IL-1 FAMILY MEMBERS IN HEALTH AND DISEASE

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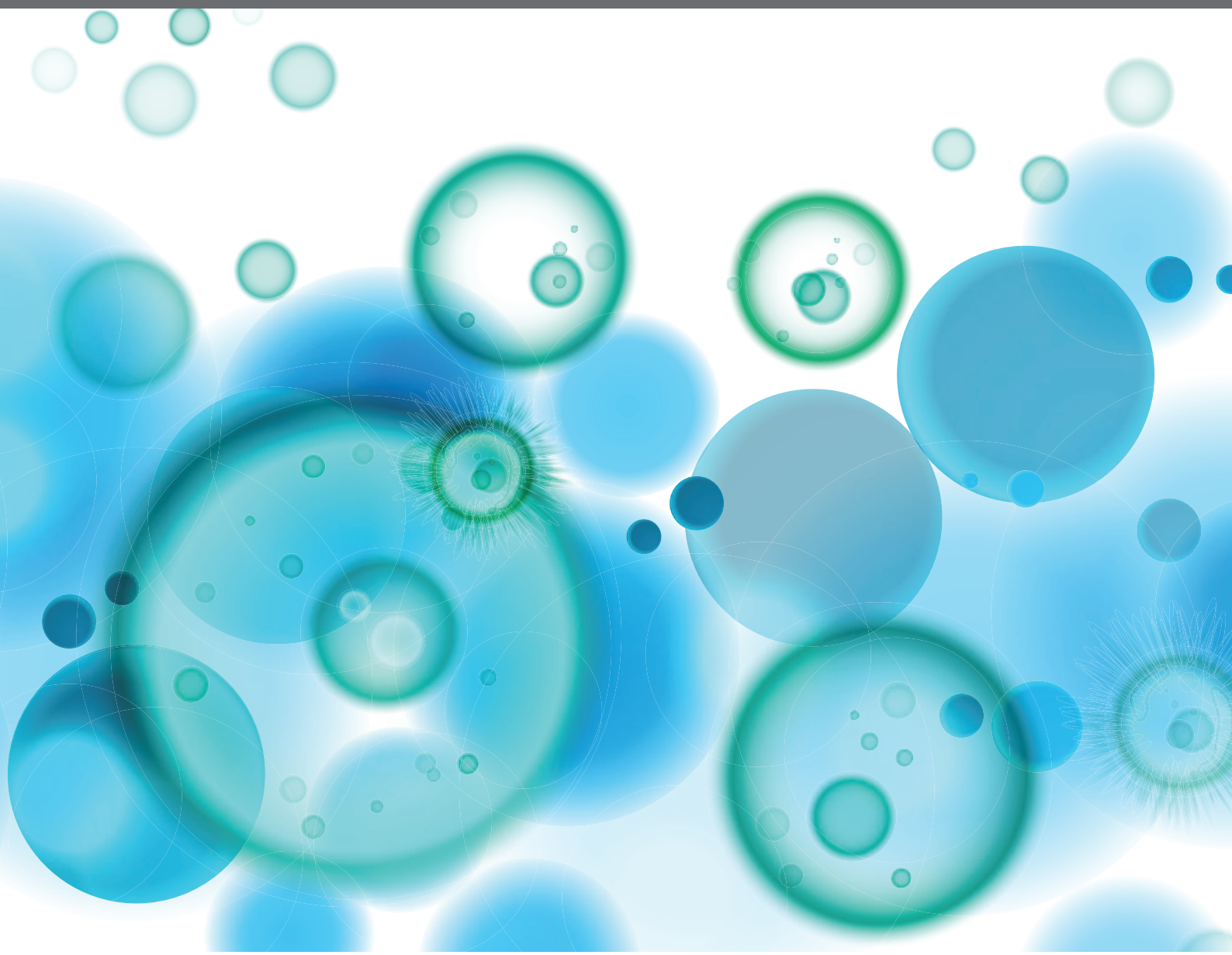

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IL-1 FAMILY MEMBERS IN HEALTH AND DISEASE

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EDITORIAL
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Editorial: IL-1 Family Members in Health and Disease

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Editorial on the Research Topic

IL-1 Family Members in Health and Disease

In 1985, two distinct cDNAs encoding proteins sharing human Interleukin-1 (IL-1) activity were described, thus defining the first two members of the IL-1 family—IL-1α and IL-1β. These potent pro-inflammatory cytokines have been the subject of much research in the area of fever and inflammation, as well as for their roles in a myriad of inflammatory associated diseases. Over the years the family expanded to include cytokines with both pro- and anti- inflammatory properties, including IL-18, IL-33, IL-36, IL-37, IL-38, IL-1 receptor antagonist (IL-1Ra), and IL-36Ra. In addition to the 11 identified cytokine members of this family, there are now multiple discrete receptor family members, that form 4 functional receptor complexes able to activate downstream signaling cascades, as well as several decoy and inhibitory receptors.

The inflammatory functions of these cytokines have been well-defined for the long established family members but, in the case of some of the newer members such as IL-36, IL-37, and IL-38, their physiological roles are still being elucidated. For the pro-inflammatory cytokines, their primary functions involve activating signaling cascades and mediating the inflammatory response to a wide variety of signals, thereby co-ordinating innate and adaptive immune responses. To achieve this, the IL-1 family cytokine binds to its cognate receptor which, in the case of all family members, then causes recruitment of a specific accessory receptor. This interaction then allows for recruitment of the signaling adaptor protein MyD88, the common adaptor to all family members. MyD88 engagement to the receptor complex subsequently results in the activation of the downstream kinases, the IRAK family of proteins. Ultimately, through recruitment and activation of additional signaling intermediates, key transcription factors, such as AP-1 and NFκB, become activated translocate to the nucleus and result in transcription of a myriad of immune and inflammatory genes. The importance of these protein:protein interactions and this pathway has long been understood to hold the key to regulation of inflammation by IL-1 family members.

As is well-known, however "with great power comes great responsibility." Therefore, given the ability of IL-1 family members to so potently drive and upregulate the inflammatory response, it is possibly not surprising that in recent years many members of this family have been identified as being critical for the development of diverse inflammatory and allergic diseases with much work focusing on IL-1 itself. Elevated plasma levels of IL-1, together with data from *in vitro* studies and murine models, have resulted in an association of IL-1 with an array of auto-inflammatory and autoimmune diseases. Perhaps the most illuminating data on the importance of IL-1 in the exacerbation of disease states has been gained from studies inhibiting the mechanism of action of these cytokines. Autoinflammatory conditions such as familial Mediterranean fever are characterized by recurrent bouts of fever in conjunction with debilitating local and systemic inflammation. These have proven to be responsive to IL-1β inhibition following administration of the naturally occurring IL-1 Receptor antagonist (IL-1Ra). Similarly, administration of IL-1Ra (now known by its generic name of anakinra) or administration of mAbs developed to target

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IL-1beta such as canikinumab, have also exhibited therapeutic efficacy in a range of diseases including type-2 diabetes, gout and rheumatoid arthritis. Indeed anakinra is now an approved therapy for the auto-inflammatory condition, Cryopyrin-Associated Periodic Syndromes and the autoimmune disease Rheumatoid arthritis, whilst canakinumab is an approved therapy for numerous auto-inflammatory conditions. It seems likely that in the coming years these therapies will be extended as approved treatments for additional diseases, as the therapeutic efficacy of targeting IL-1 in diseases ranging from neovascular disease to cancer is now being demonstrated in clinical trials. Indeed, as is highlighted by the recent findings from the CANTOS trial, IL-1β inhibition provides protection from the development of lung cancer.

The therapeutic potential of these family members is not solely limited to IL-1. IL-18 has been linked with a multitude of disease pathologies from retinal neovascular disease to inflammatory bowel disease. In terms of therapeutic targeting of IL-18, there is a current emphasis on inhibition of IL-18 function in type 2 diabetes, whilst the therapeutic benefit of activating IL-18 is being explored for both cancer and neovascular retinal disease. The function of IL-33, was first comprehensively explored in Th2 cells and asthma, given the high level of expression of its receptor in these cells, but has since been linked to other pathological conditions. To date however, therapeutic manipulation of IL-33 is still mainly centered around its role in asthma and allergic disease, with recent reports from a phase 2 clinical trial run by Regeneron and Sanofi demonstrating positive results for treatment of asthma with inhibition of IL-33. Like IL-1, IL-18, and IL-33, the more recently characterized IL-36 similarly plays a role in a variety of inflammatory diseases. Over activity of IL-36, has been implicated in psoriasis with negative outcomes. In contrast, administration of IL-36 in murine cancer models appears to be efficacious in the reduction of tumor growth. Phase 1 clinical trials targeting IL-36 signaling in a rare form of psoriasis (general pustular psoriasis) have shown positive results with this compound now progressing to phase 2. The role of IL-37 is the least understood with both pro and anti-inflammatory functions assigned to it.

In fact, opposing functions for many in this cytokine family have been shown, and as such it is clear that caution will have to be exercised with respect to the ongoing development of drugs targeting this family. Whereas, both IL-18 and IL-36 have demonstrated clear roles in the pathogenesis of certain auto-immune conditions, both have been seen to play a positive role in cancer. Additionally, both IL-1 α and IL-33 function as extracellular cytokines and as nuclear transcription factors, with their roles in the nucleus still poorly understood, Targeting of these cytokines therefore, may cause significant unwanted by-stander effects meaning that drug development will need to be nuanced to avoid unwanted interference in homeostatic roles. Therefore, while this family of cytokines appears to present excellent targets for many diseases, continued study and a deeper understanding of the pleiotropy of their functions is required. The substantial impact that drugs targeting this family of cytokines have the potential to provide for human health is evidenced by the sheer breadth of disease types that IL-1 family cytokines regulate. This is highlighted in this special topic and demonstrates that drugs developed to therapeutically manipulate this family of cytokines have the potential to cast a very wide net.

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MINI REVIEW
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IL-33 Mediated Inflammation in Chronic Respiratory Diseases—Understanding the Role of the Member of IL-1 Superfamily

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Interleukin 33 (IL-33) is an alarmin cytokine from the IL-1 family. IL-33 is localized in the nucleus and acts there as a gene regulator. Following injury, stress or cell death, it is released from the nucleus, and exerts its pro-inflammatory biological functions via the transmembrane form of the ST2 receptor, which is present mainly as attached to immune cells. In recent years, IL-33 became a focus of many studies due to its possible role in inflammatory disorders. Among respiratory disorders, the contribution of IL-33 to the development of asthma, in particular, has been most identified. Increased level of IL-33 in lung epithelial cells and blood serum has been observed in asthma patients. The IL-33/ST2 interaction activated the Th2 mediated immune response and further production of many pro-inflammatory cytokines. Single nucleotide polymorphisms in the IL-33 gene cause a predisposition to the development of asthma. Similarly, in chronic pulmonary obstructive disease (COPD), both increased expression of IL-33 and the ST2 receptor has been observed. Interestingly, cigarette smoke, a key inducer of COPD, not only activates IL-33 production by epithelial and endothelial cells, but also induces the expression of IL-33 in peripheral blood mononuclear cells. Knowledge regarding its contribution in other respiratory disorders, such as obstructive sleep apnea, remains greatly limited. Recently it was shown that IL-33 is one of the inflammatory mediators by which levels in blood serum are increased in OSA patients, compared to healthy control patients. This mini review summarizes current knowledge on IL-33 involvement in chosen chronic respiratory disorders and proposes this interleukin as a possible link in the pathogenesis of these diseases.

Keywords: asthma, COPD—Chronic obstructive pulmonary disease, OSA (Obstructive sleep apnea), IL-33, inflammation

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INTRODUCTION

The inteleukin-1 (IL-1) superfamily of cytokines plays a pivotal role in both innate and adaptive immunity by regulating host defense, inflammation and injury (1, 2). Similarly to the transforming growth factor, the Smad (TGF-Smad) superfamily, members of the IL-1 superfamily are associated with chronic pulmonary disorders and fibrosis (3).The IL-1 superfamily includes 11 members:

IL-1α, IL-1β, IL-1 receptor antagonist (IL-1Ra), IL-18, IL-33, IL-36α, IL-36β, IL-36γ, IL-36Ra, IL-37, and IL-38 (4, 5). All family members are characterized by a similar gene structure, in some cases having identical intron positioning and amino acids sequences, enabling folding of the protein into the three dimensional structure of a 12-stranded-β-barrel (6). Furthermore, all genes encoding members of IL-1 other than IL-18 and IL-33 occupy approximately a 400 kB interval on chromosome 2 (7). All the biologically active cytokines of the family are extracellular molecules, while their precursors are primarily intracellular with an exception of IL-1Ra, which encodes classical signal peptide. IL-1β and IL-18 achieve biological activity only after their precursors are cleavaged from the pro-domain by inflammasome. In the case of IL-36 α, β, γ, IL-36Ra, and IL-33, the full-length molecules already possess biological activity, however, following the cleavage of the N-terminus they obtain full biological potency (8, 9). Origin and function is not the only characteristic feature uniting the cytokines within IL-1 super family, as they also relate to each other by transduction pathways and receptor structure, all consisting of three Ig-like domains and Toll/IL-1R domain (1).

IL-33

IL-33, one of the most recently discovered members of IL-1 superfamily, is an alarmin cytokine promoting inflammatory responses (5). Originally it was known as a nuclear factor from high endothelium venules (HF-HEV) (10), and only later has its cytokine activity been defined (11). It mainly expresses itself through epithelial and endothelial cells, fibroblasts-like cell and myofibroblasts (10–13).

Differently to most of the members of IL-1 superfamily, the gene encoding IL-33 is localized on the short arm of chromosome 9, at 9p41.1 (14). The gene is comprised of 8 exons, poses interferon stimulated response element (ISRE), and several gamma interferon activation sites (GAS) in the promotor area (15). It is constitutively expressed in its pro form of 270 amino acids (full length IL-33; f-IL 33), with its molecular mass of 30 kD being stored in the nucleus of cells where it acts as a nuclear regulator (10, 11, 16). This function is facilitated by its N-terminal nuclear domain, which contains a chromatin binding motif that binds to histones (17, 18). Two other domains building up IL-33 are the IL-1-like cytokine domain, and the central domain (11, 19). IL-33 has been identified as a dual function cytokine, also possessing a full bioactivity as an alarmin upon its release from a cell (20). Furthermore, f-IL-33 serves as a substrate for serine proteases of neutrophils and mast cells. It results in shorter forms of a peptide comprised of IL-1 like domain and part of central domain, forming a mature form of IL-33 (m-IL-33). It is estimated that bioactivity of m-IL-33 increases ∼10–30 times compared to f-IL-33 (8, 19). During apoptosis caspase-3 and caspase-7 cleave f-IL-33, resulting in peptide, which lacks biological activity (9). It has been shown that the expression of IL-33 can be increased following the exposure to cigarette smoke (21). Other factors that can induce the same effect include viral infection and exposure to allergens such as pollen, chitin, fungi, or *Alternaria* spp. (22–28). The main mechanism in which the activity of IL-33 is limited is sequestration by soluble isoform of its suppression of tumorigenicity 2 (sST2) receptor that acts as a decoy, inhibiting the expansion of IL-33 mediated inflammation (29–31). Regulation of IL-33 activity also includes oxidation of cysteine residues following extracellular release and formation of disulphide bridges, resulting in the inactivation of IL-33 (24).

IL-33 signaling pathway begins with binding of the cytokine to the ST2 receptor. This transmembrane form of the receptor is generated by the same mRNA as the sST2 and the distinction is made through a different promoter and alternative splicing of the transcript (32, 33). Following the attachment of the protein to ST2, the corrector IL-1 receptor accessory protein IL-1RAcP is recruited and further heterodimeric signaling complex is formed, which involves myeloid differentiation primary response protein 88 (MYD88), IL-1R-assosiated kinase 1, and 4 (IRAK1, IRAK4), as well as tumor necrosis factor (TNF) receptor-assosiatedreceptor-6 (TRAF6) (11, 34, 35). This results in nuclear factor κB (NFκB) transcription and activation of mitogen-activated protein kinases (MAPK), including c-Jun N-terminal kinases (JNK) and p38 (11), which then in a signaling pathway drive processes such as proliferation, cell survival, cytokine secretion (IL-4, IL-5, and IL-13) and amphiregulin (AREG) expression (36–38). Only ST2$^+$ cells are affected by IL-33 stimulation. The type of response differs depending on the type of stimulated cell, as T helper 2 (Th2) cells respond by the secretion of Il-5 and IL-13 (11, 39), while mast cells release a different profile of cytokines, including IL-4, IL-5, and IL-6 (40). On the other hand, neutrophils following IL-33 stimulation migrate through chemotaxis (41). In the case of eosinophils, their response consists of degranulation and reactive oxygen species (ROS) production (36, 42, 43), while macrophages in response to IL-33 develop an alternatively activated phenotype (44, 45). IL-33 has also been shown to activate NFκB and p38 in regulatory lymphocytes T (Tregs), which further leads to the expansion of ST2$^+$ Tregs (46). Additionally, IL-33 stimulates TGF-β-mediated Treg differentiation (47). Activation of Tregs through MYD88 has been observed only in the IL-33/ST2 independent manner (48). It remains unknown if Tregs function can be affected in IL-33/ST2- MYD88 dependent pathways (49). Main directions of IL-33 molecular function in induction and maintenance of chronic respiratory disorders: asthma, COPD, and OSA are shown in **Figure 1**.

Abbreviations: AHI, apnea-hypopnea index; AREG, amphiregulin; BAL, bronchoalveolar lavage; COPD, chronic obstructive pulmonary sleep apnea; CRP, C-reactive protein; CXCL, chemokine (C-X-C motif) ligand; EMT, epithelial-to-mesenchymal transition; f-IL-33, full length interleukin-33; GAS, gamma interferon activation sites; GWS, genome-wide studies; IAK, interleukin-1 receptor associated kinase; IL, interleukin; IL-1Ra, interleukin 1 receptor antagonist; IL-1RacP, interleukin 1 receptor accessory protein; IL-36Ra, interleukin 36 receptor antagonist; ILC, innate lymphoid cell; ISRE, interferon stimulated response element; JNK, c-Jun N-terminal kinases; HF-HEV, nuclear factor from high endothelial venules; MAPK, mitogen-activated protein kinases; m-IL-33, mature interleukin 33; MHCII, major histocompatibility complex II; MYD88, myeloid differentiation primary response protein 88; NFκB, nuclear factor κB; OSA, obstructive sleep apnea; PDGF, platelet-derived growth factor; ROS, reactive oxygen species; SNP, single nucleotide polymorphism; sST2, suppression of tumorigenicity 2 soluble receptor; ST2, suppression of tumorigenicity 2 transmembrane receptor; TCR, Th2 cell receptor; TGF-β, transforming growth factor β; Th2, T helper 2 cell; TIR, Toll-like/interleukin 1receptor; TNF, tumor necrosis factor; TRAF6, tumor necrosis factor receptor-assosiatedreceptor-6.

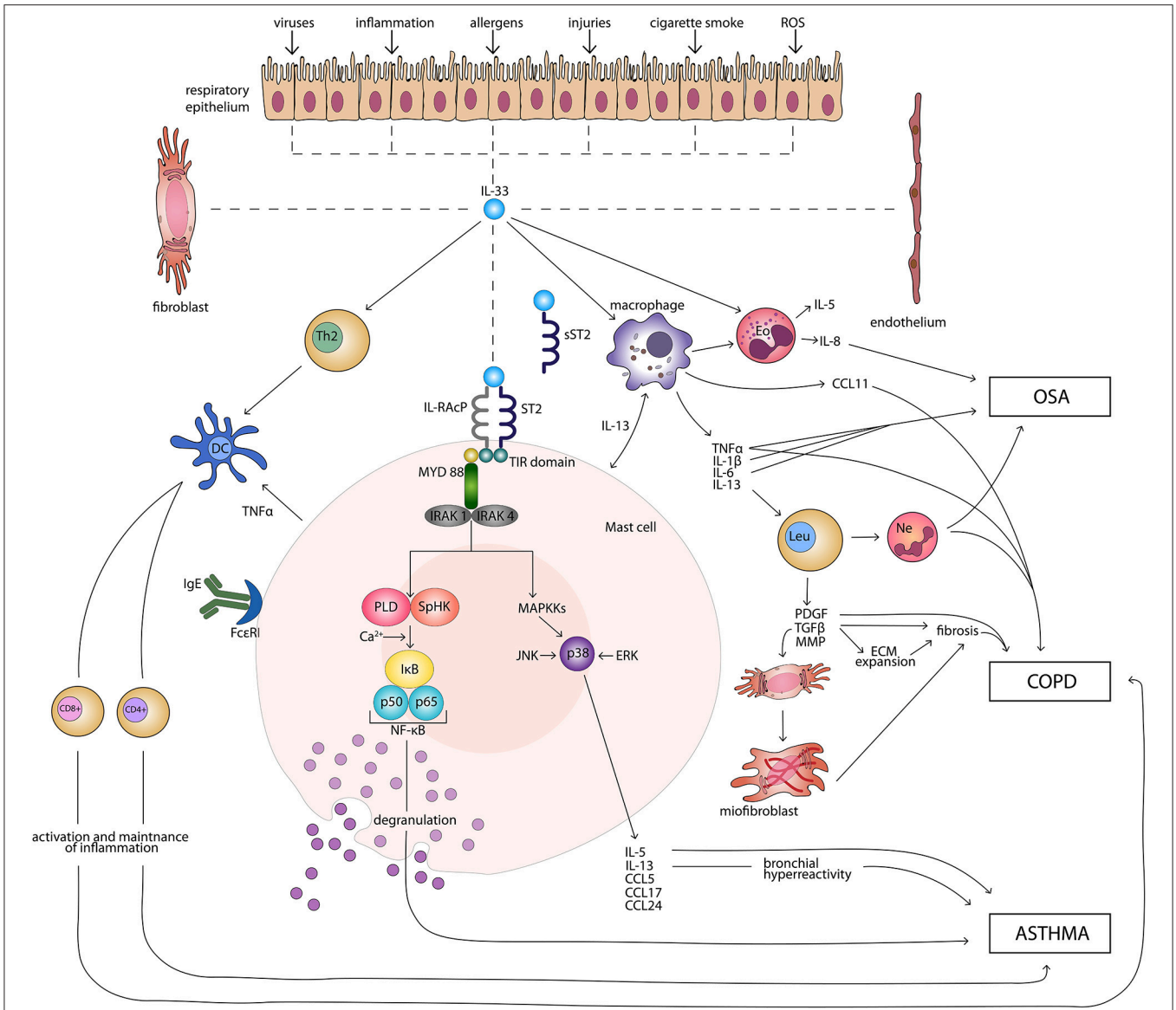

FIGURE 1 | Main directions of IL-33 molecular function in induction and maintenance of chronic respiratory disorders: asthma, COPD, and OSA. CD4+, CD4+ T cell; CD8+, CD8+ T cell; CCL, CC-chemokine ligand; COPD, chronic obstructive pulmonary disease; DC, dendritic cell; ECM, extracellular matrix; Eo, eosinophil; ERK, extracellular signal-regulated kinase; FcεRI, high-affinity receptor for the Fc region of immunoglobulin E; IgE, immunoglobulin E; IκB, inhibitor of κB; IL, interleukin; IL-RAcP, interleukin 1 receptor accessory protein; IRAK, interleukin-1 receptor associated kinase; JNK, c-Jun N-terminal kinases; Leu, leucocyte; MAPKKs, mitogen-activated protein kinase kinases; MMP, matrix metalloproteinases; MYD 88, myeloid differentiation primary response protein 88; Ne, neutrophil; NF-κB, nuclear factor κB; OSA, obstructive sleep apnea; PDGF, platelet-derived growth factor; PLD, phospholipase D; ROS, reactive oxygen species; SpHK, sphingosine kinase; ST2, suppression of tumorigenicity 2 transmembrane receptor; sST2, suppression of tumorigenicity 2 soluble receptor; Th2, T helper 2 cell; TIR, Toll-like/interleukin 1receptor; TGF-β, transforming growth factor β; TNFα, tumor necrosis factor α.

ASTHMA

Among chronic respiratory disorders, the IL-33 contribution to disease development has been furthest identified in bronchial asthma. Increased levels of IL-33 in lung epithelial cells and blood serum has been observed in asthma patients. In several genome wide association (GWA) studies it has been established that both genes encoding IL-33 and its receptor IL1RL1 are susceptibility loci for asthma (50–55). The GWA studies have identified single nucleotide polymorphisms (SNPs) associated with asthma in general, as well as in different clinical phenotypes (56–59). A significant connection was identified, for example, between SNP of IL-33—rs3939286—and atopic asthma, as well as eosinophil blood count (60). Similar observations were made for multiple SNPs in the gene encoding ST2 receptor. One example of the SNP of IL1RL1 that predisposes to both atopic and non-atopic asthma and is connected with eosinophilia, causing an increased level of serum IgE and airway hyperresponsiveness,

is rs950880 for non-Islandic population (in Islandic population it is rs1420101) (60, 61). The SNP (rs10204137) in the Toll-like/IL-1 (TIR) domain of ST2 can enhance the IL-33/ST2L pathway through further cascade (62). What is very interesting, and highlights the role of IL-33 in the development of asthma, is a rare loss of function mutation (rs146587587-C), in which premature STOP codon causes truncation of the last 66 amino acids of the cytokine. This results in a reduced eosinophil blood count and protection against asthma (63).

Several mechanisms in which IL-33 participates in the development of asthma have been described.

It has been shown that IL-33 promotes lung fibrosis and bronchial remodeling, causing further advancement of asthma (64, 65). IL-33 participates in the process of fibrosis by directly activating cells to produce the profibrotic factors as well as by inducing inflammation. Following damage, cells secrete IL-33, which activates a response from cells that possess IL1RL1 receptor. In a Th2 mediated immune response, innate lymphoid cells (ILC) and Th2 cells produce pro-inflammatory cytokines (IL-4, IL-5, and IL-13) (66), which have been identified as important mediators of fibrosis (67, 68). IL-4 and IL-13 are responsible for recruitment of basophils and eosinophils that cause differentiation of fibroblasts to myofibroblasts and collagen deposition (69). Same cytokines induce macrophage polarization to an alternatively activated profibrotic phenotype (44). IL-5 secreted by Th2 cells also takes part in the recruitment and activation of eosinophils, which are the source of profibrotic factors such as TGF-β, PDGF, and IL-13 (70). Interestingly, it has been shown that IL-33 can induce IL-5-producing T cells and promote airway inflammation independent of IL-4, which was shown in IL-4 deficient mice (45).The increased tissue fibrosis can be enhanced by interaction between major histocompatibility complex II (MHCII) of ILCs and Th2 cell receptor (TCR), which activates Th2 cells. Additionally, IL-33 itself recruits basophil, eosinophils, and mast cells to the local inflammatory site and activities them into the production of profibrotic factors (71). Moreover, it was observed that IL-33 directly promotes fibroblasts to produce fibronectin 1 and collagen type 1, factors involved in airway remodeling (65). Interestingly, it has been shown that IL-33, similarly to TGF-β, not only activates the fibroblasts, but also promotes epithelial-to-mesenchymal transition (EMT), which is associated with overexpression of profibrotic markers (72, 73). IL-33 can also contribute to the development of allergen driven airway inflammation by dysregulating Treg and impairing immunologic tolerance to inhaled antigens. Treg cells in lungs exposed to IL-33 not only upregulate their expression of Th2 transcription factor GATA binding protein 3 (GATA3) and ST2, but also enhance production of type 2 cytokines. In effect, Tregs lose their ability to supress effector T cells resulting in development of airway inflammation (74).

Bronchial hyperreactivity is typical in the course of asthma and IL-33 has been identified as inducing this process. It has been shown that through the activation of IL-5 and IL-13 secretion, IL-33 promotes airway hyperresponsiveness (75). It has been suggested that this is dependent on smooth muscle cells (76, 77). Other possible mechanisms involve irritation caused by secretion of serotonin by mast cells following the IL-33 activation (78). Feedback circuit involving both IL-33 and ILCs responsible for bronchial hyperactivity and persistence of asthma has been suggested based on the animal model of the disease (79).

CHRONIC OBSTRUCTIVE PULMONARY DISEASE

Chronic pulmonary obstructive disease (COPD), is a progressive inflammatory condition, in which an increased expression of IL-33 and the ST2 receptor, similarly to asthma, has been observed (27, 80). Cigarette smoke has been identified as the key inducer of COPD. In the mouse model of cigarette smoke-induced COPD, both IL-33 and the ST2 receptor expression was increased in lungs (27, 81, 82). Inhibition of cigarette-induced pathogenic changes in lungs have been observed following introduction of anti-IL-33 antibody (81), which suggests an important role of IL-33 in the pathogenesis of COPD. Furthermore, mice treated with IL-33 developed histopathological changes in the lungs such as lining hypertrophy, goblet cell hypertrophy, and mucus hypersecretion, which are typical for COPD patients (11). Interestingly, cigarette smoke not only activates IL-33 production by epithelial and endothelial cells, but also induces the expression of IL-33 in peripheral blood mononuclear cells (PBMC) (82) and peripheral blood lymphocytes (PBL) (80). This systemic response trough activation of the immune system might enhance processes involved in the development of COPD. Additionally, cigarette smoke alters the distribution of the expression of IL-33 receptor ST2 (21). Decreased expression of the receptor was observed on ILC2s, while macrophages and natural killer cells presented elevated expression of this receptor. This change significantly amplified type I proinflammatory response within the lungs, exaggerating exacerbations of COPD in the course of infections (21). Infections play an important role in progression of COPD and greatly increase the risk of death. Lipopolysaccharide (LPS) has been shown to enhance IL-33 expression and release not only in epithelial cells, but also in PBMC and PBL (80). This suggests that IL-33 is an important mediator of immunological response during inflammations and exacerbations in the course of COPD. It has been widely established that COPD patients suffer from chronic inflammation, which leads to alveolar disruption. It has been shown that IL-33 induces IL-6 and IL-8 production and release in lung epithelial and endothelial cells (82, 83). The same two interleukins have been observed to be increased in bronchoalveolar lavage (BAL) and the lungs of COPD patients, compared to healthy controls (84–86). This causes an influx of neutrophils to the lungs which, through the secretion of elastases and proteases, cause lung tissue damage, further resulting in lung fibrosis and decreased lung function (87). Additionally, it has been shown that IL-33 is involved in the development of eosinophilic airway inflammation in non-atopic COPD patients (88). Moreover, IL-33 plays a part in mucus production, advancing the inflammatory process and decreasing respiratory capacity of COPD patients (27). What is more, IL-33 increases vascular endothelial permeability, which further intensifies inflammatory effect (89). It has been shown that IL-33

can directly contribute to lung tissue damage trough triggering cortactin degradation mediated apoptosis in alveolar epithelial cells (90).

OBSTRUCTIVE SLEEP APNEA

Obstructive sleep apnea (OSA) is a chronic condition characterized by recurrent pauses in breathing during sleep caused by collapse of the upper airways. The disorder is highly associated with chronic low-grade systemic inflammation. The most prominent inflammatory mediators present in OSA patients include IL-1, IL-6, and CRP (91). Literature concerning IL-33 in OSA patients is greatly limited. In recent studies, it has been observed that IL-33 is one of the inflammatory mediators causing levels in blood serum to be increased, in comparison to healthy controls (92). Interestingly, increased IL-33 levels have also been shown in the saliva of OSA patients (93). This emphasizes the contribution of IL-33 to systemic inflammation in this group of patients. Substantial obesity is a well-known risk factor for OSA (94). Excessive amounts of central adipose tissue also contributes to levels of systemic inflammation among OSA (95). Adipocytes have been identified to possess ability to produce IL-33 (96), therefore adipose tissue in OSA patients offers an extensive source of IL-33. Additionally, intermittent hypoxia contributes to systemic inflammation (97). It can possibly affect the IL-33 production too, however the mechanisms of this relationship are not known yet. As described before, IL-33 highly contributes to the development of chronic inflammatory diseases such as asthma and COPD. Several studies have shown increased prevalence of these diseases among OSA patients compared to the general population (98–100). This suggests that OSA patients are vulnerable to the development of comorbidities of inflammatory etiology which are not only limited to respiratory disorders but also others, such as diabetes or psoriasis (97, 101–103). It has been shown that OSA with severe co-morbid asthma intensifies airway remodeling and is associated with more frequent exacerbations (104, 105). Even though IL-33 has not been investigated as a mediator in a group suffering from these two co-morbid diseases, it can be hypothesized that this alarmin enhances airway remodeling and fibrosis occurring in the course of asthma due to its increased level caused by co-morbid OSA. Similar observations have been made regarding co-morbid OSA and COPD, as the BAL fluid of patients with the overlapping syndromes showed a significantly increased proportion of neutrophils, higher TNFα concentrations, and IL-8 levels than that of COPD. Co-morbid OSA exacerbated the course of COPD (106). Yet again IL-33 was not investigated in the study. However, it may again be assumed that systemic inflammation present in OSA with increased IL-33 levels intensifies processes involved in the development and progression of COPD.

IL-33 AS A POTENTIAL THERAPEUTIC TARGET

The murine models of both asthma and COPD suggest that IL-33 and the ST2 receptor might be prominent new therapeutic targets for these chronic inflammatory respiratory diseases. In a murine model of asthma, the use of anti-IL-33 antibody reduced Th2 cytokines production by ILC2 (107), while the antibody blocking ST2 receptor caused a decrease of interleukin 4 expression in the lungs of mice undergoing ovalbumin challenge and extenuated airway hyperresponsiveness (108). In another study a decrease in eosinophil count in BAL fluid and reduced airway hyperresponsiveness to methacholine was observed following anti-IL-33 antibody and sST2 receptors treatment (109). It has been shown, *in vitro*, that pre-treatment with anti-ST2 antibody supressed the production of fibronectin 1 and type I collagen by human lung fibroblasts (65). Anti-IL-33 antibody has also been shown to significantly inhibit cigarette smoke induced lung inflammation as, following the treatment, neutrophil and macrophage infiltration along with cytokines (IL-1β, TNF, and IL-17) expression decreased (81).

Several clinical trials targeting either IL-33 or its receptor in asthma and COPD are ongoing at the moment. In COPD two trials concerning IL-33/ST2 have reached phase 2. One investigates the effects of anti-IL-33 monoclonal antibody compared with placebo, on the annualized rate of moderate-to-severe acute exacerbations of COPD over up to 52 weeks of treatment (ClinicalTrials.gov: NCT03546907). The second evaluates the efficacy of anti-ST2 antibody vs. placebo on the frequency of moderate-to-severe exacerbations of COPD (ClinicalTrials.gov: NCT03615040). At phase 2 of the asthma clinical trial, the anti-IL-33 receptor monoclonal antibody is investigated in subjects with moderately severe asthma, and is compared to the placebo, fluticasone propionate/salmeterol combination and fluticasone propionate (ClinicalTrials.gov: NCT03207243). Another clinical trial concerning anti-IL-33 antibody in asthma patients is in phase 1 and compares it to the placebo Dupilumab and fluticasone propionate (ClinicalTrials.gov: NCT03112577) (**Table 1**).

CONCLUSION

Analysis of the pleiotropic effects of IL-33 on multiple immunological cells (macrophages, mastocytes), as well as

TABLE 1 | Summary of ongoing clinical trials targeting IL-33 pathway in COPD and asthma.

Condition	Intervention	ClinicalTrials.gov identifier	Comparison	Phase
COPD	Anti-IL-33 monoclonal antibody	NCT03546907	Placebo	2
COPD	Anti-ST2 antibody	NCT03615040	Placebo	2
Asthma	Anti-IL-33 receptor monoclonal antibody	NCT03207243	Placebo/fluticasone propionate with salmeterol/ fluticasone propionate	2
Asthma	Anti-IL-33 monoclonal antibody	NCT03112577	Placebo/Dupilumab/ anti-IL-33 antibody with Dupilumab/fluticasone propionate	1

neurological cells of medulla oblongata, dorsal root ganglion, antigen-induced arthritis system, carrageen, and formalin, shows that this alarmin plays curtail, yet not fully known role in mediating inflammation, especially in chronic inflammatory pulmonary diseases such as asthma, COPD, and OSA. Taken into consideration the engagement in this process, in particular of mastocytes and their secretion of CXCL2, 4, 8, and other cytokines, there is no doubt regarding the etiopathogenic role of IL-33 in the development of asthma in response to various stimuli damaging bronchial epithelial cells. Additionally, IL-33 intensifies recruitment of eosinophils, macrophages, and Th2 lymphocytes, which again confirms its inflammatory role. Particularly important in the context of aggravation of chronic inflammation and progression of respiratory disease is IL-33 mediated influx of neutrophils and macrophages. As these cells secrete IL-1β, TNF-α, and release proteases (elastases, metalloproteinases, cathepsins, and proteinases), it is only a logical consequence that IL-33 is involved in development of lung emphysema and chronic bronchial inflammation. It is worth mentioning that IL-33 expression is enhanced following exposure to cigarette smoke, which correlates with increased number of lymphocytes CD8+, resulting in the release of perforin and granzymes. However, surprisingly, this model of COPD development was only confirmed during experiments on mice and was observed following the introduction of anti-IL-33 antibody. Among discussed respiratory disorders, the role of IL-33 in OSA is the least known. The alarmin indirectly increases the expression of IL-1, IL-6, CRP, and enhances systemic inflammation present in OSA. Importantly, adipocytes have been identified to possess the ability produce IL-33, which in case of OSA patients seems crucial as the vast majority of them are obese, making adipose tissue an considerable source of the interleukin. However, the effect it exerts is not yet fully understood, and further research is needed, especially focusing on the mRNA expression of chosen genetic markers and animal models. Nevertheless, IL-33 through the intensification of TGF-β expression is involved in the stimulation of fibrosis and bronchial remodeling, including EMT, which might further contribute to the development and comorbidity of asthma and OSA.

The mechanisms of IL-33 activity described in this short review highlight its important role in the development of multidirectional inflammation. Therefore, direct or indirect blockade of this cytokine might greatly increase conservative therapy quality among patients with chronic respiratory diseases. At the moment, there are clinical trials involving anti-IL-33 antibodies which are very promising. Nonetheless, authors of this review indicate a necessity to undertake new research focusing on the development of peptide inhibitors for the ST2 receptors. The establishment of several interface regions between IL-33 and ST2 receptor, based on the available complex crystallographic structure, enables the creation of peptide library *in silico* through bioinformatics methods. Creation of peptide domain for the interface of IL-33 may be a universal method of safe, non-toxic and well-tolerated treatment for patients suffering from chronic inflammatory diseases of the respiratory system. At present, there is no investigation carried out in the direction suggested by the authors.

AUTHOR CONTRIBUTIONS

AG and MP created the concept of the paper. AG conducted the literature research and wrote the manuscript. PK, AA, PB, and MP revised the paper.

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IL-33 and IL-18 in Inflammatory Bowel Disease Etiology and Microbial Interactions

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The IL-1 cytokines are a newly expanded family, with each of its 11 members playing an important role in health and disease. Typically acting as pro- or anti-inflammatory mediators of first-line innate immunity, their production is particularly important in the context of mucosal defenses, through handling breach of the delicate epithelial barrier and mediating a local immune response to invading pathogens. Mucosal immunity is often aberrantly orchestrated in intestinal diseases, such as Inflammatory Bowel Disease (IBD). Various studies have pointed to IL-1 cytokines as being important players in IBD with context-dependent roles, either through promoting auto-inflammatory mechanisms, or alleviating disease through protection against breach of pathogens across the epithelial barrier. This mini-review will succinctly examine the role of IL-1 family members in IBD, with a special focus on the recently described IL-33 as well as IL-18, and will explore the disease models within which these cytokines have been studied. Furthermore, we will examine the evidence of interplay of these cytokines with the gut microbiota, with hopes of summarizing our current knowledge of these family members and their potential for unraveling novel molecular mechanisms of IBD pathology.

Keywords: IBD, IL-18, IL-33, colitis, microbiota

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INTRODUCTION

The IL-1 family of cytokines primarily act on innate immunity to initiate inflammation in the face of local insult, thus playing a particularly important role in the pathophysiology of mucosal diseases. Members of the IL-1 family of cytokines play important, yet context-dependent roles in intestinal homeostasis and inflammation. In this review we will examine two IL-1 family cytokines, IL-33 and IL-18, in the context of mucosal immunity and with a particular focus on the pathogenesis of IBD, a chronic inflammation of the intestinal mucosa. This review will explore their role in host immunity, and describe associations of these cytokines with the host microbiota, a major component in IBD etiology. Together this review will summarize our knowledge of these newly described cytokines, and present an outlook on their individual and complex roles in IBD (**Table 1**).

IL-33

IL-33: An Alarmin in Mucosal Immunity

The IL-1 family member IL-33 plays a unique and essential role in mucosal, front-line immunity. Previously known as IL-1F11, IL-33 is a relatively newly described cytokine, with origins tracing

TABLE 1 | Known effects of IL-18 and IL-33 with implications for IBD pathogenesis.

Cytokine	Experiment	Observed effect	Implication for cytokine in IBD
IL-18			
	Activity blocked by IL-18 binding protein	Reduced small intestinal pathology caused by *T. gondii* infection *ex vivo* (25)	Detrimental
	Activity blocked by IL-18 binding protein	Attenuated DSS-colitis (26)	Detrimental
	Increased expression	Decreased butyrate producers in microbiota, with subsequent exacerbation of colitis (27)	Detrimental
	Knock-out	Protected against DNBS-induced disease in both single KO and double KO with IL-1β (28)	Detrimental
	Overexpression in enterocytes	GI tract overexpression promoted eosinophilic inflammation in rats (29)	Detrimental
	Targeted inhibition	Inflammatory mucositis alleviated in mice (30)	Detrimental
	Receptor knock-down	Protected against DSS-induced colitis in mice (31)	Detrimental
	Treatment with recombinant IL-18	Increased neutrophil transmigration across $Caco_2$ monolayer through Occludin loss (32)	Detrimental
IL-33			
	Deletion of nuclear sequestration signal	Lethal inflammation dependent on signaling through ST2 (33)	Detrimental
	Knock-out	Impaired recovery from extended DSS-colitis in mice (34)	Protective
	Receptor knock-out	Reduction in myeloid precursors of inflammation (35)	Detrimental
	Receptor signaling blockade	Alleviation of colitis in SAMP mice (23)	Detrimental
	Treatment with recombinant IL-33	Alleviation of TNBS colitis in mice through polarization of homeostatic M2 macrophages (19)	Protective
	Treatment with recombinant IL-33	Alleviation of chronic colitis in mice, reduced bacterial translocation (36)	Protective
	Treatment with recombinant IL-33	Reduced colitis severity in mice in an IL-10 dependent manner (37)	Protective
	Treatment with recombinant IL-33	Aggravated acute colitis (24)	Detrimental

back to 2005 (1). It was discovered after the characterization of its cognate receptor, suppressor of tumorigenicity 2 (ST2) (2). IL-33/ST2 signaling not only acts as a front-line herald of tissue damage, but also links innate and adaptive immunity at the host mucosae through potent induction of a type 2 response in T cells, innate lymphoid cells (ILCs) and macrophages (3–5). Despite potentially playing an important role as a mediator of mucosal immunity, and being suggested as a drug target for various disorders, there are currently no IL-33-based therapies for intestinal disease. This presents an interesting opportunity for study of this cytokine and its role in IBD.

The most well-characterized aspect of IL-33 biology is its role as an alarmin: a molecular "fire-alarm" at the barrier tissues of the body, driving inflammatory and fibrotic processes during acute mucosal breach due to cell injury (6). IL-33 is constitutively expressed in epithelial and endothelial cells, and following translation is stored as a full-length, biologically active molecule in the nucleus where it binds to chromatin (7). Following lysis of the cell through destructive mechanisms, IL-33 in the nucleus is immediately available to act as an early signifier of damage, through recruitment of neutrophils, eosinophils, natural killer (NK) cells, and by amplifying a type 2 (Th2, ILC2, M2-like macrophage) response in order to initiate fibrosis and wound healing (8, 9). Interestingly, not only being important for primed release of the cytokine, sequestration of IL-33 in the nucleus allows it to act as a transcriptional regulator, where it can bind to the p65 subunit of NFκB to activate endothelial cells (10). Unlike other members of the IL-1 family, IL-33 does not require processing through an inflammasome in order to achieve biological activity and in fact is inactivated by caspase cleavage (11). However, N-terminal cleavage by neutrophil elastase and cathepsin G proteases, which are found in the microenvironment during inflammation, can increase its potency (12). This again highlights the primary role of IL-33 in orchestrating the response to cellular destruction.

IL-33 in Intestinal Disease

Expression of IL-33 and its receptor ST2 has been well-established in the GI tract, being an important amplifier of innate immunity at the gut mucosa (13). While IL-33 is expressed largely at the mucosae and in myofibroblasts, its receptor is expressed mainly on immune cells, such as ILC2s, Tregs, T helper cells, and CD8+ T cells (14) This allows IL-33/ST2 signaling to act as a bridge between tissue damage and immune system orchestration, which may be a critical component in intestinal immunity. In an experiment whereby the N-terminus of IL-33 was altered such that it could not associate with chromatin, the result was the formation of a whole-body inflammatory response with splenomegaly, increased lymph node infiltration and indeed development of colitis (7). This response was then ablated by knock-down of the ST2 receptor. Not only does this demonstrate the importance of nuclear sequestration of IL-33 but also highlights the potency of IL-33/ST2 signaling in the acute inflammatory response and a potential role in intestinal disease.

IL-33 presents an interesting role in IBD, which is perhaps complicated by the divergent immune pathophysiologies of

Crohn's disease (CD) and Ulcerative Colitis (UC). Studies of patient biopsies have shown an increase in IL-33 levels in patients with active IBD, in particular UC, compared with healthy controls (15). Interestingly, UC-associated IL-33 is found in myofibroblasts, which tend to localize at the base of inflamed ulcerations during disease (16). Furthermore, blockade of IL-33/ST2 signaling has been shown to alleviate active disease, suggesting a pathogenic role for the cytokine (17).

In contrast with these findings, IL-33-deficient mice have been shown to be highly susceptible to colitis and colorectal cancer, which would suggest a role as an important protective mediator of intestinal immunity (18). This offers a contradictory and complex role for IL-33 in IBD. This is evidenced by results from various mouse models of disease, including but not limited to the most widely used methods of dextran-sodium sulfate (DSS)-induced and trinitrobenzene sodium (TNBS)-induced colitis. While DSS-induced colitis is largely T cell independent and mediated by chemical damage, TNBS colitis development is dependent on induction of a Th1 response. Given its association with boosting type 2 immunity, IL-33 may act as a "balancing" cytokine in TNBS colitis. This has been shown in a model of TNBS colitis, whereby recombinant IL-33 administration was shown to attenuate disease development through induction of M2-like macrophage polarization (19). Furthermore, IL-33 has been found to ameliorate TNBS colitis, in a manner that was Foxp3 dependent, through promoting a Th2 and Treg response (20). Agreeing with this, IL-33 has been shown to enhance Foxp3+ Treg cell expansion in the intestine via TGF-β (21). Attempting to clarify this, another study employed the use of SAMP colitis mice, which are characterized by development of T cell-driven enteric inflammation. SAMP mice show an early Th1 stage, which is followed by establishment of a chronic Th2-mediated disease (22). UC-like disease in SAMP mice was found to correlate with expression of full-length IL-33 in IECs, and blockade of the IL-33/ST2 signaling pathway was beneficial (23). This demonstrates a somewhat complex role for IL-33 in IBD, wherein its effector role may be determined by the T cell response pattern and intrinsic differences in CD and UC immunology.

In the context of DSS-induced colitis, IL-33 plays a varying role depending on the temporal stage of the disease. For instance, one particular study showed that administration of recombinant IL-33 exacerbated acute colitis, but ameliorated colitis in a chronic model of disease in a manner dependent on amphiregulin-EGFR signaling (24). IL-33 induced neutrophil influx during both stages of disease, which may have contributed to its exacerbating effects on acute colitis through nitric oxide (NO) immunopathology, but was shown by the authors of the study to reduce translocation of pathobionts across the impaired epithelium during chronic disease. This divergence, even through use of the same disease agent, points toward IL-33 expression patterns as an important tool for study of the early and late immune response in IBD, suggesting that despite its role in promoting acute inflammation, it may act to limit chronic inflammation in long-term disease.

IL-18

IL-18 as a Pro-inflammatory Regulator

The role of IL-18 in intestinal disease is largely related to its activity in regulating pro-inflammatory responses. Discovered in 1989 in mouse serum following challenge with bacterial LPS, IL-18 was first identified as a booster of IFNγ activity produced by monocytic cells (38). To compare it with its sister cytokines, IL-18 most similarly resembles IL-1β, in both structure and their association with the inflammasome, a proteolytic complex through which IL-1β and IL-18 precursor molecules become biologically active by caspase-1 cleavage. GI commensals are critically important in regulating intestinal inflammasome assembly, and this has been demonstrated in new-born mammals that feature progressive microbial colonization, accompanied by intestinal barrier formation and immune system maturation. This perhaps further highlights a potential role for aberrant IL-18 in IBD, a disease characterized by an excessive inflammatory response to microbial products. Furthermore, loss-of-function single nucleotide polymorphisms (SNPs) in the IL-18 gene result in an imbalance of the Th1/Th2 response, which promotes host susceptibility to CD (39). Other meta-analysis studies have supported this observation (40).

IL-18 is generally a pro-inflammatory mediator, and its production may be a key etiological factor for patients with IBD (41, 42). Pro-IL-18 is produced by a wide range of cell types, including epithelial cells, myeloid cells and lymphocytes, and following inflammasome activation carries out a wide range of effector functions, including promoting the production of IFNγ, priming of NK cell cytotoxicity (43) and stimulating the differentiation of Th1 cells (44). Despite this, the NLRP6 inflammasome itself is a known regulator of colonic homeostasis, predominantly expressed in intestinal epithelial cells (IECs) with a key role in mucosal renewal, proliferation and secretion (45). Interestingly, deficiency in the NLRP6 inflammasome is detrimental in DSS-induced colitis, in a manner related to insufficient IL-18. Likewise, caspase-1 deficiency is linked to increased DSS severity. These reports would suggest that while IL-18 is a pro-inflammatory mediator, its baseline activity is important for intestinal integrity through unknown mechanisms.

On the other hand, IL-18 has been also shown to contribute to the breakdown of the mucosal barrier, provoking inflammation and amplifying damage elicited to the intestinal epithelium during disease. Clinical studies have correlated increased epithelial secretion of IL-18 with increased severity of IBD (46). This study was supported by Nowarski et al., where deletion of IL-18 receptor (IL-18R) from IECs shielded mice from DSS-induced colitis (31). Transgenic mice deficient in IL-1β, IL-18 or both cytokines protected against TNBS colitis induction in mice (28). Thus, the double knockout increased the protective effects against intestinal inflammation, perhaps due to the inhibition of two converging inflammatory pathways. IL-18 production is elevated in IECs following infection with human immunodeficiency virus (HIV), causing IEC apoptosis through the activation of caspase-1 and caspase-3 (47), two programmed cell death proteases. This study also described how IL-18 disrupts the tight junctions that maintain intestinal

epithelium integrity. Collectively, these results suggest that IL-18 overproduction generally contributes to an increase in the permeability of the intestinal monolayer, exacerbating intestinal inflammation. Contribution to inflammation in such a regard presents an opportunity to alleviate IBD symptoms, which is being exploited through development of a monoclonal antibody against IL-18. This is currently in phase one of clinical trials (clinicaltrials.gov ID: NCT01035645).

IL-18 AND IL-33 IN HOST-MICROBE INTERACTIONS

The current working hypothesis for the etiology of IBD focuses on the loss of host tolerance for the resident microbiota. As such, an exploration of the role of IL-18 and IL-33 should critically examine their known associations with the microbiota, and the ways in which this may alter host pathology during IBD. The role of IL-18 in this regard is well-established in the literature. Indeed, intestinal IL-18 levels progressively increase over the first 5 weeks of post-natal development, harmonizing with microbial colonization (48). Levy et al., demonstrate how gut microbiota bi-products, such as metabolites, influence NLRP6 inflammasome signaling, IL-18 epithelial cell secretion and downstream secretion of anti-microbial peptides (AMPs) (49). This study is supported by Elinav et al., where NLRP6-inflammasome deficient mice had impaired production of IL-18 and concomitant dysbiosis, characterized by an expansion of the bacterial phyla Bacteroidetes (*Prevotellaceae*) and TM7 (50). This deficiency in NLRP6 and IL-18 results in a failure to produce AMPs, which are essential in mediating pathogenic bacterial clearance. Recently, NLRP6 was shown to prevent the colonization of IBD-inducing bacteria, the mucolytic *A. muciniphila*, which can induce colitis in both specific pathogen free and germ free (GF) $IL10^{-/-}$ mice. This is mediated by IL-18 (51).

Interestingly, GF mice colonized with a complex human gut-derived microbiota see a transient increase in IL-18 without tissue damage or inflammation, suggesting an ongoing homeostatic response (52). IL-22 is a classic Th1-cell-associated pro-inflammatory cytokine that exacerbates ileitis following infection with protozoa, e.g., *Toxoplasma gondii* (53). Interestingly, IL-22 knockout mice were associated with reduced production of Th1-promoting IL-18. IL-22 not only influenced the expression of IL-18 messenger RNA (mRNA) in IECs after infection with *Toxoplasma gondii* or *Citrobacter rodentium* infection, but was found to promote a homeostatic production of active IL-18 in the ileum (54), which not only is crucial for host defense but also contributes to inflammation, while assisting in microbial clearance. This suggests a double-edged sword role for IL-18, whereby it contributes to inflammation but in doing so, protects the host from pathogenic invasion.

Conversely, very little is currently known about IL-33 crosstalk with the gut microbiota, however some studies have suggested a potential for interaction. One such study in SAMP mice showed that IL-33 is induced in ex-GF mice following transplantation of commensals (55). Furthermore, IL-33 expression has a number of known interactions with the microbial-sensing Toll-like receptors (TLRs), and is indeed induced by pathogen-associated molecular patterns (PAMPs), perhaps as a protective mechanism of the host (56). Further highlighting its potential for enabling host-microbiota crosstalk, IL-33/ST2 interactions can repress the TLR2 pathway through sequestration of the adaptor molecule MYD88 (57).

The susceptibility of IL-33 deficient mice to colitis has been shown to be a result of the ability of IL-33 to promote IgA production from B cells, which notably plays a major role in maintaining microbial homeostasis in the intestine (18). This suggests that IL-33 may indirectly alter the microbiota to protect against colitis through promotion of IgA production, which is already known to be a protective factor in IBD. Supporting this, IL-33 deficient mice developed dysbiosis, characterized by increased levels of mucolytic and colitogenic bacteria, which drastically altered the microbial landscape of the gut making the mice more susceptible to colitis. This effect was found to be dependent on IL-1α release, and colitis susceptibility in IL-33 deficiency could be reversed through either reconstitution of a homeostatic microbiota or by IL-1α ablation. This provides a potential role for IL-33 to quell the colitogenic effects of its more sinister sibling cytokines.

CLOSING REMARKS

In summation, the IL-1 family of cytokines are regulators of mucosal immunity in a manner that appears highly context-dependent. IL-18 itself is a pro-inflammatory mediator capable of exacerbating disease, despite evidence that it can promote homeostasis in some circumstances. The role of IL-33 in IBD is more complex, and perhaps related to disease stage. While both of these cytokines promote early pro-inflammatory responses to effect front-line protection against mucosal breach and pathogenic invasion, their baseline expression is indeed important for the maintenance of overall intestinal integrity. As a result, poorly timed or excessive production of bioactive IL-1 family members may provide a key step in IBD development, with vast potential for therapeutic intervention.

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MW, AO, and SC developed, wrote, and revised the content in this manuscript.

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REVIEW
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Divergent Roles for the IL-1 Family in Gastrointestinal Homeostasis and Inflammation

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Inflammatory disorders of the gastro-intestinal tract are a major cause of morbidity and significant burden from a health and economic perspective in industrialized countries. While the incidence of such conditions has a strong environmental component, in particular dietary composition, epidemiological studies have identified specific hereditary mutations which result in disequilibrium between pro- and anti-inflammatory factors. The IL-1 super-family of cytokines and receptors is highly pleiotropic and plays a fundamental role in the pathogenesis of several auto-inflammatory conditions including rheumatoid arthritis, multiple sclerosis and psoriasis. However, the role of this super-family in the etiology of inflammatory bowel diseases remains incompletely resolved despite extensive research. Herein, we highlight the currently accepted paradigms as they pertain to specific IL-1 family members and focus on some recently described non-classical roles for these pathways in the gastrointestinal tract. Finally, we address some of the shortcomings and sources of variance in the field which to date have yielded several conflicting results from similar studies and discuss the potential effect of these factors on data interpretation.

Keywords: cytokine, inflammation immunomodulation, gastrointestinal, interleukin-1, inflammatory bowel conditions

TOLEROGENIC MECHANISMS OF THE GASTROINTESTINAL TRACT:

The gastrointestinal tract (GIT) is the largest internal organ in the human body with an estimated surface area in excess of 400 m^2 and has evolved to meet several physiological and biological needs. However, in order to meet these demands, part of the evolutionary process of the GIT has been to facilitate colonization by a vast repertoire of mutually beneficial micro-organisms which aid in the metabolism of certain dietary components and are of fundamental importance to the overall well-being of the host. These microbes, which include fungi, bacteria, viruses, and bacteriophages, are collectively referred to as the commensal microbiota or microflora and together with the

metabolites they produce form the host microbiome. In addition to aiding in the breakdown and absorption of dietary factors, these microbes also actively compete with enteric pathogens for essential nutrients and environmental niches and facilitate the development and maturation of a robust mucosal immune repertoire. Indeed studies with germ-free mice have highlighted that the lack of a commensal microbiota in these animals results in significant barrier defects and enhanced intestinal permeability. However, given their constant presence and the enormity of the antigenic burden faced by the GIT, robust mechanisms have evolved to prevent aberrant effector immune responses to these innocuous antigens (**Figure 1**).

While distinct regions of the GIT differ in terms of their anatomical, physiological and immunological make-up, consistent throughout is the presence of a single layer of columnar intestinal epithelial cells (IECs) which act as a physical blockade between the luminal compartment and the typically sterile underlying sub-epithelial layers (1). In an effort to preserve homeostasis, these IECs are typically hypo-responsive to innate stimuli such as the bacterial pathogen-associated molecular pattern (PAMP) lipopolysaccharide (LPS) (2, 3). This is achieved in part by the compartmentalization and spatio-temporal organization of various pathogen recognition receptors (PRRs), including several toll-like receptors (TLRs), in these cells. Indeed the expression of several TLRs is of restricted to the baso-lateral cell membrane IECs, away from the plethora of antigens to which the apical surface of these cells is exposed.

The term "brush border" has been used to describe the physical appearance of the small intestinal epithelium as the columnar enterocytes have protrusions called microvilli on their apical surface. These microvilli not only aid in physiological processes such as peristalsis and mucous sloughing, but also provide additional surface area for the absorption of dietary nutrients. Furthermore, these villous protrusions harbor digestive enzymes and transporter channels which are essential for host metabolism and osmotic homeostasis. Overlying the microvilli is a thick glycocalyx consisting of mucins and other glycoproteins which helps to prevent colonization of the intestinal epithelium by commensal bacteria and potential pathogens alike. Under steady-state conditions in a healthy GIT, adjacent IECs are closely associated to one another by tight junction proteins, thereby preventing the passive transfer of antigen-bearing material from the microbe-rich lumen into the comparatively sterile sub-epithelial layer. As a result, the sampling of luminal contents does not occur randomly but is instead primarily restricted to highly specialized microfold or M cells, so called due to their unique morphological features which includes short, loosely packed microvilli and a lack of surface glycocalyx. These cells are located in the follicle associated epithelium (FAE) which overlies lymphoid structures known as Peyer's patches (PPs), one of the primary immune-inductive sites of the gut-associated lymphoid tissue (GALT). Their differentiation and functional maturation from leucine-rich repeat containing G protein-coupled receptor positive (Lgr^+) stem cells is driven by the receptor activator of NF-κB ligand (RANKL), expression of which is typically restricted to stromal cells situated directly beneath the FAE. Signaling of RANKL through its receptor RANK, which is expressed by all epithelial cells, induces the functional maturation of M cells through up-regulation of the transcription factor Spi-B (4, 5). As a result, somewhere in the region of 10% of epithelial cells of the murine FAE can be identified as M cells at steady state (6). In addition to M cells, a certain degree of antigen sampling has also been reported to occur via goblet cell passages as well as directly from the lumen by $CX3CR1^+$ mononuclear phagocytes which have been shown to extend their dendrites through epithelial tight junctions without compromising barrier integrity (7–10).

As one of the primary portals of entry for potentially harmful pathogens, the immune system of the GIT must be capable of responding rapidly in the event of a barrier breach. Integral to this initial response are a number of T cell subsets which reside within, or in close proximity to, the epithelial layer and are therefore referred to as intra-epithelial lymphocytes (IELs). These IELs can be broadly sub-divided into two main groups, conventional T cell receptor (TCR) αβ cluster of differentiation 4 positive (CD4+) or CD8αβ-expressing cells ($TCR\alpha\beta^+$ $CD4^+$ or $TCR\alpha\beta^+$ $CD8\alpha\beta^+$ respectively), or unconventional T cells expressing an invariant heterodimeric TCR consisting of gamma and delta chains - γδ T cells - or $TCR\alpha\beta^+$ cells expressing a homodimeric CD8αα co-receptor, both of which have a divergent ontogeny to conventional IELs (11). In addition to these IELs, the GIT harbors an abundance of other atypical lymphoid cells which are primed to respond rapidly to various stimuli and include lineage negative (Lin^-) innate lymphoid cells (ILCs), as well as non-classical major histocompatibility complex (MHC)-restricted invariant natural killer T (iNKT) cells and mucosal-associated invariant T (MAIT) cells, which recognize antigens presented by cluster of differentiation 1d (CD1d) or major histocompatibility complex-related molecule 1 (MR1) (12–15). Collectively, these cells serve as the first responders to microbial colonization or epithelial damage by rapidly secreting cytokines which in turn stimulate the production of anti-microbial peptides (AMPs) or bolster epithelial integrity (16–18). However, in order to prevent aberrant effector immune responses to the plethora of innocuous stimuli to which the GIT is constitutively exposed, it is essential that all of the aforementioned processes are tightly regulated so that immune quiescence can prevail under steady-state conditions. Furthermore, the lamina propria (LP) of the GIT is populated by highly specialized tolerogenic dendritic cells (DCs), which preferentially drive the differentiation of anti-inflammatory regulatory T cells (T_{REGS}) via their expression of vitamin A metabolizing enzymes and transforming growth factor beta (TGFβ)-activating integrins (19–23). Disruption in one or more of these aforementioned tolerogenic mechanisms can result in a number of gastrointestinal disorders including, but not limited to, bacterial overgrowth, food intolerance and/or inflammatory bowel disease (IBD). The latter of these conditions consists clinically of Crohn's Disease (CD) and Ulcerative Colitis (UC), two distinct afflictions both characterized by chronic relapsing and remitting inflammation of the entire GIT or colon, respectively. Recent reports have identified particularly high incidences of IBD in Europe and North America, with an estimated incidence of 300–500 per 100,000 individuals (24).

The identification of environmental or genetic factors correlating with disease susceptibility and the elucidation of the exact mechanisms responsible for disease pathogenesis is essential for the future development of efficacious therapies.

The interleukin (IL)-1 family of cytokines and receptors have been extensively studied in the context of IBD as a result of their known role in the etiology of several inflammatory disorders. Therapeutic targeting of these pathways has been investigated for the amelioration of IBD symptoms, with variable results observed to date. Here we shall discuss the current literature as it pertains to the IL-1 family and its dichotomous roles as both a regulator of intestinal immune homeostasis and driver of inflammatory responses.

THE IL-1 FAMILY

The IL-1 family consists of 11 distinct members comprising the immunomodulatory cytokines, IL-1α, IL-1β, IL-18, IL-36α, IL-36β, IL-36γ, and IL-33 in addition to four natural antagonists—IL-1Ra, IL-36Ra, IL-37, and IL-38 as outlined in **Table 1**. Several of the family members exhibit remarkable sequence homology to one another and contain several conserved primary structural features as outlined in **Figure 2**. Indeed the genes encoding almost all of these cytokines, with the exception of *IL33* and *IL18*, cluster to a small section of chromosome 2 in humans (25). IL-1 family cytokines are produced by a vast repertoire of immune cells including monocytes, DCs, macrophages, natural killer (NK) cells, activated T and B cells, as well as non-hematopoietic cells including epithelial cells and keratinocytes in response to pathogen- and damage-associated molecular patterns (PAMPs and DAMPs), as well as other cytokines, in particular Tumor Necrosis Factor (TNF) (26).

As reviewed extensively elsewhere, receptors of the IL-1 family share remarkable structural similarity to those of the Toll-like receptors (TLRs). As a result, these two families are often grouped together as the Toll/IL-1 Receptor (TIR) superfamily (27). **Figure 3** highlights some of these similarities, with each receptor consisting of an extracellular ligand-binding domain, a transmembrane helix and an intracellular TIR domain which is essential for signal transduction. Additionally, several members of both families use myeloid differentiation primary response 88 (MyD88) as an adaptor protein for downstream signal propagation and they all utilize the IL-1 receptor accessory protein (IL-1RAcP). Specific sequence residues in the extracellular immunoglobulin-like domain of IL-1 family receptors confers specificity for their cognate ligands (28). The outcome of signaling through several IL-1 receptors (IL-1Rs) is similar to MyD88-dependent TLR signaling, due to the significant homology between the IL-1R and the TLRs which both contain cytosolic TIR domains capable of interacting with MyD88 (29). The binding of IL-1 to the type I IL-1R (IL-1R1), enables the receptor complex to recruit and bind to IL-1RAcp (30). This dimerization event is essential for the subsequent transduction of the IL-1 signal downstream, culminating in the expression of several IL-1-dependent pro-inflammatory cytokines (31, 32). Furthermore, signaling molecules such as the IL-1R-associated kinases (IRAKs) and TNF receptor-associated factor 6 (TRAF6), also present in many of the TLR signaling pathways, are also essential for IL-1 signaling. Therefore, it is not surprising that signaling through both TLRs and the IL-1R culminates in the expression of genes encoding pro-inflammatory cytokines and chemokines through activation of nuclear factor kappa B (NFκB) and mitogen-activated protein (MAP) kinases.

Signaling through the IL-1R transduces a multitude of signals many of which are capable of modulating both local and systemic immune responses. IL-1β is a highly pleiotropic cytokine and prior to the introduction of the interleukin

TABLE 1 | Summary of the IL-1 family of cytokines in humans.

Cytokine:	Receptor	Accessory protein	Antagonist	Source	Targets
IL-1α/β (*IL1A/IL1B* or *IL1F1/IL1F2*)	IL-1RI (*IL1R1*)	IL-1RAcP (*IL1RAP*)	IL-1Ra (*IL1F3*), IL-1RII (*IL1R2*), sIL-1RII, SIGIRR (*SIGIRR*)	IECs, DCs, mø, $CD4^+$ T cells	DCs, stem cells, neutrophils, ILC3s, mø, monocytes, TCRαβ, TCRγδ, B cells
IL-18 (*IL18* or *IL1F4*)	IL-18Rα (*IL18R1*)	IL-18Rβ (*IL18RAP*)	IL-18BP (*IL18BP*)	IECs, mø, DCs	Neutrophils, mø, NK, endothelial, smooth muscle, T and B cells
IL-33 (*IL33* or *IL1F11*)	ST2 (*IL1RL1*)	IL-1RAcP (*IL1RAP*)	sST2	IECs, mast cells, DCs, endothelial cells, astrocytes, cardiomyocytes	ILC2s, $CD4^+$, $CD8^+$ T cells, keratinocytes
IL-36α, β, γ (*IL36A/B/G* or *IL1F6/IL1F8/IL1F9*)	IL-36R (*IL1RL2*)	IL-1RAcP (*IL1RAP*)	IL-36Ra (*IL1F5*), IL-38	Epithelial cells, keratinocytes, DCs, mø	Keratinocytes, monocytes, DCs, $CD4^+$ T cells
IL-37 (*IL37* or *IL1F7*)	IL-18Rα (*IL18R1*)	SIGIRR (*SIGIRR*)	N/A	NK cells, monocytes, B cells	DCs, T cells, endothelial cells
IL-38 (*IL1F10*)	IL-36R (*IL1RL2*)	IL-1RAcP (*IL1RAP*)	N/A	Apoptotic mø, cancerous cells	T cells, endothelial cells, mø

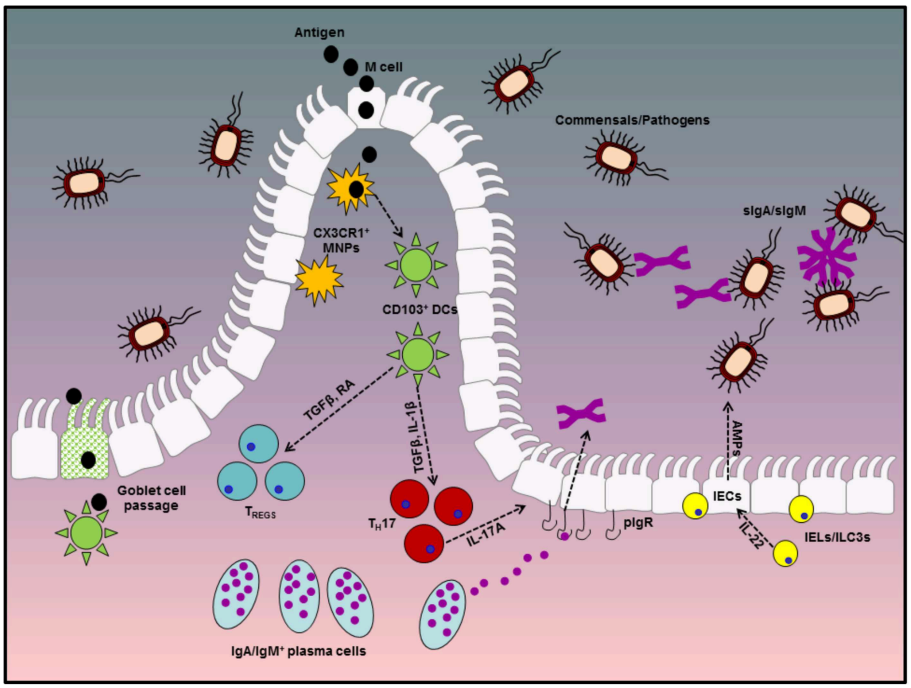

FIGURE 1 | Diagrammatic representation of tolerogenic mechanisms in the gastrointestinal tract. Homeostasis at the gastrointestinal mucosa involves several specialized immune cells and cross-talk with symbiotic microbes in the lumen. The first line of defense is provided by antibodies in the gut lumen, which are transported across the epithelial barrier via the pIgR, in conjunction with IEC-derived AMPs, production of which is stimulated by IEL or ILC-derived IL-22. Beneath the epithelial layer, the intestinal lamina propria is home to mononuclear phagocytes which can capture antigen either directly from the lumen via trans-epithelial extension of dendrites or through M cell passages. These MNPs can in turn pass antigenic material to migratory $CD103^+$ DCs which favor a tolerogenic immune response through their ability to metabolize vitamin A into retinoic acid and activate latent TGFβ, both of which are involved in the differentiation of T_{REGS} and IgA class switch recombination in B cells. T_{REGS} and IgA-secreting plasma cells play fundamental roles in remaining tolerant to innocuous antigens from diet and the microbiota and dysregulated responses in either of these factors significantly enhances susceptibility to enteric inflammatory disorders.

nomenclature, IL-1β was given the name leukocyte activating factor (LAF) due to its broad range of downstream effects. Not only is it a key cytokine involved in both chronic and acute inflammation (33, 34), it is also capable of increasing the expression of adhesion molecules on endothelial cells, inducing the functional maturation of DCs, driving the recruitment and activation of lymphocytes and NK cells while also serving as an endogenous pyrogen with the potential to cause fever (26). In addition to these classical pro-inflammatory functions, several IL-1 family cytokines are capable of modulating downstream effector T cell responses by directing their differentiation down different pathways. For example, IL-1β is known to drive the differentiation of T_H17 cells whereas IL-18 and IL-33 favor the expansion of T_H1 and T_H2 cells, respectively (35, 36). Additionally, these cytokines can also elicit stimulate the production of cytokines from various ILCs and IELs which constitutively express receptors for IL-1 family cytokines. IL-1β is capable of driving robust IL-17 secretion from both γδ T cells and type 3 ILCs (ILC3) whereas IL-18 and IL-33 can induce the rapid secretion of type 1 or type 2 cytokines from ILC1s and ILC2s, respectively (37). Owing to these pro-inflammatory capabilities, together with the diverse nature of cells expressing one or more IL-1 family receptors, the activities of IL-1 family cytokines are tightly regulated and all, apart from IL-1Ra, are initially synthesized as biologically inactive precursors which require post-translational modifications before they can signal via their cognate receptors. Maturation of IL-1β, IL-18, and IL-37 requires their pro-forms to be cleaved by inflammatory caspases while post-translational cleavage events are also a feature of IL-1α, IL-33 and the IL-36 sub-family (38). Additionally, the active cytokines themselves are endogenously regulated by a network of naturally occurring antagonists and/or decoy receptors capable of blocking pro-inflammatory signal transduction by IL-1 family members, thereby providing an additional layer of regulation governing the activities of this pleiotropic family of cytokines (39).

