Host-Pathogen Interactions

Interleukin-36 cytokines may overcome microbial immune evasion strategies that inhibit interleukin-1 family signaling

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Pathogens deploy immune evasion strategies to successfully establish infections within their hosts. Naturally, the host responds by acquiring mechanisms to counter these strategies. There is increasing evidence that the three interleukin-36 (IL-36) cytokines, IL-36α, IL-36β and IL-36γ, play important roles in host immunity. With a focus on the skin as a target for microbial and viral invasion, the current knowledge of IL-36 functions is reviewed. Furthermore, the hypothesis that the IL-36s have evolved to counteract virulence factors is presented using viruses as an example. The IL-36s are related to IL-1α, IL-1β, IL-18, and IL-33. Numerous viruses affecting the skin have developed immune evasion strategies that neutralize IL-1α, IL-1β, or IL-18 signaling or combinations of these pathways. Through small differences in activation mechanisms and receptor utilization, it is possible that IL-36 signaling may proceed unhindered in the presence of these viral inhibitors. Thus, one physiological function of the IL-36s may be to counteract microbial immune evasion.

**Introduction to the Interleukin-1 Family**

The interleukin-1 (IL-1) cytokines IL-1α and IL-1β are pleiotropic cytokines that promote a diverse range of inflammatory, innate, and adaptive immune responses. Their agonism of the IL-1 receptor 1 (IL-1R1) can be inhibited by the endogenous receptor antagonist IL-1Ra [reviewed in (1)]. IL-18 is a related cytokine that is also known to play important roles in immune responses [reviewed in (2)]. Around the turn of the millennium, the IL-1 family suddenly grew from these 4 founding members to 10 (Table 1). A few years later, IL-33 (Table 1) was also recognized as part of the family (3). Because of the timing of discovery by multiple groups, these IL-1 family members were initially given many different names. To facilitate data sharing and comparisons, a new nomenclature was proposed (4) and approved by the committees for human and mouse gene nomenclatures (www.genenames.org and www.informatics.jax.org/mgihome/nomen/). Using the guidelines established by these committees, the new family cytokines were named IL-1F5 to IL-1F10, and the human and mouse genes were named IL1F5 to IL1F10 and Il1f5 to Il1f10, respectively (4). As functions for these new cytokines started to emerge, a second round of renaming was proposed (5). This new nomenclature (Table 1) was approved by the Human Genome Organisation (HUGO) Gene Nomenclature Committee (www.genenames.org/cgi-bin/genefamilies/set/601) and adopted in sequence databases, such as the National Center for Biotechnology Information; however, the mouse gene names have remained unchanged. To avoid confusion, the approved terminology used for the human genes and proteins will be used here for both the human and mouse orthologs.

These new IL-1 family members were included in the group due to 12 to 55% sequence identity to the founding members (IL-1α, IL-1β, IL-1Ra, and IL-18) and the shared presence of a characteristic barrel-like structure comprising 12 β strands. With the exceptions of IL18 and IL33, which are located on human chromosomes 11 and 9, respectively; the genes encoding the members of this family are located on human chromosome 2 within an about 360 kilo-base pair region at 2q14.1 (Fig. 1A). Their sequence similarity and clustering suggest that they arose from a common ancestral gene through multiple duplication events (Fig. 1B). Evolution is driven by initially random genetic changes, such as gene duplication events, which will be preserved if they afford a survival or reproductive advantage. In this context, IL-37 is interesting because it is present in primates (humans, monkeys, and lemurs), bats, cattle, pigs, and a limited number of rodents (beavers, marmots, and naked mole rats) but is absent in mice. Hence, it appears that IL-37 was lost in some species during the radiation of the rodents. This could suggest that IL-37 (mouse gene Il1f7) did not provide an essential advantage in a subset of rodent species. In contrast, the other IL-1 family members appear to be preserved throughout the mammals, suggesting important beneficial functions.

Although great advances have been made in understanding the functional importance of some of the most recently recognized members of the IL-1 family, such as IL-33 [reviewed in (6)], the physiological functions of others remain enigmatic. Here, I review insights into the biology and functions of IL-36α, IL-36β, and IL-36γ (referred to collectively as the IL-36s). Numerous skin tropic viruses have acquired immune evasion strategies that inhibit IL-1 or IL-18 signaling, or both, yet do not affect signaling elicited by the related IL-36 cytokines. I propose the hypothesis that the three IL-36s have been preserved during evolution to, in part, counteract immune evasion mechanisms targeting IL-1 and IL-18 signaling. Such functions could explain why many of these pathogens initially establish skin infections but later are well controlled in the immune-competent host.

