Regulation and function of interleukin-36 cytokines in homeostasis and pathological conditions

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ABSTRACT

IL-36α, IL-36β, and IL-36γ are members of the IL-1 family of cytokines that signal through a common receptor composed of IL-36R and IL-1R/AcP to activate NF-κB and MAPKs, such as p38 and JNK, and promote inflammatory responses. IL-36Ra is a natural antagonist of the 3 IL-36 agonists that binds to IL-36R and inhibits binding of the agonistic ligands. These cytokines are expressed predominantly by epithelial cells and act on a number of cells, including immune cells, epithelial cells, and fibroblasts. Processing of the N terminus is required for full agonist or antagonist activity for all IL-36 members. The role of IL-36 has been demonstrated extensively in the skin, where it can act on keratinocytes and immune cells to induce a robust inflammatory response and is implicated strongly through functional and genetic evidence in the pathology of psoriatic disorders. Emerging data also suggest a role for this cytokine family in pulmonary physiology and pathology. Although much has been learned about the biochemistry of IL-36 and its role in various tissues, it is clear that we are at an early stage in our understanding of the full biology of these cytokines. *J. Leukoc. Biol.* 97: 645–652; 2015.

Introduction

IL-36 cytokines, which are members of the IL-1 superfamily, include 3 agonistic cytokines, namely IL-36α, IL-36β, and IL-36γ, previously known as IL-1F6, IL-1F8, and IL-1F9, and a natural inhibitor, IL-36Ra, previously termed IL-1F5. As their function became better elucidated, and it was clear that they exhibited true cytokine activity, they were given the designation of IL-36 [1]. All 3 cytokines signal through the same heterodimeric receptor to induce the same signaling cascade, leading to activation of NF-κB and MAPKs. IL-36 and its receptor are

expressed in several tissues, including skin, lung, and gut, and induce inflammatory responses in these tissues. IL-36 has activity on epithelial cells, as well as specific immune cells, inducing cellular activation and secretion of cytokines and chemokines, leading to recruitment and activation of a variety of immune cells. Here, we review the current state of knowledge of IL-36 biochemistry and biology and its role on immune cell activation and recruitment.
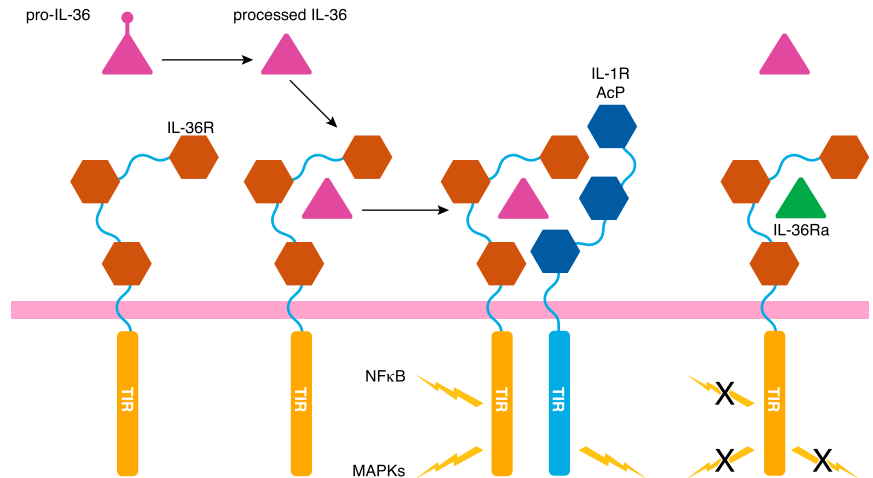
IL-36 EXPRESSION AND BIOCHEMISTRY

IL-36 cytokines were first discovered in sequence databases as a result of their homology with IL-1α and IL-1β [2]. Similar to other cytokines in the IL-1 family, all 3 agonistic cytokines signal through a heterodimeric receptor comprised of IL-36R [previously termed IL-1RL2 or IL-1R-related protein 2 (IL-1Rrp2)] and IL-1R/AcP (**Fig. 1**). The AcP is shared among IL-1α, IL-1β, and IL-33, whereas the primary receptor-binding subunit, IL-36R, is unique to IL-36. Upon binding to IL-36R, IL-36α, IL-36β, or IL-36γ recruits AcP, resulting in engagement of cytoplasmic TIR domains from each of the membrane-spanning receptors, which is required for initiation of downstream signaling events. IL-36 activates similar downstream signaling pathways as other IL-1 family members, including MAPKs and the transcription factor NF-κB [3]. Similar to IL-1, there is also an IL-36Ra (previously known as IL-1F5) that acts in a manner analogous to IL-1Ra. IL-36Ra binds to IL-36R and prevents binding of IL-36α, IL-36β, and IL-36γ; however, IL-36Ra binding to IL-36R does not induce the recruitment of AcP and therefore, does not initiate a signaling response [4] (Fig. 1). In this manner, IL-36Ra acts as a natural antagonist of all 3 agonistic IL-36 ligands. Recently, the first crystal structure for an IL-36 agonist, IL-36γ, was reported [5], whereas the structure for IL-36Ra was published several years before [6]. IL-36γ and IL-36Ra have similar structures and exhibit the typical β-trefoil-fold observed in all other IL-1 family members consisting of 12-β-strands connected by 11 loops [5]. Through use of these structures and the solved crystal structures