The IL-1 family cytokines are typically associated with pro-inflammatory responses and IL-1β has been identified as a key mediator of inflammation-induced pathology in several autoimmune and auto-inflammatory conditions including experimental autoimmune encephalomyelitis (EAE)—the murine model of multiple sclerosis (MS)—and obesity-associated airway hyper-reactivity (40–42). As a result, these cytokine pathways have emerged as candidate targets for several conditions, including IBD (43). Indeed therapeutic blockade of the IL-1R via subcutaneous administration of a recombinant IL-1Ra—anakinra (Kineret®)—is licensed for the treatment of rheumatoid arthritis (RA) in patients who fail to respond to

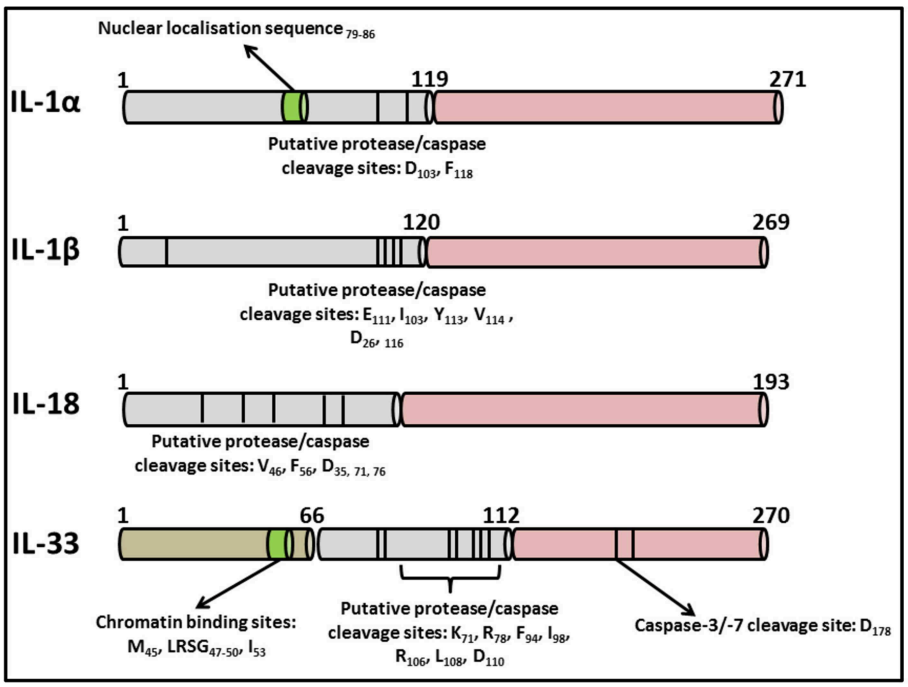

FIGURE 2 | Primary structures of IL-1 family cytokines. Diagrammatic representation of the primary structures of well characterized cytokines belonging to the IL-1 superfamily. While all contain consensus cleavage sites for caspases and/or inflammatory proteases, IL-1α and IL-33 also contain nuclear localization sequences in their N-terminal domains enabling them to traffic to the nucleus from where they can regulate inflammatory gene transcription. Activation of these cytokines via post-translational cleavage events has been extensively reviewed elsewhere (38).

anti-TNF and in individuals afflicted by cryopyrin-associated periodic syndromes (CAPS) (44). Moreover, canakinumab (Ilaris®), is a monoclonal antibody targeting IL-1β which has demonstrated efficacy in the treatment of systemic onset juvenile idiopathic arthritis (So-JIA) (45). However, while anakinra demonstrates a degree of efficacy in systemic inflammation, it has proven ineffective for the treatment of IBD (46, 47). Therefore, it is evident that the role(s) of IL-1 family members in the GIT extend beyond the elicitation of inflammatory responses and these cytokines are also required for the maintenance of optimal barrier function. As outlined below, the role of the IL-1 family in GI homeostasis or IBD pathogenesis depends on factors including disease state, genetic predisposition due to gene polymorphisms, concomitant stimuli derived from the microbiota and the relative abundance of natural antagonists. Furthermore, both CD and UC are associated with different immunological profiles, with the former characterized by a robust T_H1 response while the latter induces an immune signature more akin to a T_H2-type response. Thus, when discussing the contributions of certain cytokines or other immunological factors in the context of IBD, it is important to note that their roles may differ not only between CD and UC but also depend on the stage of the disease. Herein we will discuss the roles of the IL-1 family members and their associated signaling pathways in the context of IBD and highlight the potential manipulation of this cytokine network for therapeutic purposes.

IL-1α AND IL-1β

IL-1, the first non-interferon cytokine to be discovered, consists of two different isoforms—IL-1α and IL-1β–both of which utilize the same IL-1 receptor (IL-1RI) and have similar biological signaling pathways and functions despite sharing only 26% sequence homology to one another (48). Upon cytokine binding to IL-1RI, hetero-dimerization of the receptor chain with the IL-1R accessory protein (IL-1RAcP) facilitates receptor complex formation, thereby providing a platform to which the adaptor protein MyD88 can bind and transduce signals to downstream kinases in a manner analogous to MyD88-dependent TLR signaling. As previously alluded to, a common feature of almost all IL-1 family members, with the exception of IL-1Ra, is a requirement for post-translational processing. This is true of both IL-1α and IL-1β, the precursors of which are cleaved by a variety of enzymes. However, while the pro-form of IL-1β is functionally inert and incapable of binding to IL-1RI, full-length IL-1α is bioactive (49, 50). Cleavage of pro-IL-1β is carried out by an enzyme first designated as IL-1β converting enzyme (ICE) and later renamed caspase-1, which forms part of a multi-protein complex known as the inflammasome (51, 52). In contrast, whereas processing of full-length IL-1α augments its bioactivity, this process occurs independently of caspase-1 (53). A seminal study by Afonina et al. has demonstrated that several inflammatory proteases, including calpain, elastase, granzyme B and mast cell chymase, are all capable of cleaving IL-1α and

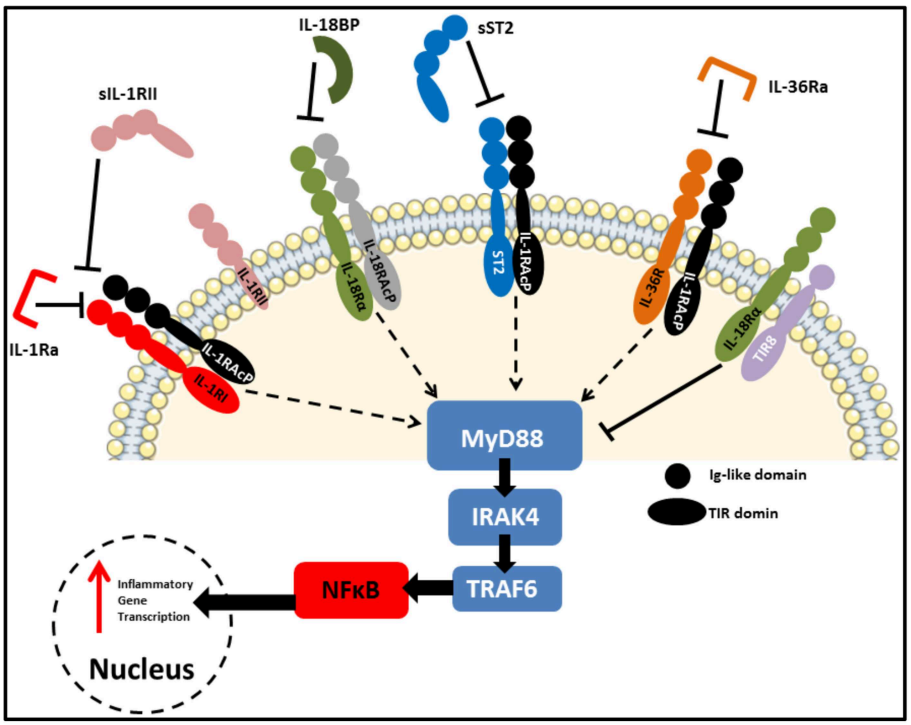

FIGURE 3 | IL-1 family receptors and negative regulators. Receptors of the IL-1 family bear close resemblance to TLRs in that they consist of an extracellular ligand-binding domain, a transmembrane helix and an intracellular TIR domain which mediates MyD88-dependent signal transduction. Due to the highly pleiotropic nature of IL-1 family cytokines, several natural antagonists and decoy receptors have been identified which ensure tight regulation of their functions. These inhibitory molecules can target the receptors themselves, as is the case for IL-1Ra and IL36Ra, or the active cytokines to prevent them from binding to their cognate receptors. Examples of the later include IL-18BP, as well as soluble and membrane-bound IL-1RII and soluble ST2. Finally, SIGIRR (TIR8) acts as an inhibitory receptor by sequestering intracellular kinases required for signaling via engaged IL-1 family receptors.

thus enhancing its affinity for the IL-1RI (54). In addition to this dichotomy in the activation states of their respective pro-forms, the two isoforms also differ in terms of their cellular localization. While IL-1α can typically be found in the cytosol both constitutively and following infection or cell damage, from where it can localize to the nucleus to modulate gene transcription in an IL-1RI-independent manner, IL-1β exhibits no intracellular functionality and must be actively secreted from the cell in response to stimuli (55). However, should the cell undergo inflammatory cell death, IL-1α is released into the extracellular environment and functions as an alarmin, signaling via IL-1RI expressing cells to initiate inflammation (56, 57). The bioactivity of this extracellular IL-1α can also be greatly potentiated by inflammatory proteases (54).

The first identified and to date best characterized endogenous regulator of IL-1 bioactivity is IL-1Ra, a natural antagonist of IL-1RI which is typically produced simultaneously with IL-1 as a means to restrict its signaling potential and prevent excessive stimulation of pro-inflammatory responses. The importance of this receptor antagonism is most evident in patients with a deficiency in the IL-1 receptor antagonist (DIRA), a condition which results in dysregulated IL-1 signaling culminating in severe auto-inflammatory responses characterized by the development of swollen joints and pustular rashes (58). Blockade of this aberrant IL-1 signaling can be achieved via the administration of recombinant IL-1Ra, Anakinra, resulting in a complete abrogation of inflammatory sequelae. Pre-clinically, mice deficient in IL-1Ra ($Il1rn^{-/-}$) are highly susceptible to several autoimmune diseases including psoriasis, diabetes and encephalomyelitis and are known to spontaneously develop arthritis. Recently, Rogier et al. have identified that the severity of this latter condition has a strong microbial component and could be attributed to dysbiosis amongst the commensal microbiota intrinsic to $Il1rn^{-/-}$ mice. Absence of IL-1Ra resulted in aberrant type-17 responses both at the intestinal mucosa and systemically and was transferable to wild-type mice via fecal transfer or co-habitation. However, this IL-17-mediated auto-inflammation could be significantly attenuated through the use of antibiotics targeting the IL-17-driving bacteria, thus indicating that in addition to directly antagonizing IL-1 bioactivity, IL-1Ra also plays a critical role in maintaining a homeostatic and balanced microbiota in the GIT (59).

In addition to IL-1Ra, the biological functions of IL-1 are further regulated by soluble and membrane-bound analogs of the IL-1R called IL-1RII. IL-1RII, whether expressed on the cell surface or in its soluble form (sIL-1RII), serves as a decoy receptor which is capable of binding to IL-1β with greater affinity than IL-1RI. Importantly, IL-1RII is the only

receptor of the IL-1 family which lacks an intracellular TIR domain and is thus incapable of initiating an intracellular signaling cascade (60). Furthermore, it is bound by IL-1Ra with far lower affinity than IL-1RI and so does not interfere with the natural antagonistic properties of the former (61). IL-1RII also competes with IL-1RI for binding to the IL-1RAcP and in doing so limits positive signal transduction via IL-1 responsive receptors. Interestingly, although up-regulated by anti-inflammatory factors such as glucocorticoids, IL-1RII expression is reduced by pro-inflammatory stimuli including LPS and TNFα, suggesting that the onset of inflammation may alleviate the regulation of the IL-1 pathway by IL-1RII (62, 63). An additional inhibitory receptor of the IL-1 family is single immunoglobulin IL-1 receptor-related molecule (SIGIRR), otherwise known as TIR8, the extracellular domain of which can interact with IL-1RI, thereby preventing its dimerization with the IL-1RAcP. Furthermore, the intracellular TIR domain functions as a decoy for components of the IL-1 signaling pathway such as TRAF6 and IRAK1, thereby sequestering them to limit their participation in active IL-1 signaling via functional IL-1Rs (64). This receptor appears to be of particular importance in the GIT as both IECs and intestinal DCs from SIGIRR-deficient mice exhibit a skewed immunological profile characterized by a constitutive up-regulation of inflammatory genes and enhanced responsiveness to TLR ligands. Moreover, *Sigirr*$^{-/-}$ mice were found to exhibit enhanced susceptibility to both intestinal inflammation and carcinogenesis in dextran sulfate sodium (DSS)-induced colitis and azoxymethane (AOM)/DSS colo-rectal cancer (CRC) models, respectively (65, 66). Interestingly, SIGIRR has more recently been shown to block T_H17 cell proliferation by antagonizing IL-1 signaling and glycolytic metabolism, a finding which may have particular importance in the context of spontaneous colitis which develops in *Il10*$^{-/-}$ mice and in which T_H17 cells can mediate significant pathology (67). Thus, it would appear that the expression of this IL-1 family receptor by both hematopoietic and non-hematopoietic cells in the GIT is integral to the maintenance of immune homeostasis.

IL-1α in its precursor form, released from damaged IECs following onset of colitis, acts as a classical alarmin by initiating local inflammation. Interestingly, exogenous administration of anti-IL-1α antibodies, but not IL-1Ra, has been shown to ameliorate intestinal inflammation and increase repair and recovery of the epithelial barrier following DSS-induced colitis (68). This important finding points to dichotomous roles for IL-1α and IL-1β in the context of intestinal inflammation, with the former acting as a mediator of disease pathogenesis. More recently, the protection against chemically-induced colitis conferred by IL-1α deficiency has recently been linked to compositional alterations in the steady-state microbiota of *Il1a*$^{-/-}$ mice and was shown to be abrogated following co-habitation of these animals with wild-type mice prior to the onset of colitis (69). Thus, it appears that in the context of IBD, IL-1α may contribute to disease pathogenesis in an indirect fashion by modulating the environmental niche occupied by commensal bacteria in the GIT.

While the role of IL-1α as a driver of inflammation has been reviewed elsewhere (70), the majority of work pertaining to IL-1 in the context of IBD has focused on IL-1β, levels of which are elevated in IBD (71–74). Mononuclear cells, predominantly macrophages of monocytic origin, residing within the LP are the primary source of these increased concentrations of IL-1 found in inflamed tissue biopsies during active IBD, with the extent to which these levels are elevated correlating with overall disease severity (75). Almost all cells at the intestinal mucosa express IL-1Rs and thus have the ability to respond to IL-1 stimulation. Therefore, deviations away from the concentrations of IL-1 found at steady-state has the potential to impact upon diverse populations of cells including those of myeloid, lymphoid and non-hematopoietic lineages which could significantly alter the immunological profile of the GIT. Indeed constitutive, intestinal macrophage-derived IL-1β signals produced in response to commensal stimuli has been deemed essential for the generation of homeostasis-promoting T_{REGS} under steady-state conditions in a recent study by Sonnenberg and colleagues. This response was found to be dependent on IL-1β-responsive ILC3s, which could drive the differentiation of T_{REGS} via their production of the proliferative cytokine IL-2. Ablation of IL-1 signaling specifically on ILC3s abrogated this induction of T_{REGS} and oral tolerance to dietary antigen. Interestingly, reduced production of IL-2 by ILC3s was found to be a feature of patient's with active CD, suggesting that this pathway may be of clinical importance and targetable (76).

In addition to IBD, elevated concentrations of IL-1β are also found in patients with colo-rectal cancer (CRC) and typically correlate with a poor prognosis. This is because in addition to its classical pro-inflammatory functions, IL-1β can also drive metastasis by promoting vascularization and cellular extravasation. Moreover, metastatic cancerous cells have been reported to express more IL-1RI than healthy bystander cells, suggesting that IL-1 can be utilized by these cells to facilitate further growth of the tumor (77). Despite this, therapeutic inhibition of IL-1 has so far proven ineffective for the treatment of IBD, suggestive of diverse roles for the IL-1 family in the maintenance of intestinal homeostasis and disease pathogenesis. Indeed in the aforementioned study by Bersudsky et al. neutralization of IL-1α, but not exogenous administration of rIL-1Ra or anti-IL-1β antibodies, ameliorated intestinal inflammation following DSS administration (68). Rather than simply quantifying circulating or mucosal concentrations of IL-1α or IL-1β in IBD patients to gather information pertaining to the stage or severity of disease flare-up, a more reliable and predictive readout has been described in which the ratio of IL-1Ra:IL-1 is measured. As reported by Casini-Raggi et al. an imbalance in the relative abundance of either of these compounds may be indicative of a perturbation of intestinal homeostasis and their work demonstrated that this ratio was shown to correlate strongly with disease severity amongst the cohort of IBD patients evaluated in their study (78). This observation is particularly noteworthy as it has previously been reported that in order to efficiently inhibit IL-1 signaling, IL-1Ra must be present in an approximate 100 fold excess compared to IL-1 (79).

Furthermore, a simultaneous analysis of circulating and intestinal IL-1-associated factors may be more informative.

IL-1 and Mucosal Antibody Secretion

In addition to its well established pro-inflammatory functions, IL-1β has more recently been shown to play an important, seemingly non-redundant role, in the differentiation of T_H17 cells and activation of IL-17 secreting innate cells in the GIT under steady-state conditions (80, 81). This manifestation of IL-1 signaling was observed by Shaw et al. in a study in which they reported both a significant reduction in the number of intestinal T_H17 cells as well as a decrease in local IL-17 production in the absence of IL-1R and/or MyD88 signaling (80). More recently, Basu et al. have provided further mechanistic insight into how IL-1 regulates the generation of T_H17 cells *in vitro* and *in vivo*, a function which is linked to the dual role of transforming growth factor beta (TGF-β) in the differentiation of both T_H17 and inducible T_{REG} cells (82, 83). Using the T_H17 cell-inducing enteric pathogen *Citrobacter rodentium*, these studies demonstrated that IL-1 was necessary to overcome retinoic acid-mediated inhibition of T_H17 cell differentiation by enhancing signal transducer and activator of transcription 3 (STAT3) activation, an effect facilitated via repression of its negative regulator, suppressor of cytokine signaling 3 (SOCS3). While phosphorylation of STAT5 in response to retinoic acid was not affected by concurrent IL-1 signaling, this sustained inhibition of SOCS3 enabled accessory T_H17-driving cytokines, including IL-6, to induce further STAT3 phosphorylation which in turn increased T_H17 cell frequency (84).

Since their discovery, the role of T_H17 cells and their signature cytokine IL-17A in auto-inflammatory disease settings has been the subject of extensive research and in a number of cases, IL-1β has been identified as a critical factor responsible for driving IL-17-mediated disease pathogenesis. For example, T_H17 cells have been identified as mediators of disease pathogenesis in experimental autoimmune encephalomyelitis (EAE)—the mouse model of multiple sclerosis (MS)—and blockade of IL-17 signaling results in a marked reduction in disease severity in this model. Moreover, *Il1r1*$^{-/-}$ mice exhibit markedly reduced inflammation suggesting that the auto-reactive T_H17 cells induced during EAE are dependent on IL-1 for their differentiation (41). More recent work has also implicated IL-1-responsive and IL-17-secreting γδ T cells in the pathogenesis of EAE, re-affirming this IL-1/IL-17 axis as a key propagator of inflammatory sequelae (85). Thus, it appears that blockade of IL-1 signaling and, by extension, the inflammasome from which much IL-1 is derived, might be prime targets for therapeutic strategies aimed at reversing the clinical symptoms of autoimmune inflammatory diseases characterized by hyper-active type 17 responses.

Ivanov et al. has shown that of 80–90% of IL-17 producing cells in the SI-LP are TCRαβ$^+$ CD4$^+$ T_H17 cells, which require instruction via MyD88-dependent IL-1 signaling for their differentiation *in vivo* (86). Thus, it is plausible that inhibiting IL-1 signaling, or its production via inhibition of the inflammasome, may result in a significant reduction in the number and frequency of intestinal T_H17 cells. While this may be beneficial in an acute inflammatory setting, the complete abrogation of IL-17 responses may have detrimental consequences for homeostatic processes which depend on intact IL-17 signaling. Such concerns are particularly pertinent when one considers the recently determined role for IL-17 in the maintenance of polymeric immunoglobulin receptor (pIgR) expression on mucosal epithelia (87, 88). This receptor, which is highly expressed on the basolateral membrane of IECs, facilitates the transport of dimeric immunoglobulin A and pentameric immunoglobulin M (dIgA and pIgM, respectively) across the epithelial barrier and into the lumen of the GIT and is therefore essential for intestinal homeostasis as demonstrated by studies in *Pigr*$^{-/-}$ mice which exhibit enhanced susceptibility to DSS-induced colitis (89). Mice lacking the IL-17 receptor display comparable susceptibility to *Pigr*$^{-/-}$ mice in this model owing to diminished epithelial pIgR expression and significantly reduced luminal IgA concentrations (87). Moreover, these animals harbor a dysbiotic microbiota characterized by an outgrowth of "colitogenic" segmented filamentous bacteria (SFB) which can closely adhere to the apical surface of IECs and exacerbate intestinal inflammation by driving excessive T_H17 responses (90). Under steady-state conditions, these pathobionts are sequestered in the lumen where they are found to be bound by IgA as depicted in **Figure 1**. This coating of these microbes by endogenous antibodies is viewed as an essential process to remain tolerant to their presence and IgA exerts a profound influence over microbiota composition (91, 92). Interestingly, IL-1β itself has recently been shown to directly influence not only the transport, but also the production of IgA within the GIT in two independent studies. By developing a murine primary IEC system, Moon et al. demonstrated that IL-1β supported IgA transcytosis by directly regulating pIgR expression by IECs whereas Jung et al. identified eosinophil-derived IL-1β as a critical factor for optimal IgA production in the small intestine. Taken together, these studies therefore further highlight the importance of IL-1β as a regulator of homeostasis within the GIT (93, 94).

IL-18

Initially referred to as interferon gamma inducing factor (IGIF), IL-18 was first identified as a potentiator of type 1 responses and in combination with IL-12 can stimulate T_H1 responses and NK cell functionality (95–97). Like IL-1β, IL-18 is initially translated as a high molecular weight pro-form which is incapable of binding to its cognate receptor and thus must undergo post-translational cleavage. Also like IL-1β, the cleavage of pro-IL-18 occurs in a caspase-1-dependent manner upon assembly of the inflammasome, thereby yielding the mature cytokine which can then be secreted from the activated cell. Although caspase-1 is the only protease shown to definitively process pro-IL-18 into its bioactive form, the finding that *Casp1*$^{-/-}$, but not *Casp8*$^{-/-}$, macrophages are capable of secreting IL-18 upon stimulation suggests that caspase-8 may also play a role in its activation (98, 99). Furthermore, other proteases suggested to act in extracellular processing of pro-IL-1β, including mast cell

chymase and granzyme B, have also been reported to cleave full length IL-18 (100, 101).

Similar to the antagonism of IL-1β by IL-1Ra, IL-18 is also regulated by an endogenous inhibitor called IL-18 binding protein (IL-18BP). However, unlike IL-1Ra which antagonizes the IL-1RI, IL-18BP binds directly to mature IL-18 with high affinity thereby preventing it from engaging the IL-18R on target cells. Crucially, IL-18BP does not bind to the pro-form of IL-18 and therefore only serves as a specific inhibitor for the active cytokine (102). Although expressed constitutively in certain tissues such as the spleen and GIT, IL-18BP levels are greatly enhanced in response to inflammatory stimuli including TNFα and IFNγ (103). Indeed work characterizing the expression of IL-18BP in human epithelial cells has demonstrated that these cells secrete elevated concentrations of IL-18BP in response to IFNγ (104, 105). Therefore, it appears that the production of this antagonist coincides with the onset of inflammation and in a manner analogous to IL-1Ra, serves to limit the signaling of its target cytokine to prevent excessive activation of the IL-18 pathway.

The constitutive expression of IL-18 by IECs suggests a role in the maintenance of GI homeostasis. Interestingly, enhanced production of IL-18, specifically by LP-resident macrophages, is a feature of CD (106, 107). While early pre-clinical studies identified IL-18 as a mediator of inflammation-induced pathology in colitis models, more recent work by a number of groups using various inflammasome-deficient mice has suggested that administration of recombinant IL-18 (rIL-18) can overcome such deficiencies and restore protection from chemically-induced colitis, with the majority of these studies concluding that this is attributed to enhanced epithelial regeneration and barrier repair in response to IL-18 (108–110). Furthermore, ILC3-derived IL-22 has been shown to drive pro-IL-18 expression by IECs, thus conferring protection from intestinal inflammation induced by the murine enteric pathogen *Citrobacter rodentium* (111). In addition to acting upon cells in the epithelial layer, IL-18 has also been shown to modulate effector CD4 responses in the GIT. A recent study by Harrison et al. reported that IEC-derived IL-18 was essential for ensuring balance between colonic T_H17 and T_{REG} differentiation at steady-state and that this equilibrium was essential for the maintenance of homeostasis (112). Interestingly, in this context IL-18 acted as both an activatory and inhibitory factor, signaling via the IL-18R to promote the differentiation of $Foxp3^+$ T_{REGS} while simultaneously directly antagonizing IL-1RI-dependent T_H17 differentiation (112). However, this assumed barrier protective role for IL-18 in the GIT has been challenged in a recent study by Nowarski et al. in which they demonstrated that IL-18 interfered with goblet cell differentiation and maturation with detrimental consequences. They demonstrated that conditional deletion of *Il18r* in epithelial cells ($Il18r^{\Delta IEC}$) conferred protection against DSS-induced colitis and could reverse the inflammatory phenotype exhibited by mice deficient in the IL-18BP (113). However, these results are in contrast to a previous report by the same group in which they associated defects in the mucus barrier of $Nlrp6^{-/-}$ mice and resulting susceptibility to both DSS- and *C. rodentium*-induced colitis with reduced levels of IL-18 (114, 115). In light of these conflicting data, at this stage it is difficult to determine the precise contribution of IL-18 in the context of IBD as the cytokine may play dichotomous roles depending on the stage of disease or its target cells. Furthermore, as is the case with IL-1Ra, IL-18BP levels are also increased in IBD and while the ratio of IL18:IL-18BP appears to correlate with the severity of inflammatory episodes in individuals with eczema and asthma, this has not been established in the context of IBD (116, 117). However, since GWAS studies have linked mutations in the IL-18 pathway with IBD susceptibility, it would appear that IL-18 is a key constituent of the host response to intestinal inflammation and therefore of interest therapeutically (118–120).

IL-33

IL-33 is unique amongst the IL-1 family cytokines in that it preferentially induces type 2 immune responses (121). First discovered in humans in 2003 where it was found to be constitutively expressed in the nucleus of cells in barrier tissues throughout the body, IL-33 was further characterized by Schmitz et al. as a cytokine that induced T_H2 cytokines and eosinophilia *in vivo* via binding to what was then the orphan cytokine receptor T1/ST2 (ST2) (121, 122). Whilst best known for its pathogenic role in asthma and allergy, its presence in the gut epithelium and its role as an "alarmin" released during tissue damage has highlighted it as a potential target in IBD.

IL-33 is constitutively expressed by non-haematopoietic cells in barrier tissues, including the gut mucosa (122–125). It is primarily expressed by epithelial and endothelial cells but expression also occurs in activated fibroblasts and myofibroblasts, which, in the gut, includes peri-cryptal fibroblasts (126). While mRNA expression of IL-33 has been widely reported in immune cells during inflammation, the functional consequence(s) of this is unknown and IL-33 remains primarily viewed as an epithelial-derived cytokine (123, 127, 128). Like other IL-1-family members, IL-33 is transcribed as a pro-form, referred to as full length-IL-33 (FL-IL-33), a 30 KDa protein containing a N-terminal chromatin-binding motif responsible and a C-terminal IL-1-like domain which mediates its cytokine activity (122, 123, 129). Akin to IL-1α, IL-33 lacks a signal sequence for secretion and owing to its chromatin-binding moiety, FL-IL-33 is found exclusively in the nucleus of viable cells, from where it may be capable of fulfilling a secondary role as a transcriptional repressor. Indeed its expression is associated with epithelial cell maturity and quiescence (129, 130). Interestingly, loss of the nuclear localization domain of IL-33 leads to ST2-dependent lethal inflammation in mice; suggesting firstly that IL-33 is a highly inflammatory cytokine, and secondly that its sequestration in the nucleus is a regulatory mechanism which limits its activity (131). Bound up in chromatin and with no described mechanism of active release, the presence of IL-33 in the extracellular space is thought to be dependent on passive release from dead or damaged cells (132). In this respect, IL-33 has been viewed as an "alarmin", a signal of tissue damage (125, 133, 134). Diverse stimuli

including bee venom, allergens such as *Alternaria* extract, the adjuvant alum as well as the physical damage associated with helminth infections have all been shown to induce release of IL-33 (123).

Unlike other IL-1-family members, with the exception of IL-1α, the pro-form of IL-33 is biologically active. However, while FL-IL-33 can bind and signal through ST2, a processed form of the cytokine consisting solely of the C-terminal IL-1-like domain, exhibits 10–30 times greater potency (135–137). This proteolytic cleavage of FL-IL-33 is achieved by many of the same enzymes responsible for the extracellular cleavage of IL-1α including neutrophil-derived cathepsin G and elastase, in addition to mast cell chymase, tryptase and granzyme B (136, 137). Thus, activity of IL-33 is greatest when it is released into an environment which already contains an inflammatory cell infiltrate.

In contrast to the processing of the pro-forms of IL-1β and IL-18 into the bioactive cytokines, cleavage of FL-IL-33 by caspases including caspase-1, −3 and −7 render it incapable of binding to ST2, thus limiting the inflammatory potential of apoptotic cells (133, 134, 138). The half-life of IL-33 is typically very short and the cytokine is often undetectable within hours of its release. It has been proposed that this rapid disappearance may be a result of natural oxidation of the cytokine in the extracellular space, leading to the formation of disulphide bridges and a conformational change that prevent binding to ST2 (139).

The receptor for IL-33, ST2, is constitutively expressed on a variety of cells of both myeloid and lymphoid origin including mast cells, T_H2 cells, ILC2s and tissue-resident T_{REGS}. In addition, although macrophages and eosinophils possess low basal levels of ST2, both can rapidly up-regulate its expression once activated (127, 132, 140, 141). The ST2 protein exists as two membrane bound splice variants ST2L and ST2V, and a soluble isoform (sST2) which lacks a transmembrane domain and is released from the cell surface (142). sST2, whose expression is controlled by activation of a secondary promoter site for ST2, is produced by immune cells during inflammation and acts as a decoy receptor, analogous to sIL-1RII, thereby limiting the biological activity of IL-33 (143).

Once bound by mature IL-33, ST2 recruits IL-1AcP to initiate signal transduction which follows classical IL-1 signaling and involves MyD88, TRAF6 and IRAK1/4 leading ultimately to activation of the NFκB and MAP-Kinase pathways (121, 123, 141, 144). The intracellular kinases downstream of ST2 appear to differ slightly depending on cell type, with NFκB activation being required for the production of pro-inflammatory cytokines such as IL-6 and TNF-α by mast cells whereas MAP-Kinase appears to be the dominant pathway engaged in T cells and ILC2s (127). Analogous to its role in IL-1RI signaling, SIGIRR is capable of inhibiting IL-33 signaling at this stage by competing with ST2 for binding to IL-1RAcP (145).

The canonical role of IL-33 is in the elicitation of type 2 immunity and it is integral to the innate response to certain helminth infections (140, 146–150). In addition, GWAS studies have proposed an association of certain mutations in IL-33 and ST2 with susceptibility to allergy and asthma (151).

IL-33 directly activates mast cells independently of IgE and is an important factor promoting the antigen-independent production of IL-5 and IL-13 by tissue-resident ILC2s and T_H2 cells (140, 152). Here, the effects of IL-33 often synergize and overlap with those of other epithelial-derived type 2 cytokines including thymic stromal lymphopoietin (TSLP) and IL-25 (148). Recently, a role for IL-33 in the elicitation of regulatory immune responses has also been uncovered with the identification of a population of ST2-expressing tissue-resident T_{REGS}, the suppressive function of which is enhanced by IL-33 (153). Furthermore, IL-33 release following tissue damage in mice has been shown to be required for the initiation of effective wound healing (154, 155). Thus, IL-33 may be best viewed as a highly-regulated cytokine which alerts the immune system to tissue damage but which also promotes the induction of responses that resolve inflammation.

As mentioned, IL-33, like IL-1α, is constitutively expressed by IECs and is released following tissue damage (142, 156–158). Exogenous IL-33 increases gut epithelial cell proliferation and mucus production (159). Moreover, there is some evidence that IL-33 signaling may affect gut motility via acting upon the enteric nervous system (160). It was reported that IL-33-deficent mice have a defect in IgA production and an altered microbiota (142, 160).

The fact that IL-33 promotes type 2 immune responses has implications for its potential differential role in CD vs. UC. While IL-33 is not associated with CD, which is typically characterized by T_H1 and/or T_H17-dominated inflammatory responses in the gut, it has been associated with UC in which a pronounced T_H2 profile is normally observed (161). Indeed concentrations of IL-33 are increased in the colon and serum of UC patients, leading to its identification as a potential pharmacological target (158, 162, 163). Interestingly, IL-33 is increased during disease flare-ups and decreased following anti-TNF-α-induced remission (164). Furthermore, IL-33 was found to induce T_H2-associated cytokine secretion from mesenteric lymph node-derived cells of UC patients (158). However, it remains to be determined whether IL-33, in the context of UC, functions as a read-out of tissue damage or direct mediator of disease pathogenesis.

The role of IL-33 in pre-clinical models of IBD is unclear, with various studies demonstrating both protective and pathologic roles for IL-33. In support of the latter, Sedholm et al. reported that levels of IL-33 were enhanced in the intestinal epithelium of both DSS- and 2,4,6-trinitrobenzene sulphonic acid (TNBS)-treated animals. In both models, recombinant IL-33 (rIL-33) exacerbated the severity of the inflammation whereas ST2-deficient animals were somewhat protected (163). Two further studies corroborated the protective phenotype observed in *Il1rl1*$^{-/-}$ mice and the exacerbating effect of IL-33 in the DSS model, with one study showing a role for IL-4 in mediating the damaging effects of IL-33 (157, 165). The apparent dichotomy was reflected in a study by Sattler et al. where exogenous administration of rIL-33 exacerbated spontaneous colitis in *Il10*$^{-/-}$ mice, while concomitantly driving the expansion of a population of IL-10-producing B cells in wild-type mice. Moreover, these IL-10^{+} B cells were shown

to constrain colitis upon transfer into *Il10*$^{-/-}$ recipients, thereby further demonstrating the duality of IL-33 (166). To complicate the picture further, Groß et al. showed a time-dependent effect of IL-33 on DSS-induced colitis, where IL-33 worsened acute disease but led to improved recovery if administered following removal of DSS from the drinking water (167).

In direct contrast to these findings, others have found a protective role for IL-33 in experimental colitis. Two independent studies demonstrated that exogenous IL-33 attenuated TNBS-induced colitis, protective phenotypes which were attributed to the induction of either T_H2/T_{REGS} or M2 macrophages, respectively (168, 169). Similarly, Seo et al. reported a protective role for IL-33-activated regulatory macrophages in DSS-induced colitis using cell transfers (170). Furthermore, in a seminal study describing a protective role for IL-33 in colitis, Malik et al. demonstrated that *Il33*$^{-/-}$ mice were more susceptible to DSS-induced tissue damage and colitis-associated tumorigenesis, phenotypes which were both due to elevated concentrations of IL-1α in the absence of IL-33 and which could be ameliorated via abolition of IL-1α signaling (160). Interestingly, the authors demonstrated that these susceptibility phenotypes had a strong microbial element and that *Il33*$^{-/-}$ mice exhibited reduced concentrations of intestinal IgA and harbored a dysbiotic microbiota, characterized by an increased abundance of the colitogenic bacterium *Akkermansia muciniphilia*. Selective reduction of this bacterium using a specific antibiotic regimen or equilibration of the microbiota via co-habitation with wild-type animals successfully attenuated the exacerbated disease symptoms otherwise observed in *Il33*$^{-/-}$ mice (160).

Some of the inconsistencies might in part be explained by the intermittent use of *Il1rl1*$^{-/-}$ vs. *Il33*$^{-/-}$ mice in several studies, the timing of administration of IL-33, or the use of different rIL-33 proteins, many of which differ in a unique way from FL-IL-33 with regard to their peptide sequence or post-translational modifications.

The interaction of IL-33 with T_{REGS} and T_H17 cells in the gut is of particular interest given their reciprocal roles in colitis. Indeed the expression of ST2 is characteristic of colonic T_{REGS}, the suppressive function of which can be enhanced by IL-33. Interestingly, IL-23, a key T_H17-promoting cytokine, has been shown to antagonize this IL-33-mediated increase in T_{REG} activity (171). In addition to modulating mucosal T_H17 responses indirectly via T_{REGS}, recent work by Pascual-Reguant et al. identifying ST2 on the surface of intestinal T_H17 cells has suggested that IL-33 may also act upon these cells directly to constrain their effector functions. Using a murine model of anti-CD3-induced colitis, they showed that ST2-expressing T_H17 cells exhibited a reduced capacity for proliferation and secretion of IL-17A but enhanced IL-10 production in response to IL-33 (156). Furthermore, ligands of the epidermal growth factor receptor (EGFR), including amphiregulin, may be important in accentuating the effects of IL-33 in the gut. ST2 and EGFR form a signaling complex which is required to mediate the effect of IL-33 on T_H2 cells (172). Considering EGFR ligands have been implicated in tissue regeneration in UC, their interplay with IL-33 in IBD may have a role in protection from disease (173). In this respect, Monticelli et al. have reported that amphiregulin, produced by ILC2 in response to IL-33, conferred protection against DSS-induced colitis by enhancing tissue repair (159).

It must be noted that the conflicting role of IL-33 as reported in pre-clinical models of IBD extends beyond the gut and IL-33 has been found to have both protective and exacerbating effects in systemic models of autoimmunity including EAE (174, 175). Thus, similar to other IL-1 members, IL-33 likely plays a dichotomous role in inflammatory disorders by initiating inflammation following its release from damaged cells while also inducing type 2 or regulatory responses that lead to resolution of inflammation and tissue repair.

IL-36

Although initially described as an extension of the prototypical IL-1 family, within the last decade IL-36 cytokines have been designated as a distinct sub-family which signal through their own specific receptor—IL-36R (176–178). Similar to IL-1, the IL-36 subfamily consists of distinct isoforms—IL-36α, β and γ–and a natural antagonist—IL-36Ra (179). The three isoforms signal via the IL-36R complex which shares the accessory protein IL-1RAcP with other IL-1 family members and similarly contains an intracellular TIR domain, thus enabling its interaction with cytoplasmic MyD88 to propagate downstream intracellular signals (28, 180). Although these cytokines lack classical caspase cleavage sites, post-translational cleavage of IL-36α, -β, and -γ, as well as IL-36Ra, at the N-terminal has been shown to dramatically enhance their potency *in vitro* (181). Furthermore, the lack of signal sequence suggests that these cytokines are not actively secreted from the endoplasmic reticulum and neutrophil-derived proteases implicated in extracellular cleavage of full length IL-1α and IL-1β, namely cathepsin G, elastase and proteinase-3, have been proposed as activators of the IL-36 sub-family members (182, 183). Thus, in a manner analogous to IL-1α and IL-33, the release of these cytokines may be a consequence of inflammatory cell death enabling them to acts as alarmins and initiate inflammatory cascades by binding to IL-36R-expressing cells. Due to this potential to accelerate inflammatory responses, IL-36 cytokines, like other IL-1 family members, are tightly regulated. Their activity is primarily limited by IL36Ra, which shares 52% sequence homology with and performs an analogous function to IL-1Ra. As previously mentioned, patients with IL-1Ra deficiencies develop systemic auto-inflammation characterized by swollen joints and pustular rashes (58). A similar disorder results from a rare inactivating mutation in the gene encoding IL-36Ra, *IL36RN*, and is manifested as a severe inflammation of the skin referred to as generalized pustular psoriasis (GPP) (184). Additionally, further intrinsic regulation comes in the form of IL-38, another IL-1 family member with a high degree of homology with both IL-36Ra (43%) and IL-1Ra (41%) and which is capable of binding to the IL-36R (185).

IL-36 cytokines are expressed either constitutively or in response to inflammatory stimuli by human and mouse cells including DCs, monocytes/macrophages, T cells and epithelial cells. IL-36R-expressing DCs can in turn respond to IL-36 by up-regulating prototypical maturation markers and cytokine production in a manner analogous to TLR stimulation (186). Thus, in this context, rather than function as a typical IL-1 family member, IL-36 primes DCs for subsequent modulation of effector responses. Indeed IL-36β has been shown to synergize with IL-12 to preferentially drive the expansion and activation of T_H1 cells from naïve precursors (187, 188). Two recent, independent studies have indicated that levels of IL36α and IL-36γ are up-regulated in the colons of both IBD patients and mice in which intestinal inflammation is induced by DSS (189, 190). In the former of these two studies, Russell et al. demonstrated that *Il1rl2*$^{-/-}$ mice were significantly less susceptible to both DSS- and *C.rodentium*-induced colitis, phenotypes which the authors attributed to a skewed T helper cell profile in the GIT of these animals. However, these results are in contrast to a study by Medina-Contreras et al. in which *Il1rl2*$^{-/-}$ mice exhibited impaired resolution of inflammatory lesions and sub-optimal wound healing compared to littermate controls (191). Of note, the elevated levels of IL-36 cytokines observed in this study were not observed in germ free mice, leading the authors to postulate a possible role for the microbiota in intestinal IL-36 signaling. Scheibe et al. recently reported that treatment with IL-36 accelerated colonic wound healing by stimulating IEC proliferation and that this required MyD88 signaling. Importantly, by generating bone marrow chimeras, the authors demonstrated that IL-36R in the hematopoietic compartment was dispensable for this improved healing suggesting that the expression of the IL-36R by non-hematopoietic IECs or epithelial progenitors likely regulates this process (190). However, as is the case with several of the other IL-1 family members, further studies will be required to fully resolve the potential role of this subfamily of cytokines in IBD. In particular, in-depth characterization of the composition of the respective microbiotas of *Il1rl2*$^{-/-}$ and *Il1rl2*$^{+/+}$ mice in parallel will help to determine whether dysbiosis, as suggested by Medina-Contreras et al. may underlie some of the phenotypes reported in these animals. Furthermore, although Scheibe et al. identified the expression of IL-36R in the non-hematopoietic compartment as an essential component of the colonic wound healing process, more in-depth studies using conditional knock-out animals will be necessary for elucidating the cell-specific effects of these cytokines. Finally, epidemiological studies investigating potential links between the *IL36RN* mutation or the ratio of IL36Ra/IL38:IL-36 and susceptibility to IBD may identify one or more of these factors as candidate biomarkers for the disease.

IL-37

IL-37 is an anti-inflammatory member of the IL-1 cytokine family, production of which is increased in a number of inflammatory conditions including psoriasis, RA and IBD. In inflamed tissue sections from UC and CD patients, IL-37 concentrations were reported to be enhanced, with follow-up *in vitro* assays revealing that this increased production was driven by TNFα as part of an auto-inhibitory feedback loop to limit TNFα-induced inflammatory responses (192). In this regard, IL-37 may perform a similar function to IL-1Ra and IL-18BP, the expression of which is also up-regulated during active IBD. In contrast, in patients with microscopic colitis, *IL37* gene expression was found to be reduced compared to healthy individuals with the former also exhibiting elevated levels of chemokines driving cellular infiltration into the intestinal LP (193, 194). Although five isoforms of IL-37 exist in humans, IL-37a-e, with IL-37b being the best characterized and most studied to date, there is no murine homolog, hence the limited literature pertaining to the role of this cytokine in inflammatory conditions. However, studies using transgenic mice expressing human IL-37b have demonstrated that these mice are more resistant to DSS-induced colitis (195, 196). The literature regarding IL-37 processing and signaling is similarly scarce, however a putative caspase-1 cleavage site has been reported which is conserved across four of the isoforms (197, 198). Similar to IL-1α and IL-33, it appears that IL-37 is also capable of exerting its biological effects both intra- and extra-cellularly. In the former scenario IL-37 translocates to the nucleus from where it can inhibit pro-inflammatory gene transcription whereas extracellular IL-37 engages IL-18Rα in association with SIGIRR to initiate an anti-inflammatory cascade (198–203). The ability of IL-37 to translocate to the nucleus appears to be caspase-1-dependent and is lost in cells lacking the inflammasome components ASC or NLRP3 (199). While the administration of recombinant IL-37 or a mimetic as an anti-inflammatory therapeutic may be a promising strategy for the treatment of auto-inflammatory conditions, further work will be required to identify the precise role of each of the IL-37 isoforms in the context of both inflammation and homeostasis and epidemiological studies might reveal whether any mutations or SNPs exist which could link alterations in this cytokine to disease susceptibility or severity.

IL-38

In humans, the gene encoding IL-38 (*IL1F10*) is located within the IL-1 gene cluster on chromosome 2p13, adjacent to the genes encoding IL-1Ra and IL-36Ra (204). Indeed, as already alluded to, the IL-38 protein shares remarkable sequence and structural homology with these natural IL-1 family antagonists—41% homologous to IL-1Ra and 43% to IL-36Ra. Perhaps unsurprisingly, IL-38 is therefore a regulatory cytokine capable of binding to several receptors and modulating the downstream function(s) of multiple pro-inflammatory cytokines, in particular those associated with a T_H17 response. Pro-inflammatory cytokine secretion following *in vitro* stimulation of peripheral blood mononuclear cells (PBMCs) and macrophages, with TLR agonists was found to be markedly reduced when IL-38 was present. Additionally, production of the chemo-attractant IL-8 following treatment of PBMCs with IL-36γ was found to be abrogated in the presence of IL-38 to a similar extent as

IL-36Ra (205). Thus, it appears that the function of IL-38 is analogous to that of other natural IL-1 family antagonists and similar to these molecules, it has been reported that IL-38 concentrations are elevated in patients afflicted by certain inflammatory conditions including RA, psoriasis, systemic lupus erythematosus (SLE), chronic obstructive pulmonary disease (COPD) and IBD. However, while many of these observations are based on serum cytokine concentrations, such read-outs are not always indicative of the response at distant sites and do not always correlate with disease severity. Indeed despite elevated IL-38 levels in the serum of patients with psoriasis, Palomo et al. have recently reported that IL-38-deficiency does not affect the severity of skin inflammation in a murine psoriasis model. Using an imiquimod-induced psoriasis model, they demonstrated that local *Il36rn*, but not *Il38* levels, were increased in the inflamed skin following topical application of Aldara™ and disease severity and progression was comparable between *Il1f10*$^{-/-}$ mice and wild-type littermate controls (206). In contrast, *Il36rn*$^{-/-}$ mice exhibited exacerbated inflammatory sequelae leading to the author's conclusion that in this model at least, IL-38 was dispensable for regulating local inflammatory responses in the skin and could not compensate for a lack of IL-36Ra in *Il36rn*$^{-/-}$ mice. However, a recent study by Han et al. demonstrated that IL-38 could indeed ameliorate imiquimod-induced skin inflammation by limiting IL-17 production from γδ T cells. Here the authors showed that *Il1f10*$^{-/-}$ mice exhibited prolonged, type-17-mediated skin inflammation, a phenotype which could be reversed via exogenous administration of recombinant human IL-38 (207).

Enhanced *Il38* levels have been reported in the colon of DSS-treated mice and also in samples obtained from CD patients where *IL38* levels were higher in inflamed colonic biopsies compared to non-inflamed tissue (208). Further studies will be necessary to determine whether IL-38 deficiency or blockade of IL-38 might result in unrestrained effector T cell responses and exacerbated intestinal inflammation. Moreover, given its role at limiting the production of T_H17 differentiation factors, together with the recently uncovered role for T_H17 cells and their signature cytokine IL-17A in the maintenance of intestinal homeostasis and composition of the microbiota, whether *Il1f10*$^{-/-}$ mice exhibit microbial dysbiosis has yet to be established and warrants further investigation (87, 90).

INFLAMMASOMES IN IBD

In light of the counterintuitive results obtained from studies investigating the inhibition of IL-1 signaling as a potential treatment for IBD, what has become strikingly apparent is that the inflammatory processes and markers associated with pro-inflammatory conditions in the periphery do not always hold true within the GIT. As previously alluded to, caspase-mediated cleavage of the pro-forms of IL-1β and IL-18 typically occurs as part of a larger complex termed the inflammasome. These high molecular weight, multi-protein complexes serve as platforms for the recruitment and activation of pro-inflammatory caspases, such as caspase-1 and−5 (209, 210). As a result, they are critical components of the inflammatory response to noxious stimuli as they bridge the initial sensing of PAMPS or DAMPs and subsequent stimulation of innate immune mechanisms with the downstream modulation of stimulus-specific adaptive immunity. Although several such complexes exist, each inflammasome is structured around a core set of elements including a sensor protein, an adaptor molecule and an inflammatory caspase. While inflammasomes can be broadly sub-divided into two distinct, but functionally similar groups on the basis of their sensor proteins—those of the Nod-like receptor (NLR) and PYHIN families—we will focus on the former for the purpose of this review due to the extensive literature pertaining to their role(s) in IBD.

The NLR family includes NLRP1, NLRP2, NLRP3, NLRP5, NLRP6, NLRP7, NLRC4, NLRP12, NLRP14, NOD1, NOD2, and absent in melanoma 2 (AIM2) and plays essential roles in pathogen recognition, the maintenance of homeostasis and embryonic development (211, 212). The impetus for studying the NLR family in the context of IBD was the identification of SNPs within the peptidoglycan-sensing *NOD2* gene which have been associated with enhanced susceptibility to CD (213, 214). Subsequent genome-wide association studies (GWAS) have identified several other mutations in NLR family members as risk factors correlating with IBD susceptibility. One of these members, NLRP3, has been the focus of the majority of inflammasome-based research and is therefore the best characterized of the NLR complexes identified to date. Recently however, there is accumulating evidence suggesting important roles for other NLR family members, in particular NLRP6, in the maintenance of intestinal homeostasis. Indeed the consequences of deficiencies in various inflammasome components across a diverse range of chemically- and pathogen-induced colitis models has been extensively reviewed elsewhere (215). Of the aforementioned NLRs, AIM2 as well as NLRP1, NLRP3, NLRP6, and NLRC4 are involved in the formation of inflammasomes. Although they differ in terms of their pattern of expression and the stimuli to which they respond, structurally they are similar and consist of a specific NLR, the apoptosis-associated speck-like protein containing a CARD (ASC) adaptor protein and an inflammatory caspase, typically caspase-1. Canonical inflammasome activation is primed by engagement of an innate receptor such as a TLR on the cell surface and fully initiated upon receipt of a secondary signal which can be in the form of an exogenous stimulus or an intracellular event such as the efflux of potassium as depicted in **Figure 4**, resulting in the association of ASC with the NLR via their respective pyrin domains. This interaction triggers the formation of the ASC speck, a multimeric complex made-up of several ASC dimers which acts as a scaffold to which caspase-1 monomers can bind via their CARD domains. This interaction of caspase-1 with ASC induces an auto-cleavage event in the enzyme which converts it from its catalytically inactive, zymogen form, into a proteolytically active enzyme capable of cleaving pro-IL-1β and pro-IL-18 (209). In the case of IL-1β, caspase-1-mediated cleavage of the 31 kD pro-form yields

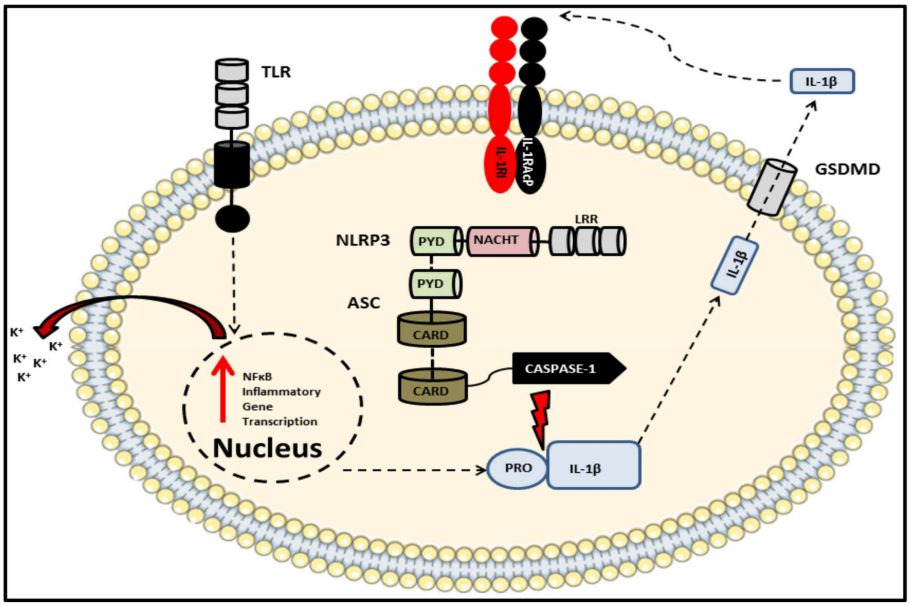

FIGURE 4 | Schematic of the NLRP3 inflammasome complex. The prototypical inflammasome-forming NLR consisting of the sensory component NLRP3 bound via PRYIN domain interactions with the adaptor protein ASC, which in turn associates with caspase-1 via their respective CARD domains. An initial stimulus, such as a TLR ligand, primes the inflammasome via the ligand sensing LRR domain, thereby initiating oligomerization of NACHT domains and activation of the inflammasome which results in the production of cytokines including pro-IL-1β and pro-IL-18. A subsequent second signal, such as potassium ion efflux, induces the autocatalysis and activation of caspase-1. This autocatalytic event enables the enzyme to cleave its substrates which include both pro-IL-1β and pro-IL-18 as well as the pore-forming protein gasdermin D. Once released into the extracellular space, these bioactive cytokines can go on to signal in an auto- or paracrine manner.

a 17 kD bioactive cytokine which can then be actively secreted and bind to its cognate receptor on target cells.

In addition to its classical role in the functional maturation of pro-IL-1β and pro-IL-18, assembly of the inflammasome in this way can also trigger a form of cell death known as pyroptosis which is characterized by the activation of inflammatory caspases such as caspase-1,−5, and−11 (216–218). This leads to cleavage of gasdermin D (GSDMD) which, like pro-IL-1β and pro-IL-18, contains consensus caspase cleavage sites. Cleaved GSDMD, upon trafficking to the cell membrane induces cell death by forming oligomeric pores in the lipid bilayer (219). The resulting release of intracellular contents into the extracellular matrix via these pores is what distinguishes pyroptosis from quiescent apoptotic cell death, in which dying cells release membranous blebs containing their contents which are subsequently engulfed by nearby phagocytes. It is however important to highlight that unlike spontaneous cell death via necrosis, pyroptosis is programmed and thus restricted to the activated cell. Indeed pyroptotic cell death is an efficient host defense mechanism as it abolishes the intracellular niche required by certain pathogens to facilitate their replication (220).

However, an important caveat to these mechanistic studies is that for the most part they have been carried out *in vitro* using specific protocols whereby signal one is provided in the form of a TLR agonist such as LPS, followed at a defined amount of time later by a second stimulus that induces the release of mature cytokines. In reality, the *in vivo* scenario is likely to be very different to this controlled experimental setting as both signals are likely to be provided simultaneously and the concentrations of each stimulus is likely to differ greatly. This is particularly true of the GIT where perturbations in homeostatic mechanisms expose inflammasome-expressing IECs and LP-resident myeloid cells to a plethora of PAMPs derived from commensal microbes. Indeed disruption of the microbiota following treatment with broad-spectrum oral antibiotics has recently been shown to enhance the production of pro-inflammatory cytokines by intestinal macrophages in response to TLR ligation *ex vivo* (221). The overall composition of the microbiota itself is another confounding variable in animal studies which is likely to differ greatly depending on host genotype, supplier, husbandry practices and the environment in which the animals are housed. Certain commensal populations can have a profound influence on disease severity in DSS-induced colitis models and can exert host protective or deleterious effects by modulating the inflammatory responses elicited (86, 222–224). Indeed the significance of the microbiota as a key factor involved in colitis severity has been highlighted in a recent study by by Britton et al. in which colonization of gnotobiotic mice with fecal microbiotas from IBD patients significantly increased disease severity in a T cell transfer model of colitis compared with mice colonized with microbiotas from healthy control subjects. This exacerbated inflammatory disease phenotype correlated with marked alterations in the CD4+ T cell compartment of the GIT in mice receiving an IBD-associated or healthy microbiota (225).

Despite the clearly defined role of the NLR inflammasomes in the maturation of inflammatory cytokines and initiation of inflammatory cell death by pyroptosis, several studies modeling inflammasome deficiency through the use of *Pycard*$^{-/-}$ mice have shown that these animals are intrinsically hyper-susceptible to chemically-induced colitis and exhibit several symptoms characteristic of severe intestinal inflammation including enhanced weight loss, diarrhea, rectal bleeding, colonic shortening and mortality. Furthermore, barrier integrity is compromised in these mice and they have been shown to exhibit elevated levels of serum endotoxin as well as bacterial translocation compared to wild-type mice (109). Importantly, *Pycard*$^{-/-}$ mice are deficient in all inflammasome complexes and the severity of the inflammation they exhibit is greater than that of strains lacking individual NLRs. Thus, despite functioning as drivers of inflammatory responses in response to diverse stimuli, it appears that the NLR inflammasomes confer a degree of natural protection against the onset of gastro-intestinal inflammation. One of the major co-morbidities associated with IBD is the enhanced risk of developing gastro-intestinal tumors knowns as colitis-associated cancer (226). Interestingly, long before the characterization of the inflammasome and description of caspase-dependent pyroptosis, ASC deficiency had been associated with a poor prognosis in both human breast and gastric cancers (227). Furthermore, more recent studies have demonstrated that *Pycard*$^{-/-}$ mice also display enhanced tumor burden and metastasis compared to wild-types in a murine CRC model which involves exposure to AOM prior to DSS administration (228). Similar results were reported on *Casp1*$^{-/-}$ mice in both DSS-colitis and AOM-DSS CRC models (110, 228–230). Despite, the evidence pointing to a protective role for inflammasomes in IBD, there are a number of caveats which must be considered before definitive conclusions can be drawn. Firstly, while the majority of the aforementioned studies have utilized the DSS-colitis model to study acute intestinal injury, the same results are not seen in models of spontaneous colitis in *Il10*$^{-/-}$ mice, which develop intestinal inflammation characterized by aberrant T_H17 responses, resulting in significantly elevated colonic concentrations of IL-17. Intriguingly, this phenotype can be reversed and the inflammation resolved by inhibiting IL-1 signaling or caspase-1 (231). Indeed, IL-1β, produced in response to microbial stimuli derived from commensal bacteria, plays a non-redundant role in the differentiation of homeostasis-promoting T_H17 cells in the GIT (80). Thus, although protective in chemically-induced colitis models, it appears that IL-1 exacerbates T_H17-mediated intestinal inflammation. Such findings are particularly interesting when one considers the recent description of inflammasome- and IL-1RI-expressing T_H17 cells in the aforementioned study by Martin et al. These cells were found to be capable of self-propagation through autocrine IL-1 signaling and drove significant pathology in EAE (232). However, the existence of such cells within the GIT has yet to be reported.

A further layer of complexity stems from the recent discovery that the *Casp1*$^{-/-}$ mice used in several of the early seminal studies investigating the role of the inflammasome in IBD are also deficient in caspase-11 (*Casp11*$^{-/-}$), the murine homolog of human caspases−4 and−5 (233). Subsequent studies have also reported a protective role for caspase-11 in DSS-colitis using *Casp11*$^{-/-}$ mice which exhibit exacerbated barrier destruction and increased intestinal permeability despite retaining fully functional caspase-1 (108, 234). Rather than full ablation of IL-1β and IL-18 as occurs in *Pycard*$^{-/-}$ and *Casp1*$^{-/-}$ mice, *Casp11*$^{-/-}$ mice merely have reduced concentrations of these cytokines and exogenous administration of recombinant IL-18 restores protection to wild-type levels. In line with previous results outlining protective roles for the inflammasome in CRC, caspases−4 and−5 have also been reported to be up-regulated in IBD patients (235). Indeed a recent study has also identified caspase-11 as protective in the AOM-DSS model. In their study, Flood et al. reported heightened susceptibility and exacerbated tumor growth in *Casp11*$^{-/-}$ mice compared to their littermate controls. The enhanced tumor burden was associated with reduced levels of both IL-1β and IL-18 which resulted in reduced STAT1 signaling and sub-optimal anti-tumor type I immunity (236). Given that IL-1 cytokines derived from activated inflammasomes evidently play a fundamental role in determining the severity of various gastro-intestinal disorders, consideration must also be given to the composition of the commensal microbiota in these mouse strains. For example, Demon et al. reported a marked reduction in the relative abundance of *Prevotellaceae* in their *Casp11*$^{-/-}$ mice, levels of which were normalized following co-habitation with wild-type mice but did not affect overall disease severity (234). Additionally, both *Pycard*$^{-/-}$ and *Casp1*$^{-/-}$ mice had also previously been reported to possess alterations in microbiota composition compared to wild-types (115). Due to these confounding variables, it is difficult to attribute a precise role to caspase-1 in the context of inflammasome-mediated protection from intestinal inflammation. However, a recent study by Flavell et al. has challenged the accepted dogma and provided evidence for a pro-inflammatory and pro-tumorigenic role for caspase-1 in the GIT. By generating mice deficient in either caspase-1 or caspase-11 only, the authors demonstrated that the expression of caspase-1 within non-hematopoietic cells was responsible for exacerbating chemically-induced intestinal inflammation and tumorigenesis independently of the microbiota or caspase-11 (237). While these findings may help to explain why specific blockade of caspase-1 had previously been reported to ameliorate inflammatory symptoms in murine spontaneous colitis models, the exact functions of specific NLR inflammasomes in IBD remains incompletely resolved.

Further confusion has arisen recently regarding the role of the NLRP6 inflammasome in the GIT. Initial work by Elinav et al. reported a protective role for this sensor in experimental colitis models and identifying a dysbiotic microbiota intrinsic to *Nlrp6*$^{-/-}$ mice characterized by an expansion of *Prevotellaceae* (114, 115). However, a recent study by Mamantopoulus et al. suggested these reported phenotypes to be artifacts resulting from so-called "legacy" effects pertaining to husbandry procedures. They demonstrated that in contrast to earlier reports, neither NLRP6 nor the adaptor protein ASC exerted influence on the make-up of the host's microbiota, thus highlighting the importance of properly controlled experimental protocols when

investigating the impact of specific immunological factors on microbial ecology (238, 239). Contrasting phenotypes have also previously been reported from DSS-induced colitis studies in *Nlrp3*$^{-/-}$ mice. While early work by Bauer et al. reported a pathologic role for NLRP3, several subsequent studies have intimated that this inflammasome sensor is in fact protective in this model (110, 240). Recently, Macia et al. have shown that dietary fiber induces non-hematopoietic activation of the NLRP3, but not NLRP6, inflammasome resulting in enhanced caspase-1 activity, IL-18 secretion and improved disease outcome while an activating mutation in *Nlrp3* - *Nlrp3*^{R258W} - was associated with increased resistance to colitis and CRC due to inflammasome-IL-1β-dependent remodeling of the microbiota (241, 242). In addition to these well characterized complexes, the less studied sensor NLRP1 has also been shown to affect the microbiota (243). However, in contrast to the assumed barrier-protective role for NLRP3, overt NLRP1 signaling was found to exacerbate inflammatory sequelae in response to DSS. Moreover, the expression of *NLRP1* was found to be increased in inflamed colonic biopsies from UC patients, suggesting a primarily pathologic role for this sensor (243).

In light of these and other reports, it has become abundantly clear that when discussing the contribution(s) of inflammasomes and IL-1 family members in the pathogenesis of IBD, the impact of the indigenous microbiota cannot be overlooked and has played a somewhat under-appreciated role in determining disease severity in earlier work. While recent studies have begun to shed light on this issue in an effort to explain the variable phenotypes reported to date, previous work on the role of the NLRP3 inflammasome and IL-1 itself may need to be re-visited. Furthermore, the implementation of standardized practices for studies related to inflammasome-deficiency and the microbiota in IBD has been championed by several recent review articles in an effort to alleviate some potential sources of inter-study variance (244–246).

PERSPECTIVES

The use of monoclonal antibody-based therapies to specifically target and inhibit pro-inflammatory cytokines has been a highly efficacious and beneficial intervention for patients with chronic inflammatory disorders. Indeed several cytokine-targeted therapies, including those targeting the IL-1 and IL-17A pathways, are used clinically to treat conditions such as RA, MS and psoriasis. However, it has frequently been observed that these highly specific and efficacious treatments do not ameliorate symptoms of IBD and in many instances have led to an exacerbation of the disease symptoms. While the exact mechanisms underlying these failures have yet to be fully elucidated, it is highly plausible that such interventions could interfere with normal physiological processes, such as the active transport of secretory antibodies at the mucosae, in which such cytokines play integral roles. Moreover, the impact of prolonged or repeated use of such therapeutics on adaptive immunity or the composition of the commensal microbiota has not been investigated for the most part. Indeed, it is only due to the improvements of next generation sequencing techniques that the influence of the microbiota on IBD and other auto-inflammatory conditions has come to be fully appreciated. Thus, although IL-1 family cytokines remain attractive targets for IBD therapeutics, in order to fully resolve their role in IBD, additional studies utilizing cell-type-specific conditional knockout animals in which standardized protocols to limit the influence of inter-strain variances in the commensal microbiota are implemented will be required.

AUTHOR CONTRIBUTIONS

CM and CF drafted the manuscript following initial discussions with EL and prepared the figures and tables. EL edited the manuscript and provided input on content, figures and perspectives.

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IL-1 Family Members in Cancer; Two Sides to Every Story

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The IL-1 family of cytokines currently comprises of seven ligands with pro-inflammatory activity (IL-1α and IL-1β, IL-18, IL-33, IL-36α, IL-36β, IL-36γ) as well as two ligands with anti-inflammatory activity (IL-37, IL-38). These cytokines are known to play a key role in modulating both the innate and adaptive immunes response, with dysregulation linked to a variety of autoimmune and inflammatory diseases. Given the increasing appreciation of the link between inflammation and cancer, the role of several members of this family in the pathogenesis of cancer has been extensively investigated. In this review, we highlight both the pro- and anti-tumorigenic effects identified for almost all members of this family, and explore potential underlying mechanisms accounting for these divergent effects. Such dual functions need to be carefully assessed when developing therapeutic intervention strategies targeting these cytokines in cancer.

Keywords: interleukin-1 (IL-1), inflammation, cancer, IL-18, IL-33, IL-36 family interleukins

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INTERLEUKIN-1 SUPERFAMILY

Introduction

The importance of inflammation in cancer is now well established (1–3). In some cancers, the inflammatory conditions precede the development of malignancy, e.g., chronic bronchitis is a major risk factor for lung cancer. Alternatively, aberrant signaling due to oncogenic mutations in tumors can result in a chronic inflammatory state developing both proximal to, and within, the tumor. This chronic inflammation acts to inhibit the anti-tumorigenic immune response, normally mediated by cells such as M1 macrophages, NK cells and $CD8^+$ T cells. Tumor cells themselves can also directly induce an immunosuppressive microenvironment through recruitment and activation of specific immune cell subtypes such as myeloid derived suppressor cells (MDSC), M2 macrophages and T regulatory cells, further promoting tumorigenesis (2, 4, 5). Understanding the complexity of immunomodulation by tumors is important for the development of effective immunotherapies. Cytokines, such as Interleukin-1 (IL-1) are central mediators of the interactions between cells in the inflammatory tumor microenvironment. The IL-1 family now includes seven ligands with pro-inflammatory activity (IL-1α and IL-1β, IL-18, IL-33, IL-36α, IL-36β, IL-36γ) as well as anti-inflammatory cytokines (IL-37, IL-38). Several members of this family, such as IL-1β and IL-18, have been extensively investigated in cancer with both pro- and anti-tumorigenic functions ascribed to these cytokines. In contrast, far less is currently understood concerning the role of more recently identified members of this family in cancer such as IL-33 and IL-37, although such data that is available also indicates that these may have both pro- and anti- tumorigenic effects. In this review, we will explore the dual functions of these cytokines in the tumorigenic process.

INTERLEUKIN-1 (IL-1)

As IL-1 was the original defining member of its family, its physiological function and indeed, pathophysiological function, has been extensively studied and reviewed (6). The triumvirate of cytokines grouped under the heading of IL-1 includes two of the activator cytokines IL-1α and IL-1β, as well as an inhibitory cytokine, the IL-1 receptor antagonist. IL-1α and IL-1β bind the same receptor, the type 1 IL-1 receptor (IL-1R), recruiting both the IL-1R accessory protein and the adaptor protein MyD88 to the receptor complex, resulting in activation of the downstream signaling cascade and ultimately in the activation of a myriad of immune and inflammatory genes (7). Whilst these cytokines activate through the same pathway, they are separately encoded proteins with a low level of sequence homology and well characterized divergent biological processes, cellular localization and mechanisms of activation. Both IL-1α and IL-1β exist as pro-forms and cleaved forms, but whereas both forms of IL-1α are biologically active, only the cleaved form of IL-1β acts as a pyrogen. IL-1α is grouped in a category of dual function cytokines (with IL-33 and IL-37), as it is located both within the nucleus of the cell where it plays a role in transcription (8), and also as a functional membrane bound cytokine. In addition, IL-1α is released from necrotic cells allowing it to function as an "alarmin." In contrast, the processing and bioavailability of IL-1β is very tightly controlled. We now know that IL-1β requires a "two-signal" process to become activated (9), with the initial priming signal triggering transcription of the gene and the second signal, resulting in inflammasome activation, allowing caspase-1 mediated cleavage and activation of IL-1β. It is therefore, not surprising that these two cytokines, so often grouped together, have been shown to have different physiological and biological effects in many human diseases and indeed, with relevance to this review, in different cancer types (10). Also, as with so many of this family of cytokines, divergent pro- and anti- tumorigenic effects have been reported for both. Here we will detail some of these alternate functions ascribed to both cytokines.

IL-1α

Tumor-Promoting Effects of IL-1α

Whilst there is no shortage of data supporting a role for IL-1β in tumorigenesis, there are relatively fewer studies directly examining IL-1α in cancer development and progression. IL-1α expression has been reported to be significantly increased in head and neck squamous carcinoma (HNSCC) patients with distant metastasis as compared to those without metastasis (11). A similar correlation between IL-1α expression and distant liver metastasis has been reported in patients with gastric carcinoma (12). High levels of IL-1α expression in cancers have also been linked to tumor dedifferentiation (13) and lymphangiogenesis (14). Associations between single nucleotide polymorphisms in the IL-1α gene and cancer risk have been identified (15) with carriers of the rs17561 missense mutation showing reduced susceptibility to clear cell ovarian cancer.

