song H, Kim TS, Yoon D, Kim CW, Bang SI, et al. Interleukin-18 is a critical factor for vascular endothelial growth factor-enhanced migration in human gastric cancer cell lines. *Oncogene* (2007) **26**(10):1468–76. doi:10.1038/sj.onc.1209926
130. Kim J, Kim C, Kim TS, Bang SI, Yang Y, Park H, et al. IL-18 enhances thrombospondin-1 production in human gastric cancer via JNK pathway. *Biochem Biophys Res Commun* (2006) **344**(4):1284–9.
131. Carrascal MT, Mendoza L, Valcarcel M, Salado C, Egilegor E, Telleria N, et al. Interleukin-18 binding protein reduces b16 melanoma hepatic metastasis by neutralizing adhesiveness and growth factors of sinusoidal endothelium. *Cancer Res* (2003) **63**(2):491–7.
132. Zhang B, Wu KF, Cao ZY, Rao Q, Ma XT, Zheng GG, et al. IL-18 increases invasiveness of HL-60 myeloid leukemia cells: up-regulation of matrix metalloproteinases-9 (MMP-9) expression. *Leuk Res* (2004) **28**(1):91–5.
133. Cho D, Song H, Kim YM, Houh D, Hur DY, Park H, et al. Endogenous interleukin-18 modulates immune escape of murine melanoma cells by regulating the expression of Fas ligand and reactive oxygen intermediates. *Cancer Res* (2000) **60**(10):2703–9.
134. Babar M, Ryan AW, Anderson LA, Segurado R, Turner G, Murray LJ, et al. Genes of the interleukin-18 pathway are associated with susceptibility to Barrett's esophagus and esophageal adenocarcinoma. *Am J Gastroenterol* (2012) **107**(9):1331–41. doi:10.1038/ajg.2012.134
135. Wei YS, Lan Y, Liu YG, Tang H, Tang RG, Wang JC. Interleukin-18 gene promoter polymorphisms and the risk of esophageal squamous cell carcinoma. *Acta Oncol* (2007) **46**(8):1090–6. doi:10.1080/02841860701373595
136. Nikiteas N, Yannopoulos A, Chatzitheofylaktou A, Tsigris C. Heterozygosity for interleukin-18 -607 A/C polymorphism is associated with risk for colorectal cancer. *Anticancer Res* (2007) **27**(6B):3849–53.
137. Haghshenas MR, Hosseini SV, Mahmoudi M, Saberi-Firozi M, Farjadian S, Ghaderi A. IL-18 serum level and IL-18 promoter gene polymorphism in Iranian patients with gastrointestinal cancers. *J Gastroenterol Hepatol* (2009) **24**(6):1119–22. doi:10.1111/j.1440-1746.2009.05791.x
138. Antillon M, Cominelli F, Lo S, Moran M, Somberg K, Reynolds T, et al. Effects of oral prostaglandins on indomethacin-induced renal failure in patients with cirrhosis and ascites. *J Rheumatol Suppl* (1990) **20**:46–9.
139. Jacobs C, Young D, Tyler S, Callis G, Gillis S, Conlon PJ. In vivo treatment with IL-1 reduces the severity and duration of antigen-induced arthritis in rats. *J Immunol* (1988) **141**(9):2967–74.
140. van der Meer JW, Barza M, Wolff SM, Dinarello CA. A

low dose of recombinant interleukin 1 protects granulocytopenic mice from lethal gram-negative infection. *Proc Natl Acad Sci U S A* (1988) **85**(5):1620–3. doi:10.1073/pnas.85.5.1620

141. Takagi H, Kanai T, Okazawa A, Kishi Y, Sato T, Takaishi H, et al. Contrasting action of IL-12 and IL-18 in the development of dextran sodium sulphate colitis in mice. *Scand J Gastroenterol* (2003) **38**(8):837–44. doi:10.1080/00365520310004047
142. Zaki MH, Boyd KL, Vogel P, Kastan MB, Lamkanfi M, Kanneganti TD. The NLRP3 inflammasome protects against loss of epithelial integrity and mortality during experimental colitis. *Immunity* (2010) **32**(3):379–91. doi:10.1016/j.immuni.2010.03.003
143. Bauer C, Duewell P, Mayer C, Lehr HA, Fitzgerald KA, Dauer M, et al. Colitis induced in mice with dextran sulfate sodium (DSS) is mediated by the NLRP3 inflammasome. *Gut* (2010) **59**(9):1192–9. doi:10.1136/gut.2009.197822
144. Siegmund B. Interleukin-18 in intestinal inflammation: friend and foe? *Immunity* (2010) **32**(3):300–2. doi:10.1016/j.immuni.2010.03.010
145. Elinav E, Strowig T, Kau AL, Henao-Mejia J, Thaiss CA, Booth CJ, et al. NLRP6 inflammasome regulates colonic microbial ecology and risk for colitis. *Cell* (2011) **145**(5):745–57. doi:10.1016/j.cell.2011.04.022
146. Sussman DA, Santaolalla R, Strobel S, Dheer R, Abreu MT. Cancer in inflammatory bowel disease: lessons from animal models. *Curr Opin Gastroenterol* (2012) **28**(4):327–33. doi:10.1097/MOG.0b013e328354cc36
147. Salcedo R, Worschech A, Cardone M, Jones Y, Gyulai Z, Dai RM, et al. MyD88-mediated signaling prevents development of adenocarcinomas of the colon: role of interleukin 18. *J Exp Med* (2010) **207**(8):1625–36. doi:10.1084/jem.20100199
148. Zaki MH, Vogel P, Body-Malapel M, Lamkanfi M, Kanneganti TD. IL-18 production downstream of the Nlrp3 inflammasome confers protection against colorectal tumor formation. *J Immunol* (2010) **185**(8):4912–20. doi:10.4049/jimmunol.1002046
149. Kumar S, McDonnell PC, Lehr R, Tierney L, Tzimas MN, Griswold DE, et al. Identification and initial characterization of four novel members of the interleukin-1 family. *J Biol Chem* (2000) **275**(14):10308–14. doi:10.1074/jbc.275.14.10308
150. Pan G, Risser P, Mao W, Baldwin DT, Zhong AW, Filvaroff E, et al. IL-1H, an interleukin 1-related protein that binds IL-18 receptor/IL-1Rrp. *Cytokine* (2001) **13**(1):1–7. doi:10.1006/cyto.2000.0799
151. Kumar S, Hanning CR, Brigham-Burke MR, Rieman DJ, Lehr R, Khandekar S, et al. Interleukin-1F7B (IL-1H4/IL-1F7) is processed by caspase-1 and mature IL-1F7B binds to the IL-18 receptor but does not induce IFN-gamma production. *Cytokine* (2002) **18**(2):61–71. doi:10.1006/cyto.2002.0873
152. Bufler P, Azam T, Gamboni-Robertson F, Reznikov LL, Kumar S, Dinarello CA, et al. A complex of the IL-1 homologue IL-1F7b and IL-18-binding protein reduces IL-18 activity. *Proc Natl Acad Sci U S A* (2002) **99**(21):13723–8. doi:10.1073/pnas.212519099
153. Nold MF, Nold CA, Lo C, Li S, Rudloff I, Zepp JA, et al. Interleukin 37 employs the IL-1 family inhibitory receptor SIGIRR and the alpha chain of the IL-18 receptor to suppress innate immunity [abstract]. *Cytokine* (2013).
154. Sharma S, Kulk N, Nold MF, Graf R, Kim SH, Reinhardt D, et al. The IL-1 family member 7b translocates to the nucleus and down-regulates proinflammatory cytokines. *J Immunol* (2008) **180**(8):5477–82.
155. Boraschi D, Lucchesi D, Hainzl S, Leitner M, Maier E, Mangelberger D, et al. IL-37: a new anti-inflammatory cytokine of the IL-1 family. *Eur Cytokine Netw* (2011) **22**(3):127–47. doi:10.1684/ecn.2011.0288
156. Nold MF, Nold-Petry CA, Zepp JA, Palmer BE, Bufler P, Dinarello CA. IL-37 is a fundamental inhibitor of innate immunity. *Nat Immunol* (2010) **11**(11):1014–22. doi:10.1038/ni.1944
157. Baekkevold ES, Roussigne M, Yamanaka T, Johansen FE, Jahnsen FL, Amalric F, et al. Molecular characterization of NF-HEV, a nuclear factor preferentially expressed in human high endothelial venules. *Am J Pathol* (2003) **163**(1):69–79. doi:10.1016/S0002-9440(10)63631-0
158. Wood IS, Wang B, Trayhurn P. IL-33, a recently identified interleukin-1 gene family member, is expressed in human adipocytes. *Biochem Biophys Res Commun* (2009) **384**(1):105–9. doi:10.1016/j.bbrc.2009.04.081
159. Moussion C, Ortega N, Girard JP. The IL-1-like cytokine IL-33 is constitutively expressed in the nucleus of endothelial cells and epithelial cells in vivo: a novel "alarmin?" *PLoS ONE* (2008) **3**(10):e3331. doi:10.1371/journal.pone.0003331
160. Chackerian AA, Oldham ER, Murphy EE, Schmitz J, Pflanz S, Kastelein RA. IL-1 receptor accessory protein and ST2 comprise the IL-33 receptor complex. *J Immunol* (2007) **179**(4):2551–5.
161. Bulek K, Swaidani S, Qin J, Lu Y, Gulen MF, Herjan T, et al. The essential role of single Ig IL-1 receptor-related molecule/Toll IL-1R8 in regulation of Th2 immune response. *J Immunol* (2009) **182**(5):2601–9. doi:10.4049/jimmunol.0802729
162. Jones LA, Roberts F, Nickdel MB, Brombacher F, McKenzie AN, Henriquez FL, et al. IL-33 receptor (T1/ST2) signalling is necessary to prevent the development of encephalitis in mice infected with *Toxoplasma gondii*. *Eur J Immunol* (2010) **40**(2):426–36. doi:10.1002/eji.200939705
163. Huang X, Du W, Barrett RP, Hazlett LD. ST2 is essential for Th2 responsiveness and resistance to *Pseudomonas aeruginosa* keratitis. *Invest Ophthalmol Vis Sci* (2007) **48**(10):4626–33. doi:10.1167/iovs.07-0316
164. Wagenaar JF, Gasem MH, Goris MG, Leeflang M, Hartskeerl RA, van der Poll T, et al. Soluble ST2 levels are associated with bleeding in patients with severe Leptospirosis. *PLoS Negl Trop Dis* (2009) **3**(6):e453. doi:10.1371/journal.pntd.0000453
165. Cayrol C, Girard JP. The IL-1-like cytokine IL-33 is inactivated after maturation by caspase-1. *Proc Natl Acad Sci U S A* (2009) **106**(22):9021–6. doi:10.1073/pnas.0812690106
166. Luthi AU, Cullen SP, McNeela EA, Duriez PJ, Afonina IS, Sheridan C, et al. Suppression of interleukin-33 bioactivity through proteolysis by apoptotic caspases. *Immunity* (2009) **31**(1):84–98. doi:10.1016/j.immuni.2009.05.007
167. Talabot-Ayer D, Lamacchia C, Gabay C, Palmer G. Interleukin-33 is biologically active independently of caspase-1 cleavage. *J Biol Chem* (2009) **284**(29):19420–6. doi:10.1074/jbc.M901744200
168. Pennock JL, Grencis RK. The mast cell and gut nematodes: damage and defence. *Chem Immunol Allergy* (2006) **90**:128–40.
169. Dudeck A, Suender CA, Kostka SL, von Stebut E, Maurer M. Mast cells promote Th1 and Th17 responses by modulating dendritic cell maturation and function. *Eur J Immunol* (2011) **41**(7):1883–93. doi:10.1002/eji.201040994
170. Suto H, Nakae S, Kakurai M, Sedgwick JD, Tsai M, Galli SJ. Mast cell-associated TNF promotes dendritic cell migration. *J Immunol* (2006) **176**(7):4102–12.
171. Nakae S, Suto H, Berry GJ, Galli SJ. Mast cell-derived TNF can promote Th17 cell-dependent neutrophil recruitment in ovalbumin-challenged OTII mice. *Blood* (2007) **109**(9):3640–8. doi:10.1182/blood-2006-09-046128
172. Hepworth MR, Danilowicz-Luebert E, Rausch S, Metz M, Klotz C, Maurer M, et al. Mast cells orchestrate type 2 immunity to helminths through regulation of tissue-derived cytokines. *Proc Natl Acad Sci U S A* (2012) **109**(17):6644–9. doi:10.1073/pnas.1112268109
173. Hepworth MR, Maurer M, Hartmann S. Regulation of type 2 immunity to helminths by mast cells. *Gut Microbes* (2012) **3**(5):476–81. doi:10.4161/gmic.21507
174. Miller AM, Xu D, Asquith DL, Denby L, Li Y, Sattar N, et al. IL-33 reduces the development of atherosclerosis. *J Exp Med* (2008) **205**(2):339–46. doi:10.1084/jem.20071868
175. Oboki K, Ohno T, Kajiwara N, Arae K, Morita H, Ishii A, et al. IL-33 is a crucial amplifier of innate rather than acquired immunity. *Proc Natl Acad Sci U S A* (2010) **107**(43):18581–6. doi:10.1073/pnas.1003059107
176. Grobeta P, Doser K, Falk W, Obermeier F, Hofmann C. IL-33

attenuates development and perpetuation of chronic intestinal inflammation. *Inflamm Bowel Dis* (2012) **18**(10):1900–9. doi:10.1002/ibd.22900

177. Rani R, Smulian AG, Greaves DR, Hogan SP, Herbert DR. TGF-beta limits IL-33 production and promotes the resolution of colitis through regulation of macrophage function. *Eur J Immunol* (2011) **41**(7):2000–9. doi:10.1002/eji.201041135
178. Imaeda H, Andoh A, Aomatsu T, Uchiyama K, Bamba S, Tsujikawa T, et al. Interleukin-33 suppresses Notch ligand expression and prevents goblet cell depletion in dextran sulfate sodium-induced colitis. *Int J Mol Med* (2011) **28**(4):573–8. doi:10.3892/ijmm.2011.718
179. Duan L, Chen J, Zhang H, Yang H, Zhu P, Xiong A, et al. IL-33 ameliorates experimental colitis through promoting Th2/Foxp3(+) Treg responses in mice. *Mol Med* (2012) **18**:753–61. doi:10.2119/molmed.2011.00428
180. Mucida D, Park Y, Kim G, Turovskaya O, Scott I, Kronenberg M, et al. Reciprocal TH17 and regulatory T cell differentiation mediated by retinoic acid. *Science* (2007) **317**(5835):256–60. doi:10.1126/science.1145697
181. Coombes JL, Siddiqui KR, Arancibia-Carcamo CV, Hall J, Sun CM, Belkaid Y, et al. A functionally specialized population of mucosal CD103+ DCs induces Foxp3+ regulatory T cells via a TGF-beta and retinoic acid-dependent mechanism. *J Exp Med* (2007) **204**(8):1757–64. doi:10.1084/jem.20070590
182. Mattioli B, Mattioli B, Pastorelli L, De Salvo C, Corridoni D, Garg RR, et al. IL-33-dependent induction of intestinal profibrotic gene expression and myofibroblast hypertrophy: potential role in inflammatory-associated gut fibrosis. *Gastroenterology* (2011) **140**(5):S844–5.
183. De Salvo C, Wang X-M, Mattioli B, Pastorelli L, Garg RR, Chowdhry S, et al. Tu1950 pathogenic role of IL-33-mediated eosinophil infiltration and function in experimental IBD. *Gastroenterology* (2012) **142**(5):S885.
184. Sun P, Ben Q, Tu S, Dong W, Qi X, Wu Y. Serum interleukin-33 levels in patients with gastric cancer. *Dig Dis Sci* (2011) **56**(12):3596–601. doi:10.1007/s10620-011-1760-5
185. De Vita F, Orditura M, Galizia G, Romano C, Infusino S, Auriemma A, et al. Serum interleukin-10 levels in patients with advanced gastrointestinal malignancies. *Cancer* (1999) **86**(10):1936–43. doi:10.1002/(SICI)1097-0142(19991115)86:10<1936::AID-CNCR9>3.3.CO;2-0
186. Sharma A, Rajappa M, Saxena A, Sharma M. Cytokine profile in Indian women with cervical intraepithelial neoplasia and cancer cervix. *Int J Gynecol Cancer* (2007) **17**(4):879–85. doi:10.1111/j.1525-1438.2007.00883.x
187. Dinarello C, Arend W, Sims J, Smith D, Blumberg H, O'Neill L, et al. IL-1 family nomenclature. *Nat Immunol* (2010) **11**(11):973. doi:10.1038/ni1110-973
188. Towne JE, Garka KE, Renshaw BR, Virca GD, Sims JE. Interleukin (IL)-1F6, IL-1F8, and IL-1F9 signal through IL-1Rrp2 and IL-1RAcP to activate the pathway leading to NF-kappaB and MAPKs. *J Biol Chem* (2004) **279**(14):13677–88. doi:10.1074/jbc.M400117200
189. Debets R, Timans JC, Homey B, Zurawski S, Sana TR, Lo S, et al. Two novel IL-1 family members, IL-1 delta and IL-1 epsilon, function as an antagonist and agonist of NF-kappa B activation through the orphan IL-1 receptor-related protein 2. *J Immunol* (2001) **167**(3):1440–6.
190. Blumberg H, Dinh H, Trueblood ES, Pretorius J, Kugler D, Weng N, et al. Opposing activities of two novel members of the IL-1 ligand family regulate skin inflammation. *J Exp Med* (2007) **204**(11):2603–14. doi:10.1084/jem.20070157
191. Zhou X, Krueger JG, Kao MC, Lee E, Du F, Menter A, et al. Novel mechanisms of T-cell and dendritic cell activation revealed by profiling of psoriasis on the 63,100-element oligonucleotide array. *Physiol Genomics* (2003) **13**(1):69–78.
192. Johnston A, Xing X, Guzman AM, Riblett M, Loyd CM, Ward NL, et al. IL-1F5, -F6, -F8, and -F9: a novel IL-1 family signaling system that is active in psoriasis and promotes keratinocyte antimicrobial peptide expression. *J Immunol* (2011) **186**(4):2613–22. doi:10.4049/jimmunol.1003162
193. Muhr P, Zeitvogel J, Heitland I, Werfel T, Wittmann M. Expression of interleukin (IL)-1 family members upon stimulation with IL-17 differs in keratinocytes derived from patients with psoriasis and healthy donors. *Br J Dermatol* (2011) **165**(1):189–93. doi:10.1111/j.1365-2133.2011.10302.x
194. Carrier Y, Ma HL, Ramon HE, Napierata L, Small C, O'Toole M, et al. Inter-regulation of Th17 cytokines and the IL-36 cytokines in vitro and in vivo: implications in psoriasis pathogenesis. *J Invest Dermatol* (2011) **131**(12):2428–37. doi:10.1038/jid.2011.234
195. Ramadas RA, Li X, Shubitowski DM, Samineni S, Wills-Karp M, Ewart SL. IL-1 Receptor antagonist as a positional candidate gene in a murine model of allergic asthma. *Immunogenetics* (2006) **58**(10):851–5.
196. Ramadas RA, Ewart SL, Medoff BD, LeVine AM. Interleukin-1 family member 9 stimulates chemokine production and neutrophil influx in mouse lungs. *Am J Respir Cell Mol Biol* (2011) **44**(2):134–45. doi:10.1165/rcmb.2009-0315OC
197. Ramadas RA, Ewart SL, Iwakura Y, Medoff BD, LeVine AM. IL-36alpha exerts pro-inflammatory effects in the lungs of mice. *PLoS ONE* (2012) **7**(9):e45784. doi:10.1371/journal.pone.0045784
198. Vigne S, Palmer G, Martin P, Lamacchia C, Strebel D, Rodriguez E, et al. IL-36 signaling amplifies Th1 responses by enhancing proliferation and Th1 polarization of naive CD4+ T cells. *Blood* (2012) **120**(17):3478–87. doi:10.1182/blood-2012-06-439026
199. Gresnigt MS, Rosler B, Jacobs CW, Becker KL, Joosten LA, van der Meer JW, et al. The IL-36 receptor pathway regulates *Aspergillus fumigatus*-induced Th1 and Th17 responses. *Eur J Immunol* (2012) **43**(2):416–26. doi:10.1002/eji.201242711
200. Blanchard C, Stucke EM, Rodriguez-Jimenez B, Burwinkel K, Collins MH, Ahrens A, et al. A striking local esophageal cytokine expression profile in eosinophilic esophagitis. *J Allergy Clin Immunol* (2011) **127**(1):e1–7. doi:10.1016/j.jaci.2010.10.039
201. van de Veerdonk FL, Stoeckman AK, Wu G, Boeckermann AN, Azam T, Netea MG, et al. IL-38 binds to the IL-36 receptor and has biological effects on immune cells similar to IL-36 receptor antagonist. *Proc Natl Acad Sci U S A* (2012) **109**(8):3001–5. doi:10.1073/pnas.1121534109
202. Costelloe C, Watson M, Murphy A, McQuillan K, Loscher C, Armstrong ME, et al. IL-1F5 mediates anti-inflammatory activity in the brain through induction of IL-4 following interaction with SIGIRR/TIR8. *J Neurochem* (2008) **105**(5):1960–9. doi:10.1111/j.1471-4159.2008.05304.x