**Signaling mechanisms and endogenous inhibition**

IL-1α and IL-1β signal through their common cell surface receptor IL-1R1, which, upon ligand binding, recruits the transmembrane IL-1R accessory protein, IL-1RAP (Fig. 2A) (7–9). Formation of this heterodimer allows intracellular recruitment of the adaptor protein MyD88 and subsequent mobilization of the IL-1R–associated kinases (IRAK1, IRAK2, and IRAK4, collectively called IRAKs) (8, 10–16). These kinases, in turn, through several additional signaling factors, lead to phosphorylation and degradation of the nuclear factor kB (NF-kB) inhibitor IkB and activation of the mitogen-activated protein kinase (MAPK) pathways involving extracellular signal–regulated kinase, c-Jun N-terminal kinase, and p38 [Fig. 2A; reviewed in (17)]. The final step in these direct signaling pathways involves translocation of activated transcription factors,
such as activator protein 1 (AP-1) and NF-xB, to the nucleus, where they activate cell type–specific gene expression (Fig. 2A). IL-18 and IL-33 stimulate gene expression via the same intracellular pathway through their respective receptor–accessory protein complexes IL-1R1–IL-1RAP and IL-1R1–IL-1RAP (Fig. 2A and Table 1) (2, 3, 18, 19).

The receptor for IL-36α, IL-36β, and IL-36γ is IL-1R–like 2 (IL-1RL2; also often referred to as IL-36R) (20, 21). Like the receptors for IL-1 and IL-33, IL-1RL2 uses IL-1RAP as a receptor–accessory protein and thus engages the same signaling cascade as IL-1, IL-18, and IL-33 (Fig. 2A). Endogenous activation of the IL-36–IL-1RL2 pathway was first demonstrated in a human ovarian tumor cell line (21) and later confirmed in a mouse macrophage cell line (22) and several primary human cell types, including synovial fibroblasts, articular chondrocytes (23), bronchial epithelial cells (24), keratinocytes (25, 26), and colonic subepithelial myofibroblasts (27). Evidence from in vitro cellular activation and gene expression analyses suggest that the three IL-36s have comparable activities (21, 25, 27–31).

The agonist activities of the IL-1 family cytokines can be neutralized by both endogenous factors and pathogen effectors in various ways to ensure restraint of the resulting inflammatory responses. In the context of this review, the most important endogenous inhibitors are the IL-1R type 2 (IL-1R2) and IL-18 binding protein (IL-18BP). The gene encoding IL-1R2 is located between IL1R1 and IL1R1 on chromosome 2 (2q12) in humans. IL-1R2 binds IL-1α and IL-1β; however, it lacks the intracellular domain required for recruitment of MyD88 and IRAKs and therefore cannot initiate intracellular signaling (Fig. 2B) (32). Because IL-1R2 association with IL-1α and IL-1β prevents the IL-1s from binding to the signaling receptor IL-1R1 (Fig. 2B), IL-1R2 is considered a decoy receptor (32). This decoy activity may occur while the receptor is associated with the cell membrane or in the extracellular space after proteolytic cleavage and release of the extracellular domain (33, 34). IL-1R2 may also divert IL-1RAP away from the IL-1–IL-1R1 complex, thereby rendering the latter inactive (35, 36). A somewhat similar mechanism of signaling inhibition involves IL-18BP (Fig. 2B), which is a secreted protein that acts as a decoy for IL-18 (37). However, unlike IL-1R2, which is related to IL-1R1 in sequence, the gene encoding IL-18BP, located at 11q13 in humans, appears unrelated to IL1R1 (37). Four IL-1 family members, IL-1α, IL-36α, IL-37, and IL-1F10, also act as endogenous inhibitors of signaling. IL-1Rα and IL-36αa act as competitive antagonists of IL-1 and IL-36, respectively, by binding to the receptors (Fig. 2B) in a manner that does not allow the recruitment of IL-1RAP (20, 38–42). Because two intracellular domains—one from the receptor and one from the receptor–accessory protein—are required for MyD88 docking (Fig. 2A), the IL-1α–IL-1R1 and IL-36αa–IL-1R2 complexes do not initiate signaling (Fig. 2B). IL-18 signaling can be disrupted through the formation of an IL-37–IL-18BP–IL-1RAP complex, which, as described above, does not signal due to the absence of a heterodimeric MyD88 docking platform (Fig. 2B) (43). An alternative mechanism of signaling inhibition involves binding of IL-37 to IL-18R1 and a single immunoglobulin domain IL-1R–related protein, SIGIRR (44–47). In this complex, SIGIRR appears to have dual functions (44–47): It prevents the recruitment of MyD88 and subsequent engagement of downstream pathways (Fig. 2B), and it activates alternative signaling cascades that stimulate anti-inflammatory mechanisms. SIGIRR can also inhibit IL-1 and IL-33 signaling [reviewed in (48)].