Both *in vitro* and *in vivo* evidence have supported this clinical data. Interestingly, both forms of IL-1α have been shown to have potential tumor promoting effects (16) as the pro-piece, nuclear form of IL-1 has been shown to facilitate the growth of acute T-lymphocytic leukemia cells through the activation of NF-κB and SP1. Stable overexpression of IL-1α in the normally non-metastatic IL-1α negative pancreatic carcinoma cell line MIaPaCa-2 enhanced NF-κB production and cellular invasion *in vitro* (17), and resulted in liver metastasis following orthotopic injection in murine models. Moreover, co-culture of pancreatic ductal adenocarcinoma cells with cancer-associated fibroblasts (CAFs) resulted in marked upregulation of multiple inflammatory factors including IL-1α. Inhibition of IL-1α alone reduced the crosstalk-induced production of these inflammatory factors (18), indicating a key role for IL-1α in the formation and maintenance of the inflammatory tumor environment. Pancreatic cancer cell-derived IL-1α was similarly shown to promote hepatocyte growth factor secretion by fibroblasts (19), promoting pancreatic cancer invasion, proliferation and angiogenesis. IL-1α expression was also essential for tumor development following implantation of the 3-MCA-induced fibrosarcoma cell line (20), with findings from this study highlighting an important role for IL-1α in controlling immune-surveillance of the developing tumor. A similar role for Il-1α was recently demonstrated in breast cancer (21). Breast tumor-derived IL-1α, acting on tumor-infiltrating myeloid cells, induced the expression of thymic stromal lymphopoietin (TSLP), which was not only a critical tumor survival factor, but is also required for the metastatic spread of the tumor cells. These studies define a novel IL-1α-TSLP-mediated crosstalk between tumor-infiltrating myeloid cells and tumor cells in the control of metastatic breast cancer. Finally, recent elegant work examining the relationship between HER2 expression in breast cancer, inflammation and expansion of cancer stem cells (CSCs) highlighted an essential role for IL-1α in this process (22). These authors demonstrate that HER2 expression in the tumor drives a positive feed-forward activation loop, inducing the expression of IL-1α and IL-6. This, in turn, increases activation of NF-κB and STAT3, allowing for the generation and maintenance of CSCs. IL-1α- deficient mice show delayed MMTV-Her2-induced tumorigenesis, reduced inflammation and reduced numbers of CSCs. Moreover, pharmacologic blockade of IL-1α signaling reduced the population of CSCs in the tumors, and improved chemotherapeutic efficacy (22).

Given these tumor-promoting functions for IL-1α, studies have investigated whether it represents a potential therapeutic target for cancer therapy. MABp1 (XBiotech Inc.) is a naturally occurring human IL-1α neutralizing antibody which has shown promising outcomes in several cancer types (23, 24). Also, a recent randomized phase III trial by Kurzrock et al. showed patients with advanced colorectal cancer and lower levels of circulating IL-1Ra are more responsive to treatment with bermekimab, an antibody targeting IL-1α. Levels of IL-1Ra were shown to be reflective of the potentiated response from antibody administration (25). Further work in this field will identify whether mechanisms for neutralizing IL-1α can be optimized and made viable for cancer therapy.

Tumor-Suppressive Effects of IL-1α

In contrast to the above findings, studies have shown that, particularly transient, overexpression of IL-1α may have anti-tumorigenic effects. Both fibrosarcoma and lymphoma cells were induced to express IL-1α in a transient manner. Of note, in this model IL-1α was expressed in the cytosol and not excreted. The IL-1α-expressing tumor cells were seen to predominantly not result in tumor development. Furthermore, if tumors did occur, they subsequently regressed. Tumor regression was observed to be mainly mediated by $CD8^+$ T cells, with some involvement of NK cells and macrophages (26, 27). Moreover, stimulation of multiple cancer cell lines, including MCF-7 breast cancer, A375 melanoma, prostate stem cells and murine primary mammary cells, with IL-1α inhibited cell proliferation by causing G0–G1 cell cycle arrest. (28–30). More recently, a study investigating the role of the IL-1R in tumorigenesis indicated that IL-1R signaling could suppress mammary tumor cell proliferation in the MMTV-PyMT breast cancer mouse model (31). This phenotype was shown to be IL-1α dependant, indicating that IL-1α-IL1R signaling may be tumor-suppressive in PyMT-driven breast cancer.

IL-1β

Tumor-Promoting Effects of IL-1β

Evidence supporting a pro-tumorigenic role for IL-1β across all cancer types has been accumulating over many years and has been extensively reviewed in recent times (30). Solid tumors in which IL-1β has been shown to be upregulated include breast, colon, lung, head and neck cancers, and melanomas, and patients with IL-1β producing tumors generally exhibit a poorer prognosis (32). Multiple strands of both *in vitro* and *in vivo* evidence point toward a tumor-promoting function for IL-1β. Fibrosarcoma cells, genetically modified to constitutively excrete mature IL-1β, show increased growth, invasiveness and angiogenesis (33). Indeed, clear associations have been demonstrated between IL-1β and angiogenesis in multiple tumor types (34, 35). In a transgenic model of Myc-dependent carcinogenesis, IL-1β has been characterized as the principal effector molecule in the onset of angiogenesis (36). Mechanistically, this was seen to be mediated via matrix metalloproteinase (MMP) -induced sequestration of extracellular matrix–associated vascular endothelial growth factor (VEGF), followed by its ligation to VEGFR2 on endothelial cells. Moreover, in a model of melanoma, myeloid cells was shown to produce IL-1β (37), which subsequently activated endothelial cells to produce VEGF and other proangiogenic factors, modulating the inflammatory microenvironment of the tumor, allowing for enhanced angiogenesis and tumor progression.

As could be predicted based on its ability to enhance angiogenesis, IL-1β has also been shown, in multiple models across multiple cancer types, to enhance tumor cell metastasis. In both murine and human breast cancer models, tumor progression was associated with the activation of inflammasome components and subsequent elevated levels of IL-1β at primary and metastatic sites. Mice deficient for inflammasome components exhibited significantly reduced tumor growth and lung metastasis (38). Similar findings have been shown for the NLRP3 inflammasome in lymphangiogenesis and metastasis (39). Macrophage-dependent lymphangiogenesis was triggered upon inflammasome activation and required IL-1β production. NLRP3 expression in tumor-infiltrating macrophages also correlated with survival, lymph node invasion, and metastasis of mammary carcinoma patients (39). The role of IL-1β in driving expression and production of downstream pro-tumorigenic cytokines appears to play a key role in this process. IL-1β induces IL-6 production in breast cancer cells, as well as additional cytokines and growth factors including TGF-β, TNF-α, and EGF. Indeed, these cytokines and growth factors seem to potentiate the effect of IL-1β on IL-6 expression. In breast cancer cells, epithelial-to-mesenchymal (EMT) and stem-cell-like phenotypes were attenuated by either anti-IL-6 or anti-IL-1β antibody treatment (40). A similar causal link between IL-1β and IL-22 has recently been established (41). IL-22 is now emerging as a cytokine with potent tumor-promoting properties. It has recently been shown that IL-22 production in humans is dependent on activation of the NLRP3 inflammasome with the subsequent release of IL-1β from both myeloid and T cells. The IL-1 receptor antagonist Anakinra abrogates IL-22 production and reduces tumor growth in a murine breast cancer model (40).

Based on these, and many other (42), findings, IL-1β is now viewed as an attractive target for pharmacological intervention. Several agents are available including IL-1Ra (Anakinra) which inhibits both IL-1α and IL-1β signaling, neutralizing antibodies to IL-1β (Canakinumab), as well as inflammasome inhibitors. It has recently been shown that administration of inhibitory antibodies targeting IL-1β results in the augmentation of anti-tumor cell immunity in a murine model of breast cancer. A synergistic effect of anti-IL-1β and anti-PD-1 treatment with antibodies was capable of altering TME cell populations in favor of an anti-tumor phenotype (43). In clinical trials, Anakinra has been shown to inhibit IL-6 production and enhance the progress-free survival of patients with indolent myeloma (44). Moreover, a recent comprehensive study convincingly demonstrated that Anakinra may also be beneficial in the treatment of breast cancer (43). In this study, production of IL-1β in primary breast cancer tumors was linked with advanced disease. IL-1β was shown to originate from tumor-infiltrating $CD11c^+$ myeloid cells with production triggered by cancer cell-derived TGFβ. Administration of anakinra prevented breast cancer progression in humanized mouse model. Moreover, a pilot clinical trial wherein 11 patients with $Her2^-$ metastatic breast cancer were treated with daily subcutaneous Anakinra for a median duration of 4 months, in combination with one of the standard chemotherapeutics, demonstrated that high risk $Her2^-$ breast cancer patients would benefit from reducing levels of IL-1β and treatment with anakinra (45, 46). Excitingly, a recent phase three clinical trial (CANTOS) (45) demonstrated that an inhibitory antibody targeting IL-1β (canakinumab) could significantly reduce the incidence and mortality of lung cancer. Although this trial involving 10,500 patients was not initially designed to study lung cancer as an end-point, analysis revealed that there was a 56% reduction in incident lung cancer in the canukinumab-treated group as compared to the placebo group.

As these patients were extensively screened prior to recruitment, the indication is that inhibition of IL-1β reduced the progression, invasiveness and metastatic spread of early stage lung cancers that were undiagnosed at time of recruitment. These data emphasize the importance of this cytokine in chronic inflammation in lung tumorigenesis, and its validity as a therapeutic target.

Tumor Inhibiting Effects of IL-1β

The divergent and conflicting role of the IL-1 cytokines is particularly apparent when investigating IL-1β. The majority of these studies highlight the ability of IL-1β to induce both Th1 and Th17 responses and thus to induce anti-tumorigenic effects. Exogenous injection of IL-1β resulted in tumor regression, as long as the tumors were of a sufficient size, and the mice were not deficient in T cells (47). Haabeth and colleagues also demonstrated that IL-1β drives activation of tumor specific Th1 responses, thus protecting against B cell myeloma and lymphoma (48, 49). Contrasting tumor-inhibiting effects have also been ascribed to IL-1β in the case of colorectal cancer (CRC). Inhibition of the NLRP3 inflammasome was seen to reduce tumor burden in the murine model of colitis-associated cancer, with the increased tumor burden correlating with attenuated levels of IL-1β and IL-18 at the tumor site (50). An insight into the underlying mechanism concerning the dichotomous role of IL-1β in CRC was recently provided. Dmitrieva-Posocco and colleagues examined the impact of IL-1 signaling in different cell types within the CRC microenvironment (51). Analysis of epithelial cell specific IL-1R1 deletion revealed a decrease in CRC tumor multiplicity, slower proliferation of early tumor seeds, and decreased activation of NF-κB. T cell specific ablation of IL-1R1 similarly decreased tumor-elicited inflammation dependent on IL-17 and IL-22, thereby reducing CRC progression. In contrast, IL-1 signaling in myeloid, particularly neutrophil populations, was shown to be potently anti-tumorigenic due to their role in controlling the local microbiota populations. These authors demonstrate that IL-1R signaling in myeloid cells is responsible for keeping specific species/genera of tumor infiltrating microbes at bay, thus preventing local, tumor-specific dysbiosis and excessive amounts of pro-tumorigenic inflammatory cytokines. Interesting interactions between primary tumors and distant metastatic sites in breast cancer, involving IL-1β, have also recently been elegantly described. These authors demonstrate that certain primary tumors elicit a systemic inflammatory response involving IL-1β-expressing innate immune cells that then infiltrate distant metastatic microenvironments. At the metastatic site, IL-1β maintains metastatic cells in a ZEB1-positive differentiation state, preventing them from establishing themselves. In line with these findings (52), the authors further demonstrate that inhibition of the IL-1 receptor relieves the differentiation block and results in metastatic colonization, and that among patients with lymph node-positive breast cancer, high primary tumor IL-1β expression is associated with better overall survival and distant metastasis-free survival. Contrasting this, however, Li et al have provided evidence indicating an opposing role of IL-1β through ZEB1 activation resulting in the promotion of stemness and invasion of colon cancers cells (53). It is clear, therefore, that these dichotomous findings regarding IL-1β need to be carefully considered when developing anti-cancer therapies designed to inhibit IL-1β. The divergent roles of IL-1β in cancer are shown in **Figure 1**.

INTERLEUKIN-18

Interleukin-18 (IL-18) (also known as IFN-γ-inducing factor) was first identified in 1989 (54) as a factor that enhanced IFN-γ production from anti-CD3-stimulated Th1 cells, especially in the presence of IL-12. Subsequent isolation and characterization of the cytokine determined that its structure was similar to that of IL-1 (55). Similar to IL-1β, IL-18 is synthesized as an inactive 24kDa pro-peptide that is activated by proteolytic cleavage by caspase-1 in the NLRP3 inflammasome, generating the mature, bioactive, 18 kDa molecule. The IL-18 receptor (IL-18R) consists of the inducible component IL-18Rα (IL-1 receptor-related protein [IL-1Rrp]) and the constitutively expressed component IL-18Rβ (IL-1R accessory protein-like [IL-1RAcPL]), and activates a similar pathway to that of IL-1 (55). The activity of IL-18 is suppressed following binding by the high affinity, naturally occurring IL-18 binding protein (IL-18BP), which prevents IL-18 binding to its receptor. IL-18 is expressed by many cell types and plays an important role in both the innate and adaptive immune response (56).

Tumor-Promoting Effects of IL-18

Numerous studies have investigated the role of IL-18 in cancer, with both pro- and anti-tumorigenic functions identified (56). Some of the first evidence that IL-18 can promote tumor growth came from the observation of elevated expression of IL-18 by tumor cells or elevated levels of IL-18 in the serum of cancer patients, with elevated levels of IL-18 associated with poor prognosis (57–59). These high levels of IL-18 may play a role in angiogenesis, tumor cell migration, invasion and metastasis. Indeed, studies in gastric cancer showed that IL-18 induces the expression of the pro-angiogenic factor, vascular endothelial growth factor (VEGF) (60). VEGF, in turn, can increase the expression and processing of IL-18 (61, 62), with IL-18 increasing cell migration directly through filamentous-actin polymerization and tensin downregulation (61, 62). Consistent with cell migration and angiogenesis being key steps in tumor metastasis, suppression of IL-18 expression in gastric cancer cells resulted in a reduction in both primary tumor growth and metastasis *in vivo*. Moreover, angiogenesis was greatly reduced in tumors lacking IL-18 relative to control tumors (60).

Additional mechanisms proposed for the migratory and metastatic ability of IL-18 involve alterations in expression of adhesion molecules and tight junction proteins (63–65). For instance, administration of IL-18BP significantly suppressed melanoma cell metastasis to the liver by preventing IL-18-induced expression of adhesion molecules such as vascular cell adhesion molecule-1 (VCAM-1) on hepatic sinusoidal endothelial cells (63). Exogenous IL-18 also enhanced the migration of breast cancer cells (65), and this was due to suppression of expression of the tight junction proteins, in particular, claudin-12.

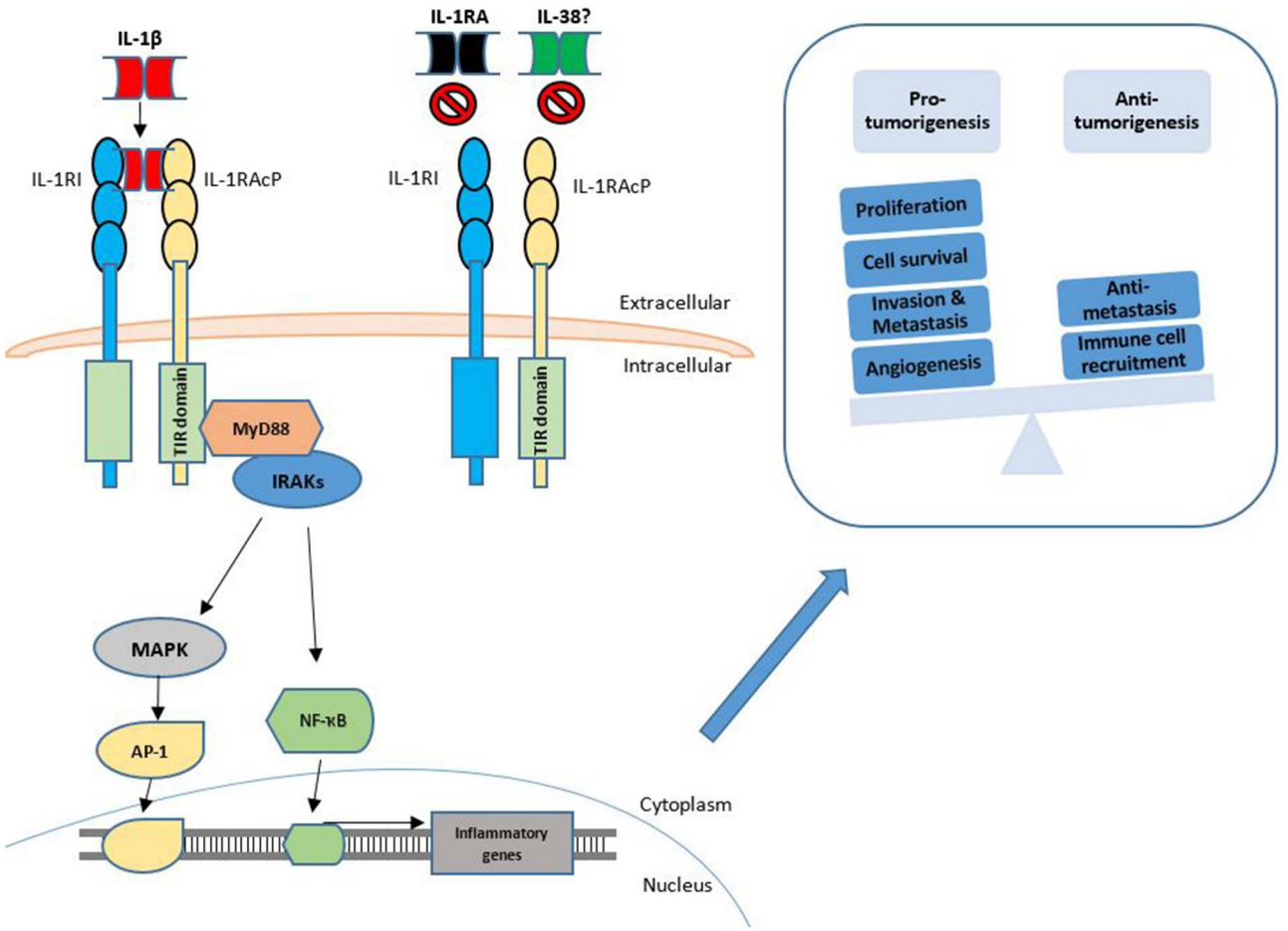

FIGURE 1 | Functions of IL-1β signaling in cancer. The IL-1 signaling pathway is activated through the binding of IL-1α or IL-1β to the IL-1RI. This is followed by dimerization of IL-1R1 with IL-1RAcP. This interaction may be competitively inhibited by the IL-1RA and IL-38. Agonist binding is followed by engagement of further signaling intermediates with subsequent activation of transcription factors such as NF-κB and AP-1. Transcription of inflammatory genes is upregulated which may then result in pro-tumorigenic or anti-tumorigenic phenotypes.

Other tumor-promoting effects associated with IL-18 include evasion of the anti-tumor immune response. Natural Killer (NK) cells, if appropriately activated, play an important role in tumor rejection. However, NK cells can also have immunosuppressive effects. Administration of recombinant IL-18 was shown to up regulate the expression of PD-1 by conventional NK cells, and to convert them into a subset with immunosuppressive characteristics (66, 67). Silencing of IL-18 or blockade of IL-18 activity with IL-18BP, in turn, led to the restoration of NK cell-dependent immune surveillance in tumors (67). IL-18 has also been implicated in the generation of myeloid derived suppressor cells (MDSC). MDSC are potent suppressors of the anti-tumor immune response, with administration of IL-18 shown to increase the population of MDSC in multiple myeloma and in a murine model of melanoma (68, 69). Together, these studies suggest that targeting IL-18 may represent a potential mechanism to overcome tumor-associated immunosuppression.

However, phase 2 clinical trials for metastatic melanoma by GSK utilizing a recombinant human IL-18 (Iboctadekin) demonstrated insufficient clinical benefit (70). It is possible, however, that combining an IL-18 targeting strategy with additional chemotherapeutic reagents could be a better approach in the treatment of patients with cancer. Indeed, a dose-escalation study of recombinant Iboctadekin in combination with rituximab (anti-CD20) was well tolerated and showed increased production of IFN-γ, GM-CSF and chemokines in a pilot study of 5 patients with non-Hodgkin's lymphoma (71).

Tumor-Inhibiting Effects of IL-18

IL-18, however, has also be shown to activate the anti-tumor immune response, resulting in the suppression of tumor growth and metastasis in multiple tumor types. For instance, administration of recombinant IL-18 has been shown to activate $CD4^+$ T cells and/or NK cell immune responses, suppressing the growth and metastasis of melanoma cells *in vivo* (72). Consistent with this, individuals with low NK cell activity have an increased risk of developing cancer (73), with high numbers of

intratumoral NK cells often correlated with improved prognosis for cancer patients (74, 75).

These opposing effects described for IL-18 in tumorigenesis could be due to the level of IL-18 administered and/or present in the tumor microenvironment (67). For instance, low doses of IL-18 were shown to accelerate tumor progression (66), in part through the induction of Kit^+ immunosuppressive NK cells, while daily administration of IL-18 induced inflammation and increased the anti-tumor response, thus suppressing tumor growth. The function of IL-18 in cancer could also depend on cytokines present at the site of NK cell activation and maturation. Recent studies have shown that incubation of NK cells with IL-18, IL-12, and IL-15 results in the generation of cytokine-induced memory-like (CIML) NK cells, which have enhanced cytokine (IFN-γ) production and exhibit potent anti-tumor effects against both hematological (76) and non-hematological malignancies (77). Studies aimed at establishing protocols for the *in vitro* generation of tumor-suppressive NK cells determined that radiation therapy greatly increased the number and function of the transferred NK cells, and was essential for the anti-tumor activity of transferred CIML NK cells (78). Thus, harnessing cytokine-induced memory-like NK cell responses could represent a promising translational immunotherapy approach for patients with malignant disease.

Given the studies demonstrating an anti-tumorigenic function for IL-18, numerous strategies are being investigated aimed at increasing the level of IL-18 in the tumor microenvironment and thus to augment the anti-tumor immune response. This includes the engineering of oncolytic adenoviruses co-expressing IL-18 and IL-12 (79). Intratumoral administration of these adenoviruses improved the anti-tumor immune response in the B16-F10 murine melanoma model, in part though the generation of an effective T-cell mediated immune response. Bacteria have also been used to deliver IL-18 into the tumor microenvironment to augment the T-cell mediated immune response (80). IL-18-producing attenuated *Salmonella typhimurium* inhibited the growth of primary subcutaneous tumors as well as pulmonary metastases in immunocompetent mice challenged with syngeneic multidrug-resistant clones of murine carcinoma cell lines, without overt toxicity to normal tissues.

INTERLEUKIN-33

Initially identified as a nuclear factor, IL-33 was later determined to be a ligand for the receptor ST2 (Fit-1/IL-33R/ IL1RL1). A decoy receptor, sST2, has been found to be integral to the regulation and attenuation of the IL-33/ST2 axis, similar to that of IL1-R2 in interleukin-1 signaling (81, 82). IL-33 is expressed by a diverse range of cells, with the strongest expression observed in non-haematopoietic cells including endothelial cells, epithelial cells, keratinocytes, fibroblasts, fibrocytes and smooth muscle cells. Its receptor, ST2, is strongly expressed on the surface of fibroblasts and hematopoietic cells such as T helper type 2 (Th2) lymphocytes, ILC2s and mast cells. The IL-33/ST2 pathway has been investigated in many inflammatory diseases and dysregulation of this pathway has been particularly implicated in Th2-mediated conditions such as allergic diseases (83). The IL-33/ST2 signaling axis has been linked to processes involved in wound healing such as angiogenesis, production of matrix components, to fibrosis, and to modulation of immune populations (84). Given the critical importance of such pathways in tumor formation, links between the IL-33/ST2 signaling axis and tumorigenesis have recently been identified, with multiple research groups implicating IL-33 as a key player in the mediation of neoplastic transformation, tumor growth and metastasis in various cancers including breast, gastric, colorectal and lung (85). In parallel with other IL-1 family members, both pro- and anti-tumorigenic roles have been reported for IL-33 in cancer.

Tumor Promoting Effects of IL-33

Several studies have examined expression of IL-33 and ST2, and their association with patient prognosis. IL-33 and ST2 are both elevated in comparison to adjacent healthy tissue in breast cancer, with serum levels of IL-33 and its decoy receptor soluble ST2 (sST2) also elevated. These expression changes also correlated with markers of poor prognosis (86). Similarly, expression of IL-33 was found to be increased when compared to adjacent healthy tissue in patients with both non-small-cell lung carcinoma (NSCLC) (87) and epithelial ovarian cancer (88). Maywald et al showed expression of IL-33 to be induced in colorectal cancer at both mRNA and protein levels in patient-derived cell lines, adenocarcinoma biopsies and in the systemic circulation of CRC patients as compared to adjacent non-tumor tissue (89).

Much of the early work examining IL-33 in tumorigenesis focussed on the role of IL-33 in breast cancer. Deletion of ST2 in BALB/c mice bearing mammary carcinoma attenuated tumor growth and metastasis (90). This was accompanied by increased serum levels of IL-17, IFN-γ and TNF-α, and decreased IL-4. Suppressing sST2 reduced ErbB2-induced cell motility in breast cancer cells (91). In a subsequent study (92), administration of exogenous IL-33 to breast cancer-bearing mice was seen to enhance tumor growth and metastasis. The mechanism proposed to be responsible for the enhanced tumor growth involves the ability of IL-33 to cause an increase in the number of infiltrating immunosuppressive immune cells and innate lymphoid cells to the tumors, drive the tumorigenic process. Subsequent *in vitro* and *in vivo* work on multiple cancer types have confirmed these findings, with a similar IL-33-induced immuno-suppressive microenvironment reported to play a tumor-promoting role in, amongst others, CRC, gastric cancer, lung cancer, head and neck cancers, pancreatic cancers and cervical cancer (93).

A role for IL-33 in the classic tumorigenic processes such as EMT, proliferation, migration and invasion, has now been extensively studied (84). Stromal CAFs are the main type of non-immune cells in the tumor microenvironment (TME), with CAFs known to interact with cancer cells to promote tumor proliferation. IL-33-producing CAFS have been implicated as a critical mediator in CAF-induced invasiveness in head and neck squamous cell carcinoma (HNSCC) (94). In this cancer type, administration of IL-33 promotes cancer cell migration and invasion through induction of EMT. In oral squamous cell carcinoma (OSCC), an interplay between a long non-coding (lnc)-RNA strongly upregulated in CAFs and IL-33 was recently

established. IL-33 was found to be co-expressed with this lnc-CAF, resulting in elevation in the expression of CAF markers, conversion of the normal fibroblast phenotype to stromal carcinoma-related fibroblasts and enhanced tumor growth. This was achieved by the lnc-CAF promoting IL-33 stability (95). Intestinal epithelial cells, in turn, have been shown to use epidermal growth factor (EGF) as a key factor to modulate IL-33 production in cells. Inhibition of the EGF receptor resulted in lower levels of IL-33 transcripts, with increased EGF stimulation resulting in increased expression of ST2 and IL-33 (96). IL-33 has also been shown to promote tumor cell growth, colony formation and expression of Ki-67 and proliferating cell nuclear antigen (PCNA) in a PGE_2 dependant manner (97).

Regarding tumor cell metastasis, *in vitro* assays have shown a direct ability of IL-33 to stimulate migration and invasion of the lung cancer cell line A549 (98). Moreover, Andersson and colleagues recently demonstrated that in mouse and human fibroblast-rich pancreatic cancers, genetic deletion of IL-33, ST2 or MMP-9 markedly blocked metastasis (99). This appeared to be due, in part, to modulation of the tumor microenvironment by IL-33-producing CAFs, which caused the transition of tumor-associated macrophages from an M1 (anti-tumorigenic) to an M2 (pro-tumorigenic) phenotype. Moreover, the decoy receptor sST2 has been found to have decreased expression in high-metastatic cells compared with low-metastatic human and mouse CRC cells (100). sST2 was found to negatively regulate tumor growth and the metastatic spread of CRC through modification of the tumor microenvironment. The expression of the IL-33 receptor, ST2L, has also been implicated in metastasis (101). Low-metastatic cells but not high-metastatic cells derived from Lewis lung carcinoma were found to express functional ST2L. IL-33 was seen to augment the cell death of ST2L-positive low-metastatic cells, but not of the ST2L-negative high-metastatic cells, suggesting that IL-33 enhances lung cancer progression by selecting for more malignant cells in the tumor microenvironment. Combined these data indicate the potential therapeutic benefit of blocking this pathway in tumorigenesis.

Tumor-Inhibiting Effects of IL-33

Alongside the above listed pro-tumorigenic roles ascribed to the IL-33/ST2 pathway are several strands of evidence implicating a divergent role for IL-33 in the inhibition of tumorigenesis. Many reports focusing specifically on tumoral expression of IL-33 and ST2 have reported decreasing expression of these proteins with increasing tumor stage. In cervical intraepithelial neoplasia (CIN) tissues, IL-33 protein and mRNA levels in cervical tissues were significantly lower in severe CIN as compared to those patients with mild or no CIN (102). Levels of IL-33 have also been shown to negatively correlate with tumor stage in multiple myeloma patients (103) and lung cancer patients (104). Our laboratory also demonstrated that ST2L expression is decreased in human CRC tissue as compared to adjacent non-tumor tissue, with lower ST2L expression correlating with poorer patient prognosis (105). A similar pattern of expression has been demonstrated in metastases. IL-33 expression was shown to be reduced in many carcinomas upon their transition to the metastatic form of the disease (106). These authors described low tumoral expression of IL-33 as an immune biomarker associated with recurrent prostate and kidney renal clear cell carcinomas. Taken together these studies indicate that downregulation of the IL-33/ST2 axis in epithelial cells is tightly associated with tumorigenesis and metastasis, suggesting that retaining IL-33/ST2 expression in these cells might inhibit cancer progression.

A key theme emerging in the field of the anti-tumorigenic effects of IL-33 is the ability of Il-33 to induce Type 1 immune responses. Given the fact that the focus of IL-33/ST2 research was, for many years, related to its expression and involvement in type 2 immune cells and responses, this is something of a paradigm shift. Gao et al. investigated the effect of overexpressing IL-33 in both B16 melanoma cells and 4T1 breast cancer cells (107). Intradermal injection of these cells resulted in a greatly decreased tumor growth rate. Characterization of immune cells in the IL-33-expressing tumors revealed a local increase in $CD8^+$ T cells and NK cells, with increased expression of type 1 effectors such as IFN-γ, IL-12 and granzyme B. Moreover, IL-33 was shown to regulate local tumor-associated myeloid cells and enhance the expression of MHC II as a result of the type I immune response (108). Similarly, suppression of ST2 in colon cancer cells was seen to enhance tumor growth and reduce $CD8^+$ T cell infiltration into the tumors (105).

Administration of exogenous IL-33 has also been shown to have potent tumor protective effects. Augmentation of the immune response by systemic administration of recombinant IL-33 was shown to inhibit leukemia growth and prolong the survival of leukemia bearing mice (109). This was mediated via an increase in $CD8^+$ T cell and IFN-γ. Similarly, administration of recombinant IL-33 inhibited the growth of established melanoma tumors by activating myeloid dendritic cells and tumor associated $CD8^+$ T cells (110). Exogenous administration of IL-33 has also been shown to limit cellular metastasis (111). Injection of IL-33 into mice-bearing subcutaneous B16-F10 melanoma cells resulted not only in significant attenuation of primary tumor growth but also in a reduction in pulmonary metastasis. Finally, introduction of IL-33 into metastatic tumors reduced the levels of circulating tumor cells and boosted immune recognition against metastatic tumors *in vivo* (106). Collectively, these data indicate that when high levels of IL-33 are administered, potent anti-tumor effects are observed.

Taken together, there can be no doubt that whilst IL-33 drives tumorigenesis in certain cancers and cell types much caution must be exerted surrounding future development of this cytokine as a therapeutic target.

INTERLEUKIN-36

The IL-36 cytokines are a recently described subset of the IL-1 family of cytokines (112). The three members of this family, IL-36α, IL-36β, and IL-36γ, all share the same receptor complex, which is composed of the IL-36 receptor (IL36R/IL1RRP2/IL1RL1) and the IL1 Receptor accessory protein. A biological inhibitor to this complex has also been identified, the IL-36R antagonist (IL-36Ra). The IL-36 cytokines and their receptor are expressed by several tissues, particularly the lung, skin and intestine, as well as by immune cells such as monocytes, macrophages, dendritic cells and T cells (112, 113). Activity of the IL-36 cytokines relies on proteolytic cleavage, with,

proteases released by neutrophils, in particular cathepsin G and elastase, recently identified shown to be potent activators of the IL-36 cytokines (114).

Similar to other IL-1 family members, IL-36 cytokines are important activators of the inflammatory response, stimulating both innate and adaptive immune responses (113). Whilst these cytokines have been shown to play an important role in autoimmune diseases, in particular in the pathogenesis of psoriasis and Inflammatory Bowel Diseases, few studies to date have investigated their role in cancer. Indeed, the majority of studies to date have focused on IL-36γ, with few, if any, investigating the role of IL-36α or IL-36β in cancer.

Tumor-Inhibiting Effects of IL-36

One of the first studies identifying a role for IL-36γ in cancer determined that IL-36γ was anti-tumorigenic in breast cancer and melanoma (115). In particular, IL-36γ was shown to enhance the effector functions of $CD8^+$ T cells, NK cells and γδ T cells, to transform the tumor microenvironment into one favoring tumor destruction, and ultimately to have profound anti-tumor effects, suppressing both tumor growth and metastasis. Chen et al. investigated this anti-tumorigenic role of IL-36γ (116). The group developed and showed a polymer-based delivery system that allowed the co-delivery of IL-36γ expression plasmid with doxorubicin (Dox). This showed that micelles loaded with IL-36γ and Dox significantly reduced the metastatic spread of breast cancer cells to the lung. This was associated with an enhanced type I immune response together with a reduction in the levels of immunosuppressive myeloid-derived suppressor cells in the lung. Moreover, injection of bioactive IL-36γ into the tumor microenvironment was also shown to delay tumor progression (117), via with the rapid recruitment of T cells and the formation of tertiary lymphoid organs (TLOs). TLOs form at sites of persistent inflammation and provide a specialized microenvironment for priming of naïve T cell into effector cells, with the presence of TLOs in a broad range of cancer types associated with improved clinical prognosis (118).

Subsequent studies in human colon cancer demonstrated the presence of IL-36γ in a variety of cell types within the tumor microenvironment, including immune cells, in particular in classically activated or M1 macrophages, tumor cells and vascular/perivascular cells (119). Interestingly, expression of the IL-36 receptor antagonist, IL-36RN, was associated with elevated expression of immune checkpoint molecules such as PD-1, PD-L1, and CTLA-4 which are well-characterized inhibitors of the anti-tumor immune response. Given that the binding affinity of IL-36γ to the IL-36R is ∼ 100–1000-fold greater than IL-36RN, the ability of exogenously administered IL-36γ to augment the anti-tumor immune response and suppress tumor growth, may be, in part, due to a reversal in suppression mediated by these checkpoint inhibitors.

Of the remaining members of this family, IL-36α and IL-36β, to date, three studies in the literature have suggested that IL-36α may also have tumor-suppressive effects. IL-36α was shown to suppress the growth of ovarian cancer cells (120). Moreover, reduced expression of IL-36α has also been shown to be associated with poor prognosis in colon (121) and hepatocellular cancer (122). However, the mechanisms underlying this suppressive function are unknown. As such, the role of IL-36α or IL-36β in cancer remains poorly defined.

Interestingly, in contrast to other members of this family, to the best of our knowledge, no studies to date have determined that any of the IL-36 cytokines have tumor-promoting effects. Given the paucity of studies concerning IL-36α and IL-36β in tumorigenesis, further work is required to delineate the role of these family members in cancer and future studies may identify alternate tumor promoting effects of these cytokines. The known functions of IL-36 signaling in cancer are summarized in **Figure 2**.

INTERLEUKIN-37

Similar to IL-1α and IL-33, IL-37 is a dual-function cytokine that can function as either a nuclear transcription factor or can activate signaling pathways by binding to its receptor on the cell surface (123, 124). The gene for IL-37 undergoes alternative splicing, resulting in the expression of five splice variants (IL-37a–e), although IL-37c and IL-37e are thought to encode non-functional proteins (123). IL-37b is the most abundant and most studied isoform, with almost all experimental published investigations focused on this isoform (124).

Expression of IL-37 is induced by pro-inflammatory stimuli, with IL-37 being synthesized as a precursor that is cleaved in the cytosol by caspase-1, generating mature IL-37. Both the precursor and mature forms are secreted by cells, and both forms are biologically active (124). Extracellular IL-37 binds to IL-18 receptor α (IL-18Rα; also used by IL-18), and recruits IL-1 receptor 8 (IL-1R8/TIR-8/SIGIRR) for the activation of intracellular signaling pathways, while the intracellular mature form of IL-37 binds to SMAD-3 in the cytoplasm, and then traffics to the nucleus. In contrast to the inflammatory function seen with other IL-1 family members, IL-37 is consistently an anti-inflammatory cytokine, with both extracellular and nuclear IL-37 functioning to suppress innate and adaptive immune responses by inhibiting the production of pro-inflammatory cytokines and chemokines (125, 126).

Tumor-Promoting Effects of IL-37

While most studies investigating a role for IL-37 in cancer have determined that IL-37 is an anti-tumorigenic cytokine, some studies have suggested that IL-37 may also have pro-tumorigenic functions. Consistent with a role in tumor growth and metastasis, high levels of IL-37 in the serum of patients with ovarian cancer was associated with poor prognosis, poor overall survival and the progression-free survival (127). Similarly, IL-37 protein expression was shown to be increased during the malignant transformation of the oral mucosa, being elevated to precancerous lesions as well as in OSCC (128). However, IL-37 expression was lower in OSCC cells with lymph node metastasis than in those without metastasis, suggesting that IL-37 may play a role in cellular transformation, but once cells become malignant, IL-37 functions to inhibit the tumorigenic process.

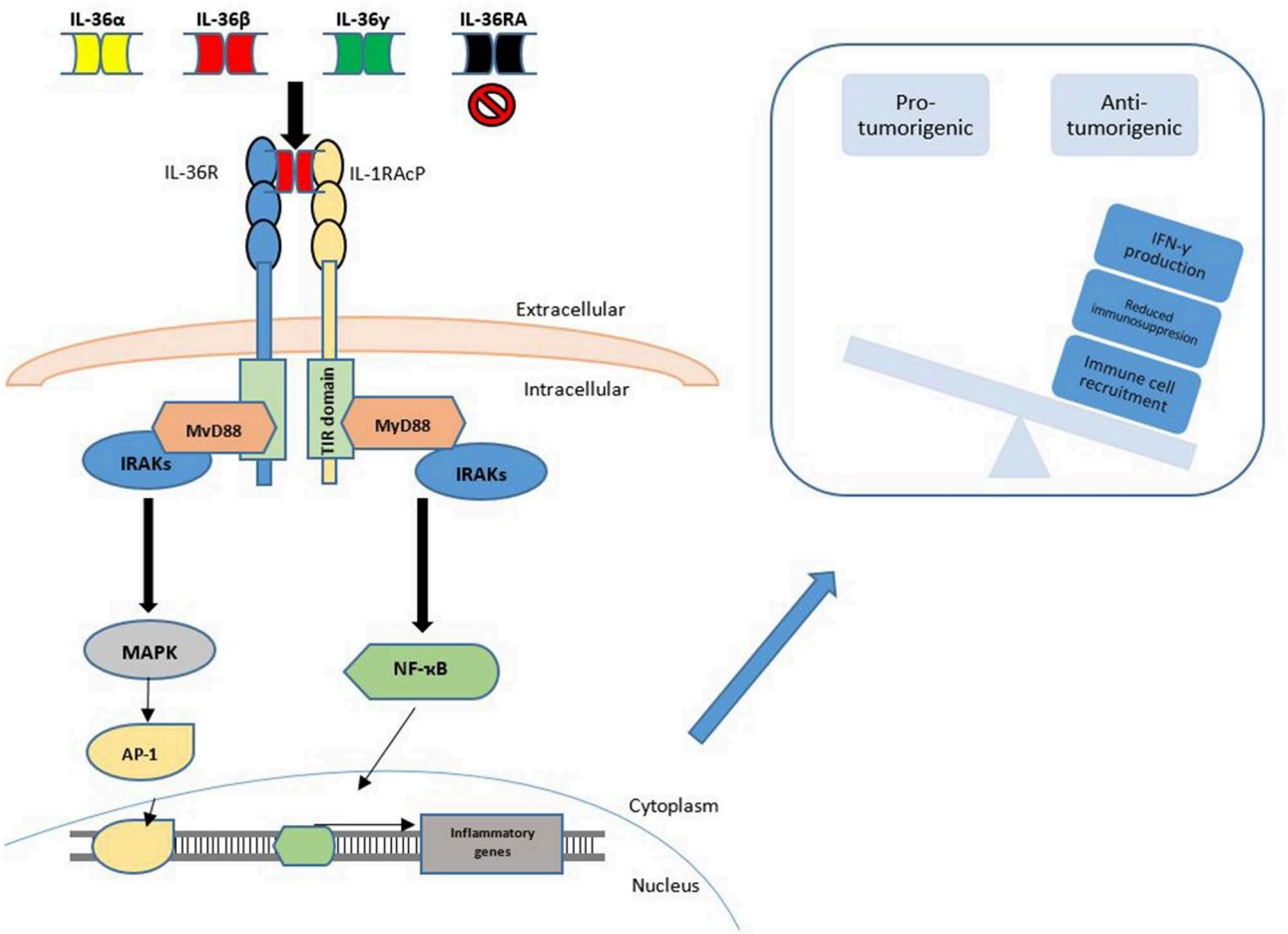

FIGURE 2 | IL-36 Signaling in cancer. IL-36 signaling is achieved through binding of the IL-36α, IL-36β or IL-36γ cytokines to the IL-36 Receptor (IL1RL2), along with the transmembrane accessory protein, IL-1RAcP. This binding may be inhibited through competitive binding of IL-36RA or IL-38. Agonist binding is followed by subsequent engagement and phosphorylation of intracellular signaling molecules to activate transcription factors such as NF-κB and AP-1. This promotes the observed anti-tumorigenic phenotype, which may be achieved by increased IFN-γ production, reduced immunosuppression and increased immune cell recruitment to the local tumor microenvironment.

Moreover, a recent study determined that IL-37 is a potent pro-angiogenic cytokine (129). IL-37 was shown to be upregulated by endothelial cells under hypoxic conditions, and to promote endothelial cell proliferation, capillary formation, migration and vessel sprouting from aortic rings. Given that tumor growth and metastasis depends on angiogenesis, the ability of IL-37 to promote the formation of new blood vessels in hypoxia regions of tumors could represent an important pro-tumorigenic function for IL-37. However, whether IL-37 functions as a pro-angiogenic factor in tumors is currently unknown.

Tumor-Inhibiting Effects of IL-37

Given its ability to suppress the production of inflammatory factors, it is unsurprising that the majority of studies investigating the role of IL-37 in cancer have shown that IL-37 is decreased in cancer, with low levels associated with poor prognosis (130–133). Moreover, studies have determined that IL-37 can suppress tumor progression, migration, invasion and metastasis in multiple cancer types, including fibrosarcoma (134), hepatocellular carcinoma (HCC)(135), cervical cancer (136), lung cancer (137) and colon cancer (138). Consistent with these studies, intratumoral injection of IL-37 or over-expression of IL-37 by tumor cells inhibited tumor growth (130, 132, 134). Several mechanisms have been proposed, including suppression of STAT3 signaling (130, 136, 137). Activated STAT3 is known to increased cell proliferation and invasion, and to promote oncogenic inflammation, with IL-37 shown to inhibit both the expression and phosphorylation of STAT3. IL-37 has also been shown to inhibit epithelial-mesenchymal transition (137, 139, 140), which plays an important role in metastasis. Additional mechanisms proposed for the anti-metastatic function include inhibition of Rac1 activation (133), with the intracellular mature form of IL-37, but not its extracellular form, markedly inhibiting the migration of multiple tumor types.

IL-37 has also been shown to affect immune cell recruitment to the tumor microenvironment (132). IL-37 expression positively correlated with the density of tumor infiltrating $CD57^{+}$ NK cells

in HCC tumors, which were significantly associated with better overall survival in HCC patients. Moreover, overexpression of IL-37 by hepatic tumor cells resulted in enhanced local $CD57^+$ NK cell infiltration of the tumors and suppressed tumor growth *in vivo* (132). This relationship between high intratumoral levels of NK cells and increased survival has been shown in several cancer types (141). Moreover, given that IL-37 suppresses inflammatory cytokine production, IL-37 could potentially alter

TABLE 1 | Pro- and anti-tumorigenic functions of IL-1 family members in cancer.

	Function	Mechanism	Tumor Type
IL-1α	Pro-tumorigenic	Angiogenesis	Pancreatic cancer (19), Lung cancer (14)
		Cell growth	ALL (16)
		Dedifferentiation	Breast cancer (13)
		Invasion & Metastasis	HNSCC (11), Gastric Cancer (12), Pancreatic cancer (17, 19), Breast cancer (21)
		Proliferation	Pancreatic cancer (19), Breast Cancer (21, 22)
		Tumor-promoting inflammation	Pancreatic cancer (17), Fibrosarcoma (18)
	Anti-tumorigenic	Immune cell recruitment	Fibrosarcoma (25), lymphoma (26)
		Proliferation	Breast Cancer (27), Melanom, Prostate Cancer (28)
IL-1β	Pro-tumorigenic	Angiogenesis	Fibrosarcoma (32), Lung cancer (34), Pancreatic cancer (35), Melanoma (36), Breast cancer (38)
		Cell growth	Fibrosarcoma (32)
		Invasion & Metastasis	Breast cancer (37–39), Lung cancer (45), CRC (50)
		Proliferation	Breast cancer (38), Fibrosarcoma (32)
		TME modulation	Breast cancer (37)
	Anti-tumorigenic	Immune cell recruitment	Fibrosarcoma (46), lymphoma (48), B-cell myeloma (47)
		Metastasis	Breast cancer (51)
IL-18	Pro-tumorigenic	Angiogenesis	Gastric cancer (58)
		Invasion & Metastasis	Melanoma (61, 62), HCC (55)
		Immune evasion	Melanoma (64, 65, 67), Colon Carcinoma (64, 65) Myeloma (66)
		Migration	HCC (61), Breast cancer (63)
	Anti-tumorigenic	Immune cell modulation	Melanoma (70, 71, 78), CRC7 (3), Lung cancer (74), Myeloid leukaemia (75), Ovarian Cancerm (76), Breast Cancer (79)
IL-33	Pro-tumorigenic	Cell growth	OSCC (96), Colon cancer (97), CRC (98, 101)
		Immune evasion	Breast Cancer (91), HNSCC (95), Pancreatic Cancer (92), Cervical Cancer (92)
		Invasion & Metastasis	Lung Cancer (86, 99, 102), Breast cancer (89–91), Ovarian Cancer (87), CRC (88, 101)
		Migration	HNSCC (95), Lung Cancer (99)
		TME modulation	CRC (88, 93, 94), Gastric Cancer (92), Lung Cancer (94), Pancreatic Cancer (92)
	Anti-tumorigenic	Immune modulation	Cervical Cancer (103) Multiple Myeloma (104), Lung Cancer (105),
		Metastasis	CRC (106), Melanoma (108, 111), Breast Cancer (108) Prostate Cancer (107), Renal Clear Cell Carcinoma (107)
IL-36	Pro-tumorigenic	N/A	N/A
	Anti-tumorigenic	Metastasis	Breast Cancer (117)
		TME modulation	Breast Cancer (116, 117), Melanoma (116), Fibrosarcoma (118), Colon cancer (118, 120)
IL-37	Pro-tumorigenic	Cell transformation	OSCC (129)
		Metastasis	Ovarian Cancer (128)
	Anti-tumorigenic	TME modulation	Renal Cell Carcinoma (132), HCC (133, 136), Fibrosarcoma (135), Colon Cancer (139)
		Proliferation	Cervical Cancer (137), Lung Cancer (140),
		Invasion & Metastasis	Cervical Cancer (131, 137), Lung cancer (131, 134, 138), Gallbladder Cancer (141), Breast Cancer (131), Liver adenocarcinoma (131), Prostate Cancer (131)
		Migration	Lung Cancer (140), Gallbladder cancer (141), OSCC (123)
IL-38	Pro-tumorigenic	TME modulation	Lung Cancer (150)
	Anti-tumorigenic	Invasion	Lung Cancer (151)
		Migration	Lung Cancer (151)
		Proliferation	Lung Cancer (151)

the tumor microenvironment in favor of one that promotes tumor rejection. For instance, IL-37 reduces the secretion by both immune cells (142) and tumor cells (133) of IL-6, IL-1β and tumor necrosis factor alpha, cytokines known to play a role in tumor progression.

Finally, consistent with IL-37b being the most abundant isoform of IL-37, studies investigating tumor expression of the IL-37 splice variants identified IL-37b as the isoform expressed by breast, liver, lung and prostate cancer, with low levels of IL-37b significantly associated with poor prognosis (130). Whether this variant of IL-37 is important in suppressing tumor growth and metastasis in all forms of cancer is unknown.

Taken together, the major function of this member of the IL-1 family is to suppress the development and growth of tumor cells, and suggest that it represents a novel target for the treatment of neoplastic disease.

INTERLEUKIN-38

IL-38 is the most recent member of the IL-1 family to be identified. It was discovered by two independent research groups in 2001 (143, 144). IL-38 shares structural features with both IL-1Ra and IL-36Ra, suggesting that it might act as an IL-1 family antagonist. Indeed, one of the first described biological functions of IL-38 was blocking the activation of the IL-36R, similar to that of IL-36Ra (145). In addition to binding to the IL-36R, studies have also proposed that IL-38 can bind to IL-1R1, or can interact with one of the inhibitory co-receptors of the IL-1R family, namely SIGIRR, TIGIRR1 and/or TIGIRR2 (146, 147). Similar to other family members, IL-38 is also processed by proteolytic cleavage, although there is currently a lack of consensus on the cleavage site.

IL-38 is expressed in several tissues including tonsils, placenta, heart and brain, and has been implicated in a variety of diseases including cardiovascular and autoimmune disease. In general, IL-38 polymorphisms are associated with increased susceptibility for auto-inflammatory diseases such as spondyloarthritis, rheumatoid arthritis, and psoriatic arthritis, suggesting a role of IL-38 suppressing chronic inflammation (147). Consistent with this, overexpression of IL-38 attenuated the severity of experimental arthritis, in part by decreasing the production of pro-inflammatory cytokines by macrophages and synovial fibroblasts (148).

Tumor-Promoting Effects of IL-38

Few studies to date have investigated the role of IL-38 in cancer. One of the first studies investigating expression of IL-38 in cancer demonstrated that tumor expression of IL-38 was increased in multiple cancer types, and that high expression of IL-38 was associated with poor prognosis in lung adenocarcinoma (149).

Tumor-Inhibiting Effects of IL-38

In contrast, Wang et al. demonstrated that IL-38 expression is decreased in NSCLC alone relative to adjacent non-tumor tissue (150), with lack of IL-38 expression correlating with poor prognosis. Administration of recombinant IL-38 inhibited β-catenin expression and reduced the proliferation, migration and invasion of lung cancer cells *in vitro*, while increasing cell death. Consistent with a role for IL-38 in suppressing lung cancer, overexpression of IL-38 suppressed NSCLC development *in vivo* and increased the sensitivity to chemotherapeutic drugs. The reason for these divergent results is unclear, but may be due to differential processing of the precursor form of IL-38 by the different tumor types. Studies comparing the function of full-length IL-38 vs. truncated IL-38 in macrophages (151) demonstrated that high concentrations of the truncated form decreased IL-1β-induced IL-6 production in macrophages. However, the full-length form of IL-38 increased IL-6 production at these concentrations, suggesting the biological function of IL-38 is affected by processing *in vivo*. Although IL-38 is well characterized as an anti-inflammatory cytokine (147), the role of IL-38 in cancer remains to be determined.

CONCLUSIONS

The IL-1 superfamily is a diverse family of cytokines, most of whose members have been shown to have dual functions in tumorigenesis with both pro- and anti-tumorigenic roles being ascribed (**Table 1**). The role of these cytokines varies greatly depending on the tissues and organs involved, the inflammatory background and the stage of the cancer. An additional factor determining the pro- or anti- tumorigenic effects of these cytokines appears to be whether the cytokines are produced by the tumor-infiltrating immune populations or by the tumor cells. Finally, it is possible that the levels of the cytokines present within the tumor microenvironment may have a role in determining the outcome of the effect on tumorigenesis. For example, when the pro-inflammatory cytokines are produced at persistent, chronic low levels, often by the tumor itself, this could play a role in shaping an immune-suppressive tumor microenvironment, facilitating a chronic inflammatory state and enabling tumor growth and spread. In line with this, results from the CANTOS trial imply that suppressing such chronic inflammatory effects of these cytokines over an extended period may have significant benefits in terms of limiting cancer development and progression. In contrast, exogenous administration of high levels of these cytokines has been shown to often have a potent anti-tumorigenic effect due to recruitment and activation of Type 1 immune responses. Further work is required in this field to determine the levels of these cytokines within both normal and tumor tissue.

There is clear potential for the development of agents to target these cytokines, whose administration, most likely as a combination therapy with checkpoint inhibitor proteins, will activate the anti-tumor immune response and lead to tumor regression. Given the complexity and the dual functions associated with these proteins, the development of these cytokines as therapeutic agents will require extensive knowledge and understanding of the specific functions of each member of this family in each individual cancer type. Finally further work regarding the more novel members of this family is required to firmly establish their role in tumor development and progression.

AUTHOR CONTRIBUTIONS

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

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REVIEW
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Structural Basis of IL-1 Family Cytokine Signaling

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Interleukin-1 (IL-1) family cytokines are key signaling molecules in both the innate and adaptive immune systems, mediating inflammation in response to a wide range of stimuli. The basic mechanism of signal initiation is a stepwise process in which an agonist cytokine binds its cognate receptor. Together, this cytokine-receptor complex recruits an often-common secondary receptor. Intracellularly, the Toll/IL-1 Receptor (TIR) domains of the two receptors are brought into close proximity, initiating an NF-κB signal transduction cascade. Due to the potent inflammatory response invoked by IL-1 family cytokines, several physiological mechanisms exist to inhibit IL-1 family signaling, including antagonist cytokines and decoy receptors. The numerous cytokines and receptors in the IL-1 superfamily are further classified into four subfamilies, dependent on their distinct cognate receptors—the IL-1, IL-33, and IL-36 subfamilies share IL-1RAcP as their secondary receptor, while IL-18 subfamily utilizes a distinct secondary receptor. Here, we describe how structural biology has informed our understanding of IL-1 family cytokine signaling, with a particular focus on molecular mechanisms of signaling complex formation and antagonism at the atomic level, as well as how these findings have advanced therapeutics to treat some chronic inflammatory diseases that are the result of dysregulated IL-1 signaling.

Keywords: structure, IL-1, IL-33, IL-36, IL-18

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INTRODUCTION

During the hunt for the fever-inducing molecule produced by lymphocytes in the second half of the last century, interleukin 1 (IL-1) was discovered (1). Originally given different names, such as leukocytic pyrogen and lymphocyte activating factor, a unifying nomenclature was introduced and it was named interleukin for its capacity to communicate between leukocytes (2). Later, it was discovered that IL-1 exerts its effects on a much broader set of cells, not only leukocytes (3). The purified cytokine had unprecedented activity even at pM levels (1). During biochemical characterization, it was soon realized that these IL-1 purifications contained, in fact, two proteins of similar molecular weight, later named IL-1α and IL-β, that work through the same receptor on cells, inducing similar immunological effect (4). Cloning of the corresponding genes and subsequent recombinant expression of the proteins paved the way for detailed molecular studies.

With the improvement of genome analysis during the 1990s, the number of genes that could be identified as IL-1-like cytokines grew substantially. Now, 11 cytokines and 10 receptors are considered members of this cytokine family (**Tables 1**, **2**) (5). For the majority of cases, the genomic identification of IL-1 family members preceded the discovery of their immunological function. In fact, there are still cytokines whose modes of action are not entirely clear (e.g., IL-37, IL-38) and receptors whose ligands and/or function are not yet fully described (e.g., SIGIRR, IL-1RAPL1/2).

Structural biology has been instrumental in answering some of the central questions concerning IL-1 family cytokine signaling. For example, despite the similarity in function, sequence identity of the mature cytokines IL-1α and IL-1β is only 25%. With the solution of the X-ray crystal structures of both cytokines, it became evident that both mature cytokines share an overall fold, explaining the ability to engage the same receptor (4, 6). Later, the structure of the naturally occurring antagonist cytokine IL-1 receptor antagonist (IL-1Ra) was found to exhibit the same fold as both agonist cytokines, IL-1α and IL-1β. Comparison of the IL-1 receptor (IL-1RI) bound to IL-1β and IL-1Ra, combined with earlier mutagenesis work, revealed how IL-1Ra can compete with IL-1β for binding its primary receptor yet prevent engagement of the co-receptor IL-1 receptor accessory protein (IL-1RAcP) (7, 8) and, thus, inhibit IL-1 signaling. Another long-standing question was how the binary receptor-cytokine pair can engage its co-receptor IL-1RAcP, the final step of signal initiation. It was not until the structure of the ternary complex of IL-1β with a decoy receptor, IL1RII, and its co-receptor, IL-1RAcP, was published that this was finally clarified (9).

Guided by structural studies, we now have a detailed picture of the general mechanisms of signal activation and inhibition in the IL-1 cytokine family, of which we describe key features in more detail in the following sections.

SIGNALING

All members of the IL-1 family are extremely potent modulators of inflammation. Hence, their activities are regulated on several levels, including gene transcription, expression as inactive proforms, secretion and binding at the receptor level. Almost all cytokines of the IL-1 family are expressed as proforms with N-terminal domains of varying length from more than 100 amino acids (IL-33) to just a single amino acid (IL-36Ra). Proteases remove the N-terminal amino acids, creating mature, signaling-competent cytokines (10, 11). Once active cytokines are secreted, they can bind to their cognate cell surface receptors and initiate signaling. Within the IL-1 family, the mechanism of signal initiation is highly conserved.

Typical agonist signaling is initiated by a cytokine, such as IL-1β, binding its cognate receptor IL-1RI with nM affinity (**Figure 1**). Upon binding, a shared co-receptor, IL-1RAcP, is recruited by binding to the composite surface of the cytokine and primary receptor complex, resulting in the creation of a ternary complex; the binding affinity of IL-1RAcP is approximately 100-fold weaker than that of the IL-1β/IL-1RI complex. Through a single transmembrane helix spanning the plasma membrane, the ectodomains of these receptors are attached to Toll/interleukin-1 receptor (TIR) domains that reside in the cytoplasm. As the trimeric complex containing the cytokine, primary receptor, and accessory protein is formed, the cytoplasmic TIR domains of the two receptors are brought together to elicit downstream signaling via Myd88-dependent signaling pathways.

At the receptor level, signaling can be regulated by antagonistic cytokines. These bind to the primary receptor yet do not allow the accessory receptor to form the trimeric complex, thus prohibiting IL-1 signaling (**Figure 1E**). This can also be achieved by decoy receptors (**Figures 1D,F**). These receptors bind the cytokine but lack the intracellular TIR domain necessary for signaling, thereby neutralizing agonist cytokines.

One hallmark of IL-1 signaling is the redundancy of cytokines capable of binding the same cognate receptor (12). For instance, the primary receptor IL-1RI binds IL-1α, IL-1β, and IL-1Ra and the inhibitory receptor IL-1RII binds the same three cytokines, albeit with different affinities (13). The four IL-36 cytokines (the agonists IL-36α, IL-36β, and IL-36γ, and the antagonist IL-36Ra) share IL-36R as their single primary receptor. The most promiscuous receptor is IL-1RAcP, the co-receptor for three primary receptors, one decoy receptor and six agonist cytokines, binding eight different cytokine/receptor pairs altogether.

CYTOKINES

The high-resolution structures of IL-1α, IL-1β, IL-1Ra, IL-33, and IL-36γ, IL−18, IL-37, and IL-38 cytokines have all been determined by either X-ray crystallography or solution state NMR. These cytokines all possess a conserved β-trefoil conformation and a central hydrophobic core composed of 12 β-sheets, six of which (β1, β4, β5, β8, β9, and β12) form an anti-parallel β-barrel (**Figure 2**) (14). The β-trefoil consists of six β-hairpins and, using the structure of IL-1β by way of example, the naming of the β-sheets starts consecutively from the N-terminus for all IL-1 family cytokines (**Figure 2A**). While this structural motif is conserved among the cytokines, their sequence identity is low, even for members that bind the same primary receptor. Due to the inherent affinities for their primary receptors, these cytokines function at picomolar levels in order to elicit their downstream effects.

IL-1

IL-1 is an extremely potent inflammatory cytokine that is involved in myriad immunological responses, spanning both innate and adaptive immunity (15). Of the cytokines that bind the primary receptor IL-1RI, there are two similar yet distinct molecules, IL-1α and IL-1β, which are encoded by different genes. The IL-1α precursor gene is expressed constitutively in cells, including kidney, liver, lung, endothelial cells, astrocytes, and the epithelium of the gastrointestinal track (3). Unlike IL-1β, IL-1α is already active in its primary precursor form and acts as an alarmin by eliciting a signaling cascade through IL-1RI. The crystal structure of IL-1α has been determined at a resolution of 2.7 Å. (4). Similar to other cytokines within the IL-1 family, IL-1α is composed of 12 β-strands in a β-trefoil architecture.

TABLE 1 | List of IL-1 family cytokines with their respective nomenclatures, Uniprot IDs, alternative names, domains, and structures by PDB code.

Cytokines	Uniprot	Alternative name	Main Domain	Structure	Structure	Structure	Structure	Structure
IL1-F1	P01583	IL-1α	113-271	2ILA (Cα only)	2KKI (NMR)			
IL1-F2	P01584	IL-1β	117-269	2I1B	6I1B(NMR)	1ITB (with IL-1RI)	4DEP (with IL-1RI/IL-1RAcP)	3O4O (with IL-1RII/ IL1-RAcP)
IL1-F3	P18510	Il-1Ra, anakinra	26-177	1ILR	1IRP (NMR)			
IL1-F4	Q14116	IL-18	37-193	2VXT (with antibody)	1J0S (NMR)	3F62 (with IL-18BP)	3WO4 (IL-18R/IL-18RAcP/IL-18)	3WO3 (IL-18R/IL-18RAcP/IL-18)
IL-1F5	Q9UBH0	IL-36Ra	1-155	1MD6 (murine)	4P0J (chimera)	4P0K (chimera)	4P0L (chimera)	
IL-1F6	Q9UHA7	IL-36α	1-158					
IL-1F7	Q9NZH6	IL-37	46-218	5HN1				
IL-1F8	Q9NZH7	IL-36β, IL1-H2	1-157					
IL-1F9	Q9NZH8	IL-36γ	1-169	4IZE				
IL1-F10	Q8WWZ1	IL-38	1-152	5BOW				
IL-1F11	O95760	IL-33, NF-HEV	112-270	2KLL (NMR)	4KC3 (ST2/IL-33)			

TABLE 2 | List of IL-1 family cytokine receptors with their respective nomenclatures, Uniprot IDs, alternative names, domains, and structures by PDB code.

Receptor	Uniprot	Alternative name	Structure	Structure	Structure
PRIMARY RECEPTORS					
IL-1R1	P14778	IL-1RI, CD121a	1ITB (with IL-1β)	1G0Y (with antagonistic peptide)	4DEP (IL-1β/IL-1RI/ IL-1RAcP)
IL-1R2	P27930	IL-1RII, CD121b	3O4O (IL-1β/IL-1RII/IL-1RAcP)		
IL-1R4	Q01638	IL-33R, ST2, IL1-RL1, DER4, T1, IL-1R4	4KC3 (ST2/IL-33)	5VI4 (ST2/IL-1RAcP/IL-33)	
IL-1R5	Q13478	IL-18Rα, IL-1Rrp, IL-1Rrp1	3WO4 (IL-18/IL-18Rα/IL-18Rβ)	3WO3 (IL-18/IL-18Rα)	4R6U (IL-18/IL-18Rα)
IL-1R6	Q9HB29	IL-36R, IL-1RL2, IL-1Rrp2,			
CO-RECEPTORS					
IL-1R3	Q9NPH3	IL-1RAcP	4DEP (IL-1β/IL-1RI/IL-1RAcP)	3O4O (IL-1β/IL-1RII/IL-1RAcP)	
IL-1Rb		IL-1RAP			
IL-1R7	O95256	IL-18Rβ, AcPL	3WO4 (IL-18/IL-18Rα/IL-18Rβ)	3WO3 (IL-18/IL-18Rα)	4R6U (IL-18/IL-18Rα)
IL-1R8	Q6IA17	SIGGIR, TIR8			
IL-1R9	Q9NZN1	IL-1RAPL1 OPHN4, TIGIRR-2	5WY8 (IL-1RAPL1/PTPRdelta 1T3G (TIR)	4M92 (peptide 207-222 in complex with N33/Tusc3)	
IL-1R10	Q9NP60	IL-1RAPL2, TIGIRR-1			

Unlike IL-1α, IL-1β is expressed in a more limited number of cell types and must be processed from its precursor form to become an active agonist in IL-1 signaling. IL-1β is transcribed by monocytes, macrophages, and dendritic cells following Toll-like receptor (TLR) activation by pathogen-associated molecular patterns (PAMPs) or cytokine signaling. IL-1β is also transcribed in the presence of itself in a form of auto-inflammatory induction (15, 16). The inactive IL-1β precursor needs to be processed by caspase-1 cleavage, which in turn requires activation by danger-associated molecular patterns (DAMPs).

While active IL-1β and IL-1α have a sequence identity of only 25%, their overall structures are highly similar with an overall root mean square deviation (RMSD) of 1.54 Å over all Cα positions (17). The β4/5 and β11/12 (**Figure 2A**) loops of the cytokine are instrumental for their function and differ between agonist and antagonist cytokines, as discussed below.

IL-33

IL-33, the latest addition to the IL-1 superfamily, was discovered over a decade ago and is now clearly defined as a key component of innate and adaptive immune responses (18, 19). The IL-33 receptor, ST2, had been discovered previously and was considered an orphan receptor in the absence of any known ligand. ST2 was first used as a marker to differentiate T helper 2 (Th2) from T helper 1 (Th1) cells as it was selectively expressed on the former. Later, the ST2/IL-1RAcP signaling complex was shown to exist on group 2 innate lymphoid (ILC2) cells, helper and regulatory T cells, mast cells, basophils, eosinophils, NKT cells, and NK cells (20). As such, this cytokine is instrumental in immune defense against parasites and viruses (21–23).

Similar to IL-1α, IL-33 is biologically active in its nuclear form and is expressed constitutively in tissues, although subsequent cleavage by proteases can increase its potency (24, 25). Conversely to IL-1β, IL-33 is inactivated by caspase-1

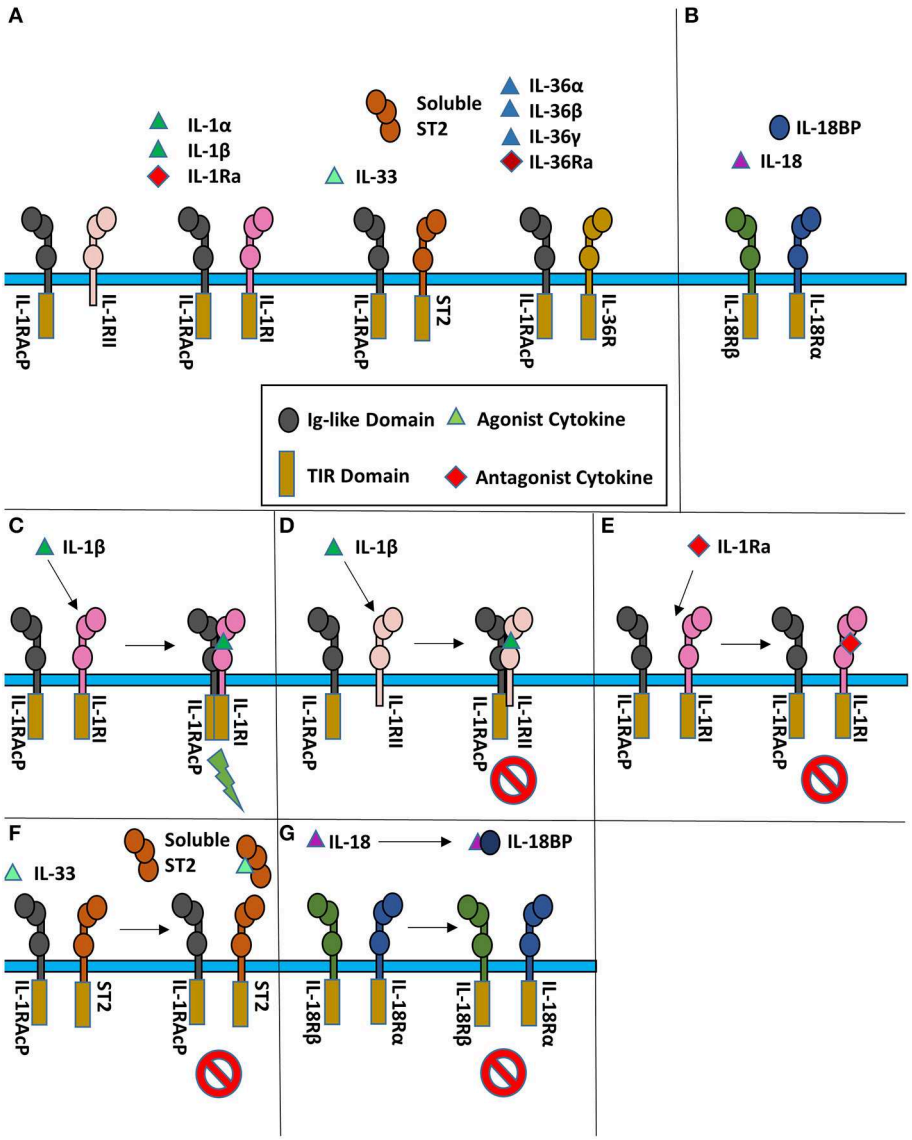

FIGURE 1 | Cartoon representation of IL-1 super family. **(A)** Cytokines of the IL-1 subfamily are above their respective receptor binding partners. **(B)** IL-18 has a different co-receptor, IL-18Rβ, and is a part of the IL-18 subfamily. **(C)** Upon binding of IL-1β to IL-1RI, IL-1RAcP is recruited to initiate signaling. **(D)** When IL-1β binds IL-1RII, no signaling occurs as IL-1RII lacks a cytoplasmic TIR domain. **(E)** When the IL-1R receptor antagonist (IL-1Ra) binds IL-1RI, the IL-1RAcP is not recruited, leading to no signaling. **(F)** IL-33 signaling can be inhibited by sequestration of the cytokine by the soluble ST2 receptor. **(G)** IL-18 can be sequester by the IL-18 binding protein (IL-18BP) to inhibit signaling.

cleavage during apoptosis (26). It acts as an alarmin critical to innate and adaptive immune defenses. IL-33 also plays an important role in allergic inflammation. Upon allergen induced activation, IL-33 protein levels increase beyond its basal levels (27–29). Unlike IL-1, there is no known antagonist cytokine to downregulate this activation. Instead, ST2 also exists in a soluble form (soluble ST2, sST2) that contains only the ectodomain of the receptor, composed of three immunoglobulin (Ig) fold domains, with no transmembrane helix (**Figure 1F**). As this decoy receptor is released, excess IL-33 may be sequestered to limit IL-33 driven inflammation.

The IL-33 fold was first predicted by a computational screen based on structural alignments of IL-1 family cytokines and fibroblast growth factor (FGF) β-trefoil cytokines; its three-dimensional structure was then determined by NMR (30). Similar to both IL-1α and IL-1β, IL-33 is a 12-stranded β-barrel surrounding a hydrophobic core in a β-trefoil configuration. There are two alpha helices (α1 and α2) that precede β-strands β8 and β12 (**Figure 2B**). The β 4/5 loop of IL-33 is substantially longer than those of other IL-1 family cytokines with an additional 10 amino acids (30).