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Figure 1. Biochemistry. IL-36 is produced originally as a precursor (pro-IL-36) protein. Processing of the N terminus of IL-36 must occur before it can bind to the IL-36R with high affinity. Binding of IL-36 to IL-36R induces recruitment of the coreceptor AcP that brings the crucial TIR domains of the 2 receptor chains in close enough proximity to enable signaling, leading to induction of transcription factors, including NF-κB and MAPKs. IL-36Ra acts as an antagonist as it binds IL-36R and prevents binding of agonistic IL-36 ligands to the receptor. Unlike IL-36, IL-36Ra binding does not lead to recruitment of AcP and the generation of a heterodimeric signaling complex and therefore, does not initiate signaling.

of IL-1β in complex with IL-1R1/AcP or IL-1R2/AcP, the interaction of IL-36γ or IL-36Ra with IL-36R/AcP was modeled. Based on the model, chimeric versions of IL-36γ and IL-36Ra were generated and examined for binding to the IL-36R/AcP heterodimer, as well as for functional activity. IL-36 agonists follow grossly similar principles as IL-1 but use distinct, specific interactions for activation. Likewise, whereas the mode of action of IL-36Ra is similar to IL-1Ra, the exact mechanism of antagonism is markedly different. Modeling showed that the $\beta4/5$ and $\beta11/12$ loops in IL-36 likely interact directly with AcP. The largest conformational differences between IL-36Ra and IL-36γ are found in these 2 loops, and superimposing IL-36Ra onto the ternary complex of IL-1 leads to significant clashes with AcP. These data provide the first pieces of structural data to explain how IL-36 agonists lead to recruitment of AcP, whereas IL-36Ra does not and therefore, is an antagonist [5].

The IL-36 cytokines are clustered on human chromosome 2 in the same locus that includes the genes for all other IL-1 family cytokines except for IL-18 and IL-33. The IL-36R also lies in the IL-1R family locus on human chromosome 2 and is flanked by IL-1R1 and IL-1R2 on the centromeric side and IL-33R, IL-18R, and IL-18RAP (accessory protein) on the telomeric side [7–9]. IL-36 cytokines, such as all IL-1 family members except for IL-1Ra, are synthesized without a signal peptide and therefore, are not secreted via the endoplasmic reticulum-Golgi pathway. It is unclear how IL-36 cytokines are released from cells; however, it is clear that post-translational processing is required for full agonist or antagonist activity of all IL-36 members. In the absence of processing, IL-36Ra exhibits no antagonist activity, whereas the full-length IL-36 agonists are several of orders of magnitude less active than their properly truncated forms [4]. Truncation of IL-36 members must occur at precisely the right location to generate the active form, whereas removal of 1 more or less amino acids results in a protein with activity comparable with the full-length protein. The enzyme or enzymes required for processing of IL-36 cytokines are unknown, and there are no aspartate residues or other features suggesting a cleavage site for caspase-1. In addition, there is very little sequence homology among IL-36 isoforms in the region around the cleavage site, suggesting that it is unlikely that a single enzyme is responsible for processing all 3 IL-36 agonists [8, 9]. Whereas it is clear that

processing of IL-36 must occur for full agonist or antagonist activity, as of yet, there is no compelling evidence that truncation of these ligands occurs in humans under pathologic conditions. When and how processing of IL-36 ligands occurs are active areas of research.

Expression of IL-36 cytokines is found at low levels in many different tissues, most notably the skin, esophagus, tonsil, lung, gut, and brain. IL-36 can also be expressed by immune cells, including monocytes/macrophages and T cells [10–12]. IL-36 family members are strongly up-regulated in the skin by many agents, including cytokines, TLR agonists, or by pathologic conditions, such as psoriasis [13, 14]. Bronchial epithelial cells express IL-36 cytokines, and IL-36γ, in particular, is highly induced in response to a number of stimuli, including cytokines, bacteria, rhinovirus infection, and smoke [15–18]. IL-36α mRNA is produced by adipose tissue-associated macrophages, and its expression levels are enhanced upon stimulation with LPS in vivo [19]. The IL-36R, on the other hand, is widely expressed at low levels and does not seem to be highly regulated [3, 10].