The final IL-1 family member acting as an endogenous inhibitor is IL-1F10, which is often referred to as IL-38, a term yet to be officially accepted by the gene nomenclature committees. The biological activity of IL-1F10 resembles that of IL-36Ra (Fig. 2B) (49). IL-1 and IL-33 activity can also be neutralized by several proteins generated through alternative splicing. The two soluble IL-1R1Ps, sIL-1R1P and sIL-1R1P–β, are alternative splice variants of IL1RAP (7, 50, 51), whereas soluble IL-1R1 (sIL-1R1) is a splice variant of

Table 1. The receptors, receptor–accessory proteins, and endogenous inhibitors of the IL-1 family of cytokines. IL-1 family members (in bold) are grouped into four subfamilies. The receptors and receptor–accessory proteins that transduce signals from each family member are listed, as well as inhibitors of signaling. The nomenclature used here follows that of the HUGO Gene Nomenclature Committee for ILs (www.genenames.org/cgi-bin/genefamilies/set/601) and IL receptors (www.genenames.org/cgi-bin/genefamilies/set/602).

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<th>Agonist subfamily</th>
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Fig. 1. Chromosomal organization and phylogenetic relationships of human IL-1 family members. (A) Chromosomal locations and organization of the genes encoding the human IL-1 family members. Arrows indicate the direction of transcription. (B) Protein phylogeny of the 11 human IL-1 family members. The human IL-1α (NP_000566.3), IL-1β (NP_000567.1), IL-1Ra (NP_776213.1), IL-1B (NP_001553.1), IL-33 (NP_254274.1), IL-36α (NP_055255.1), IL-36β (NP_775270.1), IL-36γ (NP_062564.1), IL-36Ra (NP_036407.1), IL-37 (NP_055242), and IL-1F10 (NP_775184.1) protein sequences were aligned, and a Cobalt cladogram was generated using COBALT and default settings at www.ncbi.nlm.nih.gov/tools/cobalt/re_cobalt.cgi.

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**Fig. 2.** IL-1 family signaling pathways and endogenous inhibitors. (A) Signaling from the IL-1 family receptors takes place through the following sequence of events: The IL-1 family agonists—IL-1α (blue), IL-1β (red), IL-33 (pink), and IL-36γ (yellow)—bind to their relevant receptors—IL-1s to IL-1R1, IL-18 to IL-18R1, IL-33 to IL-1RL1, and IL-36s to IL-1RL2. This causes the appropriate receptor accessory protein to be recruited to form a heteromeric receptor–accessory protein complex. MyD88 binds to the intracellular domains of the newly formed receptor complex heterodimer, and IRAKs are recruited to the complex. Through several intermediate steps, the MAPK pathway is activated, and IκB is degraded. The transcription factors AP-1 and NF-κB translocate to the nucleus and stimulate target gene expression. (B) Numerous endogenous inhibitors prevent the assembly of functional receptor–accessory protein complexes through various mechanisms. Shapes are for illustration purposes only, and do not reflect actual protein structures or mechanisms of interaction.

***IL1RL1*** (52–54). These soluble proteins are secreted from cells due to the inclusion of a signal peptide and absence of the transmembrane and intracellular regions present in IL-1RAP and IL-1RL1, respectively (7, 50–54). The sIL-1RL1 acts as a decoy by binding IL-33, thereby preventing the latter from associating with the full-length, membrane-bound receptor (Fig. 2B) (55). The sIL-1RAs function by (i) preventing the association of the IL-1–IL-1R1 (Fig. 2B) and IL-33–IL-1RL complexes with the membrane-bound IL-1RAP required for signaling (50, 56–58) and (ii) enhancing the affinities of IL-1R2 for IL-1α and IL-1β (Fig. 2B) and sIL-1RL1 for IL-33 (Fig. 2B) (58, 59). Because of the common use of IL-1RAP for signaling (Fig. 2A), the sIL-1RAs are also expected to inhibit IL-36 signaling (Fig. 2B).