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

Received: 06 April 2013; accepted: 24 June 2013; published online: 09 July 2013.
*Citation: Lopetuso LR, Chowdhry S and Pizarro TT (2013) Opposing functions of classic and novel IL-1 family members in gut health and disease. Front. Immunol. **4**:181. doi: 10.3389/fimmu.2013.00181*
This article was submitted to Frontiers in Inflammation, a specialty of Frontiers in Immunology.

frontiers in
IMMUNOLOGY

REVIEW ARTICLE
published: 31 October 2013
doi: 10.3389/fimmu.2013.00351

The central role of anti-IL-1 blockade in the treatment of monogenic and multi-factorial autoinflammatory diseases

***Silvia Federici*[1], *Alberto Martini*[1,2] *and Marco Gattorno*[1]*

[1] 2nd Division of Pediatrics, G. Gaslini Institute, Genoa, Italy
[2] Department of Pediatrics, University of Genoa, Genoa, Italy

Edited by:
Cecilia Garlanda, Istituto Clinico Humanitas, Italy

Reviewed by:
Joost Frenkel, University Medical Center Utrecht, Netherlands
Michaël Hofer, Centre Hospitalier Universitaire Vaudois, Switzerland

****Correspondence:***
Marco Gattorno, 2nd Division of Pediatrics, G. Gaslini Institute, Largo G. Gaslini 5, 16147 Genoa, Italy
e-mail: marcogattorno@ospedale-gaslini.ge.it

Inherited autoinflammatory diseases are secondary to mutations of proteins playing a pivotal role in the regulation of the innate immunity leading to seemingly unprovoked episodes of inflammation. The understanding of the molecular pathways involved in these disorders has shed new lights on the pattern of activation and maintenance of the inflammatory response and disclosed new molecular therapeutic targets. Cryopyrin-associated periodic syndrome (CAPS) represents the prototype of an autoinflammatory disease. The study of the pathophysiological consequence of mutations in the cryopyrin gene (*NLRP3*) allowed the identification of intracellular pathways responsible for the activation and secretion of the potent inflammatory cytokine interleukin-1β (IL-1β). It became clear that several multi-factorial inflammatory conditions display a number of pathogenic and clinical similarities with inherited autoinflammatory diseases. The dramatic effect of interleukin-1 (IL-1) blockade in CAPS opened new perspectives for the treatment of other inherited and multi-factorial autoinflammatory disorders. Several IL-1 blockers are now available on the market. In this review we outline the more recent novelties in the treatment with different IL-1 blockers in inherited and multi-factorial autoinflammatory diseases.

Keywords: IL-1β, autoinflammatory diseases, periodic fevers, inflammasome, treatment

INTRODUCTION

The Autoinflammatory Syndromes are a number of different conditions characterized by episodes of inflammation secondary to an activation of the innate arm of the immune response, in the absence of high-titer auto-antibodies or antigen-specific T cells (1). The term "Autoinflammatory diseases" was originally referred to a limited number of rare inherited diseases identified as *periodic fevers*. Under this term were gathered some monogenic diseases featured by periodic or recurrent episodes of systemic inflammation causing fever often associated with rash, serositis, lymphadenopathy, arthritis, and other clinical manifestations. These disorders were secondary to mutations of genes coding for proteins that play a pivotal role in the regulation of the inflammatory response. In the following years the discovery of new genes and novel inherited conditions allowed to clarify that the possible clinical presentation of these new diseases was much wider and that the term of periodic fevers was associated to a reductivist view of the clinical phenotype possibly associated to this group of disorders (**Table 1**).

In the meanwhile, the pathogenetic insights derived from studies on these rare disorders allowed a better understanding of mechanisms responsible for the induction and maintenance of inflammation and have set the basis for the identification of molecular targets for treatment. Due to the enormous interest raised by the identification of *NLRP3* Inflammasome (2) and by the dramatic response to interleukin-1 (IL-1) blockers observed in patients presenting a gain of function mutation of *NLRP3* gene (cryopyrin-associated periodic syndromes, CAPSs) (3–5), IL-1, is now considered the pivotal pro-inflammatory cytokine in these disorders. The availability of specific IL-1 targeting agents has revealed a pathological role of IL-1-mediated inflammation in a growing list of multi-factorial diseases in which a deregulation of the same intracellular pathway probably occurs. Aim of the present review is to provide a state of the art of the treatment of inherited and multi-factorial autoinflammatory diseases with IL-1 blockers.

ANTI-IL-1 AGENTS

There are two related but distinct IL-1 genes, *IL1A* and *IL1B*, encoding IL-1α and IL-1β, respectively. The IL-1α precursor is constitutively present, in an active form, in most of the cells of healthy individuals. Under disease conditions, IL-1α moves to the cell's surface membrane where it can activate adjacent cells bearing the IL-1 receptor (6, 7). Conversely IL-1β is a product of a limited type of cells (e.g., blood monocytes, tissue macrophages, and dendritic cells). In physiological conditions IL-1β is in an inactive form and requires a series of intracellular events to be activated. In normal conditions both IL-1α and IL-1β bind to type 1 IL-1 receptor (IL-1R1) and to the adaptor protein IL-1RAcP in order to trigger signal transduction (**Figure 1**).

Targeting IL-1 started in 1993 with the introduction of a recombinant non-glycosylated form of the naturally occurring IL-1 receptor antagonist (IL-1Ra, Anakinra), which blocks the activity of both IL-1α and IL-1β. IL-1Ra competes with free IL-1α and IL-1β for the IL-1R1 binding but not with the adaptor protein, thus preventing signal transduction. Anakinra has a short terminal half-life ranging from 4 to 6 h and it is administered subcutaneously daily. Other IL-1 blockers have been successively

Table 1 | Clinical classification of inherited and multi-factorial autoinflammatory diseases (AID).

	Inherited AID (gene, transmission)	Multi-factorial AID
CLINICAL PRESENTATION		
Recurrent episodes of inflammation	FMF (*MEFV*, AR) TRAPS (*TNFRSF1A*, AD) MVK (*MVK*, AR)	PFAPA Recurrent idiopathic pericarditis Mollaret syndrome (recurrent meningitis)
Systemic inflammation with urticarial rash	CINCA/NOMID (*NLRP3*, AD) Muckle–Wells/FCAS (*NLRP3*, AD) FCAS2 (*NLRP12*, AD)	SoJIA Adult onset Still disease Schnitzler's syndrome Delayed pressure urticaria
Sterile inflammation of skin/bone/joints	PAPA (*CD2BP1*, AD) DIRA (*IL1RN*, AR) DITRA (*IL36RN*, AR) Majeed syndrome (*LPIN2*, AR) CAMPS (*CARD14*, AD) Blau's syndrome (*CARD15*, AD)	CRMO SAPHO Gout and pseudogout HLA-B27 spondyloarthropathy Reactive arthritis Sweet syndrome Generalized pustular psoriasis Hallopeau acrodermatitis
Panniculitis/lipodystrophy	Nakajo–Nishimura (*PSMB8*, AR) JMP (*PSMB8*, AR) CANDLE syndrome (*PSMB8*, AR)	Neutrophilic panniculitis Erythema nodosum and panniculitis
Inflammatory bowel disease	Early-onset inflammatory bowel disease (*IL10*, *IL10RA*, *IL10RB*)	Crohn's disease
Hemophagocytic lymphohistiocytosis	FHL1 (Unknown) FHL2 (*PFR1*/perforin 1, AR) FHL3 (*UNC13D/Munc* 13-4, AR) FHL4 (*STX11*/syntaxin 11, AR) FHL5 (*STXB2*/syntaxin binding protein, AR)	SoJIA-associated MAS Infection-associated MAS

FMF, familial Mediterranean fever; FCAS, familiar cold autoinflammatory syndrome; MWS, Muckle–Wells syndrome; TRAPS, TNF-receptos associated periodic syndrome; MVK, mevalonate kinase deficiency; CINCA, chronic infantile neurological cutaneous and articular; PAPA, pyogenic sterile arthritis, pyoderma gangrenosum, and acne; JMP, joint contractures, muscle atrophy, microcytic anemia, and panniculitis-induced childhood-onset lipodystrophy; CANDLE, chronic atypical neutrophilic dermatosis with lipodystrophy and elevated temperature; DIRA, deficiency of the IL-1 receptor antagonist; DITRA, deficiency of IL-36 receptor antagonist; CAMPS, CARD14-mediated pustular psoriasis; FHL, familial hemophagocytic lymphohistiocytosis; MAS, macrophage activation syndrome; AR, autosomal recessive; AD, autosomal dominant.

developed. Rilonacept is a protein consisting of the extracellular domains of humanized IL-1 type 1 receptor and the IL-1 receptor accessory protein fused with the Fc portion of IgG1. Rilonacept which has a terminal half-life of 6.3–8.6 days and is administered once weekly, binds IL-1β and IL-1α with high affinity and powerfully inhibits IL-1 activity.

Canakinumab is a fully human anti-interleukin-1β (IL-1β) monoclonal antibody that selectively blocks IL-1β with high affinity and does not cross-react with IL-1α or IL-1Ra. Binding to IL-1β prevents the cytokine from the interaction with its receptor and, thus, blocks the inflammatory signaling cascade. Compared to other IL-1 blockers, Canakinumab provides a longer plasma half-life (21–28 days).

Recently a novel compound called Gevokizumab has been developed. Gevokizumab is a IgG2 humanized mAb that modulates IL-1β bioactivity by reducing the affinity for its IL-1RI:IL-1RAcP signaling complex. It binds to a single IL-1β epitope where residues critical for binding have been identified. It has a long plasma half-life, which would allow once-monthly administration. Some clinical trials are ongoing in osteoarthritis, non-infectious uveitis, Pyoderma Gangrenosum, and Diabetes mellitus[1].

Other therapeutic approaches, including IL-1α neutralization, a therapeutic vaccine targeting IL-1β, and a chimeric IL-1Ra, are in early clinical trials. Moreover, orally active small-molecule inhibitors of IL-1 production, such as Caspase 1 inhibitors, have been developed and are being tested (8, 9).

MONOGENIC AUTOINFLAMMATORY DISEASES

CRYOPYRIN-ASSOCIATED PERIODIC SYNDROME

Familiar Cold Autoinflammatory Syndrome (FCAS), Muckle–Wells Syndrome (MWS), and Chronic Infantile Neurological Cutaneous and Articular Syndrome (CINCA) or Neonatal Onset Multi-systemic Inflammatory Disease (NOMID) are three diseases originally described as distinct entities that turned out to belong to the wide clinical phenotype of disorders due to mutations of *NLRP3* gene (NOD-like receptor 3, previously known as

[1]http://clinicaltrials.gov

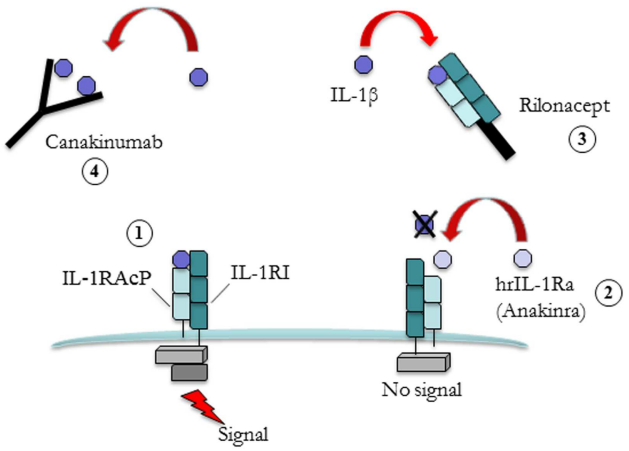


FIGURE 1 | Different strategies for IL-1 blockade. Free interleukin (IL)-1b binds to type 1 IL-1 receptor (IL-1R1) and to the adaptor protein IL-1RAcP leads to signal transduction (1). Human recombinant IL-1 receptor antagonist (hrIL-1Ra, Anakinra) (2) competes with free IL-1β for the binding with IL-1R1 but not with the adaptor protein, thus preventing signal transduction. Rilonacept (3) is a fusion protein comprising the extracellular domains of the IL-1β receptor (IL-1RI) and adaptor protein (IL-1RAcP) attached to a human IgG molecule. Its action is to bind to circulating IL-1β. The same mechanism of action is also valid for Canakinumab (4), a fully humanized anti-IL-1 monoclonal antibody.

Cold-Induced Autoinflammatory Syndrome 1, *CIAS1*). Mutations in this site determine a gain of function of the protein with subsequent increased secretion of IL-1β. FCAS represents the milder phenotype and presents with cold-induced fever, urticaria-like rash, and constitutional symptoms. MWS represent an intermediate form and manifests with fever, urticarial rash, sensorineural hearing loss, and arthritis usually not related to cold exposure. CINCA patients, instead, display the worst clinical picture characterized by fever, urticarial rash, epiphyseal overgrowth of the long bones, and central nervous system involvement (mental retardation, chronic aseptic meningitis, increased intracranial pressure, papilledema, cerebral atrophy, sensorineural hearing loss). Almost 1/3 of patients with CAPS may develop amyloidosis that seem to be more frequent in MWS than in FCAS or CINCA patients.

The active form of cryopyrin (NLRP3) is involved in the assembly of an intracellular multi-protein complex (called inflammasome) that play a pivotal role in the activation of Caspase 1, a cytoplasmatic enzyme responsible for the activation and secretion of the biological active 17 kD form of IL-1β (10).

The massive secretion of active IL-1β observed in cryopyrin-mutated individuals, suggested that anti-IL-1 treatment could represent an effective therapy (10).

Initial isolated case reports showed the dramatic effects of Anakinra in the control of rash and other systemic manifestations in MWS (3), FCAS (4), and CINCA (5, 11, 12) patients.

The long-term efficacy and safety of Anakinra in pediatric CAPS patients has been described in two distinct cohorts of patients (13, 14).

These two studies indicate that Anakinra treatment is safe and effective in the long term and should be initiated early, before irreversible lesions have developed.

Sibley et al. recently published an open label, long-term follow-up study on a cohort of 26 CINCA/NOMID patients treated with Anakinra 1–5 mg/kg/day for at least 36 months (15). Twenty-one out of 26 patients carried mutations in *NLRP3* gene and all of them had an active disease at baseline.

Aim of the study was to evaluate the efficacy and safety of 36 and 60 months of IL-1-blocking therapy in controlling systemic and organ-specific inflammation and in preventing the progression of organ damage.

Sustained improvements in diary scores, parent's/patient's and physician's global scores of disease activity, parent's/patient's pain scores, and inflammatory markers were observed during all the period of the study. Despite a general good control of clinical manifestations (including hearing loss, ocular manifestations, and headache) and laboratory parameters, a few patients displayed a persistent even if mild inflammation of CNS. Anti-IL-1 treatment did not prevent the progression of the bone involvement. Overall Anakinra was well tolerated and no major adverse effects were observed.

Kuemmerle-Deschner et al. reported the long-term safety and efficacy of Anakinra in pediatric and adult patients affected by MWS. A rapid and persistent control of constitutional symptoms and organ manifestations was observed (16).

The efficacy of Rilonacept (160 mg/weekly) on CAPS-related clinical manifestations have been shown in two sequential placebo-controlled studies performed in patients with FCAS or MWS (17). The treatment was generally well tolerated. Site reactions in 1/3 of patients and mild upper respiratory infections were the most common adverse events (AEs). In 2008, the FDA in USA has approved its use in CAPS as an orphan drug in adult and children above the age of 12.

The first evidence for the efficacy of Canakinumab was obtained by a 48-week, double-blind, placebo-controlled, randomized withdrawal study, involving 35 MWS patients receiving a subcutaneous dose of 150 mg (or 2 mg/kg) every 8 weeks (18).