IMMUNE EFFECTS OF IL-36

As for other IL-1 cytokine family members, IL-36 plays significant roles in host immunity by stimulating innate and adaptive immune responses (**Fig. 2**). In particular, IL-36 exerts strong, stimulatory effects on DCs that play a critical role in the interface between innate and adaptive immune responses. Mouse BMDCs express high levels of IL-36R and produce IL-12 and IL-23, as well as IL-1β, TNF-α, IL-6, CCL1, CXCL1, and GM-CSF, upon IL-36 stimulation [11]. These cytokines and chemokines can stimulate Th1 and Th17 responses, as well as innate responses. Importantly, the stimulatory effect of IL-36 is relatively unique, as other IL-1 family cytokines, including IL-1 itself, have little effect on murine BMDCs. In addition, IL-36 has a modest maturing effect on DCs, up-regulating MHCII antigens, as well as CD40, CD80, and CD86, to a level comparable with that achieved by LPS stimulation. Finally, BMDCs themselves constitutively express IL-36γ and up-regulate IL-36α upon stimulation by various agents, including IL-36 itself. The production of IL-36 by BMDC suggests the presence of positive autocrine and paracrine IL-36/IL-36R amplification loops.
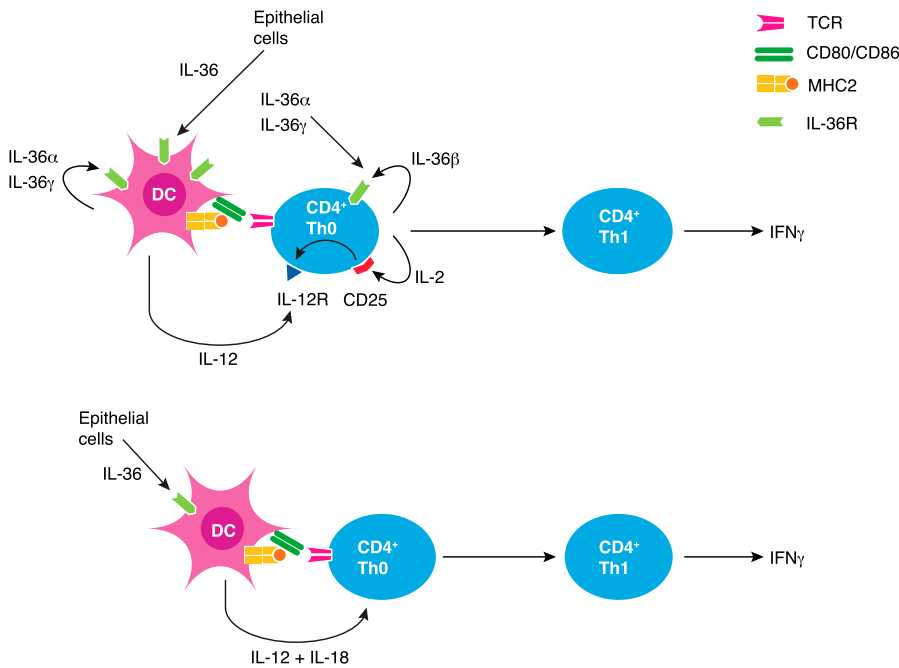
Figure 2. Effects of IL-36 cytokines on DCs and T cells. In mouse cells, IL-36R is constitutively expressed on DCs and Th0 cells. IL-36, produced by keratinocytes or other epithelial cells, activates DCs to secrete IL-12. IL-36 is also produced by activated DCs that may further stimulate DCs in an autocrine manner. IL-36, produced by Th0 cells, activates Th0 cells. By a synergistic effect, IL-36 and IL-12 induce Th1 polarization, leading to IFN-γ secretion. The formation of a positive-feedback loop created by IL-36/IL-36R leads to sustained IFN-γ-mediated immune responses (upper). Human DCs but not CD4$^+$ T cells, constitutively express IL-36R. IL-36, produced by keratinocytes or other epithelial cells, activates DCs to secrete IL-12 and IL-18. In DC–T cell allogenic cultures, IL-36 induces Th1-polarization and IFN-γ secretion (lower).

Human monocytes express IL-36R, however to a much lower extent than MDCs. IL-36 agonists stimulate the production of several cytokines by human MDCs, including IL-1, IL-6, IL-23, IL-12, and IL-18, and stimulate MDC maturation by enhancing the cell-surface expression of CD83, CD86, and MHCII molecules (refs. [20, 21] and unpublished data). MDCs, cultured in the presence of IL-36, were able to drive the proliferation of allogeneic T cells [20], as well as the production of IFN-γ [21]. Isolated blood neutrophils showed no expression of IL-36R and failed to respond to IL-36 stimulation. Production of IL-36γ and IL-36Ra is stimulated in PBMCs, cultured in the presence of *Aspergillus fumigatus*. The induction of IL-36γ was dependent on dectin-1/spleen tyrosine kinase (Syk) and TLR4 signaling. Inhibition of IL-36R signaling by IL-36Ra reduced the production of IFN-γ and IL-17 by infected PBMCs, suggesting that IL-36 contributes to the production of these cytokines in response to *A. fumigatus* [12]. MDC and M1-polarized macrophages produced IL-36γ in response to a combination of IL-1, TNF-α, and IFN-γ, and this effect was dependent on expression of the endogenous T-bet [22].