**Cytokine activation mechanisms**

Most of the IL-1 family members lack signal peptides for conventional protein secretion and, consequently, are produced in the cytosol either constitutively or in response to inflammatory mediators. Although the full-length forms of IL-1α and IL-33 are biologically active, IL-1β and IL-18 require proteolytic processing to generate the active forms, an event typically associated with extracellular release [reviewed in (6, 60)]. The most extensively studied protease involved in this process is caspase-1. Caspase-1 is activated upon recruitment to an inflammasome (Fig. 3A) comprising multiple copies of the adaptor protein apoptosis-associated speck-like protein (ASC) and sensor proteins [reviewed in (60)]. The sensor proteins are involved in directly or indirectly detecting cellular stress and microbrial infections and can be divided into three main groups (Fig. 3A): NOD (nucleotide-binding oligomerization domain)–like receptors (NLRs), absent in melanoma 2-like receptors (ALRs), and RIG-1 (retinoic acid–inducible gene I)–like receptors (RLRs) (60). In addition to cleaving pro–IL-1β and pro–IL-18, caspase-1 and the related caspase-4, caspase-5, and caspase-11 also cleave gasdermin D (61–63), which, in turn, oligomerizes in the plasma membrane to form pores (64–69) through which mature IL-1β and IL-18 can be released (Fig. 3A). The formation of these pores also leads to cell death by pyroptosis (61–69). Additional release mechanisms may be present (Fig. 3A); however, these are less well understood. Activation of the inflammasomes and caspase-1 has proven important not only for host immunity against bacteria, viruses, and fungi but also for development and progression of immune-mediated diseases (60). In addition to caspase-1, several other proteases, including cathepsin G, chymase, chymotrypsin, elastase, proteinase-3, granzyme 4, and a staphylococcal...
protease, have been shown or predicted to cleave IL-1β within a region of 10 amino acids that includes the caspase-1 cleavage site [Fig. 3B; reviewed in (70)]. The physiological importance of many of these potential additional activation mechanisms is largely unknown; however, they would be expected to take place outside the cells due to the extracellular localization of these proteases.

Like the IL-1s and IL-18, the IL-36s lack signaling peptides for conventional protein secretion (71, 72). When the IL-36s were first discovered (71, 72), they were believed to be synthesized as mature cytokines, IL-1β and IL-18, are released through the newly formed gasdermin D pores (solid black arrow). Caspase-1 also cleaves gasdermin D (GSDMD), thereby releasing the N-terminal domain (GSDMD-NT) from autoinhibition by the C-terminal domain (GSDMD-CT). GSDMD-NT translocates to the cell membrane, oligomerizes, and forms pores. Caspase-1 has not been determined.

**Biological Functions of IL-36**

**Role in immunity against microbes**

When and where a protein is produced can provide important clues to its function. Hence, from the time of their discovery, several of the newest additions to the IL-1 family were suspected to have a role in innate or adaptive immunity. The abundance of IL36A mRNA increases in the proliferating keratinocytes of mouse skin infected with herpes simplex virus-1 (HSV-1) (72) and human monocytes treated with LPS (71). In addition, LPS-activated monocytes (71) were found to have increased expression of the IL36B mRNA. Subsequent studies have demonstrated an increase in IL-36 transcripts and proteins in epithelial sites (Fig. 3B) do not resemble caspase-1 sites (73, 74). In vitro, neutrophil-derived cathepsin G, elastase, and proteinase-3 can truncate full-length recombinant IL-36 and IL-36Ra proteins (74–76); however, whether this processing is activating or inactivating is currently controversial (74–76). Note that several of these proteases have been reported to also cleave IL-1β (Fig. 3B). Recently, it was shown that extracellular cathepsin S released from lysosomes in epithelial cells, including keratinocytes, cleaves IL-36γ at the critical Ser18 residue (Fig. 3B), leading to enhanced activity of the cytokine (74). Whether intracellular processing resembling that of IL-1 and IL-18 occurs to facilitate the extracellular release of IL-36s is unknown. However, in keratinocyte cultures, high calcium concentrations, which promote terminal differentiation, appear to cause intracellular cleavage of IL-36γ (26) at a site further downstream than the predicted activation site (73). The protease(s) involved and the functional importance of this processing have not been determined.