These positive results were confirmed in a 2-years open label study in which the response to treatment was analyzed in 166 patients (109 Canakinumab-naive and 57 roll-over patients) with FCAS ($n = 30$), MWS ($n = 103$), or NOMID/CINCA ($n = 32$) (19). The study showed that a complete response was achieved in 85 of 109 Canakinumab-naive patients (78%; 79/85 patients within 8 days, and 5 patients between days 10 and 21). Of 141 patients with an available relapse assessment, 90% did not relapse, their CRP/SAA levels normalized (<10 mg/l) by day 8, and remained in the normal range thereafter. Median treatment duration was 414 days (29–687 days). Notably, upward adjustments of dose or frequency were needed in 24.1% patients mostly children with a severe CAPS phenotype (CINCA/NOMID). Predominant AEs were infections (65.7%) of mostly mild-to-moderate severity. Serious AE reported in 18 patients (10.8%) were mainly infections and were responsive to standard treatment. The majority of patients (92%) reported as having no injection-site reactions and only 8% of patients reported mild-to-moderate reactions. Patients receiving vaccinations (15%) showed normal immune response. Based on these studies, Canakinumab has been approved in many countries for all forms of CAPS in patients older than 4 years. The first experience on the use of Canakinumab in daily clinical practice in 13 pediatric CAPS patients has been

recently reported (20). Globally, patients with a mild-intermediate MWS phenotype display a complete control of disease activity maintaining the initial dosage of 2 mg/kg (or 150 mg) every 8 weeks, independently from their age. Conversely, the majority of CINCA patients required to increase the dosage up to 4 mg/kg (or 300 mg) and progressively increase the frequency of dosing (20).

FAMILIAL MEDITERRANEAN FEVER

Familial Mediterranean fever (FMF) is the most frequent among hereditary recurrent inflammatory disorders. It presents with an autosomal recessive pattern of inheritance and is due to mutations in the *MEFV* (Mediterranean Fever) gene encoding pyrin (also called marenostrin) (21, 22).

Colchicine represents the treatment of choice for FMF (23). Nonetheless, approximately one third of the patients have a partial remission and about 5–10% are non-responders; another 2–5% do not tolerate the drug mainly due to gastrointestinal symptoms (24). Data from a large international registry (Eurofever) showed that almost 40% of FMF patients display an incomplete response to colchicine, by means of persistent presence of fever attacks or persistent elevation of acute-phase reactants (25).

Before the advent of colchicine, reactive AA amyloidosis represented the most frequent and severe complication of the disease. It occurred in almost 60–75% of patients over the age of 40 with a poor prognosis. Amyloidosis usually presents in those patients with severe attacks starting early in infancy but it may develop even in those patients without clear inflammatory episodes. The genetic background, the presence of high penetrance mutations, environmental factors, and the presence of SAA1 gene haplotype seem to influence the development of amyloidosis too. Even if the use of Colchicine dramatically reduced the incidence of amyloidosis, a relevant number of patients still present this long-term complication (26).

Recent evidences have shown that pyrin is able to interact with some components of the *NLRP3* Inflammasome (e.g., ASC and Caspase 1), raising the hypothesis of a possible role of this protein as a negative regulator (27, 28) or as an inducer of IL-1β secretion (29–31). Omenetti et al. have recently reported that *MEFV*-mutated monocytes display an increased IL-1β secretion (32).This over-secretion is correlated to the number and penetrance of *MEFV* mutations (32), confirming the presence of a dose effect of *MEFV* mutations already suggested by studies on FMF animal models (29) and patients (33).

Indeed, the use of IL-1 targeting drugs in colchicine-resistant FMF patients was recently proposed as a valid therapeutic strategy (27, 34). Anakinra and Canakinumab, have been reported to be generally effective in case reports and non-controlled series in more than 30 patients with colchicine-resistant or intolerant FMF (35–44).

Blocking IL-1 has been used with good results even in a small cohort of patients with AA amyloidosis and chronic renal failure (44). In the majority of cases Colchicine was maintained after the introduction of Anakinra even if at a lower dose.

Normally SAA is completely degraded in the lysosome. It seems that, in patients with AA amyloidosis, the process of degradation may be impaired. High levels of SAA due to inflammatory burden thus worsen the accumulation of this substance. A good control of the disease by IL-1 blockers with persistent SAA value in the range of normality, may avoid the worsening of amyloidosis and may allowed the progressive degradation of the fibrils previously accumulated.

Recently, Hashkes et al. reported the results of a randomized, double-blind, single-participant alternating treatment study for Rilonacept in 14 colchicine-resistant or intolerant FMF patients (45). Participants were aged 4 years or older and were required to have an estimated mean of 1 or more FMF attacks per month for 3 months before screening and 1 or more attacks per month during screening despite receiving adequate colchicine treatment. Colchicine intolerance was defined as the inability to tolerate the drug at that dose controlling attacks to fewer than 1 per month. Patients were treated with Rilonacept 2.2 mg/kg (maximum, 160 mg) or equal volume of placebo, both given once weekly by subcutaneous injection. Rilonacept significantly reduced the frequency of FMF attacks versus placebo and ameliorated the physical Health related quality of life.

TNF-RECEPTOR ASSOCIATED AUTOINFLAMMATORY SYNDROME

TNF-receptor associated autoinflammatory syndrome (TRAPS) is a rare dominantly inherited disorder, caused by mutations in the p55 TNF Receptor (or TNFR1), encoded by the TNF Receptor Super Family 1A (*TNFRSF1A*) gene (46). Fever episodes are usually prolonged (1–3 weeks) and are usually accompanied by serositis, arthritis, a skin rash with underlying fasciitis, and periorbital edema. Reactive amyloidosis develops in almost 14–25% of TRAPS patients and it seems to correlate with the severity and lasting of fever episodes.

Mutations of TNFR1 lead to a misfolding of the protein that is thus accumulated in the ER and cytoplasm. This leads to a response to the unfolded protein with consequent inappropriate cytokine secretion (47). Mitochondrial reactive oxygen species promote production of pro-inflammatory cytokines and are elevated in TNFR1-TRAPS (48). Moreover, TRAPS patients display an exhaustion of the autophagy system due to the intracellular overload of the unfolded mutated protein that represents a further "stress signal" for the cells (49). This may lead to the activation of the NLRP3 Inflammasome, consistent with the over-secretion of active IL-1β observed in TRAPS patients (49).

The inflammatory attacks are usually responsive to high-dose corticosteroids but side effects limit their use especially in patients with frequent relapses or nearly continuous symptoms (chronic course). The use of immunosuppressive drugs have been reported to be ineffective (50). Since the molecular defect of p55 TNFR is also associated with an impaired shedding of the receptor from the membrane surface, the use of Etanercept (Enbrel), was originally proposed (46).

In a recent study Bulua et al. (51) reported the experience of 15 TRAPS patients enrolled in a prospective, open label, dose-escalation study using Etanercept. The treatment significantly attenuated the total symptom score, as well as reduced the frequency of symptoms and the values of acute-phase reactants during asymptomatic periods. However, during a 10-year follow-up period, most of the patients discontinued treatment mainly

due to the lack of efficacy, with a median duration of treatment of 3.3 years.

Of note, the use of anti-TNF monoclonal antibodies (infliximab and adalimumab) has been shown to worsen the inflammatory manifestations in TRAPS patients (52–54).

On the other hand, some anecdotic observations have suggested an excellent response to Anakinra in some patients (53, 55).

In 2008, the first small cohort of TRAPS patients treated with Anakinra was described (56). Patients were treated with Anakinra 1.5 mg/kg/day. All patients had a dramatic response, with disappearance of symptoms and normalization of acute-phase reactants. During the following year the patients were treated continuously with Anakinra and did not experience any disease-related clinical manifestations or any increase in acute-phase reactants (56).

Interestingly, data from the Eurofever registry recently showed the better performance of IL-1 blockade on anti-TNF treatment in TRAPS patients. In fact, even if Etanercept was beneficial in 32 of the 37 patients, only 11 (30%) experienced a complete response. Conversely, Anakinra was able to induce a complete response (absence of clinical manifestations and normalization of acute-phase reactants) in 26 of 33 patients (79%) and a partial response in five others (25). The effect of Anakinra in patients carrying low-penetrance mutations (e.g., R92Q) seems less striking in respect to those observed in patients with mutations affecting cysteine residues (57). The same good results have been preliminary reported in one TRAPS patients treated with the anti-IL-1 monoclonal antibody (Canakinumab) (58).

Interim data of an open label 4-months study with Canakinumab and of 5-months of follow-up involving 20 active TRAPS patients have been recently presented, showing the complete control of clinical manifestations and persistent normalization of acute-phase reactants (59).

These data support the pivotal role of IL-1β in the pathogenesis of TRAPS, but need to be confirmed in a larger number of patients.

MEVALONATE-KINASE DEFICIENCY

Mevalonate-kinase deficiency (MKD) is due to mutations in the mevalonate-kinase (MVK) gene (60, 61). MVK is an essential enzyme in the isoprenoid biosynthesis pathway which produces several molecules involved in different cellular processes (62). The severe reduction of the enzymatic activity leads to a severe multi-systemic disease, named mevalonic aciduria. The partial enzymatic defect is associated with a normal mental and physical development, but is characterized by recurrent fever attacks.

Almost 25% of MKD patients present more than 12 attacks per years in their second decade of life (17.8% of patients >20 year old) with a severe impact on quality of life in some patients (63). Unlike what happen in the other monogenic periodic fevers, amyloidosis do not represent a frequent complication of MKD. The first case of AA amyloidosis was reported in 2004 (64) and few other patients in the following years (65). van der Hilst et al. (63) reported a frequency of 2.9% of amyloidosis in a group of 103 patients coming from The International hyper-IgD syndrome (HIDS) database.

Fever attacks usually respond to the administration of a single or a few steroids doses. However, due to the high frequency of fever episodes, some patients may need almost continuous treatment.

Colchicine, cyclosporine, thalidomide, and statins are not effective (63). The efficacy of biologic treatments is largely anecdotal and still controversial. Anti-TNF therapy (Etanercept) has been found to reduce the frequency and intensity of fever attacks in some patients (66–68) but not in others (69). In the same line a positive response was also observed after the use of Anakinra (70–72). The same variable response was also observed in the International HIDS registry (63).

Galeotti et al. reported the results of a retrospective study aimed at evaluating the effects on disease activity of an anti-IL-1 treatment in a group of 11 genetically confirmed MKD patients (73). In this study daily Anakinra (nine patients) or Canakinumab injections every 4–8 weeks (six patients, in four cases following Anakinra treatment) were associated with complete remission in four cases and partial remission in seven.

The rationale for the use of IL-1 blockers is found in studies from a Dutch group (74) in which the Authors show that a shortage of isoprenoid end products due to the defective function of mevalonate kinase contributes to an increased secretion of IL-1β by MK-deficient peripheral blood mononuclear cells.

In a recent paper Ter Haar et al. analyzed MKD response to treatment in 67 patients coming from The Eurofever International Registry[2] (25). Anakinra was effective in 24 (89%) of 27 patients treated, inducing a complete remission in six (22%) of them. Etanercept was effective in 11 (65%) of 17 treated patients, with only one complete response.

An Open label, Multicenter, Pilot Study of 6-month Canakinumab Treatment With up to 6-month Follow-up in Patients With active HIDS is now ongoing to evaluate the efficacy, the safety, and the pharmacokinetics (PK)/pharmacodynamics (PD) of Canakinumab treatment in patients with HIDS (see text footnote 1).

BLAU'S SYNDROME OR NOD2 GENE-ASSOCIATED PEDIATRIC GRANULOMATOUS ARTHRITIS

Blau's syndrome is the genetic form of what was previously known as early-onset sarcoidosis and is due to mutations of the NACHT domain of the gene *CARD15* (or *NOD2*). It is characterized clinically by the triad of arthritis, skin rash, and uveitis, and histologically by the presence of non-caseating epithelioid granulomas in the affected sites that represent the hallmark of the disease. Other, less frequent clinical symptoms, such as camptodactyly, intermittent fever, cranial neuropathies, and malignant hypertension, have also been reported (75). The clinical course is variable, but in many cases the prognosis is poor, with severe disabilities and sequelae in a high percentage of patients. Eye involvement is frequently progressive and can lead to panuveitis and severe complications up to blindness.

An international registry was established in 2005 to collect both patients affected by Blau syndrome and its sporadic counterpart of early-onset sarcoidosis that share with the former an identical phenotype. Rose et al. (76) reported the results of the registry

[2]http://www.printo.it/eurofever/

1 year after its creation. In the paper the authors aimed to define the spectrum of the clinical phenotype and establish the mutation frequency and variants in patients with "pediatric granulomatous arthritis." Up to date this work represent the largest collection of patients (33 pts) with both sporadic or familiar pedigrees.

Anti-TNF monoclonal antibodies have been shown to be effective in some anecdotal reports (77, 78). Martin et al. did not observe evidence for increased IL-1β production in cells obtained from five subjects with Blau syndrome compared with healthy control (79). In their study the Authors treat two cases with recombinant human IL-1 receptor antagonist without an evident clinical response. On the other hand few case reports have shown the effectiveness of IL-1 blockade (75, 80).

PYOGENIC STERILE ARTHRITIS, PYODERMA GANGRENOSUM, AND ACNE SYNDROME

Pyoderma gangrenosum and acne (PAPA) syndrome is a rare autosomal dominant inherited autoinflammatory syndrome characterized by pyogenic sterile arthritis (usually occurring after minor trauma or even spontaneously) less frequently accompanied by PAPA. It is associated with dominant missense mutations in the proline-serine-threonine phosphatase-interacting protein 1 (*PSTPIP1*) gene (81, 82). Around 30 cases have been described in the literature so far. PAPA syndrome is generally responsive to oral or intra-articular glucocorticoids (81, 82) but anti-TNF has been reported to be effective in a few patients too (83, 84).

Interestingly, *PSTPIP1* is able to interact with pyrin, the protein mutated in FMF (85). It has also been proposed that pyrin and *PSPTPIP1* form a tri-molecular complex with ASC that is able to directly recruit and activate Caspase 1 (31).

Mutated *PSTPIP1* has increased interaction with pyrin and the effect of this interaction is similar to that of mutated pyrin, resulting in decreased apoptosis and elevated IL-1 levels (85) thus setting the basis for the use of IL-1 blockade in PAPA patients.

Up to date anecdotal cases of treatment with Anakinra (both as maintenance therapy or at occurrence) has been reported with good results particularly on articular manifestations (86–88). More uncertain is the effectiveness of Anakinra on cutaneous manifestations.

Geusau et al. have recently reported the successful treatment of a patient with a PAPA-like syndrome with Canakinumab (89). This patient, despite the unusual presence of an homozygous substitution in exon 11 (c.773G > C p.Gly258Ala) in a disease with an autosomal recessive pattern of inheritance, had a clinical phenotype clearly consistent with a PAPA syndrome. He was mainly affected by cutaneous manifestations (PAPA fulminans) since the age of 14 and, despite the absence of arthritis he had suffered from early childhood of episodes of arthralgia, painful joints, and fever not responding to antibiotics. Moreover he had elevated inflammatory markers and white blood cell count with relative neutrophilia. Notably, the substitution found in this case had been previously described in an heterozygous symptomatic PAPA patient[3]. Soon after the first injection of Canakinumab the patient displayed a complete remission of the acne and a complete normalization of acute-phase reactant. Canakinumab was administered every 9 weeks and no flares were observed in a 9-months period apart from slight flares of acne at the end of 8 weeks, occasionally accompanied by a moderate increase in C-reactive protein and serum amyloid A levels (89).

In line with this experience a Phase II Multi Center Open Label Pilot Study to assess a potential effect of Canakinumab on Pyoderma Gangrenosum is ongoing (see text footnote 1).

DEFICIENCY OF THE INTERLEUKIN-1-RECEPTOR ANTAGONIST

Deficiency of the interleukin-1-receptor antagonist (DIRA) is a recently identified autoinflammatory disorder characterized by a severe systemic inflammation beginning at birth with persistent inflammation and papular pustulosis. A severe inflammatory bone involvement (multifocal osteomyelitis, periostitis) is also observed. The patients display deleterious truncating or missense mutations in the interleukin-1-receptor antagonist (*IL1RN*) gene (90). Deletions at chromosome 2q13, which encompasses several IL-1 family members, including *IL1RN* have been reported too (91). As a result of these mutations, no interleukin-1-receptor antagonist protein is secreted, with subsequent unopposed action of IL-1. The patients show a dramatic response to the substitutive treatment with recombinant IL-1 receptor antagonist (Anakinra) (90). Up to date only 16 cases have been described in literature.

MAJEED'S SYNDROME

The Majeed's syndrome is an autosomal recessive, autoinflammatory disorder characterized by the triad of chronic recurrent multifocal osteomyelitis (CRMO), congenital dyserythropoietic anemia, and inflammatory dermatosis. The disease was firstly described in three related Arab children by Majeed and co-workers (92) and subsequently associated to mutation of the *LPIN2* gene (93). A recent report describe the over production of IL-1 by monocytes of two brothers carrying *LPIN2* mutations and the good response to anti-IL-1 treatment (94), thus showing the possible pivotal involvement of this cytokine also in this condition.

MULTI-FACTORIAL DISORDERS

The identification of genetically determined inflammatory diseases characterized by a predominant involvement of innate immunity in respect to the adaptive branch of the immune response (lack of pathogenic auto-antibodies and antigen-specific T cells) represented a major breakthrough in the field of inflammatory diseases in the last decade. It became clear that several multi-factorial inflammatory conditions were much more similar to the recently identified inherited autoinflammatory diseases rather than to the classical autoimmune rheumatic diseases (RA, SLE, etc.). Indeed, many multi-factorial disorders present with the same clinical manifestations observed in inherited autoinflammatory diseases (**Table 1**) and also share the same pathways of activation of innate immunity.

In fact, it was observed that NLRP3 Inflammasome can be activated not only by rare, gain of function mutations in the *NLRP3* gene but also by a large variety of endogenous or exogenous stimuli leading to an over-activation of cells of innate immunity in different circumstances (95–97). These findings represented the rationale for the successful use of the anti-IL-1 blockers in many multi-factorial inflammatory diseases.