In mice and humans, members of IL-1 family drive the development of CD4$^+$ T cell adaptive responses. IL-1β, IL-18, and IL-33 influence CD4$^+$ T cell responses and their polarization into Th17, Th1, and Th2 CD4$^+$ effector cells, respectively, although the selectivity of these responses may be modulated by the cytokine environment. More specifically, in addition to IL-1 cytokines, the presence of cytokines stimulating the JAK and STAT pathways is required to fully stimulate the polarization of CD4$^+$ T cells in the different Th subsets [10, 23–28]. IL-1 has been shown to promote the differentiation of naïve CD4$^+$ T cells into Th17 cells [24, 29, 30] and to promote expansion of IL-17-secreting memory CD4$^+$ T cells [31]. IL-18 synergizes with IL-12 as an amplifying signal to induce Th1 responses [32]. Although IL-33 is also an IL-1 family member, its function on the adaptive immune response is distinct from IL-18 in that it predominantly

drives Th2 responses [33]. The subset-specific effects of IL-1, IL-18, and IL-33 are a result, in part, of selective expression of their receptors, including IL-1R1 in Th17, IL-18Rα in Th1, and ST2 (IL-33R) in Th2 cells, respectively (reviewed in ref. [10]). Regarding IL-36R expression, there is an important difference between human and mouse cells. In the human, T cells do not express IL-36R and fail to respond to IL-36 [20]. However, as described above, indirect responses to IL-36 stimulation, in particular, IFN-γ secretion, have been observed in T cell/MDC allogeneic cocultures [21]. In contrast, mouse T cells express IL-36R and respond directly and indirectly to IL-36 stimulation. Indeed, IL-36α, IL-36β, and IL-36γ have a direct effect on mouse CD4$^+$ T cells and splenocytes, inducing the production of several proinflammatory chemokines and cytokines, including IFN-γ, IL-4, and IL-17 [11]. The critical difference between IL-36 and other IL-1 family members is that IL-36R is expressed predominantly by naïve CD4$^+$ T cells, whereas the receptors for other IL-1 family members are present on polarized CD4$^+$ T cell subsets, as described above. Consequently, IL-36, but not other IL-1 family cytokines, potently induces IL-2 production, survival, and cell division in TCR-activated, naïve T cells (Th0 cells). This finding suggests a pivotal role for IL-36 in priming early immune responses in mice [34].

In addition, IL-36 has a role in the induction of T cell polarization. IL-36β, like IL-18, acts in combination with IL-12 to induce the in vitro differentiation of Th0 cells into IFN-γ-producing Th1 cells [34]. The combinations of IL-12 with IL-36β or IL-18 induced T-bet and IL-12Rβ2 mRNA expression. Interestingly, IL-12Rβ2 mRNA levels were much higher when Th0 cells were primed in the presence of IL-12 and IL-36β than with IL-12 and IL-18, pointing toward a more efficient cooperation between IL-36 and IL-12 in Th0 cells. IL-36β was constitutively expressed in Th0 cells, and interestingly, Th1 polarization, induced by IL-12 and IL-18, was severely impaired in IL-36R$^{-/-}$ Th0 cells, thus indicating that endogenous IL-36β

plays a prominent role in IL-12-mediated Th1 polarization, even when it is apparently stimulated by IL-18. The mechanism by which IL-36 induces the polarization of Th0 cells into the Th1 subset has been explored recently in vitro by use of IL-2$^{-/-}$ Th0 cells. In the absence of IL-2, the combination of IL-12 and IL-36β failed to induce IL-12Rβ2, and the production of IFN-γ was decreased markedly. Thus, the influence of IL-36 on Th1 differentiation is completely dependent on IL-2 induction. In contrast, the effect of IL-36 on Th0 cell proliferation and survival is largely independent of IL-2 [34]. The role of IL-36 in Th1 responses can also be seen in vivo. IL-36R$^{-/-}$ mice have decreased Th1 cell responses, including lower IFN-γ, TNF-α, and NO production by ex vivo-stimulated splenocytes, 4 weeks after in vivo *Mycobacterium bovis* BCG infection, suggesting that endogenous IL-36 signaling is required to obtain efficacious Th1 responses against mycobacteria [34]. This effect is, however, not essential for host survival and bacterial clearance. Indeed, mice infected with *M. bovis* BCG did not exhibit any differences regarding survival, even after several months of observation. Furthermore, in contrast to IL-1R1-deficient and particularly, TNF-α-deficient mice, IL-36R-deficient mice did not show increased susceptibility to *Mycobacterium tuberculosis*-induced lethality compared with wild-type mice (unpublished data). In addition, the severity of experimental models of autoimmune diseases, such as antigen-induced arthritis and collagen-induced arthritis, was independent of IL-36R signaling [35]. Taken together, these results indicate that the role of IL-36 in these models is redundant to other cytokines, including other IL-1 family members.