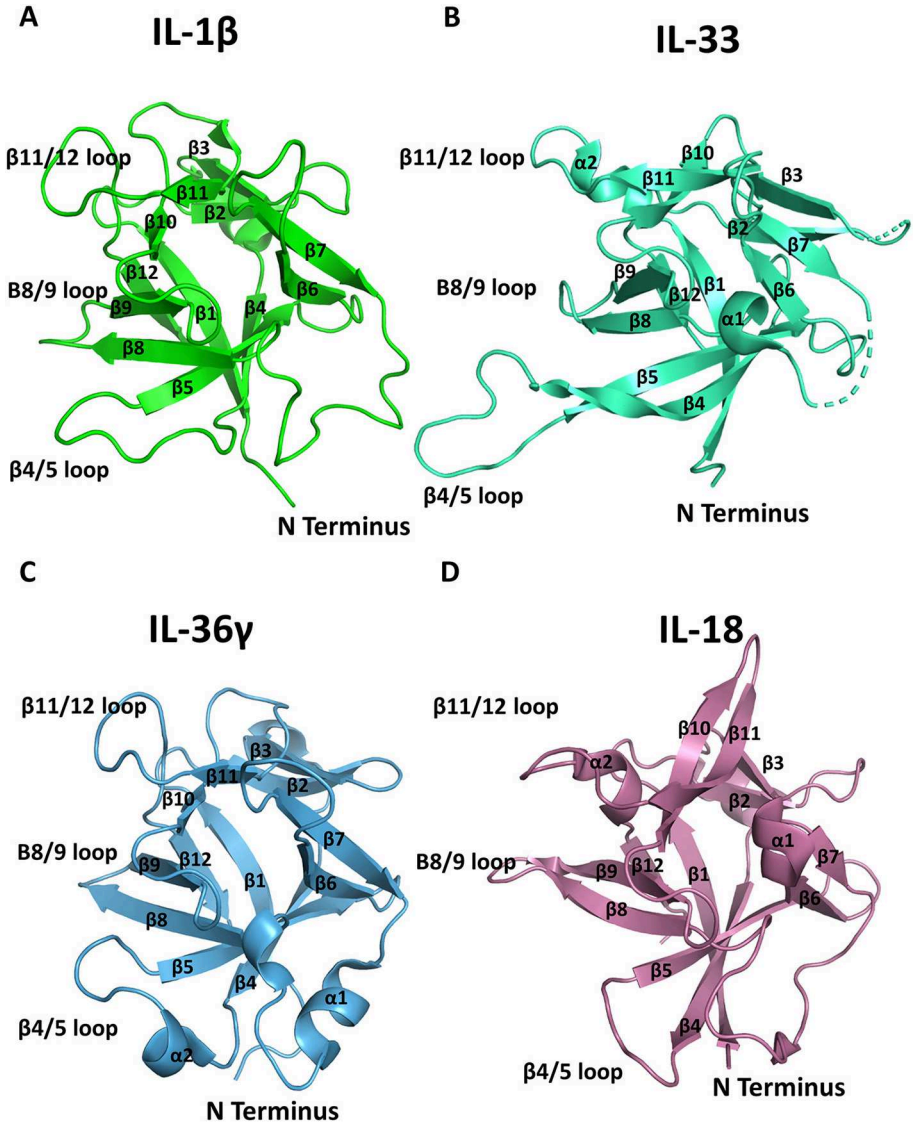

FIGURE 2 | Representation of cytokine structures. **(A)** The structure of IL-1β with its β-sheets and key loops labeled. **(B)** The structure of IL-33 with its β-sheets and key loops labeled. **(C)** The structure of IL-36γ with its β-sheets and key loops labeled. **(D)** The structure of IL-18 with its β-sheets and key loops labeled.

IL-36

The IL-36 receptor (IL-36R) is the most promiscuous primary receptor in the IL-1 family; it binds three agonist cytokines, IL-36α, IL-36β, and IL-36γ, as well as a single antagonist cytokine, or receptor antagonist, IL-36Ra. IL-36R, and the IL-36 cytokines were discovered separately through genome screening (31, 32). Both remained orphaned until their functional dependence was shown (33). While IL-36α, IL-36β, IL-36γ, and IL-36Ra all lack an N-terminal secretion signal, they are secreted by an unidentified mechanism as they function, putatively, extracellularly (34).

While relatively new to the IL-1 family, the broader immunological role of the IL-36 cytokines began to be elucidated after the discovery that their N-terminus must be processed precisely for higher affinity binding to its cognate receptor (35). CD4+ T cells, upon activation, may be stimulated by IL-36 agonist cytokines to induce IL-2 production, proliferate, and be subsequently polarized for a Th1 response (36). In addition to T cells, IL-36 cytokines are involved in the regulation of dendritic cells (37). IL-36γ is the only agonist cytokine of the IL-36 subfamily for which a high-resolution structure has been determined (**Figure 2C**) (38).

IL-18

IL-18 is best known for its capacity to induce IFN-γ and is expressed by macrophages, epithelial cells, such as keratinocytes, and dendritic cells (34). Reminiscent of IL-1β, IL-18 must be processed from its 23 kDa proform by caspase-1 into its 18 kDa active form (39). The architecture of IL-18 is grossly similar to those of the other IL-1 family member cytokines (**Figure 2D**).

BINARY COMPLEXES

The formation of the binary complex is a key step in the initiation of a functioning signaling complex. Through structural studies, much has been elucidated concerning these interactions. The primary receptors share a similar overall molecular architecture and bind their respective cytokines in conserved binding sites (**Figure 3**). Due to these similarities, we use the structure of IL-1β bound to IL-1RI here to highlight the commonalities between all three binary complex structures.

IL-1β/IL-1RI

The ectodomain of the primary receptor IL-1RI contains three Ig-like domains (D1, D2, and D3) that form two distinct binding sites, A and B, that together drive its interactions with IL-1 cytokines (**Figure 3B**). The primary receptor adopts an architecture that resembles a grasping hand in how it binds the cytokine. D1 and D2 are tightly packed against each other and, together, their contiguous molecular surface constitutes binding site A. Between D1/D2 and D3, there is a 6 amino acid linker lacking secondary structure. D3 is a single Ig domain and forms binding site B (8). IL-1β, in total, has a buried surface area within the interface of IL-1RI of 1932 Å^2 over 47 residues. When divided between respective binding sites, IL-1β has a buried surface area of ∼1,000 Å^2 over 25 amino acids at site A (8). In site B, formed by the D3 domain of the receptor, there is a nearly equivalent sized interface to the D1/D2-IL-1β interface over 21 amino acids (8). Five of the six β-sheets from the Ig fold of D3 are involved in this interface. Additionally, there is a hydrogen bond between IL-1β with the linker between D1/2 and D3, falling outside the canonical sites A and B.

IL-33/ST2

IL-33 binds its primary receptor, ST2, with an affinity of 450 pM (30). While a crystal structure of the binary complex IL-33/ST2 was determined in 2013 (40), a previous NMR structure of IL-33 alone had been published in 2009 (30). IL-33 exhibits very little conformational change upon binding ST2; there is an RMSD of 1.2 Å over all Cα atoms between ST2-bound and unbound IL-33. As with IL-1RI, ST2 is composed of three Ig domains, all of which interact with IL-33, that can be further divided into site A and B, analogous to IL-1RI/IL-1β (40).

While the overall D1/D2 architecture is conserved between the known structures of IL-1β/IL-1RI and IL-33/ST2, the orientation of the D3 domain relative to D1/2 is not, resulting in an RMSD of 4.51 Å between the two receptors. Together, IL-33 and ST2 share a buried surface area of roughly 1,700 Å^2. When divided into the respective sites, site A of ST2 has a buried surface area of 940 Å^2 contributed by 30 residues. The vast majority of this interface has a positive electrostatic potential (**Figure 3D**). On the D3 of ST2, binding site B, there is a buried surface area of 818 Å^2 over 22 residues. Binding site B, in contrast to site A, has a lower electrostatic potential and relies heavily on salt bridges between ST2 and IL-33.

IL-18/IL-18Rα

Shortly after the publication of the IL-33/ST2 crystal structure, the binary complex of IL-18 with its primary receptor IL-18Rα was published (41). IL-18Rα, like the other primary receptors within the IL-1 family, is composed of three Ig-like domains that can be grouped into two respective parts, D1/D2 and D3. In comparison to the IL-1β/IL-1RI and IL-33/ST2 binary structures, the IL-18/IL-18Rα structure has a similar overall architecture of its domains. As with the other cytokine/primary receptor pairs, IL-18 binds to IL-18Rα at two distinct sites, encompassing all three Ig domains of the primary receptors (**Figure 3F**). In total, IL-18 has a buried surface area with IL-18Rα of 1,650 Å^2 over 49 residues. From the perspective of IL-18Rα, binding site A has a buried surface area of 890 Å^2 over 30 AA. This is composed mainly by residues on the β1/2 loop, β2 and β3 strands, and β10/11 loops of IL-18 (**Figure 2D**). As shown by the crystal structures, hydrophilic interactions dominate this interaction. When considering binding site B on IL-18Rα, there are six residues from IL-18 that make up the composition of this interface. On IL-18Rα, site B is composed of 22 amino acids. This, in total, results in a buried surface area of roughly 600 Å^2 (41).

TERNARY COMPLEXES

The available ternary complex structures of the IL-1 family members all share common structural motifs. This is not wholly surprising. For one, a feature of the IL-1 family is the redundancy of binding partners. Highly variable cytokine sequences result in a common secondary structure. In turn, these cytokines can bind to the same primary receptors at nM affinity (e.g., similar affinities of IL-1α and IL-1β for IL-1RI). The primary and secondary structures are both composed of three Ig-like domains. The binding of the cytokine to the primary receptor creates a composite surface for the recruitment of the secondary receptor. This allows cytoplasmic TIRs to aggregate for a MyD88 signaling cascade. Even for cytokines that share a common secondary-receptor, these protein complexes are able to interact differently with the secondary receptor at the same key areas. This allows the same co-receptor, IL-1RAcP, to be a key mediator of vastly different immunologic outcomes.

IL-1 Ternary Complexes

The first high-resolution ternary structure in the IL-1 family determined was the inhibitory complex of IL-1β with the decoy receptor IL-1RII and IL-1RAcP (9). Shortly thereafter, the signaling-competent ternary complex IL-1β/IL-1RI/IL-1RAcP was determined (42). The two ternary complexes display high structural similarity to one another with an RMSD of 1.8 Å between their Cα atoms. These structures have greatly informed our understanding of IL-1 family signaling mechanisms by revealing the interactions necessary for the recruitment of the accessory protein.

The overall architecture of the binary complex IL-1β/IL-1RI remains predominately unchanged when the secondary receptor is recruited to form the trimeric complex IL-1β/IL-1RI/IL-1RAcP, with an RMSD of 1.4 Å (42). The binding of the cytokine to its cognate receptor allows for a composite surface

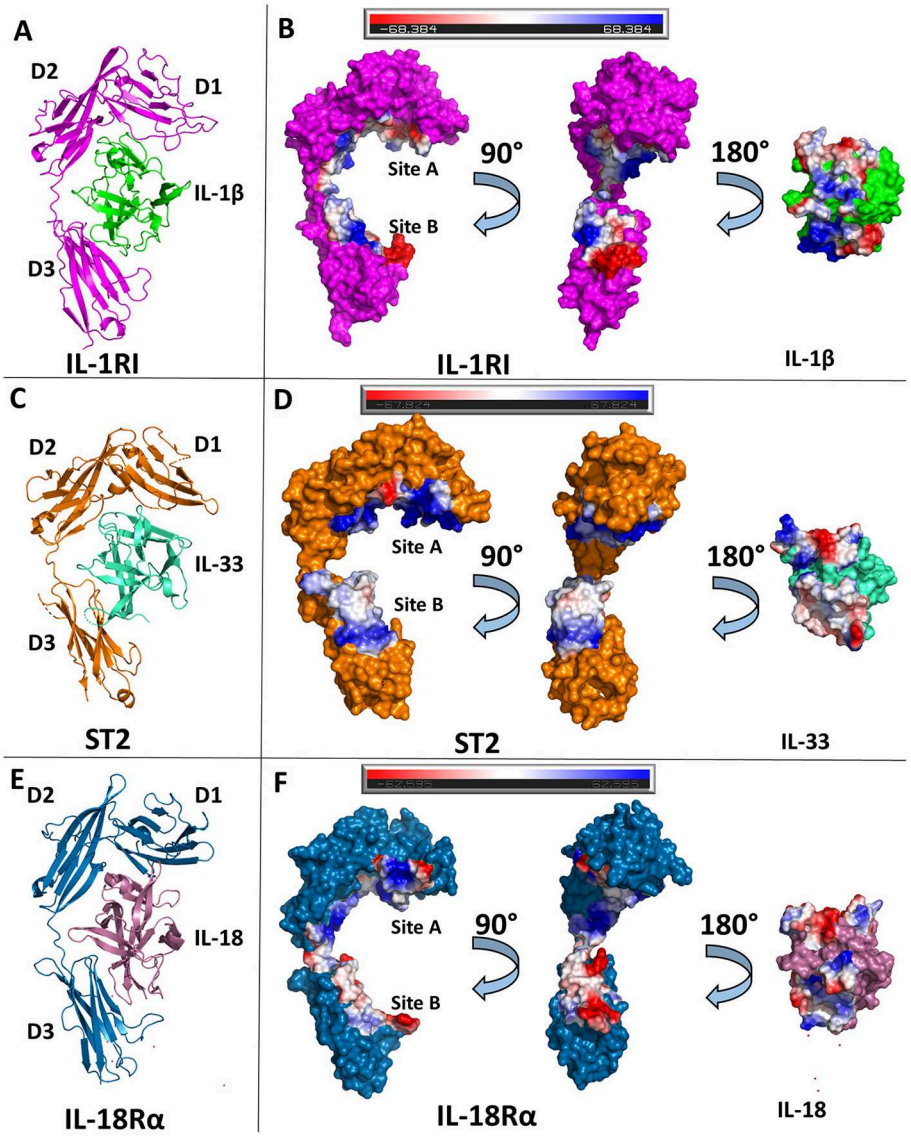

FIGURE 3 | Structures of binary cytokine/receptor complexes. **(A)** Cartoon of IL-1β/IL-1RI binary complex (pdb: 1itb). **(B)** Surface of both IL-1β and IL-1RI with respective interfaces showing electrostatic potential. **(C)** Cartoon of IL-33/ST2 binary complex (pdb: 4kc3). **(D)** Surface of both IL-33 and ST2 with respective interfaces showing electrostatic potential. **(E)** Cartoon of IL-18/IL-18Rα binary complex (pdb: 3wo3). **(F)** Surface of both IL-18 and IL-18Rα with respective interfaces showing electrostatic potential.

between IL-1β/Il-1RI to recruit the accessory protein with sub-μM affinity, as demonstrated by surface plasmon resonance (SPR) (9). No IL-1 cytokine has appreciable affinity for IL-1RAcP on its own in the absence of its cognate receptor.

Previous to the structural determination of these trimeric complexes, the precise orientation of the accessory protein to its respective binary complex was unknown, although attempts to discern its interaction with the binary complex was modeled (43). Similar to its counterpart IL-1RI, the accessory protein is composed of three Ig-like domains whose D1/2 domains are juxtaposed to each other. As seen in **Figure 4B**, however, the accessory protein binds with its backside to IL-1β/IL-1RI, making extensive contact with IL-1RI in the D2 domain and, to a lesser extent, to the D3 domain. D1 of the accessory protein is located far from the interface and makes no contacts with the binary complex (**Figure 4A**). The interface corresponding to site A and B in the primary receptors is not involved in binding the binary cytokine/receptor complex. As had been previously described in mutagenesis studies of IL-1β and IL-1Ra, the β4/5 and the β11/12 loops of these cytokines were crucial for their opposing agonist/antagonist functions (44, 45). In subsequent SPR studies, IL-1Ra with loop swaps for IL-1β β4/5 and β11/12 rescued binding of the IL-1Ra/IL-1RI complex to the accessory protein, albeit with a lower affinity (i.e., μM) than the inherent sub-μM

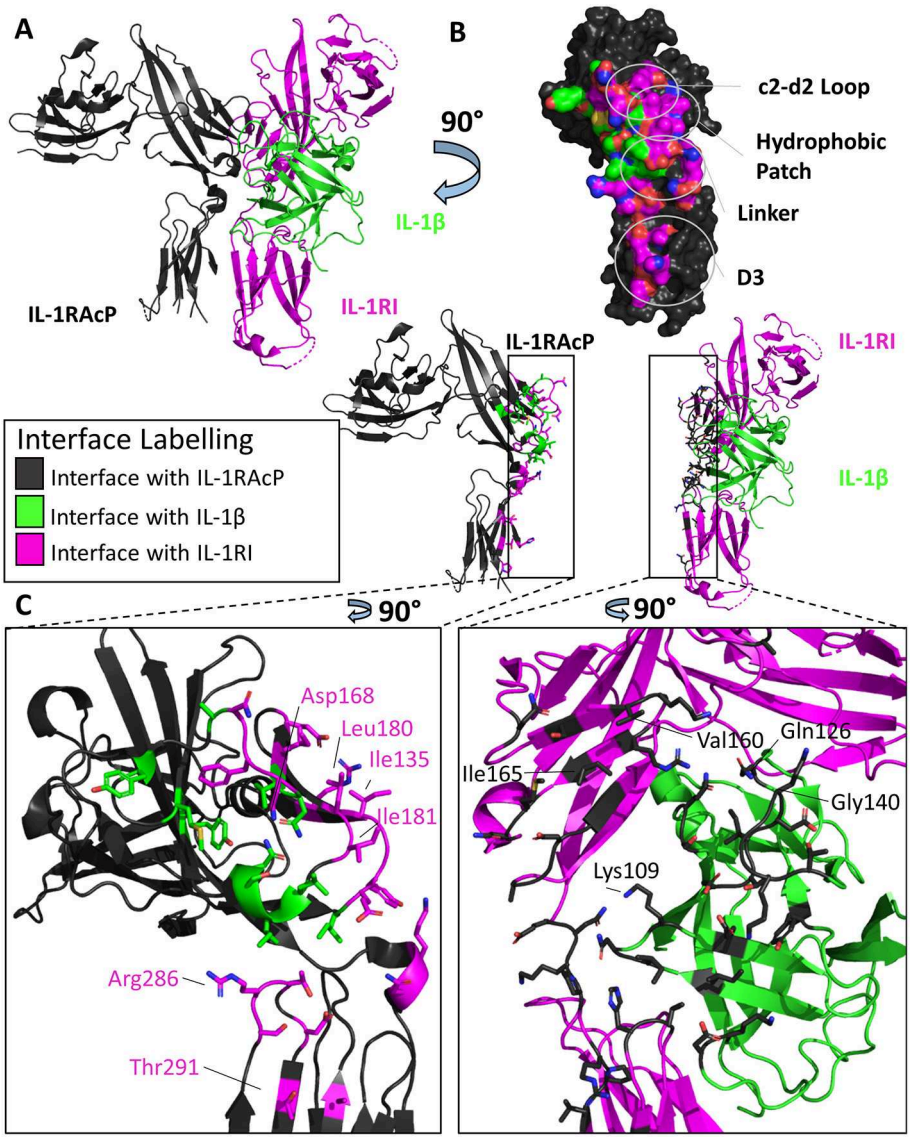

FIGURE 4 | Structure of the IL-1β/IL-1RI/IL-1RAcP ternary complex. **(A)** Cartoon of IL-1β/IL-1RI/IL-1RAcP ternary complex (pdb: 4dep). **(B)** Surface of IL-1RAcP with interface to binary complex labeled and colored according to its binding partner. **(C)** Cartoon of IL-1RAcP and IL-1β/IL-1RI with residues involved in interface shown as sticks colored according to its binding partner.

affinity the IL-1RAcP has for the IL-1β/IL-1RI binary complex (9), providing a molecular mechanism by which agonist and antagonist cytokines of the IL-1 family function to either recruit IL-1RAcP or not, respectively.

IL-33 Ternary Complex

As both the IL-1β/IL-1RI and IL-33/ST2 binary structures described previously had high structural homology when aligned, it was originally thought that these complexes would recruit their shared accessory protein, IL-1RAcP, in a similar fashion (40). When the X-ray crystal structure of the IL-33/ST2/IL-1RAcP was finally determined (**Figure 5A**), however, the diverse ways in which IL-1 family cytokines could recruit IL-1RAcP were finally appreciated (46).

There are many similarities between the two signaling-competent ternary complexes of IL-1β/IL-1RI/IL-1RAcP and IL-33/ST2/IL-1RAcP. Both ternary complexes contain the same overall structure, with an RMSD of 3.2 Å. Additionally, both binary complexes engage IL-1RAcP on the backside of IL-1RAcP, interacting with the D2 and D3 of the co-receptor. As with IL-1β, the β4/5 and β11/12 of IL-33 loops are at the interface with IL-1RAcP, with a buried surface area of 201 Å^2 while having a 54 Å^2 buried surface area to ST2 (**Figure 5B**).

These two binary complexes engage key regions of the IL-1RAcP differently, however. On IL-1RAcP, there exist four distinct areas that interact with both the IL-1β/IL-1RI and IL-33/ST2 binary complexes: the c2-d2 loop region, the hydrophobic patch, the linker region and the D3 region. The c2-d2 region is

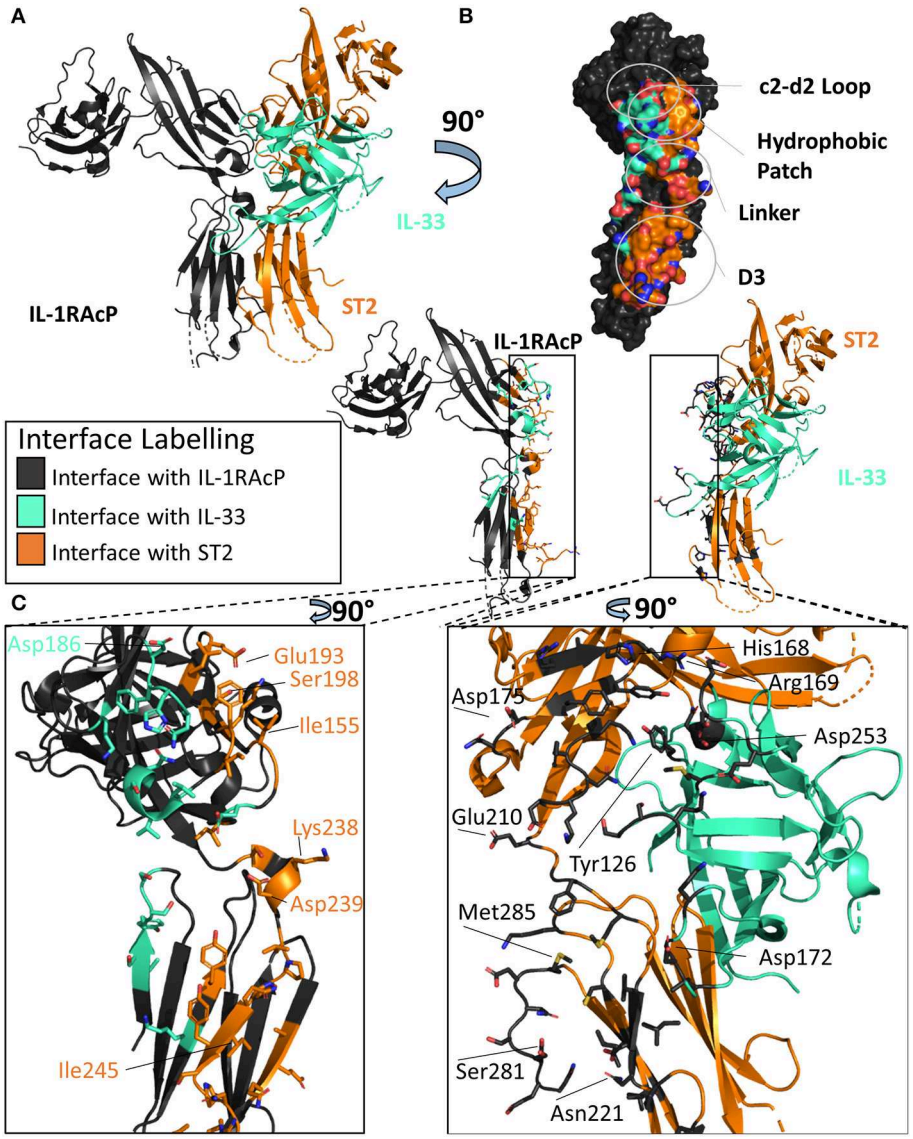

FIGURE 5 | Structure of the IL-33/ST2/IL-1RAcP ternary complex. **(A)** Cartoon of IL-33/ST2/IL-1RAcP ternary complex (pdb: 5vi4). **(B)** Surface of IL-1RAcP with interface to binary complex labeled and colored according to its binding partner. **(C)** Cartoon of IL-1RAcP and IL-33/ST2 with residues involved in interface shown as sticks colored according to its binding partner.

a loop connecting strands c and d in the D2 domain of the IL-1RAcP (**Figures 4B**, **5B**). These strands exhibit conformational plasticity, a trait that allows them to interact with both binary complexes in distinct ways to accommodate their inherent differences. For both, however, there exists a network of hydrogen bonds that interacts with the respective binary complexes. The hydrophobic patch is a region on IL-1RAcP that makes hydrophobic interactions with both binary complexes. An important residue within the IL-1RAcP is Ile155, which engages both the cytokine and primary receptor in the case of IL-1β/Il-1RI through shared hydrophobicity and engages ST2 through van der Waals contacts to Asp175 of ST2. The linker region encompasses residues that reside between D2 and D3 of the IL-1RAcP and engage both binary complexes through different composite surfaces. Lastly, the D3 region of the IL-1RAcP engages both binary complexes differently. The D3 of ST2 is rotated by 60 degrees around the long axis of the domain, thereby presenting a larger surface for the D3 of IL-1RAcP than for the IL-1β/IL-1R1 complex (**Figures 4C**, **5C**).

There exist subtle differences in the overall domain positioning upon ternary complex formation as well. For IL-1β/IL-1RI, the D1/2 domain is rotated 3.7 degrees away from the interface with IL-1RAcP while the D3 domain rotated 6.6 degrees toward the interface, resulting in a 3 Å displacement of residues when compared to the binary complex. For IL-33/ST2, the D1/2 domain in contrast rotates toward the IL-1RAcP

interface, while the D3 domain similarly rotated by 9 degrees toward the IL-1RAcP D3 domain (46).

To further elucidate key differences between the IL-1RI and ST2 ternary complexes, hydrogen-deuterium exchange coupled to mass spectrometry (HDX-MS) was conducted to assess intrinsic protein flexibility in the ternary complex (46). For ST2, a major peptide fragment that differed in flexibility upon co-receptor binding was peptide 166-172$_{ST2}$ that lay directly on a hydrophobic patch with the IL-1RAcP. Similarly, in the IL-1β/IL-1RI/IL-1RAcP ternary complex, fragment 129-142$_{IL-1RI}$, part of a hydrophobic patch, exhibited reduced flexibility, indicative of binding of IL-1RAcP (46).

A key difference in the HDX-MS data that highlighted the intrinsic differences between recruitment of IL-1RAcP by the binary complex involved loops β4/5 and β11/12 of the cytokine. The β4/5 and β11/12 loops of IL-1β make key interactions with the IL-1RAcP. IL-1Ra has differences within these loops that do not allow the recruitment of the Il-1RAcP for a functioning ternary complex. This importance does not translate to the IL-33 ternary structure, however. In the β11/12 regions, this loop was highly shielded from exchange in the IL-1β ternary complex; the overall exchange was half (46). As both the β4/5 and β11/12 loops of IL-33 showed a large amount of exchange over a long time scale, these areas are clearly not tightly engaged in the ternary structure.

Through extensive alanine scanning mutagenesis, hotspots of IL-1RAcP recruitment for both IL-1β/IL-1RI and IL-33/ST2 were identified (46). It was from these studies that inherent differences were highlighted between these respective binary complexes. IL-1RI displayed a narrow distribution of binding energy, localized to key residues; ST2 displayed a broader distribution of binding over more residues.

Although interacting with the same key regions of IL-1RAcP, it is clear that there exist marked differences in the recruitment of the IL-1RAcP between the IL-1β and IL-33 ternary complexes. While for IL-lβ/IL-1R1/IL-1RAcP, the cytokine is the main driving force in the interaction with the co-receptor, for IL-33/ST2/IL-1RAcP it is the primary receptor (**Figure 6**). In this case, the cytokine seemingly only arrests the primary receptor ST2 in a conformation that enables its interaction with IL-1RAcP (46).

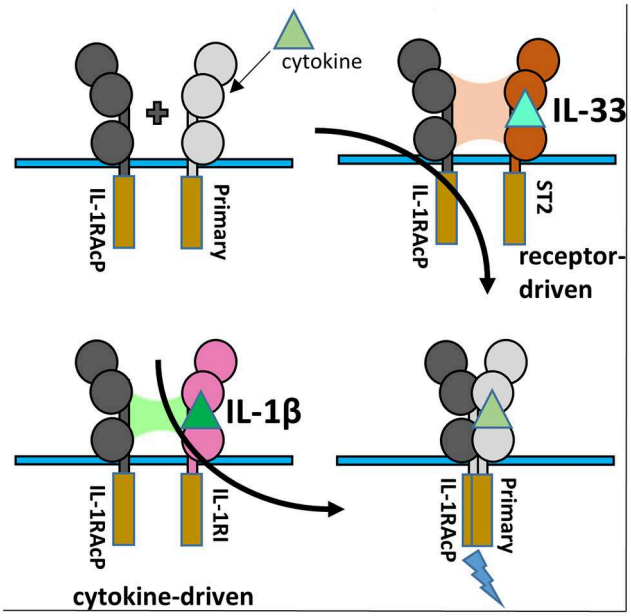

FIGURE 6 | IL-1RAcP Recruitment by IL-1β/IL-1RI and IL-33/ST2 binary complexes. **(A)** Cartoon of the differences inherent in IL-1RAcP recruitment by IL-1β/IL-1RI and IL-33/ST2 binary complexes.

IL-18 Ternary Complex

The IL-18 ternary complex provided new insight into the function of IL-1 family members that do not share the IL-1RAcP (47). The IL-18 ternary complex forms when the binary complex IL-18/IL-18Rα is recognized by the IL-18Rβ secondary receptor (**Figure 7A**). As with other ternary complexes, the IL-18Rβ will not bind IL-18Rα prior to formation of the binary IL-18/IL-18Rα complex (48).

The overall architecture of the IL-18/IL-18Rα/IL-18Rβ structure is highly similar to that of the ternary complexes involving IL-1β. The cytokine binds the primary receptor and presents a shared surface for the secondary receptor. The secondary receptor is composed of three Ig-like domains, interacting with the binary complex through the D2 and D3 domains. IL-18 binds IL-18Rα with a low nM affinity (20–40 nm), while the recruitment of the IL-18Rβ forms the larger ternary complex at high nM affinity (49).

This high affinity complex has multiple interactions with both the cytokine and the IL-18Rα chain. For the cytokine, there are 12 residues in the interface with the IL-18Rβ chain, composing a buried surface area of 453 Å^2. This constitutes 5.3% of the solvent accessible area of the cytokine. The entirety of this interface is composed of hydrogen bonds over those 15 residues on IL-18. For the IL-18Rβ, this interface is roughly 380 Å^2 in size and is composed of 11 residues. This is reminiscent of IL-1β's role in the formation of the IL-1 ternary complex with Il-1RAcP.

IL-18Rα grasps IL-18 in a similar fashion to the IL-1β/IL-1RI binary complex and has a similar orientation of the accessory protein for the respective IL-18Rβ. On ternary complex formation, the binary complex does not change significantly, with an RMSD of 0.7 Å. Additionally, the co-receptor IL-18Rβ adopts an orientation reminiscent of IL-1RAcP in the IL-1β ternary complex. The RMSD between these ternary structures is 4.6 Å.

There are, however, major differences in the positions of the D2 and D3 domains to the IL-18Rβ in comparison the IL-1β ternary complex. IL-18Rα D2 supplies two loops at the interface of IL-18Rβ D2, namely B2 and E3, that interact together. In addition to electrostatic interactions seen in IL-1β/IL-1RAcP, the IL-18/18Rβ interface has aromatic interactions that contribute to the affinity of the ternary complex. The β4/5 loop of IL-18, however, does not interact with IL-18Rβ as it does within the IL-1β/IL-1RAcP interaction (**Figure 7B**).

Recognition of the binary complex IL-18/IL-18Rα by IL-18Rβ is mediated by numerous interactions (**Figure 7C**). In the

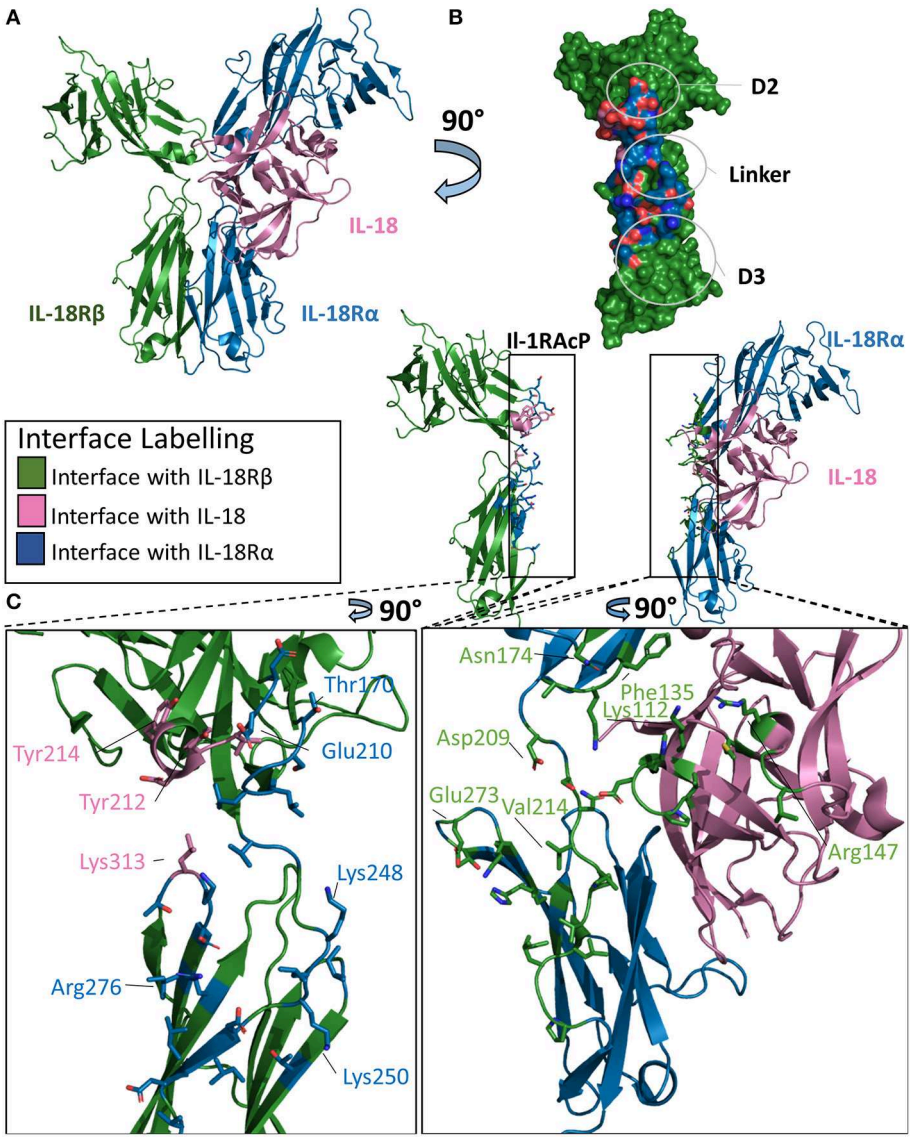

FIGURE 7 | Structure of the IL-18/IL-18Rα/IL-18Rβ ternary complex. **(A)** Cartoon of IL-18/IL-18Rα/IL-18Rβ ternary complex. **(B)** Surface of IL-18Rβ with interface to binary complex labeled and colored according to its binding partner. **(C)** Cartoon of IL-1RAcP and IL-18/IL-18Rα with residues involved in interface shown as sticks colored according to its binding partner.

crystal structure, Tyr212 of IL-18Rβ makes aromatic interactions, most likely pi-stacking from a 3.4 Å distance, with IL-18 in a core concave area of the D2 interactions shared between IL-18 and IL-18Rα (47). Overall, there are 14 residues from IL-18Rα that interact with IL-18Rβ, resulting in a buried surface area of 511 Å^2. Conversely, 25 residues of IL-18Rβ recognize IL-18Rα, constituting an interface surface area of 802 Å^2.

Analysis of the interface size of the three ternary complex structures revealed that the size of the interface formed by the cytokine together with the primary receptor D1/2 domains and secondary receptor is constant (~2,000 Å^2), while the overall interface size varies from about 2,500 Å^2 for IL-1β/IL-1RII/IL-1RAcP to over 3,700 Å^2 for IL-33/ST2/IL-1RAcP due to the different contribution by the primary receptor D3 domain (46). In contrast, the interface in the IL-18 complex with its unique coreceptor IL-18Rβ has the overall smallest interface between the secondary receptor and binary cytokine/primary receptor complex (2,400 Å^2). Although the supramolecular structures are inherently similar, their exist differences between the three known ternary structures.

PHYSIOLOGICAL MECHANISMS OF IL-1 SIGNALING INHIBITION

As aberrant inflammation can lead to a myriad of pathological effects, regulation of these signaling systems is crucial for a

functioning immune system. Dysregulated IL-1 signaling can mediate numerous auto-inflammatory diseases (50). There are several mechanisms to regulate IL-1 family signaling effectively (**Figure 1**). Within the IL-1 family of cytokines, there exist antagonist cytokines (IL-1Ra & IL-36Ra), immunosuppressive cytokines (IL-37 & IL-38), decoy receptors (sST2 and IL-1RII) and the IL-18 binding protein (IL-18BP).

Antagonists and Immunosuppressive Cytokines

Antagonist cytokines function by binding the primary receptor and prohibiting the recruitment of the secondary receptor. This inhibits signaling by occupying the binding pocket of the primary receptor, thus not allowing an agonist cytokine the opportunity to bind. Antagonist cytokines within the IL-1 family include the IL-1 receptor antagonist (IL-1Ra), whose cognate receptor is IL-1RI, and the IL-36 receptor antagonist (IL36Ra), whose cognate receptor is IL-36R.

IL-1Ra

The IL-1Ra structure was solved to a resolution of 2.1 Å by X-ray crystallography in 1994 (51). IL-1Ra is a 17 kDa polypeptide composed of 12 β-strands and two very short 3–10 helices, similar in architecture to IL-1α and IL-1β (**Figure 8A**) (4, 52).

IL-1Ra is an antagonist cytokine; it can occupy the binding pocket of IL-1RI without eliciting any downstream signaling (53, 54). IL-1Ra is capable of binding IL-1RI with equal affinity to IL-1RI as IL-1β, thus competing with IL-1 signaling. IL-1Ra preferentially binds IL-1RI over IL-1RII, thus not binding the decoy receptor in what would be a non-productive mechanism of inhibition. As previously stated, IL-1β binds IL-1RI at two distinct sites, sites A and B. IL-1Ra, however, binds predominantly site A, as determined by extensive mutagenesis (55). Differences also exist between Il-1β and IL-1Ra s in the β4/5 loops of the cytokines, key mediators of interaction with IL-1RAcP. The total RMSD of IL-1Ra and IL-1β is only 0.90 Å, however.

IL-36Ra

Like IL-1Ra, IL-36Ra binds to its primary receptor and does not allow a functioning signaling complex to be formed (35). To date, many studies have shown that IL-36Ra is able to inhibit IL-36γ stimulated NF-κB signaling (33, 38, 56). The structure of murine IL-36Ra was first published in 2003 to a resolution of 1.6 Å (57). Like other cytokines within this family, IL-36Ra is composed of 12 β-strands that fold into a β-trefoil conformation (**Figure 8B**). The largest differences between IL-36γ and IL-36Ra were the β4/5 and β 11/12 loops (**Figure 8C**). To investigate the structural determinants of IL-36Ra, the loops from IL-36Ra were swapped into IL-36γ. In the case of β11/12, the inclusion of this loop from IL-36Ra into IL-36γ led to a 14-fold decrease in binding affinity and a 1,000-fold decrease in activity during *in vitro* assays (38). Swapping the β4/5 loops of IL-36Ra into IL-36γ led to a 10-fold decrease in signaling and only a slight decrease in binding affinity, highlighting that β4/5 loops may not make as crucial of interactions with the IL-1RAcP as does the β11/12 loops of IL-36γ.

IL-38

While the functional role of IL-38 continues to be elucidated, the structure has been deposited in the Protein Data Bank (PDB 5BOW). IL-38 was first cloned and added as a member for the IL-1 superfamily in 2001 (58). It is associated with the clinical manifestations of systemic lupus erythematosus (59). It is predominately expressed in the skin and in proliferating B cells. This cytokine lacks a signal peptide, is 152 AA in length, and does not contain any caspase-1 cleavage sites. Functionally, IL-38 inhibits *Candida albicans*-induced Th17 responses in PMBCs (60). It has been suggested that IL-38 acts through IL-36R in a fashion similar to IL-36Ra, although low affinity binding to IL-1RI has also been reported (58, 60). Moreover, a truncation variant lacking the first 19 amino acids was reported to act through IL-1RAPL1 (61). This N-terminal truncation would lead to a complete loss of beta strand 1 and part of beta strand 2 (**Figure 9A**), which likely leads to an unstable, misfolded and/or dysfunctional protein. How exactly IL-38 exerts its anti-inflammatory actions remains to be clarified, although it has been suggested IL-38 could recruit one of the inhibitory co-receptors of the IL-1 family, namely SIGGIR, TIGIRR1, and/or TIGIRR2 (62).

As with the other cytokines within the IL-1 family, IL-38 shares certain canonical traits. It has 12 β-strands connected by 11 loops, organized into a β-trefoil configuration (**Figure 9A**). Based on sequence comparison, IL-38 is related to two other well-known antagonists, IL-1Ra and IL-36Ra, with sequence identities of 39 and 43%, respectively (60). Comparison of their X-ray structures show that IL-38 has an RMSD of 1.23 Å to IL-1Ra and 0.96 Å to IL-36Ra. Superposition of IL-38 and IL-36Ra reveal that loop of β4/5, important for antagonism of IL-1Ra and IL-36Ra, is nearly identical to that of IL-36Ra (**Figure 9B**), further indication of IL-38's immunomodulatory role.

IL-37

IL-37 was first identified within the IL-1 gene cluster in 2001 (63). There are five isoforms of IL-37, IL-37a-e; IL-37b is the largest with five of the six exons from the locus (64). IL-37 has proven to be a potent anti-inflammatory cytokine (65). Like IL-1β, it requires caspase 1 cleavage for activation. Transgenic expression of IL-37 abrogates inflammation in the presence of endotoxin in mice that naturally lack IL-37 (66). Upon stimulation in RAW cells, IL-37 was highly potent in reducing TNF, MIP-2, and IL-1α levels (64). Functionally, IL-37 works in two distinct ways: by trafficking to the nucleus and blocking Smad3 activation after LPS stimulation, or by interacting with the receptor chain IL-18Rα and the single Ig domain receptor SIGIRR (67). This was highlighted by the silencing of IL-18Rα and, ultimately, the reduction of the anti-inflammatory properties of IL-37 (65). Additionally, over-production of IL-37 protected mice from endo-toxemia, colitis, obesity and metabolic syndrome, spinal cord injury, and myocardialischemia (68).

In 2017, the structure of the cytokine domain of IL-37b, the best characterized isoform, was determined to 2.25 Å (**Figure 9C**) (67). IL-37 crystallized as a homodimer (**Figure 9D**). This head-to-head dimer architecture is unique to the IL-1 family cytokine structures discussed, although IL-37 still retains its 12 β-strands

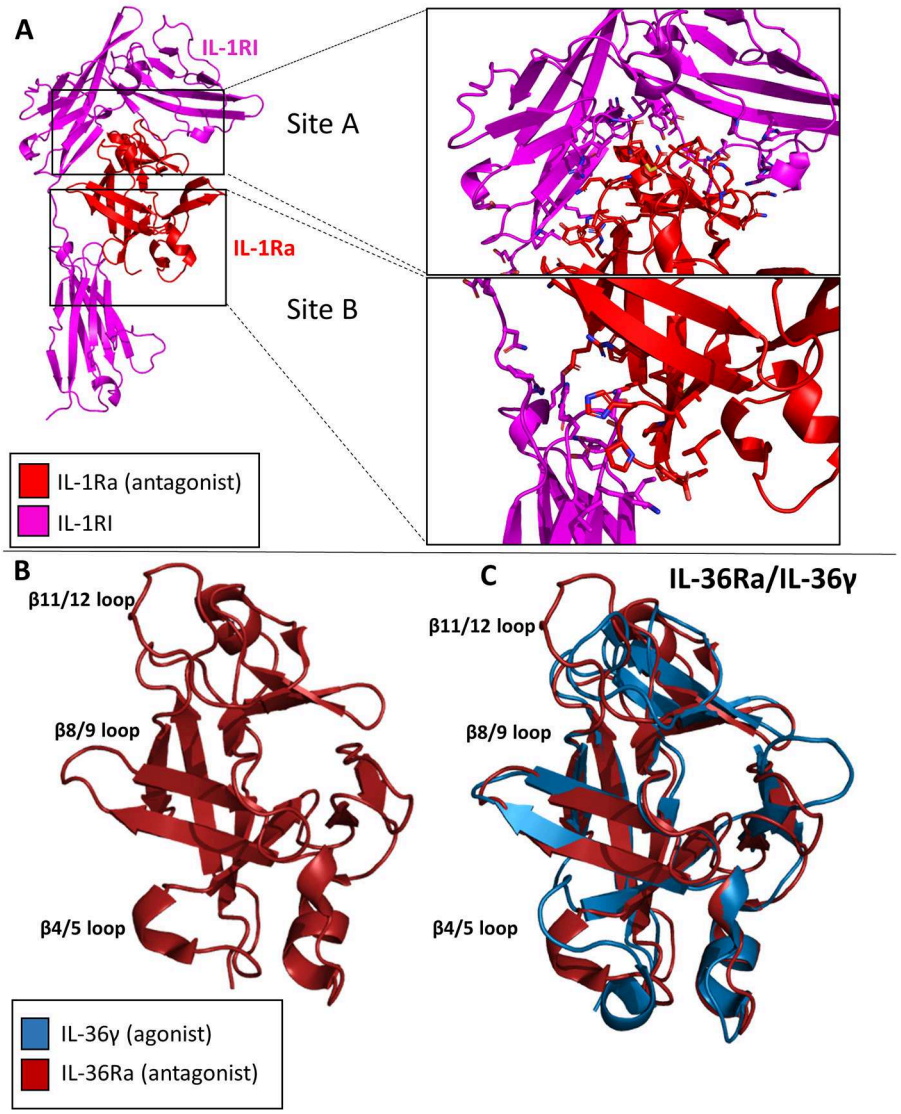

FIGURE 8 | Crystal structures of IL-1 family antagonists. **(A)** IL-1Ra bound to IL-1RI (pdb: 1IRA). Site A and Site B are enlarged to display the larger interface for Site A by IL-1Ra than for Site B, contrary to the binding mechanism of IL-1β. **(B)** Crystal structure of Il-36Ra. **(C)** Crystal structure of IL36Ra aligned to crystal structure of Il-36γ.

and 3 helices to form the prototypical β-trefoil seen in IL-1 family cytokines. While IL-37 shares a common receptor IL-18Rα with IL-18, it is only 19% identical in sequence to IL-18. The RMSD of the Cα atoms between these two cytokines, however, is 1.64 Å (67).

Decoy Receptors

Three decoy receptors exist in the IL-1 family: IL-1RII, sST2, and IL-18BP. While these decoy receptors all mimic the primary receptor, the mechanisms by which they inhibit signaling differ.

IL-1RII

IL-1α and IL-1β signaling may be inhibited in two ways, by the antagonist cytokine IL-1Ra (described above) and by the decoy receptor IL-1RII. IL-1RII is similar to IL-1RI, as it is composed extracellularly of three Ig-like domains and attached to the plasma membrane by a single transmembrane α-helix. IL-RII differs, however, in that it lacks an intracellular TIR domain (**Figure 1D**) (69). As IL-1RII can bind IL-1 agonists, it subsequently may recruit the IL-1RAcP for the creation of the IL-1 ternary complex. As both the cytoplasmic TIRs of the primary and secondary receptor are necessary for the initiation of a signaling cascade, no signaling can occur (70).

sST2

sST2, like its membrane bound homolog ST2, is composed of three Ig-like domains. It differs, however, as it is not attached to the plasma membrane and, as the name implies, is soluble. sST2

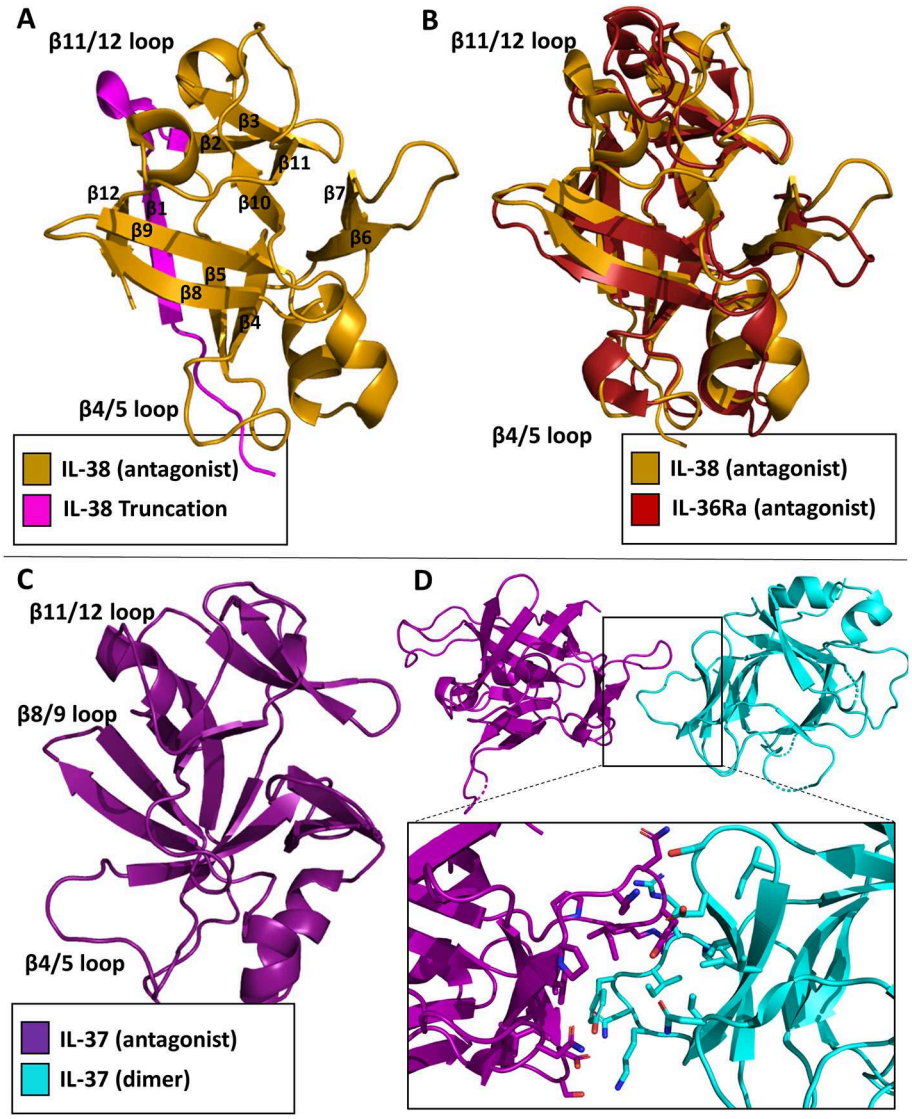

FIGURE 9 | Inhibitory cytokine crystal structures. **(A)** IL-38 crystal structure (pdb: 5BOW) The truncation variant missing the first 19 amino acids can be visualized by the exclusion of the magenta portion of the protein. **(B)** Overlay of the IL-38 crystal structure with IL-36Ra (pdb: 1md6). **(C)** IL-37 crystal structure (5HN1). **(D)** Homo-dimer crystal structure of IL-37 with a focus on the dimer interface.

functions by sequestering free IL-33, thus not allowing it to bind cell-surface expressed ST2 and IL-1RAcP (**Figure 1F**). sST2 levels have been correlated with a number of disease states associated with a Th2 response, including systemic lupus erythematosus, asthma, idiopathic pulmonary fibrosis, and sepsis (71–74).

IL-18BP

The IL-18 binding protein (IL-18BP) is a naturally occurring negative regulator of IL-18 signaling that sequesters free IL-18 and inhibits its binding to the 18Rα (**Figure 1G**). While IL-18BP is usually expressed in 20-fold higher amounts than IL-18, under certain inflammatory conditions, IL-18 may be in excess (75) IL-18BP was discovered when 500 liters of human urine was concentrated and subsequently passaged over an IL-18-agarose column (76). IL-18BP was ultimately shown to abrogate the ability of IL-18 to induce a Th1 response in mice treated with LPS (76). While a naturally produced negative-feedback mechanism within humans, IL-18BP has also been acquired by a multitude of poxviruses, including molluscum contagiosum virus (MCV) and orthopoxviruses (77).

The structure of IL-18BP has been determined to 2 Å resolution (77). IL-18BP has an Ig domain with two four-stranded β-sheets and two disulfides that hold the beta-sandwich together (**Figure 10A**). When this complex structure is overlaid with the binary IL-18 receptor, it is evident that IL-18BP clearly adopts a binding mode resembling the D3 domain of IL-18Rα (**Figure 10B**). In its binding, it lies on top of β-barrel of IL-18 and adds its hydrophobic residues to the binding pocket (77).

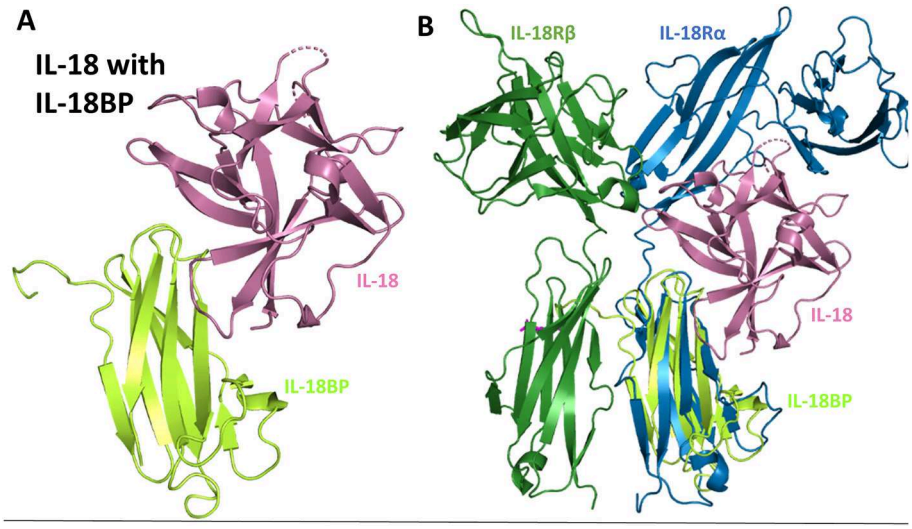

FIGURE 10 | IL-18 sequestration by IL-18BP. **(A)** Crystal structure of IL-18 bound to IL-18BP (pdb 3F62). **(B)** Overlay of IL-18/IL-18BP crystal structure with ternary IL-18/IL-18Rα/IL-18Rβ to highlight IL-18BP resembling the D3 domain of IL-18Rα.

This complex has a buried surface of 1,930 Å^2, as opposed to the 600 Å^2 shared by IL-18 and site B of IL-18Rα (77). This would allow both the human expressing the IL-18BP or a virus that has hijacked this mechanism to downregulate IL-18 induced IFN-γ production effectively.

THERAPEUTICS

Dysregulation of IL-1 family signaling can result in myriad pathologies. As such, stemming the inflammatory signals inherent to agonists within the IL-1 superfamily of cytokines is an attractive therapeutic target. To date, numerous avenues of inhibiting IL-1 family signaling have been explored.

Receptor Antagonists

One of the earliest therapeutics developed against IL-1 signaling is Anakinra, the recombinant version of IL-1Ra and the first to get FDA approval (2001) (15, 78). In an effort to enhance the therapeutic potency of IL-1Ra by leveraging the insight gained from the structures of the IL-1RI/1Ra and IL-1RI/IL-1β complexes, a chimera of IL-1β/IL-1Ra was designed that bound IL-1RI with an 85-fold increase in affinity (SPR) and ∼100-fold increase in potency *in vivo* (13). While the receptor antagonist has changes in the β4/5 and β11/12 loops of the cytokine, it preferentially binds site A while not binding site B, a contributing factor to its inability to make a functioning signaling complex. In contrast, IL-1β engages both sites A and B, but compared to IL-1Ra has a lower affinity for site A. Through rational protein engineering derived from structural knowledge gained from these binary complexes, the authors combined site A of IL-1Ra with site B of IL-1β to create a novel antagonist, EBI-005 (13, 15). The disassociation constant to IL-1RI of this chimera was 6.3×10^{-6} s^{-1} compared to 3.0×10^{-5} s^{-1} for IL-1Ra, leading to a theoretical half-life of 31 and 6.4 h, respectively (13). This potency was recapitulated *in vivo*, resulting in a 100-fold increase in potency of EBI-005 as compared to IL-1Ra (13). While these were not consecutive amino acid substitutions in the primary structure, these residues lie next to each other three dimensionally upon the axis of the β-trefoil (**Figure 11A**).

Decoy Receptors

As described above, there are three naturally occurring decoy receptors in the IL-1 superfamily: IL-1RII, sST2, and IL-18BP (3). In addition, it has been found that a soluble version of IL-1RI may exist, as evidenced by Raji cells stimulated by dexamethasone increased surface expression of IL-1RI and, ultimately, release of a soluble version of IL-1RI(79). While a soluble version of IL-1RI had been tested in clinical trials for graft vs. host disease (GVHD), human immunodeficiency virus (HIV) and rheumatoid arthritis (RA), however, those studies were halted as no therapeutic benefit was observed. It was hypothesized that soluble IL-1RI preferentially bound to IL-1Ra and thus negated any gains that might have been seen by its anti-inflammatory properties (15).

A unique solution to the soluble receptor problem was the idea of cytokine traps, most notably the fusion peptide Rilonacept (trade name Arcalyst, Regeneron Pharmaceuticals) (80, 81). Rilonacept is the inline fusion protein of IL-1RAcP, IL-1RI, and IgG-Fc creating homodimers containing two IL-1RAcP and IL-1RI molecules (**Figure 11B**). In addition to its ability to neutralize IL-1 signaling by acting as a decoy receptor, cytokine traps configured in this way have the added benefit of increased therapeutic half-life due to its fusion to an IgG Fc region (81).

DNA Aptamers

A more recent approach to countering inflammatory diseases resulting from IL-1 signaling has been the use of DNA aptamers (from the Latin "aptus," fit; and the Greek "meros," part), which

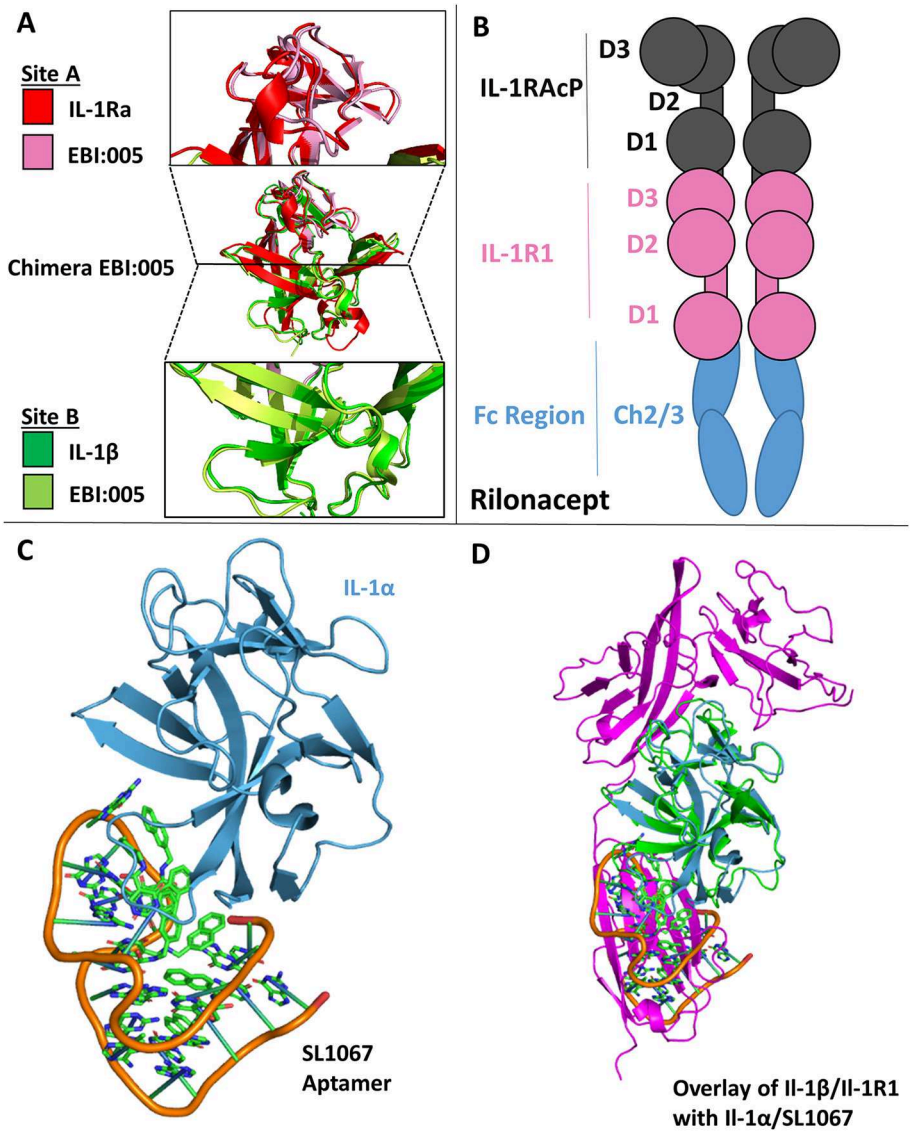

FIGURE 11 | Therapeutics. **(A)** Crystal structure of Chimera EBI-005. **(A)** Top: overlay of IL-1Ra (red) and EBI:005 chimera (pink/portion of binding Site A); Middle: overlay of IL-1Ra/EBI:005/IL-1β; Bottom: Overlay of IL-1β (green) and EBI:005 chimera (lime/portion of binding Site B). **(B)** Rilonacept cartoon with respective components labeled. **(C)** Crystal structure of IL-1α bound of SL1067 aptamer (pdb: 5 uc6). **(D)** Overlay of IL-1α/SL1067 and structure IL-1β/IL-1RI (pdb: 1itb).

are oligonucleotide fragments that can bind protein targets. The DNA aptamer SL1067 binds IL-1α and disrupts its ability to bind to its cognate receptor IL-1RI (**Figure 11C**) (82). The SL1067/IL-1α interface is composed predominately of hydrophobic moieties on both the cytokine and the aptamer, with the addition of π-interactions between amino acids and nucleotides. SL1067 binds on the surface of IL-1α that interacts with D3 of the IL-1RI. While a crystal structure of the IL-1α/IL-1RI binary complex has not been published to date, the RMSD between IL-1α and IL-1β is 1.56 Å and thus can be superimposed to the IL-1β/IL-1RI binary complex for visualization (**Figure 11D**), indicating its mechanism of action (82).

Peptides

While naturally occurring receptor antagonists work well at abrogating IL-1 signaling, an early goal of the field was to discover lower molecular weight antagonist peptides that could be delivered orally to patients. As early as 1996, numerous peptides that inhibited IL-1RI signaling had been discovered by phage display (83). One such peptide, AF10847, was crystalized with IL-1RI to determine its mechanism of antagonism (84), showing that this peptide bound site A of IL-1RI and induced a conformational change in the receptor that renders it incapable of cytokine binding. Indeed, binding site B swung ∼170 degrees away from the orientation of IL-1β binding,

thus not allowing agonist binding and at the same time demonstrating the flexibility of the D3 domain in respect to D1/2 (84).

Antibodies

An early solution to aberrant IL-1 family signaling by agonist cytokines was the development of neutralizing antibodies to these potent mediators of inflammation. AMG108 is currently an antibody licensed to AstraZeneca and targets IL-1RI. It has been shown that this antibody can block IL-1 mediated signaling and is efficacious in the treatment of osteoarthritis (85). More commonly, monoclonal antibodies have been made against agonist cytokines, such as IL-1α and IL-1β, to stem aberrant inflammatory signaling. While there are currently multiple disease states that anti-IL-1β antibodies are being used against, Canakinumab was approved by the FDA for the treatment of cryopyrin-associated periodic syndromes (CAPS) in 2009 (15). This methodology has also been applied to IL-1α, as with the case of MABp1, a mAb targeting refractory cancers (86). As inflammation is responsible for a number of pleiotropic disease states, antibodies against IL-1 family signaling is an attractive target. As there exists numerous pharmaceutical antibodies at various stages of development, the entirety of the known repertoire will not be addressed in this review.

FUTURE PERSPECTIVES

There remains much to be learned within the IL-1 family. No binary or ternary structures involving IL-36 cytokines have been determined at any resolution. As such, the interaction of IL-36 with its primary receptor and recruitment of IL-1RAcP remains poorly understood. As was learned with IL-1 and IL-33, an altogether unique mode of Il-1RAcP interaction may yet exist for IL-36 agonist cytokines. In addition, the precise mechanisms of IL-37 and IL-38 remain to be determined. IL-37 supposedly has a novel mechanism of negative regulation of itself, dimerizing above a certain concentration threshold (67). This could prove particularly difficult for solving a ternary complex of IL-37 by crystallography as reaching metastable concentrations of the respective components might prevent ternary complex formation. While preliminary data has been published concerning its function and primary receptor, high resolution data addressing its dependence on IL-18Rα and SIGIRR are yet missing.

There are two more orphan receptors in the IL-1 family, IL-1 receptor accessory protein like (IL-1RAPL) 1 and 2. Based on structural similarity, they are grouped with IL-1RAcP (87). No immunological function has yet been attributed to them. Instead, they were shown to play an important role in the neuronal system in trans-synaptic signaling (88). The ectodomain of IL-1RAPL1 binds the ectodomain of protein tyrosin phosphatase receptor δ (PTPRδ). Additionally, IL-1RAcP was shown to facilitate trans-synaptic signaling in a similar way (89). Crystal structures of both IL-1 family receptors with PTPRδ revealed that mainly the D1 domain of both IL-1AcP and IL-1RL1 was engaged by PTPRδ (87). Why IL-1RAcP functions in both the immune and nervous systems remains unclear. Notably, there is a unique isoform of IL-1RAcP only found in the nervous system (90).

Beyond the extracellular domains of IL-1 family receptors, the particulars of the intracellular signaling cascade remain somewhat of a mystery in regards to their actual mechanistic interactions with other TIRs, such as MyD88. Cytoplasmically, receptors of the IL-1 family are attached to TIR domains through a single trans-membrane helix. Analogous to the Toll-Like-Receptor (TLR) TIR domains, these mediators bind several cytoplasmic molecules to initiate intracellular signaling. To date, the structure of only a single TIR domain from the IL-1 family has been solved. The first IL-1 family TIR domain structure determined was a homo-dimer of IL-1 receptor accessory protein like 1 (IL-1RAPL1) in 2004 (91). As there are significant differences in both sequence identity and structural similarity to other known TIR domains, namely the TLR TIR domains, it suggests that TIR structural diversity allows for the diverse signal transduction that can occur. In coming years, IL-1 family TIR domain structural studies could provide a fruitful avenue of research for the field. As was learned through the ectodomains of these receptors, the differences inherent to the TIRs mediate their action and could prove attractive therapeutic targets.

Structural biology has added a wealth of information to the molecular mechanisms of IL-1 family signaling. To date, three high-resolution ternary complexes have been determined by X-ray crystallography: IL-1β/IL-1R1/IL-1RAcP, IL-33/ST2/IL-1RAcP, and Il-18/IL-18Rα/IL-18Rβ. Through these structures, the distinctive structural and functional properties of each respective ternary complex have been elucidated. As such, new avenues of antibody therapy are now clear. As previously stated, both IL-1β and IL-33 binary complexes recruit the IL-1RAcP secondary receptor differently. By targeting different solvent accessible features of IL-1RAcP, and thus different interface residues, selective inhibition of IL-1 family signaling cascades might be achieved. This could be especially useful for IL-33 as no natural antagonist cytokine exists for the primary receptor.

The field's structural biology knowledge can be leveraged in other ways as well. In addition to antagonist cytokines, it is possible to use low molecular weight peptides that bind the primary receptor and act as antagonists (84). The flexibility of the D3 domain with respect to D1/2 has been shown experimentally (40) as well as theoretically (92, 93). By using the inherent flexibility of the primary receptor by holding the primary receptor in a non-amenable conformation for cytokine binding, IL-1 family signaling can be ablated.

CONCLUSION

IL-1 family signaling is an instrumental component of an inflammatory response, ultimately helping to orchestrate both

innate and adaptive immunity to fight a myriad of pathogens. Conversely, aberrant signaling within these systems can lead to a host of auto-inflammatory disease states. By employing methods in structural biology, a wealth of information has been gained concerning how these receptor complexes function and the particulars of each subfamily system. Through the knowledge that has and will continue to be learned through structural biology, it will be possible to fully understand these member systems and, feasibly, harness it for our therapeutic benefit.

AUTHOR CONTRIBUTIONS

JF wrote the manuscript and designed the figures. SG and ES edited the manuscript and the figures.

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REVIEW
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IL-1 Family Cytokine Regulation of Vascular Permeability and Angiogenesis

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The IL-1 family of cytokines are well-known for their primary role in initiating inflammatory responses both in response to and acting as danger signals. It has long been established that IL-1 is capable of simultaneously regulating inflammation and angiogenesis, indeed one of IL-1's earliest names was haemopoeitn-1 due to its pro-angiogenic effects. Other IL-1 family cytokines are also known to have roles in mediating angiogenesis, either directly or indirectly via induction of proangiogenic factors such as VEGF. Of note, some of these family members appear to have directly opposing effects in different tissues and pathologies. Here we will review what is known about how the various IL-1 family members regulate vascular permeability and angiogenic function in a range of different tissues, and describe some of the mechanisms employed to achieve these effects.

Keywords: IL-1, IL-18, IL-33, IL-36, angiogensis, vascular permeability

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THE IL-1 FAMILY OF CYTOKINES

The interleukin-1 family (IL-1F) are pivotal regulators of the innate immune response composed of 7 agonist signaling ligands [interleukin-1 alpha (IL-1α), interleukin-1 beta (IL-1β), interleukin-18 (IL-18), interleukin-33 (IL-33), interleukin-36 alpha (IL-36α), interleukin-36 beta (IL-36β), and interleukin-36 gamma (IL-36γ)]; three receptor antagonists [interleukin-1 receptor antagonist (IL-1Ra), interleukin-36 receptor antagonist (IL-36Ra), and interleukin-38 (IL-38)] as well as one other cytokine [interleukin-37 (IL-37)] (1, 2) whose biological role remains somewhat controversial. These cytokines signal through the IL-1 receptor (IL-1R) family of transmembrane proteins, whose 11 members form six receptor chains, resulting in four functional signaling complexes (**Figure 1**). Members of the IL-1R family are grouped due to the characteristic motifs shared in their extra- and intracellular domains. The extracellular domain is typically composed of three immunoglobulin (Ig)-like domains. Interleukin-18 binding protein (IL-18BP) and Toll/IL-1R8 (TIR8) are exceptions having only a single Ig-like domain. Intracellularly, the signaling complexes are identifiable by their (**T**oll/**I**L-1 **R**eceptor) TIR-domains, which are required to recruit the adapter protein myeloid differentiation primary response protein 88 (MyD88) through homotypic interactions and initiate the downstream signaling cascades that are the consequence of IL-1F receptor activation (3). The signaling complexes and their respective ligands can be seen in **Figure 1**. Most of the IL-1 family cytokines, including IL-1, IL-18, and IL-36 are produced in inactive precursor forms, that

require N-terminal cleavage in order to become activated (4, 5). In the case of IL-1 and IL-18, this cleavage is classically performed by caspase-1, that itself is activated by the formation of an inflammasome; however alternative cleavage of pro-IL-1β via various serine proteases and matrix metalloproteinases, and cleavage of IL-18 by caspase-8 has also been reported (5, 6). The mechanisms through which other IL-1 family cytokines, such as IL-33 and IL-36, are cleaved, are still emerging, with studies thus far suggesting that neutrophil elastase and various cathepsins are the relevant proteases (7, 8). Broadly generalizing, upon IL-1 family cytokine binding to its receptor complex, the adaptor protein MyD88 is recruited to the intracellular TIR domain which forms a complex with interleukin-receptor associated kinase 4 (IRAK4), facilitating the activation of the downstream signaling cascade, canonically comprising of mitogen associated protein kinase (MAPK) and Inhibitor of κB Kinase (IKK) complexes, and resulting in the respective activation of Activator protein-1 (AP-1) and nuclear factor kappa-light-chain-enhancer of B cells (NFκB) transcription factors, driving gene transcription of IL-1F responsive genes.