[3]http://fmf.igh.cnrs.fr

A clear example is given by *systemic onset juvenile idiopathic arthritis* (*SoJIA*) and *adult onset Still's disease (AOSD)* that share with the severe form of CAPS (CINCA) the multi-systemic inflammatory involvement (**Table 1**). SoJIA and AOSD patients classically present with high spiking fever, arthritis (either with oligoarticular or severe symmetric polyarticular course), and evanescent rash. Lymphadenopathy, hepatosplenomegaly, serositis, arthralgias, and myalgias are frequently present too. Few patients with SoJIA or AOSD respond to NSAID monotherapy. A single steroid course can control disease manifestations in patients with monocyclic presentation. However the majority of the patients present a progression of the disease and develop a steroid dependency. In these patients Methotrexate or TNF-α neutralizing agents have been employed with limited success (98, 99). IL-6 has been proven to play a pivotal role in the pathogenesis of the disease (100) and IL-6 blockade represent a very effective treatment in steroid-dependent patients, as recently confirmed by a large international multicentre trial (101). On the other hand, seminal studies by Pascual et al. were able to demonstrate the presence of a clear IL-1 signature in SoJIA patients (102, 103) that, at least in a variable percentage, display an optimal response to IL-1 blockade (98, 103–105). The same observations were also anecdotally reported in AOSD patients (106, 107). A recent large international trial has shown that the use of Canakinumab is able to control the articular and systemic manifestations and allow the tapering and withdrawn of steroids in a large proportion of patients (108).

Recurrent bouts of seemly unprovoked inflammation are the most classical manifestation observed in inherited periodic fevers (**Table 1**).

PFAPA syndrome is the prototype of a multi-factorial periodic fever syndrome. Patients display recurrent, often clock-wised, spontaneous episodes of fever in the absence of proof of infections, associated with an increase of the principal acute-phase reactant lasting 4–6 days. Usually the disease onset is before the age of five and aphthous stomatitis, pharyngitis, and laterocervical lymphadenopathy represent the most characteristic symptoms. Children present normal growth and complete well-being among the episodes. The disease is usually self-remitting with the age. The use of steroid on demand and the tonsillectomy in persistent cases represent the two main therapeutic strategies. The response to Anakinra on demand represents the *in vivo* demonstration of the *in vitro* studies indicating the involvement of this cytokine in the pathogenesis of the disease (109, 110).

Idiopathic recurrent pericarditis also has many features that are consistent with an autoinflammatory disease. The first observation of the role of IL-1 blockade in this condition came from the report on the effect of Anakinra in three steroid-dependent and colchicine-resistant children (111). Pericarditis recurred when Anakinra treatment was discontinued and no further episodes occurred after it was resumed. After this report several others confirmed the good response to IL-1 blockade (112–114).

Urticarial rash associated with signs of systemic inflammation represents the classical hallmark of the milder form of CAPS. *Schnitzler's syndrome* share many clinical similarities with these conditions (**Table 1**). It is a chronic disabling inflammatory disorder, characterized by chronic urticarial rash, paraproteinemia, and systemic inflammation. Disease onset is usually observed after the age of 40 and patients can also present fever, bone pain, and arthralgias or arthritis. A higher risk of developing a lymphoproliferative disorder and AA amyloidosis in the long term has also been reported (115, 116). Anakinra was found to be effective in over 45 cases to date (117, 118) implying a pivotal pathophysiological role of IL-1. Canakinumab has been tried with optimal response too. A 9-month open label, single-arm trial demonstrate the long-term efficacy of Canakinumab in a cohort of 8 Dutch patients (119).

A prevalent neutrophilic inflammation of joints, bone, and skin is a common finding in a number of inherited autoinflammatory diseases (such as PAPA, DIRA, DITRA, etc.), but also in many multi-factorial disorders (**Table 1**).

A severe and painful arthritis characterized by a diffuse neutrophilic joint effusion is the main clinical feature of *gout and pseudogout*, two common conditions occurring in adulthood. The diseases are caused respectively by the deposition of monosodium urate (MSU) and calcium pyrophosphate dihydrate (CPPD) crystals in the joints and periarticular tissues. The finding that both MSU and CPPD crystal are able to activate the NLRP3 inflammasome (96) strongly supported the hypothesis that the inflammatory manifestations of these metabolic conditions recognize the same pathogenic mechanisms observed in inherited autoinflammatory diseases, by means of a persistent over-activation of the NLRP3 inflammasome.

IL-1 blockade has been shown to be effective in colchicine-resistant gout and pseudogout (120, 121).

Ghosh et al. described the results of Anakinra treatment in 26 patients affected from gout (122). In 67% of them, pain improved significantly within 24 h and a complete resolution of signs and symptoms of gout occurred by day 5 in 72.5% of patients. Anakinra was well tolerated and no adverse outcomes were attributed to the drug. Only one patient appeared to be refractory to this form of IL-1 inhibition (122).

In two recent 12-week randomized, multicentre, double-blind, parallel-group core studies, Canakinumab provided significant pain and inflammation relief and reduced the risk of new flares in patients with acute gouty arthritis. AEs reported more frequently with Canakinumab included infections, low neutrophil count, and low platelet count (123).

Chronic recurrent multifocal osteomyelitis and *Synovitis, Acne, Pustulosis, Hyperostosis, and Osteitis (SAPHO) syndrome* display a number of clinical similarities with inherited autoinflammatory diseases characterized by the presence of sterile osteolytic lesions, such as Majeed's syndrome and DIRA.

No uniformly effective therapeutic strategies have been established for both CRMO and SAPHO (124, 125). Among the various possible biologic treatments used so far, also IL-1 blockers have been anecdotally reported to be at least partially effective in both conditions (124, 126, 127).

Pustular psoriasis and neutrophilic dermatoses are also frequent manifestations observed in inherited autoinflammatory disease, such as DIRA, DITRA, and CARD14-mediated familial psoriasis.

Generalized pustular psoriasis is an acute form of psoriasis with erythematous, painful skin, and widespread sterile pustules associated with systemic inflammation (fever, malaise), leukocytosis,

and elevation of acute-phase reactants. The effect of IL-1 treatment have been recently described in two patients (128).

Sweet's syndrome is a neutrophilic dermatosis characterized by fever, an elevated neutrophil count, and painful erythematous cutaneous lesions. Histopathological analysis reveals a neutrophilic dermal infiltrate. Systemic corticosteroid therapy remains the mainstay of the treatment. However recently have been described few cases with a dramatic response to IL-1 blockage in patients resistant to standard treatments (129, 130).

Acrodermatitis continua of Hallopeau (ACH) is a rare, chronic disease characterized by acropustular eruptions predominantly involving the distal phalanges of the hands and feet with marked involvement of the nail bed. The sterile pustules may coalesce to form groups of lesions, which, over time, may spread proximally to involve the dorsal side of the hands, forearms, and feet. Onychodystrophy and even anonychia of the involved digits, atrophic skin changes, and osteolysis are often present causing painful and disabling lesions. In the last years cases responsive to either anti-TNF (131) and anti-IL-1 (Anakinra) treatments (132) have been described.

In conclusion, the recent advances in the identification of the molecular mechanisms leading to the severe inflammatory response observed in ultra-rare inherited autoinflammatory diseases allow to clarify that similar pathogenic mechanisms play also a crucial role in sustaining inflammation in a growing number of multi-factorial disorders. These findings led to a relevant re-thinking in the classification of the inflammatory diseases (133) and pointed out the pivotal role of IL-1 as therapeutic target in these conditions (134). The underlined importance of IL-1 in the pathogenesis of most of these conditions is reflected by the high number of clinical trials ongoing with IL-1 blockers (see text footnote 1).

REFERENCES

1. Masters SL, Simon A, Aksentijevich I, Kastner DL. Horror autoinflammaticus: the molecular pathophysiology of autoinflammatory disease (*). *Annu Rev Immunol* (2009) **27**:621–68. doi:10.1146/annurev.immunol.25.022106.141627
2. Martinon F, Burns K, Tschopp J. The inflammasome: a molecular platform triggering activation of inflammatory caspases and processing of proIL-beta. *Mol Cell* (2002) **10**(2):417–26. doi:10.1016/S1097-2765(02)00599-3
3. Hawkins PN, Lachmann HJ, McDermott MF. Interleukin-1-receptor antagonist in the Muckle-Wells syndrome. *N Engl J Med* (2003) **348**(25):2583–4. doi:10.1056/NEJM200306193482523
4. Hoffman HM, Rosengren S, Boyle DL, Cho JY, Nayar J, Mueller JL, et al. Prevention of cold-associated acute inflammation in familial cold autoinflammatory syndrome by interleukin-1 receptor antagonist. *Lancet* (2004) **364**(9447):1779–85. doi:10.1016/S0140-6736(04)17401-1
5. Lovell DJ, Bowyer SL, Solinger AM. Interleukin-1 blockade by anakinra improves clinical symptoms in patients with neonatal-onset multisystem inflammatory disease. *Arthritis Rheum* (2005) **52**(4):1283–6. doi:10.1002/art.20953
6. Kaplanski G, Farnarier C, Kaplanski S, Porat R, Shapiro L, Bongrand P, et al. Interleukin-1 induces interleukin-8 secretion from endothelial cells by a juxtacrine mechanism. *Blood* (1994) **84**(12):4242–8.
7. Kurt-Jones EA, Beller DI, Mizel SB, Unanue ER. Identification of a membrane-associated interleukin 1 in macrophages. *Proc Natl Acad Sci U S A* (1985) **82**(4):1204–8. doi:10.1073/pnas.82.4.1204
8. Dinarello CA. Anti-inflammatory agents: present and future. *Cell* (2010) **140**(6):935–50. doi:10.1016/j.cell.2010.02.043
9. Vojinovic J, Damjanov N, D'Urzo C, Furlan A, Susic G, Pasic S, et al. Safety and efficacy of an oral histone deacetylase inhibitor in systemic-onset juvenile idiopathic arthritis. *Arthritis Rheum* (2011) **63**(5):1452–8. doi:10.1002/art.30238
10. Martinon F, Tschopp J. Inflammatory caspases: linking an intracellular innate immune system to autoinflammatory diseases. *Cell* (2004) **117**(5):561–74. doi:10.1016/j.cell.2004.05.004
11. Goldbach-Mansky R, Dailey NJ, Canna SW, Gelabert A, Jones J, Rubin BI, et al. Neonatal-onset multisystem inflammatory disease responsive to interleukin-1beta inhibition. *N Engl J Med* (2006) **355**(6):581–92. doi:10.1056/NEJMoa055137
12. Gattorno M, Tassi S, Carta S, Delfino L, Ferlito F, Pelagatti MA, et al. Pattern of interleukin-1beta secretion in response to lipopolysaccharide and ATP before and after interleukin-1 blockade in patients with CIAS1 mutations. *Arthritis Rheum* (2007) **56**(9):3138–48. doi:10.1002/art.22842
13. Lepore L, Paloni G, Caorsi R, Alessio M, Rigante D, Ruperto N, et al. Follow-up and quality of life of patients with cryopyrin-associated periodic syndromes treated with anakinra. *J Pediatr* (2010) **157**(2):310–15.e1. doi:10.1016/j.jpeds.2010.02.040
14. Neven B, Marvillet I, Terrada C, Ferster A, Boddaert N, Couloignier V, et al. Long-term efficacy of the interleukin-1 receptor antagonist anakinra in ten patients with neonatal-onset multisystem inflammatory disease/chronic infantile neurologic, cutaneous, articular syndrome. *Arthritis Rheum* (2010) **62**(1):258–67. doi:10.1002/art.25057
15. Sibley CH, Plass N, Snow J, Wiggs EA, Brewer CC, King KA, et al. Sustained response and prevention of damage progression in patients with neonatal-onset multisystem inflammatory disease (NOMID) treated with anakinra. *Arthritis Rheum* (2012) **64**(7):2375–86. doi:10.1002/art.34409
16. Kuemmerle-Deschner JB, Tyrrell PN, Koetter I, Wittkowski H, Bialkowski A, Tzaribachev N, et al. Efficacy and safety of anakinra therapy in pediatric and adult patients with the autoinflammatory Muckle-Wells syndrome. *Arthritis Rheum* (2011) **63**(3):840–9. doi:10.1002/art.30149
17. Hoffman HM, Throne ML, Amar NJ, Sebai M, Kivitz AJ, Kavanaugh A, et al. Efficacy and safety of rilonacept (interleukin-1 Trap) in patients with cryopyrin-associated periodic syndromes: results from two sequential placebo-controlled studies. *Arthritis Rheum* (2008) **58**(8):2443–52. doi:10.1002/art.23687
18. Lachmann HJ, Kone-Paut I, Kuemmerle-Deschner JB, Leslie KS, Hachulla E, Quartier P, et al. Use of Canakinumab in the cryopyrin-associated periodic syndrome. *N Engl J Med* (2009) **360**(23):2416–25. doi:10.1056/NEJMoa0810787
19. Kuemmerle-Deschner JB, Hachulla E, Cartwright R, Hawkins PN, Tran TA, Bader-Meunier B, et al. Two-year results from an open-label, multicentre, phase III study evaluating the safety and efficacy of Canakinumab in patients with cryopyrin-associated periodic syndrome across different severity phenotypes. *Ann Rheum Dis* (2011) **70**(12):2095–102. doi:10.1136/ard.2011.152728
20. Caorsi R, Lepore L, Zulian F, Alessio M, Stabile A, Insalaco A, et al. The schedule of administration of Canakinumab in cryopyrin associated periodic syndrome is driven by the phenotype severity rather than the age. *Arthritis Res Ther* (2013) **15**(1):R33. doi:10.1186/ar4184
21. French FMF Consortium. A candidate gene for familial Mediterranean fever. *Nat Genet* (1997) **17**(1):25–31. doi:10.1038/ng0997-25
22. The International FMF Consortium. Ancient missense mutations in a new member of the RoRet gene family are likely to cause familial Mediterranean fever. *Cell* (1997) **90**(4):797–807. doi:10.1016/S0092-8674(00)80539-5
23. Goldfinger SE. Colchicine for familial Mediterranean fever. *N Engl J Med* (1972) **287**(25):1302. doi:10.1056/NEJM197212212872514
24. Kallinich T, Haffner D, Niehues T, Huss K, Lainka E, Neudorf U, et al. Colchicine use in children and adolescents with familial Mediterranean fever: literature review and consensus statement. *Pediatrics* (2007) **119**(2):e474–83. doi:10.1542/peds.2006-1434