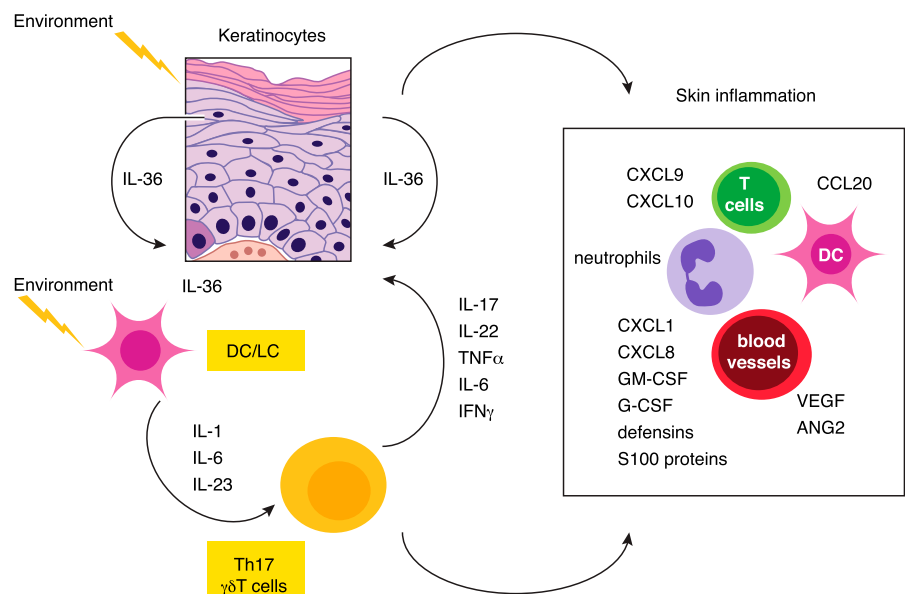## INTERPLAY BETWEEN IMMUNE CELLS AND EPITHELIAL CELLS

The role of IL-36 seems critical in the interplay between nonhematopoietic cells in tissues, such as skin, and immune cells, including T cells and resident and migratory DCs (**Fig. 3**). For example, IL-36 cytokines produced at high levels by epithelial cells, e.g., keratinocytes, may have autocrine and paracrine actions as a result of the presence of IL-36R on keratinocytes. Indeed, IL-36 induces the release of inflammatory cytokines, such as IL-6, IL-8, and TNF-α, as well as antimicrobial proteins, such as human β-defensins 2 and 3, LL37, and S100A7, by keratinocytes in culture [13, 36]. In the mouse, i.d. injection of IL-36 induces many genes, including IL-17, IL-20, IL-22, IL-23, TNF-α, IFN-γ, and a host of other cytokines, chemokines, and antimicrobial proteins [14]. IL-36α and IL-36γ are expressed primarily by keratinocytes and can be up-regulated in human keratinocytes by IL-36 in an autocrine manner. In addition, IL-36α and IL-36γ production is enhanced in skin cultures by TNF-α, IL-17, or IL-22 [13, 36]. Combinations of those molecules are even more effective. Likewise, in the mouse, i.d. injection of TNF-α or IL-23 will up-regulate IL-36 family members, and injection of combinations of TNF-α, IL-17, IL-22, IL-23, and/or IFN-γ works even better [14].

IL-36 IN SKIN

Several lines of evidence indicate that IL-36 is involved in the pathogenesis of psoriasis. Gene expression studies have found that IL-36α and IL-36γ are up-regulated in skin psoriatic lesions [37], and there was a strong correlation between expression of IL-36α and IL-36γ in human psoriatic lesions and expression of other cytokines, including IL-17, IL-23, TNF-α, and IFN-γ [13], suggesting that a positive gene expression loop might occur in psoriasis. Transgenic mice overexpressing IL-36α in keratinocytes have a strong skin phenotype at birth that resolves at weaning. This phenotype is abrogated when transgenic mice are crossed with IL-36R- or AcP-deficient mice but strongly exacerbated when IL-36α transgenic mice are crossed with IL-36Ra-deficient mice [14]. Adult mice with histologically normal-looking skin develop an inflammatory condition upon treatment with a phorbol ester, such as TPA, that is very similar to psoriasis. Importantly,

Figure 3. Potential role of IL-36 in psoriasis and inflammatory skin diseases. Environmental factors can stimulate the release of IL-36 by keratinocytes and/or DCs or Langerhans cells (LC). IL-36 stimulates keratinocytes and DCs/Langerhans cells in an autocrine and paracrine manner. IL-1, IL-6, and IL-23, secreted by activated DC/Langerhans cells, stimulate Th17 responses, as well as the release of IL-17 by dermal $\gamma\delta$ T cells, leading to an inflammatory loop with keratinocytes. The production of chemokines and proinflammatory cytokines leads to the typical pathologic manifestations of psoriasis, including the recruitment of neutrophils, DCs, and T cells and the proliferation of keratinocytes. VEGF, Vascular endothelial growth factor; ANG2, angiopoietin 2.

inhibitors of clinically relevant pathways, such as TNF-α, IL-12/23p40, IL-23, and IL-17, are highly effective at blocking the skin lesions, which ensue in the transgenic mice upon exposure to TPA, suggesting relevance to human disease and further providing evidence that IL-36 is an upstream cytokine driving production of other cytokines, such as TNF-α, IL-23, and IL-17 [14]. Imiquimod, a TLR7 agonist, which can induce psoriasis-like lesions, is an excellent IL-36 inducer. Furthermore, imiquimod induces the development of skin thickening, IL-17 production, and influx of neutrophils, macrophages, and $\gamma\delta$ T cells into the skin that was almost completely dependent on IL-36R signaling. Indeed, IL-36R-deficient mice were protected of the development of imiquimod-induced skin inflammation, whereas IL-36Ra-deficient mice exhibited a more severe phenotype [38]. One of the most compelling lines of evidence for a role of IL-36 as a driver of skin inflammation was obtained by engrafting immunodeficient mice with human psoriasis skin. Treatment of these mice with an anti-human IL-36R-neutralizing mAb led to substantial normalization of skin pathology [14].