Release of unprocessed IL-36α by macrophages can be induced in vitro by extracellular administration of lipopolysaccharide (LPS) + adenosine 5’-triphosphate (ATP) or the toxin nigericin (77). In vivo IL-36α is also externalized from inflamed skin as an unprocessed cytokine (78). Both in vitro and in vivo, this release is independent of caspase-1 (77, 78). Extracellular IL-36γ has been detected in culture medium from keratinocytes treated with ATP, the double-stranded RNA analog polyinosinic-polycytidylic acid [poly(I:C)], or the antimicrobial peptide LL-37 and from lung macrophages exposed to bacteria or bacterial components (24, 26, 31, 79, 80). Some of this released IL-36γ appears to be present in extracellular vesicles (80, 81); however, the purpose of such packaging is unknown.
and immune cells in response to a wide range of inflammatory mediators, microbial agents, and pathogen-associated molecular patterns (PAMPs). Screens using Toll-like receptor (TLR) ligands identified a subset of agents that could induce expression of one or more IL-36s in either bronchial epithelial cells or skin keratinocytes (24, 79). These ligands included double-stranded RNA [poly(I:C)], flagellin, and Mycoplasma fermentans synthetic lipopeptide (FSL-1), LPS, and zymosan and implicate the IL-36s in immunity against bacteria, fungi, and viruses (24, 79, 82). Confirmatory and complementary studies using intact microorganisms have reported increased production of IL-36γ in the lung and oral epithelial cells after exposure to rhinovirus, dust mites, or the bacterial pathogens Pseudomonas aeruginosa or Porphyromonas gingivalis (22, 83–85), in monocytes activated by LPS (86), and in macrophages infected with Mycobacterium tuberculosis (87). All three IL-36s can be induced in peripheral blood mononuclear cells by the opportunistic fungal pathogen Aspergillus fumigatus (88), whereas LPS and Burkholderia species of bacteria have been reported to increase the production of IL-36α in macrophages (89, 90). Increased abundance of IL-36α has also been observed in the blood, lungs, and peritoneal cavity during experimental sepsis in mice induced by cecal ligation and puncture. The cells producing the IL-36α were not identified (91); however, a possible source could be monocytes and macrophages because treatment with LPS or bacteria causes these cells to increase their expression of IL-36α mRNA (71, 89, 90).

Endogenous inflammatory mediators are also potent activators of IL-36 and may, in part, explain some of the changes reported in response to microorganisms. Production of the IL-36s is induced by IL-1, IL-17, IL-18, IL-22, tumor necrosis factor (TNF), and the IL-36s themselves in bronchial epithelial cells, skin keratinocytes, synovial fibroblasts, articular chondrocytes, colonic myofibroblasts, and myeloid cells (23, 24, 92–94). IL-36β appears to be the only IL-36 that is induced by interferon-γ (IFN-γ) (24, 92). Given the diverse range of agents that stimulate IL-36 production, it seems reasonable to hypothesize that the IL-36s, just like other IL-1 family members, play important roles in immune responses against microbes.

Role in leukocyte recruitment
The IL-36s can induce production of IL-1α, IL-1β, IL-6, IL-8, C-C motif chemokine ligand 2 (CCL2), CCL20, colony-stimulating factor 2 (CSF2), CSF3, CXCL1, CXCL2, CXCL3, CXCL5, and TNF cytokines in keratinocytes, lung and oral epithelial cells, monocytes, macrophages, chondrocytes, and fibroblasts in vitro (22–24, 27, 30, 78, 95, 96). In light of the IL-36 induction by PAMPs (above), this cytokine induction suggests an important role in orchestrating the positioning of immune cells at sites of infection, as previously only demonstrated for IL-1 signaling (97, 98). The first in vivo evidence for a role of the IL-36s in leukocyte recruitment came from studies using IL-36α transgenic mice. Overexpression of IL-36α in keratinocytes stimulated skin inflammation involving epidermal Langerhans cells, dermal macrophages, neutrophils, and T cells (38). This immune cell recruitment was associated with increased expression of the cytokine-encoding mRNAs IL23A (p19), CSF2 (GM-CSF), TNF-α, IL1α, CCL2 (MCP-1), and CXCL2 (MIP-2) (38). These phenotypes strongly resemble the human inflammatory skin disease psoriasis, in which expression of IL36α and IL36γ is increased [reviewed in (99)]. Similar responses seem to take place in the lungs because it has been reported that intratracheal administration of recombinant IL-36α (100) or IL-36γ (22) promotes similar chemokine production and neutrophil infiltration. Further studies using knockout mice have confirmed the role of IL-36α–IL-1RL2 signaling in promoting inflammation in a mouse model of drug-induced psoriasis (78, 101).