Abbreviations: AP-1, Activator protein-1; ALK1, Activin receptor-like kinase 1; AMD, Age-related macular degeneration; ang-1, Angiopoietin-1; BBB, Blood-brain barrier; BRB, Blood-retina-barrier; BMP-6, Bone morphogenic protein-6; BAL, Bronchoalveolar; CMVEC, Cardiac microvascular endothelial cells; CCR7, C-C chemokine receptor type 7; CNS, Central nervous system; CCL21, Chemokine (C-C motif) ligand 21; CXCL1, Chemokine (C-X-C motif) ligand 1; CXCL2, Chemokine (C-X-C motif) ligand 2; YKL-40, Chitinase-3-like protein 1; CNV, Choroidal neovascularisation; JNK, c-JUN N-terminal kinase; CEC, Corneal endothelial cells; COX-2, Cyclooxygenase-2; CsA, Cyclosporine a; CINC-1, Cytokine-induced neutrophil chemoattractant; DC, Dendritic cell; ERK 1/2, Endoplasmic-reticulum kinase-1/2; EC, Endothelial cells; eNOS, Endothelial nitric oxide synthase; ET-1, Endothelin-1; ECM, Extracellular matrix; FGF-2, Fibroblast growth factor-2; FAK, Focal adhesion kinase; HBMVEC, Human brain microvascular endothelial cells; HCMVEC, Human cereberal microvascular endothelial cells; HCAEC, Human coronary artery endothelial cells; HMVEC-d, Human dermal microvascular endothelial cells; HPAEC, Human pulmonary artery endothelial cells; HRGEC, Human renal glomerular endothelial cells; HUVEC, Human umbilical vain endothelial cells; HIMF, Hypoxia induced mitogenic factor; HIF-1α, Hypoxia-inducible factor-1α; Ig, Immunoglobulin; ICAM-1, Intercellular adhesion molecule-1; IFNγ, Interferon gamma; IL-1, Interleukin-1; IL-1F, Interleukin-1 family; IL-1R, Interleukin-1 receptor; IL-1Ra, Interleukin-1 receptor antagonist; IRAK4, Interleukin-1 receptor associated kinase 4; IL-10, Interleukin-10; IL-18, Interleukin-18; IL-18BP, Interleukin-18 binding protein; IL-33, Interleukin-33; IL-34, Interleukin-34; IL-36, Interleukin-36; IL-36Ra, Interleukin-36 receptor antagonist; IL-38, Interleukin-38; IL-6, Interleukin-6; IL-8, Interleukin-8; JAK2, Janus kinase 2; KO, Knock-out; M-CSF, Macrophage colony stimulating factor; MAPK, Mitogen-activated protein kinase; MAP2K2, Mitogen-activated protein kinase kinase 2; MS, Multiple Sclerosis; MyD88, Myeloid differentiation primary response protein 88; NLRP3, Nacht, LRR, and PYD domains-containing protein 3; NO, Nitric oxide; NDRG1, N-myc downstream regulated gene 1; NF-HEV, Nuclear factor high endothelial venules; NFκB, Nuclear factor kappa-light-chain-enhancer of B cells; OPN, Osteopontin; PTX3, Pentraxin 3; PI3K, Phosphoinositide-3 kinase; PDK1, Phosphoinositide-dependent kinase-1; Akt, Protein kinase B; PTP, Protein tyrosine phosphatase; RA, Rheumatoid arthritis; RSPO3, R-spondin 3; SMAD1, SMAD family member 1; SMAD3, SMAD family member-3; SP1, Specificity protein 1; NFSA, Spontaneous fibrosarcoma; SSeCKs, Src-suppressed C-kinase substrate; SE, Status epilepticus; Th2, T-helper type-2; TF, Tissue factor; TOLLIP, Toll interacting protein; TIR8, Toll/IL-1R 8; TIR-domain, Toll/IL-1R domain; TLR, Toll-like receptor; TGF-β, Transforming growth factor β; TNF-α, Tumor necrosis factor alpha; u-PA, Urokinase-type plasminogen activator; VCAM-1, Vascular cell adhesion molecule-1; VEGF, Vascular endothelial growth factor; VEGFR2, Vascular endothelial growth factor receptor 2; VSMC, Vascular smooth muscle cells; vWF, von Willebrand factor; ZO-1, Zonula occludens-1.

ANGIOGENESIS AND VASCULAR PERMEABILITY

Angiogenesis is the growth of new capillaries from existing blood vessels; a process controlled by complex interactions of inhibitors and activators, an appropriate balance of which is required to initiate and maintain physiological homeostasis. This process is important in development, as well as for tissue growth and repair. However, aberrant angiogenesis or neovascularisation has been linked to several malignancies, including cancer, rheumatoid arthritis (RA), blindness, and psoriasis (9). In many of these conditions, unwanted, disease-associated neovascularisation results in malformed, immature, and unstable vessels that leak plasma factors and can create an oedemic environment. A thorough understanding of the factors that influence the formation of the vasculature in addition to the maturation and integrity of the neovesssels is essential for delineating therapeutic targets for these conditions (10, 11). The process of angiogenesis is multistep, and involves the degradation of the extracellular matrix (ECM), migration, differentiation, and proliferation of endothelial cells (ECs), microtubule formation, and the sprouting of new capillary branches (12). Factors controlling angiogenesis may be categorized as indirect, in that they act via intermediary mechanisms, or direct, in that they are able to induce proliferation, migration and/or differentiation of endothelial cells directly (13).

Current therapeutic options for neovascular and oedemic disease attempt to target the pathways that coordinate angiogenesis and permeability. The best characterized of these is the vascular endothelial growth factor (VEGF) pathway. VEGF is considered the master regulator of angiogenesis and permeability, and is implicated as a driver of neovascularization and oedema in a number of diseases, hence anti-VEGF therapeutics are currently utilized in diseases ranging from age-related macular degeneration (AMD) to a number of forms of cancer (14, 15).

It has long been established that IL-1 is capable of simultaneously regulating inflammation and angiogenesis, indeed one of IL-1's earliest names was haemopoeitn-1 due to its pro-angiogenic effects (16). In fact, proteome profiling has revealed that there are considerable overlaps in pathways and biological functions regulated by IL-1β and VEGF in activated EC, in particular the mitogen activated protein kinase (MAPK) cascade is induced by both IL-1β and VEGF, and may potentially play a role in the overlapping effects caused by inflammatory and angiogenic signaling (17). Other IL-1 family cytokines are also known to have roles in mediating angiogenesis, either directly or indirectly. Of note, two of these family members appear to have directly opposing effects in different tissues and pathologies. Here we will review what is known about how the various IL-1 family members regulate vascular permeability and angiogenic function in a range of different tissues, and describe some of the mechanisms employed to achieve these effects.

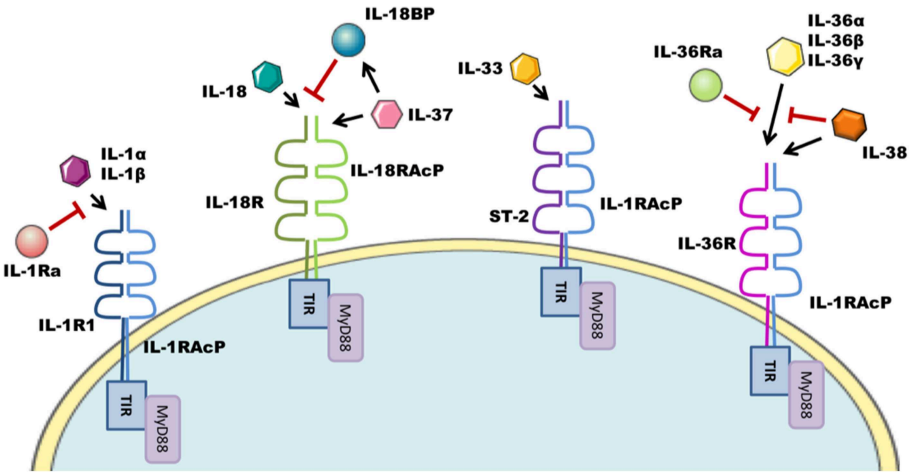

FIGURE 1 | IL-1F family signaling complexes. The IL-1R family has four signaling complexes; IL-1R1/IL-1RAcP, IL-18R/IL-18RAcP, ST-2/IL-1RAcP, and IL-36R/IL-1RAcP. IL-1α/IL-1β signal through IL-1R1/IL-1RAcP; this pathway can be antagonized by IL-1Ra. IL-18 signaling uses the IL-18R/IL-18RAcP complex. IL-18 ligand can be sequestered and thereby inhibited by IL-18BP. IL-37 also utilizes this complex. ST-2/IL-1RAcP is the receptor complex for the IL-33 cytokine. IL-36α/IL-36β/IL-36γ signal through the IL-36R/IL-1RAcP complex, which can be antagonized by IL-36Ra and IL-38.

IL-1—AN EXTENSIVE MEDIATOR OF ANGIOGENESIS

IL-1α and IL-1β ligands have been recognized for some time as cytokines that can drive an angiogenic phenotype *in vivo*. IL-1α is rarely secreted by living cells, and has been less widely studied than its homolog, IL-1β. However, there is still a wealth of literature that suggests IL-1α can play a role in mediating angiogenesis, most predominantly in cancer and in the brain (18).

One area in which IL-1α and IL-1β are thought to act synergistically is in the process of wound healing. IL-1α and IL-1β are markedly induced 12–24 h post-wound, and the levels of these cytokines return to basal levels once the proliferative stage of wound healing has been completed (19). The wound healing response involves a finely tuned, self-limiting series of cellular and molecular events orchestrated by the transient activation of specific signaling pathways. Controlled regulation of these events is essential. Failure to initiate key steps at the right time delays healing, leading to chronic wounds, while aberrant initiation of wound healing processes may produce cell behaviors that promote cancer progression. Angiogenesis is a crucial step in wound healing, as there is a substantial oxygen-demand in this highly energy-consuming process (20).

In response to a wound in the brain, a rapid induction of inflammatory cells and astrocytes occurs, IL-1 is among the inflammatory cytokines produced in this process, and it is known to stimulate astrocytosis and neovascularization (21). IL-1α is widely acknowledged to have a role as a direct regulator of angiogenesis following ischemic injury (22). Post-stroke, controlled angiogenesis is important for repairing the damaged area of the brain and is known to improve functional outcome. In post-stroke mice, IL-1α is upregulated, and IL-1α can potently induce the expression of other pro-angiogenic cytokines such as chemokine (C-X-C motif) ligand 1 (CXCL1) in brain EC's, as well as being capable of directly driving proliferation, migration and tube formation, all hallmarks of angiogenesis, in these same cells (23, 24). Such is the potency of IL-1α in this context, downstream targets of IL-1α are also being studied as potential neurotherapeutic molecules in stroke. Perlecan is one such downstream target (25, 26). When Perlecan domain V was systemically administered for 24 h post stroke in rat and mouse models, it increased VEGF levels via α5β1 integrin, and displayed neuroprotective and angiogenic effects (27).

Like IL-1α, IL-1β can mediate angiogenesis in the cerebrovascular system. At least one mechanism likely involves IL-1β upregulation of pentraxin 3 (PTX3), a marker of stroke (28). PTX3 KO mice display reduced post-stroke angiogenesis, and recombinant PTX3 can induce hallmarks of angiogenesis including cell proliferation and tube formation in the murine brain derived endothelial cells; indicating that this IL-1β regulated molecule can mediate angiogenesis and contribute to recovery after stroke (29). However, IL-1 activation in the brain is not without its downsides, and has been shown to be a major driver of neuroinflammation; responsible for activating endogenous microglia and vascular EC, allowing them to recruit peripheral leukocytes that can sustain neuroinflammation (30). In particular, IL-1β can influence neutrophil-mediated toxicity, and neutrophil recruitment to the brain appears to be IL-1β dependent. Transendothelial migration of neutrophils across IL-1β stimulated brain endothelium pushes neutrophils to acquire a neurotoxic phenotype (31).

Perhaps not surprisingly, one of the ways in which IL-1R signaling mediates angiogenesis is indirectly, via its ability to induce VEGF expression and secretion in a number of cell types (32). Under normoxia, IL-1β has been shown to induce hypoxia-inducible factor-1α (HIF-1α) at protein level, despite no change being detected in HIF-1α mRNA levels (33). Given

the recent literature on inflammation induced regulation of metabolic processes, this is likely due to IL-1 induced stabilization of the labile HIF-1α protein in response to a metabolic shift in the cell. HIF-1α mediates angiogenesis via its target gene, VEGF, which has established hypoxia regulatory elements in its promoter (34, 35). In fact, early reports demonstrated that IL-1β can drive transcription of both VEGF and its receptor VEGF-receptor 2 (VEGFR2) in cardiac myocytes and cardiac microvascular endothelial cells (CMVEC) (36) indicating that an important role for IL-1 signaling is likely that of potentiating VEGF biology. Later reports demonstrated that IL-1β KO mice have impaired VEGF-controlled mobilization of endothelial progenitor cells into the peripheral blood resulting in markedly reduced neovascularisation compared to wild type mice following ischemic injury (37). There have since been several studies that investigate which pathways are important for IL-1β mediated VEGF induction. In myocardial neovascularisation, the VEGF-dependent physiological response to myocardial ischemia, it has been demonstrated that in addition to HIF1- α stabilization, VEGF mRNA transcription, and stability are improved following incubation with IL-1β; and that this activity is dependent on specificity protein 1 (SP1) sites in the VEGF gene's promoter targeted by IL-1β-activated p38 MAPK and c-JUN N-terminal kinase (JNK) signaling (38). Similarly, treatment with IL-1β of vascular smooth muscle cells (VSMC) from small tumor vessels, also resulted in improved VEGF transcription and stability in a p38 MAPK dependent manner (39). Taken together, these findings indicate that both IL-1β induction of the p38 MAPK signaling cascade and induction of HIF1- α are important for upregulating VEGF, and that IL-1β upregulation of VEGF consequently drives neovascularisation.

It is often reported that cancers are "wounds that never heal" or even "over-healing wounds" (40, 41) and inflammation, the arm of the immune system that regulates wound healing, can be a cause of cancer. In addition to the modulating effect that IL-1 has on VEGF expression and wound healing, IL-1 has also been reported to directly regulate tumor-mediated angiogenesis (18), potentially providing an alternative or adjunct therapeutic target in these cancers. Observationally, studies have demonstrated that IL-1α is secreted by colonic, gastric, and pancreatic cancer cells and that this enhances angiogenesis *in vitro* (42–44). Others have delved further into the mechanism investigating how IL-1α is activated in cancer cells and how this effects neovascularisation and subsequent tumor growth. In gastric cancer cells, N-myc downstream regulated gene 1 (NDRG1) increased IL-1α expression, which was then capable of driving tumor angiogenesis via a c-JUN N-terminal kinase (JNK)/AP-1 dependent pathway (45). In prostate cancer, Bone morphogenic protein-6 (BMP-6) is overexpressed, and was shown to induce IL-1α in macrophages via NFκB/Smad1 signaling (46) that was capable of driving tube formation in endothelial progenitor cells. Tumor growth and neovascularisation is significantly decreased when BMP-6 is expressed in IL-1α knockout (KO) mice compared to wild type controls; indicating that IL-1α is mediating angiogenesis in this context. While it remains possible, and probable, in these latter studies that IL-1 is not working alone, but is also driving VEGF production and effecting neovascularisation in an indirect manner, it is clear that IL-1 is an inducer of angiogenesis in the tumor environment. In melanoma, IL-1α and IL-1β are both required for NFκB activation that results in the upregulation of proinflammatory cytokines IL-6 and IL-8, as well as the adhesion molecules intercellular adhesion molecule-1 (ICAM-1), vascular cell adhesion molecule-1 (VCAM-1), and tissue factor (TF) in EC's, driving these cells toward a proinflammatory phenotype that supports tumor angiogenesis (47). While in Lewis lung carcinoma, IL-1β drives tumor growth through its upregulation of VEGF and the proangiogenic chemokine (C-X-C motif) ligand 2 (CXCL2) (48). Further evidence to support the role IL-1 signaling plays in driving tumor angiogenesis can be seen when the IL-1 signaling pathway is blocked; for example through continuous delivery of IL-1Ra, which neutralizes IL-1 signaling, and has been shown to reduce angiogenesis and tumor development (49). It is also worth noting that initial findings from a major randomized trial [Canakinumab Anti-Inflammatory Thrombosis Outcomes Study (CANTOS)] studying Canakinumab, an IL-1 inhibitory antibody, reported a reduction in the number of incident cases of lung cancer in the treatment groups compared to control groups. Although the mechanisms at play in this setting remain unknown and maybe independent of IL-1 induced neovascularisation. For a deeper review of how IL-1 cytokines regulate cancer we would direct the reader to the review on IL-1 family and Cancer also in this special topic.

A common theme when examining the regulation of angiogenesis by IL-1 family members is the interplay between IL-1 family regulation of and by macrophages, and how this interplay can control angiogenic processes. For example, in Lewis lung carcinoma in addition to IL-1 receptor induction of proangiogenic chemokine CXCL2 driving tumor promoting angiogenesis, IL-1β driven neovascularization was found to be dependent on infiltrating cyclooxgenase 2 (COX-2) positive macrophages (50). Macrophages play a pivotal role in restoring tissue homeostasis after inflammation or damage, and angiogenesis is an integral process in tissue repair, so it is perhaps unsurprising to see that IL-1 family members derived from macrophages have an intrinsic ability to regulate angiogenesis. Matrigel plugs, containing the supernatants from activated macrophages, have been used to demonstrate that macrophage derived IL-1, predominantly IL-1β, attracts myeloid cells from the bone marrow, and these cells locally produced additional IL-1, which further activated macrophages and stimulated EC's to produce VEGF (51). In macrophages at least, it appears that the activation of NFκB, a target of IL-1 signaling, is crucial for VEGF production (52). In a dysregulated or diseased setting, Foucher et al. proposed a mechanism, in which IL-1α, constitutively expressed by interleukin-34 (IL-34) induced macrophages, allows these macrophages to maintain local inflammation, which contributes to aberrant angiogenesis (53).

Angiogenesis is a pathological feature of RA (54). RA synovial tissue, which is rich in blood vessels, invades the periarticular cartilage and bone, resulting in the destruction of the joint. In contrast to IL-1's direct role in mediating angiogenesis in the brain following stroke. IL-1β has no direct effect on mediating angiogenesis in RA, instead it indirectly mediates the

pathophysiological angiogenesis via several of its downstream effectors upregulating the proangiogenic mediators angiopoietin-1 (ang-1), Tie-2, and VEGF in a JNK and p38 MAPK dependent pathway (55). IL-1β also upregulates expression of VEGF and chemokine (C-C motif) ligand 21 (CCL21) in RA synovial fibroblasts, this chemokine binds to its receptor C-C chemokine receptor type 7 (CCR7) on EC's, facilitating cell migration, capillary tube formation and *in vivo* blood vessel formation (56, 57). Consequently in the clinic, anakinra, the IL-1Ra homolog treatment that blocks IL-1 signaling, has been shown to reduce neovascularisation of the pannus in RA (58, 59).

The majority of studies described have focused on the effects of IL-1 on VEGF, as the master regulator of angiogenesis and permeability, however, there is also some evidence that suggests IL-1β can regulate other pro-angiogenic growth factors in addition to VEGF. For example, stimulation of corneal endothelial cells (CEC) with IL-1β resulted in the NFκB-dependent induction of fibroblast growth factor-2 (FGF-2), which stimulates endothelial mesenchymal transformation (EndoMT) (60). EndoMT involves EC's losing their endothelial phenotype and attaining a myofibroblast or mesenchymal phenotype (61). While this is not directly related to angiogenesis, it is a cause of many fibrotic conditions and demonstrates the powerful effects IL-1β can exert on endothelial cell regulation. **Table 1** details clinical trials targeting IL-1 family cytokines in various diseases that have vascular dysfunction as an aspect of the disorder. It is noteworthy that all but one of these trials are targeting IL-1 with the intention of inhibiting its activity.

IL-18 AS AN ANGIOGENIC MEDIATOR—THE CONTEXT IS KEY

IL-18 signals through its own receptor (IL-18R) and is unique among the other family cytokines in that it has its own specific accessory protein (IL-18RAcP). IL-18 is considered a pro-inflammatory cytokine due to its potency in activating Natural Killer cells and instructing T helper cell differentiation. Like IL-1β, IL-18 requires cleavage from its inactive precursor in order to become activated; this cleavage is classically performed by the inflammasome activated caspase-1 (5, 62). IL-18 has not been studied to the extent that IL-1 has in the context of angiogenic regulation, however, the reports in the literature appear opposing, indicating that IL-18 appears capable of mediating either a pro-angiogenic signal or an anti-angiogenic signal dependent on the tissue site. In fact the first report of IL-18 involvement in vascular regulation came in 1999 and described how IL-18 negatively regulated neovascularisation *in vivo* (63), this was quickly followed by a report in 2001 demonstrating that IL-18 was pro-angiogenic as it could induce endothelial tube formation both *in vivo* and *in vitro* (64).

IL-18 as a Pro-angiogenic Cytokine

In RA, IL-18 is elevated in sera, synovial tissues and synovial fluid of patients compared to healthy controls, and IL-18 can upregulate expression of RA stimulators including the adhesion molecules ICAM-1 and VCAM-1, chemokines and VEGF *in vitro* (65, 66). These factors encourage the recruitment and activation of leukocytes as well as the formation of new blood vessels. In fact, IL-18 has been shown to stimulate human dermal microvascular endothelial cells (HMVEC-d) migration and tube formation in Matrigel plugs in a Src- and JNK- dependent manner (67). Other mechanisms that drive IL-18 mediated angiogenesis have also been elucidated in RA. Chitinase-3-like protein 1 (YKL-40) is a proinflammatory molecule that is strongly expressed in RA patients (68). YKL-40 has the ability to induce IL-18 production in osteoblasts, which in turn stimulates angiogenesis in endothelial progenitor cells in a process that requires the suppression of miR-590-3p via the focal adhesion kinase (FAK)/PI3K/Akt pathway (69). IL-18 has also been associated with IL-1β in RA patient biopsies, raising the possibility that these molecules are working in tandem to drive the aberrant angiogenesis as well as other features of the disease (70). In the rare autoimmune disorder, inflammatory myopathy, hypoxia induced mitogenic factor (HIMF) was found to increase IL-18 production in myoblasts via a phosphoinositide-dependent kinase-1 (PDK1)/PI3K/Akt signaling cascade (71). This IL-18 was secreted and drove tube formation in endothelial progenitor cells as well as driving angiogenesis in *in vivo* models.

One of the better understood ways in which IL-18 exerts angiogenic function is via its effect on macrophages. IL-18 has been shown to act synergistically with IL-10 to amplify the production of osteopontin (OPN) and thrombin, angiogenic mediators in their own right, from macrophages, a process which alters the polarization of M2 macrophages, as characterized by the increased expression of CD163 (72). CD163 could potentially be responsible for mediating cell-cell interactions between these macrophages and EC, thereby resulting in excessive angiogenesis. On a side note, in addition to mediating angiogenesis, macrophage derived IL-18 has also been reported to drive vascular remodeling in inflammageing, a process prevalent in many age-related disorders (73).

IL-18 as a Negative Regulator of Angiogenesis

Despite the variety of tissues in which IL-18 demonstrates a pro-angiogenic profile, there is clear evidence that indicates this is not the end of the story. In the eye especially, there is a significant body of work that attests to IL-18 acting as an anti-angiogenic factor in different models of disease. IL-18 was initially described as a suppressor of angiogenesis by Cao et al. who reported that IL-18 inhibited the proliferation of bovine CEC's, and suppressed mouse corneal neovascularisation (63). More recently it has been demonstrated in a clinical cohort that individuals with macular oedema due to retinal vein occlusion who have high aqueous levels of IL-18 at baseline have better visual outcomes following anti-VEGF administration than those with low levels of IL-18 at baseline (74). This may be due to IL-18's reciprocal negative regulation of VEGF that is reported in the eye (74, 75). Our own group has demonstrated that IL-18 inhibits choroidal neovascularisation (CNV) in both murine and non-human primate laser induced models of neovascular AMD. Administration of IL-18 to experimentally induced CNV

TABLE 1 | Table of clinical trials targeting IL-1F cytokines in diseases related to vascular dysfunction.

Condition	Treatment	Clinical trial.gov identifier	Status
Rheumatoid arthritis	Anakinra	NCT00037700	Phase II, completed—no results available
		NCT00117091	Phase III, completed—no results available
	ACZ855/Canakinumab	NCT00619905	Phase I/II, completed—no results available
		NCT00505089	Phase I/II, terminated—results available
		NCT00504595	Phase II, completed—results available
		NCT00487825	Phase II, completed—results available
		NCT00424346	Phase II, completed—results available
	GSK1827771	NCT00539760	Phase I, completed—no results available
Juvenile rheumatoid arthritis	ACZ855/Canakinumab	NCT00426218	Phase I/II, completed—no results available
Systemic juvenile idiopathic arthritis (Still's disease)	Anakinra	NCT00339157	Phase II/III, completed—no results available
		NCT00037648	Phase II, completed—no results available
		NCT03932344	Enrolling by invitation
	ACZ855/Canakinumab	NCT01676948	Phase III, withdrawn
		NCT00889863	Phase III, completed—results available
		NCT00886769	Phase III, terminated—results available
		NCT02296424	Phase III, completed—results submitted
		NCT00891046	Phase III, completed—results available
		NCT02396212	Phase III, completed—results submitted
	Rilonacept	NCT01803321	Phase I, completed—no results available
		NCT00534495	Phase II, completed—results available
Osteoarthritis	sc-rAAV2.5IL-1Ra	NCT02790723	Phase I, recruiting
	Anakinra	NCT00110916	Phase II, completed—no results available
	Diacerein	NCT00685542	Phase IV, completed—no results available
Corneal neovascularization	Topical IL-1Ra	NCT00915590	Phase I/II, terminated (lack of participants)—results available
Wet age-related macular degeneration	ACZ855/Canakinumab	NCT00503022	Phase I, completed—no results posted
Kawasaki disease	Anakinra	NCT02179853	Phase I/II, recruiting
		NCT02390596	Phase II, recruiting
Atherosclerosis/coronary artery disease	Anakinra	NCT01566201	Completed
	ACZ855/Canakinumab	NCT01327846	Phase III, active—not recruiting
	Rilonacept	NCT00417417	Phase II, completed—results available
Giant cell arteritis	Anakinra	NCT02902731	Phase III, not yet recruiting
Chronic renal insufficiency	Rilonacept	NCT01663103	Phase IV, completed—results available
Intracerebral hemorrhage	Anakinra	NCT03737344	Phase II, not yet recruiting
Subarachnoid hemorrhage	Anakinra	NCT03249207	Phase III, recruiting
Abdominal aortic aneurysm	ACZ855/Canakinumab	NCT02007252	Phase II, terminated (lack of efficacy)—results available
Behcet's disease	Anakinra	NCT01441076	Phase I/II, completed—results available
	GSK1070806	NCT03522662	Phase II, not yet recruiting

reduced the volume of the CNV lesion significantly compared to vehicle control in both murine and non-human primate models, again adding to the growing data that, in the eye at least, IL-18 has anti-angiogenic properties (75, 76). This finding is further strengthened by experiments that demonstrated in the JR5558 mouse model, which spontaneous develops bilateral CNV and retinal angiomatus proliferation, IL-18 administered via interperitoneal injection suppressed both progression and enhanced regression of the spontaneous CNV (75). Similarly, in $VEGF^{hyper}$ mice, that spontaneously develop CNV lesions physiologically similar to those observed in AMD, the ablation of IL-18 expression resulted in a significant increase in the volume and number of lesions formed, when compared to the $VEGF^{hyper}$ phenotype alone or in combination with inhibition of IL-1β or NLRP3 (77).

In line with data emerging from studies on the eye, IL-18 has also been shown to inhibit neovascularisation in murine fibrosarcoma and suppress growth, an anti-angiogenic function that suggests it may have a role in some contexts as a tumor suppressor (63). IL-18 also suppressed tumor growth and metastasis in implanted Lewis lung cancer, an effect it achieved by downregulating VEGF, thereby suppressing angiogenesis (78). This data implies that IL-18 down regulation of VEGF is not unique to the eye but is observed in non-ocular tissue as well.

Even in the case of macrophage derived IL-18, the pro-angiogenic effects of which have been referred to above;

there are instances where IL-18 has been shown to negatively regulate angiogenesis. IL-18 produced by the tumor induced M1 macrophages in spontaneous fibrosarcoma (NFSA) tumors, cause the destruction of EC's *in vitro*, and are suspected to result in the necrosis of NFSA tumors in part by enhancing macrophage phagocytosis (79). This conclusion is supported by research that shows the macrophage derived IL-18 strongly inhibited blood vessel formation in the tumors *in vivo* (80). However, there is abundant evidence to suggest that in many cancers, IL-18 is more harmful than helpful in regard to tumor angiogenesis and metastasis (81). In human gastric cancer cell lines, IL-18 increased cell migration directly, as well as enhancing VEGF induced migration (82). This study also demonstrated positive feedback between IL-18 and VEGF, with VEGF enhancing IL-18 production in a manner dependent on endoplasmic-reticulum kinase-1/2 (ERK 1/2) phosphorylation.

The diametrically opposing reports of IL-18 effector function in the context of angiogenesis are intriguing and it is not clear why or how this can be the case. The overwhelming evidence that IL-1 is pro-angiogenic and the fact that due to their shared processing via caspase-1 cleavage, IL-18 and IL-1 tend to be found together may provide some context to speculate for instance that the balance of molar concentrations of IL-18 and IL-1 *in vivo* may influence the environment such that the ability of IL-18 comes down in favor of an anti-angiogenic signal vs. a pro-angiogenic signal. Alternatively, the timing of IL-18 signaling may be critical, or there may be a threshold of relative abundance of pro-angiogenic signals that IL-18 cannot overcome. These remain conjecture; however, one thing that is clear is that further research into the mechanisms of action of IL-18 in mediating angiogenesis and the relative contributions of macrophage vs. endothelial cell signals are needed in order to help understand how this cytokine can promote both pro-and anti-angiogenic signals.

IL-33 IN THE CONTEXT OF NEOVASCULAR REGULATION

IL-33 is an IL-1 family member, widely expressed in a number of different tissues, that signals via the ST-2/IL-33 receptor and IL-1RAcP complex and induces T-helper type-2 (Th2) associated cytokines (83, 84). IL-33 is expressed in a wide variety of tissues, including stomach, lung, central nervous system (CNS) and skin, however in these tissues it appears to be confined to endothelial, smooth muscle and epithelial cells (83). The method by which IL-33 is activated is a matter of some debate; originally, like IL-1 and IL-18, it was thought to be cleaved by caspase-1 for activation, however others have suggested that secreted full length IL-33 is itself active and that the caspase-1 cleavage results in the inactivation of the cytokine (85). IL-33 can however, be processed by various serine proteases, into a more biologically active mature form (86, 87). It has also been reported that IL-33 can behave as a nuclear factor; it is the same molecule that was originally called nuclear factor high endothelial venules (NF-HEV), and *in vivo* it associates with heterochromatin and has potent transcriptional repressor properties (88). In EC's, nuclear IL-33 is a marker of quiescence, which is lost in a Notch dependent manner during angiogenesis (89).

On a mechanistic level, similar to the other IL-1 family members, it has not been clearly delineated how soluble IL-33 exerts its effects on angiogenesis. The majority of reports indicate that IL-33 promotes a pro-angiogenic phenotype, however others report that IL-33 signaling has an anti-angiogenic function. Urokinase-type plasminogen activator (u-PA) plays a pivotal role in extracellular proteolysis, and is thought to be involved in the modulation of angiogenesis. IL-33 can upregulate u-PA at both mRNA and protein level in human coronary artery cells and human umbilical vain endothelial cells (HUVEC) in a time- and concentration-dependant manner (90). However, it has yet to be explored whether blocking this function of IL-33 alters its ability to drive angiogenesis in EC.

As a Th2 promoting cytokine, IL-33 has been implicated in asthma pathogenesis. Angiogenesis is a feature of airway remodeling in asthma, and it has been demonstrated that IL-33 can increase the expression of blood vessel von Willebrand factor (vWF), as well as angiogenic factors such as angiogenin when administered nasally to a murine asthma surrogate model (91). IL-33 has also been shown to induce microvessel formation by human EC *in vitro* in a concentration dependent manner (91). In diabetic mice, IL-33 was found to improve wound healing and re-epithelization of skin wounds by promoting new ECM deposition and neovascularisation, and was capable of inducing expression of VEGF and vWF (92). In a similar manner, hypoxic human pulmonary artery endothelial cells (HPAEC), increase s IL-33/ST-2 expression and in this cell type IL-33 enhances proliferation, adhesiveness and spontaneous angiogenesis. This process is dependent on HIF-1α, the aforementioned VEGF transcription factor, as when HIF-1α is knocked down, IL-33 does not mediate its pro-angiogenic effects on HPAEC (93). This report implies IL-33 may be mediating angiogenesis by upregulating VEGF expression through HIF-1α. Nitric oxide (NO) production in EC is transiently regulated by VEGF and Ang-1, in turn NO can modulate the angiogenic function of these factors. It has been shown that IL-33 can activate the PI3K/Akt/endothelial nitric oxide synthase (eNOS) signaling cascade in a TRAF-6 dependent manner; however, the same study demonstrated that despite IL-33's pro-angiogenic properties, it did not stimulate the levels of VEGF in the manner of IL-1β (94).

Like IL-1 and IL-18 there are some reports that demonstrate IL-33 acting as a pro-angiogenic mediator in tumors. Tumor derived IL-33 is capable of inducing tumor angiogenesis by activating EC (95). Currently, the pervading opinion is that IL-33, like IL-18, can have both pro- and anti-tumorigenic functions, as reviewed by Fournie et al. (96) and Brint et al. in this special topic.

One context where IL-33 appears to have anti-angiogenic properties, like IL-18, is in the eye. IL-33 and ST-2 are expressed constitutively in human and murine retina and choroid tissues. Like IL-18, IL-33 has been shown to protect against angiogenesis in the eye. IL-33 appears to regulate tissue remodeling inhibiting CNV formation in an ST-2 dependent manner when injected intravitreally into mouse eyes (97). To date, in contrast to IL-1, IL-33 is not a target in the clinic for diseases with dysregulated angiogenesis (clinicaltrials.gov).

IL-36, IL-37, AND IL-38—THE STORY EMERGING SO FAR

There are three IL-36 isoforms—IL-36α, IL-36β, and IL-36γ, which signal through the IL-36R and IL-1RAcP complex. As with other IL-1 family members, IL-36 cytokines require cleavage for activation, however this is not performed by caspase-1. Instead the IL-36 cytokines are cleaved by the neutrophil proteases cathepsin G and elastase (7), other reports also indicate that cathepsin-S can cleave the IL-36γ isoform (8). To date, IL-36 has been widely studied in psoriasis, where its proinflammatory effects are strongly associated with disease pathology. Psoriasis lesions show a developed vascular network, with wide, leaky capillaries, and it has been demonstrated that IL-36γ, which is commonly found at high levels in these lesions, can activate both HUVEC and HMVEC-d (98, 99). IL-36γ enhanced angiogenesis in these HUVECs in a VEGF-dependent manner, and conditioned media from macrophages incubated with IL-36γ could activate EC and induce ICAM-1 expression (100). These early studies suggest that at least in psoriasis IL-36γ is acting as a pro-angiogenic mediator. IL-36γ is a recognized T-bet target in myeloid cells, and T-bet can instigate decreases in tumor growth by promoting dendritic cell (DC)-mediated ectopic lymphoid organogenesis in a manner that is dependent on IL-36γ (101, 102). There is the mounting evidence that suggests IL-36 cytokines can act as tumor suppressors, although it is yet to be deciphered whether this function has any connection to angiogenic regulation (103, 104).

IL-37 is considered to be an anti-inflammatory cytokine, as it has the capability to suppress MyD88-dependent inflammatory cytokine expression (105–107). Currently, IL-37 is thought to signal through the IL-18R and a yet unidentified accessory protein (108). IL-37 can act as an intracellular cytokine; it can translocate to the nucleus and interact with SMAD family member-3 (SMAD3) to exert anti-inflammatory function (109). IL-37 has the ability to bind TGF-β, which is a pivotal regulator of both developmental and patho-physiological angiogenesis (110). When IL-37 binds to TGF-β, it enhances binding of IL-37 to the activin receptor-like kinase 1 (ALK1) receptor complex and allows IL-37 to signal through ALK1 to activate pro-angiogenic responses (111). IL-37 is expressed and secreted in EC and upregulated under hypoxic conditions, where it enhances cell proliferation, capillary formation, migration, and vessel sprouting from aortic rings with potency comparable to that of VEGF (112). Interestingly, it appears that IL-37's ability to induce the tube formation characteristic of angiogenesis is strongly dose-dependent. Lower doses of 1 and 10 ng/ml could potently drive tube formation in HUVEC, along with other angiogenic characteristics including proliferation and endothelial migration, but little change occurs upon the higher 100 ng/ml dose, which interestingly was reported by another group to suppress tube formation in the same cell type (112, 113).

IL-37, like many of the other IL-1 family members, has been found in higher levels in serum of RA patients vs. healthy control, and the levels are also higher in patients with more advanced disease (114). It has not been decisively established what function this IL-37 plays yet, but it is worth noting that the other IL-1 family cytokines are known to be pro-angiogenic mediators in RA. Due to IL-37's role as an anti-inflammatory cytokine there is speculation that this elevation may be trying to counter the proinflammatory cytokine profile seen in RA.

IL-38 is a relatively novel IL-1 family member, that signals through the IL-36R and has been shown to have anti-inflammatory properties. In a murine model of oxygen induced retinopathy, treatment with IL-38 reduced the size of neovascular regions, indicating the attenuation of angiogenic processes *in vivo* (115). IL-38 was also shown to attenuate proliferation, migration, and tube formation of EC in a dose dependent manner. IL-38 is strongly expressed in synovial tissues from RA patients, and as IL-38 KO mice show greater RA severity in auto-antibody induced RA, it is tempting to suggest that IL-38 acts as an inhibitor of RA pathogenesis (116).

REGULATION OF VASCULAR PERMEABILITY BY IL-1 FAMILY CYTOKINES

The vascular endothelium forms a barrier that regulates the flow of molecules, as well as leukocyte, entry into tissues. To regulate vascular permeability is to regulate the cell-cell junctions between EC. This cell-cell junction has several complexes that regulate and contribute to its integrity, including tight junctions and adherens junctions. Molecules like VEGF can regulate vascular permeability by promoting signaling cascades that alter the composition of the junction. However, it is note-worthy that not all mediators of angiogenesis are also able to alter vascular permeability, and indeed there are well-recognized examples of pro-angiogenic mediators acting as anti-permeability factors, thereby helping to improve the vascular integrity, as is the case for Ang-1 (117). It is well-established that IL-1 family cytokines can alter the permeability of vasculature, and, as is the case with angiogenesis, the different IL-1 family cytokines vary in their functionality, with some members displaying tissue dependent effects.

IL-1 IS A POTENT INDUCER OF VASCULAR PERMEABILITY

With respect to IL-1, it appears that despite IL-1α inducing increases in permeability (118, 119), this isoform has slighter effects than IL-1β, at least *in vitro* (120). One of the mechanisms by which IL-1β has been shown to alter the permeability of the vasculature is by regulating the expression of cell-cell junction components. Of note, treatment with IL-1β can result in the loss of β-catenin and VE-cadherin specifically at the endothelial cell border, which results in small holes and gaps forming between cells (121). IL-1β can also induce vascular permeability indirectly through induction of pro-permeability factor R-spondin 3 (RSPO3) (121). Combined treatment of IL-1β and RSPO3 can synergistically increase permeability in human coronary artery endothelial cells (HCAEC), HPAEC, human cerebral microvascular endothelial cells (HCMVEC),

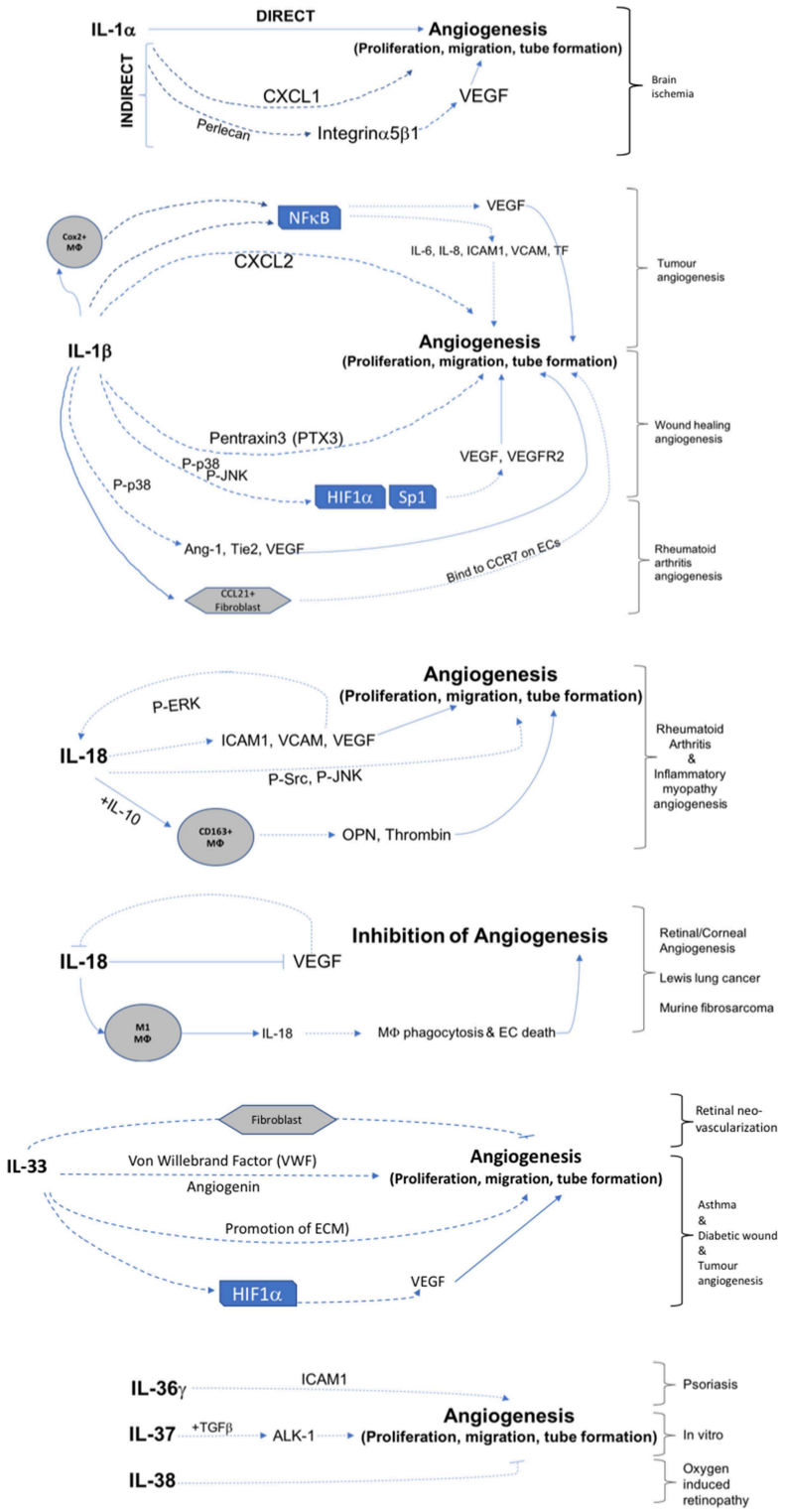

FIGURE 2 | Graphical representation of IL-1F cytokine regulation of angiogenesis. IL-1 F cytokines regulate angiogenesis either by promotion of proliferation, migration, and tube formation or by inhibiting these steps. Direct mechanisms are depicted by solid arrows, indirect mechanisms are depicted by broken arrows. Brackets on the right hand side of the figure indicate mechanisms associated with specific angiogenic diseases. Transcription factors are shown in boxes; *P*, indicates phosphorylation event; Mϕ, macrophage.

human brain microvascular endothelial cells (HBMVEC), and HMVECd (122).

The largest body of evidence characterizing IL-1's role in regulating vascular permeability comes from literature on the blood-brain barrier (BBB). The endothelium and the associated astrocytes control the balance between barrier stability and permeability via production of factors that regulate vessel plasticity (123) and recombinant IL-1β increases the permeability of the BBB in rats when injected intracranially (124). Interestingly, evidence that suggests IL-1β injection into the striatum of juvenile brains results in a neutrophil dependent increase in BBB permeability, but this effect is not recapitulated in mature animals (125). This study demonstrated that in the juvenile animals the blood vessels that recruited neutrophils displayed loss of the tight junction proteins occludin and zonula occludens-1 (ZO-1) and redistribution of vinculin, an adherens junction component involved in the linkage of integrin adhesion molecules to the actin cytoskeleton (125). Electron microscopy further demonstrated that cell-cell adhesions in these cells are morphologically different to control brain endothelial junctions, implying that IL-1β mediated leukocyte recruitment can result in junction disorganization and BBB breakdown. Additionally, IL-1β intracerebral administration in adult rats induces meningitis, however in juvenile rats that are between 2 and 6 weeks old IL-1β has the secondary effect of increasing BBB permeability (126). Chemokines, including cytokine-induced neutrophil chemoattractant (CINC-1), are induced following the intracerebral administration of IL-1β, and this induction produces a far more intense neutrophil response in juvenile rats compared to their adult counterparts (127). The IL-1β mediated breakdown in BBB integrity in juvenile rats could be attenuated by using a CINC-1 neutralizing antibody, indicating that the difference in response between adult and juvenile mammals may be due to differences in sensitivity to IL-1β upregulated CXC chemokines. The finding that these processes are applicable only to juvenile animals is reflective perhaps of the fact that children are more inclined to encounter permanent damage and mortality than adults after trauma or inflammation in the brain.

In encephalitis, parenchymal brain inflammation, the ratio of IL-1Ra to IL-1β in the cerebrospinal fluid can be indicative of patient outcomes, with a higher ratio of IL-1β to IL-1Ra indicative of poor patient outcome, and associated with reduced integrity of the BBB (128). Loss of BBB integrity is also an early significant event in Multiple Sclerosis (MS) and has been proposed through microarray analysis to induce expression of HIF-1α and VEGF-A in astrocytes, as well as potently downregulating an important maturation and stability factor for BBB integrity gravin/src-suppressed C-kinase substrate (SSeCKs) (129). In co-cultures of astrocytes and HBMVEC, endothelin-1 (ET-1) could induce IL-1β in astrocytes, and this IL-1β was demonstrated to be the effector of ET-1 induced BBB permeability (130).

In a manner akin to its effect on the BBB, IL-1β can alter the permeability of the blood-retina-barrier (BRB), by negatively regulating the tight junctions of the retinal vascular endothelium (131). Mice exposed to retinal cryopexy, followed by intraperitoneal administration of [^{3}H]mannitol allowed radioactive leakage to be compared between the retina and the lung/kidney (132). This technique demonstrated that IL-1β caused relatively rapid breakdown of the BRB, and that this breakdown in barrier integrity was more prolonged in nature than that induced by VEGF alone. In a transgenic mouse model, where IL-1β was overexpressed in the lens, there was an early peak of VEGF at P5-P7, which was determined to be IL-1β dependent and coincided with the onset of BRB breakdown, which suggests that similar to angiogenesis, IL-1β can exert some

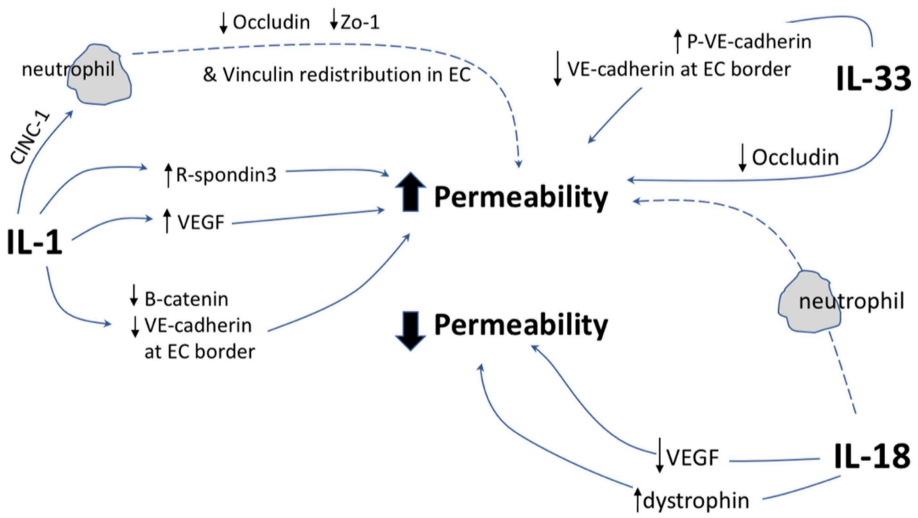

FIGURE 3 | Graphical representation of IL-1F cytokine regulation of vascular permeability. IL-1 F cytokines regulate vascular permeability, direct mechanisms are depicted by solid arrows, indirect mechanisms are depicted by broken arrows. EC, endothelial cell; P, indicates phosphorylation event. ↑ indicates upregulation of protein; ↓ indicates down regulation of protein.

of its regulatory effects on vascular permeability by inducing VEGF expression (133).

In addition to its effects in brain and retinal endothelium, IL-1β induces lung vascular permeability in an integrin αvβ6 dependent manner in a lung injury model (134) and contributes to significant increases in the vascular permeability in dengue, the most virulent haemorrhagic disease worldwide (135). Interestingly though, the potent effect of IL-1β on barrier integrity is not ubiquitous to all endothelial cell types. The glomerular capillaries differ from most endothelium types due to their formation of large fenestrated areas that comprise 20–50% of the entire endothelial surface; the result of this is that human renal glomerular endothelial cells (HRGEC) are naturally more permeable than other endothelial cell types. Treatment of HRGEC with IL-1β therefore results in a modest increase in permeability compared to that seen in HUVEC following the same treatment (136). In addition to the wealth of data on IL-1 effects on the BBB and BRB, this may indicate that the primary effect of IL-1 is on regulating the tight junctions and adherens junctions that are not found in fenestrated capillary beds.

REGULATION OF VASCULAR PERMEABILITY BY OTHER IL-1 FAMILY CYTOKINES

As with angiogenesis, the other IL-1 family members have also been shown to have a level of influence over vascular permeability, although for the more novel members of the family—IL-36, IL-37, and IL-38—their effects, if any, remain to be examined. However, there are studies, all be they limited, that demonstrate that IL-18 and IL-33 can regulate vascular permeability effectively.

Like IL-1, IL-33 has also been shown to regulate the permeability of EC, by altering VE-cadherin. IL-33 increased the permeability of HUVEC, as demonstrated in a [^{14}C] sucrose permeability assay *in vitro* and the Miles vascular permeability assay *in vivo*. This increase in permeability coincided with a reduction in the localization of VE-cadherin to the cell-cell junction (94). The authors of this study also demonstrated that IL-33 treatment resulted in the phosphorylation of VE-cadherin in a VEGF-independent manner; VE-cadherin phosphorylation is known to correlate with the loosening of adherens junctions, which is associated with transendothelial permeability. Further studies have demonstrated that IL-33 can also decrease expression of tight junction proteins, such as occludin, in order to decrease the barrier integrity of HUVEC (137).

Similar to its effects on neovascularisation, IL-18 can either promote or restrict permeability in a context dependent manner. IL-18 is upregulated in inflamed lungs, and the mature IL-18 cytokine can be found in bronchoalveolar (BAL) fluid. In a rat model of lung inflammation, caused by deposition of IgG immune complexes, intratracheal administration of IL-18 was shown to significantly increase lung vascular permeability, as well as increasing neutrophil and inflammatory cytokine presence in BAL fluids (138). However, in the retina, IL-18 administration reduces vascular leakage in a spontaneous mouse model of bilateral neovascularisation as measured by fluorescein angiography (75) and likewise in the brain it has been demonstrated that IL-18 can protect against BBB disruption. Status epilepticus (SE) is an epileptic condition in which multiple consecutive epileptic fits occur with no recovery of consciousness between them; resulting in an increase in BBB permeability in limited cerebral regions, this increase in permeability is followed by vasogenic oedema. IL-18 is upregulated following SE in local astrocytes, microglia and macrophages, and it has been shown by infusing recombinant IL-18 into the rat piriform cortex that this IL-18 reduces vasogenic oedema formation, potentially through its upregulation of dystrophin and its possible mediation of BBB permeability (139).

CONCLUDING REMARKS & QUESTIONS FOR FUTURE RESEARCH

This review has demonstrated that IL-1 cytokine family signaling clearly has an influential role in mediation of angiogenesis and vascular permeability during disease processes, see **Figures 2**, **3** for graphical representations. For the original members of the family—IL-1α and IL-1β–their proangiogenic effects have been characterized widely and many attempts have been made to understand the drivers and resulting signaling cascades at a molecular level. In the case of IL-18 and IL-33, although their ability to influence angiogenesis is undeniable, questions remain about how they promote angiogenesis in some tissues and yet inhibit it in others. For the more recently discovered members of the family—IL-36, IL-37, and IL-38—studies are beginning to surface that demonstrate that they too, most likely possess the ability to regulate angiogenesis in a number of tissues, yet it remains to be seen as to whether they have the singular effects of IL-1 or the more nuanced effects observed for IL-18 and IL-33. There is equally clear evidence that demonstrates IL-1 can expansively regulate increased permeability of the vasculature throughout the body. IL-18 and IL-33 have also demonstrated that they can alter the cell-cell junction of EC, thereby altering permeability. Whether IL-36, IL-37, and IL-38 can also exert sway over vascular permeability akin to the other IL-1 family members is unknown and remains to be explored. In conclusion, the IL-1 family have potent and far reaching effects on the regulation of angiogenesis and vascular permeability, that are accomplished through both direct and indirect mechanisms.

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EF wrote the manuscript. SD wrote and edited the manuscript.

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Interleukin-1 Receptor Antagonist Protects Newborn Mice Against Pulmonary Hypertension

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Pulmonary hypertension secondary to bronchopulmonary dysplasia (BPD-PH) represents a major complication of BPD in extremely preterm infants for which there are currently no safe and effective interventions. The abundance of interleukin-1 (IL-1) is strongly correlated with the severity and long-term outcome of BPD infants and we have previously shown that IL-1 receptor antagonist (IL-1Ra) protects against murine BPD; therefore, we hypothesized that IL-1Ra may also be effective against BPD-PH. We employed daily injections of IL-1Ra in a murine model in which BPD/BPD-PH was induced by antenatal LPS and postnatal hyperoxia of 65% O_2. Pups reared in hyperoxia for 28 days exhibited a BPD-PH-like disease accompanied by significant changes in pulmonary vascular morphology: micro-CT revealed an 84% reduction in small vessels (4–5 μm diameter) compared to room air controls; this change was prevented by IL-1Ra. Pulmonary vascular resistance, assessed at day 28 of life by echocardiography using the inversely-related surrogate marker time-to-peak-velocity/right ventricular ejection time (TPV/RVET), increased in hyperoxic mice (0.27 compared to 0.32 in air controls), and fell significantly with daily IL-1Ra treatment (0.31). Importantly, *in vivo* cine-angiography revealed that this protection afforded by IL-1Ra treatment for 28 days is maintained at day 60 of life. Despite an increased abundance of mediators of pulmonary angiogenesis in day 5 lung lysates, namely vascular endothelial growth factor (VEGF) and endothelin-1 (ET-1), no difference was detected in *ex vivo* pulmonary vascular reactivity between air and hyperoxia mice as measured in precision cut lung slices, or by immunohistochemistry in alpha-smooth muscle actin (α-SMA) and endothelin receptor type-A (ET_A) at day 28. Further, on day 28 of life we observed

cardiac fibrosis by Sirius Red staining, which was accompanied by an increase in mRNA expression of galectin-3 and CCL2 (chemokine (C-C motif) ligand 2) in whole hearts of hyperoxic pups, which improved with IL-1Ra. In summary, our findings suggest that daily administration of the anti-inflammatory IL-1Ra prevents the increase in pulmonary vascular resistance and the pulmonary dysangiogenesis of murine BPD-PH, thus pointing to IL-1Ra as a promising candidate for the treatment of both BPD and BPD-PH.

Keywords: pulmonary hypertension, bronchopulmonary dysplasia, interleukin-1 receptor antagonist, pulmonary vascular resistance, neonatal immunity, anti-inflammatory therapy, interventional immunology, preterm infants

INTRODUCTION

Improved survival of preterm infants with gestational ages as low as 23 weeks exposes ever more preterm infants to bronchopulmonary dysplasia (BPD). BPD is a severe inflammatory lung disease that affects up to 15,000 preterm infants each year in the USA (1, 2), with the highest incidence of 35–68% in the 0.5–1 kg birth weight group (3, 4). The diminished lung function of the preterm infant calls for intensive care with mechanical ventilation and oxygen supplementation, which each contribute to the development of the multifactorial pathophysiology of BPD (5) and to health complications that persist into adulthood (6, 7).

Pulmonary hypertension secondary to BPD (BPD-PH) can be considered the gravest complication of BPD as it substantially worsens the prognosis of afflicted infants. BPD-PH can eventually over-tax the right ventricle and limit cardiac output. Occurring in 15–30% of BPD patients (8), the deleterious consequences of BPD-PH are far worse than those of systemic hypertension, with a survival of just 50% 2 years after diagnosis in the severe cases (9).

The risk factors for PH, which overlap with those for BPD, include low gestational age, fetal growth restriction, oligohydramnios, prolonged mechanical ventilation, and oxygen dependency (9, 10). In normal lung development, alveolarization, and vascularization of the distal lung saccules run in parallel (11). BPD disrupts development by reducing the number of alveoli, and therefore the overall volume of lung tissue. In addition, it also causes a significant dysangiogenesis that markedly reduces the cross-sectional area of the pulmonary vascular bed and thereby increases pulmonary arterial blood pressure (9, 12, 13). The ongoing afterload of the pulmonary circulation eventually causes hypertrophy and remodeling of the right ventricle as well as of the walls of the pulmonary vessels, and subsequently a narrowing of the vascular lumen that further limits blood flow (14–16).

Strong evidence implicates inflammation as a key player in BPD and BPD-PH (17–19). Mediators such as VEGF and endothelin-1 are increased and lead to the development of immature and leaky capillaries (20, 21). Recognizing that BPD and BPD-PH are inflammatory diseases, in 2006 the American Academy of Pediatrics issued an urgent call for new anti-inflammatory BPD therapies (22), but with no safe and effective therapy forthcoming, in 2014 the call was renewed (23).

Our research has focused on inhibition of IL-1 as a potential therapy. We previously showed that the endogenous inhibitor of IL-1, interleukin-1 receptor antagonist (IL-1Ra) (24), ameliorates murine BPD induced by perinatal inflammation and hyperoxia (19, 25, 26). To advance the prospects for an anti-inflammatory treatment for pulmonary injury and cardiovascular complications, we extended our earlier study by examining whether the benefit IL-1Ra provides against BPD extends to the pulmonary vasculature and thereby also improves pulmonary vascular resistance, cardiac inflammation and fibrosis. In this study, we confirm that IL-1Ra not only improves alveolar structure (19) but also lung vascularization, thus ameliorating BPD-PH. Hence, IL-1 is a promising target for therapeutic intervention in inflammatory neonatal diseases such as BPD and BPD-PH.

MATERIALS AND METHODS

Murine Model of BPD-PH

We used a previously published two-hit model to induce BPD/BPD-PH in mice pups (19, 26). Briefly, on day 14 of gestation (embryonic day 14; E14) pregnant C57BL/6J mice were given an intra-peritoneal (i.p.) injection of 150 μg/kg of lipopolysaccharide (LPS) to mimic maternal systemic inflammation (e.g., chorioamnionitis). Pups delivered naturally at term (G19-21) when their lungs are at the saccular stage of lung development (27), which is at an equivalent stage to the lungs of extremely preterm human infants (23–29 weeks' gestational age) who are at most risk of developing BPD (28). Our intention was to study the impact of BPD-PH during the alveolar stage of lung development, which spans postnatal day 5 to day 28 (d5-d28) in the mouse, similar to 32 weeks' gestation to 2–3 years of age in the human. Thus, we chose day 28 as the first time-point to assess the pulmonary vasculature and PH in our mice. To assess long-term outcomes, we chose a second time-point of day 60 in mice, which represents ∼20 years in human age (29).

Within 24 h after birth, pups and dams were randomized into treatment groups of daily subcutaneous (s.c.) injections for 28 days of 10 mg/kg IL-1Ra or s.c. injections with an equal volume of saline (vehicle) and then exposed to either gas with a FiO_2 of 0.21 (room air) or 0.65 (hyperoxia) for 28 d, for a total of four experimental groups: air saline, air IL-1Ra, hyperoxia saline, and hyperoxia IL-1Ra. Dams had unlimited access to food and water and were rotated between room air and hyperoxia groups in a 3 day cycle, to reduce effects from exposure to hyperoxia. In

the long-term study group, daily treatment with IL-1Ra ceased at day 28 and animals of all groups were left in room air from day 28 until day 60 of life. Experiments for all timepoints were performed at a temperature of 22°C and humidity of 50–60% and light was cycled in a 12 h day/night rhythm. At day 5 pups were humanely euthanized by decapitation and at day 28 or day 60 by cervical dislocation.

Murine Echocardiography

At 28 d, mice were anesthetized with isoflurane (3% isoflurane mixed with 0.5 L/min 100% O_2 to induce anesthesia and then 1–1.5% isoflurane mixed with 0.5 L/min 100% O_2 to maintain anesthesia). All echocardiography evaluations were performed by a single operator using the Vivid 7 advantage cardiovascular ultrasound system (GE Medical Systems, Milwaukee, WI, USA). 2-D guide M-mode echocardiographic examination of the left ventricle was performed using a 13-MHz linear transducer (i13L probe; General Electric Co) at a sweep speed of 100 mm/s. The mouse was placed on a heated pad and time of anesthesia was <10 min for all animals (30). Time-to-peak-velocity (TPV) expressed relative to right ventricular ejection time (RVET) (TPV/RVET) was calculated as an index of right ventricular (RV) function and pulmonary artery pressure, to which it is inversely correlated. Left ventricular (LV) function was determined by measuring internal diameters at end-diastole and systole (LVIDd, LVIDs) and fractional shortening.

X-Ray Micro-Computed Tomography, Image Acquisition, and Analysis

After cervical dislocation, 28 day lungs were intubated via the trachea and the lungs were fixed with 4% PFA (pH 7.4, instilled at a pressure of 20 cmH_2O). The lung was then removed from the thorax, kept in 4% PFA for a minimum of 24 h, and then stored in 70% ethanol. For *ex vivo* X-ray micro computed tomography (thereafter referred to as CT) the right superior lobe was stained in Lugol's iodine solution (31, 32) for a minimum of 24 h and then washed in 70% ethanol and embedded in agarose for imaging.

CT scans were conducted with the X-ray photon energy tuned to 35 keV using the "Ruby" detector in the 3B enclosure of the Imaging and Medical beamline (IMBL) at the Australian Synchrotron, which was designed and fabricated by the Laboratory for Dynamic Imaging, Division of Biological Engineering, Monash University. During the experiment, the system was tuned to produce 2,560 × 2,160 pixel images giving a field of view of 15 × 12 mm with 6.0 μm pixel size with the measured resolution of 20.1 μm. CT data acquisition for the samples consisted of 1,800 projections over a 180° axis. The scans included 40 images of each background (no sample in the beam) and dark-field (beam is off) contrasts both before and after the sample acquisition. The exposures were 180 ms per projection, and accumulated time taken to scan a single sample was ∼6 min. Each image projection was then processed by subtracting electronic noise and background (sample—dark field/background—dark field) using the median of all 40 frames of the background and dark-field images.

Reconstruction of a vertical series of CT cross-section slices of the samples was then performed utilizing a Filtered Back-Projection algorithm on the processed images with phase retrieval and ring artifact suppression filter as necessary. Processing was performed on the MASSIVE high performance cluster (33) using XLI software (34). Reconstructed slices were stored as 32-bit float-point TIFF images and further converted into 8-bit integer volumes for 3D rendering and analysis. Images were rendered using Drishti (35). The software tools used in the pipeline include several open-source projects: ImageMagick (36) for noise removal, cropping and image format conversion, and CTas (37) for the background and dark-field removal.

Image stacks were saved as 8-bit 3D.tif image stacks and displayed as 3D volumes in Imaris (Bitplane AG) for data quality checks. The image stacks then underwent several pre-processing steps prior to the final branching analysis. Pre-processing of the images was necessary to fill the larger diameter blood vessels so that the Imaris Filament Tracer can detect the vessel inside rather than the vessel membranes. Edge detection of large vessels was performed in Fiji (38) by running two subsequent difference-of-Gaussian (DoG) algorithms with increasing diameter ($r_1 = 2$, $r_2 = 5$), followed by thresholding, binarizing, and despeckling on each plane of the image stack. Subsequently, Matlab (MathWorks Inc.) was used to fill large vessels by further despeckling, skeletonizing, removing skeleton fragments, then detecting the tips of the skeleton, and closing with nearest neighbor algorithm and filling all fully-enclosed areas. The original and the filled image stacks was then loaded in Imaris as two channels, a smoothed surface was created around the filled data set and used to remove potential artifacts of the filling algorithm in the red channel. In a final step, Imaris Filament Tracer was used to detect large vessels ("dendrites") in the filled channel, followed by the detection of small vessels ("spines") in the original image stack (green channel). Overlaying the original volume with the analyzed filament network provided a visual quality check of the branching analysis.

For statistical visualization and analysis of the obtained filament data, multiple data processing steps were performed to ensure comparability of individual lung data sets as well as comparability between different treatment groups. Firstly, for each lung, all detected vessels were grouped into defined ranges of vessel diameters. Detected vessels with <4 μm diameter were discarded from subsequent analysis due to resolution limitations of the applied imaging technique. Absolute number of vessels were binned by diameter size and expressed as percentage of the total number of vessels per lung lobe. To take into account differences between the treatments on vessel number, each group was then normalized to the mean of the total vessel count of the air vehicle group.

Precision Cut Lung Slices (PCLS)

PCLS were prepared from 28 day old mice as previously described, with minor modifications (39). Immediately after euthanasia, mice were dissected to expose the heart and trachea. The right ventricle of the heart was injected with warmed gelatin (∼1 ml, 8% in 1X HBSS/HEPES). The trachea was cannulated with a catheter containing two ports (24G Intima; Becton

Dickinson, North Ryde, NSW, Australia). The lungs were then inflated with warmed agarose (∼1.2 ml, 2% in 1X HBSS/HEPES), followed by a bolus of air (∼0.4 ml). The agarose was solidified in 1X HBSS/HEPES at 4°C for 15 min, and the left lobe isolated and mounted on a vibratome (Compresstome; Precisionary Instruments, Greenville, NC). PCLS (150 μm thickness) were cut and maintained overnight in DMEM containing 1% penicillin–streptomycin solution (37°C, 5% CO_2). PCLS were transferred to 1X HBSS/HEPES and mounted in customized chambers (∼100 μl) where an artery was selected (∼120 μm diameter). Using a gravity-fed system, PCLS were perfused with U46619 (3–1000 nM) or ET-1 (1–100 nM). Arteries were visualized under phase–contrast microscopy (Nikon Eclipse Ti-U [Nikon Instruments Inc., Melville, NY]; Pulnix CCD camera model TM-62EX [Jai, Miyazaki, Japan]). Changes in artery lumen area were captured in the form of digital images (744 × 572 pixels) recorded in time lapse (0.5 Hz) using image acquisition and analysis software (Video Savant; IO Industries, Inc., London, ON, Canada). Images were converted to TIFF files and analyzed in ImageJ using a grayscale threshold to distinguish between the artery lumen and surrounding tissue, with lumen area in each image calculated by pixel summation.

Lung and Heart Preparation, Histology, and Immunohistochemistry (IHC)

After cervical dislocation, 28 day old mice were intubated via the trachea and the lung was inflated and fixed with 4% paraformaldehyde (PFA; pH 7.4, instilled at 20 cmH_2O pressure). Thereafter both the lung and heart were removed, further fixed in 4% PFA for ≥2 h, and processed for paraffin embedding.

Paraffin embedded lungs were cut into 4 μm sections for immunohistochemistry (IHC). Expression of the contractile marker α-smooth muscle actin (α-SMA) or ET_A (endothelin receptor) were detected after antibody incubations by biotinylation with peroxidase. IHC sections were scanned on an Aperio Scanscope (ePathology Solutions) and analyzed by Aperio positive pixel count algorithm as intensity of strong positive staining divided by area (μm^2) (25).

4% PFA-fixed hearts were cut in the mid-coronal plane before paraffin embedding. Heart paraffin blocks were then sectioned at 4μm thickness. Sirius Red staining for tissue collagen was performed as previously described (40). Briefly sections were incubated in 0.1% Sirius Red in saturated picric acid (Sigma-Aldrich) for 15 min, dehydrated and mounted in Depex and subjected to systematic digital analysis of entire sections using ImageJ software. Cardiac interstitial collagen content was quantified as a percentage of total myocardial area, excluding blood vessels. Quantitation was performed by an investigator blinded as to the identity of the samples using particle counting in ImageJ.

Murine Protein Analysis

At day 5, lungs were harvested, washed in ice-cold PBS, snap frozen in liquid nitrogen and stored at −80°C. For analysis, the lungs were homogenized in lysis buffer (41) using an Ultra Turrax homogenizer. The homogenate was centrifuged for 10 min at 14,000 × g and the supernatants were assayed for protein. ELISAs for VEGF-A and endothelin-1 (R&D Systems) were performed according to the manufacturer's instructions. All results were normalized to total protein concentration by using the Pierce BCA Protein Assay (Thermo Fisher Scientific).

Quantitative RT-PCR

At day 28, hearts were removed and washed in PBS and snap frozen in liquid nitrogen and stored at −80°C. Total RNA was isolated from hearts using TriReagent (Sigma-Aldrich Co. MO) and Ambion DNA-free DNA treatment used to remove contaminating DNA following the manufacturer's instructions. RNA was isolated using the RNA Mini Kit (Bioline), quantified with a NanoDrop (ND-100) spectrophotometer (Thermo Fisher Scientific), and assessed to have a 260:280 ratio of ∼2.0. First strand cDNA synthesis was performed using the Life Technologies SuperScriptIII First-Strand Synthesis Kit (Invitrogen, MA). Heart RNA was analyzed by BioMark HD digital PCR (Fluidigm) using TaqMan primer probes listed in **Supplementary Table 1**. Gene expression values were normalized to the most stably expressed housekeeping gene, actin beta (*Actb*), across our samples. Relative expression was quantified using the $\Delta\Delta C_T$ method (42).

In vivo Cine-Angiography and Surgical Preparation

At day 60 of age, mice were imaged via synchrotron radiation. For surgical preparation general anesthesia was induced with pentobarbital (1:10 diluted solution, 60 mg/kg). Subsequently, mice were intubated for artificial ventilation (6 μl/g tidal volume and ∼170–190 breaths/min; AccuVent200 Small Animal Ventilator, Notting Hill Devices, Melbourne, Australia) and the right jugular vein was cannulated with 24-gauge Angiocath (Becton Dickinson, NJ, USA) following modification as previously described by Sonobe et al. (43), so that the tip of the catheter was placed in the right atrium or right ventricle. The right carotid artery was cannulated with a polyurethane catheter (Instech Solomon FunnelCath™ PUFC-C30-10) for arterial blood pressure and heart rate monitoring throughout experiments. Body temperature was maintained at 38°C with a thermostatically controlled heating pad. Anesthesia was maintained via additional intraperitoneal boluses of pentobarbital (20 mg/kg/h). Blood pressure was recorded via a disposable pressure transducer (MLR0699, AD Instruments, NSW, Australia) from the carotid arterial line. The signal was digitized at 1,000 Hz and recorded with CHART software (version 6.0, AD Instruments, NSW, Australia) to obtain mean arterial pressure (MAP) and heart rate (HR). The mouse was taped securely on a thin acrylic board in a supine position during surgery. Following surgical preparation, the board was then set in a vertical position in front of the Ruby X-ray detector.

X-rays at 34 keV (energy bandwidth 25–120 eV) and a flux of 6×10^{11} photons/mm^2/s passed through the mouse chest and were recorded on X-ray detector (Imaging and Medical Beamline, Australian Synchrotron, Melbourne, Victoria, Australia) with a resolution of 16-bit at 30 ms intervals. High-resolution images were stored in a digital frame memory system

with 1,024 × 1,024 pixel format with a 9.9 μm pixel size. Between recordings the X-ray beam was blocked with filters. The pulmonary angiograms were acquired with the long axis of the lung aligned vertically within the X-ray beam with an imaging field of ∼10 × 10 mm. After verification of the placement, contrast agent was remotely injected through the jugular vein catheter as a bolus (90–120 μl over 2 s; Iomeron 350; Bracco-Eisai) using a syringe pump (PHD-2000, Harvard Apparatus, Holliston, MA, USA). Image acquisition was initiated right before iodine contrast injection and ∼100 frames were recorded for each scan whilst ventilation was briefly interrupted at end inspiration to eliminate movement artifacts. After completion of the imaging protocol, animals were terminally anesthetized (pentobarbital, 100 mg/kg i.v).