GPP is a severe, life-threatening form of psoriasis. Marrakchi and colleagues [39] discovered a causative mutation in IL-36Ra leading to a leucine-to-proline change at position 27 in a Tunisian family with GPP, which resulted in a mutant protein with reduced potency and stability. A complementary study was published by Onoufriadis et al. [40], who discovered 3 unrelated cases of GPP, where all carried missense mutations in IL-36Ra. Both groups demonstrated enhanced IL-36 responses in patients' cells consistent with decreased function of a natural regulator of IL-36 activity [39, 40]. A variety of missense and nonsense changes have now been described in European and Asian patients [41], with 13 pathogenic variants currently reported. However, not all GPP patients have mutations in IL-36Ra, and in particular, patients with GPP and concomitant psoriasis vulgaris are much less likely to contain *IL-36RN* disease alleles [42]. Altogether, these results indicate that IL-36 may play an important role in psoriasis, as well as in other inflammatory skin pathologies. Indeed, IL-36, released by activated or damaged keratinocytes, can activate dermal DC, leading to the secretion of proinflammatory cytokines. TNF-α and IL-17A can markedly enhance the stimulatory function of IL-36 on cultured keratinocytes [13]. These interactions between keratinocytes and DCs constitute a proinflammatory loop that can lead to the recruitment of T cells, neutrophils, and DCs (Fig. 3).

In addition to psoriasis, the role of IL-36 has been studied in the pathogenesis of allergic contact dermatitis, ACD. The mRNA levels of all 3 IL-36 agonists, but not IL-36Ra, was enhanced in ACD-involved skin. By immunohistochemistry, IL-36 agonists were detected in epidermal layers. Of note, IL-1β, IL-1Ra, and IL-33 levels were also increased in the same skin samples. Ex vivo administration of IL-36Ra into uninvolved skin biopsies of ACD patients led to a significant reduction of IL-36 and IL-8 mRNA levels [43]. Recently, a comparative genome-wide expression study was carried out in patients affected with psoriasis and eczema, thus allowing the comparison within the same patients of gene transcript levels in psoriasis and eczema lesions to autologous, uninvolved skin. The results showed that enhanced IL-36α and IL-36γ expression was observed exclusively in psoriasis lesions. In addition, confirmatory studies performed in

3 different cohorts of patients showed that IL-36γ, as well as 14 other genes, provided a molecular signature that was able to distinguish psoriasis from eczema [44]. Taken together, these findings suggest that IL-36 cytokines are particularly involved in the pathogenesis of psoriasis.

IL-36 IN LUNG AND OTHER TISSUES

Emerging data suggest a strong role for IL-36 cytokines in the lung. Bronchial epithelial cells produce IL-36 in response to cytokines, such as TNF-α, IL-1β, and IL-17, and following challenge with cigarette smoke condensate [15, 16]. IL-36γ is strongly induced in human PBECs by infectious agents, including *Pseudomonas aeruginosa* and rhinovirus, and induction of IL-36γ by rhinovirus was stronger in PBECs from asthmatic donors than from controls [17, 18]. Rhinovirus infections are frequently associated with asthma exacerbations, including increased severity of respiratory symptoms and decreased lung function, and a strong increase in neutrophilic inflammation in the upper airways. i.n. Administration of IL-36α or IL-36γ to mice induces a rapid influx of neutrophils into the BALF [45, 46]. Therefore, it is reasonable to postulate that IL-36 contributes to neutrophil recruitment into the lung in the context of asthma exacerbations associated with rhinovirus infection. Neutrophil accumulation in the airways contributes to the pathogenesis of a number of pulmonary diseases in addition to asthma, including COPD, acute respiratory distress syndrome and cystic fibrosis. Smoke is the causative agent of COPD, and expression of IL-36α, IL-36β, and IL-36γ is increased in human bronchial epithelial cells in response to cigarette smoke condensate, suggesting that IL-36 could contribute to the influx of neutrophils into the lung in COPD [16].