25. Ter Haar N, Lachmann H, Özen S, Woo P, Uziel Y, Modesto C, et al. Treatment of autoinflammatory diseases: results from the Eurofever registry and a literature review. *Ann Rheum Dis* (2012) **72**(5):678–85. doi:10.1136/annrheumdis-2011-201268
26. Akar S, Yuksel F, Tunca M, Soysal O, Solmaz D, Gerdan V, et al. Familial Mediterranean fever: risk factors, causes of death, and prognosis in the colchicine era. *Medicine* (2012) **91**(3):131–6. doi:10.1097/MD.0b013e3182561a45
27. Chae JJ, Wood G, Masters SL, Richard K, Park G, Smith BJ, et al. The B30.2 domain of pyrin, the familial Mediterranean fever protein, interacts directly with caspase-1 to modulate IL-1beta production. *Proc Natl Acad Sci U S A* (2006) **103**(26):9982–7. doi:10.1073/pnas.0602081103
28. Papin S, Cuenin S, Agostini L, Martinon F, Werner S, Beer HD, et al. The SPRY domain of pyrin, mutated in familial Mediterranean fever patients, interacts with inflammasome components and inhibits proIL-1beta processing. *Cell Death Differ* (2007) **14**(8):1457–66. doi:10.1038/sj.cdd.4402142
29. Chae JJ, Cho YH, Lee GS, Cheng J, Liu PP, Feigenbaum L, et al. Gain-of-function pyrin mutations induce NLRP3 protein-independent interleukin-1beta activation and severe autoinflammation in mice. *Immunity* (2011) **34**(5):755–68. doi:10.1016/j.immuni.2011.02.020
30. Gavrilin MA, Abdelaziz DH, Mostafa M, Abdulrahman BA, Grandhi J, Akhter A, et al. Activation of the pyrin inflammasome by intracellular *Burkholderia cenocepacia*. *J Immunol* (2012) **188**(7):3469–77. doi:10.4049/jimmunol.1102272
31. Yu JW, Fernandes-Alnemri T, Datta P, Wu J, Juliana C, Solorzano L, et al. Pyrin activates the ASC pyroptosome in response to engagement by autoinflammatory PSTPIP1 mutants. *Mol Cell* (2007) **28**(2):214–27. doi:10.1016/j.molcel.2007.08.029
32. Omenetti A, Carta S, Delfino L, Martini A, Gattorno M, Rubartelli A. Increased NLRP3-dependent interleukin 1β secretion in patients with familial Mediterranean fever: correlation with MEFV genotype. *Ann Rheum Dis* (2013). doi:10.1136/annrheumdis-2012-202774. [Epub ahead of print].
33. Federici S, Calcagno G, Finetti M, Gallizzi R, Meini A, Vitale A, et al. Clinical impact of MEFV mutations in children with periodic fever in a prevalent western European Caucasian population. *Ann Rheum Dis* (2012) **71**(12):1961–5. doi:10.1136/annrheumdis-2011-200977
34. Fernandes-Alnemri T, Wu J, Yu JW, Datta P, Miller B, Jankowski W, et al. The pyroptosome: a supramolecular assembly of ASC dimers mediating inflammatory cell death via caspase-1 activation. *Cell Death Differ* (2007) **14**(9):1590–604. doi:10.1038/sj.cdd.4402194
35. Alpay N, Sumnu A, Caliskan Y, Yazici H, Turkmen A, Gul A. Efficacy of anakinra treatment in a patient with colchicine-resistant familial Mediterranean fever. *Rheumatol Int* (2010) **32**(10):3277–9. doi:10.1007/s00296-010-1474-6
36. Belkhir R, Moulonguet-Doleris L, Hachulla E, Prinseau J, Baglin A, Hanslik T. Treatment of familial Mediterranean fever with anakinra. *Ann Intern Med* (2007) **146**(11):825–6. doi:10.7326/0003-4819-146-11-200706050-00023
37. Calligaris L, Marchetti F, Tommasini A, Ventura A. The efficacy of anakinra in an adolescent with colchicine-resistant familial Mediterranean fever. *Eur J Pediatr* (2008) **167**(6):695–6. doi:10.1007/s00431-007-0547-3
38. Gattringer R, Lagler H, Gattringer KB, Knapp S, Burgmann H, Winkler S, et al. Anakinra in two adolescent female patients suffering from colchicine-resistant familial Mediterranean fever: effective but risky. *Eur J Clin Invest* (2007) **37**(11):912–4. doi:10.1111/j.1365-2362.2007.01868.x
39. Kuijk LM, Govers AM, Frenkel J, Hofhuis WJ. Effective treatment of a colchicine-resistant familial Mediterranean fever patient with anakinra. *Ann Rheum Dis* (2007) **66**(11):1545–6. doi:10.1136/ard.2007.071498
40. Meinzer U, Quartier P, Alexandra JF, Hentgen V, Retornaz F, Kone-Paut I. Interleukin-1 targeting drugs in familial Mediterranean fever: a case series and a review of the literature. *Semin Arthritis Rheum* (2011) **41**(2):265–71. doi:10.1016/j.semarthrit.2010.11.003
41. Ozen S, Bilginer Y, Aktay AN, Calguneri M. Anti-interleukin 1 treatment for patients with familial Mediterranean fever resistant to colchicine. *J Rheumatol* (2011) **38**(3):516–8. doi:10.3899/jrheum.100718
42. Petropoulou AD, Robin M, Socie G, Galicier L. Transmission of familial Mediterranean fever mutation after bone marrow transplantation and successful treatment with anakinra. *Transplantation* (2010) **90**(1):102–3. doi:10.1097/TP.0b013e3181d84cc3
43. Roldan R, Ruiz AM, Miranda MD, Collantes E. Anakinra: new therapeutic approach in children with familial Mediterranean fever resistant to colchicine. *Joint Bone Spine* (2008) **75**(4):504–5. doi:10.1016/j.jbspin.2008.04.001
44. Stankovic SK, Delmas Y, Urena TP, Peltier J, Pelle G, Jeru I, et al. Dramatic beneficial effect of interleukin-1 inhibitor treatment in patients with familial Mediterranean fever complicated with amyloidosis and renal failure. *Nephrol Dial Transplant* (2012) **27**(5):1898–901. doi:10.1093/ndt/gfr528
45. Hashkes PJ, Spalding SJ, Giannini EH, Huang B, Johnson A, Park G, et al. Rilonacept for colchicine-resistant or -intolerant familial Mediterranean fever: a randomized trial. *Ann Intern Med* (2012) **157**(8):533–41. doi:10.7326/0003-4819-157-8-201210160-00003
46. McDermott MF, Aksentijevich I, Galon J, McDermott EM, Ogunkolade BW, Centola M, et al. Germline mutations in the extracellular domains of the 55 kDa TNF receptor, TNFR1, define a family of dominantly inherited autoinflammatory syndromes. *Cell* (1999) **97**(1):133–44. doi:10.1016/S0092-8674(00)80721-7
47. Simon A, Park H, Maddipati R, Lobito AA, Bulua AC, Jackson AJ, et al. Concerted action of wild-type and mutant TNF receptors enhances inflammation in TNF receptor 1-associated periodic fever syndrome. *Proc Natl Acad Sci U S A* (2010) **107**(21):9801–6. doi:10.1073/pnas.0914118107
48. Bulua AC, Simon A, Maddipati R, Pelletier M, Park H, Kim KY, et al. Mitochondrial reactive oxygen species promote production of proinflammatory cytokines and are elevated in TNFR1-associated periodic syndrome (TRAPS). *J Exp Med* (2011) **208**(3):519–33. doi:10.1084/jem.20102049
49. Bachetti T, Chiesa S, Castagnola P, Bani D, Di Zanni E, Omenetti A, et al. Autophagy contributes to inflammation in patients with TNFR-associated periodic syndrome (TRAPS). *Ann Rheum Dis* (2012) **72**(6):1044–52. doi:10.1136/annrheumdis-2012-201952
50. Hull KM, Drewe E, Aksentijevich I, Singh HK, Wong K, McDermott EM, et al. The TNF receptor-associated periodic syndrome (TRAPS): emerging concepts of an autoinflammatory disorder. *Medicine (Baltimore)* (2002) **81**(5):349–68. doi:10.1097/00005792-200209000-00002
51. Bulua AC, Mogul DB, Aksentijevich I, Singh H, He DY, Muenz LR, et al. Efficacy of etanercept in the tumor necrosis factor receptor-associated periodic syndrome (TRAPS). *Arthritis Rheum* (2012) **64**(3):908–13. doi:10.1002/art.33416
52. Drewe E, Powell RJ, McDermott EM. Comment on: failure of anti-TNF therapy in TNF receptor 1-associated periodic syndrome (TRAPS). *Rheumatology (Oxford)* (2007) **46**(12):1865–6. doi:10.1093/rheumatology/kem231
53. Sacré K, Brihaye B, Lidove O, Papo T, Pocidalo MA, Cuisset L, et al. Dramatic improvement following interleukin 1beta blockade in tumor necrosis factor receptor-1-associated syndrome (TRAPS) resistant to anti-TNF-alpha therapy. *J Rheumatol* (2008) **35**(2):357–8.
54. Siebert S, Amos N, Lawson TM. Comment on: failure of anti-TNF therapy in TNF receptor 1-associated periodic syndrome (TRAPS). *Rheumatology (Oxford)* (2008) **47**(2):228–9. doi:10.1093/rheumatology/kem243
55. Simon A, Bodar EJ, van der Hilst JC, van der Meer JW, Fiselier TJ, Cuppen MP, et al. Beneficial response to interleukin 1 receptor antagonist in traps. *Am J Med* (2004) **117**(3):208–10. doi:10.1016/j.amjmed.2004.02.039
56. Gattorno M, Pelagatti MA, Meini A, Obici L, Barcellona R, Federici S, et al. Persistent efficacy of anakinra in patients with tumor necrosis factor receptor-associated periodic syndrome. *Arthritis Rheum* (2008) **58**(5):1516–20. doi:10.1002/art.23475
57. Lachmann HJ, Papa R, Gerhold K, Obici L, Touitou I, Cantarini L, et al. The phenotype of TNF receptor-associated autoinflammatory syndrome (TRAPS) at presentation: a series of 158 cases from the Eurofever/EUROTRAPS international registry. *Ann*

Rheum Dis (2013). doi:10.1136/annrheumdis-2013-204184
58. Brizi MG, Galeazzi M, Lucherini OM, Cantarini L, Cimaz R. Successful treatment of tumor necrosis factor receptor-associated periodic syndrome with Canakinumab. *Ann Intern Med* (2012) **156**(12):907–8. doi:10.7326/0003-4819-156-12-201206190-00027
59. Gattorno M, Obici L, Meini A, Tormey V, Abrams K, Davis N, et al. Efficacy and safety of Canakinumab in patients with TNF receptor associated periodic syndrome. *Arthritis Rheum* (2012) **64**(10):S322.
60. Drenth JP, Cuisset L, Grateau G, Vasseur C, van de Velde-Visser SD, de Jong JG, et al. Mutations in the gene encoding mevalonate kinase cause hyper-IgD and periodic fever syndrome. International Hyper-IgD Study Group. *Nat Genet* (1999) **22**(2):178–81. doi:10.1038/9696
61. Houten SM, Kuis W, Duran M, de Koning TJ, van Royen-Kerkhof A, Romeijn GJ, et al. Mutations in MVK, encoding mevalonate kinase, cause hyperimmunoglobulinaemia D and periodic fever syndrome. *Nat Genet* (1999) **22**(2):175–7. doi:10.1038/9691
62. Houten SM, Frenkel J, Waterham HR. Isoprenoid biosynthesis in hereditary periodic fever syndromes and inflammation. *Cell Mol Life Sci* (2003) **60**(6):1118–34.
63. van der Hilst JC, Bodar EJ, Barron KS, Frenkel J, Drenth JP, van der Meer JW, et al. Long-term follow-up, clinical features, and quality of life in a series of 103 patients with hyperimmunoglobulinemia D syndrome. *Medicine (Baltimore)* (2008) **87**(6):301–10. doi:10.1097/MD.0b013e318190cfb7
64. Obici L, Manno C, Muda AO, Picco P, D'Osualdo A, Palladini G, et al. First report of systemic reactive (AA) amyloidosis in a patient with the hyperimmunoglobulinemia D with periodic fever syndrome. *Arthritis Rheum* (2004) **50**(9):2966–9. doi:10.1002/art.20490
65. Lachmann HJ, Goodman HJ, Andrews PA, Gallagher H, Marsh J, Breuer S, et al. AA amyloidosis complicating hyperimmunoglobulinemia D with periodic fever syndrome: a report of two cases. *Arthritis Rheum* (2006) **54**(6):2010–4. doi:10.1002/art.21901
66. Demirkaya E, Caglar MK, Waterham HR, Topaloglu R, Ozen S. A patient with hyper-IgD syndrome responding to anti-TNF treatment. *Clin Rheumatol* (2006) **26**(10):1757–9. doi:10.1007/s10067-006-0501-1
67. Takada K, Aksentijevich I, Mahadevan V, Dean JA, Kelley RI, Kastner DL. Favorable preliminary experience with etanercept in two patients with the hyperimmunoglobulinemia D and periodic fever syndrome. *Arthritis Rheum* (2003) **48**(9):2645–51. doi:10.1002/art.11218
68. Breda L, Nozzi M, Di Marzio D, De Sanctis S, Gattorno M, Chiarelli F. Recurrent pericarditis in hyper-IgD syndrome. *Clin Exp Rheumatol* (2009) **27**(4):695.
69. Marchetti F, Barbi E, Tommasini A, Oretti C, Ventura A. Ineffi cacy of etanercept in a child with hyper-IgD syndrome and periodic fever. *Clin Exp Rheumatol* (2004) **22**(6):791–2.
70. Cailliez M, Garaix F, Rousset-Rouviere C, Bruno D, Kone-Paut I, Sarles J, et al. Anakinra is safe and effective in controlling hyperimmunoglobulinaemia D syndrome-associated febrile crisis. *J Inherit Metab Dis* (2006) **29**(6):763. doi:10.1007/s10545-006-0408-7
71. Rigante D, Ansuini V, Bertoni B, Pugliese AL, Avallone L, Federico G, et al. Treatment with anakinra in the hyperimmunoglobulinemia D/periodic fever syndrome. *Rheumatol Int* (2006) **27**(1):97–100. doi:10.1007/s00296-006-0164-x
72. Lequerré T, Vittecoq O, Pouplin S, Klemmer N, Mejjad O, Daragon A, et al. Mevalonate kinase deficiency syndrome with structural damage responsive to anakinra. *Rheumatology (Oxford)* (2007) **46**(12):1860–2. doi:10.1093/rheumatology/kem258
73. Galeotti C, Meinzer U, Quartier P, Rossi-Semerano L, Bader-Meunier B, Pillet P, et al. Efficacy of interleukin-1-targeting drugs in mevalonate kinase deficiency. *Rheumatology (Oxford)* (2012) **51**(10):1855–9. doi:10.1093/rheumatology/kes097
74. Frenkel J, Rijkers GT, Mandey SH, Buurman SW, Houten SM, Wanders RJ, et al. Lack of isoprenoid products raises ex vivo interleukin-1beta secretion in hyperimmunoglobulinemia D and periodic fever syndrome. *Arthritis Rheum* (2002) **46**(10):2794–803. doi:10.1002/art.10550
75. Aróstegui JI, Arnal C, Merino R, Modesto C, Antonia Carballo M, Moreno P, et al. NOD2 gene-associated pediatric granulomatous arthritis: clinical diversity, novel and recurrent mutations, and evidence of clinical improvement with interleukin-1 blockade in a Spanish cohort. *Arthritis Rheum* (2007) **56**(11):3805–13. doi:10.1002/art.22966
76. Rose CD, Wouters CH, Meiorin S, Doyle TM, Davey MP, Rosenbaum JT, et al. Pediatric granulomatous arthritis: an international registry. *Arthritis Rheum* (2006) **54**(10):3337–44. doi:10.1002/art.22122
77. Becker ML, Martin TM, Doyle TM, Rose CD. Interstitial pneumonitis in Blau syndrome with documented mutation in CARD15. *Arthritis Rheum* (2007) **56**(4):1292–4. doi:10.1002/art.22509
78. Milman N, Andersen CB, Hansen A, van Overeem HT, Nielsen FC, Fledelius H, et al. Favourable effect of TNF-alpha inhibitor (infliximab) on Blau syndrome in monozygotic twins with a de novo CARD15 mutation. *APMIS* (2006) **114**(12):912–9. doi:10.1111/j.1600-0463.2006.apm_522.x
79. Martin TM, Zhang Z, Kurz P, Rose CD, Chen H, Lu H, et al. The NOD2 defect in Blau syndrome does not result in excess interleukin-1 activity. *Arthritis Rheum* (2009) **60**(2):611–8. doi:10.1002/art.24222
80. Simonini G, Xu Z, Caputo R, De Libero C, Pagnini I, Pascual V, et al. Clinical and transcriptional response to the long-acting interleukin-1 blocker Canakinumab in Blau syndrome-related uveitis. *Arthritis Rheum* (2013) **65**(2):513–8. doi:10.1002/art.37776
81. Lindor NM, Arsenault TM, Solomon H, Seidman CE, McEvoy MT. A new autosomal dominant disorder of pyogenic sterile arthritis, pyoderma gangrenosum, and acne: PAPA syndrome. *Mayo Clin Proc* (1997) **72**(7):611–5. doi:10.1016/S0025-6196(11)63565-9
82. Wise CA, Gillum JD, Seidman CE, Lindor NM, Veile R, Bashiardes S, et al. Mutations in CD2BP1 disrupt binding to PTP PEST and are responsible for PAPA syndrome, an autoinflammatory disorder. *Hum Mol Genet* (2002) **11**(8):961–9. doi:10.1093/hmg/11.8.961
83. Cortis E, De Benedetti F, Insalaco A, Cioschi S, Muratori F, D'Urbano LE, et al. Abnormal production of tumor necrosis factor (TNF) – alpha and clinical efficacy of the TNF inhibitor etanercept in a patient with PAPA syndrome [corrected]. *J Pediatr* (2004) **145**(6):851–5. doi:10.1016/j.jpeds.2004.08.001
84. Tofteland ND, Shaver TS. Clinical efficacy of etanercept for treatment of PAPA syndrome. *J Clin Rheumatol* (2010) **16**(5):244–5. doi:10.1097/RHU.0b013e3181e969b9
85. Shoham NG, Centola M, Mansfield E, Hull KM, Wood G, Wise CA, et al. Pyrin binds the PSTPIP1/CD2BP1 protein, defining familial Mediterranean fever and PAPA syndrome as disorders in the same pathway. *Proc Natl Acad Sci U S A* (2003) **100**(23):13501–6. doi:10.1073/pnas.2135380100
86. Brenner M, Ruzicka T, Plewig G, Thomas P, Herzer P. Targeted treatment of pyoderma gangrenosum in PAPA (pyogenic arthritis, pyoderma gangrenosum and acne) syndrome with the recombinant human interleukin-1 receptor antagonist anakinra. *Br J Dermatol* (2009) **161**(5):1199–201. doi:10.1111/j.1365-2133.2009.09404.x
87. Demidowich AP, Freeman AF, Kuhns DB, Aksentijevich I, Gallin JI, Turner ML, et al. Brief report: genotype, phenotype, and clinical course in five patients with PAPA syndrome (pyogenic sterile arthritis, pyoderma gangrenosum, and acne). *Arthritis Rheum* (2012) **64**(6):2022–7. doi:10.1002/art.34332
88. Dierselhuis MP, Frenkel J, Wulffraat NM, Boelens JJ. Anakinra for flares of pyogenic arthritis in PAPA syndrome. *Rheumatology (Oxford)* (2005) **44**(3):406–8. doi:10.1093/rheumatology/keh479
89. Geusau A, Mothes-Luksch N, Nahavandi H, Pickl WF, Wise CA, Pourpak Z, et al. Identification of a homozygous PSTPIP1 mutation in a patient with a PAPA-like syndrome responding to Canakinumab treatment. *JAMA Dermatol* (2013) **149**(2):209–15. doi:10.1001/2013.jamadermatol.717
90. Aksentijevich I, Masters SL, Ferguson PJ, Dancey P, Frenkel J, van Royen-Kerkhoff A, et al. An autoinflammatory disease with deficiency of the interleukin-1-receptor antagonist. *N Engl J Med* (2009) **360**(23):2426–37. doi:10.1056/NEJMoa0807865