Image analysis was performed with the use of ImageJ software (44). To eliminate background structure and enhance vessel visibility the frame obtained just before iodine injection was subtracted from frames acquired after injection. Subsequently angiograms were median filtered (2-pixel radius) for clarity. Each vessel was manually marked with a color-coded dot (green-1st

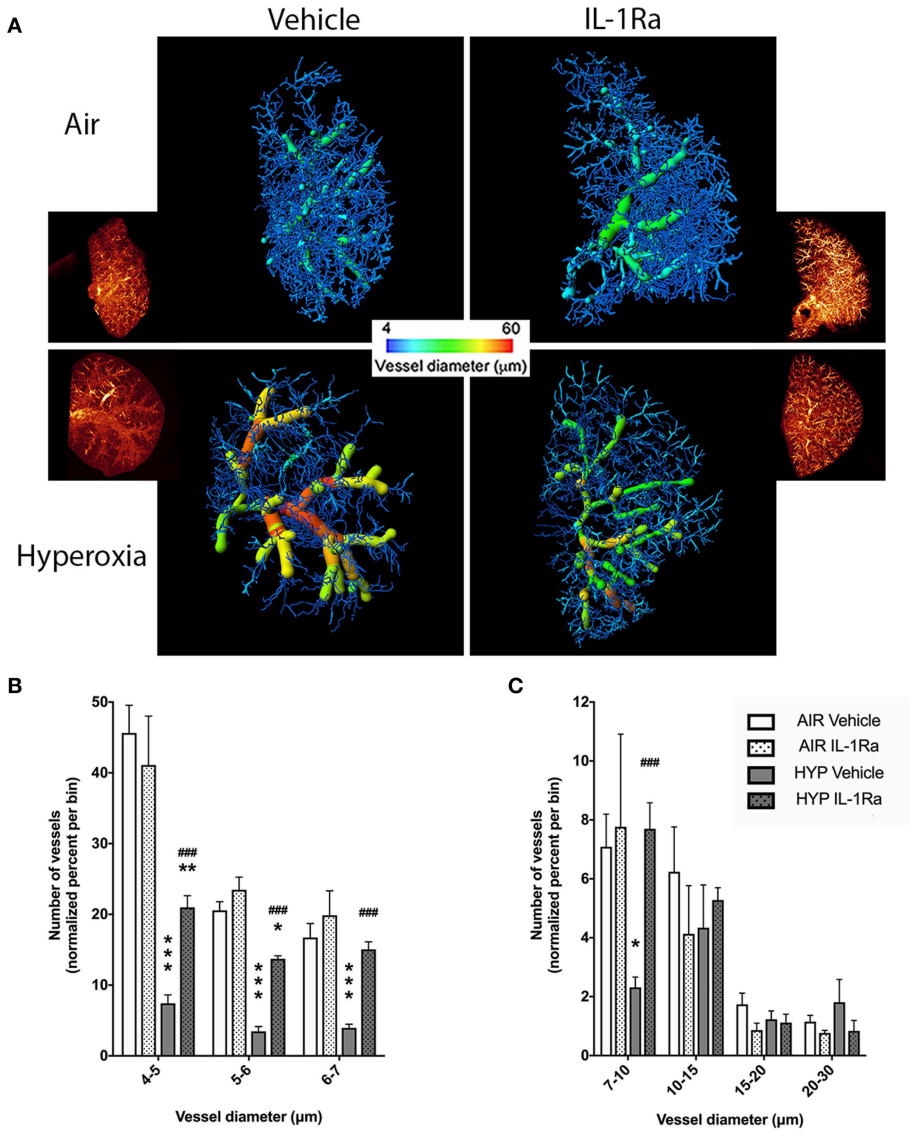

FIGURE 1 | Pulmonary vascular injury caused by perinatal inflammation and postnatal hyperoxia is rescued by IL-1Ra. Pregnant C57BL6/J dams were injected i.p. with LPS at day 14 of gestation. Within 24 h after birth, pups were randomized to either 65% O_2 (hyperoxia) or 21% O_2 (room air). Pups also received daily s.c. injections of IL-1Ra or vehicle. At day 28, lungs were fixed and stained, and micro-CT imaging was performed. **(A)** One representative image of each group is shown; inset shows reconstructed lung volumes and larger image shows the filament filling color-coded for diameter size. Quantification of the number of vessels in the lung grouped by vessel diameter: **(B)** small vessels, 4–7 μm and **(C)** medium vessels, 7–30 μm. Vessel number was normalized to percent of total vessels per bin. Data are shown as mean ± SEM. $n = 3$–10 per group. $^{*}P < 0.05$, $^{**}P < 0.01$, and $^{***}P < 0.001$ for air vehicle vs. hyperoxia vehicle; $^{\#\#\#}P < 0.001$ for hyperoxia vehicle vs. hyperoxia IL-1Ra.

generation, yellow-2nd, and orange-3rd) and all dots were counted automatically by ImageJ.

Statistical Analysis

Data sets (raw data) were first tested for normality and equal variance (P-value to reject = 0.05), then analyzed by GraphPad Prism 7 (GraphPad Software) with one-way analysis of variance (ANOVA) and Tukey's multiple comparisons test. *In vivo* synchrotron angiography and TPV/RVET were analyzed by Student's t-test. Expression of α-SMA and ET_A were measured as strongly positive pixel count/area. For concentration-response curves, contraction was calculated as % reduction in initial artery area, and pEC_{50} and maxima obtained from non-linear regression curve fit using Prism. For all measures, data was expressed as mean ± standard error of the mean (SEM) and considered significantly different when $P < 0.05$.

RESULTS

IL-1Ra Restores the Pulmonary Vascular Structure Impaired by Antenatal LPS and 65% O_2

Our clinically relevant double-hit BPD/BPD-PH model combines perinatal inflammation and 28 days of postnatal hyperoxia which leads to a severe BPD-like lung disease with a pronounced change in lung morphology as seen in BPD infants. Having shown in our model that IL-1Ra prevents the loss of alveoli that is characteristic of BPD (19, 26), the aim of the current study was to determine if blocking inflammation with IL-1Ra for 28 days ameliorates rarefaction of the pulmonary vascular bed in mice with BPD and thereby also reduces pulmonary hypertension. By using high resolution *ex vivo* X-ray micro CT scanning at the Australian Synchrotron, we quantified the number of pulmonary vessels in the right superior pulmonary lobe of day 28 mice (**Figure 1A**). To illustrate the distribution of blood vessel numbers by diameter as well as the relative abundance of vessels, the vessel numbers were converted into percentages binned by diameter, then normalized to the air vehicle group (**Figures 1B,C**). Our results revealed a marked reduction in the number of microvessels in the lung from pups reared in hyperoxia compared to pups housed in room air for 28 days (e.g., −84% for capillaries sized 4–5 μm in diameter, −83% for 5–6 μm, −76% for 6–7 μm; **Figure 1B**). Treatment with IL-1Ra conferred significant protection from BPD-PH-associated loss in the small and medium vessels (**Figures 1A–C**). In summary, our findings suggest that if commenced early in life, daily treatment with IL-1Ra protects the pulmonary vascular bed from the injury induced by perinatal LPS and postnatal hyperoxia.

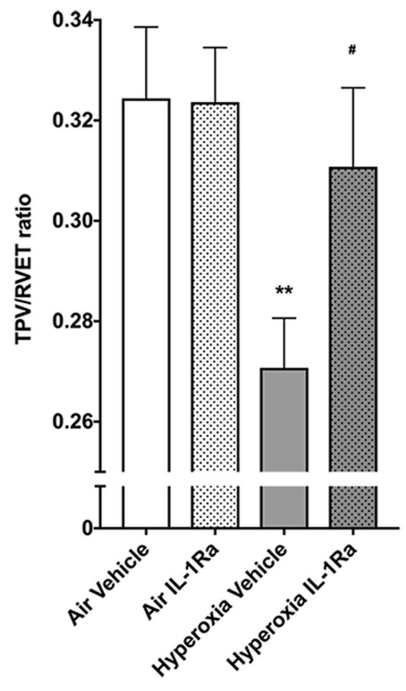

FIGURE 2 | Mice treated with IL-1Ra are protected from the BPD-PH-associated increase in pulmonary vascular resistance. Echocardiography was performed on each of the experimental groups on day 28 of life in the same animals shown in **Figure 1**. TPV/RVET ratio, an index of RV function, was measured; $n = 12–20$ per group. Data are shown as mean ± SEM; $^{**}P < 0.01$ for air vehicle vs. hyperoxia vehicle; $^{\#}P < 0.05$ for hyperoxia vehicle vs. hyperoxia IL-1Ra.

IL-1Ra Improves Pulmonary Vascular Resistance: Physiological Function and Molecular Aspects

The changes we observed in the number of vessels in the pulmonary vascular bed at 28 days of life in BPD mice begged the question whether this tissue injury had functional consequences. Accordingly, we subjected the same mice that subsequently underwent synchrotron analysis to non-invasive echocardiography, the diagnostic tool of choice in preterm infants for diagnosing pulmonary hypertension (45). Specifically, we assessed pulmonary vascular resistance in 28 days-old mice using the surrogate parameter time-to-peak-velocity/right ventricular ejection time (TPV/RVET) ratio, where the TPV/RVET ratio is inversely related to pulmonary vascular resistance. We found a significant reduction in TPV/RVET from 0.32 in pups housed in room air to 0.27 in the hyperoxia control group (**Figure 2**). The reduction in TPV/RVET caused by hyperoxia was completely prevented in hyperoxic pups receiving daily treatment with IL-1Ra, which had a TPV/RVET ratio of 0.31, similar to the room air control pups (**Figure 2**).

We then assessed whether additional mechanisms beyond vascular rarefaction could be contributing to this hyperoxia-induced increase in pulmonary vascular resistance we observed *in vivo*. To determine if pulmonary artery reactivity to vasoconstrictors was increased by hyperoxia, we initially performed *ex vivo* experiments using precision-cut lung slices (PCLS) from either 28 days-old room air or hyperoxia-housed pups, without IL-1Ra treatment. We visualized intrapulmonary artery responses to endothelin-1 (ET-1), a key contractile mediator often elevated in pulmonary

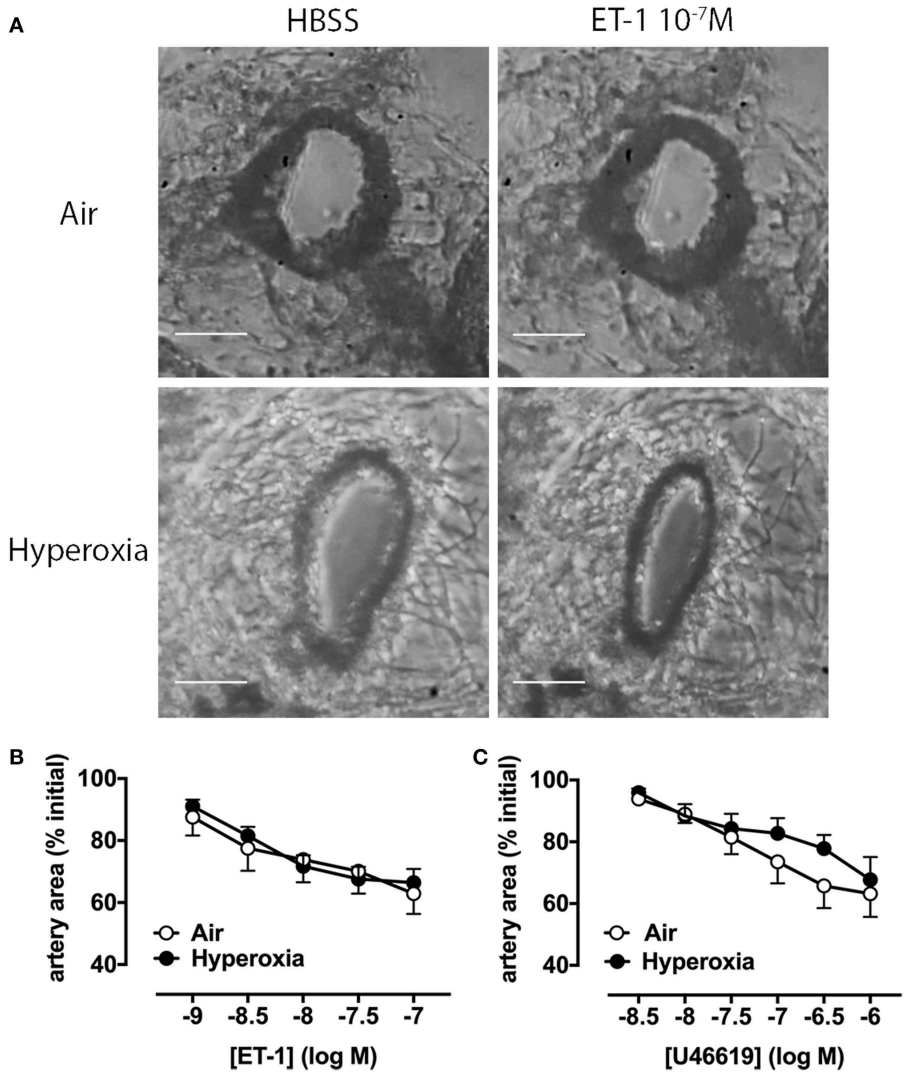

FIGURE 3 | Pulmonary artery contraction in response to ET-1 and U46619 is not altered by hyperoxia in PCLS. Pulmonary artery reactivity was assessed in PCLS prepared from 28 days-old mice after exposure to antenatal LPS and postnatal hyperoxia. **(A)** One representative image per group is depicted, showing an artery during perfusion with HBSS (i.e., vehicle) or ET-1. Concentration-response curves for ET-1 **(B)** and U46619 **(C)** are expressed as percent initial artery area; scale bars are 100 μm. Data are shown as mean ± SEM, $n = 7$–9 per group.

arterial hypertension, and to the thromboxane mimetic U46619 (**Figure 3**).

Representative images show that a maximally effective concentration of ET-1 (10^{-7} M) reduces luminal area by ∼40% in arteries from both air and hyperoxia groups (**Figure 3A**). Averaged data show reductions in artery area during perfusion with increasing ET-1 or U46619 concentrations at 10 min intervals (**Figures 3B,C**). A representative video of PCLS prepared from a hyperoxic mouse, showing intrapulmonary artery constriction to ET-1 (1–100 nM), with increasing concentrations added at 10 min intervals, where 1 s corresponds to 2 min in real time has been provided in the supplementary material (**Supplementary Video 1**; the airway is shown on the left, the artery on the right). In PCLS from air control mice, ET-1 was ∼10-fold more potent than U46619, but both agonists caused a similar maximum % reduction in artery luminal area (ET-1 43 ± 6%, $n = 8$; U46619 38 ± 8%, $n = 8$). Interestingly, neither the potency of either agonist, nor its maximum response, was significantly altered by hyperoxia (**Figures 3B,C**), suggesting that hyperoxia does not alter vascular reactivity.

In addition, we assessed whether hyperoxia altered the abundance of vascular smooth muscle (determined by measuring α-SMA), since an increase in smooth muscle has the potential to augment vasoconstriction and thus pulmonary artery resistance *in vivo*. Given that we observed increases in α-SMA staining in the adjacent airways of pups exposed to hyperoxia (25), we were somewhat surprised to find no

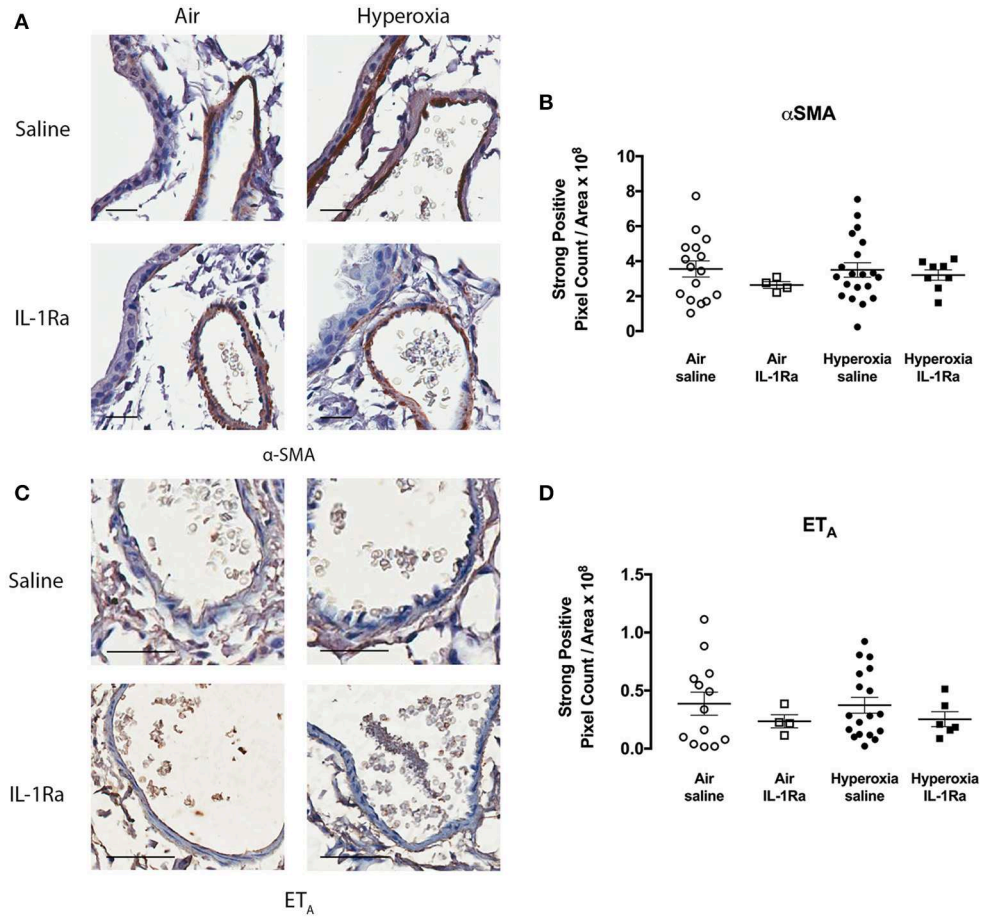

FIGURE 4 | α-SMA and ET_A abundance in pulmonary arteries is not altered following hyperoxia. Immunohistochemistry for α-SMA and ET_A was performed in lung sections from the air vehicle and hyperoxia vehicle groups. Each representative image for α-SMA **(A)** shows an airway on the left and an artery on the right. Scale bars = 100 μm. Representative images for ET_A **(C)** show an artery only. Scale bar = 50 μm. Staining was analyzed using the Aperio positive pixel count algorithm and expressed as intensity of strongly positive staining divided by area (μm^2) for α-SMA **(B)** and ET_A **(D)**. Data are shown as mean ± SEM, n = 13–20 mice per group.

difference in α-SMA staining by immunohistochemistry in the pulmonary arteries between air and hyperoxia groups (**Figures 4A,B**). IL-1Ra treatment also did not alter α-SMA abundance in the pulmonary arteries in both air and hyperoxia, when compared to air vehicle mice. We also investigated the receptor ET_A, which mediates contraction in response to ET-1. No alterations in ET_A abundance on vascular smooth muscle could be observed in the hyperoxia vehicle, and IL-1Ra-treated air and hyperoxia groups, when compared to air vehicle (**Figures 4C,D**).

Perinatal inflammation and postnatal exposure to 65% O_2 resulted in a significant increase in pulmonary vascular resistance *in vivo*, but we found neither an increase in vascular smooth muscle abundance, nor a change in the constrictive response of pulmonary arteries *ex vivo*, nor altered abundance of ET_A in the pulmonary arteries. This being the case, the effects of IL-1Ra treatment on these parameters were not explored further. Instead, we focused on the earlier timepoint of day 5, at which molecular changes occur that affect the development of the pulmonary vascular bed (11).

Murine lungs on experimental day 5 were assessed for protein abundance of vascular endothelial growth factor (VEGF) and ET-1, mediators known to affect pulmonary angiogenesis. We found that exposure to 65% O_2 moderately, but significantly, increased VEGF-A (1.4-fold, **Figure 5A**), the member of the VEGF family with the greatest impact on physiological and pathophysiological functions in the lung (21). Moreover, ET-1 increased 1.4-fold (**Figure 5B**) in hyperoxia vehicle pups compared to room air vehicle pups. Blockade of inflammation by daily injections of IL-1Ra over 5 days prevented the increase in VEGF-A and ET-1 (**Figures 5A,B**).

In summary, daily treatment of BPD mice with IL-Ra improves vascular resistance at day 28 of life, exerts a beneficial effect on early life vascular markers and restores subsequent vascular development.

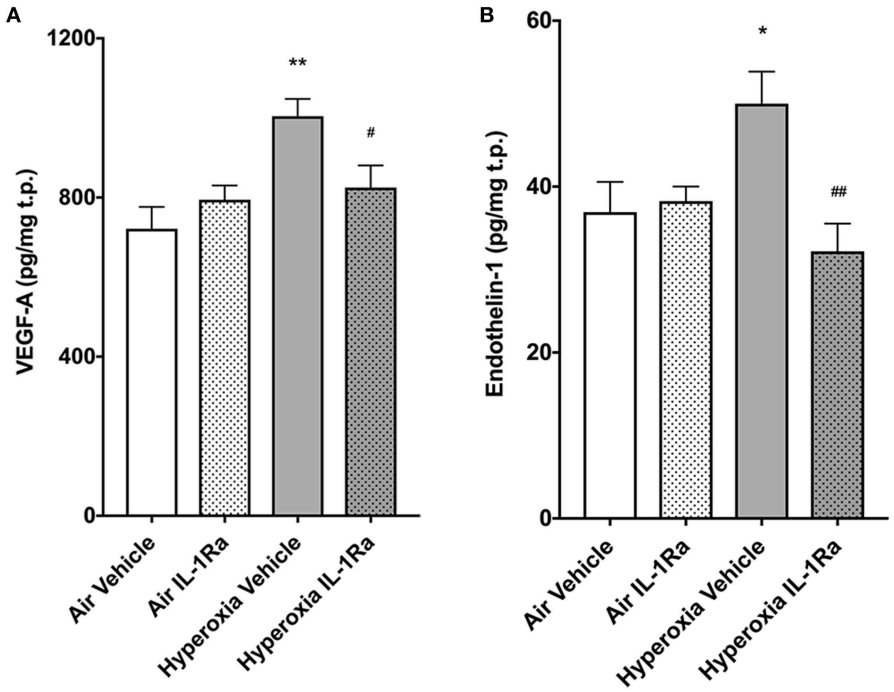

FIGURE 5 | IL-1Ra prevents increases in VEGF and ET-1 on day 5 of BPD-PH. The abundance of VEGF-A **(A)** and ET-1 **(B)** was determined by ELISA in d5 lungs exposed to prenatal LPS and postnatal hyperoxia or room air. Data shown as means normalized to total protein (t.p.) ± SEM. $n = 9$–13 per group; $^{*}P < 0.05$ and $^{**}P < 0.005$ for room air vehicle vs. hyperoxia vehicle; $^{\#}P < 0.05$ and $^{\#\#}P < 0.005$ for hyperoxia vehicle vs. hyperoxia IL-1Ra.

IL-1Ra Protects From Cardiac Inflammation

To evaluate the impact of perinatal LPS and hyperoxia on myocardial remodeling and function, we next assessed left ventricular (LV) function by echocardiography and a set of established markers for cardiac inflammation and fibrosis at 28 days. The left ventricle diameter at the end of diastole (LVIDd) and systole (LVIDs) showed no change in the presence of hyperoxia or IL-1Ra (**Supplementary Figures 1A,B**). Fractional shortening was calculated as a measure of LV function and similarly did not show regulation by either hyperoxia or IL-1Ra (**Supplementary Figure 1C**). Given that inflammation is associated with structural remodeling in many tissues, including the heart, we also investigated cardiac fibrosis, as measured by Sirius Red-stained collagen in whole hearts (46, 47). Fibrosis was significantly, 2.2-fold increased in mice exposed to hyperoxia in comparison with their counterparts reared in room air (**Figures 6A,B**). Daily treatment with IL-1Ra reduced collagen abundance in the hyperoxia group, although this difference failed to reach significance ($P = 0.054$). In addition, we assessed changes in the expression of markers of cardiac inflammation and fibrotic remodeling by RT-PCR. *Lgals3*, which encodes the protein Mac-2/galectin-3, is predominantly expressed by activated macrophages and known to regulate inflammatory and fibrotic responses in the heart (48), was markedly increased (2.9-fold) by exposure to hyperoxia when compared to air vehicle. This increase was reduced by 50% in daily IL-1Ra-treated hyperoxic mice (**Figure 6C**).

Consistent with the data for *Lgals3* expression, the macrophage recruitment chemokine (C-C motif) ligand 2 (CCL2, also called MCP-1) was also, albeit non-significantly, increased 1.8-fold by hyperoxia, and *Ccl2* expression was significantly lower (-59%) in hearts continuously exposed to hyperoxia but treated with IL-1Ra (**Figure 6D**). B-type natriuretic peptide (BNP; gene name *Nppb*) is released by cardiomyocytes in response to stretch caused by increased ventricular blood volume. Although hyperoxia did not increase *Nppb* expression compared to hearts from air-breathing controls, treatment with IL-1Ra reduced *Nppb* expression in both air and hyperoxia (39% decrease compared to hyperoxia vehicle, **Figure 6E**).

Taken together, these data suggest that prenatal inflammation accompanied by postnatal hyperoxia do not modify LV function at day 28 but have a detrimental effect on the myocardium.

Early Life IL-1Ra Treatment Confers Long-Term Benefits on the Pulmonary Vasculature

Next, we investigated whether the beneficial effects of IL-1Ra on the pulmonary vascular bed lasted beyond day 28. A 60 day experimental endpoint was selected, representing early

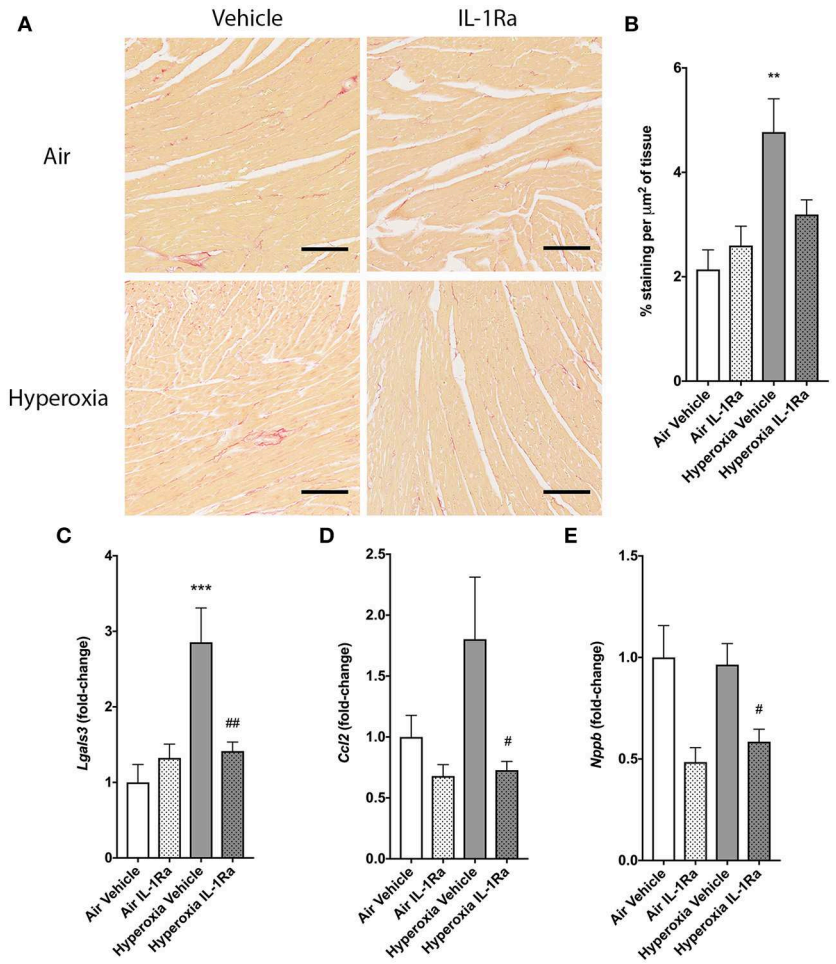

FIGURE 6 | Effects of IL-1Ra on the heart in murine BPD-PH. At day 28 of the BPD-PH model, hearts were stained with Sirius Red and whole heart slices were analyzed. **(A)** One representative image per group is depicted. Scale bars, 100 μm; $n = 7$–10 per group. **(B)** Quantification of Sirius Red staining in $n = 7$–10 pups per group. Data shown as mean ± SEM. **(C–E)** Real-time PCR was performed on whole heart homogenates for **(C)** *Lgals3*, **(D)** *Ccl2* and **(E)** *Nppb*. Results were normalized to *Actb* and depicted as fold-change relative to the lowest expressed gene ± SEM. $^{**}P < 0.01$ and $^{***}P < 0.001$ for air vehicle vs. hyperoxia vehicle; $^{\#}P < 0.05$ and $^{\#\#}P < 0.005$ for hyperoxia vehicle vs. hyperoxia IL-1Ra.

adulthood, i.e., ∼20 years of age, in the human (49). We ceased IL-1Ra treatment on day 28, housed pups of the four experimental groups in normal husbandry conditions until day 60, then performed *in vivo* cine-angiography at day 60. We observed that the vascular development was substantially compromised in hyperoxia vehicle adult mice when compared to air vehicle mice (**Figure 7A**). A video of the live *in vivo* cine-angiography for a representative hyperoxia vehicle mouse at day 60 is provided as supplementary material (**Supplementary Video 2**; the video first shows the cine-angiography in real time, then replayed at one third of the speed). Early life treatment with IL-1Ra ameliorated this vascular growth arrest: the green square in **Figure 7A** highlights a well-developed distal vascular region of a 60 days-old mouse treated daily with IL-1Ra for 28 days, which compares favorably to the red square, indicating a region with poor perfusion of distal blood vessels in a representative hyperoxia vehicle pup. Quantification of the overall visible vessel number (**Supplementary Figure 2**) revealed that in each branching generation vessel numbers were markedly reduced in the hyperoxia vehicle group (−18% in generation 2 to −21% in generation 3, **Figure 7B**). IL-1Ra rescued the number of pulmonary blood vessels when compared to vehicle-treated hyperoxia pups (**Figure 7B**) in generations 2 and 3 (30 and 48%).

Thus, the pulmonary vascular injury induced by perinatal inflammation and 28 days of hyperoxia in our murine BPD model persists into early adulthood. Importantly, early life treatment with IL-1Ra confers lasting protection after treatment has ceased.

DISCUSSION

Previous work by us (19, 26) and others (50), showed the protective properties of IL-1Ra on alveologenesis in a murine model of BPD. In addition, we also observed that overall number

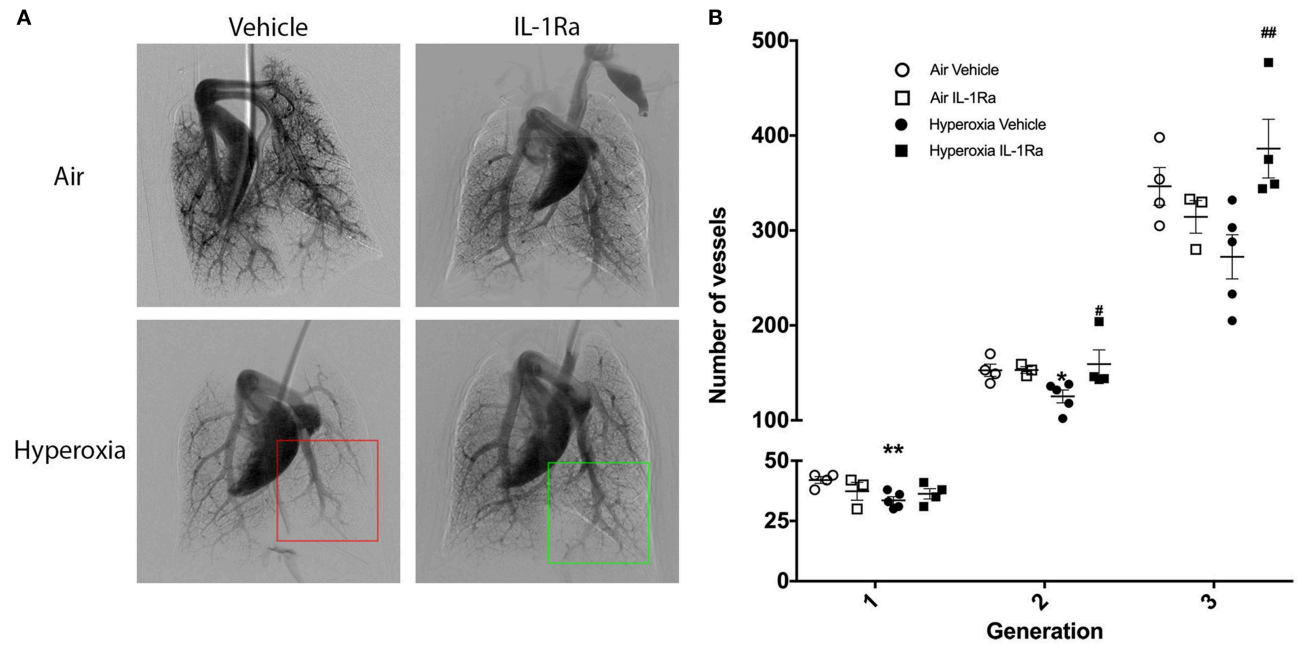

FIGURE 7 | Sustained treatment effects of IL-1Ra on day 60 of life. The pulmonary vasculature of 60 days-old BPD-PH mice was visualized by cine-angiography. **(A)** One representative synchrotron radiation angiogram of the pulmonary vasculature per group is depicted. The red squares highlight poor regional perfusion of distal blood vessels in hyperoxia vehicle mice and the green square shows the amelioration of vascular development afforded by IL-1Ra. **(B)** Quantification of absolute blood vessel number; $n = 3$–5 mice per group. Data are mean ± SEM. $^{*}P < 0.05$ and $^{**}P < 0.005$ for air vehicle vs. hyperoxia vehicle; $^{\#}P < 0.05$ and $^{\#\#}P < 0.005$ for hyperoxia vehicle vs. hyperoxia IL-1Ra.

of $CD45^{+}$ immune cells was up to three times lower in the hyperoxia group when compared to air controls and there was increased activation of macrophages and DCs ($CD11b^{+}$ and $GR1^{+}$ cells); IL-1Ra treatment partially restored these values back to normal. Here, we set out to investigate the effects of perinatal LPS and postnatal hyperoxia on the pulmonary vasculature and the heart of the neonatal mouse, as well as potential beneficial actions of IL-1Ra in preventing the development of BPD-PH (51). The major findings in our preclinical model of BPD-PH are that prophylaxis with IL-1Ra prevents the increase in pulmonary vascular resistance *in vivo* and limits the loss of pulmonary vascular density at both day 28 and 60 of life. Additionally, we revealed that IL-1Ra reduces VEGF and ET-1 at day 5 in the lung. Moreover, we demonstrated that treatment with IL-1Ra reduces the fibrotic effect observed in the heart in BPD-PH.

To the best of our knowledge, our study is the first to demonstrate a beneficial effect of IL-1Ra on vascular resistance *in vivo* in the setting of experimental BPD-PH, as exemplified by echocardiography improving the TPV/RVET ratio. In contrast to the marked changes in RV function that reflect pulmonary vascular dysfunction, LV function (fractional shortening) was not changed at 28 days, highlighting the selective impact of BPD on the RV at this stage of the disease. These data add substantially to the evidence implicating IL-1 in the pathogenesis of PH in animals and humans (52–56). Moreover, studies investigating IL-1Ra as a treatment option for PH are sparse. In an adult rat inflammatory PH model, daily IL-1Ra treatment brings about a significant reduction of PH (57), and neonatal piglets have been shown to be protected from neonatal PH when treated with IL-1Ra (58). A recent single-arm, open label study has reported IL-1Ra successfully treats human adult PH (59).

Exposure to high oxygen concentrations and to mechanical ventilation leads to an early rise in VEGF, followed by a decline in both animals and humans developing BPD (60–64). Prenatal chronic conditional overexpression of VEGF-A in the murine lung increases early life mortality, causes alveolar remodeling, and increases inflammation (65). Based on these studies, we focused our investigation on VEGF-A and found it to be increased on day 5 in murine BPD lungs when compared to air lungs. In IL-1Ra-treated hyperoxic pups, VEGF-A abundance was similar to that in control air pups. Hence, elevated VEGF-A may contribute to the disruption of early life pulmonary vascular development, and IL-1Ra reverses this effect. Our findings of early elevated VEGF moreover resemble neonatal hyperoxia studies in rodents as well as data in human infants, showing that regulation of VEGF isoforms and its receptors in hyperoxia is dependent on timing and oxygen concentrations (60–63).

It is well-known that pro-inflammatory cytokines including IL-1β increase VEGF abundance in a variety of cell types, including lung cells (66–69). Furthermore, several lines of evidence implicate IL-1 in BPD. A study in 1,062 preterm infants (70), as well as smaller studies (71–73), report an association between BPD or death and increased abundance of IL-1β. Additionally, IL-1Ra modulates endothelial cell

(EC) proliferation (74) and thereby might contribute to EC protection, re-growth and vessel healing after vascular injury. In more general terms, increased abundance of IL-1 in early lung development induces pulmonary VEGF production and secretion, thereby promoting dysangiogenesis. Our findings suggest that vascular remodeling can be prevented by immediate IL-1Ra treatment after birth, just as we reported previously for alveolar changes in BPD (26).

ET-1 plays an important role in maintaining normal pulmonary circulation in early life and is known to be elevated by inflammation and hyperoxia in lung and plasma (75, 76) in rodent models of disease. ET-1 also increases microvascular permeability *ex vivo* in rat lungs (77). Abnormally elevated ET-1 has been studied in plasma and tracheobronchial aspirates of infants suffering from BPD-PH for more than two decades (20, 78, 79). These findings are consistent with our data showing increased ET-1 protein in day 5 lungs exposed to antenatal inflammation and postnatal hyperoxia. Here, we show that IL-1Ra treatment restored ET-1 in lung lysates back to air control levels. Since ET-1 is known to be increased directly and indirectly via IL-1 (80), we infer that one of the mechanisms of IL-1Ra function is to block the induction of ET-1 by IL-1. Considering the data available, we conclude that IL-1Ra protects newborn mice from the dysangiogenesis promoted by ET-1 and VEGF-A immediately after birth.

We assessed the involvement of increased vascular reactivity in BPD-PH by measuring the reactivity of intrapulmonary arteries *ex vivo* using PCLS from pups on day 28 of life. We have previously used this approach to establish that airway contraction in response to methacholine *ex vivo* is increased in PCLS from the same model (25). However, although in the current study vascular density and pulmonary vascular resistance were altered by hyperoxia *in vivo*, there was no difference in *ex vivo* vasoconstriction to ET-1 or U46619. Consistent with these findings, the abundance of ET_A receptors that mediate the effects of ET-1 on vascular tone was not altered, and α-SMA, a marker of remodeling of the pulmonary arteries associated with increased constriction, was not elevated. Other studies have shown that isolated pulmonary arteries from BPD rats exhibit increased vasoreactivity to U46619 (81) and in a 14 day BPD rat model increased vasoconstriction was associated with PH (82). It is possible that the absence of an increase in arterial α-SMA in hyperoxic pulmonary arteries in our mouse model could explain these discrepancies. However, increased α-SMA associated with vascular smooth muscle thickening in pulmonary arteries of hyperoxic mice was also reported in a study which had applied 85% O_2 (83). We have shown that 85% O_2 represents a more severe model of BPD (19) when compared to the 65% O_2 we applied here; thus, a lower O_2 level could account for the absence of *ex vivo* vascular reactivity and markers of vascular remodeling in our study.

The molecular mechanisms associated with right ventricular dysfunction and failure in BPD-PH are poorly understood, and therapeutic options are limited. Hence, we also investigated effects of our BPD model on tissue remodeling processes in the heart. Despite no change in LV function or dimensions being detected, our data show that exposure to LPS and hyperoxia induces tissue fibrosis and that IL-1Ra reversed these changes, indicating an important contribution by IL-1 to the fibrotic process (84). *Lgals3* and *Ccl2* have both been implicated in a variety of processes associated with heart failure, including myofibroblast proliferation, fibrogenesis, tissue repair, inflammation, and ventricular remodeling (85, 86), and both were therefore investigated in our model. Our mechanistic studies indicate that *Lgals3* and Ccl2 were both induced by hyperoxia and significantly reduced by daily treatment with IL-1Ra, pointing to an IL-1-dependent mechanism. These data support the concept that neonatal hearts share some common inflammatory pathways with adult hearts to promote cardiac tissue remodeling (87).

Our data showed that cardiac *Nppb* expression was not increased by hyperoxia, but IL-1Ra was able to reduce mRNA expression in both air and hyperoxia. Given that there were no changes in cardiac dimensions in any group, as we measured by echocardiography, our data suggest that IL-1Ra treatment might only reverse LPS-induced expression of *Nppb* in the 28 day heart as all groups were exposed to antenatal inflammation at E14. It is important to note that whole hearts were used for analysis and therefore potential differences between the left and right ventricle could not be detected. However, inflammatory mediators can induce BNP mRNA expression (*Nppb*). Binding of LPS to its receptor and stimulation of downstream pathways, including p38MAPK activation and induction of RAC1 and GATA elements, results in transactivation of the BNP promoter (88). Other studies report that TNF and IL-1β can selectively stimulate BNP at the transcriptional and translational levels in cardiomyocytes (89). Therefore, we can conclude that IL-1Ra treatment improves antenatal LPS induced long term *Nppb* expression and potential hemodynamic changes in the 28 day old heart.

In summary, previous studies and our own results point to the conclusion that decreases in vascular density and therefore an increase in vascular resistance, rather than changes in vascular reactivity, could be the cause of BPD-PH. This finding could explain why treatment of BPD-PH with vasodilators has shown limited efficacy in preventing BPD and/or BPD-PH in human infants (90), and therapeutic efforts to restore the vascular density of the lung should also be considered. IL-1Ra exerts beneficial effects by blocking IL-1 and indirectly by inhibiting VEGF and ET-1, thereby improving pulmonary vascular density and pulmonary vascular resistance. Although further research is needed to prove these hypotheses, we suggest that IL-1Ra could represent a supportive therapeutic to restore pulmonary homeostasis in neonatal cardiopulmonary disease.

DATA AVAILABILITY

All datasets generated for this study are included in the manuscript and/or the **Supplementary Files**.

ETHICS STATEMENT

The animal studies, imaging experiments, and protocols were carried out in accordance with the recommendations and approvals of the Animal Review Board MMCA of Monash University and the Australian Synchrotron animal ethics committee.

AUTHOR CONTRIBUTIONS

All authors were involved in drafting the article or revising it critically for important intellectual content, and all authors approved the final version to be published. CB and CN-P had full access to all of the data in the study and take responsibility for the integrity of the data and the accuracy of the data analysis. JP, MN, and CN-P study conception and design. CB, MKo, EL, KE, AS, IR, DS, HT, MKr, SC, AM, JP, and CN-P acquisition of data. CB, MKo, EL, KE, AM, PB, MY, JB, JP, MN, and CN-P analysis and interpretation of data. MC, JB, JP, and CN-P supervision. JB, JP, MN, and CN-P funding acquisition.

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SUPPLEMENTARY MATERIAL

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fimmu.2019.01480/full#supplementary-material

Supplementary Video 1 | Video of PCLS prepared from a hyperoxic mouse, showing intrapulmonary artery constriction to ET-1 (1–100 nM), with increasing concentrations added at 10 min intervals. The airway is shown on the left and the artery is shown on the right. One second corresponds to 2 min in real time.

Supplementary Video 2 | Video of the live *in vivo* cine-angiography for a representative hyperoxia vehicle mouse at day 60. Videos depict cine-angiography in real time and then replayed at one third of the speed.

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REVIEW
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IL-1 Family Members Mediate Cell Death, Inflammation and Angiogenesis in Retinal Degenerative Diseases

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Inflammation underpins and contributes to the pathogenesis of many retinal degenerative diseases. The recruitment and activation of both resident microglia and recruited macrophages, as well as the production of cytokines, are key contributing factors for progressive cell death in these diseases. In particular, the interleukin 1 (IL-1) family consisting of both pro- and anti-inflammatory cytokines has been shown to be pivotal in the mediation of innate immunity and contribute directly to a number of retinal degenerations, including Age-Related Macular Degeneration (AMD), diabetic retinopathy, retinitis pigmentosa, glaucoma, and retinopathy of prematurity (ROP). In this review, we will discuss the role of IL-1 family members and inflammasome signaling in retinal degenerative diseases, piecing together their contribution to retinal disease pathology, and identifying areas of research expansion required to further elucidate their function in the retina.

Keywords: retinal degeneration, IL-1 (interleukin-1), age-related macular degeneration (AMD), inflammation, cytokine, inflammasome, IL-1b, photoreceptor cell death

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IL-1 FAMILY MEMBERS

Introduction to the IL-1 Family

The interleukin-1 (IL-1) family is a central mediator of innate immunity and inflammation [reviewed by Dinarello (1)]. IL-1 family members have been widely associated with both the development and progression of inflammatory diseases, and in particular have been linked to neurodegenerative and neuroinflammatory diseases such as Alzheimer's disease (2–5), stroke (6), cerebral ischemic cell death (7), Multiple Sclerosis (8, 9), Parkinson's disease (10, 11), Down syndrome (3), and retinal degenerative diseases including Age-Related Macular Degeneration (AMD).

The IL-1 family of cytokines has 11 members, which are further subdivided into three groups; the IL-1, IL-18, and IL-36 subfamilies. The IL-1 cytokine subfamily includes agonists (IL-1α, IL-1β, and IL-33) as well as receptor antagonist, IL-1Ra; the IL-18 subfamily comprises agonists IL-18 and IL-37, and the IL-36 subfamily is made up of agonists IL-36α, β, γ, and receptor antagonists IL-36Ra and IL-38 (1). In addition, there are 10 members of the IL-1 receptor (IL-1R) family which are able to bind specific IL-1 ligands in combination with a co-receptor, and perform pro- and anti-inflammatory functions (1).

IL-1β, IL-18, and IL-1α are the most widely researched IL-1 family members associated with retinal degenerative diseases (**Table 1**), having pro-inflammatory actions, and in the case of IL-18, a role in angiogenesis (49, 50). IL-1β and IL-1α are known to exert similar biological effects (51), acting on IL-1R, eliciting pro-inflammatory actions following activation. However, unlike IL-1α which is both constitutively expressed and active in its 31 kDa pro-form, IL-1β is only produced in its inactive 35 kDa pro-form following priming signals, such as pathogen- or damage-associated molecular patterns (PAMPs or DAMPs), and is only subsequently cleaved to its 17 kDa active form following inflammasome activation in damaged or diseased states (1, 2, 52–54). While IL-1α is suggested to act early in inflammation by inducing neutrophil immune cell recruitment, IL-1β is thought to act in the later phase of macrophage recruitment to damaged tissue (51).

Conversely, IL-18, which acts on the IL-18Rα/β receptor, is both constitutively expressed in its pro-form, but cleaved into its active form following inflammasome activation (51). Interestingly, IL-18 has been reported to have both anti- and pro-inflammatory actions, but is also more widely known for its angiogenic roles (49, 50). In addition to known pro-inflammatory activities, IL-1 family members can also participate in anti-inflammatory pathways, with certain IL-1 family members (IL-33 and IL-1α) having dual functions, being able to bind to DNA or the cell membrane receptor and elicit differential effects (1, 46, 55, 56).

In this review, we will discuss the role of IL-1 family members in retinal degenerative diseases, piecing together their contribution to retinal disease pathology, and identifying areas of research expansion required to further elucidate their function in the retina. Furthermore, we will elaborate on some of the mechanisms of IL-1β activity in degeneration, the most highly studied IL-1 family member in the retina.

Inflammation in Retinal Degenerative Diseases

The retina is part of the central nervous system (CNS) and is a specialized sensory tissue lining the posterior surface of the eye. Photoreceptors, specialized light-sensing retinal cells, have the ability to convert light into electrical signals, which are transmitted to the brain via the optic nerve. Both inherited and acquired retinal degenerative diseases can occur when retinal homeostasis is disrupted. This is caused by a combination of genetic mutations (57), the accumulation of reactive oxygen species (ROS) (58), and inflammation in aging (59, 60). The progression of both inherited and acquired retinal degenerative diseases share several features in common, including chronic increases in both oxidative stress and inflammation (59, 61, 62). Increased activation, migration, and recruitment of resident microglia and blood-borne macrophages are characteristic of progressive photoreceptor degeneration in AMD (16, 17, 63–66), diabetic retinopathy (67, 68), retinitis pigmentosa (69–72), glaucoma (73–75), and retinopathy of prematurity (44).

Microglia and macrophages are the primary leukocyte populations found in the retina during disease, and one critical mechanism by which these cells cause damage in retinal degenerations is through activation of the inflammasome. The inflammasome is an oligomer protein complex that leads to the maturation and secretion of two IL-1 family members, IL-1β and IL-18, into the extracellular environment (76). The assembly and activation of the NOD-like receptor pyrin domain-containing 3 (NLRP3) inflammasome, the most well-characterized inflammasome, is stimulated by several mechanisms, including Toll-like receptor (TLR) signaling and purinergic receptor signaling (76), the latter which is activated by extracellular ATP released by dying cells (76, 77). The migration and recruitment of microglia and macrophages is associated with an increased production of chemokines and cytokines, including IL-1β, as well as complement activation, which leads to progressive photoreceptor degeneration [reviewed in Ambati et al. (78) and McMurtrey and Tso (79)].

AGE-RELATED MACULAR DEGENERATION

AMD Disease Pathogenesis

AMD is the leading cause of irreversible blindness in the Western World, primarily affecting the aging population. The estimated prevalence is expected to be 288 million worldwide by 2040, posing a significant global economic burden (80). Although neovascular "wet" AMD currently is treated using anti-vascular endothelial growth factor (VEGF) intravitreal injections to prevent choroidal neovascularisation (CNV) (81, 82), early "dry" AMD and late-stage atrophic dry AMD are currently untreatable. In dry AMD, there is a gradual loss of retinal pigment epithelium (RPE) cells and photoreceptors in the outer retina, leading to the development of a retinal lesion in the specialized macular region, responsible for central high visual acuity, which progressively expands over time (83). The cumulative loss of outer retinal cells and the expansion of the atrophic lesion results in a large drop in visual function in patients with the disease (84). Development of a 2 mm lesion in the foveal region within the macula can result in legal blindness (85).

Immune-based therapies are being explored as the most likely drug candidates for clinical trials, due to the significance of immunological processes in the pathogenesis of AMD. This includes a number of complement system inhibitors to control this major inflammatory pathway such as APL-2 (Apellis Pharmaceuticals) for the treatment of advanced dry AMD (78, 86). Dysregulation of the immune system is critically linked to the development of advanced dry AMD, including the recruitment and activation of resident microglia to the outer retina, and the persistent accumulation of subretinal macrophages recruited from the vasculature, which together are the primary immune cells of the retina under damage conditions (64, 66, 87–91). It has been demonstrated that retinal microglia and macrophages are centrally involved in AMD pathogenesis, including production of various innate immune system components such as complement (65), chemokines (92), and IL-1β (18). This suggests that these microglia and macrophages are major therapeutic targets for

TABLE 1 | Tissue-specific expression of IL-1 family members associated with retinal degenerative diseases.

Name	Receptor	Mechanism	Retinal Degeneration	References	Tissue	Tags per million (TPM)	Confidence (z-score)	Enhanced expression (TPM)
IL-1α	IL-1RI	Pro-inflammatory	Dry AMD DR Glaucoma	(12, 13) (14) (15)	Retina Brain	0 1.1	1 (1.1) 3 (4.4)	Tonsils (22)
IL-1β	IL-1RI	Pro-inflammatory	Dry AMD Wet AMD Glaucoma RP ROP DR	(16–18) (12, 19, 20) (21) (22, 22, 23) (24–26) (27–29)	Retina Brain	3.8 9.7	3 (4.4) 4 (7)	Spleen (78)
IL-18	IL-18Rα	Pro-inflammatory	Wet AMD Dry AMD RP ROP Glaucoma	(30–33) (34–37) (23) (38–40) (41)	Retina Brain	12 12	2 (2.6) 3 (4.1)	*Expressed in all* Esophagus (167)
IL-1Ra	IL-1RI	Antagonist for IL-1α, IL-1β	DR ROP	(27, 28, 42, 43) (39, 44)	Retina Brain	1.7 0.8	N/A 3 (3.8)	Tonsils (1553)
IL-33	ST2	Pro-inflammatory	Dry AMD Wet AMD	(45) (46)	Retina Brain	N/A N/A	N/A N/A	N/A N/A
IL-36α	IL-1Rrp2	Pro-inflammatory	N/A	N/A	Retina Brain	0 0	N/A N/A	Tonsils (391)
IL-36β	IL-1Rrp2	Pro-inflammatory	N/A	N/A	Retina Brain	N/A N/A	N/A N/A	N/A N/A
IL-36γ	IL-1Rrp2	Pro-inflammatory	N/A	N/A	Retina Brain	N/A N/A	N/A N/A	Tonsils (24)
IL-36Ra	IL-1Rrp2	Antagonist for IL-36α, IL-36β, IL-36γ	N/A	N/A	Retina Brain	0 0	N/A N/A	Tonsils (25)
IL-37	Unknown	Anti-inflammatory	DR	(47)	Retina Brain	N/A N/A	N/A N/A	N/A N/A
IL-38	IL-1Rrp2	Anti-inflammatory	ROP	(48)	Retina Brain	N/A N/A	N/A N/A	N/A N/A

Tags Per Million (TPM) provided from the human protein ATLAS, provided by FANTOM5 consortium as part of the human protein Atlas (https://www.proteinatlas.org/). Confidence scores provided from the Tissues Expression database (https://tissues.jensenlab.org/About). Main RNA tissue category (Enhanced Expression) is shown in TPM. "Expressed in all" means all tissues investigated produced that molecule (IL-18).

the treatment of dry AMD, in which progressive atrophic lesion expansion is promoted by activation of these cells.

IL-1α as a Potential Initiator of the Inflammasome in AMD

The release of interleukin-1α (IL-1α), a 31 kDa constitutively expressed member of the IL-1 family, is known to be both inflammasome-dependent and independent (93, 94). However, in a positive feedback loop, IL-1α is also known to prime the assembly of the NLRP3 inflammasome in the retina, with inflammasome priming using IL-1α in RPE cells increasing the damage caused by blue light-induced oxidative stress (95). In this model, the accumulation of lipofuscin, lipid-containing pigment granules that build up during aging, occurs in the photoreceptor outer segments causing oxidative damage (95). IL-1α stimulation altered the cell death profile of damaged RPE cells from apoptosis to pyroptosis, an inflammatory cell death pathway dependent on inflammasome activation (95). Other studies have indicated that IL-1α is a danger signal, or "alarmin," released from stressed or dying RPE cells, leading to the secretion of other pro-inflammatory cytokines from these cells (96, 97). As RPE cell death is central to the progression of AMD, IL-1α has been suggested as a therapeutic target for controlling sterile retinal inflammation. In AMD patients, serum levels of IL-1α amongst other cytokines are significantly higher compared to healthy control patients (12), a trend which was also observed in plasma in a rat model of ischemia/reperfusion injury (13). These data indicate that IL-1α could be biomarker for retinal diseases.

The Role of IL-1β in AMD

Interleukin-1β (IL-1β) is a pro-inflammatory cytokine produced as a 35-kDa precursor, however following inflammasome activation is cleaved by protease enzyme Caspase-1 (CASP1) into a 17-kDa active form (51, 98, 99). Active IL-1β has known roles in initiating and propagating sterile inflammation, including macrophage recruitment (100), activation of the pro-inflammatory cytokine interleukin-6 (IL-6) (101) and modulating chemokine expression (18), which in retinal degenerative diseases such as AMD are characteristic pathogenic features that ultimately result in progressive photoreceptor cell death. Although inflammasome signaling is thought to play both protective and detrimental roles in wet and dry AMD due to the

production of IL-18 (30, 31, 34, 102–106), the synthesis of mature IL-1β is well-established in the pathogenesis of both forms of AMD (19, 107). IL-1β dysfunction has been associated with excessive inflammation in retinal degenerations using animal models (16–18, 20, 70, 108, 109), including those modeling key features of dry AMD.

However, the mechanisms behind inflammasome-mediated production of active IL-1β and the subsequent induction of photoreceptor cell death and retinal damage is unclear. Although IL-1β has not been conclusively linked to dry AMD in human patients (103, 110), the role of IL-1β in AMD pathogenesis has been investigated both *in vivo* in rodent dry AMD models, as well as *in vitro*. An increase in IL-1β was found in the vitreous of rats injected intravitreally with drusen component amyloid beta (Aβ) (111, 112), a toxic peptide aggregate known to accumulate in neurodegenerative diseases such as Alzheimer's disease [reviewed in Murphy and LeVine (113)], as well as in dry AMD (114, 115). In photo-oxidative damage models that mimic several facets of dry AMD pathogenesis (116), it has been found that the gene and protein expression of IL-1β was up-regulated in the photo-oxidative damaged rodent retina (18, 108, 117–119). It was demonstrated that inhibition of IL-1β using both small interfering RNA (siRNA) and a neutralizing antibody was able to ameliorate retinal degeneration, reducing immune cell recruitment to the outer retina, and production of chemokines (*Ccl2*, *Cxcl1*, *Cxcl10*) from both Müller glia and RPE cells, ultimately slowing photoreceptor cell death in retinal degeneration (18).

In addition, others have demonstrated that using a photo-oxidative damage model, cone segment degeneration was correlated with an increased infiltration of IL-1β-expressing mononuclear phagocytes (16). Notably, while mononuclear phagocyte accumulation remained, cone degeneration was abolished following IL-1β inhibition (16). Although the mechanism of IL-1β-dependent photoreceptor cell death is unclear, both photoreceptor degeneration and the associated presence of accumulated mononuclear phagocytes has been reported in dry AMD by the same group (16, 17, 63).

In contrast to most recent literature, previous studies have reported a dose dependent effect of IL-1β, with low doses (5 μg/ml) conferring retinal protection and photoreceptor rescue in The Royal College of Surgeons (RCS) rats (120, 121), a strain with inherited retinal degeneration (122). It is possible that a high dose of IL-1β provides an additional priming signal via the IL-1R signaling axis to amplify the expression of inflammasome components, inducing assembly of the inflammasome and perpetuating photoreceptor cell death. Further investigation into mechanisms by which IL-1β causes photoreceptor cell death may shed light on the protective vs. detrimental roles of IL-1β in the progression of dry AMD and in other retinal degenerations where the gradual inflammatory-mediated loss of photoreceptors is a key feature of disease.

We will further detail the localization and pro-inflammatory functions of IL-1β in retinal degenerations later in this review, focusing on its role in dry AMD pathogenesis.

Role of IL-18 in Angiogenesis and Neovascular AMD

Mature interleukin-18 (IL-18) is a pro-inflammatory cytokine produced through inflammasome activation (98, 99). The role of IL-18 in retinal degenerations including AMD is controversial in the current literature [reviewed in Campbell et al. (49, 50)], with some sources highlighting a detrimental role for IL-18 in the progression of dry AMD (34, 103), whilst there is also evidence of a protective role of IL-18 for wet AMD, in which angiogenesis and neovascularisation contributes to disease progression (30, 31).

The involvement of IL-18 in angiogenesis and neovascularisation has been well-established demonstrating that IL-18 has a role in the formation of blood vessels in the retina (123). It was observed that in early development, IL-18 knockout mice demonstrated abnormal vessel formation and retinal overexpression of angiogenic factors including VEGF (123). A series of studies by Doyle et al. built upon these foundations demonstrating that IL-18 knockout mice had smaller laser-induced CNV volumes than wild type controls (31). Incubation with recombinant IL-18 reduced VEGF expression in immortalized RPE cells (ARPE-19) and brain-derived microvascular endothelial cells (bEnd.3), indicating a mechanism by which smaller CNV volumes in the IL-18 knockout mice may have occurred (31). The follow up studies by Doyle et al. investigated the potential of IL-18 treatments as therapeutics for wet AMD, including in non-human primates, demonstrating its ability to reduce the pathogenic hallmarks of wet AMD (30, 105). A separate study also found that IL-18 displayed protective effects against neovascularisation (32), in which IL-18 levels increased in the eye after treatment with anti-VEGF, and in mice with ischemic/reperfusion (IR) injury, suppression of VEGF caused an increase in IL-18^{+} myeloid cells. Additionally, an injection of IL-18 reduced CNV (32). Another study demonstrated that NLRP3 and IL-1β signaling promoted VEGF-induced CNV formation, and a deficiency in IL-18 had the same effect on CNV lesion development (33). These studies support the findings from Doyle and colleagues in regards to the anti-angiogenic properties of IL-18 (31).

However, in dry AMD, the literature points to a different contribution of IL-18 in retinal degenerations. A study involving dry AMD patients with the complement factor H (CFH) Y402H polymorphism demonstrated that systemic levels of IL-18 were elevated in patients with the at-risk CC variant for the polymorphism, alongside increased systemic IL-1β (35). Ijima et al. also found that serum IL-18 levels in dry AMD patients were higher than age-matched control patients (34). A series of studies using both mouse models and patients with dry AMD demonstrated that *Alu* RNA mediates RPE degeneration through activation of NLRP3 and IL-18 in these cells (36, 103), possibly through activation of Caspase-8 (CASP8) (37). The same group found a critical role for IL-18 in wet AMD (124).

Overall, the literature surrounding the role of IL-18 in AMD indicates a potential dual role of this pro-inflammatory cytokine in modulating retinal damage in

neovascular retinal diseases including wet AMD, which may act differently to its involvement in dry AMD pathogenesis. It is possible that local concentrations of IL-18, like that of IL-1β, determine the protective and detrimental effects of this cytokine in the retina. Further investigations are required to elucidate the mechanisms by which IL-18 may have a role in regulating inflammation in retinal degenerations, including through the induction of interferon-gamma (IFN-γ) (125), a cytokine thought to play a role in AMD (126).

IL-33 and Cytokine Production in AMD

The agonist interleukin-33 (IL-33) is known to have a key role in both innate and adaptive immunity, activating NFκB and MAPK inflammatory signaling pathways and inducing cytokine release [reviewed in Liew et al. (127)]. IL-33 has been shown to be induced upon Aβ stimulation in RPE cells, where it led to a regulation of IL-1β, IL-6, IL-8, and TNFα (128). Other studies have found that IL-33 is upregulated in activated RPE cells in laser-induced CNV, regulating angiogenesis, tissue remodeling and wound healing (46). These studies suggest that IL-33 regulation in the eye may affect critical cytokine signaling pathways involved in retinal degenerations. However, not only RPE cells have been implicated in IL-33 upregulation in retinal damage, with Müller cells also found to produce IL-33 in late-stage dry AMD donor retinas within the lesion area (45). It was shown that IL-33 production by Müller cells was able to induce the expression of other chemokines and cytokines (45), including CCL2, which has been shown to be produced primarily by Müller cells in retinal degenerations to recruit macrophages into the site of damage (129, 130). Together, these studies indicate that IL-33 signaling by RPE and Müller cells may regulate the migration of microglia and the influx of macrophages into the retina following photoreceptor and RPE damage, by influencing cytokine production.

IL-1Ra in RPE Cells

Interleukin-1 receptor antagonist (IL-1Ra) is an anti-inflammatory competitive receptor antagonist that acts to inhibit IL-1α and IL-1β binding to IL-1R preventing their inflammatory activities. Although the role of IL-1Ra has not been established in AMD pathogenesis, several studies have linked the expression of IL-1Ra to RPE cells. In human RPE cells, intracellular and secreted IL-1Ra was detected in both unstimulated and IL-1β-stimulated RPE cell cultures (131). In another study, late passage human RPE cells had an increased level of IL-1Ra compared to early passage cultures, possibly to prevent inflammation whilst under additional stress in culture (132). These authors also found that older (aged 70+) donor eyes had an increased expression of IL-1Ra in the RPE cells compared to younger donor eyes (132). Further, in mouse cultured RPE cells, inhibiting IL-1Ra expression led to a failure to suppress mature dendritic cell activation (133). These studies investigating an anti-inflammatory role for IL-1Ra expressed by RPE cells could have implications for AMD, where RPE dysfunction is a crucial mechanism for onset of retinal degeneration.

DIABETIC RETINOPATHY

DR Disease Pathogenesis

Diabetic retinopathy (DR) is a major complication of diabetes, with loss of vision that arises due to unregulated high blood sugar levels, causing damage to the blood vessels and in most cases results in diabetic macular edema (DME) due to the breakdown of the blood-retinal barrier (BRB) and fluid leakage. DR can progress into two forms; non-proliferative, in which blood vessels leak or become blocked, forming microaneurysms that result in oxygen starvation to areas of the retina; or proliferative diabetic retinopathy (PDR), a more severe form characterized by neovascularisation, retinal scar tissue, and retinal detachment leading to blindness [reviewed in Duh et al. (134)]. While treatment options exist, injections of corticosteroids to reduce neovascularisation and edema are frequent and not without side effects (135). Inflammation has been implicated in the pathogenesis of DR, with increased leukocyte levels and adhesions to the vasculature, activated microglia and increased cytokine levels subsequently resulting in compromised and leaky blood vessels [reviewed in Altmann and Schmidt (68) and Tang and Kern (136)].

Inflammasome Activation in DR

Inflammasome-mediated activation and consequent secretion of IL-1β and IL-18 is thought to play a major role in DR disease pathogenesis, with gene and protein levels of inflammasome components NLRP3, CASP1, ASC, IL-1β, and IL-18 all elevated in the peripheral blood mononuclear cell population in both non-proliferative and PDR patients, compared to controls (137). The role of the inflammasome in the pathogenesis of DR has also been investigated in rodent models of DR, and is highlighted via the use of methylene blue in streptozotocin (STZ)-induced diabetic rats, demonstrating following treatment, NLRP3 inflammasome activation including levels of IL-1β and IL-18 was reduced along with an increase in the thickness of retinal layers, and reduced permeability of the BRB (138). Additionally, in diabetic rat retinas treated with lentiviral vectors encoding *Nlrp3* short hairpin RNA (shRNA), there was a significant reduction of inflammasome components (CASP1, IL-1β, and IL-18), which correlated to a decrease in vasculature permeability when compared to diabetic controls (138). This effect was also shown following induced hyperglycaemia in diabetic rats with and without minocycline treatment, a tetracycline antibiotic, along with vascular permeability and retinal vascular apoptosis following treatment (139). Protein expression of IL-1β and IL-18 were also found to be increased in the vitreous of DR patients (137). While this finding does contrast with other reports showing that there was no change in IL-1β protein levels in the vitreous of PDR patients compared to controls, they did report increased levels of CASP1 and IL-18 in the vitreous, supporting a role of inflammasome-mediated cytokine release in DR (140). Increased levels of VEGF were also found in this study, supporting an angiogenic role of IL-18 as seen in many other retinal degenerative diseases (141).

Angiogenic Role of IL-18 in DR

In further support of the angiogenic role of IL-18 in DR, PDR eyes with the highest levels of fulminant neovessel formation also had higher levels of IL-18 than inactive neovessel controls (141). Furthermore, CASP1 levels were reduced following anti-VEGF treatment (bevacizumab) (141). IL-18 was significantly upregulated in the serum from patients with Diabetes Mellitus Type 2 with background retinopathy, with the serum inducing a higher rate of neovascularisation when injected intradermally to mouse skin samples (mouse cutaneous angiogenesis test) compared to control serum (142). Higher serum levels of IL-18 have also been reported in Type 1 diabetic patients, half of which had a form of DR (143). While no analysis between patients with and without retinopathy was investigated in this study, the authors do remark that there was no association found between IL-18 levels and microvascular changes, however further investigation is required (143). Overall evidence suggests that IL-18 plays an important role in neovascular changes characteristic of DR, and may therefore represent a valuable therapeutic target.

IL-1β in Cell Death in DR

The role of IL-1β in DR progression has also been widely investigated, with IL-1β protein levels found to be significantly increased in the vitreous (24, 140, 144, 145), as well as in aqueous humor (146) of DR patients. However, while IL-1β levels were shown to be significantly increased in serum from PDR patients (145), other studies have shown no change (14, 147), with this discrepancy possibly due to the different levels of breakdown of the BRB.

While systemic IL-1β inhibition using canakinumab in patients with PDR did not have an effect on neovascularisation (148), a non-statistically significant reduction in edema was evident in DME patients (148). In support of IL-1β inhibition potentially leading to more efficacy in DME than in PDR, it has been found that patients with DME have an IL-1Ra/IL-1β ratio that is 13 times higher than in PDR patients (149).

Rodent studies investigating the role of IL-1β on DR pathogenesis have also shown IL-1β to be upregulated following STZ-induced diabetes, and significantly reduced following IL-1β inhibition by anti-inflammatory cyclosporin-A administration (150), as well as following a multiple anti-oxidant diet (151) and pituitary adenylase cyclase activating peptide (152). IL-1β was also upregulated in isolated retinal vessels, compared to control rats, as well as in bovine retinal vascular endothelial cells (BREC) (153). It has also been demonstrated that following intravitreal injection of IL-1β, along with increased TUNEL-positive capillary cells in retinal microvessels, the formation of acellular capillaries had increased two-fold, both characteristic early features seen in DR pathology (151). Taken together, human and rodent models of DR suggest a role for IL-1β in cell death in this disease.

Role of IL-1Ra in DR

The role of IL-1Ra in the progression of DR is largely unknown, however the few studies that have investigated its role largely support an anti-inflammatory or protective role. A study investigating the risk factors for the development of DR in Type 2 diabetes patients found that IL-1Ra levels in serum were negatively correlated with disease presence, with low serum levels of IL-1Ra hypothesized to be a risk marker for DR progression (27). In another study, IL-1Ra levels were found to be significantly increased in the tears of diabetic patients without retinopathy compared to those with retinopathy (42), and reduced in the plasma of diabetic patients compared to controls (43). These lower levels of IL-1Ra in diabetic patients without DR could indicate a heightened risk for developing DR, as it has been suggested that increased IL-1Ra production in diabetes could be a compensatory response to the heightened auto-immune state in diabetic patients (42). Taken together, these studies indicate a protective mechanism for IL-1Ra in preventing DR onset, as low levels of IL-1Ra could suggest that inflammation may propagate due to increased IL-1β activity.

In patients with DR however, IL-1Ra expression patterns appear to be less clear, with IL-1Ra levels along with IL-1α shown to be unchanged in the serum compared to controls (129, 132). Furthermore, following intravitreal injection of anti-VEGF agent bevacizumab in 8 patients with DR, IL-1Ra along with several other cytokines were found to be significantly lower in the vitreous than in controls (154). It is possible that as VEGF inhibition reduced neovascularisation and inflammation, IL-1Ra upregulation was not required. In addition, using the STZ-induced diabetes rat model, retinas exposed to hyperglycaemia showed significantly increased levels of IL-1ra as well as IL-1β, and their transmembrane receptors IL-1r type 1 and IL-1r type II, compared to controls (28), along with major changes in retinal architecture including compromised BRB integrity, and thinning of the ganglion cell layers (28). Evidence from studies investigating IL-1Ra levels in DR patients and rodent models could suggest an overburdening of compensatory IL-1Ra antagonist activities in more severe inflammatory states, highlighting that IL-1Ra could be a therapeutic target to prevent IL-1β and IL-18 propagation. Further investigations are still however necessary to fully elucidate the role of IL-1Ra in DR pathogenesis.

Potential Pro-angiogenic Role of IL-37 in DR

IL-37, an anti-inflammatory cytokine in the IL-1 family, is known to inhibit the innate immune system in several models of inflammation, including hepatitis, colitis, and psoriasis (155–157). In the retina, Zhao et al. have shown that IL-37 is involved in the pathogenesis of PDR, with IL-37 levels elevated in PDR patients (47). This was also correlated with an induction of VEGF-A and pro-angiogenic cytokine angiopoietin (Ang2), indicating a potential role for IL-37 in neovascular retinal conditions (47). The authors of this study report that following IL-37 treatment in a monkey chorioretinal vessel endothelial cell line (RF/6A), tube formation and branching points were increased (85.3 and 71.4%, respectively) along with cell proliferation, compared to PBS controls (47). IL-37 has been suggested to have pro-angiogenic roles similar to IL-18 in other diseases (158, 159), able to signal through the IL-18Ra.

In another study, an upregulation of IL-37 was demonstrated in HLA-B27-associated acute anterior uveitis (AAU), inflammation of the anterior eye, which was associated with an inhibited production of a number of cytokines including IL-1β, IL-6, TNF-α, and IFN-γ (160). Further investigation into the role of IL-37 in the regulation of cytokine signaling in retinal degenerative diseases may reveal novel insights into the anti-inflammatory nature of IL-37.