IL-36 has been implicated in mouse models of experimental allergic asthma. IL-36γ was increased in the lungs of mice following sensitization and challenge with the house dust mite, as well as in A/J mice following challenge with OVA [45, 47]. A/J mice are much more sensitive to development of asthmatic-like symptoms in response to OVA challenge than C3H/HeJ mice, which are relatively asthma resistant. Genetic mapping of loci involved in this differential sensitivity identified the genomic region containing the genes for IL-1Ra, IL-1F10, IL-36Ra, IL-36β, IL-36α, and IL-36γ [48]. Several noncoding polymorphisms between A/J and C3H/HeJ mice were found in the *Il36g* gene (encoding for IL-36γ) [47], whereas no functional sequence variants were identified in IL-1Ra between the 2 mouse strains. There was also a clear increase in IL-36γ expression in the asthma-susceptible A/J mice compared with the resistant C3H/HeJ mice, with no difference in IL-1Ra expression between the 2 strains. These data suggest polymorphisms in IL-36γ may be important in conferring increased susceptibility to allergic asthma in A/J mice.

i.n. Administration of rIL-36γ has been used to investigate the role of IL-36 in the lung. Twice-daily i.n. delivery of IL-36γ to the lungs of mice for 2 days resulted in epithelial cell hypertrophy, infiltration of immune cells into the space around the airways, and alveolar spaces and mucus production [45]. Interestingly, lung resistance was increased 24 h after a single intratracheal

challenge with IL-36γ, which was accompanied by an increased cellularity in the BALF, consisting of predominantly neutrophils and some lymphocytes. Accordingly, IL-36γ administration led to a rapid increase in the neutrophil chemoattractants CXCL1 and CXCL2. i.n. Challenge of IL-36γ did not induce eosinophil infiltration into the lung even after multiple doses, suggesting that IL-36 induces lung pathology independent of eosinophils. IL-1α was also increased following i.n. challenge with IL-36γ, and i.n. administration of IL-1α leads to many of the same responses in the lung as IL-36γ, including rapid neutrophil influx. However, the IL-36γ effects were found to be independent of IL-1 induction, as they were unchanged in IL-1α$^{-/-}$IL-1β$^{-/-}$ mice [46].

IL-36 is produced by bronchial epithelial cells in the lung in response to a number of challenges. Epithelial cells and the adjacent fibroblasts can respond to IL-36, and IL-36 has been shown to induce NF-κB and MAPKs as well as expression of the neutrophil chemokines IL-8 and CXCL3 and the Th17 chemokine CCL20 [15]. The IL-36R is also expressed on CD11c+ alveolar macrophages, which are the predominant immune cell found in the naïve mouse lung, suggesting that these cells are poised to respond to IL-36 [46]. Whereas it has not yet been shown directly that alveolar macrophages respond to IL-36, splenic CD11c+ APCs respond to IL-36 through the induction of cytokines and neutrophil-attracting chemokines [46]. Therefore, the working model is that IL-36 is produced from bronchial epithelial cells in response to insult, including infection, cytokines, or exposure to cigarette smoke. IL-36 then acts on the epithelial cells, as well as the adjacent fibroblasts and alveolar macrophages, to induce inflammatory cytokines and chemokines, leading to an influx of neutrophils and lymphocytes into the lung and an increase in airway responsiveness and mucus production (**Fig. 4**).
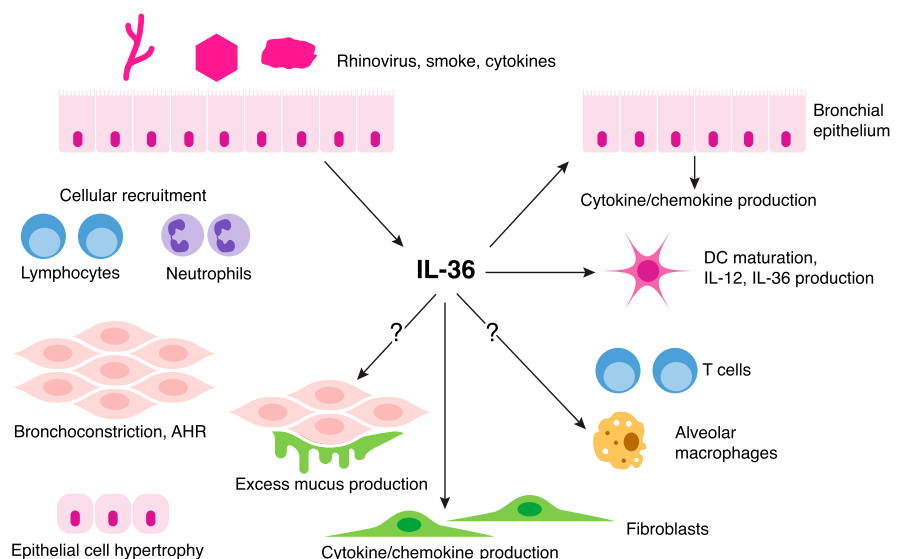
The expression of IL-36 in human pulmonary disease has not been thoroughly investigated, and there are only a few reports examining IL-36 expression in human lung. IL-36γ mRNA expression was increased in biopsies of recurrent respiratory papillomas and expression levels correlated with disease severity [49]. IL-36 levels were also evaluated as part of a study investigating >500 inflammatory mediators in patients with COPD and healthy controls [50]. In this study, there was a general down-regulation of systemic inflammatory mediators in patients with COPD who were undergoing acute exacerbations, including a decrease in IL-36α expression; however, levels of these mediators were not examined in the lung tissue itself.