91. Reddy S, Jia S, Geoffrey R, Lorier R, Suchi M, Broeckel U, et al. An autoinflammatory disease due to homozygous deletion of the IL1RN locus. *N Engl J Med* (2009) **360**(23):2438–44. doi:10.1056/NEJMoa0809568
92. Majeed HA, Kalaawi M, Mohanty D, Teebi AS, Tunjekar MF, al-Gharbawy F, et al. Congenital dyserythropoietic anemia and chronic recurrent multifocal osteomyelitis in three related children and the association with Sweet syndrome in two siblings. *J Pediatr* (1989) **115**(5 Pt 1):730–4. doi:10.1016/S0022-3476(89)80650-X
93. Ferguson PJ, Chen S, Tayeh MK, Ochoa L, Leal SM, Pelet A, et al. Homozygous mutations in LPIN2 are responsible for the syndrome of chronic recurrent multifocal osteomyelitis and congenital dyserythropoietic anaemia (Majeed syndrome). *J Med Genet* (2005) **42**(7):551–7. doi:10.1136/jmg.2005.030759
94. Herlin T, Fiirgaard B, Bjerre M, Kerndrup G, Hasle H, Bing X, et al. Efficacy of anti-IL-1 treatment in Majeed syndrome. *Ann Rheum Dis* (2013) **72**(3):410–3. doi:10.1136/annrheumdis-2012-201818
95. Cassel SL, Eisenbarth SC, Iyer SS, Sadler JJ, Colegio OR, Tephly LA, et al. The Nalp3 inflammasome is essential for the development of silicosis. *Proc Natl Acad Sci U S A* (2008) **105**(26):9035–40. doi:10.1073/pnas.0803933105
96. Martinon F, Petrilli V, Mayor A, Tardivel A, Tschopp J. Gout-associated uric acid crystals activate the NALP3 inflammasome. *Nature* (2006) **440**(7081):237–41. doi:10.1038/nature04516
97. Masters SL, O'Neill LA. Disease-associated amyloid and misfolded protein aggregates activate the inflammasome. *Trends Mol Med* (2011) **17**(5):276–82. doi:10.1016/j.molmed.2011.01.005
98. Gattorno M, Piccini A, Lasiglié D, Tassi S, Brisca G, Carta S, et al. The pattern of response to anti-interleukin-1 treatment distinguishes two subsets of patients with systemic-onset juvenile idiopathic arthritis. *Arthritis Rheum* (2008) **58**(5):1505–15. doi:10.1002/art.23437
99. Quartier P, Taupin P, Bourdeaut F, Lemelle I, Pillet P, Bost M, et al. Efficacy of etanercept for the treatment of juvenile idiopathic arthritis according to the onset type. *Arthritis Rheum* (2003) **48**(4):1093–101. doi:10.1002/art.10885
100. de Benedetti BF, Martini A. Targeting the interleukin-6 receptor: a new treatment for systemic juvenile idiopathic arthritis? *Arthritis Rheum* (2005) **52**(3):687–93. doi:10.1002/art.20946
101. De Benedetti F, Brunner HI, Ruperto N, Kenwright A, Wright S, Calvo I, et al. Randomized trial of tocilizumab in systemic juvenile idiopathic arthritis. *N Engl J Med* (2012) **367**(25):2385–95. doi:10.1056/NEJMoa1112802
102. Allantaz F, Chaussabel D, Stichweh D, Bennett L, Allman W, Mejias A, et al. Blood leukocyte microarrays to diagnose systemic onset juvenile idiopathic arthritis and follow the response to IL-1 blockade. *J Exp Med* (2007) **204**(9):2131–44. doi:10.1084/jem.20070070
103. Pascual V, Allantaz F, Arce E, Punaro M, Banchereau J. Role of interleukin-1 (IL-1) in the pathogenesis of systemic onset juvenile idiopathic arthritis and clinical response to IL-1 blockade. *J Exp Med* (2005) **201**(9):1479–86. doi:10.1084/jem.20050473
104. Nigrovic PA, Mannion M, Prince FH, Zeft A, Rabinovich CE, van Rossum MA, et al. Anakinra as first-line disease-modifying therapy in systemic juvenile idiopathic arthritis: report of forty-six patients from an international multicenter series. *Arthritis Rheum* (2011) **63**(2):545–55. doi:10.1002/art.30128
105. Quartier P, Allantaz F, Cimaz R, Pillet P, Messiaen C, Bardin C, et al. A multicentre, randomised, double-blind, placebo-controlled trial with the interleukin-1 receptor antagonist anakinra in patients with systemic-onset juvenile idiopathic arthritis (ANAJIS trial). *Ann Rheum Dis* (2011) **70**(5):747–54. doi:10.1136/ard.2010.134254
106. Fitzgerald AA, Leclercq SA, Yan A, Homik JE, Dinarello CA. Rapid responses to anakinra in patients with refractory adult-onset Still's disease. *Arthritis Rheum* (2005) **52**(6):1794–803. doi:10.1002/art.21061
107. Giampietro C, Ridene M, Lequerre T, Costedoat CN, Amoura Z, Sellam J, et al. Anakinra in adult-onset Still's disease: long-term treatment in patients resistant to conventional therapy. *Arthritis Care Res (Hoboken)* (2013) **65**(5):822–6. doi:10.1002/acr.21901
108. Ruperto N, Quartier P, Wulffraat N, Woo P, Ravelli A, Mouy R, et al. A phase II, multicenter, open-label study evaluating dosing and preliminary safety and efficacy of Canakinumab in systemic juvenile idiopathic arthritis with active systemic features. *Arthritis Rheum* (2012) **64**(2):557–67. doi:10.1002/art.33342
109. Kolly L, Busso N, von Scheven-Gete A, Bagnoud N, Moix I, Holzinger D, et al. Periodic fever, aphthous stomatitis, pharyngitis, cervical adenitis syndrome is linked to dysregulated monocyte IL-1beta production. *J Allergy Clin Immunol* (2013) **131**(6):1635–43. doi:10.1016/j.jaci.2012.07.043
110. Stojanov S, Hoffmann F, Kéry A, Renner ED, Hartl D, Lohse P, et al. Cytokine profile in PFAPA syndrome suggests continuous inflammation and reduced anti-inflammatory response. *Eur Cytokine Netw* (2006) **17**(2):90–7.
111. Picco P, Brisca G, Traverso F, Loy A, Gattorno M, Martini A. Successful treatment of idiopathic recurrent pericarditis in children with interleukin-1beta receptor antagonist (anakinra): an unrecognized autoinflammatory disease? *Arthritis Rheum* (2009) **60**(1):264–8. doi:10.1002/art.24174
112. Camacho-Lovillo M, Mendez-Santos A. Successful treatment of idiopathic recurrent pericarditis with interleukin-1 receptor antagonist (anakinra). *Pediatr Cardiol* (2013) **34**(5):1293–4. doi:10.1007/s00246-013-0663-y
113. Scardapane A, Brucato A, Chiarelli F, Breda L. Efficacy of an interleukin-1β receptor antagonist (anakinra) in idiopathic recurrent pericarditis. *Pediatr Cardiol* (2012). doi:10.1007/s00246-012-0532-0. [Epub ahead of print].
114. Vassilopoulos D, Lazaros G, Tsioufis C, Vasileiou P, Stefanadis C, Pectasides D. Successful treatment of adult patients with idiopathic recurrent pericarditis with an interleukin-1 receptor antagonist (anakinra). *Int J Cardiol* (2012) **160**(1):66–8. doi:10.1016/j.ijcard.2012.05.086
115. de Koning HD, Bodar EJ, van der Meer JW, Simon A. Schnitzler syndrome: beyond the case reports: review and follow-up of 94 patients with an emphasis on prognosis and treatment. *Semin Arthritis Rheum* (2007) **37**(3):137–48. doi:10.1016/j.semarthrit.2007.04.001
116. Lipsker D, Veran Y, Grunenberger F, Cribier B, Heid E, Grosshans E. The Schnitzler syndrome. Four new cases and review of the literature. *Medicine (Baltimore)* (2001) **80**(1):37–44. doi:10.1097/00005792-200101000-00004
117. Besada E, Nossent H. Dramatic response to IL1-RA treatment in longstanding multidrug resistant Schnitzler's syndrome: a case report and literature review. *Clin Rheumatol* (2010) **29**(5):567–71. doi:10.1007/s10067-010-1375-9
118. de Koning HD, Bodar EJ, Simon A, van der Hilst JC, Netea MG, van der Meer JW. Beneficial response to anakinra and thalidomide in Schnitzler's syndrome. *Ann Rheum Dis* (2006) **65**(4):542–4. doi:10.1136/ard.2005.045245
119. de Koning HD, Schalkwijk J, van der Ven-Jongekrijg J, Stoffels M, van der Meer JW, Simon A. Sustained efficacy of the monoclonal anti-interleukin-1 beta antibody Canakinumab in a 9-month trial in Schnitzler's syndrome. *Ann Rheum Dis* (2012) **145**(6):851–5. doi:10.1136/annrheumdis-2012-202192
120. McGonagle D, Tan AL, Shankaranarayana S, Madden J, Emery P, McDermott MF. Management of treatment resistant inflammation of acute on chronic tophaceous gout with anakinra. *Ann Rheum Dis* (2007) **66**(12):1683–4. doi:10.1136/ard.2007.073759
121. Molto A, Ea HK, Richette P, Bardin T, Liote F. Efficacy of anakinra for refractory acute calcium pyrophosphate crystal arthritis. *Joint Bone Spine* (2012) **79**(6):621–3. doi:10.1016/j.jbspin.2012.01.010
122. Ghosh P, Cho M, Rawat G, Simkin PA, Gardner GC. The treatment of acute gouty arthritis in complex hospitalized patients with anakinra. *Arthritis Care Res (Hoboken)* (2013) **65**(8):1381–4. doi:10.1002/acr.21989
123. Schlesinger N, Alten RE, Bardin T, Schumacher HR, Bloch M, Gimona A, et al. Canakinumab for acute gouty arthritis in patients with limited treatment options: results from two randomised, multicentre, active-controlled, double-blind trials and their initial extensions. *Ann Rheum Dis* (2012) **71**(11):1839–48. doi:10.1136/annrheumdis-2011-200908
124. Eleftheriou D, Gerschman T, Sebire N, Woo P, Pilkington CA, Brogan PA. Biologic therapy in refractory chronic non-bacterial osteomyelitis of childhood. *Rheumatology (Oxford)* (2010)

49(8):1505–12. doi:10.1093/rheumatology/keq122
125. Nguyen MT, Borchers A, Selmi C, Naguwa SM, Cheema G, Gershwin ME. The SAPHO syndrome. *Semin Arthritis Rheum* (2012) **42**(3):254–65. doi:10.1016/j.semarthrit.2012.05.006
126. Colina M, Pizzirani C, Khodeir M, Falzoni S, Bruschi M, Trotta F, et al. Dysregulation of P2X7 receptor-inflammasome axis in SAPHO syndrome: successful treatment with anakinra. *Rheumatology (Oxford)* (2010) **49**(7):1416–8. doi:10.1093/rheumatology/keq074
127. Wendling D, Prati C, Aubin F. Anakinra treatment of SAPHO syndrome: short-term results of an open study. *Ann Rheum Dis* (2012) **71**(6):1098–100. doi:10.1136/annrheumdis-2011-200743
128. Viguier M, Guigue P, Pages C, Smahi A, Bachelez H. Successful treatment of generalized pustular psoriasis with the interleukin-1-receptor antagonist anakinra: lack of correlation with IL1RN mutations. *Ann Intern Med* (2010) **153**(1):66–7. doi:10.7326/0003-4819-153-1-201007060-00030
129. Delluc A, Limal N, Puechal X, Frances C, Piette JC, Cacoub P. Efficacy of anakinra, an IL1 receptor antagonist, in refractory Sweet syndrome. *Ann Rheum Dis* (2008) **67**(2):278–9. doi:10.1136/ard.2006.068254
130. Kluger N, Gil-Bistes D, Guillot B, Bessis D. Efficacy of anti-interleukin-1 receptor antagonist anakinra (Kineret(R)) in a case of refractory Sweet's syndrome. *Dermatology* (2011) **222**(2):123–7. doi:10.1159/000326112
131. Dini V, Barbanera S, Romanelli M. Efficacy of adalimumab for the treatment of refractory paediatric acrodermatitis continua of hallopeau. *Acta Derm Venereol* (2013) **93**(5):588–9. doi:10.2340/00015555-1540
132. Lutz V, Lipsker D. Acitretin- and tumor necrosis factor inhibitor-resistant acrodermatitis continua of hallopeau responsive to the interleukin 1 receptor antagonist anakinra. *Arch Dermatol* (2012) **148**(3):297–9. doi:10.1001/archdermatol.2011.2473
133. McGonagle D, McDermott MF. A proposed classification of the immunological diseases. *PLoS Med* (2006) **3**(8):e297. doi:10.1371/journal.pmed.0030297
134. Dinarello CA. Interleukin-1 in the pathogenesis and treatment of inflammatory diseases. *Blood* (2011) **117**(14):3720–32. doi:10.1182/blood-2010-07-273417

Conflict of Interest Statement: Marco Gattorno and Alberto Martini has received honoraria for meeting presentations from Novartis and SOBI. The Gaslini hospital to which Alberto Martini and Marco Gattorno work as full-time employees have received contributions to support PRINTO and Eurofever research activities from Bristol-Myers Squibb, Abbott, Novartis, Roche, Centocor, ACRAF, Pfizer, and Xoma.

Received: 14 June 2013; accepted: 14 October 2013; published online: 31 October 2013.
*Citation: Federici S, Martini A and Gattorno M (2013) The central role of anti-IL-1 blockade in the treatment of monogenic and multi-factorial autoinflammatory diseases. Front. Immunol. **4**:351. doi: 10.3389/fimmu.2013.00351*
This article was submitted to Inflammation, a section of the journal Frontiers in Immunology.

frontiers in
IMMUNOLOGY

REVIEW ARTICLE
published: 15 July 2013
doi: 10.3389/fimmu.2013.00182

IL-1 and T helper immune responses

Veronica Santarlasci, Lorenzo Cosmi, Laura Maggi, Francesco Liotta and Francesco Annunziato*

Department of Experimental and Clinical Medicine, University of Florence, Florence, Italy

Edited by:
Alberto Mantovani, University of Milan, Italy

Reviewed by:
Massimo Gadina, National Institutes of Health, USA
Hideki Ogura, Osaka University, Japan

****Correspondence:***
Francesco Annunziato, Department of Experimental and Clinical Medicine, University of Florence, V.le Pieraccini 6, Florence 50134, Italy
e-mail: f.annunziato@dmi.unifi.it, francesco.annunziato@unifi.it

CD4 T cells play a critical role in mediating adaptive immunity to a variety of pathogens as well as in tumor immunity. If not adequately regulated, CD4 T cells can be also involved in autoimmunity, asthma, and allergic responses. During TCR activation in a particular cytokine milieu, naïve CD4 T cells may differentiate into one of several lineages of T helper (Th) cells, including Th1, Th2, and Th17, as defined by their pattern of cytokine production and function. IL-1, the prototypic proinflammatory cytokine, has been shown to influence growth and differentiation of immunocompetent lymphocytes. The differential expression of IL-1RI on human CD4 T cell subsets confers distinct capacities to acquire specific effector functions. In this review, we summarize the role of IL-1 on CD4 T cells, in terms of differentiation, activation, and maintenance or survival.

Keywords: Th17 cells, Th2 cells, Th1 non-classic, IL-1RI and T cells, IL-1 and T cells

T HELPER CELL SUBSETS

CD4+ T helper (Th) lymphocytes represent a heterogeneous population of cells that play an essential role in adaptive immunity. These cells include effector cells, which are devoted to protection against pathogens, and regulatory T cells (T_{regs}), which protect against effector responses to autoantigens, and also to exogenous antigens when they become dangerous for the host. The term Th derived from the observation that these cells were critical for helping B cells to produce antibodies in the primary response (humoral immunity). CD4+ T cells were also found to be responsible for the so called cell-mediated immunity, or delayed-type hypersensitivity, which is characterized by reaction involving activation of macrophages. The distinct protective function of different effector CD4+ T lymphocytes, enables the best type of response according to the nature of the invading microorganism. Th1 cells produce high levels of IFN-γ and are responsible for both phagocyte activation and the production of opsonizing and complement-fixing antibodies, thus playing an important role in protection against intracellular pathogens. Th2 cells produce IL-4, IL-5, IL-9, and IL-13. Th2 cells, because of their ability to produce IL-4 and IL-13, can induce IgE class switching by B lymphocytes, enabling in this way mast cells and basophils sensitization and possible subsequent activation. In addition, IL-5 produced by Th2 cells has specific activity on differentiation, activation, and recruitment of eosinophils. Finally, IL-9 has an important role in the proliferation and accumulation of mast cells and the induction of mucus production by cells of the respiratory tract and the gut (1–4). Because of all the mentioned characteristics, Th2 cells are effective in the protection against helminthes (5). The more recently discovered Th17 subset is characterized by the production of IL-17A, IL-17F, IL-8, IL-21, and IL-22. Th17 cells play a critical role in the recruitment, activation, and migration of neutrophil granulocytes, both directly, through IL-8 production (6) and indirectly, by inducing, via IL-17, the production of colony stimulatory factors (CSF) and CXCL8 (7) in tissue resident cells. Because of their unique ability to recruit neutrophils, the main protective function of Th17 cells appears to be the clearance of extracellular pathogens, including fungi (8, 9). The distinctive features of the various CD4 effector/regulatory subpopulations are determined largely by the set of transcription factors they express and the genes they transcribe. The induction of the distinctive patterns of gene expression is dependent on the milieu of microenvironmental cytokines during the antigen-mediated activation of a naïve T cell.

In addition to their protective functions against invading pathogens, Th1, Th2, and Th17 cells contribute to the development of human disorders: Th1 and Th17 cells have been thought to be involved in the pathogenesis of organ-specific autoimmune diseases, as well as other chronic inflammatory disorders, such as Crohn's disease (CD), psoriasis, and rheumatoid arthritis (RA); Th2 cells certainly play a central role in the development of allergic disorders (10–12).

IL-1 FAMILY OF CYTOKINES

Although the original IL-1 family comprised only IL-1α and IL-1β, the IL-1 family has expanded considerably in the last few years and nowadays comprises 11 members (IL-1α, IL-1β, IL-1RA, IL-18, IL-33, IL-36α, IL-36β, IL-36γ, IL-36RA, IL-37, and IL-38) which have similar gene structure. All these cytokines use heterodimeric receptors for signaling. IL-1 (α and β) binds to IL-1RI, IL-33 to T1/ST2 and IL-36 (α, β, and γ) to IL-1Rrp2, and subsequently, they recruit the same coreceptor IL-1R accessory protein (IL-1RAcP). IL-18 signals through the IL-18Rα associated to the coreceptor IL-18Rβ. On receptor binding, all IL-1 family cytokines activate similar intracellular signals. The signal is initiated with recruitment of the adaptor protein MyD88 to the Toll-IL-1 receptor (TIR) domain. Several kinases are then phosphorylated, NF-κB translocates to the nucleus, and the expression of a large portfolio of inflammatory genes takes place (13) IL-1 receptor antagonist (IL-1RA) and IL-36RA, act as natural inhibitors for the biologic activities of IL-1 (α and β) and of IL-36 (α, β, and γ), respectively.

In addition to signaling receptors, also decoy receptors and inhibitory receptors for IL-1 cytokine family members had been described. One of these is the IL-1RII that does not signal because it lacks the TIR cytoplasmic domain. IL-1RII binds IL-1β with

higher affinity than IL-1RI but does not transduce a signal, acting therefore as a decoy receptor (14). The IL-1RII–IL-1β complex is able to bind to IL-1RAcP, in this way the decoy receptor also serves to sequester the accessory receptor from participating in IL-1 signaling from the IL-1RI. Other receptors characterized by the ability to deliver inhibitory signals in response to IL-1 family members are SIGIRR and IL-1RAcPb (15, 16).

Each member of the IL-1 family, IL-1Ra is the unique exception, is first synthesized as a precursor without a clear signal peptide for processing and secretion, and none are found in the Golgi. IL-1α and IL-33 are similar in that their precursor forms can bind to their respective receptor and trigger signal transduction. The precursor forms of IL-18 and IL-1β do not bind their respective receptors, are not active, and require cleavage by either intracellular caspase-1 or extracellular neutrophilic proteases (17).

Since the discovery of this family of cytokines their "immunostimulant activity" was evident, but wasn't that clear on which and how this cytokine could interact on different T lymphocytes. The present review will focalize exclusively on two members of the IL-1 family of cytokines, IL-1α and IL-1β, and it will discuss, on the basis of the last 30 years literature, their involvement in the differentiation, activation, and maintenance or survival of the different Th cell subsets.