RETINITIS PIGMENTOSA

RP Disease Pathogenesis

Retinitis pigmentosa (RP) is an inherited form of retinal dystrophy characterized by initial rod photoreceptor degeneration, secondary cone degeneration, and retinal pigment deposits (161, 162). RP presents as a loss of peripheral vision, resulting in tunnel vision and night blindness, which in some cases ultimately progresses to full blindness (161, 162). RP has a varied etiology, including a range of non-syndromic types, as well as syndromic and systemic types, and it is caused by inherited or acquired mutations in over 50 different genes including rhodopsin (*RHO*) (163). In this disease, there is an inflammatory component to disease pathogenesis, with both increased microglial and macrophage activity (69–72) and increased levels of chemokines and cytokines found in patient and rodent models (22, 164). However, it is unclear if this increased inflammatory state is causative or a consequence of this currently untreatable disease, and the exact role that members of the IL-1 family play.

Inflammasome-Mediated Cell Death in RP

Microglial activation can occur in both RP and late-onset retinal degeneration (L-ORD) and is a consequence of a bystander effect of rod photoreceptor cell death, causing further adjacent photoreceptor death including cones (165). Bystander photoreceptor cell death has been reported in other RP studies, including Zhao et al. that demonstrated that microglial phagocytosis of healthy photoreceptors in the retina adjacent to dying cells was evident in the rd10 mouse model of RP (70), which is a model of autosomal recessive retinitis pigmentosa where rod degeneration occurs from P18 (166). These microglia were found to express IL-1β (70). Another study demonstrated that in P23H rhodopsin mutant rats, a model of autosomal dominant retinitis pigmentosa, differential cell death pathways existed in rod and cone photoreceptors (167). It was suggested that while rod cell death occurs via heightened RIP1/RIP3/DRP1-axis mediated necroptosis, cone cell death only occurs subsequently due to bystander cell death pathways via activation of the ATP-binding P2X7 receptor and NLRP3 inflammasome activation (167). This was supported by further data that showed preserved viability of cone photoreceptors on an NLRP3-deficient mouse strain that possesses the P23H mutation (167).

Furthermore, inflammasome components were measured in three early-onset (rcd1, xlpra2, and erd) and one late-onset (xpra1) canine model of RP, with *Nlrp3*, *Casp1*, *Asc*, *Il-1b*, *Il-1ra,* and *Il-18* gene expression all upregulated in the most aggressive early-onset model, rcd1, gradually rising from the induction phase of the disease at 3 weeks and peaking in expression during the chronic cell death phase at 16 weeks (23). The expression of these inflammasome genes was also upregulated significantly in the xlpra2 early-onset model, however not until 7–16 weeks and *Il-1β* was upregulated in the late-onset model from 16 weeks. However, on examining protein expression levels, there was only a change in active IL-1β levels in the rcd1 and xpra2 models at 16 and 7 weeks, respectively. In comparison, pro-IL-18 levels were significantly reduced in both models as well as erd, with active bands not detected at all. Taken together, these results suggest an involvement of inflammasome-mediated IL-1β coinciding with photoreceptor cell death in early-onset RP (23).

This idea is supported by a study using rd10 mice, showing that increased photoreceptor cell death was correlated with increased CASP1 protein expression (168). Vitreous levels in rd10 mice, as well as patients with RP, showed increased levels of IL-1β along with reduced visual fields compared to wild type and idiopathic epiretinal membrane patient controls, respectively (22, 22), while there was no change in IL-1α levels in RP patients compared to controls, indicating that IL-1β and the inflammasome may play a role in RP.

Despite strong upregulation of inflammatory genes and IL-1β in animal models and human studies with RP, to our knowledge the other members of the IL-1 family have not been studied in the progression or onset of this disease.

GLAUCOMA

Glaucoma Disease Pathogenesis

Glaucoma defines a heterogeneous group of visual disorders that arises from compression of the optic nerve due to elevated intraocular pressure (169). Glaucoma is the leading cause of blindness in the world, and currently there is no cure. Furthermore, due to the gradual onset of vision loss, many patients are unaware they have developed this disease. In addition to optic nerve damage, glaucoma is characterized by degeneration of the retinal ganglion cells (RGC) and their axons (170, 171), a layer at the front of the retina responsible for the transmission of collated visual information to the optic nerve. There are three forms of Glaucoma, open-angle, closed angle and secondary-glaucoma, with open-angle glaucoma further divided into high or low pressure forms, named primary open-angled glaucoma (POAG) and normal tension glaucoma, respectively (169). Elevated intraocular pressure (IOP) in glaucoma can be caused by impaired aqueous outflow, either anatomically obstructed in closed glaucoma, or in open glaucoma can be caused by defective trabecular meshwork (TM) including dysregulated function of tight junctions or by build-up of plaque-like materials (15). Along with genetic and environmental risk factors, as in most retinal degenerative disorders, oxidative stress, and inflammation are believed to contribute to disease pathogenesis, augmenting IOP via the infiltration of immune cells through a leaky or impaired BRB surrounding the optic nerve, which ultimately results in RGC death and axonal injury (73, 170, 172).

IL-18 in Glaucoma

IL-1 family members have been shown to play a role in glaucoma pathogenesis, with IL-18 expression increasing with age in the ciliary body, iris and aqueous humor of DBA/2J mice, a model of pigmentary glaucoma that naturally presents with increased IOP, RGC loss, and pigmentary dispersion (41). Levels of IL-18 appeared to precede classical pathological symptoms of this disease, peaking in expression in the iris, ciliary body and aqueous humor at 6 months. The authors therefore hypothesized that IL-18 could be a marker indicating disease onset (41).

IL-1α and IL-1β in Glaucoma

Patients with POAG have been found to have significantly increased gene levels of IL-1β in their blood and significantly increased IL-1β protein expression in the aqueous humor compared to healthy controls (21), however in tears from POAG patients was not significant from healthy controls (173). *Il-1α* and *Il-1β* mRNAs were found to be increased in the TM in glaucomatous eyes compared to controls, acting in a feedback loop to control endothelial leukocyte adhesion molecule 1 (ELAM-1), an early marker of atherosclerotic plaque that forms in glaucoma (15). Furthermore, treatment with IL-1Ra, an IL-1R antagonist, downregulated the expression of ELAM-1 (15). These studies indicate that IL-1α and IL-1β may be involved in glaucoma pathology.

In rodent models of glaucoma, IL-1β is demonstrated to cause an increase in RGC death, hypothesized to be activated via a TLR4-NLRP1/NLRP3-CASP8-axis in an acute IOP glaucoma model in mice. In both $Tlr4^{-/-}$ mice and CASP8-inhibited mice, there was reduced IL-1β production and preserved RGC health (174). Using the same IOP model in both mice and rats, another group demonstrated significantly high mRNA levels for inflammasome components *Nlrp3*, *Casp1*, *Asc,* and *Il-1β*, peaking at 1 day post-insult, however suggested that this increase in inflammatory genes was primed via the P2X7 receptor (175). P2X7-inhibited and $P2X7^{-/-}$ mice did not demonstrate the same increase in IL-1β following damage, while the use of the P2X7 agonist bzATP promoted a surge of IL-1β again at 1 day post-insult (175). The mechanism by which P2X7-mediated IL-1β secretion occurs in glaucoma has been suggested by this group and others to occur in response to stretch and swell mechanical stresses from increased IOP (175, 176).

There exists wide speculation that given pathological similarities between glaucoma and Alzheimer's disease, a gene cluster of IL-1 polymorphisms may indicate increased risk of developing glaucoma (177). To support this idea, a study showed that the IL-1α (−889C/T) polymorphism increased IL-1 gene expression, which was associated with amyloid-β deposits that are known to accumulate in RGCs in glaucoma models (178). However, independent studies into IL-1 gene cluster polymorphisms such as C/T polymorphism in the promoter region of IL-1α, IL-1α (−889) T allele, and two C/T polymorphisms in IL-1β, rs16944 (−511 C/T) and rs1143634 (+3953C/T), have reported conflicting information on POAG and normal-tension glaucoma (NTG) disease susceptibility (179–185), promoting a meta-analysis to investigate the relationship between these polymorphisms and glaucoma risk factor. From the meta-analysis, it was concluded that there was no association between these polymorphisms and POAG or NTG development (177).

RETINOPATHY OF PREMATURITY

ROP Disease Pathogenesis

Retinopathy of prematurity (ROP) is the leading cause of severe visual impairment and blindness in infants, that arises due to premature birth and results in underdeveloped vasculature and retinal detachment [reviewed in Shah et al. (186)]. ROP has been considered to have two phases of disease; incomplete vascularisation of the retina creating a hypoxic environment, and as a consequence, leading to neovascularisation and proliferative retinopathy (187).

IL-18 as a Regulator of Neovascularisation in ROP

Qiao et al. determined that the expression of IL-18 was reduced in a mouse model of oxygen-induced retinopathy (OIR) (188), in which supplemental oxygen induces incomplete vascularisation of the retina, indicating that IL-18 is able to regulate neovascularisation in retinal degenerations, suggesting possible repercussions in other neovascular retinal diseases such as ROP. In humans, the development of ROP was correlated with an early decline in systemic IL-18 levels, but in later periods, correlated with increasing IL-18 levels in whole blood from 877 ROP patients (38). Incomplete retinal vascularisation during the first phase of ROP may be linked to these changes in IL-18.

IL-1β and Choroidal Toxicity in ROP

In mouse models of OIR, IL-1β has been shown to be associated with choroidal involution, a characteristic feature of ROP (39). In this study, IL-1β was found to be increased in both the RPE and choroid, inducing toxicity in the choroid and leading to retinal and choroidal degeneration. These effects were ameliorated following IL-1β inhibition through administration of an IL-1R antagonist (39). Additionally, in a pre-term birth mouse model that induces chorioamnionitis, IL-1β was injected between the two fetal membranes on day 11 of gestation, and following birth, retinas were shown to exhibit high levels of pro-inflammatory genes accompanied by a persistent infiltration of mononuclear phagocytes in the retina (40). This was accompanied by thinning of the choroid and underdevelopment of retinal vessels. Upon antenatal administration of a non-competitive IL-1R agonist, these effects were prevented, highlighting a novel antenatal role of IL-1β on retinal vascular development (40).

In humans, levels of IL-1β were found to be unchanged and were below detectable levels in a multiplex bead cytokine array of vitreous samples from ROP and control patients (189). Further investigation into the expression levels of IL-1β is warranted, especially in the RPE and choroid.

Role of IL-1Ra in ROP

Few studies have investigated the role of IL-1Ra in ROP pathogenesis, however this competitive antagonist was found in significantly high levels in the vitreous and tears of ROP

babies, along with increased levels of VEGF, complement component proteins, and matrix metalloproteinase 9 (MMP9) (44). Furthermore, there was an increase in activated microglia/macrophages in the vitreous from ROP babies (44). As ROP is characterized by abnormal retinal vasculature development and inflammation, it is possible that IL-1Ra levels were increased in these patients as a compensatory mechanism to prevent IL-18 angiogenic effects and IL-1β-induced cell death as described in other sections. Further work is necessary to understand the role and therapeutic potential of IL-1Ra in ROP.

IL-38 as a Novel Anti-angiogenic Factor in ROP

IL-38 is the newest member of the IL-1 family, classified under the IL-36 subfamily and has been reported to have roles in inflammation propagation in diseases such as rheumatoid arthritis, psoriasis and systemic lupus erythematosus [reviewed in Xu and Huang (190)]. IL-38 however has been largely unreported in retinal degenerations. A recent study however describes a role for IL-38 in ROP, where in a mouse model of OIR, a significantly higher level of IL-38 was found in OIR mouse retinas compared to controls (48). In addition, following IL-38 local and systemic injections in these OIR mice, angiogenesis was significantly reduced in the retinas compared to controls along with pro-inflammatory cytokine IL-1β levels (48). This was subsequently demonstrated in a cell culture model, in which VEGF-treated cells administered IL-38 had slowed wound healing following a scratch test, attenuated vascular tube formation, and reduced proliferation, processes which were eliminated with the addition of anti-IL-38 (48). It is therefore possible that IL-38 administration to ROP babies could help prevent pathogenic neovascularisation and inflammation. Further investigation is necessary to elucidate whether IL-38 may play a role in other retinal degenerative diseases, particularly in neovascular retinal diseases such as wet AMD and DR.

OTHER RETINAL DISORDERS

Stargardt Macular Dystrophy

Stargardt macular dystrophy (STGD) is a common form of inherited macular dystrophy that leads to juvenile macular degeneration caused by an inherited autosomal recessive mutation in the *ABCA4* gene. STGD affects 1:10,000 adults and children and is characterized by progressive central vision loss resulting from lesion development in the macular region of the retina [reviewed in Tanna et al. (191) and Fujinami et al. (192)]. Although little is known about the IL-1 family members and STGD pathology, the involvement of microglia has been characterized by Kohno et al. in a *Abca4*/*Rdh8* double knockout mouse model, where activation of microglia occurred through the TLR4 signaling pathway (72), and in the same model expressed the chemokine CCL3 (193), a macrophage-inflammatory protein known to be involved in the progression of retinal degeneration (92, 194, 195). Further investigations into the role of IL-1 family members in STGD may elucidate novel inflammatory mechanisms at play during retinal degeneration in this disease.

Retinal Vein Occlusion

Branch and central retinal vein occlusion (RVO) occurs when there is abnormal arteriovenous (A/V) crossing with vein compression and obstruction, causing degenerative changes in the vessel wall [reviewed in Laouri et al. (196) and Rehak and Rehak (197)]. Inflammation is involved in the pathology of RVO, with microglial activation and macrophage recruitment associated with an increase in pro-inflammatory cytokine production in an experimental branch RVO model (198), as well as increased levels of chemokines and cytokines including CCL2 and IL-6 in the vitreous of patients with branch RVO and macular edema (199). IL-1 family members have also been thought to play a role in disease pathogenesis in human RVO patients with retinal ischemia and recurrent macular edema, where IL-1α was significantly elevated in the aqueous humor (200), similar to in AMD patient serum (12) and in the plasma of rat ischemia/reperfusion injury (13). It has been found that RVO patients also have an increase in vitreal levels of IL-1β (19, 201), however was not elevated in the aqueous humor (202). The role of other IL-1 family members in RVO is yet to be explored.

Retinal Detachment

A retinal detachment is a break between the neurosensory retina and the RPE, leading to fluid accumulation under the retina and sudden vision loss in the rhegmatogenous form [reviewed in Ghazi and Green (203)]. Retinal detachment can occur as a symptom of other retinal degenerative diseases including DR (134). Without prompt reattachment, retinal detachments can lead to starvation of the photoreceptors due to separation from their choroidal oxygen supply, resulting in photoreceptor cell death. Although inflammation (204), microglial migration (205), and monocyte infiltration (206) has been thought to play a role in retinal detachment, novel findings suggest that microglia may actually mediate photoreceptor cell death following retinal detachment, potentially by phagocytosing cell debris that may cause retinal damage (207). In patients with retinal detachments, elevated levels of IL-1β have been detected in the vitreous or retina (109, 208, 209), indicating a role for IL-1β in disease pathogenesis. In support of this, a study involving a mouse retinal detachment model showed that photoreceptor cell death was reduced when IL-1β and CASP1 were inhibited, as well as in *Nlrp3*$^{-/-}$ mice with retinal detachment (109), also indicating a role for inflammasome activation in this disease. The role of other IL-1 family members in retinal detachment require further investigation.

Autoimmune Uveoretinitis

Experimental autoimmune uveoretinitis (EAU) is a T cell-mediated autoimmune disease that is used as a model for human posterior segment uveitis, including sympathetic ophthalmia, birdshot chorioretinopathy, Vogt-Koyanagi-Harada disease, and Behçet's disease (210). Rodent EAU is induced by immunization with uveitogenic retinal proteins including the retinal soluble antigen (S-Ag) and the interphotoreceptor retinoid-binding protein (IRBP) (211). Mononuclear phagocytes have been identified to play a role in EAU, with microglial migration evident in the earlier phases of EAU and subsequent macrophage

recruitment in the later phases (212). Several IL-1 family members have also been linked to the development of EAU, including IL-33 and IL-1β (213, 214), with the role of other IL-1 family members generally unknown in this disease. The expression of IL-33 was elevated in the inner nuclear layer of EAU mice compared to naïve mice (213). Interestingly, administration of IL-33 led to a decrease in EAU severity in wild type mice, alongside a reduction in T cells, IFN-γ, and IL-17 production (213), indicating that IL-33 induced a protective effect against the adaptive immune system despite its classical role as an inducer of T cell activation (127).

IL-1β has also been found to increase the severity of EAU, with the systemic delivery of recombinant IL-1β elevating EAU symptoms when administered during the priming phase of the immune response in EAU, and a decrease in EAU severity when a neutralizing antibody for IL-1β was delivered (214). IL-1β levels were found to be significantly elevated in the aqueous humor and supernatants of posterior eyecups from EAU rats (215), indicating increased production and dysfunction of IL-1β, which has been shown to cause BRB breakdown by opening the retinal vascular endothelial tight junctions in EAU (216). Another study reported that IL-1β was secreted by neutrophils, macrophages and dendritic cells in an EAU model (217). In this study, IL-1R-deficient mice had reduced severity of EAU alongside a reduction in immune cell recruitment into the retina (217), supporting other studies describing the protective effect of IL-1β neutralization in EAU (214).

OTHER IL-1 FAMILY MEMBERS IN THE EYE

The role of other IL-1 family members in retinal degenerations remains elusive, with agonists IL-36 (α, β, and γ) and receptor antagonist IL-36Ra not being investigated in the retina, to our knowledge. Although many members of the IL-1 family have not been investigated in the retina, in patients with HLA-B27-associated AAU, changes in IL-1 family members were detected in the aqueous humor (218). Significantly higher levels of several IL-1 family members, including IL-1β, IL-18, IL-1Ra, IL-36Ra, and IL-37 was observed in AAU patient aqueous humor compared to controls (218). This study indicates that other IL-1 family members including IL-36Ra may also contribute toward ocular inflammation and may play a role in retinal degenerative diseases. In support of this, IL-36Ra has been thought to play a role in *Pseudomonas aeruginosa* keratitis, a severe corneal ulceration, with its downregulation leading to an increased severity of disease (219).

IL-36 (α, β, and γ) and IL-36Ra have been shown to play a role in the pathogenesis of other inflammatory diseases [reviewed in Ding et al. (220) and Walsh and Fallon (221)]. IL-36 cytokines have been well characterized in psoriasis, a chronic inflammatory skin condition, where the three IL-36 agonist ligands (α, β, and γ) were found to be upregulated in skin lesions [reviewed in Towne and Sims (222)]. Subsequently, a mouse model of psoriasis was created using an overexpression of IL-36α (223). IL-36 activity (IL-36 α, β, γ, or IL-36Ra) has also been linked to the pathogenesis of several autoimmune conditions, including colitis (224, 225), systemic lupis erythematosus (226), Primary Sjögren's syndrome (227) and psoriatic and rheumatoid arthritis (228, 229). Autoantibody production has also been associated with retinal degenerations such as autoimmune retinopathy (AIR) and AMD [reviewed in Morohoshi et al. (230)], and so it is possible that the IL-36 signaling axis could play a role. Several other mechanisms of IL-36 activity may also be relevant to retinal degenerative diseases; for example, it has been found that the IL-36 receptor (IL-36R) is constitutively expressed by several types of immune cells, including macrophages (231), and that IL-36α may also be expressed by macrophages (225). IL-36 agonist ligands have been shown to stimulate the production of chemokines (224) and cytokines including IL-18 (232) and IL-6 (229), also heavily involved in retinal degenerative diseases. A study showed that after stimulation with IL-1β, IL-36α, IL-36β, or IL-36γ, there was an overlap between differentially expressed genes in epidermal keratinocytes, including cytokine and chemokine production and leukocyte recruitment genes (233). The study also indicated a role for the MyD88 adaptor protein in shared IL-1β/IL-36 responses (233).

In the CNS, neuronal and glial cells have been shown to express IL-36β (234), with microglia and astrocytes thought to express IL-36R (235). However, in an experimental autoimmune encephalomyelitis (EAE) mouse model, although it was demonstrated that IL-36γ was expressed by neutrophils leading to microglial activation, IL-36γ or IL-36R deficiency did not change the severity of EAE compared to wild type controls (236). This indicates that the role of the IL-36 subfamily members in CNS diseases is unclear, and further investigation is required to determine whether IL-36 (α, β, and γ) and IL-36Ra are expressed by the retina, and if they play a role in retinal disease pathogenesis.

IL-1β MECHANISMS OF ACTION IN RETINAL DEGENERATIONS

IL-1β has been the most widely studied IL-1 family member in retinal degenerative diseases, due to its broad range of pro-inflammatory functions. However, several important questions surrounding IL-1β in retinal degenerations, particularly in AMD, remain unclear; (1) IL-1β as a potential biomarker of retinal disease; (2) which inflammatory pathways it mediates; (3) which retinal cell types produce, express or secrete IL-1β; and (4) as IL-1β has no N-terminal secretory signal (237), how this unconventionally secreted protein is released from its producing cell. Therefore, this section of the review aims to summarize the current literature surrounding these themes and highlight gaps in our knowledge surrounding the role of IL-1β, particularly in the context of dry AMD.

IL-1β as a Biomarker for Diagnosis of Retinal Degenerations

The analysis of pro-inflammatory cytokine IL-1β as a diagnostic biomarker and therapeutic target have been investigated in both ocular tissues and fluids, as well as in serum from patients

with retinal degenerative diseases. Pro- and active- forms of IL-1β have been found to be upregulated in the vitreous humor (19, 22, 24–26, 109, 140, 145, 149), aqueous humor (21, 238), retina (209), and serum (12, 145, 239) of patients with retinal degenerations such as wet AMD (12, 19, 239), diabetic macular edema (149, 238), retinal detachment (109, 208, 209), RVO (19, 201), glaucoma (21), retinitis pigmentosa (22), and diabetic retinopathy (19, 24–26, 140, 144, 145, 240). However, very few studies have reported IL-1β expression levels in intraocular fluid, serum or retinal tissue in human patients with dry AMD, with reports of no significant change in IL-1β levels in AMD (mostly dry AMD patients), retinitis pigmentosa, and glaucoma, using a multiplex immunoassay system (164). In another study, no significant increase in *IL-1β* gene expression was found in the RPE of patients with geographic atrophy (GA) (103), and a non-significant increase in IL-1β levels in the aqueous humor of dry AMD patients compared to healthy controls (241). In a retrospective case-controlled study of polymorphisms in interleukin genes of nearly 500 late-stage Taiwanese dry AMD patients and controls, no single nucleotide polymorphisms (SNPs) in the IL-1β gene were found associated with the development of AMD (110), indicating little association exists between dysfunctional IL-1β gene expression and dry AMD. This suggests strongly that the dysregulation of the *IL-1β* gene might not be as important as the control mechanism which regulates its protein expression and subsequent activation through inflammasome and CASP1-mediated activation.

Further investigations into pro- and active-IL-1β levels in serum, ocular fluid, and retina in human AMD patients, particularly in dry AMD, would be of interest to determine if this pro-inflammatory cytokine may be useful as a biomarker or therapeutic target for dry AMD.

Induction of Chemokine Production by IL-1β

Chemokines, or chemotactic cytokines, provide activation and directional cues following retinal injury to recruit immune cells to the site of damage, and are known to be regulators of leukocyte activation and recruitment in AMD (242), and have been associated with progressive retinal degeneration in mouse models of AMD (92, 129, 193, 194, 243–245). IL-1β has been implicated in the modulation of chemokine secretion via mediating NF-kB nuclear translocation allowing the genes to be subsequently transcribed (246).

Our previous work has shown that at 12 h post-injection of recombinant IL-1β into the rat eye, there was induction of retinal *Ccl2*, *Cxcl1,* and *Cxcl10*, key chemokines involved in leukocyte recruitment (18). This was accompanied by a significant increase in recruited macrophages into the retina through the optic nerve. Another study using ultrastructural analysis indicated that following IL-1β intravitreal injection into Lewis rats, the recruitment of mononuclear phagocytes into the retina was identified from 4 h after injection peaking at 24–48 h, accompanied by a breakdown of the BRB, edema and a higher inflammatory state (247). These studies indicate that IL-1β induction may be a mechanism by which microglia and macrophages are recruited into the damaged photoreceptor layer (18), and potentially facilitate photoreceptor cell death via phagocytosis (70). This finding is supported by a transcriptome-wide analysis of AMD retinas which showed that *Ccl2*, *Cxcl1*, *Cxcl10,* and *Cxcl11* were all upregulated in AMD retinas compared to healthy controls (248).

The Role of IL-1β in Other Inflammatory Pathways

Several other pathways associated with AMD pathogenesis may be also affected by IL-1β production in the retina, which may lead to retinal cell death. Interleukin-6 (IL-6), a pro-inflammatory cytokine associated with pathogenesis of AMD (63, 92, 249, 250), as well as in a model of ocular toxoplasmosis (251), and has been shown *in vitro* to be regulated by IL-1β, following IL-1β-dependent activation of the p38 MAPK/NF-kB pathway (252). Regulation of NF-kB by IL-1β has also been demonstrated in a mouse model of DR following intravitreal injection of IL-1β, with concomitant increases in oxidative stress levels (8OHG and nitric oxide) and increased TUNEL-positive capillary cells, which are characteristic features of this disease (151). Adeno-associated virus (AAV) vector-mediated gene transfer of IL-1β, which was injected intravitreally, demonstrated the greatest ocular inflammatory effect on the eye even at low-dose levels, compared to AAV vectors expressing IL-6 or IL-17A (253). This led to an upregulation of inflammatory factors CXCL1, CCL2, MMP-9, VCAM-1, VEGFA, IL-6, and IL-17A, reduced photoreceptor thickness, increased cellular infiltrates, and damage to the overall structural integrity of the posterior eye (253).

Matrix metalloproteinases (MMPs), responsible for the protein degradation of the extracellular matrix (ECM) (254), have also been linked to IL-1β in retinal degenerations, with wet AMD patients carrying SNPs in *MMP-1* and *MMP-7* genes found to have a higher serum concentration of IL-1β (239). Associations between MMPs and the IL-1 family have also been found in other retinal degenerations, with increased levels of MMP-1, MMP-9, MMP-12, and IL-1β found in the vitreous of patients with POAG (21), as well as elevated MMP-9 and IL-1Ra observed in the vitreous and tears of ROP infants (44). Further, in optic-nerve induced retinal damage, increased levels of MMP-9 promoted RGC loss, which was ameliorated by an intravitreal injection of IL-1Ra (255).

Finally, the complement cascade, comprised of three pathways to trigger the lysis of pathogens, apoptotic cells and clearance of foreign debris (256, 257), may also be influenced by inflammasome signaling and may alter the level of IL-1β production in retinal degeneration. It is well established that dysregulation of the complement cascade is a critical factor in AMD pathogenesis [reviewed in Anderson et al. (258)]. Doyle et al. has demonstrated that complement component 1q (C1q), the initiator of the classical pathway, may activate the NLRP3 inflammasome in drusen using a carboxyethylpyrrole (CEP)-adducted model of dry AMD (31). We have shown that classical complement deficient ($C1qa^{-/-}$) mice that had undergone photo-oxidative damage had a significant reduction in IL-1β protein expression in the progressive atrophic stages

of degeneration in this model, which was associated with a reduction in inflammasome activation (259). Other complement components, including C3a (260, 261) and C5a (262), have also been thought to prime IL-1β expression by retinal cells.

Cells Expressing IL-1β in the Retina

IL-1β has been widely reported to be expressed by cells of haematopoietic lineage (51), which in the retina encompasses resident microglia as well as infiltrating macrophages. Rodent models of retinal degenerations including dry AMD and retinitis pigmentosa, support this notion, with IL-1β localization shown to be expressed primarily by infiltrating macrophages in the outer retina and subretinal space (16, 18, 108, 109, 263). This localization pattern has also been demonstrated in non-retinal neural tissues, with IL-1β expressed in resident microglia and infiltrating macrophages of the brain following ischemic stroke (264) and in the developing cerebellum (265).

Additionally, there is scarce literature on the localization of IL-1β to any other retinal cell type in both human retinas and animal models. Recently, Chaurasia et al. localized the expression of IL-1β protein to unspecified cells in the inner retina in the Akimba mouse model of PDR (266). In other studies, intravitreal injection of NMDA induced neurotoxicity and IL-1β stimulation in Müller cells (267, 268), as well as in RGCs (268). Many studies, however, have induced the expression of IL-1β in a range of immortalized and primary retinal cell cultures lines following inflammasome stimulation (269, 270), with the majority of the literature focused on investigating the activation of the NLRP3 inflammasome in the RPE (36, 103, 104). Various *in vitro* models of retinal degenerations, using mostly RPE and microglia/macrophages in culture, have shown increased gene and/or protein expression levels of IL-1β in response to oxidative stress and inflammatory stimulations such as 4-hydroxynonenal (HNE), an end product of lipid peroxidation (271), lipofuscin components including A2E (272–275), Aβ (276–278), lysosome destabilization (104), lipopolysaccharide (LPS)-stimulated microglia-conditioned medium (279), and complement components (31, 280, 281). Taken together, these *in vitro* models, using inflammatory or oxidative stress signals characteristically found in the pathogenesis of retinal diseases, highlight potential IL-1β upregulation pathways, however lack the complexity that *in vivo* testing accounts for such as cell-to-cell

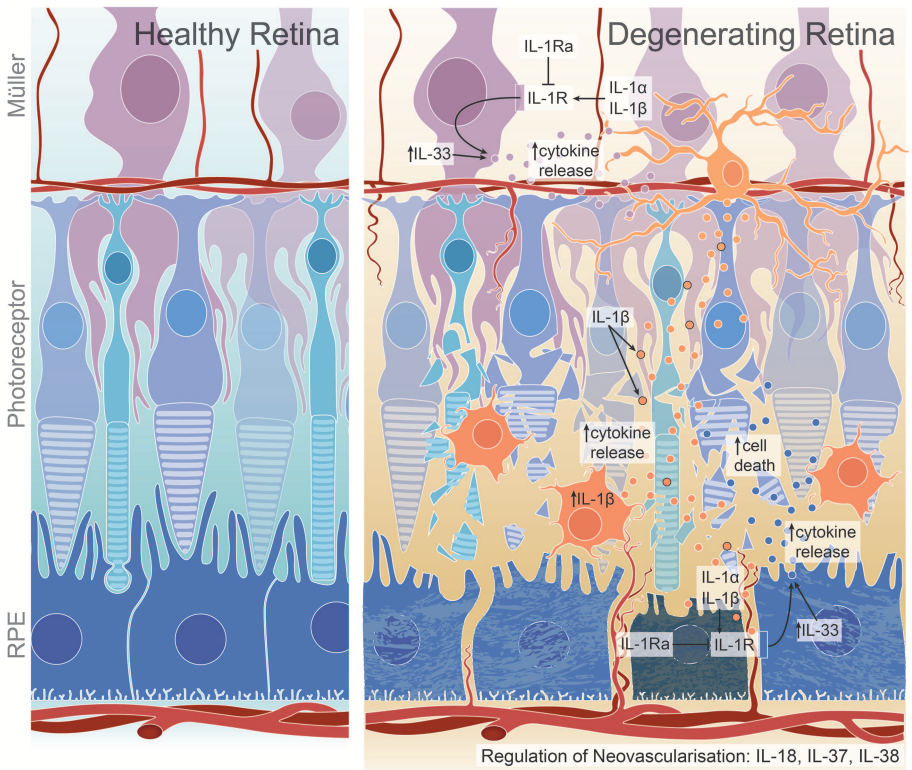

FIGURE 1 | Proposed roles of IL-1 family members on cell death, inflammation, and angiogenesis in the degenerating retina. IL-1β production by activated microglia and macrophages may lead to increased chemokine and cytokine release from Müller and RPE cells, promoting further macrophage recruitment to the damaged site and ultimately resulting in photoreceptor and RPE cell death (16, 18, 108, 109). This may occur through IL-1R expression on Müller and RPE cells (18), through which IL-1α may also exert its inflammatory functions (96, 97). IL-1Ra, a competitive antagonist for IL-1R, is dysregulated in retinal degenerations (27, 44). IL-33, a less-characterized IL-1 ligand in the retina, may play a role in cytokine regulation, specifically in dry AMD pathogenesis (45, 128). IL-18, IL-37 and IL-38 all have reported roles in regulating neovascularisation; however, have been shown to have both pro- or anti- angiogenic effects, with IL-18 dysregulation conferring protection against neovascularisation in wet AMD (31), but detrimental effects in dry AMD (35), DR (141), and potentially ROP (188). Although not widely characterized, IL-37 may play a pro-angiogenic role in DR (47) while IL-38 is suggested to have anti-angiogenic roles in ROP (48).

interactions and retinal signaling, transport, and regulatory pathways. Furthermore, while it has been widely considered to be the predominant retinal cell type expressing the inflammasome, NLRP3 activation in the RPE has not been conclusively proven to be responsible for propagating IL-1β release and inflammatory-mediated cell death in retinal degenerations (282).

Movement of IL-1β

There exists some discrepancy between the investigations into inflammasome activators in the RPE, and the well-reported localization of IL-1β in microglia and macrophages (16, 18, 108, 109, 263). Although it is possible and documented for the transmission of gene transcripts between the gene-producing and gene- or protein-expressing cell types (283–286), this phenomenon has not been investigated nor reported for IL-1β in the retina. However, it has been reported that extracellular vesicle encapsulation and transfer of CASP1, along with ASC and IL-1β secreted from monocytes, was able to induce a "cell death message" in vascular smooth muscle cells, a process that was inhibited using CASP1-specific inhibitor ac-YVAD-cmk (287). This process is further supported by work in pulmonary vascular endothelial cell injury showing that following LPS stimulation, active CASP1 was packaged in microparticles, along with cleaved gasdermin D, an inflammasome-dependent pyroptotic pore, and was able to stimulate endothelial cell death (288). It is therefore possible that this phenomenon could exist in the retina, with extracellular vesicle transfer of inflammasome components to microglia following receptor activation in the RPE or other host cell types. A study in which ARPE-19 cells were subject to blue-light photo-stimulation (488 nm) in culture support this hypothesis, demonstrating exosomal release with increased levels of inflammasome components CASP1, IL-1β, and IL-18 compared to unstimulated controls (289). This possibility also highlights the flaws in using only single-cell culture-based models, as it limits the ability to fully understand cell-to-cell communication and transport pathways, and prevents localization and uncovering the mechanism of how this pro-inflammatory cytokine is activated and secreted in the retina. Investigating the transport pathway of these inflammatory components using gene and protein detection methods simultaneously, as well as in the presence of gene inhibitors such as siRNA, or the use of co-culture *in vitro* systems, could shed more light on these essential cellular interactions.

CONCLUSIONS

Synergy exists between the development and progression of various retinal degenerative diseases, and the dysregulation of IL-1 family members, which contribute to either immune cell recruitment, retinal cell death, or dysfunctional angiogenesis (**Figure 1**). These hallmark pathogenic features are evident in both acquired and inherited forms of retinal degenerations, and are strongly correlated to the activation of the two most characterized IL-1 family members, IL-1β, and IL-18. Clear trends exist between the role of IL-1β as a regulator of cytokine production and cell death across many retinal diseases including AMD, DR, RP, glaucoma and ROP, and IL-18, which modulates neovascular aspects of these diseases. As these two pro-inflammatory cytokines are secreted in an inflammasome-dependent manner, it is well-documented that the inflammasome may play a key role in disease pathogenesis.

The role of other IL-1 family members in the retina, comprising IL-1α, IL-1Ra, IL-37, and the IL-36 subfamily (including IL-38), however, is less clear. While few studies have been performed on these members in the retinal diseases discussed in this review, taken together, evidence suggests that these cytokines may also play a regulatory role in mediating cell death, inflammation and angiogenesis in the retina. It therefore appears that the IL-1 family members may all contribute toward these major pathogenic features that typify retinal degenerations. Further investigations into the lesser-known IL-1 family members in both the retina and other neural tissues is however necessary to uncover novel mechanisms by which they may act.

While IL-1β is the most widely investigated and characterized IL-1 family member in retinal degenerative diseases including AMD, there is the limitation of testing in appropriate *in vivo* models that mimic retinal inflammasome activation, with the majority of investigative studies performed in cell culture-based systems. Although single cell culture experiments can shed light on inflammatory pathways that are active in individual retinal cell types, in order to fully elucidate the role that IL-1β plays in intercellular communication in diseases, *in vivo* testing and the use of retinal co-culture systems is necessary.

Finally, while each IL-1 family member has primarily been investigated independently of the other members, it would be worthwhile to determine how these IL-1 family members work together and how they influence each other, given the crossover between their functions in cell death, inflammation and angiogenesis. This includes IL-1Ra regulation of IL-1β, both IL-18 and IL-37 performing angiogenic functions, and a potential IL-1β/IL-36 signaling axis, briefly described in this review. Localizing IL-1 family members, as well as their receptors, will shed light on the cellular expression of these cytokines, and may elucidate novel mechanisms of action for regulating the progression of retinal degenerations.

AUTHOR CONTRIBUTIONS

YW, SM, RN, and NF wrote and edited the manuscript. RA-B prepared the summary figure for publication.

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REVIEW
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Interleukin-1 Family Cytokines: Keystones in Liver Inflammatory Diseases

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The pyrogenic property being the first activity described, members of the interleukin-1 superfamily (IL-1α, IL-1β, IL-18, and the newest members: IL-33, IL-36, IL-37, and IL-38) are now known to be involved in several inflammatory diseases such as obesity, atherosclerosis, cancer, viral and parasite infections, and auto-inflammatory syndromes as well as liver diseases. Inflammation processes are keystones of chronic liver diseases, of which the etiology may be viral or toxic, as in alcoholic or non-alcoholic liver diseases. Inflammation is also at stake in acute liver failure involving massive necrosis, and in ischemia-reperfusion injury in the setting of liver transplantation. The role of the IL-1 superfamily of cytokines and receptors in liver diseases can be either protective or pro-inflammatory, depending on timing and the environment. Our review provides an overview of current understanding of the IL-1 family members in liver inflammation, highlighting recent key investigations, and therapeutic perspectives. We have tried to apply the concept of trained immunity to liver diseases, based on the role of the members of the IL-1 superfamily, first of all IL-1β but also IL-18 and IL-33, in modulating innate lymphoid immunity carried by natural killer cells, innate lymphoid cells or innate T-αβ lymphocytes.

Keywords: interleukin-1 family cytokines, liver diseases, inflammation, innate immunity, trained immunity, invariant natural killer T-cells, natural killer cells, innate lymphoid cells

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THE EXPANDING FAMILY OF INTERLEUKIN (IL)-1 CYTOKINES AND THEIR RECEPTORS

From "Endogenous Pyrogens" to the IL-1 Superfamily of Cytokines

The story of the IL-1 superfamily of cytokines began in the 1940s when Menkin (1), Beeson (2), and then Atkins (3) described a group of proteins, named "endogenous pyrogens," responsible for fever, produced by leucocytes, and able to mediate multiple biological activities (4–6). By the late 1970s, the term "interleukin" was being used to describe the pleiotropic factors implicated in inflammatory immune response and the designation "IL-1" was then used to define the factors secreted by macrophages (4, 6). The discovery of IL-1, and its two forms IL-1α and IL-1β, is considered the birth of "cytokine biology," and it led to the discovery of more members. This

is how, over the years, IL-18, IL-33, IL-36, IL-37, and IL-38, which share several functional and structural properties, have been added to and included in the IL-1 superfamily of cytokines.

IL-1 Sub-Families

IL-1 superfamily (7) is divided into three sub-families according to the length of the N-term pro-pieces: IL-1 sub-family (IL-1α, IL-1β and IL-33, IL-1Ra), IL-18 sub-family (IL-18 and IL-37), and IL-36 sub-family (IL-36 α, β, γ, and IL-38) (**Table 1**). Among these cytokines, in each sub-family one may distinguish "pro-inflammatory" members such as IL-1α, IL-1β, IL-33 or IL-18 from "anti-inflammatory" members such as IL-1Ra, IL-36Ra, IL-37, or IL-38 [**Table 1**, and for reviews: (7–9)].

All of their receptors are heteropolymers of which at least one sub-unit is a member of the family of IL-1 receptors (IL-1R), which are characterized by extracellular immunoglobulin-like domains and an intracellular Toll/Interleukin-1R (TIR) domain within their cytoplasmic tail. Upon cell stimulation, IL-1R sub-units are oligomerized through the TIR domains. Secondly, MyD88 binds to the TIR domain, triggering nuclear factor-κB (NF-κB) translocation to the nucleus and activation of mitogen-activated protein kinases (MAPK) such as p38 and JNK, thereby leading to pro-inflammatory responses.

The combination of receptor sub-units conditions the type of signal transduced. For example, while association of IL-1RAcP (chain common to IL-1 and IL-1R3 receptors in the new nomenclature; **Table 1**) with the IL-1R1 sub-unit transduces an activation signal following binding of IL-1α or IL-1β, association of IL-1RAcP with IL-1R2 constitutes an IL-1β receptor that transduces an inhibitory signal.

IL-1RAcP is also a sub-unit of the IL-33 and IL-36 receptors, which associate IL-1RAcP with the IL-1RL1 chain (also called ST2: "suppression of tumorigenicity 2", T1/ST2 or IL-1-R4) and with the IL-1Rrp chain (also called IL-36 R or IL-1R6), respectively.

IL-1R8 (SIGIRR for Single Ig IL-1 related receptor), another receptor of the IL-1 family, has no known ligands but transduces an inhibitory signal and has been described as a checkpoint for terminal maturation and acquisition of the effector functions of NK cells (10). Mantovani et al. have suggested that IL-1R8 interferes with the TIR-domain oligomerization of the IL-1 family receptors engaged by a ligand agonist, thereby leading to blocked recruitment of the MyD88 adapter (9, 11). Lastly, IL-1R8 is a sub-unit of the IL-37 receptor. IL-37 can reduce production of the pro-inflammatory cytokines IL-1α and IL-1β, IL-1Ra, IL-8, IL-17, IL-23, tumor necrosis factor (TNFα), and interferon γ (IFNγ) through IL-18Rα and IL-1R8 (SIGIRR) (12).

It is important to note that there exist soluble forms of the IL-1, sIL-1R1, and sIL-1R2 receptors that possess the inhibitory functions of IL-1. On the same token, IL-18BP (Binding Protein), which consists in a single IgG domain such as the transmembrane receptor IL-1R8, is a soluble factor interfering with the liaison of IL-18 and its receptor. IL-18BP possesses a particularly strong affinity with IL-18. Seric concentrations of IL-18BP in a healthy individual generally range from 2,000 to 4,000 pg/ml, which means that in steady state circulation, there is always a major excess of IL-18BP compared to IL-18. In a pathological situation, however, the anti-inflammatory effects of IL-18BP are liable to be attenuated due to the fact that IL-18BP likewise binds to IL-37. While blocking the anti-inflammatory functions of IL-37, this association is liable to render excessive the active free form of IL-18.

As regards the IL-33 receptor, there exists a soluble free form of the chain specific to the ST2 receptor, sST2. It is derived from alternative splicing of the ST2 mRNA (13). The biological relevance of the sST2 chain has remained elusive: Is it, as suggested in some studies, a decoy receptor enabling IL-33 neutralization, or is it simply the signature of the bringing into play of the IL-33/ST2 axis, with the seric sST2 concentrations being correlated with the severity of the pathology under consideration (13–16).

All in all, these different elements demonstrate the existence of cross-regulation between the different IL-1 family cytokines, of which each member possesses a counter-regulatory receptor or ligand.

IL-1 Superfamily Members Differentially Depend on Processing Mechanisms to Function

IL-1 superfamily members (cytokines and receptors) contribute to a wide spectrum of immunological and inflammatory responses. Most of their members lack a signal peptide and are not immediately secreted. They are found diffusely in the cytoplasm as precursors containing cleavage sites and the difference that exists between members in terms of expression and proteolytic processing impact their respective roles *in vivo*. Indeed, to be biologically active, full-length precursors of IL-1β, IL-18, and IL-37 require intracellular processing, which depends on caspase-1, and the activation of the NOD-like receptor family, pyrin domain containing 3 (NLRP3)-inflammasome, required for conversion of the procaspase-1 into the active caspase-1. While pro-IL-36 and pro-IL-38 also need to be cleaved to

Abbreviations: AID, induced cytidine deaminase; ACLF, HBV acute-on-chronic liver failure; ALD, alcoholic liver disease; ALF, acute liver failure; Areg, Amphiregulin; BP, binding protein; CHB, chronic HBV; ConA, concanavalin A; DAMP, damage-associated molecular pattern; DT2, type 2 diabetes; EMT, epithelial-mesenchymal transition; ERK, extracellular signal-regulated kinases; HAV, hepatitis virus A; HBV, hepatitis virus B; HCV, hepatitis virus C; HCC, hepatocellular carcinoma; HFD, high fat diet; HIF, hypoxia-inducible factor; ICAM-1, intercellular adhesion molecule 1; IκB, inhibitor of kappa B; IL, interleukin; IL-1Ra, IL-1 receptor antagonist; IL-1R, IL-1 receptor; IL-1RAcP, IL-1 receptor accessory protein; IFNγ, interferon γ; ILC, innate lymphoid cell; iNKT, invariant Natural Killer T; IR, ischemia-reperfusion; IRS-1, insulin receptor substrate-1; KC, Kupffer cells; LSEC, liver sinusoidal endothelial cells; MAPK, mitogen-activated protein kinases; miR, microRNA; NAFLD, non-alcoholic fatty liver disease; NF-κB, nuclear factor- κB; NLRP3, NOD-like receptor family, pyrin domain containing 3; NLRP6, NOD-like receptor family, pyrin domain containing 6; NAPQI, N-acetyl-*p*-benzoquinone-imine; NK, natural killer; PAMP, pathogen-associated molecular pattern; PMN, polymorphonuclear neutrophils; PR3, proteinase 3; ROS, Reactive oxygen species; SIGIRR, single Ig IL-1 related receptor; ST2L, suppression of tumorigenicity 2 ligand; sST2, soluble ST2; TAM, Tumor-associated macrophages; TCR, T-cell receptor; Th, T helper; TLR, toll-like receptor; TLR/IL-1R, TIR domain TLR/IL-1R; TNF, tumor necrosis factor; Treg, T regulatory; TXNIP, thioredoxin-interacting protein; WT, wild-type.

TABLE 1 | IL-1 cytokine superfamily characteristics.

Cytokine name	Specific receptor chain name	Alternative receptor name	Co-receptor	Alternative co-receptor name	Function	Inhibitory ligand/Interference ligand
IL-1α, IL-1β	IL-1R1	CD121a	IL-1R3	IL-1RAcP	Pro-inflammatory	IL-1Ra, sIL-1R1
IL-1β	IL-1R2	CD121b	IL-1R3	IL-1RAcP	Anti-inflammatory	sIL-1R2
IL-18	IL-1R5	IL-18Rα, IL-2Rrp, CD218a	IL-1R7, and/or IL-R8 (inhibitory)	IL-18Rβ, IL-18RAcP, CD218b	Pro-inflammatory	IL-18BP
IL-33	IL-1R4	T1/ST2, ST2L, IL-1RL1, IL-33R	IL-1R3 and/or IL-R8 (inhibitory)	IL-1RAcP	Pro-inflammatory	sST2 (sIL1R4)
IL-36α, IL-36β, IL-36γ	IL-1R6	IL-1Rrp2, IL-1RL2, IL-36R	IL-1R3	IL-1RAcP	Pro-inflammatory	IL-36Ra
IL-36Ra	IL-1R6	IL-1Rrp2, IL-1RL2, IL-36R	IL-1R3	IL-1RAcP	Anti-inflammatory	
IL-37	IL-1R5	IL-18Rα, IL-2Rrp, CD218a	IL-1R8	TIR8, SIGIRR	Anti-inflammatory	
IL-38	IL-1R6	IL-1Rrp2, IL-1RL2, IL-36R	IL-1R9	IL-1RAPL, TIGIRR-2	Anti-inflammatory	
Not known	NA		IL-1R8	TIR8, SIGIRR	Anti-inflammatory	
Not known	NA		IL-1R9 and IL-1R10	IL-1RAPL, TIGIRR-2 TIGIRR, TIGIRR-1	Not known	

IL-1RAcP, Interleukin-1 Receptor Associated Protein; IL-1RAPL, Interleukin-1 Associated Protein Like 1/2; IL-1Ra, Interleukin-1 antagonist; IL-18BP, IL-18 Binding Protein; SIGIRR, Single Ig Interleukin-1 Related Receptor; TIGIRR, Three Ig Interleukin-1 Related Receptor; NA, not applicable.

generate a mature active form, the underlying mechanism does not depend on caspase-1 and remains unclear.

Remarkably, IL-1α and IL-33 are considered dual-function cytokines, meaning that in addition to their function as a classical cytokine, full-length IL-1α and full-length IL-33 (as part of damage-associated molecular pattern or DAMP) act as an "alarmin." IL-1α and IL-33 precursors are constitutively expressed in the nuclei of epithelial and endothelial cells from different organs such as kidneys, liver, and lung. Moreover, the nuclear location of IL-33 seems to correspond to a storage function, a supposition confirmed by observation showing that mice expressing IL-33 from which the nuclear anchoring area has been eliminated have a fatal systemic inflammatory response syndrome, which is due to the absence of cellular retention of IL-33 (17). As regards IL-1α, a steady-state shuttle of cytokines between nucleus and cytoplasm has been described (18). In the event of a pro-apoptotic signal, IL-1α leaves the cytoplasm, is closely bound with the chromatin complex, and has no pro-inflammatory biological activity. By contrast, in the event of a necrotic signal, IL-1α leaves the nucleus and moves toward the cytoplasm, as the necrotic cell releases pro-inflammatory IL-1α (18). To this day no similar phenomenon has been documented regarding IL-33.

Consequent to tissue damage, biologically active full-length IL-1α and IL-33 are rapidly released by necrotic cells to alert the immune system to the danger, resulting in rapid production of pro-inflammatory mediators and infiltration of polymorphonuclear neutrophils (PMN) initially followed by monocytes/macrophages to the insult site.

With the exception of IL-1α and IL-33, cytokine precursors of the IL-1 family generally require intracellular processing to generate active forms. However, extracellular processing also occurs, involving different proteases. Protease driven from PMN, including elastase and cathepsin G, as well as chymase from mast cells and macrophages, proteinase 3 from PMN and macrophages, granzyme B from natural killer (NK) cells, and meprims from epithelial cells, are able to convert pro-IL-1β, pro-IL-18, pro-IL-36 α, β, and γ into their corresponding fully active forms. Active pro-IL-33 can also be cleaved extracellularly by these proteases to generate a super active form (**Figure 1**).

Immune Cell Targets of IL-1 Superfamily Members

IL-1 Superfamily Members and Myelopoiesis Control

While IL-1 superfamily members have a role in the activation and mobilization of monocytes and in the recruitment of PMN, they also fulfill a critical function in the induction of emergency hematopoiesis leading to sustained myeloid M1 skewing. In this setting, IL-1α and IL-1β are both liable to be produced by the medullar environment. However, emergency hematopoiesis and its pro-inflammatory component are strongly associated with IL-1β, which mainly acts systemically (see section Concept of Trained Immunity and Innate Immunological Memory: The Place of the IL-1 Cytokine Family: trained immunity), whereas IL-1α in its role as cytokine/alarmin seems to exercise juxtracrine action (9, 19–21).

Cytokines of the IL-1 Superfamily and Control of T Helper 1 Effectors and NK Cells

IL-18 and IL-1 are implicated in the genesis of type 1 responses through the recruitment and differentiation of T helper (Th)1 lymphocytes and the activation of NK cells and of innate

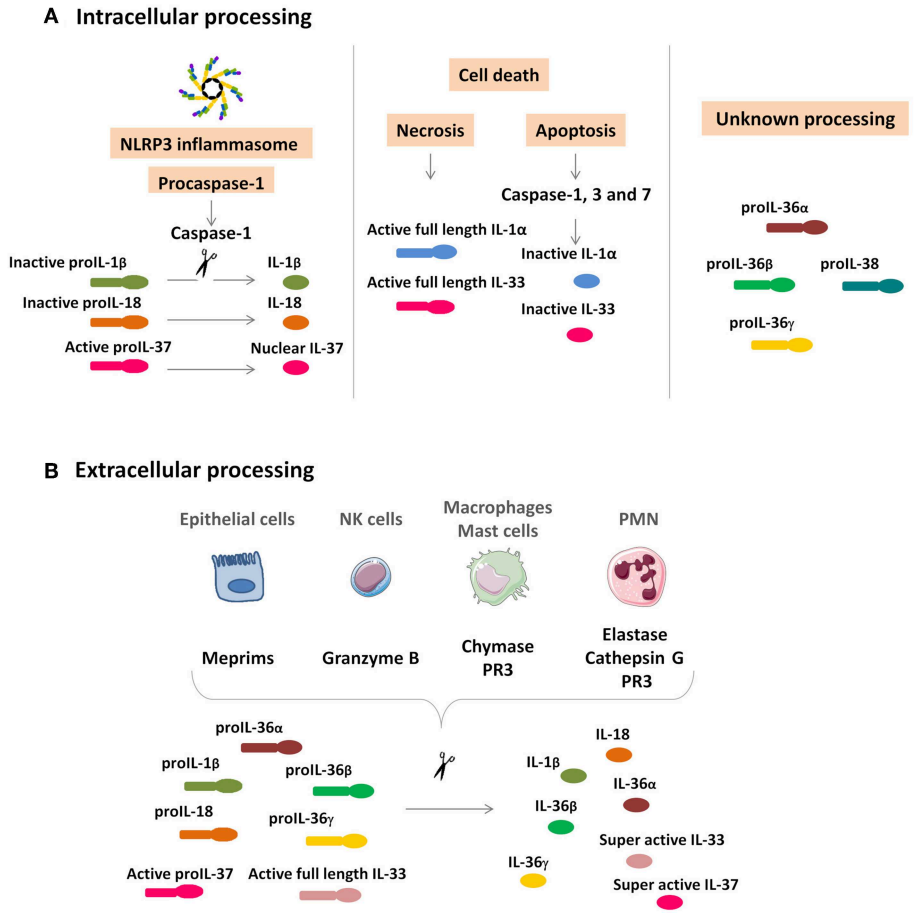

FIGURE 1 | IL-1 cytokine family intracellular and extracellular processing. **(A)** *Intracellular processing.* Caspase-1 cleavage from the NLRP3 inflammasome activates pro-IL-1β and pro-IL-18 and inactivates pro-IL-37, which is translocated in the nucleus. IL-1α and IL-33 are rapidly released by damaged necrotic cells in their functionally active full-length precursors and are inactivated by caspase-1, 3, and 7 cleavage. Intracellular processing of IL-36α, IL-36β, IL-36γ, and IL-38 are still unknown. **(B)** *Extracellular processing.* Epithelial cells, NK cells, macrophages, mast cells, and PMN secrete proteases to activate IL-1β, IL-18, IL-33, IL-36α, IL-36β, and IL-36γ. Unlike the intracellular processing, the cleavage of IL-33 and IL-37 results in a superactive form. NK, natural killer; PMN, polymorphonuclear neutrophils; PR3, proteinase 3.

lymphoid cells (ILC)1, particularly by inducing IFNγ production. In a mouse model, absence of IL-18 production or of its mature form leads to loss of anti-tumoral activity, loss that is partially objectified by the absence of the FasL-dependent cytotoxic function by hepatic NK cells, a circumstance favoring pulmonary and colorectal cancers (22).

The existence of antigen-specific and/or NK memory cells was recently described [for review (23)]. Remarkably, these are liver-resident NK cells serving as the support for memory responses in mice and, quite probably, in humans as well (23–25). That much said, in men as well as mice there exist several models suggesting that IL-18 is a factor implicated in the generation of antigen-specific and/or NK memory cells (26). However, study of mice genetically deficient in IL-18 or IL-18Rα shows that IL-18 is not essential in this function (24).

IL-1R8 seems to be an antagonist of IL-18 insofar as the effector/cytotoxic function of NK cells increase pronouncedly in mice genetically deficient in IL-1R8. Remarkably, these mice are resistant to the induction of hepatocellular carcinoma (HCC) or hepatic metastases of colorectal or pulmonary cancer. IL-1R8 at least partially exercises its antagonistic effects on the effector functions of NK cells by interfering with the IL-18/IL-18Rα/Myd88 signaling pathway. In humans, IL-37, of which the receptor consists in the IL-1R8 and IL-18Rα sub-units, is suppressive with regard to NK CD56(dim)CD57(+)FcεRγ(+)NKG2C(−) cells.

So it is that the cytokine/receptor couples IL-18/IL-1R5 and IL-37/IL-1R8 have opposed effects in the effector/cytotoxic functions of NK/cytotoxic cells.

According to organism site or the tissue, the effects of IL-1R8 favor or disfavor the emergence of infections and/or cancers. For example, IL-1R8 represents not only a protective factor as concerns the development of some colon cancers (11), infectious complications of keratitis (27), and acute *Pseudomonas aruginosa* pneumopathies (28), but also a promoting factor as concerns mammary cancer and HCC (10, 29). One interpretation of these

opposed effects is that in the absence of IL-1R8, an exacerbated inflammatory response (bringing into play the different cell types sensitive to IL-18/IL-33 and IL-37) favors solid tumorigenesis and tissue lesions during infections, while censoring of cancers in liver, particularly hepatic metastases, is partially counteracted by IL-1R8.

IL-18BP (Binding Protein) is also involved and blocks IL-18 action and seems critical as a means of limiting deleterious hepatic NK responses in hepatic aggression models (30, 31) (see Acetaminophen-Induced ALF and Post-Viral Hepatitis A ALF).

IL-1α/β and Th17 Lymphocyte Differentiation

IL-1 superfamily members are cytokines of critical importance in control of IL-17 production and in differentiation of Th17 lymphocytes and ILC3 (9, 32, 33). This function of IL-1 superfamily members is synergic with that of IL-23. As a result, IL-1R1-deficient mice are lacking in differentiation of Th17 lymphocytes/ILC3 and protected from experimental autoimmune encephalitis induction (34, 35).

The Complex Effects of IL-33 on the Dynamics of Immune Response

- Pro-Th2 effects: IL-33 is a key factor in ILC2 and M2 macrophage differentiation/maturation and also intervenes in Th2 lymphocyte differentiation. From that standpoint, IL-33 has a critical role in the immune responses of mucus epitheliums, as has been demonstrated in mouse models of bronchial hyper-reactivity, pulmonary allergies, and pulmonary response to *A fumigatus* (13). Remarkably, IL-33 on ILC2 induces expression of amphiregulin (Areg), a factor implicated in tissue repair (36, 37).
- The facilitating effects of Th1/cytotoxic/NK cell response: In addition to its effects on type 2 immunity, IL-33 plays a role in control of T cytotoxic and NK cell responses. Associated with IL-12, IL-33 induces IFNγ production by NK cells and seems to have a spectrum of action similar to that of IL-18 (25, 38, 39). According to the timing of stimulation and its chronicity at the level of the bronchial mucus, IL-33 will either favor a type 2 response by means of ILC2 or, on the contrary, induce a pro-Th1 NK cell response, as in the case of chronic lung inflammation due to tobacco in chronic obstructive pneumopathy disease (40). In a context of antiviral response, IL-33 serves as an amplification factor of effector cytotoxic T CD8 cell response by favoring the expansion of short live effector cells (41). Lastly, IL-33, in combination with IL-12, induces IFNγ production by invariant natural killer T (iNKT) cells (38, 42). From this standpoint, we may note the role of the IL-33/iNKT/IFNγ axis in the genesis of renal ischemia-reperfusion (IR) injury in animal models and in humans following transplantation (43–45).
- A role in the recruitment and acquisition of T regulatory lymphocytes' innate competences IL-33 is implicated in the recruitment of T regulatory (Treg) lymphocytes during renal IR injury (46), and is associated with improved renal IR injury tolerance. Furthermore, the eutrophic functions of Treg lymphocytes present in visceral adipose tissue are dependent on the production of IL-33 by the stromal cells in adipose tissue (47, 48). Lastly, IL-33, and to a certain extent IL-18, are liable to induce Areg production independently of the T cell receptor (TCR) (innate Treg function), thereby contributing to restorative functions, particularly those of the epitheliums, and also favoring tumor promotion and growth (36, 49).

Concept of Trained Immunity and Innate Immunological Memory: the Place of the IL-1 Cytokine Family

A series of recent studies has shown that non-specific stimulation of the immune system (pathogenic agent through *Mycobacterium tuberculosis* or BCG, oxidized low-density lipoproteins, Western diets…) induces modification of the pro-inflammatory immune response program driven by or dependent on myeloid cells (monocytes/macrophages) (19–21, 50, 51). Acquisition of this imprint depends on IL-1β. This inflammatory emergency hematopoiesis is implicated in the genesis of atherosclerosis lesions, metabolic syndrome, NAFLD or induction of tumor transformation, in particular by a promoting effect of epithelial-mesenchymal transition (EMT). It is the demonstration that this phenomenon leads to the constitution of an innate memory of cells of myeloid origin that led to the proposal of the new concept of "trained immunity." We have attempted here to apply the concept of trained immunity to liver diseases, based on the role of the IL-1 superfamily, first of all IL-1β but also IL-18 and IL-33, in modulating innate lymphoid immunity carried by NK cells, ILC or innate T-αβ lymphocytes.

CONTRIBUTION OF IL-1 SUPERFAMILY OF CYTOKINES TO HEPATIC DISEASES

Constitutive and Inducible Expression of Members of the IL-1 Superfamily in the Liver

In steady-state, the liver expresses the IL-1α and IL-33 cytokines/alarmins, IL-18 cytokine, the receptors functioning as decoys or inhibitors of these three cytokines: IL-1Ra, IL-18BP, and IL-1R2. While pro-IL-1α is expressed in hepatocytes, pro-IL-18 is expressed in Kupffer cells (KC). IL-33 is constitutively expressed in liver vascular endothelial and liver sinusoidal endothelial cells (LSEC) (52). Such constitutive expression may have major implications during liver injuries in which cell death by necrosis is predominant and a rapid immune response is required.

Expression in steady-state in liver also occurs for anti-inflammatory factors such as IL-1Ra, which antagonizes IL-1-related functions and modulates a variety of immune and inflammatory responses (53) or IL-18BP, of which the spontaneous production neutralizes locally produced basal IL-18. The sources of IL-18BP in the liver seem to be the hepatocytes themselves, KC and hepatic stellate cells (HSC) (31). The soluble form of IL-1 receptor accessory protein (IL-1RAcP) is constitutively produced by the liver and forms a complex with the

soluble IL-1RII, which binds and neutralizes IL-1β (8, 54). IL-33 and ST2 are also constitutively expressed in the normal liver (55).

Under pathological conditions that drive liver damage, IL-1 superfamily members are up-regulated. IL-1 and IL-1Ra/IL1-R1 are expressed in almost all cells, especially in activated non-parenchymal cells such as KC. As an alarmin, IL-33 is rapidly released from LSEC and vascular endothelial cells following carbon tetrachloride or Concanavalin A (ConA)-induced hepatitis (52) to mediate a pro-inflammatory response. In response to these danger signals, IL-33 may also be newly secreted in hepatocytes and/or HSC. Its specific receptor ST2 (IL-1 receptor-like 1) and co-receptor IL-1RAcP constitutively expressed on innate immune cells contribute to a rapid immune response.

Acute Liver Failure

Acute liver failure (ALF) is defined as the rapid development (within days or weeks) of severe liver injury with impaired liver function and hepatic encephalopathy (56). ALF is a life-threatening condition and refers to a wide variety of causes, among which toxin/drug-induced (usually acetaminophen) liver damage or viral hepatitis (hepatitis A, B, and E) are most common in the United States and Europe (56, 57).

The Contributions of Experimental Models

In vivo studies in animals have shown the existence of mechanisms common to the different ALF models. So it is that cell death by necrosis results in rapid IL-1α precursor release, upregulation of IL-1β and IL-18 leading to tissue injury through the IL-1R/IL-18R-MyD88 pathway (58–60) with massive upregulation of anti-inflammatory molecule IL-1Ra (61). Following ALF, IL-1α, IL-1β, and IL-18 all up-regulate the pro-inflammatory process through a dramatic decrease in hepatic inhibitor of kappa B (IκB) levels and NF-κB pathway activation, leading to IL-6 and TNFα secretion, which contributes to apoptosis, and ultimately to liver damage and animal death (60).

Lipopolysaccharide-induced ALF

In the study by Tsutsui et al. (62) KC were shown to be the main producers of IL-18, acting not in a caspase-1-dependent manner but upon Fas Ligand stimulation, thereby increasing IFNγ and TNFα production, which causes substantial acute liver damage. Furthermore, Yan et al. demonstrated a correlation between IL-1 and MMP9 expression implicated in extracellular matrix degradation, sinusoidal collapse, leading to parenchymal cell death, and loss of liver function after ALF induction (63). Inhibition of the IL-1 pathway (using adenovirus IL-1Ra) before the induction of ALF led to a significant reduction in plasma levels of hepatic enzymes and to animal survival improvement (64). Lastly, in humans, treatment by recombinant human IL-1Ra (Anakinra) improved survival of patients with acute liver injury in a post-septic situation (65).

Acetaminophen-induced ALF

In the acetaminophen (acetyl-*p*-Aminophenol, APAP)-induced acute liver injury model, KC were shown to be the

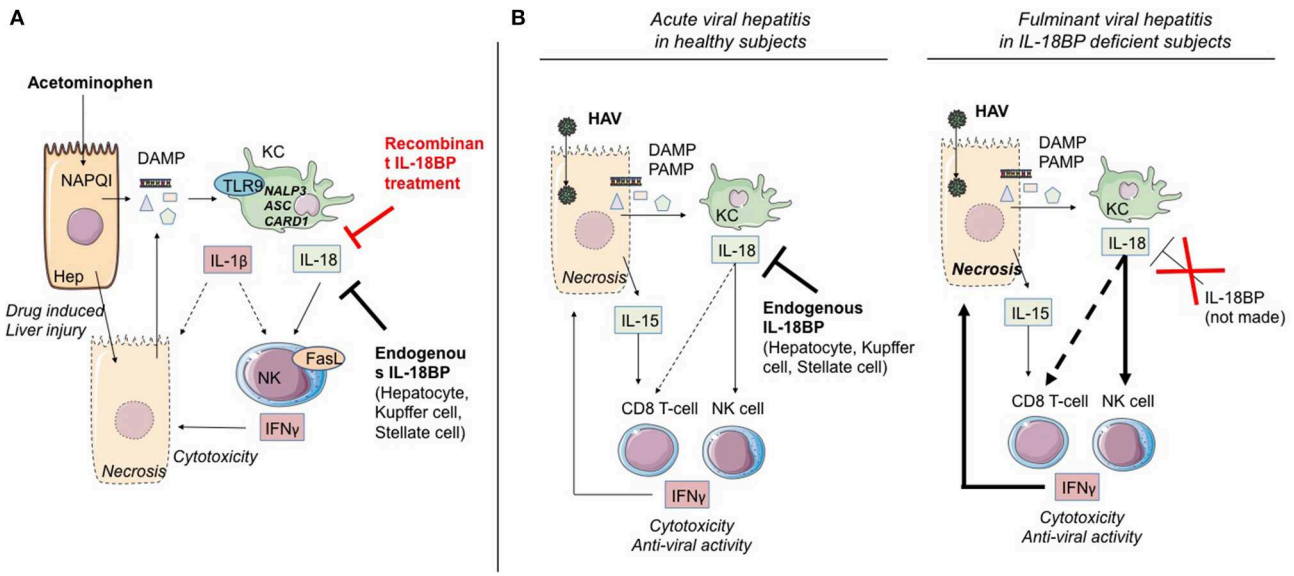

FIGURE 2 | IL-1α/β and IL-18 members are involved in ALF. **(A)** *Acetaminophen-induced ALF*. NAPQI (N-acetyl-*p*-benzoquinone-imine) is a toxic acetaminophen metabolite generated by hepatic cytochrome Cype2e1 and Cyp1a2. Under NAPQI action, injured and necrotic centrilobular hepatocytes secrete DAMP. Among them, ATP and TLR9 receptor ligand induce IL-1β and IL-18 production by Kupffer cells. Both cytokines drive NK and iNKT cell killing function through FasL and IFN-γ production. This inflammatory process can be blocked either by endogenous IL-18BP production by non-damaged hepatocytes, Kupffer and stellate cells, or by recombinant IL-18BP treatment. **(B)** *Critical role of IL-18 in HAV fulminant hepatitis.* HAV-infected hepatocytes produce IL-15 as well as PAMP and DAMP which, in turn, activate IL-18 production by Kupffer cells. IL-18 activates IFN-γ production by NK cells and CD8 T-cells with innate functions, hence promoting cytotoxicity toward infected hepatocytes. In healthy infected subjects (left), endogenous IL-18BP blocks the deleterious IL-18 action and modulates NK cell and probably CD8 T-cell activation. In IL-18BP deficient subjects (right), there is no inhibition of the deleterious effects of NK cell and CD8 T-cell activation. ALF, acute liver failure; KC, Kupffer cells; NK, natural killer; Hep, hepatocyte; bounded arrow, presumably yes.

main producers of IL-1β and IL-18, in a TLR9-dependent induction and NALP3-ASC-caspase-1-dependent manner increasing/inducing IFN-γ and TNF-α production by Th1 and NK cells, which causes substantial acute liver damage (30, 62, 66) (**Figure 2A**). Overexpression of IL-18, known to induce FasL expression on NK cells and CD4(+) T cells, also increased expression of Fas on hepatocytes, which were thereby sensitized to NK cytotoxicity. These Fas/Fas Ligand interactions induced hepatocyte apoptosis, massive periportal fibrosis, inflammation, and severe liver failure (67). Remarkably, IL-18-deficient mice were resistant to ALF induction by APAP, and blocking of IL-1β by a neutralizing antibody reduced ALF severity (68). Moreover, using the same model, it was recently shown that treatment of mice by IL-18BP, which blocks the binding of IL-18 to its receptor, provided protection from induction of hepatic lesions, thereby confirming the critical role of IL-18/IL-18R-NK cells (30).

To further explore the mechanisms involving the IL-33/ST2 axis in ALF, Antunes et al. (69) used a mouse model of APAP-IL (-induced liver) failure and showed that liver necrosis was associated with massive IL-33 and chemokine release. Non-parenchymal liver cells were the main sensors of IL-33, and the inflammatory response triggered by IL-33 was amplified by liver PMN infiltration. In this model, IL-33 may have acted in synergy with IL-18 in the recruitment of NK cells.

IL-36 is induced after treatment with acetaminophen, presumably in hepatocytes, and up-regulates chemokine ligand 20, a chemokine implicated in tissue protection and repair. Administration of the antagonist IL-36Ra aggravates liver damage and disturbs tissue recovery (70).

Poly-I:C-induced ALF

In a mouse model of ALF induced by poly(I:C), IL-33 expression was up-regulated and correlated with severe liver injury. Interestingly, iNKT cell-deficient mice exhibited protection against poly(I:C)-induced hepatitis accompanied by an increased number of IL-33-positive hepatocytes compared with wild-type (WT) controls (52, 71).

Liver IR injury

Ischemia, followed by reperfusion at the time of liver graft implantation, leads to what is called IR injury. Understanding of the mechanisms involved could help to identify novel therapeutic targets in view of improving organ survival. In mice and rats, warm ischemia followed by reperfusion represents the most common model to study IR injury.

On the one hand, IL-1β and the NLRP3 inflammasome are implicated in the lesions of warm IR injury *via* high-mobility group box 1, NF-κB and toll-like receptor (TLR)4 (72). Reactive oxygen species (ROS) mediate inflammasome activation in KC (73). On the other hand, it has been suggested that NLRP3 could be involved in IR injury independently of inflammasomes through PMN recruitment (74). Sadatomo et al. (75) showed in a murine model that macrophages secreted pro-IL-1β, which is activated by neutrophils-derived proteinases. Interaction between neutrophils and macrophages promoted IL-1β maturation and causes IL-1β-driven inflammation in the IR liver.

Regarding the role of IL-33 and its receptor ST2 in warm IR injury, Yazdani et al. (76) demonstrated in a warm IR injury murine model (confirmed in human liver resection specimens) that IL-33 release from LSEC increases sterile inflammation and results in PMN extracellular trap formation, while administration of recombinant IL-33 during IR exacerbates hepatotoxicity and inflammation. One may note that iNKT cells mediate hepatic IR injury by promotion of intrahepatic PMN influx (77), similarly to what has been shown in models of IR injury involving other organs, such as the kidney (43). Interestingly, preconditioning with intra-peritoneal injection of IL-33 (55) showed that IL-33 has a protective effect on hepatocytes, with decreased liver IR injury *via* the activation of NF-κB, p38 MAPK, cyclinD1, and Bcl-2. Sakai et al. (78) used a mouse model of IR with injection of recombinant IL-37 at the time of reperfusion. They demonstrated that IL-37 protects against IR injury by reducing pro-inflammatory cytokine and chemokine production by hepatocytes and KC, and suppression of PMN activity.