There is evidence that IL-36 may play a role in other tissues, including the joint, kidney, adipose tissue, brain, and gut. IL-36α, IL-36β, IL-36Ra, and IL-36R are expressed in human synovial tissues, and IL-36 induces cytokine production from human synovial fibroblasts [51, 52]. No difference was seen in the level of IL-36β between healthy controls and rheumatoid arthritis or osteoarthritis patients in synovial fluid or serum [51], whereas increased levels of IL-36α have been reported in joints of patients with psoriatic and rheumatoid arthritis [52]. Inhibition of IL-36 signaling by use of an IL-36R-neutralizing antibody or in some cases, IL-36R null mice had no impact in several mouse models of arthritis, including collagen-induced arthritis, antigen-induced arthritis, and the human TNF-transgenic mouse arthritis model driven by TNF overexpression [35, 53]. The lack of impact of IL-36 cytokine inhibition in mouse models of arthritis is likely a result of redundancy with other IL-1 family members, most notably, IL-1, which plays a dominant role in rodent arthritis models. It is unclear whether this redundancy is similar in human arthritides. Of note, the magnitude of the stimulatory effect of IL-36 in synovial fibroblasts and articular chondrocytes was markedly lower than those of IL-1 [51], suggesting that IL-36 is probably not a key player in human arthritis also.

The role of IL-36 cytokines has also been studied in adipose tissue and kidney. IL-36α is expressed in human adipose tissue, primarily in the stromal vasculature but also in the adipocytes themselves [19]. Adipocytes responded to IL-36 through induction of IL-6 and IL-8 and a decrease in the expression of peroxisome proliferator-activated receptor-γ, suggesting

Figure 4. Potential role of IL-36 in the lung. IL-36 is produced by bronchial epithelial cells in response to a number of damage signals, including viral infection, smoke, and inflammatory cytokines. IL-36 can then act on the bronchial epithelial cells and adjacent fibroblasts and alveolar macrophages to induce secretion of cytokines and chemokines, leading to recruitment of immune cells, including neutrophils and lymphocytes, ultimately resulting in induction of epithelial cell hyperplasia, airway hyper-reactivity (AHR), and excess mucus production.

a reduction in adipocyte differentiation. Expression of IL-36 is increased in several mouse models of kidney disease, including chronic glomerulonephritis, where the number of IL-36α-positive tubules correlated with proteinuria, fibrosis score, and the presence of tubulointerstitial lesions, and in the streptozotocin-induced diabetic model, where the presence of IL-36α-positive tubules correlated with the magnitude of renal damage [54]. IL-36 is also known to be expressed in the gut and brain; however, the function of IL-36 cytokines in these tissues has yet to be thoroughly explored.

CONCLUSIONS

IL-36α, IL-36β, and IL-36γ signal through the same receptor and appear to have the identical effects on cells and in vivo, which calls into question the need for 3 ligands. There is evidence that the expression patterns for IL-36 ligands are different, with IL-36γ as the most highly expressed and inducible in skin and lung. In addition, there is very little sequence homology among the 3 ligands around the site of cleavage, suggesting differential mechanisms for producing the active form. Therefore, whereas all IL-36 ligands appear to have the same activity, their expression and activation are likely differentially regulated. More work needs to be done to address the regulation of IL-36 cytokines at the expression level, as well as to define and understand the forces governing their truncation. Whereas it is clear that processing these ligands must occur for their activity, the identity of the enzymes involved in the regulation of this process is unknown. Demonstration that truncation of IL-36 ligands occurs in vivo and under certain circumstances and the search for the proteases involved are active areas of research.

Recent data have emerged demonstrating that IL-36 cytokines play a crucial role in the skin, most dramatically evidenced by the genetic variants in *IL36RN*, leading to the life-threatening disease GPP, the marked up-regulation of IL-36 cytokines in psoriatic lesional tissue, and the ability of IL-36R neutralization to decrease the inflammation and skin thickening of human psoriatic tissue when engrafted onto mice. Despite convincing evidence for a role for these cytokines in skin pathology and the strong ability to induce inflammatory responses in the lung, it is not yet clear whether IL-36 plays a role in pathologic conditions in the lung, such as asthma or COPD, and whereas IL-36 cytokines are expressed in other tissues, including the kidney, brain, and gut, it is unclear what role they play in the physiology or pathophysiology of these tissues. In addition, whereas IL-36 clearly acts on DCs to enhance their maturation and production of cytokines and acts on naïve T cells in mice, the full role of these cytokines on human immune cells is not yet clear. Can IL-36 act on human $\alpha\beta$ T cells or $\gamma\delta$ T cells if provided in the right context? Likewise, the role of IL-36 on macrophages, in particular, of different subtypes and activation states has not been thoroughly explored. Similar to its fellow family members—IL-1, IL-18, and IL-33—IL-36 likely plays a prominent role in human health, and further studies assessing the expression and activity of IL-36 in the context of disease will likely uncover additional roles for these cytokines.

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