The IL-1α precursor is produced constitutively in all epithelial cells and fibroblasts and can be also found on the surface of several cells, particularly on monocytes and B lymphocytes. The primary sources of IL-1β are the blood monocytes, tissue macrophages, and dendritic cells; B lymphocytes and NK cells also produce IL-1β (18).

IL-1 AND THE TH2 IMMUNE RESPONSE

In the 80s the first studies taking in account the direct effects of the prototypic proinflammatory cytokine, IL-1, on T lymphocytes were published (19–21). These studies indicated IL-1 as a cytokines possibly influencing growth and differentiation of immunocompetent lymphocytes. IL-1 costimulatory role for T cells was at that time attributed to two complimentary effects: (1) IL-1 can enhance transcription and secretion of the T cell growth factor IL-2; (2) IL-1 stimulates the expression of the membrane receptors for IL-2. The combination of these complimentary effects of IL-1 on T cells could explain its T-cell stimulating function. In 1988, Lichtman and colleagues (20) were the first to evaluate IL-1α costimulatory function on, at that time recently discovered, murine Th1 and Th2 cells. In this study, the authors demonstrated that only Th2 cells express high affinity receptors for IL-1 and that, accordingly, only this cell subset proliferate in response to IL-1α, whereas Th1 cells do not.

Few years later, Taylor-Robinson and colleagues (22), examining the expression of selected interleukin receptors by cloned CD4+ T cells specific for the murine malaria parasite *Plasmodium chabaudi* representative of the Th1 and Th2 subsets, found that while IL-1RI was constantly expressed by Th2 clones, its expression by the Th1 clones was either negligible or undetectable. Since then, the scientific community assumed that just the Th2 cell subset expresses IL-1RI, but lacking to confirm this data on human cells. Considering the pathogenic role of Th2 cells in allergic diseases, IL-1 activity was therefore investigated in several murine models of allergy. Nakae and colleagues (23) demonstrated that the ovalbumin-induced airway hypersensitivity response (AHR) in IL-1α/β-double deficient mice was significantly reduced when compared to wild type mice, whereas the response seen in IL-1RA-deficient mice was profoundly exacerbated, suggesting that IL-1 is required for Th2 cell activation during AHR. Accordingly, the authors showed that ovalbumin-specific IL-4 and IL-5 production by T cells, and IgG1 and IgE production by B cells in IL-1α/β-double deficient mice were markedly reduced compared with these responses in wild type mice. Similar results were obtained by Schmitz and colleagues (24) that investigated the role of IL-1 in models of allergic asthma using IL-1R1-deficient mice. The authors showed that in a model of mild asthma, based on repeated sensitization of mice with low doses of ovalbumin in the absence of any adjuvant, the pulmonary eosinophilic inflammation, the goblet cell hyperplasia, as well as antibody responses including IgG, IgE, and IgA were strongly reduced in IL-1R1-deficient as compared to wild type mice. In contrast, sensitization of mice in the presence of alum adjuvant, a more severe asthma model, rendered the IL-1 pathway dispensable for the development of pulmonary allergic Th2 responses. The role of IL-1 in sustaining the Th2 immune responses comes also from animal models of parasites infestation. Helmby and Grencis (25) showed that Th2 response-associated resistance to gastrointestinal nematode Trichuris muris is mediated was dependent on the presence of IL-1α and IL-1β. Indeed, they demonstrated that both IL-1α- and IL-1β-deficient mice were susceptible to chronic Trichuris muris infection and that the inability to eliminate the worms was associated with a defect in the development of a Th2 response in the mesenteric lymph nodes. Opposite data were obtained by Satoskar and colleagues (26) that found significantly increased IL-4 and IL-10 production by lymph node cells from *Leishmania* major infected IL-1RI-deficient mice when compared to wild type mice.

These findings are contradictory to the one showed by Helmby and Grencis, possibly because of differences in the type of cytokine/receptor KO utilized, the choice of experimental model, as well as the genetic background of the host.

The first description of IL-1RI expression and modulation on human T cells, however without distinguishing on which particular subsets, was made by Shirakawa and colleagues (21). Few years later Manetti and colleagues (27) analyzed the effects exerted by IL-1α on the growth and differentiation of human Th1 and Th2 cells. In this study, the authors showed that neither IL-1α nor the IL-1RA had detectable activity toward the antigen- or anti-CD3 antibody-induced proliferative response of already established Th1 or Th2 clones. However, allergen-specific T-cell lines, derived in the presence of anti-IL-1α Ab or IL-1RA, exhibited reduced and increased ability to produce IL-4 and IFN-γ, respectively. These data suggested that IL-1α was not required for the growth of already established human Th1 or Th2 clones, but it played a critical role in the development of Th2 cells, whereas Th1 development was unaffected. In light of the above mentioned data, the lack of an effect, described by Manetti, in terms of proliferative response to IL-1α on already established human Th2 cells and the reduction in the Th2 polarization in IL-1α neutralizing conditions, could be interpreted today as an indirect effect on non-Th2 subsets that are expanded in the presence of IL-1α (27).

The first data relative to the expression of IL-1RI on a particular Th cell subsets came out only in 2010. Indeed, Cosmi and colleagues (28) demonstrated the lack of IL-1-RI mRNA on human established Th2 clones, while Wang and colleagues (29) observed a slight membrane expression of the receptor on freshly enriched human CRTH2 positive cells, being CRTH2 a surface molecule selectively expressed by human Th2 cells (30, 31).

In any case neither Cosmi nor Wang analyzed the ability of the human Th2 cells to respond to IL-1, i.e., monitoring the activation signal transduction molecules downstream the IL-1RI, therefore it is not known if the receptor has functional activity.

Since the activity of IL-1 on human Th2 cells is not unequivocally established, caution is needed in considering this cytokine as potential new therapeutic target for human bronchial asthma as some studies suggest (32).

BOTH MICE AND HUMANS TH17 EXPRESS IL-1RI AND ARE MODULATED BY ITS SIGNALING

For the *in vitro* differentiation of naïve T cell into Th17 cells in the mouse, the scientific community was fairly unanimous in defining TGF-β and IL-6, as the key cytokines. Yet in 2006 Veldhoen and colleagues (33) described a synergistic role of IL-1β and TNF-α in the Th17 differentiation initiated by TGFβ and IL-6 and in the same year Sutton and colleagues (34) described a lower induction of Th17 cells in IL-1RI-deficient mice, than in wild type mice and also a resistance to experimental autoimmune encephalomyelitis (EAE). Interestingly, in models of autoimmune diseases, such as EAE and collagen-induced arthritis (CIA), the induction of the Th17 cells require the presence of a mixture of killed *Mycobacterium tuberculosis*, that has been recently discovered to induce, via dectin-1 and TLR4, the release of IL-1β (35). Therefore it's possible to speculate that IL-1β plays a pivotal role in Th17 induction. This hypothesis is confirmed observing mice deficient in caspase1-enzyme that cleaves IL-1β precursor into a mature form-, where EAE is markedly attenuated. On the other hand when IL-1β activity is unopposed, like in IL-1RA knot-out mice (C57BL/6J), causes autoimmunity and arthritis that closely resembled RA in humans (36). These data have been confirmed, observing that mice specifically deficient in endogenous IL-1RA developed an increased Th17 response, and CIA appears to be because of unrestrained IL-1 activity (37, 38), which may in turn, contribute to a more severe form of CIA. In keeping with these observations Coccia and colleagues (39) showed that IL-1β promotes intestinal inflammation by augmenting the recruitment of granulocytes and the accumulation and activation of innate lymphoid cells (ILCs) in a model of in Helicobacter hepaticus-triggered intestinal inflammation. In particular, the observation that synergistic interactions between IL-1β and IL-23 sustain innate and adaptive inflammatory responses in the gut, promoting intestinal pathology, suggests that targeting IL-1β may represent a useful therapeutic approach in IBD. To further support the possibility of IL-1β play significant role not only in the induction of Th17 phenotype, but also in their expansion and homeostatic maintenance, Sutton and colleagues reported that IL-1β can promote Th17 expansion and cytokine production *in vitro* even in the absence of TCR stimulation. The mechanisms underlying these *in vivo* phenomena became more clear since it has been described IL-1RI expression first on IL-17+CD4+ T cells of SKG mice (that spontaneously develop arthritis) (40), and later on, by the demonstration that IL-1 signaling is required for the upregulation of IRF4 and RORC (two fundamental Th17 transcription factors) during the early Th17 lineage programing and to sustain its differentiation (41).

When the differentiation process, from naïve to effector cells, was analyzed in humans, a number of evidence showed soon that a predominant role was led by IL-1β, alone or in combination with other cytokines. Annunziato and colleagues (42) described the expression of IL-1RI on Th17 cell clones derived from peripheral blood (PB) and gut specimens of Crohn's affected patients, and, accordingly, Acosta-Rodriguez and colleagues (43) was able to induce a Th17 phenotype by culturing naïve T cells in presence of IL-1β and IL-6. In particular, IL-1β was sufficient to induce the expression of RORC and production of both IL-17 and IFN-γ. Cosmi and colleagues reported that all humans IL-17-producing cells originate from CD161+ naïve CD4+ T cells of umbilical cord blood, as well as of the postnatal thymus, in response to the combined activity of IL-1β and IL-23. Confirmation that IL-1β is important in the differentiation of Th17 cells comes from studies conducted on the CD161 positive fraction of naïve CD3+CD4+ cell from the thymus as well from the cord blood of newborns where the combination of IL-1β and IL-23 allows the Th17 polarization (44, 45).

These data were recently confirmed by Lee and colleagues (46) who, demonstrated the upregulation of IL-1RI on naïve cord blood CD4+ T cell after exposure to common γ-chain cytokines (IL-7, IL-15) plus TGF-β and establishing that such condition promote the differentiation into Th17 cells upon TCR triggering and IL-1β stimulation, which is enhanced by IL-23 and blocked by IL-1RA. The same upregulation of IL-1RI was described by Raffin and colleagues (47) on PB naïve CD4+ T cell in the presence of the combination of IL-2, IL-1β, IL-23, and TGF-β.

In human disease, several clinical studies support a role for IL-1β secreted by colon lamina propria monocytes in disease activity during IBD. IL-1β levels in the colon during active phase of IBD correlated with disease activity and high levels of IL-1β were associated with active lesions (48), suggesting an important role of this cytokine in promoting localized inflammation.

A human example of IL-1β dysregulation is the heterozygous mutation of NLRP3 gene (encoding for the inflammasome component, cryopyrin) that leads to an abnormal secretion of IL-1β by monocytes, leading to different clinical inflammatory manifestations, but all hampered by inhibition of IL-1β. Indeed Lasiglie (49) analyzing 11 patients carrying this mutation (Cryopyrin-associated periodic syndromes, CAPS) observed a skewed Th17 phenotype in PB lymphocytes, as well as an increased production of IL-1β and IL-23 by monocyte-derived dendritic cells. The anti-IL-1β treatment *in vivo* reduce the secretion of IL-1β by monocytes and both IL-1β and IL-23 by monocyte-derived dendritic cells *in vitro*. The observation that IL-1RA treatment leads to a down modulation of IL-23 in PBMC of celiac patients may support the hypothesis that the over expression of IL-23 in CAPS patients is actually related to an IL-1β dependent mechanism, likely associated to the activation of the inflammasome (50). The second arm of "IL-1 system" has been enlighted in 2009 with the identification (51) of the cause of a human autoinflammatory syndrome

of skin and bone in a homozygous truncating mutations in the *IL-1RN* gene that leads to the lack of secretion of this receptor antagonist (IL-1RA), and as a consequence in an unopposed IL-1 signaling. Increased number of IL-17 secreting cells was found in biopsy samples of inflamed skin from patients with deficiency of the IL-1RA (DIRA patients); as expected treatment with Anakinra, a recombinant IL-1RA, leaded to symptoms remission.

We can conclude that many evidence in mice and humans support the concept that IL-1β, acting concurrently with other cytokines, is a key cytokine in the early phases of Th17 development, acting through its specific receptor expressed already by the naïve CD4+ Th17's precursor.

Moreover, even if IL-1β plays an important role in combating the invading pathogen as part of the innate immune response, its dysregulation is responsible for a number of autoinflammatory disorders in which Th17 cells are involved. As a consequence, its inhibition has proved therapeutically beneficial in the treatment of a spectrum of serious, yet relatively rare, heritable pathologies. This raises the possibility that anti-IL-1 therapeutics may have broader applications than previously believed, and may be utilized across diverse disease states that are linked insidiously through heightened inflammasome activity.

TH1 "NON-CLASSIC" HUMAN T CELL EXPRESS IL-1RI: A NEW POINT OF VIEW

Because of the scientific community had assumed the absence of IL-1RI expression by Th1 cells, very few works subsequently investigated the possible expression and function of this receptor on Th cell subset. Ben-Sasson and colleagues (52) were the first that described an activation effect of IL-1β on Th1 cells in a mouse model. The first data on human Th1 cells came from the study of Cosmi and colleagues mentioned before (44). In this study, the authors demonstrated that the combination of IL-1β and IL-23 was able to induce the development of Th17 cells in CD4+CD161+ cells, and also the Th1 phenotype, in both CD4+CD161+ and CD4+CD161− cell fractions. This observation leads the authors to hypothesize that also Th1 cells able to respond to IL-1β could exist. In keeping with this hypothesis, it has been recently found (53) that the CD4+CD161+ clones and inflamed tissue derived cells able to produce IFN-γ expressed IL-1RI mRNA. Interestingly, in the synovial fluid of JIA patients, the CD4+CD161+ IFN-γ-producing cells showed higher IL-1RI mRNA expression when compared to the CD4+CD161− counterpart (54). Of note, in this paper Cosmi and colleagues has highlighted the plasticity of Th17 cells showing that Th1 clones, expressing CD161 (named as "non-classic Th1 cells"), derive from an *in vivo* shifting of Th17 cells toward a Th1 phenotype. Interestingly, the authors found significantly increased levels of IL-12 in the SF of JIA patients and that Th17 cells from the PB of healthy children could be induced to shift to Th1 cells when cultured *in vitro* in the presence of JIA SF. More importantly, this effect was completely reversed by a neutralizing anti-IL-12 mAb, strongly suggesting that the shifting of Th17 cells toward the Th1 phenotype was related mainly to the activity of IL-12 present in the SF.

The late plasticity of Th17 cells to Th1 cells has been recently confirmed also in mice, where it has been found that IL-12, or the prolonged exposure to IL-23, is able to polarize Th17 cells toward the Th1 phenotype (55). Furthermore, similar results were recently reported by Nistala and colleagues (56), that showed Th17 plasticity to Th1 to be driven by the inflammatory environment in human autoimmune arthritis. Finally, very recently, the instability of the Th17 phenotype has been definitively demonstrated, at a genetic level, in mice (57).

These new data leads us to argue that up to now the scientific community overlooked this population of "non-classic Th1" cells expressing CD161 and IL-1RI (53). As mentioned before in this review many animal models of autoimmune disorders demonstrate the pivotal role of IL-1 in the pathogenesis of the disease and its relationship to Th17, but failed to look at the Th1 cells that could be affected by a lack of IL-1 signaling. In this context, the presence, and sometimes the prevalence, of Th1 cells in the inflammatory tissues have been interpreted as a protective, rather than proinflammatory, function. In humans many autoinflammatory disorders are treated blocking IL-1β [i.e., Familial Mediterranean fever (FMF), Pyogenic arthritis, pyoderma gangrenosum, acne (PAPA), CAPS, Hyper IgD syndrome (HIDS), Adult and juvenile Still disease Schnitzler syndrome, TNF receptor-associated periodic syndrome (TRAPS), Blau syndrome; Sweet syndrome, Deficiency in IL-1 receptor antagonist (DIRA)]. Initially, Anakinra (IL-1RA) was used to treat several chronic inflammatory diseases, today, these diseases are also successfully treated with neutralization by human anti-IL-1β monoclonal Abs. It's time to speculate that the improvements observed during these treatments are not only due to the general anti-inflammatory effects and to the reduction in terms of production, survival and differentiation of Th17 cells, but also on the activity on Th1 effector cells in particular Th1 expressing IL-1RI probably derived from a Th17 phenotype (42, 53).

CONCLUDING REMARKS

Since the discovery of the lack of IL-1RI on murine Th1 cells, most of the studies focused their attention to *in vivo* animal models of Th2 related diseases. Different models of mice either deficient for IL-1RI or for IL-1α/IL-1β were analyzed to verify the effects in the Th2 response; most of the studies agree with the idea that both cytokines promote proliferation and differentiation of Th2 cells *in vitro* and *in vivo*, but some other found no effects or even the opposite. The contradictory findings could be related to the different animal models used, the different protocol of disease induction, the different genetic background; furthermore the analysis conducted on *ex vivo* bulk cultures may induce to overestimate some observations that are actually side or indirect effects. As most of the conclusions made on the basis of animal models could have an impact in clinical practice, we would have expected to find many papers confirming or disproving these data on human cells. Surprisingly very few studies focused on the effects of IL-1α either IL-1β in human Th2 cells, and the findings do not enlighten if human Th2 cells express a functional IL-1RI and therefore can be modulated by these cytokines.