Observational Clinical Studies

The pro-inflammatory/anti-inflammatory balance of IL-1 family members determines the clinical outcome

Clinical observations first revealed the contribution of the IL-1 family of cytokines to the pathophysiology of ALF. Significant increase in serum levels of the pro-inflammatory cytokine IL-1α (79), IL-1β (80), IL-18 and its activator Caspase-1 (81), IL-33 (82) positively correlates with acute hepatitis in humans. Both highly significant elevation in IL-1α levels (79) and reduction in the IL-1Ra over IL-1β ratio (IL-1Ra/IL-1β) (80) have been detected in ALF patients with a fatal outcome. These findings show that the IL-1 family members brought into play are systematically associated in the event of a fatal outcome with loss of balance in the liver between inflammatory signals IL-1α/β/IL-18 and anti-inflammatory IL-1Ra.

Upregulation of IL-33 and sST2 in serum has been shown to exist in ALF and acute-on-chronic liver failure in patients (82), correlating with the intensity of necrosis as assessed by transaminase activity. Interestingly, sST2 was elevated in acute and acute-on-chronic liver failure, but not in chronic liver failure, suggesting that sST2 could be a tool to monitor the course of the disease.

Post-viral hepatitis A ALF

Less than 1% of acute hepatitis A virus (HAV) infections result in acute fulminant hepatitis. A few cases of post-AHV ALF family isolates have been reported, suggesting the role of a genetic factor implicated in the determinism of this pathology. The study by Belkaya et al. (31) on an 11-year-old patient having died from fulminant HAV hepatitis, with no previous personal of familial medical history, identified a deletion of 40 nucleotides of the gene coding IL-18BP, leading to instability at the mRNA level, and loss of the normal structure, or the absence of IL-18BP.

During the acute phases of viral hepatitis, particularly HAV, massive secretion in patients' serum of IL-18 and IL-15 has

been observed (83). Interestingly, the secretion is accompanied by the appearance of a cell population consisting in innate CD8 T lymphocytes (whose expansion and cytotoxic function are independent of TCR engagement) in the blood and the liver (83). It is highly likely that acquisition of this "innate memory" phenotype depends, as has been described above (section Cytokines of the IL-1 Superfamily and Control of T Helper 1 Effectors and NK Cells), on the joint action of IL-18 and IL-15. Belkaya et al. (31) went on to show that in the patient having succumbed to post-HAV ALF, the aforementioned absence of IL-18BP was accompanied by excessive activation of NK cells, activation that depended on IL-18. On the basis of their analysis of this clinical case, the authors hypothesized that the IL-18BP deficiency could partially explain the fulminant viral hepatitis (**Figure 2B**).

To sum up, the different findings from experimental models and from clinical situations involving ALF underline the importance of the implication of NK/T CD8 cells by means of IL-18 and IL-1 and confirm the decisive role of "negative" regulatory elements (IL-1R8, IL-18BP, IL-37) whose appearance attenuates or reduces the severity of acute hepatic inflammation (**Figure 2**).

Alcoholic Liver Diseases

Alcoholic liver diseases (ALD) includes liver manifestations due to alcohol overconsumption: fatty liver, alcoholic hepatitis, and chronic hepatitis with liver fibrosis or cirrhosis.

Alcohol acts as an "exogenous signal" on KC through TLR with activation of the inflammasome NLRP3-caspase 1 and production of IL-1β. A second "endogenous signal" leading to activation of the same pathway, is the release of two DAMP: ATP and uric acid by damaged hepatocytes because of alcohol damage (84). Upregulation of IL-1β activity leads to inflammation, steatosis, and additional damage (85, 86) (**Figure 3**).

Recently, Heo et al. (87) demonstrated in human and murine liver samples that alcohol decreases microRNA (miR)-148a through the transcriptional regulator forkhead box protein O1. TXNIP (thioredoxin-interacting protein) is a direct target of miR-148a and is overexpressed during alcoholic liver disease, activating NLRP3 inflammasome in hepatocytes, and caspase 1-mediated pyroptosis. Hepatocyte-specific delivery of miR-148a to mice abrogates alcohol-induced TXNIP overexpression and inflammasome activation (**Figure 3**).

In human patients with alcoholic liver disease, sST2 but not IL-33 was correlated to the severity of the disease (88). In the same fashion, Wang et al. (89) showed with IL-33- and ST2- deficient mice that ST2 decreases the inflammatory activation of hepatic macrophages by inhibiting NF-κB in alcoholic liver disease, in an IL-33-independent manner. However, during severe liver injury, massive cell death, and release of IL-33 triggers IL33/ST2 signaling and increases tissue damage, presumably by local activation of different cells expressing ST2, namely hepatic NK cells, iNKT cells, ILC2, and Treg lymphocytes.

During ALD, one target of IL-1β, and possibly IL-33, is the iNKT cell population (86). From this standpoint, it is interesting to note that mice genetically deficient in Jα18 T cells or CD1d, and consequently without iNKT lymphocytes, are at least partially protected from ALD (90, 91). In these models, iNKT lymphocytes are responsible for PMN influx during ALD progression.

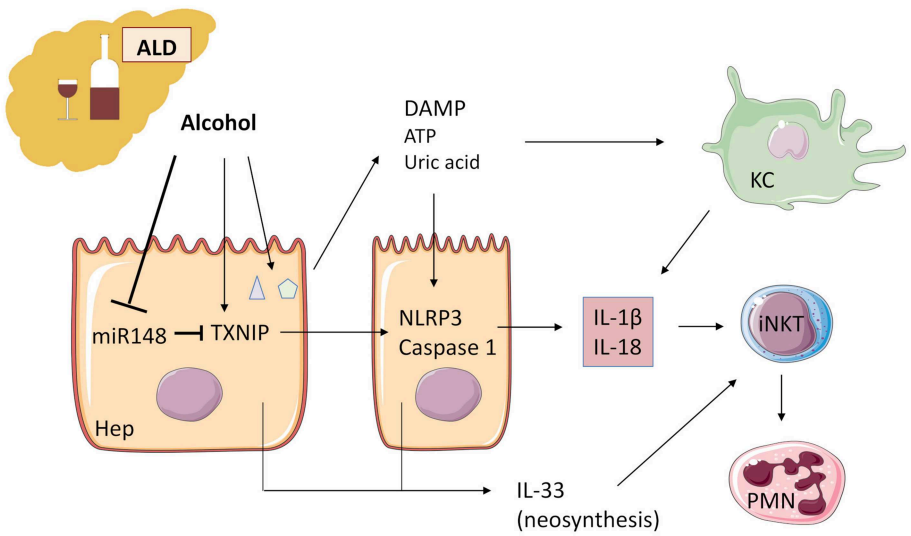

FIGURE 3 | Alcohol acts as an "exogenous signal" on Kupffer cells to activate the inflammasome NLRP3-caspase 1 and production of IL-1β in ALD. Alcohol decreases microRNA miR-148a, a negative regulator of TXNIP (thioredoxin-interacting protein), which is overexpressed during ALD. TXNIP activates NLRP3 inflammasome in hepatocytes and caspase 1-mediated pyroptosis, leading to IL-1β and IL-18 production. ATP and uric acid release by damaged hepatocytes after alcohol damage is a second "endogenous signal" leading directly and/or through Kupffer cell activation to inflammatory cytokine production. IL-1β and IL-18 recruit and activate iNKT cells that, in turn, induce PMN afflux and hepatic injury. IL-33 released by damaged and necrotic hepatocytes exacerbates iNKT cells and PMN recruitment and activation. ALD, alcoholic liver disease; KC, Kupffer cells; iNKT, invariant natural killer T-cells; PMN, polymorphonuclear neutrophils; Hep, hepatocyte.

Non-alcoholic Fatty Liver Disease

With prevalence of 30% for adults and 10% for children in Western countries, non-alcoholic fatty liver disease (NAFLD) is a growing public health issue frequently associated with morbid obesity, type 2 diabetes, and metabolic syndrome (92, 93). NAFLD is characterized by excessive fat accumulation in the liver associated with insulin resistance, and is defined by the presence of steatosis in hepatocytes. NAFLD ranges from simple steatosis or non-alcoholic fatty liver to a more serious form called non-alcoholic steatohepatitis, which is characterized by liver inflammation and tissue damage that can lead to fibrosis, cirrhosis, and hepatocellular carcinoma (94).

There is increasing evidence that the induction of inflammation and production of inflammatory mediators released from the adipose tissue of obese subjects, such as adipocytokines and classical cytokines contribute to obesity-induced NAFLD, in which IL-1α/β plays a key role (**Figure 4**) (95, 96). In fact, NAFLD is the last step of low-grade inflammation depending on the bringing into play of "trained immunity".

Free fatty acids, acting as DAMP, are capable together with TLR ligands present in gut microbiota of activating NLRP3 and NLRP6 inflammasomes, thereby inducing caspase-1 activation and the release of IL-1α, IL-1β, and IL-18 in hepatocytes (97, 98). IL-1α/β, in turn, activates inflammasome, TNFα and IL1β and IL-18 release in KC (98). Other extra-hepatic cell types such as adipocytes could also be important sources of IL-1α and IL-1β during NAFLD associated with obesity (99).

IL-1β promotes hepatic steatosis by stimulating triglyceride and cholesterol accumulation in primary liver hepatocytes and lipid droplet formation (96) and acts on LSEC to promote liver inflammation by upregulating intercellular adhesion molecule

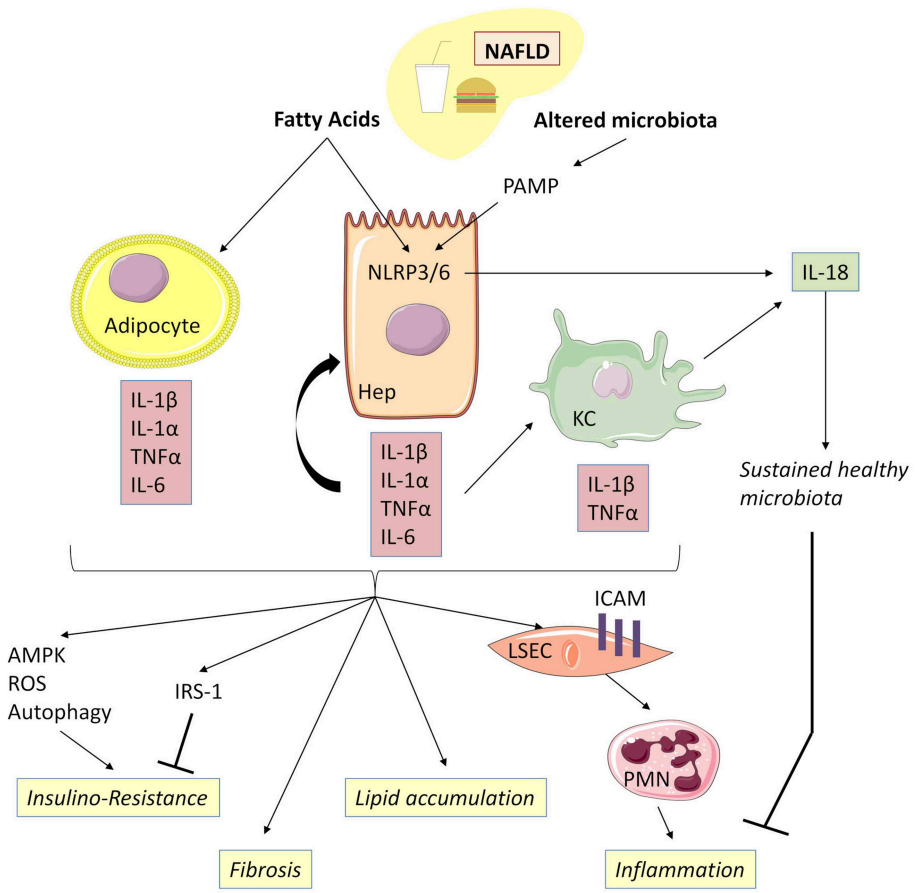

FIGURE 4 | IL-1β, together with TNFα, mediates low-grade inflammation during NAFLD. Free fatty acids, acting as DAMP, are capable together with TLR ligands of activating NLRP3 and NLRP6 inflammasomes, thereby inducing caspase-1 activation and release of IL-1α and IL-1β in hepatocytes and Kupffer cells. Adipocytes could be an important source of IL-1α and IL-β during NAFLD associated with obesity. Adipocyte-derived IL-1β has been shown to directly induce insulin resistance in hepatocyte development of insulin resistance in adipocytes enabling accumulation of lipids in the liver. IL-1β is also capable of reducing insulin receptor substrate-1 (IRS-1) expression dependently and independently of ERK activation and of inducing impairment in insulin signaling and action. IL-1β is released by hepatocytes through inflammasome activation and, in turn, amplifies inflammasome activation, TNFα, and IL-β release in Kupffer cells. Increased IL-1β and NLRP3 drive liver fibrosis in experimental models of NAFLD mice. IL-1β acts on LSEC to promote liver inflammation by upregulating ICAM-1 (intercellular adhesion molecule 1) expression, which stimulates neutrophil recruitment in the liver. IL-18 production negatively regulates NAFLD/NASH progression *via* modulation of the gut microbiota. NAFLD, Non-alcoholic fatty liver disease; KC, Kupffer cells; LSEC, liver sinusoidal endothelial cells.

1 expression, which stimulates neutrophil recruitment in the liver (100). Moreover, IL-1β produced by the liver, together with pro-inflammatory IL-6 and TNFα, contributes to the activation of resident immune cells and the recruitment of other leucocytes to the damaged liver, leading to chronic inflammation (101). Accordingly, IL-1β and/or IL-1α-deficient mice have demonstrated less diet-induced inflammation and liver fibrosis, as attested by lower serum transaminases and serum amyloid alpha concentrations, as well as decreased expression of inflammatory and fibrosis transcripts such as *IL-6*, *TNFα*, *P-Selectin*, *Cxcl1*, and *TGFβ*, as compared to WT controls (102).

IL-1α/β are upregulated in the adipose tissue of obese and insulin-resistant mice. Together with TNFα and IL-6, IL-1α/β, in turn, contributes to the development of insulin resistance in adipocytes through downregulation of insulin receptor substrate-1 expression, thereby facilitating accumulation of lipids in the liver (103). Moreover, adipocyte-derived IL-1β has been shown to directly induce, alone or in combination with TNFα, insulin resistance in hepatocytes *via* AMPK-ROS signaling and autophagy (104, 105). Accordingly, IL-1α and IL-1β -deficient mice have lower fasting glucose and insulin levels and improved insulin sensitivity (106).

Of note, IL-1α deficiency increased both hepatic and systemic cholesterol levels, suggesting that liver fat storage and inflammation are not necessarily parallel events and that some hepatic lipids might support anti-inflammatory functions (102). IL-1Ra-deficient mice exhibited severe steatosis and pericellular fibrosis containing many inflammatory cells, as observed in steatohepatitis histological lesions in humans, following 20 weeks of feeding with an atherogenic diet (107). These different observations, particularly as regards the role of IL-1Ra in NAFLD, raise the question of the interest of therapeutic utilization of human recombinant IL-1Ra (Anakinra) in the natural history of NAFLD.

Henao-Mejia et al. (108) have reported that changes of gut microbiota linked to NLRP3 and NLRP6 inflammasome deficiency were associated with increased hepatic steatosis and inflammation. Interestingly, IL-18 production negatively regulates non-alcoholic steatohepatitis progression *via* modulation of the gut microbiota. This observation strongly highlights the role of the maintenance of digestive microbiome homeostasis in the genesis of "metabolic syndrome" and NAFLD (108).

Regarding new members of the IL-1 superfamily, IL-33 has not been widely explored. In a murine model, a high fat diet (HFD)-induced steatohepatitis was associated with upregulation of IL-33 but IL-33 deficiency did not affect severity of liver inflammation or liver fibrosis, suggesting that endogenous IL-33 has no effect on the progression of fibrosis during experimental steatohepatitis (109). Treatment with recombinant IL-33 in mice attenuated diet-induced hepatic steatosis but aggravated hepatic fibrosis in a ST2-dependent manner (110). The pro-fibrotic effect of pharmacological IL-33 treatment in HFD mice was confirmed in another study (111), where galectin-3 enabled upregulation of the IL-33/ST2 pathway and production of IL-13 by peritoneal macrophages.

Autoimmune Hepatitis

Autoimmune hepatitis (AIH) is a chronic inflammatory disease of the liver of which the pathogenic mechanisms have yet to be unraveled. Treatment relies mainly on lifelong immunosuppressive therapy and liver transplantation in the final stage. Acute hepatitis induced with ConA is the most widely used mouse model of AIH (**Figure 5**). In this model, massive activation of immune cells induces massive local production of cytokines such as IL-4, IFNγ, and TNFα (112–114), which are directly implicated in hepatocyte death (113). iNKT lymphocytes are of major importance in acute hepatitis induced with Con-A, as is attested in works showing that iNKT lymphocyte-deficient mice resist hepatitis induction (115). Aside from their cytokine production, the pathogenic effect of iNKT lymphocytes depends on their cytotoxic functions, which bring into play perforin and granzymes as well as FasL/Fas and TRAIL/DR5 interactions (115, 116). Depletion of KC prior to ConA treatment attenuates hepatic injury (117). Activation of iNKT lymphocytes may consequently depend on KC, thereby ensuring a role of auto-antigen presentation. Another pathogenic mechanism may consist in activation of KC by iNKT lymphocytes, leading to production by the KC of TNFα, ROS and IL-1β depending on the activation by ROS of the NLRP3 inflammasome (118). On this subject, it is interesting to note that in this model, treatment of mice by human recombinant IL-1Ra (Anakinra) at least partially prevents hepatic lesions (118).

IL-33 is rapidly neosynthesized (6 hours) in the hepatocytes after treatment by ConA (116, 119) (**Figure 5**). Interestingly, this expression of IL-33 depends on the presence of iNKT lymphocytes and possibly on TRAIL/DR5 interactions between iNKT lymphocytes and hepatocytes (116). IL-33 production seems to moderate hepatitis severity, because: (i) IL-33-deficient mice have more heavily damaged livers than WT mice (52, 71, 116, 119, 120); (ii) injection of recombinant IL-33 renders less severe the hepatitis induced by ConA (120). Biologically, the increased severity of hepatitis lesions in IL-33-deficient mice is associated with pronounced expression of TNFα and IL-1β and is accompanied by accumulation in the liver of activated NK cells (119).

The protective action of IL-33 in the model of ConA-induced hepatitis may be attributed to the induction of anti-apoptotic factors such as BAX or Bcl2 (120). Another element associated with the protective action of IL-33 may be the recruitment in the liver of Treg lymphocytes [TCR-αβ(+)CD4(+)CD25(+)FoxP3(+)] ST2(+) (119). One is tempted to suggest that these Treg ST2(+) cells contribute to hepatic repair by expression of trophic factors such as Areg, as previously described in other tissues (36, 121).

Viral Hepatitis B, C

According to the World Health Organization, 325,000,000 persons are currently living with a chronic infection of either hepatitis virus B (HBV) or hepatitis virus C (HCV). Infection with HBV rarely leads to acute liver failure. Chronic infections with both HBV and HCV are responsible for progressive fibrosis and cirrhosis, as well as development of hepatocellular carcinoma.

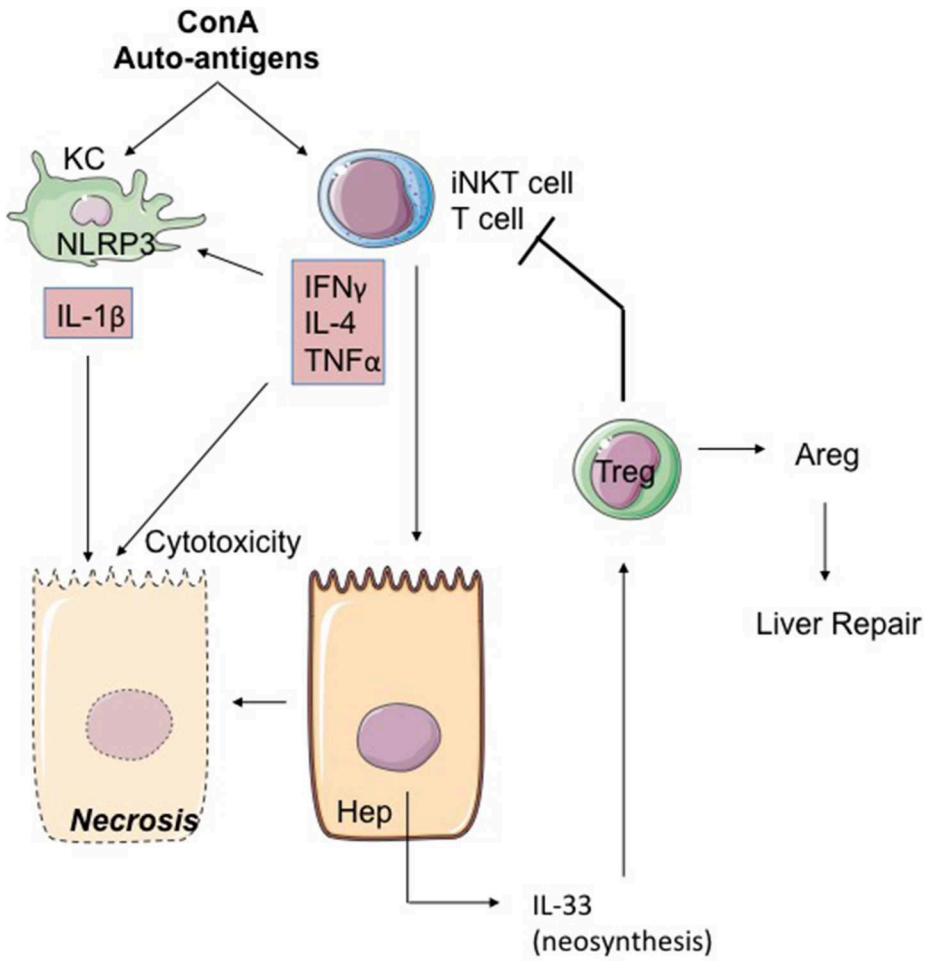

FIGURE 5 | The ConA model of hepatitis. The mitogen Concanavalin A (ConA) activates iNKT cells in a KC and iNKT cell-dependent mode. IL-4, IFNγ, and TNFα produced by hepatic activated iNKT cells activate the NLRP3 inflammasome leading to toxic IL-1β production by hepatocytes. iNKT cells could also induce IL-33 production by hepatocytes through TRAIL/DR5 interactions. Secreted IL-33 acts as a retro-negative regulator of iNKT cells by recruiting Treg. Indeed, Treg can restrain T-cell cytotoxicity and promote repair through signals like Areg. ConA, concanavalin A; Areg, Amphiregulin; KC, Kupffer cell; Hep, hepatocyte.

IL-1β and IL-18 may play a role in liver inflammation in the setting of HCV infection. Monocyte-derived human macrophages and Kupffer cells (122) produce IL-1β and IL-18 in response to HCV infection: HCV induces pro-IL-1β and pro-IL-18 in macrophages *via* the NF-κB signaling pathway. It has been shown that viral RNA can trigger MyD88/TLR7 pathway, thereby inducing IL-1β expression. HCV concomitantly activates the NLRP3 inflammasome, leading to IL-1β secretion (123).

It has been shown in humans that with their IFNγ production, iNKT lymphocytes inhibit the replication of HCV in hepatocytes (124) and that over the course of HBV infection, recruitment of iNKT lymphocytes occurs at the level of the liver (125–127). Lastly, in a model of HBV-transgenic mice, the authors showed that IL-18 induces repression of viral replication in a context depending on local recruitment of NK cells and iNKT lymphocytes (128). Interestingly, when infection becomes chronic, hepatic iNKT lymphocytes are progressively reduced in number (127), conceivably resulting in a loss of sensitivity of iNKT lymphocytes to IL-18 and/or IL-33.

From this standpoint, IL-33 expression in LSEC significantly increases in patients with chronic HBV (CHB) infection, and is likewise highlighted in the hepatocytes of lesions/inflammatory foci in patients with the most severe form of hepatitis (129). Seric IL-33 and ST2s concentrations are highly elevated compared to those in healthy donors and, interestingly enough, they are correlated with the severity of hepatic cytolysis (129, 130). In HBV acute-on-chronic liver failure (ACLF) (129), seric IL-33 and ST2s concentrations are pronouncedly higher than the concentrations observed in CHB patients. The circulating monocytes in ACLF patients present a highly activated and pro-inflammatory phenotype, which is correlated with IL-33 production. For that reason, it has been suggested that IL-33 increases inflammation and disease severity through monocyte activation, TNFα, IL-6, and IL-1β secretion. Similarly, IL-33

levels in human patients correlate with HCV RNA and liver damage (131). Such IL-33 production, particularly in the early phases of infection, can act in synergy with IL-18 and IL-1α/β in the recruitment of NK, iNKT, and T cytotoxic cells. And given the chronicity of its production, IL-33 is also liable to induce a state of systemic inflammation and, locally, a phenomenon of immuno-subversion through recruitment of Treg lymphocytes and ILC2 as opposed to recruitment and activation of NK and iNKT cells.

Fibrosis

Liver fibrosis, the final stage of most types of chronic liver diseases, characterized by the excessive accumulation of extracellular matrix proteins, including collagen, is a consequence of extracellular matrix hepatic stem cell activation. Gieling et al. (132) established that IL-1 activates hepatic stem cells that produce MMP-9 and control the progression from liver injury to fibrogenesis. Advanced liver fibrosis results in cirrhosis with progressive liver failure and development of portal hypertension.

Marvie et al. (133) showed that in chronic hepatitis, in the model induced by CCl_4, IL-33 and ST2 expression correlates with fibrosis (133). In humans suffering from chronic hepatitis, there also exists at the level of the liver a correlation between IL-33 cellular expression and fibrosis severity (133). In models of fibrosis induced by CCl_4, thioacetamide, and bile duct ligation, increased hepatic IL-33 expression in activated HSC has been confirmed. Remarkably, fibrosis lesions are absent or non-severe in IL-33-deficient mice, thereby underscoring the major role of IL-33 in the manifestations of fibrosis (134). Lastly, this study suggests that the profibrosis function of IL-33 depends on its action in the activation and expansion of liver ILC2 (134). In the absence of IL-13 produced by ILC2, CCl_4 does not induce hepatic fibrosis.

The fibrosis lesions described in the aforementioned works depend on the impact of environmental, chemical or physical stress. It may be the case that in the induction of hepatic fibrosis coming about during NAFLD, IL-33 is non-indispensable. This hypothesis is premised on the fact that in a model of hepatic fibrosis induced by HFD, there is no difference in fibrosis severity between WT and genetically IL-33-deficient mice (109).

Hepatocellular Carcinoma (See Figure 6)

As described in section Cytokines of the IL-1 Superfamily and Control of T Helper 1 Effectors and NK Cells, the IL-18/IL-18R axis is a checkpoint driven by the immunological components controlling carcinogenesis and hepatic metastases. In addition to NK lymphocytes, iNKT lymphocytes are of key importance in the censoring of hepatic metastases (135, 136). As a result, iNKT lymphocytes strongly express IL-18 and IL-33 receptors (38, 39, 137) and may consequently be implicated in the anti-tumoral effect of the IL-18/IL-18R axis. Moreover, influx in the liver of iNKT lymphocytes depends on the expression of CXCL16 by LSEC, that is to say cells of which another characteristic is nuclear expression of IL-33. The study by Ma et al. (135) shows that recruitment of anti-tumoral iNKT lymphocytes in the liver is indirectly controlled by the digestive microbiota and by the metabolites produced by the latter, metabolites that will induce CXCL16 expression by LSEC. From this standpoint, it should be pointed out that there exists an accumulation of iNKT CD4(+) cells with a type Th2 profile in HCC liver (138). Enriched with iNKT cells, its infiltration is correlated with HCC progression, attesting to the loss of effectiveness of the anti-tumoral activity of iNKT lymphocytes in a tumor niche during the natural history of HCC.

Several works in mouse models have shown that endogenous IL-33 is an adjuvant factor in anti-tumoral and anti-metastatic immunity, including at the level of the liver (139–141). The limitation of these studies resides in the utilization of metastasis models, some of which use HCC cell lines but do not reproduce the natural history of HCC.

Even though HCC may develop in healthy liver parenchyma, in most cases it develops in chronic inflammatory liver parenchyma in pathology secondary to the induction of trained immunity depending on IL-1β production (NAFLFD, ALD, Viral chronic hepatitis, hepatic fibrosis). As we previously discussed, the liver possesses its own means of induction and control of oncogenesis. Consequently, the process of transformation/oncogenesis at the hepatic level is partially dependent on IL-1β and will avail itself of amplification paths.

Recently, Zhang et al. (142) demonstrated in cell cultures, mouse models, and human samples the existence of a hypoxia-inducible factor (HIF)-1α/IL-1β signaling loop between cancer cells and tumor-associated macrophages (TAM) in a hypoxic microenvironment, resulting in cancer cell epithelial-mesenchymal transition and metastasis. When exposed to moderate hypoxia, HIF-1α accumulation up-regulates IL-1β secretion in TAM. IL-1β increases inflammatory signaling and enhances epithelial-mesenchymal transition in cancer cells through the IL-1β/HIF-1α/COX2 axis, which promotes the invasive capacities of tumoral cells in a hypoxic microenvironment.

IL-37 is an anti-inflammatory cytokine, which in numerous models seems to block the deleterious effects of IL-1α/β and IL-18. Schematically, this cytokine counteracts the induction of trained immunity. From this standpoint, it is altogether remarkable that hepatic expression of IL-37 decreases during HCC evolution and that it does so both in the tumor itself and the non-tumoral tissue adjacent to the tumor. Factors suggesting that IL-37 may possess an anti-tumoral effect are corroborated by the following points: (i) persistence of hepatic expression of IL-37 is associated during HCC with infiltration of NK CD57(+) cells. *In vitro,* the HCC cell lines expressing IL-37 attract NK CD57(+) cells which, in turn, are cytotoxic with regard to the HCC lines (143); (ii) the profusion of NK CD57(+) cells and the elevated level of hepatic expression of IL-37 are associated with improved overall survival (143); (iii) IL-37 has an anti-tumoral effect during HCC. While the JNK/pSmad3L/c-Myc pathway is associated with tumor growth during HCC, the signaling depending on IL-37 diverts pSmad3L from that platform and associates it with the suppressor of tumor p21 in a pSmad3C/p21 complex. The presence of pSmad3L is inversely correlated with overall survival (144); (iv) IL-37 is liable to induce autophagia in the HCC cell lines by inhibiting the PI3K/Akt/mTOR signaling pathway, thereby sensitizing HCC cells to apoptosis induction (145).

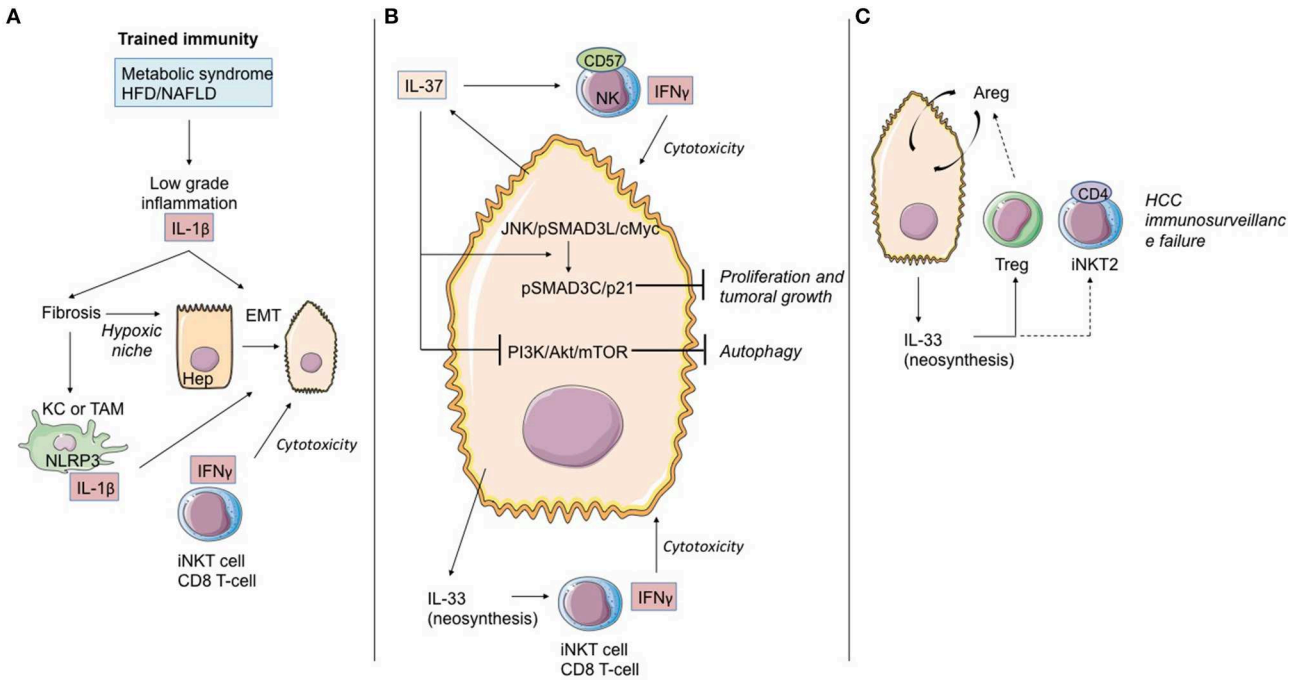

FIGURE 6 | Role of IL-1 cytokine family members in the immunological history of the hepatocarcinoma. **(A)** *IL-1β cytokine and trained immunity in initiation of hepatocarcinoma transformation.* Trained immunity induced by metabolic syndrome or HFD/NAFLD leads to low-grade inflammation linked to IL-1β and other cytokine (as TNF-α) production. These cytokines induce hepatic fibrosis, exposing hepatocytes to moderate hypoxia. HIF-1α accumulation up-regulates IL-1β secretion in TAM/KC. Locally produced IL-1β induces or enhances epithelial-mesenchymal transition in cancer cells. At that time, iNKT cells and CD8 T-cells maintain the immunosurveillance of the HCC. **(B)** *IL-37 driven-suppression of hepatocellular carcinoma.* Hepatocytes and transformed hepatocytes produce IL-37, a cytokine with several cell targets. IL-37 suppresses hepatocellular carcinoma growth by converting pSmad3 signaling from JNK/pSmad3L/c-Myc oncogenic signaling into pSmad3C/P21 tumor-suppressive signaling. IL-37 inhibits the PI3K/AKT/mTOR pathway, inducing autophagy in hepatocellular carcinoma cells. IL-37 recruits cytotoxic anti-tumoral NK cells harboring the terminal differentiation marker CD57. At that time, IL-33 neosynthesis by hepatocarcinoma cells and hepatocytes could maintain the immunosurveillance by maintaining iNKT1 cell and T CD8 T-cell activation. **(C)** *Immunosurveillance failure at the end-stage HCC*. Sustained IL-33 production by tumoral cells can recruit Treg lymphocytes and promote development of iNKT2 CD4(+) cells, both leading to anti-tumoral immunosurveillance failure. Areg, Amphiregulin; EMT, epithelial-mesenchymal transition; HCC, hepatocellular carcinoma; KC, Kupffer cells; TAM, tumor-associated macrophages; bounded arrows, presumably yes.

As regards IL-33, data from the natural history of HCC are available in humans. Yang et al. (146) detected IL-33 protein expression by immunohistochemistry in patients with HCC, liver cirrhosis, hepatitis, and normal livers. They showed that expression of IL-33 is increased in HCC and LC patients, as compared to healthy donors. In HCC patients, IL-33 is expressed by both tumoral cells and peri-tumoral tissue. Immunohistology results in this work are corroborated by quantitative RT-PCR data. A second study (147) likewise underlines IL-33 expression at the level of the tumor during HCC. IL-33 seric expression is particularly pronounced when the disease has evolved, whereas high seric concentrations of ST2s have been found to be associated with reduced overall survival of patients with HCC (148).

High expression of IL-33 could be a factor contributing to deregulation of local response, possibly increased by desensitization of iNKT and NK lymphocytes as well as the recruitment of Treg lymphocytes and ILC2 producers of AReg, a cytokine of which the function in transformation and progression of HCC has been documented (149).

THERAPEUTIC PERSPECTIVES

Although the role of some members of the IL-1 cytokine family in liver disease has been extensively studied (IL-1α, IL-1β, IL-33), leading to strong arguments favoring these molecules as potential therapeutic targets, the role of the other members (IL-36, IL-37, IL-38) remains to be elucidated. Furthermore, some members of the IL-1 family are pro-inflammatory and enhance tissue damage and inflammation, while other members are more protective/anti-inflammatory by promoting tissue regeneration and preventing tissue damage and inflammation. Hence, these cytokines represent crucial targets for liver disease therapies and could open new perspectives of potentially innovative therapeutic approaches aimed at controlling local immune response and at limiting liver injury.

Members of the IL-1 superfamily have multiple cellular sources and targets, as well as numerous natural inducers and inhibitors. The pharmacological agents that can either suppress cytokine production or block their biological actions may have potential therapeutic value against a wide variety of liver diseases.

Moreover, several members of the IL-1 cytokine family can activate (or inhibit) the same receptors. For example, IL-1RAcP is shared by IL-1α, IL-1β, IL33, and IL-36, highlighting redundancy and compensatory actions. Since IL-1α, IL-1β, IL-33, and IL-36 are implicated *in vivo* during liver diseases, the efficiency of the therapeutic approaches based on targeting a cytokine by the other members may be affected. In other words, when targeting the shared receptors, in this case IL-1RAcP may be more effective. In addition to redundancy, some cytokines in the IL-1 superfamily are known for their duality of function. For example, IL-33 has shown both pathological and hepatoprotective functions and rIL-33-ST2 therapeutic possibilities seem to depend on the presence of different inflammatory conditions, which may require either activation or inhibition of this pathway.

Modulation of IL-1β and IL-18 by Interference With Inflammasome

While some pharmacological agents against the NLRP3–caspase-1 pathway have been developed, their efficiency regarding cytokine inhibition and liver inflammation is moderate (106, 150, 151). This may be explained by the implication of additional mechanisms of extracellular activation of the cytokines. In addition to the classical NLRP3 inflammasome–caspase-1 cytokine activation pathway, serine proteases from immune cells (**Figure 1**) are also potent cytokine activators contributing to liver disease progression. The use of agents acting against those serine proteases, combined or not with anti-inflammasome-caspase-1 therapy, can be considered as a valuable therapeutic strategy and merits further investigation (152).

Manipulation of the IL-1/IL-1R Pathway in Chronic Inflammatory Hepatopathies

IL-1β is a critical mediator of trained immunity and chronic inflammatory hepatopathies such as NASH and NAFLD, raising the question of the therapeutic targeting of the IL-1/IL-1R axis.

Anakinra is the recombinant form of IL-Ra used in treatment of chronic or acute inflammatory pathologies (Muckle-Wells, adult-onset Still's disease). It effectively treats macrophage activation syndrome (MAS), as regards both MAS associated with inflammatory pathologies such as Still's disease and MAS associated with septic shock (for a review, see 5, 63). Altogether remarkably, in the latter indication, Anakinra clearly improves survival of patients with disseminated intravascular coagulation or hepatobiliary injury (65).

Several studies have suggested that utilization of Anakinra in precancerous states slows evolution of the disease, particularly in cases of smoldering myeloma (associated with Dexametasone) (153). Moreover, it is a treatment that seems beneficial in management of some cancers, particularly metastatic colorectal cancer (154). It is also remarkably well-tolerated, with relatively few adverse effects, especially infectious [for a review, see (8)].

Blockage of the IL-1/IL-1R pathway can also be achieved with a neutralizing antibody directed against IL-1β, Canakinumab, which seems beneficial in atherosclerosis prevention in high-risk patients (155).

Most of these diseases involve inflammatory pathways depending on the bringing into play of trained immunity, of which we previously noted the role/implication in the genesis of different hepatopathies. While blockage of the IL-1/IL-R pathway is a therapeutic option that may be envisioned in cases of chronic inflammatory hepatopathies, conclusive demonstration of its effectiveness has yet to be provided.

IL-18BP in ALF and Acute Viral Hepatitis

Recent demonstration of the role of the IL-18/NK cell axis in ALF genesis, particularly in cases of viral origin (31), has underscored the critical role of negative regulatory signals characterizing the pathway: IL-1R8 and IL-18BP. From this standpoint, in terms of therapeutic manipulation, IL-18BP is an excellent candidate. A human recombinant form (Tradekinig Alfa, AB2 Bioltd) is currently under evaluation in treatment of Still's disease and hemophagocytic lymphohistiocytosis syndrome, that is to say a pathology in which the IL-18/IL-18R axis seems preponderant. It has also been suggested that IL-18BP can prevent acetaminophen-mediated hepatotoxicity (30, 68). These different studies raise the issue of pharmacological targeting of IL-18 in a wide range of ALF cases.

Recombinant IL-233

In situations of acute suffering due to IR injury, targeting of the IL-33/ST2 pathway is an option of genuine interest that nonetheless raises questions of intervention timing. Its application in organ preconditioning could represent a protective strategy, given that during an IR injury phase IL-33 is likely to be implicated in the appearance of lesions.

Another strategy aimed at favoring the recruitment of Treg lymphocytes during an IR injury sequence consists in the utilization of recombinant IL-233. This chimeric IL associates IL-2 and IL-33 functions: the N-terminal residues 21–169 of the IL-2 receptor are linked by 15 amino-acyl residues to C-terminal residues 109–266 of the IL-33 receptor (156). Recombinant IL-233 enables preferential recruitment of T lymphocytes and ILC2 at the expense of iNKT lymphocytes, NK cells and ILC1. Stremska et al. (156) have shown that in a renal IR injury model, recombinant IL-233 is "formidably effective" as a means of protecting mice at post-sequence IR injury. In addition to a modified balance of recruitment between pro-inflammatory effectors (iNKT/T/NK/PMN) and anti-inflammatory effectors (Treg lymphocytes/ILC2), it is altogether probable that in this model, IL-233 promotes rapid, and effective repair signals. It is consequently relevant to ask whether this molecule may be beneficially utilized in cases of acute hepatic inflammation, especially those associated with liver transplantation.

Even though it currently remains speculative, future therapeutic utilization of recombinant IL-233 in cases of chronic inflammatory hepatopathies merits consideration. A recent study highlighted the beneficial effects of IL-233 in prevention of diabetic nephropathy in an Ob type 2 diabetes (DT2) mouse model. In this mouse model of predisposition to obesity and DT2 on account of an Ob mutation of leptin rendering the latter non-functional, IL233 treatment during or after the appearance of DT2 provides protection from diabetic nephropathy (46).

These results give rise to the question of the possible interest of a "humanized" form of this molecule in the prevention of long-term consequences of trained immunity, especially as regards the liver. However, other questions arise: Might there not ensue an effect stemming from loss of cancer censuring and, eventually, promotion of tumor growth depending on the Treg/ILC2 lymphocytes expressing Areg?

Conclusion Concerning Therapeutic Manipulation of Cytokines in the IL-1 Family

Further *in vivo* studies are needed to understand the potential benefits of targeting these shared receptors or simultaneously blocking multiple members of the IL-1 cytokine family.

Furthermore, these cytokines require enzymatic or proteolytic processing to become active, and their activators contribute to the process of liver inflammation and can therefore be potential therapeutic targets.

In conclusion, acute and chronic inflammations of the liver have various etiologies, ranging from toxic aggression (alcohol, fat diet, drugs) to viruses. Chronic liver inflammation leads to fibrosis and end-stage cirrhosis, and predisposes to the development of neoplasms such as hepatocellular carcinoma. The different members of the IL-1 superfamily of cytokines, which contribute to control of tissue homeostasis and help the liver to respond to damage and disease by promoting immune responses, have a major role in most liver inflammatory diseases and may represent potential clinical targets.

AUTHOR CONTRIBUTIONS

AR, CS, and ES contributed to literature search and editing of the review. LB, MF, AB, J-MG, and AH contributed to literature search for the review and provided writing and editing of the review.

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REVIEW
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The Roles of IL-1 Family Cytokines in the Pathogenesis of Systemic Sclerosis

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The IL-1 family consists of 11 cytokines, 7 ligands with agonist activity (IL-1α, IL-1β, IL-18, IL-33, IL-36α, IL-36β, IL-36γ) and four members with antagonistic activities [IL-1 receptor antagonist (IL-1Ra), IL-36Ra, IL-37, IL-38]. Recent articles have described that most members of IL-1 family cytokines are involved in the process of innate and adaptive immunity as well as fibrosis in systemic sclerosis (SSc). IL-1 family gene polymorphisms, abnormal expression of IL-1 and its potential role in the fibrosis process have been explored in SSc. IL-33 and IL-18 have also been discussed in the recent years. IL-33 may contribute to the fibrosis of SSc, while IL-18 remains to be researched to confirm its role in fibrosis process. There is a lack of study on the pathophysiological roles of IL-36, IL-37, and IL-38 in SSc, which might provide us new study area. Here, we aim to give a brief overview of IL-1 family cytokines and discuss their pivotal roles in the pathogenesis of SSc.

Keywords: IL-1 family cytokines, systemic sclerosis, scleroderma, fibrosis, pathogenesis

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INTRODUCTION

Systemic sclerosis (SSc) is an idiopathic autoimmune disease characterized by immune dysfunction, vasculopathy, and progressive fibrosis in skin and internal organs. Clinically, skin thickening and fibrosis are the most typical features of SSc. In patients with SSc, major causes of premature death are the pathological changes in lung, gastrointestinal tract, kidney, heart (1). However, the etiology and pathogenesis of immune abnormalities and fibrosis in SSc are poorly understood, which leads to a lack of effective treatments for SSc. The current treatment is mainly non-specific symptomatic treatment, which can only temporarily improve the condition but cannot fundamentally control the progress of fibrosis (2).

The interleukin (IL)-1 family is a group of 11 proinflammatory and anti-inflammatory cytokines. Recent findings show that expression of most IL-1 family cytokines, such as IL-1α, IL-1β, IL-18, and IL-33, was abnormal in many autoimmune diseases including SSc. Similarly, gene polymorphisms of IL-1α, IL-1β, IL-18, and IL-33 were reported to be correlated with SSc susceptibility. Therefore, in this review, we provide a brief introduction of IL-1 family cytokines biological functions, the association of IL-1 family genes and SSc and the roles of IL-1 family cytokines in the expression and pathogenesis of SSc. The IL-1 family cytokines and their roles in SSc are summarized in **Table 1**.

TABLE 1 | The role of IL-1 family members in the pathogenesis of SSc.

Common name	IL-1 family name	Receptor	Co-receptor	Potential roles in SSc or fibrosis
IL-1α	IL-1F1	IL-1R1	IL-1 RAcP (also termed IL-1R3)	Up-regulated in the lesional skin and serum. Induce the production of IL-6 and PDGF. Promote viability of SSc fibroblasts.
IL-1β	IL-1F2	IL-1R1 and IL-1R2	IL-1 RAcP	Elevated in the serum, BAL, and lesional skin. Induce IL-6 and TGF-β1, promote Th17 cell differentiation
IL-1Ra	IL-1F3	IL-1R1	NA	Up-regulated in SSc-affected fibroblasts. Induce fibroblasts differentiate into myofibroblast
IL-18	IL-1F4	IL-18Rα (also termed IL-18R1 or IL-1R5)	IL-18Rβ (also termed IL-1R7)	Elevated in serum and BAL. Pro-and anti-fibrotic effects were reported in fibrosis
IL-33	IL-1F11	ST2 (also termed IL-1R4)	IL-1 RAcP	Down-regulated in early SSc, upregulated in late SSc. Elevated in the serum. Induce M2 macrophages and ILC2 to produce IL-13 and TGF-β
IL-36α, IL-36β, IL-36γ	IL-1F6	IL-36R (also termed IL-1R6)	IL-1 RAcP	Drive fibrosis and activate the NLRP3 inflammasome
IL-36Ra	IL-1F5	IL-36R	NA	Unknown
IL-37	IL-1F7	IL-18Rα	SIGIRR (also termed IL-1R8)	Down-regulate pro-inflammatory cytokines
IL-38	IL-1F10	IL-36R	TIGIRR-2 (also termed IL-1R9)	Unknown

THE BIOLOGICAL CHARACTERISTICS OF IL-1 FAMILY CYTOKINES

The IL-1 family consists of 7 members with agonistic functions (IL-1α, IL-1β, IL-18, IL-33, IL-36α, IL-36β, and IL-36γ) and 4 members with antagonistic activities, including IL-1Ra, IL-36Ra, IL-37, and IL-38 (3). IL-1 family cytokines are divided into 3 subfamilies based on the length of precursor protein and the N-terminal pro-pieces for each precursor. The IL-1 subfamily consists of IL-1α, IL- 1β, IL-33, and possess the longest pro-pieces, composed of ∼270 amino acids. The IL-18 subfamily is comprised of IL-18 and IL-37 and also possess long pro-pieces composed of ∼190 amino acids. IL-36 subfamily comprising IL-36α, IL-36β, IL-36γ, and IL-38 possess the shortest pro-pieces of ∼150 amino acids (4).

Most IL-1 family members are commonly expressed as full-length precursors that require proteolytic processing for biologically mature forms. The full-length IL-1α is cleaved by the cysteine protease calpain, whereas IL-1β and IL-18 precursors require proteolytic cleavage by the inflammasome (5). IL-33 and IL-36 require neutrophil proteinases such as elastase and proteinase-3 for their processing (6, 7). IL-37 is cleaved by capsase-1 before maturation (8). IL-38 is bioactive as a full-length molecule.

IL-1 family cytokines activate signal transduction by the IL-1 receptor (IL-1R) family, which consists of 10 members: IL-1R1, IL-1R2, IL-1R accessory protein (IL-1RAcP), IL-18Rα, IL-18Rβ, ST2 (or IL-33R), IL-36R, single Ig IL-1R-related molecule (SIGIRR), three Ig domain-containing IL-1R related-2 (TIGGIR-2), and TIGGIR-1 (9). The receptor and co-receptor of IL-1 family are summarized in **Table 1**. With the exception of SIGIRR, which contains only one extracellular immunoglobulin (Ig) region, the other IL-1R members have three extracellular Ig regions. The intracellular domains of the IL-1R members are toll-like/IL-1R (TIR) domains. IL-1R2 is unique in IL-1R family because of lacking a TIR domain.

Pro-inflammatory cytokines of IL-1 family (IL-1α, IL-1β, IL-18, IL-33, IL-36) bind to similar conserved receptors consisting of extracellular Ig domains and intracellular TIR domains and induce cell activation through recruiting cytoplasmic myeloid differentiation primary response protein 88 (MyD88), IL-1R associated kinase 4 (IRAK4), tumor necrosis factor receptor-associated factor 6 (TRAF6), which ends up in the activation of nuclear factor-κB (NF-κB), and mitogen-activated protein kinase (MAPK) (10). IL-37 binds to the IL-18Rα and subsequently recruits SIGIRR, which does not trigger the recruitment of MyD88 (11). IL-38 mainly binds to the IL-36R. Both IL-37 and IL-38 exert anti-inflammatory effects by inhibiting NF-κB and MAPK signaling (12). IL-1Ra and IL-36 Ra, in competition with IL-1α, IL-1β, and IL-36, respectively, bind to IL-1R1 and IL-36R, and cannot recruit the co-receptor, which eventually results in the inhibition of IL-1 and IL-36 signaling (**Figure 1**).

THE ASSOCIATION OF IL-1 FAMILY GENES AND SSC

Different single nucleotide polymorphisms (SNPs) may result in the production of structurally different proteins with specific transcription rate and biological function. Investigating the correlation between SNPs of a specific gene and SSc seems to be inducible to understand the disorder's pathogenesis and find biomarkers for predicting the risk of SSc. In recent years, genome-wide association studies have revealed associations between genes encoding IL-1 family cytokines and SSc, which further supported the participation of IL-1 family cytokines in pathogenesis of the disease.

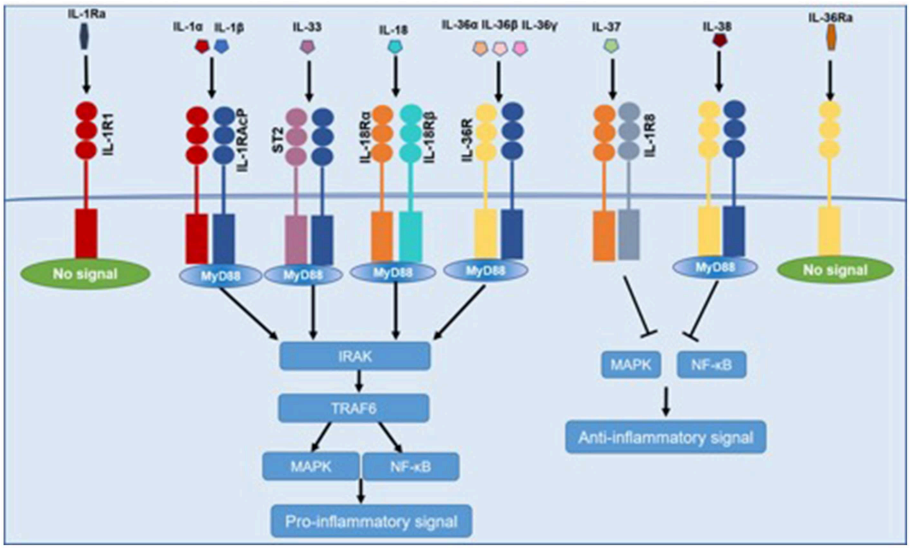

FIGURE 1 | Common signaling pathway for IL-1 family cytokines. IL-1α, IL-1β, IL-18, IL-33, and IL-36 bind to IL-1R family members, recruiting MyD88, IRAK4, TRAF6, which resulted in the activation of NF-κB and MAPK and then promoting the transcription of several inflammatory genes. IL-37 and IL-38 exert anti-inflammatory effects by inhibiting NF-κB and MAPK signaling. IL-1Ra and IL-36 Ra cannot recruit the signaling chain.

IL-1 family gene complex is located on chromosome 2q13–21. It consists of IL-1A, IL-1B, and IL-1RN. The human IL-1α (IL-1A) gene contains common SNPs including rs1800587 and rs17561, which have been reported to be linked to several autoimmune diseases in some populations. IL-1A rs1800587 was reported to be associated with SSc susceptibility in the Slovak Caucasian, Japanese, and Chinese populations (13–15). However, this association was not supported by other relevant studies (16). And in the meta-analysis, IL-1A rs17561 or rs1800587 polymorphism seems not to be statistically linked to the risk of SSc (17). Recently, a study demonstrated a significant association between the IL-1β (IL-1B) (+3962; rs1143634) SNP and the development of a severe ventilatory restriction in SSc patients. This SNP acted as an independent risk factor for restrictive lung disease along with the diffuse cutaneous subset of SSc and the presence of the anti-topoisomerase I antibody in a Cox regression model (18). However, in another study focused on Italian SSc patients, the frequency of the IL-1B rs1143634 CT genotype was significantly lower in patients with SSc compared to the control group, which indicated that this allele might be protective (16). Mattuzzi et al. described that IL-1BC-31C (rs1143627) and IL-1BC-511-T (rs16944) were significantly more frequent in SSc patients compared with controls (19). Also, Beretta determined the ability of epistatic interactions of cytokine SNPs to predict susceptibility to disease subsets in SSc. They performed the MDR analysis and showed a significant epistatic interaction among IL-1 receptor Cpst1970T, IL-6 Ant565G, and IL-10 C-819T SNPs increased the dcSSc susceptibility (20).

The association between IL-18 SNPs and SSc susceptibility was also analyzed. Results showed that IL-18 rs187238 and IL-18 rs1946518 polymorphism were not correlated with SSc susceptibility. However, ESR and dyspnea were associated with IL-18 rs187238 and IL-18 rs1946518 polymorphism, respectively (13).

TABLE 2 | Genetic polymorphisms in the IL-1 family cytokines that are associated with SSc.

IL-1 family	SNP associated with SSc	Risk/protection	References
IL-1A	rs1800587	Risk	(13–15)
	rs17561		(17)
IL-1B	rs1143634	Protection	(16)
	rs1143627	Risk	(19)
	rs16944	Risk	(19)
IL-18	rs1946518		(13)
	rs187238		(13)
IL-33	rs7044343	Risk	(21)
	rs1157505		(13)
	rs11792633		(13)
	rs1929992		(13)

Several studies focused on the association of genetic polymorphism of IL-33 in SSc patients. A multicentric preliminary study in 300 Turkish patients with SSc and 280 healthy controls showed that rs7044343 polymorphism of IL-33 gene was related to increased susceptibility to SSc (21). However, another study failed to find any association between IL-33 rs7044343 polymorphism and SSc susceptibility in Chinese population (13).

These results indicate that genetic variations of certain IL-1 family members are implicated in the pathogenesis of the disease and associate with SSc susceptibility. **Table 2** summarizes the association between SNPs and SSc.

THE EXPRESSION AND FUNCTION OF IL-1 FAMILY CYTOKINES IN SSC

The Expression and Function of IL-1α in SSc

The expression of IL-1α and IL-1β mRNA were barely detectable in unstimulated dermal fibroblasts, however, their expression was strongly increased after adding IL-1α and TNF-α. Cultured dermal fibroblasts from patients with SSc expressed higher levels of intracellular IL-1α than fibroblasts from healthy subjects (22). Immunohistochemical analysis showed that the expression of intracellular IL-1α was constitutively up-regulated in the lesion skin fibroblasts of SSc patients. The production of procollagen and IL-6 were decreased when the expression of IL-1 α was inhibited via IL-1α siRNA in SSc-affected fibroblasts. Conversely, overexpression of IL-1α through stable transfection in normal fibroblasts induced the differentiation of the SSc fibroblast phenotype (23). These evidence suggested that IL-1α could have a potential role in regulating fibroblast–myofibroblast differentiation, which is believed to be a key event in SSc. In addition, the serum level of IL-1α in SSc is controversial. Lin et al. reported that SSc patients with high serum IL-1α concentrations were more likely to have digital ulcers (24). These data emphasize the need for further research to determine the role of IL-1α in SSc pathogenesis, particularly in obliterative vasculopathy.

Endogenous IL-1α can induce fibroblast proliferation and collagen production through promoting the production of IL-6 and platelet-derived growth factor (PDGF) in SSc. Then, inhibition of endogenous IL-1α resulted in the decreased expression levels of IL-6 and PDGF in SSc fibroblasts (25). IL-6 is a critical mediator of fibrosis in SSc via inducing pro-fibrotic gene expression *in vivo*, enhancement of TGFβ1 production and by regulating TGFβ receptor (26, 27). TGF-β1 is a major regulator of fibrosis through stimulating cells undergoing epithelial-mesenchymal transition (EMT), fibroblast proliferation, ECM synthesis, and inhibition of collagenase and matrix metalloproteinases (MMP) (28, 29).

As mentioned above, IL-1α also induced PDGF, a potent chemotactic factor for inflammatory cells and TGF-β1, which can directly induce the differentiation of fibroblasts into actively EMC-producing myofibroblasts (30). What's more, in SSc fibroblasts, IL-1α can bind nuclear protein necdin in SSc fibroblasts and antagonize the function of necdin, which has an inhibitory effect on procollagen type I production (31). In addition, IL-1α and IL-1β were found to promote the viability of cultured fibroblasts and myofibroblasts from patients with SSc and this directly induced expression of N-cadherin and α-SMA, which is commonly used as a specific marker of myofibroblasts formation (32). These findings showed that IL-1 might contribute to fibroblast–myofibroblast differentiation and the myofibroblasts longevity, which are believed to be key events in SSc consequent skin fibrosis in patients with SSc.

In animal models of allergy, IL-1α and IL-1β were required for Th2 cell activation during airway hypersensitivity response (33). IL-1α and IL-1β were also proved to sustain the Th2 immune responses in parasites infestation (34). However, very few studies focused on the effects of IL-1α either IL-1β in Th2 cells in SSc. Considering the pathogenic role of Th2 cells in SSc, we would have expected to find many papers studying the pathogenic roles of IL-1α and IL-1β in SSc patients.

The Expression and Function of IL-1β in SSc

In patients with SSc, studies have observed a distinct elevation of IL-1β in the serum and bronchoalveolar lavage fluid (BAL) (35). In the lesion skin tissue of SSc patients, the expression levels of IL-1β and IL-18 were significantly up-regulated. Furthermore, there was a positive association between dermal fibrosis severity evaluated by modified Rodnan skin score (MRSS) and IL-1β and IL-18 expression, respectively (36). IL-1β has been abnormally expressed in a variety of fibrotic diseases. Studies have showed that pulmonary fibrosis induced by bleomycin, renal interstitial fibrosis resulting from unilateral ureteric obstruction, liver fibrosis in hypercholesterolemic and cardiovascular fibrosis after myocardial infarction are all attenuated in IL-1β-deficient mice (37–40).

Like IL-1 α, IL-1β also induces myofibroblast activation and fibrosis through IL-6 and TGF-β1. In addition, it has been observed that IL-1β and TGF-β2 can drive endothelial to mesenchymal transition (EMT), which is an important pathologic process in fibrosis (41). IL-1β has also been found to participate in the differentiation of Th17 cells that may play a crucial role in the development of tissue fibrosis (42).

What's more, the upstream regulation of IL-1β also has effects on the pathogenesis of SSc. The inflammasome has been found to be important in the pathogenesis of SSc by activating some IL-1 family cytokines such as IL-1β and IL-18. Inflammasomes are poly-protein complexes. Many subtypes of inflammasomes have been identified, and the nucleotide-binding domain, leucine rich repeat containing family and pyrin domain-containing 3 (NLRP3) inflammasomes are the most extensively studied in SSc. The critical function of the NLRP3 inflammasome is to activate caspase-1, which can cleave the precursors of IL-1β and IL-18 into biologically active forms. Inhibition of caspase-1 could reduce the secretion of IL-1β and IL-18 in SSc skin and lung fibroblasts. In addition, the expression of α-SMA protein was decreased in SSc dermal myofibroblasts when treated with a caspase-1 inhibitor. Furthermore, NLRP3$^{-/-}$ mice were resistant to bleomycin-induced skin fibrosis (43). One study observed the significantly increased expression of NLRP3, caspase-1, IL-1β, IL-18, and a positive correlation between the severity of dermal fibrosis and NLRP3 inflammasome in SSc lesion skin (36). Mechanistically, Artlett et al. reported that inflammasome promoted the expression of miR-155, which is critical in driving fibrosis in SSc (44). Overall, NLRP3 inflammasome and its subsequent effectors have been proven to be critical in the development of SSc, and deemed as promising candidates for targeting treatment in the clinics.

The Expression and Function of IL-Ra in SSc

IL-1Ra comprises 4 different isoforms. One isoform (sIL-1Ra) is secreted and the other three (icIL-1Ra1, icIL-1Ra2, and icIL-1Ra3) are intracellular. Both sIL-1Ra and icIL-1Ra1 mRNAs were constitutively expressed by human dermal fibroblasts,

whereas intracellular IL-1Ra was markedly up-regulated in SSc-affected fibroblasts compared to normal skin fibroblasts after stimulating with IL-1 β or TNF-α (22). When Intracellular IL-1Ra is overexpressed in cultured normal human skin fibroblasts via transfection with a viral vector, it induces a myofibroblasts phenotype characterized by increased expression of α-SMA and plasminogen activator inhibitor-1 (PAI-1), which plays a crucial role in fibrogenesis and is expressed markedly in myofibroblasts, along with decreased expression of collagenase and MMP-1 (an enzyme involved in the breakdown of ECM in the skin) (45). Collectively, these studies suggested that intracellular IL-1Ra might be relevant to the pathogenesis of fibrosis in SSc.

The Expression and Function of IL-33 in SSc

Studies showed that IL-33 played an important role in the pathogenesis of multiple autoimmune diseases, such as systemic lupus erythematosus (SLE), rheumatoid arthritis (RA) and inflammatory bowel disease (IBD) (46–48). Recently, an increasing number of studies have shown the potential role of IL-33 in SSc. In the skin biopsies from early SSc patients, the expression of IL-33 protein was down-regulated. By contrast, in patients with late stage SSc, IL-33 protein was constitutively found in most endothelial cells (49). Several studies demonstrated that serum level of IL-33 was elevated in patients with SSc compared with healthy controls. High serum level of IL-33 was positively correlated with peripheral vascular involvement, such as digital ulcers and the severity of skin sclerosis and pulmonary fibrosis (50–52).

When IL-1RAcP$^{-/-}$, ST2$^{-/-}$, and wild-type (WT) mice were treated by recombinant IL-33, IL-1RAcP$^{-/-}$, and ST2$^{-/-}$ mice did not develop cutaneous fibrosis compared to WT mice, which means that IL- 33 induces cutaneous fibrosis by type 2 immunity is ST2 and IL-1RAcP-dependent (53). IL-33 can participate in the polarization of M2 macrophages to produce IL-13 and TGF-β1, which are both profibrotic cytokine in pathological fibrosis (54). In addition, IL-33 also induced the expansion of type 2 innate lymphoid cells (ILC2s) to increase the production of IL-13 (55) (**Figure 2**).

In brief, the critical role of IL-33 in SSc pathogenesis has been elucidating. However, more studies on the precise function of IL-33 in the process of immune dysfunction, vasculopathy, and fibrosis are required in SSc.

The Expression and Function of IL-18 in SSc

Serum IL-18 levels in SSc patients were significantly higher than that in control subjects and positively correlated with the presence of anti-nuclear antibody (ANA) and clinical grades in patients with SSc, respectively (56). IL-18 levels in serum and BAL in patients with IPF were also increased compared with control subjects (57). These results indicated that IL-18 may be involved in the process of fibrosis. However, the exact mechanism of the IL-18 in fibrosis is controversial because both pro-and anti-fibrotic effects were reported in the literature.

Kitasato et al. reported that IL-18 mediates hepatic fibrosis by activating CD4+ T cells, and that this effect is blocked by anti-IL-18 treatment. Moreover, in renal fibrosis, stimulating proximal tubular cells with IL-18 could induce α-SMA, collagen

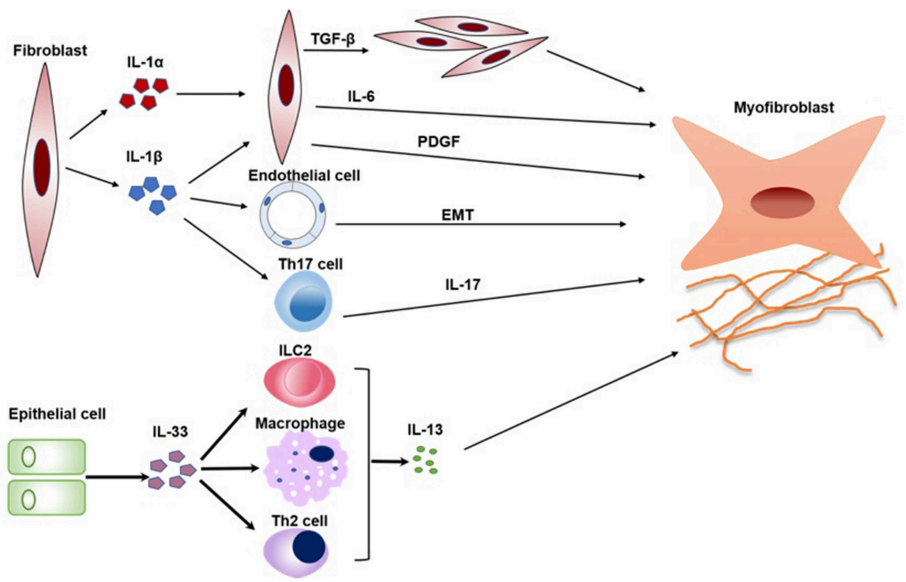

FIGURE 2 | The pathogenic roles of IL-1 family in SSc. Key mechanisms by which IL1α, IL-1β, and IL-33 promotes a variety of cells activation and transition are summarized in a schematic form. IL-1α and IL-1β can induce fibroblast proliferation and fibrosis through promoting the production of IL-6 and PDGF. In addition, IL-1β can also promote EMT and the differentiation of Th17 cells, which both play crucial roles in SSc. IL-33 induces the expansion of Th2 cells, ILC2, and M2 macrophages to increase the production of IL-13. IL-13 is a profibrotic cytokine that is sufficient for the induction of fibrosis in SSc. PDGF, platelet-derived growth factor; EMT, epithelial-mesenchymal transition; ILC2, type 2 innate lymphoid cell.

I, and fibronectin production in a dosage and time dependent fashion (58).

On the contrary, some studies have observed that IL-18 has anti-fibrotic effects. Nakatani-Okuda et al. reported that mice deficient in IL-18 developed more severe fibrosis than WT mice (59). Furthermore, Kim et al. demonstrated that IL-18 down-regulated the production of collagen in human dermal fibroblasts through the E26 transformation-specific-1 and the ERK pathway, indicating that IL-18 may have anti-fibrotic effects in patients with SSc (60). Whether IL-18 has pro-fibrotic or anti-fibrotic effects need further validation.

The Pathogenesis of IL-36, IL-37, and IL-38 in SSc

At present, increasing number of studies suggested important roles of IL-36, IL-37, and IL-38 in a variety of autoimmune diseases. However, few studies have evaluated their expression and pathophysiological roles in SSc patients. Thus, information obtained from studies of other autoimmune and fibrotic diseases may be beneficial to understand their potential effects on SSc.

IL-36 comprises 3 isoforms, IL-136α, IL-36β, and IL-36γ. At present, very limited evidence in the researches regarding IL-36 in SSc or fibrosis has been reported. IL-36α was observed to induce tubulointerstitial fibrosis in the mice model with unilateral ureteral obstruction. In IL-36 receptor knock-out mice, fibrosis was attenuated (61). In this study, recombinant IL-36α can activate the NLRP3 inflammasome. IL-36α was also elevated in the fibrotic tissue of patients with chronic pancreatitis, which further implicated IL-36 as a profibrotic cytokine (62). Several studies had shown that IL-36 was related to autoimmune diseases such as RA, SLE, and psoriasis (63, 64).

Low doses of IL-37 inhibited joint inflammation and significantly decreased synovial IL-1β, TNF-α, IL-6, CCL3, CXCL1 in an arthritis mice model (65). IL-37 also played an effective immunosuppressive role in experimental psoriasis by down-regulating pro-inflammatory cytokines such as IL-6, TNF-α, and IL-1β (66). No data about IL-37 and fibrosis has been reported so far.

IL-38 seems to play a role in the development of fibrosis. The expression of IL-38 is significant in the lungs of patients with acute idiopathic pulmonary fibrosis (67). However, further investigation is needed to explore their potential roles and their receptors in SSc.

In conclusion, the functional implications of IL-36 and IL-38 are not yet known in SSc, but similar studies in tubulointerstitial fibrosis and IPF have indicated that IL-36 and IL-38 may induce fibrosis. A similar understanding in SSc would represent a significant advance. IL-37 down-regulates pro-inflammatory and pro-fibrotic cytokines such as IL-6 and IL-1β. Therefore, whether IL-37 could play immunosuppressive and anti-fibrotic roles in SSc requires further study.

CLINICAL APPLICATION VIA INHIBITING IL-1 FAMILY CYTOKINES IN SSC

In recent years, clinical application targeting IL-1 family cytokines has been used in multiple autoimmune diseases such as RA and gout (68, 69). However, few studies have explored the clinical benefits in patients with SSc.

In a clinical trial, rilonacept, an IL-1 receptor fusion protein, did not show treatment-related efficacy in patients with SSc compared to placebo, and also failed to reduce the expression of IL-6, C-reactive protein (CRP), or CCL18 expression (70). Although anti-IL-1 therapy seems to be ineffective according to limited studies, the development of new biologics with specific IL-1 antagonists and the blocking of IL-18 or IL-33 may show potential clinical usefulness in the future.

CONCLUSION

The IL-1 family of cytokines have been shown to play a vital role in the pathogenesis of SSc, and the IL-1 family gene polymorphisms have been demonstrated to be closely related to SSc. Recent studies have investigated the abnormal expression of IL-1 and its potential role in the fibrosis process. However, many aspects of IL-1 family members in SSc remain to be elucidated. There is large room for the mechanism study of IL-1 family cytokines, especially for IL-37 and IL-38. Furthermore, researches exploring the potential benefits of simultaneously inhibiting multiple members of the IL-1 family cytokines *in vivo* are promising.

AUTHOR CONTRIBUTIONS

DX prepared the draft manuscript. RM and XW revised and finalized the article. All authors have read and approved it for publication.

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