Conversely, as we look to the relationship between IL-1β and Th17, human's studies and animal models supported both the concept that IL-1β has a fundamental role in Th17 modulation. Two genetic human diseases carrying an impairment in IL-1β either in the expression or in its regulation and showing a skewed Th17

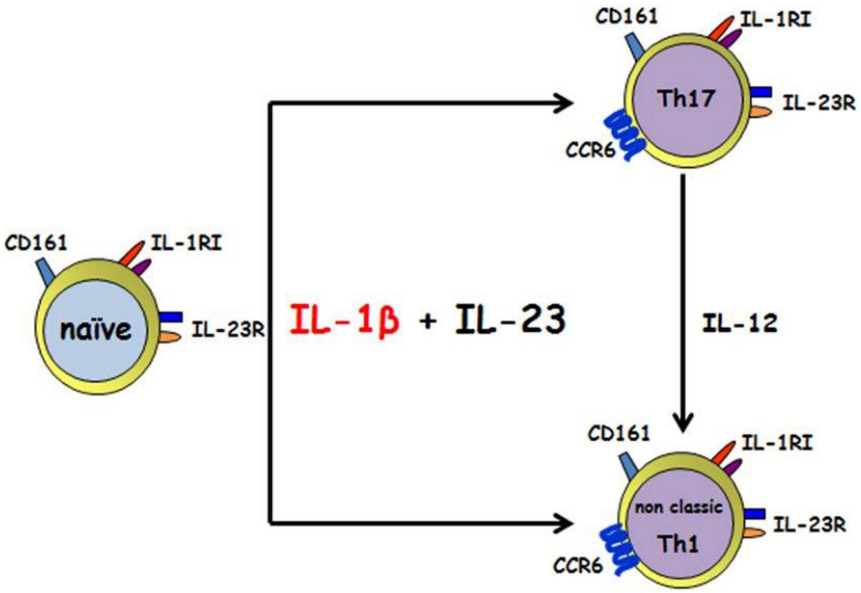


FIGURE 1 | IL-1β and IL-23 induce the differentiation of human CD161+ precursor toward both the Th17 and the non-classic Th1 phenotype. IL-1 β together with IL-23 acts on human CD4+CD161+ precursor to induce Th17 and non-classic Th1 effector cells. Th17 can shift toward a non-classic Th1 phenotype in the presence of IL-12.

phenotype, is for sure of great confirmation of *in vitro*/*ex vivo* data. The *in vitro* assays clarify that IL-1β is able to induce those transcription factors necessary for Th17 development, as soon as its own receptor is upregulated in naïve T cells upon TCR triggering in the presence of γ-chain cytokines. The cooperation with other cytokines, i.e., IL-23, IL-6, IL-21 leads to the differentiation and the stabilization of the phenotype (39, 43, 58, 59); IL-1RI expression is long lasting, maintained on effector Th17 cells and its signaling is probably responsible for their survival during inflammation. The recent discovery of Th17 plasticity toward a Th1 phenotype in the presence of an inflammatory environment is driving the scientific community to focus attention also to those Th1 highly present in many autoimmune diseases that have been so far considered protective rather than pathogenic; it is intriguing, that also a sub population of human Th1 cells expressing CD161 and deriving from Th17 (named "Th1 non-classic") do express IL-1RI and most likely respond to IL-1β (**Figure 1**). It is therefore likely that the therapeutically approaches were the IL-1β activity is blocked, like in JIA patients, are effective because acting on these Th1 CD161+ IL-1RI cells whose number correlate with some parameter disease. Moreover other autoimmune diseases, where Th1 CD161+ IL-1RI cells were increased, could benefit of an anti-IL-1 treatment.

REFERENCES

1. Annunziato F, Romagnani S. Heterogeneity of human effector CD4+ T cells. *Arthritis Res Ther* (2009) **11**:257. doi:10.1186/ar2843
2. Glimcher LH, Murphy KM. Lineage commitment in the immune system: the T helper lymphocyte grows up. *Genes Dev* (2000) **14**:1693–711.
3. Khan WI, Richard M, Akiho H, Blennerhasset PA, Humphreys NE, Grencis RK, et al. Modulation of intestinal muscle contraction by interleukin-9 (IL-9) or IL-9 neutralization: correlation with worm expulsion in murine nematode infections. *Infect Immun* (2003) **71**:2430–8. doi:10.1128/IAI.71.5.2430-2438.2003
4. Louahed J, Toda M, Jen J, Hamid Q, Renauld JC, Levitt RC, et al. Interleukin-9 upregulates mucus expression in the airways. *Am J Respir Cell Mol Biol* (2000) **22**:649–56. doi:10.1165/ajrcmb.22.6.3927
5. Romagnani S. The Th1/Th2 paradigm. *Immunol Today* (1997) **18**:263–6. doi:10.1016/S0167-5699(97)80019-9
6. Pelletier M, Maggi L, Micheletti A, Lazzeri E, Tamassia N, Costantini C, et al. Evidence for a cross-talk between human neutrophils and Th17 cells. *Blood* (2010) **115**:335–43. doi:10.1182/blood-2009-04-216085
7. Ouyang W, Kolls JK, Zheng Y. The biological functions of T helper 17 cell effector cytokines in inflammation. *Immunity* (2008) **28**:454–67. doi:10.1016/j.immuni.2008.03.004
8. Chen Y, Thai P, Zhao YH, Ho YS, DeSouza MM, Wu R. Stimulation of airway mucin gene expression by interleukin (IL)-17 through IL-6 paracrine/autocrine loop. *J Biol Chem* (2003) **278**:17036–43.
9. Kao CY, Chen Y, Thai P, Wachi S, Huang F, Kim C, et al. IL-17 markedly up-regulates beta-defensin-2 expression in human airway epithelium via JAK and NF-kappaB signaling pathways. *J Immunol* (2004) **173**:3482–91.
10. Bettelli E, Oukka M, Kuchroo VK. T(H)-17 cells in the circle of immunity and autoimmunity. *Nat Immunol* (2007) **8**:345–50. doi:10.1038/ni0407-345
11. Maggi E. The TH1/TH2 paradigm in allergy. *Immunotechnology* (1998) **3**:233–44. doi:10.1016/S1380-2933(97)10005-7
12. Monteleone I, Pallone F, Monteleone G. Th17-related cytokines: new players in the control of chronic intestinal inflammation. *BMC Med* (2011) **9**:122. doi:10.1186/1741-7015-9-122
13. Martin MU, Wesche H. Summary and comparison of the signaling mechanisms of the Toll/interleukin-1 receptor family. *Biochim Biophys Acta* (2002) **1592**:265–80. doi:10.1016/S0167-4889(02)00320-8
14. Mantovani A, Locati M, Vecchi A, Sozzani S, Allavena P. Decoy receptors: a strategy to regulate inflammatory cytokines and chemokines. *Trends Immunol* (2001) **22**:328–36. doi:10.1016/S1471-4906(01)01941-X

15. Riva F, Bonavita E, Barbati E, Muzio M, Mantovani A, Garlanda C. TIR8/SIGIRR is an interleukin-1 receptor/toll like receptor family member with regulatory functions in inflammation and immunity. *Front Immunol* (2012) **3**:322. doi:10.3389/fimmu.2012.00322
16. Yoshida T, Shiroshima T, Lee SJ, Yasumura M, Uemura T, Chen X, et al. Interleukin-1 receptor accessory protein organizes neuronal synaptogenesis as a cell adhesion molecule. *J Neurosci* (2012) **32**:2588–600. doi:10.1523/JNEUROSCI.4637-11.2012
17. Arend WP, Palmer G, Gabay C. IL-1, IL-18, and IL-33 families of cytokines. *Immunol Rev* (2008) **223**:20–38. doi:10.1111/j.1600-065X.2008.00624.x
18. Dinarello CA. Immunological and inflammatory functions of the interleukin-1 family. *Annu Rev Immunol* (2009) **27**:519–50. doi:10.1146/annurev.immunol.021908.132612
19. Herrmann F, Oster W, Meuer SC, Lindemann A, Mertelsmann RH. Interleukin 1 stimulates T lymphocytes to produce granulocyte-monocyte colony-stimulating factor. *J Clin Invest* (1988) **81**:1415–8. doi:10.1172/JCI113471
20. Lichtman AH, Chin J, Schmidt JA, Abbas AK. Role of interleukin 1 in the activation of T lymphocytes. *Proc Natl Acad Sci U S A* (1988) **85**:9699–703. doi:10.1073/pnas.85.24.9699
21. Shirakawa F, Tanaka Y, Ota T, Suzuki H, Eto S, Yamashita U. Expression of interleukin 1 receptors on human peripheral T cells. *J Immunol* (1987) **138**:4243–8.
22. Taylor-Robinson AW, Phillips RS. Expression of the IL-1 receptor discriminates Th2 from Th1 cloned CD4+ T cells specific for *Plasmodium chabaudi*. *Immunology* (1994) **81**:216–21.
23. Nakae S, Komiyama Y, Yokoyama H, Nambu A, Umeda M, Iwase M, et al. IL-1 is required for allergen-specific Th2 cell activation and the development of airway hypersensitivity response. *Int Immunol* (2003) **15**:483–90. doi:10.1093/intimm/dxg054
24. Schmitz N, Kurrer M, Kopf M. The IL-1 receptor 1 is critical for Th2 cell type airway immune responses in a mild but not in a more severe asthma model. *Eur J Immunol* (2003) **33**:991–1000. doi:10.1002/eji.200323801
25. Helmby H, Grencis RK. Interleukin 1 plays a major role in the development of Th2-mediated immunity. *Eur J Immunol* (2004) **34**:3674–81. doi:10.1002/eji.200425452
26. Satoskar AR, Okano M, Connaughton S, Raisanen-Sokolwski A, David JR, Labow M. Enhanced Th2-like responses in IL-1 type 1 receptor-deficient mice. *Eur J Immunol* (1998) **28**:2066–74. doi:10.1002/(SICI)1521-4141(199807)28:07<2066::AID-IMMU2066>3.0.CO;2-X
27. Manetti R, Barak V, Piccinni MP, Sampognaro S, Parronchi P, Maggi E, et al. Interleukin-1 favours the in vitro development of type 2 T helper (Th2) human T-cell clones. *Res Immunol* (1994) **145**:93–100. doi:10.1016/S0923-2494(94)80019-7
28. Cosmi L, Maggi L, Santarlasci V, Capone M, Cardilicchia E, Frosali F, et al. Identification of a novel subset of human circulating memory CD4(+) T cells that produce both IL-17A and IL-4. *J Allergy Clin Immunol* (2010) **125**:222–30. doi:10.1016/j.jaci.2009.10.012
29. Wang YH, Voo KS, Liu B, Chen CY, Uygungil B, Spoede W, et al. A novel subset of CD4(+) T(H)2 memory/effector cells that produce inflammatory IL-17 cytokine and promote the exacerbation of chronic allergic asthma. *J Exp Med* (2010) **207**:2479–91. doi:10.1084/jem.20101376
30. Cosmi L, Annunziato F, Galli G, Manetti R, Maggi E, Romagnani S. CRTH2: marker for the detection of human Th2 and Tc2 cells. *Adv Exp Med Biol* (2001) **495**:25–9. doi:10.1007/978-1-4615-0685-0_4
31. Nagata K, Tanaka K, Ogawa K, Kemmotsu K, Imai T, Yoshie O, et al. Selective expression of a novel surface molecule by human Th2 cells in vivo. *J Immunol* (1999) **162**:1278–86.
32. Lee JH, Wang LC, Yu HH, Lin YT, Yang YH, Chiang BL. Type I IL-1 receptor (IL-1RI) as potential new therapeutic target for bronchial asthma. *Mediators Inflamm* (2010a) **2010**:567351. doi:10.1155/2010/567351
33. Veldhoen M, Hocking RJ, Atkins CJ, Locksley RM, Stockinger B. TGFbeta in the context of an inflammatory cytokine milieu supports de novo differentiation of IL-17-producing T cells. *Immunity* (2006) **24**:179–89. doi:10.1016/j.immuni.2006.01.001
34. Sutton C, Brereton C, Keogh B, Mills KH, Lavelle EC. A crucial role for interleukin (IL)-1 in the induction of IL-17-producing T cells that mediate autoimmune encephalomyelitis. *J Exp Med* (2006) **203**:1685–91. doi:10.1084/jem.20060285
35. van de Veerdonk FL, Teirlinck AC, Kleinnijenhuis J, Kullberg BJ, van CR, van der Meer JW, et al. *Mycobacterium tuberculosis* induces IL-17A responses through TLR4 and dectin-1 and is critically dependent on endogenous IL-1. *J Leukoc Biol* (2010) **88**:227–32. doi:10.1189/jlb.0809550
36. Horai R, Saijo S, Tanioka H, Nakae S, Sudo K, Okahara A, et al. Development of chronic inflammatory arthropathy resembling rheumatoid arthritis in interleukin 1 receptor antagonist-deficient mice. *J Exp Med* (2000) **191**:313–20. doi:10.1084/jem.191.2.313
37. Koenders MI, Devesa I, Marijnissen RJ, Abdollahi-Roodsaz S, Boots AM, Walgreen B, et al. Interleukin-1 drives pathogenic Th17 cells during spontaneous arthritis in interleukin-1 receptor antagonist-deficient mice. *Arthritis Rheum* (2008) **58**:3461–70. doi:10.1002/art.23957
38. Lamacchia C, Palmer G, Seemayer CA, Talabot-Ayer D, Gabay C. Enhanced Th1 and Th17 responses and arthritis severity in mice with a deficiency of myeloid cell-specific interleukin-1 receptor antagonist. *Arthritis Rheum* (2010) **62**:452–62. doi:10.1002/art.27235
39. Coccia M, Harrison OJ, Schiering C, Asquith MJ, Becher B, Powrie F, et al. IL-1beta mediates chronic intestinal inflammation by promoting the accumulation of IL-17A secreting innate lymphoid cells and CD4(+) Th17 cells. *J Exp Med* (2012) **209**:1595–609. doi:10.1084/jem.20111453
40. Hirota K, Yoshitomi H, Hashimoto M, Maeda S, Teradaira S, Sugimoto N, et al. Preferential recruitment of CCR6-expressing Th17 cells to inflamed joints via CCL20 in rheumatoid arthritis and its animal model. *J Exp Med* (2007) **204**:2803–12. doi:10.1084/jem.20071397
41. Chung Y, Chang SH, Martinez GJ, Yang XO, Nurieva R, Kang HS, et al. Critical regulation of early Th17 cell differentiation by interleukin-1 signaling. *Immunity* (2009) **30**:576–87. doi:10.1016/j.immuni.2009.02.007
42. Annunziato F, Cosmi L, Santarlasci V, Maggi L, Liotta F, Mazzinghi B, et al. Phenotypic and functional features of human Th17 cells. *J Exp Med* (2007) **204**:1849–61. doi:10.1084/jem.20070663
43. Acosta-Rodriguez EV, Napolitani G, Lanzavecchia A, Sallusto F. Interleukins 1beta and 6 but not transforming growth factor-beta are essential for the differentiation of interleukin 17-producing human T helper cells. *Nat Immunol* (2007) **8**:942–9. doi:10.1038/ni1496
44. Cosmi L, De PR, Santarlasci V, Maggi L, Capone M, Frosali F, et al. Human interleukin 17-producing cells originate from a CD161+CD4+ T cell precursor. *J Exp Med* (2008) **205**:1903–16. doi:10.1084/jem.20080397
45. Santarlasci V, Maggi L, Capone M, Frosali F, Querci V, De PR, et al. TGF-beta indirectly favors the development of human Th17 cells by inhibiting Th1 cells. *Eur J Immunol* (2009) **39**:207–15. doi:10.1002/eji.200838748
46. Lee WW, Kang SW, Choi J, Lee SH, Shah K, Eynon EE, et al. Regulating human Th17 cells via differential expression of IL-1 receptor. *Blood* (2010b) **115**:530–40. doi:10.1182/blood-2009-08-236521
47. Raffin C, Raimbaud I, Valmori D, Ayyoub M. Ex vivo IL-1 receptor type I expression in human CD4+ T cells identifies an early intermediate in the differentiation of Th17 from FOXP3+ naive regulatory T cells. *J Immunol* (2011) **187**:5196–202. doi:10.4049/jimmunol.1101742
48. Ludwiczek O, Vannier E, Borggraefe I, Kaser A, Siegmund B, Dinarello CA, et al. Imbalance between interleukin-1 agonists and antagonists: relationship to severity of inflammatory bowel disease. *Clin Exp Immunol* (2004) **138**:323–9. doi:10.1111/j.1365-2249.2004.02599.x
49. Lasiglie D, Traggiai E, Federici S, Alessio M, Buoncompagni A, Accogli A, et al. Role of IL-1 beta in the development of human T(H)17 cells: lesson from NLPR3 mutated patients. *PLoS ONE* (2011) **6**:e20014. doi:10.1371/journal.pone.0020014

50. Harris KM, Fasano A, Mann DL. Cutting edge: IL-1 controls the IL-23 response induced by gliadin, the etiologic agent in celiac disease. *J Immunol* (2008) **181**:4457–60.
51. Aksentijevich I, Masters SL, Ferguson PJ, Dancey P, Frenkel J, van Royen-Kerkhoff A, et al. An autoinflammatory disease with deficiency of the interleukin-1-receptor antagonist. *N Engl J Med* (2009) **360**:2426–37. doi:10.1056/NEJMoa0807865
52. Ben-Sasson SZ, Hu-Li J, Quiel J, Cauchetaux S, Ratner M, Shapira I, et al. IL-1 acts directly on CD4 T cells to enhance their antigen-driven expansion and differentiation. *Proc Natl Acad Sci U S A* (2009) **106**:7119–24. doi:10.1073/pnas.0902745106
53. Maggi L, Santarlasci V, Capone M, Rossi MC, Querci V, Mazzoni A, et al. Distinctive features of classic and nonclassic (Th17 derived) human Th1 cells. *Eur J Immunol* (2012) **42**:3180–8. doi:10.1002/eji.201242648
54. Cosmi L, Cimaz R, Maggi L, Santarlasci V, Capone M, Borriello F, et al. Evidence of the transient nature of the Th17 phenotype of CD4+CD161+ T cells in the synovial fluid of patients with juvenile idiopathic arthritis. *Arthritis Rheum* (2011) **63**:2504–15. doi:10.1002/art.30332
55. Lee YK, Turner H, Maynard CL, Oliver JR, Chen D, Elson CO, et al. Late developmental plasticity in the T helper 17 lineage. *Immunity* (2009) **30**:92–107. doi:10.1016/j.immuni.2008.11.005
56. Nistala K, Adams S, Cambrook H, Ursu S, Olivito B, de JW, et al. Th17 plasticity in human autoimmune arthritis is driven by the inflammatory environment. *Proc Natl Acad Sci U S A* (2010) **107**:14751–6. doi:10.1073/pnas.1003852107
57. Kurschus FC, Croxford AL, Heinen AP, Wortge S, Ielo D, Waisman A. Genetic proof for the transient nature of the Th17 phenotype. *Eur J Immunol* (2010) **40**:3336–46. doi:10.1002/eji.201040755
58. Sha Y, Markovic-Plese S. A role of IL-1R1 signaling in the differentiation of Th17 cells and the development of autoimmune diseases. *Self Nonself* (2011) **2**:35–42. doi:10.4161/self.2.1.15639
59. Wilson NJ, Boniface K, Chan JR, McKenzie BS, Blumenschein WM, Mattson JD, et al. Development, cytokine profile and function of human interleukin 17-producing helper T cells. *Nat Immunol* (2007) **8**:950–7. doi:10.1038/ni1497

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

Received: 09 April 2013; paper pending published: 12 May 2013; accepted: 24 June 2013; published online: 15 July 2013.
*Citation: Santarlasci V, Cosmi L, Maggi L, Liotta F and Annunziato F (2013) IL-1 and T helper immune responses. Front. Immunol. **4**:182. doi: 10.3389/fimmu.2013.00182*
This article was submitted to Frontiers in Inflammation, a specialty of Frontiers in Immunology.