Function in leukocyte activation
In addition to promoting leukocyte recruitment, there is increasing evidence that the IL-36s can activate some types of immune cells. Several studies have reported that the IL-36s activate subsets of dendritic cells (DCs), including skin Langerhans cells, in vitro to increase the surface abundance of clusters of differentiation 14 (CD14), CD40, CD80, CD83, CD86, and major histocompatibility complex class 2 and the secretion of IL-1β, IL-6, IL-12, IL-18, IL-23, and TNF-α (28–30, 100, 102). These antigen-presenting cells may promote initiation of adaptive immune responses to microorganisms; however, such functions against infections remain to be demonstrated in vivo.

Additional in vitro studies have established the effects of IL-36 on T cell and natural killer (NK) cell populations. IL-36 stimulates proliferation of CD4+ T cells and their polarization toward the T helper 1 (Th1) cell fate and production of IFN-γ, IL-2, IL-4, and IL-17 (28–30, 88, 100, 102–105). Cross-communication in vivo between keratinocytes and DCs leading to Th1-related skin disease has been demonstrated in mice by inducing inflammation with the antiviral drug imiquimod (101). Furthermore, expansion and activation of γδ T cells and cytotoxic T cell and NK cell populations, leading to increased IFN-γ production, can be induced by IL-36 when cells are cotreated with additional cytokines or antibodies that target activating CD markers (105, 106). Enhanced in vivo cytotoxic immune responses against tumors genetically engineered to overexpress IL-36γ have also been reported (105). Hence, it appears that the IL-36s can promote both the activation and expansion of Th1- and cytotoxic cell–mediated immune responses, including production of the antimicrobial IFN-γ. Whereas cells isolated from IL-1RL2 knockout mice produced less IFN-γ than did cells from wild-type mice when infected with the vaccine strain Mycobacterium bovis Bacillus Calmette-Guérin (BCG) ex vivo (103), the IL-1RL2–deficient mice cleared BCG and pathogenic Mycobacterium tuberculosis as well as wild-type mice (107). Thus, an essential role for endogenous IL-36 in immunity against microbiota mediated by Th1 or cytotoxic cells, or both, remains to be demonstrated in vivo.

Several other cell types have been reported to be activated by IL-36. Pretreatment of macrophages with IL-36α enhances the ability of these cells to phagocytose and destroy Escherichia coli in vitro, which might explain how IL-36α provides protection against the outcome of sepsis induced by cecal ligation and puncture (91). In addition, the presence of IL-1RL2 on B cells (104) and the ability of IL-1RL2 to activate M2 macrophages (95) have been reported; however, the biological importance of these observations has not been examined.

Involvement in production of antimicrobial peptides
Many types of cells that are stimulated by IL-36 produce antimicrobial peptides. Keratinocytes have been reported to make cathelicidin (cathelicidin antimicrobial peptide; IL-37), β-defensin 4 (DEFB4), DEFB103, lipocalin 2, peptidase inhibitor 3, and S100 calcium-binding protein A7 (S100A7; psoriasin) (25, 31), whereas IL-36–treated vaginal and endocervical epithelial cells secrete the β-defensins DEFB4A [human β-defensin-2 (HBD-2)] and DEFB104 (HBD-4) (82). Functionally, IL-36–induced production of cathelicidin and DEFB4A has been linked to restricting the growth of M. tuberculosis in macrophages in vitro (87).

Contribution to induction of fever
IL-1β is well known to cause fever during acute infections through induction of the endogenous pyrogens IL-6 and prostaglandin E2...
[reviewed in (108)]. IL-6 stimulates the production of cyclooxygenase 2, which converts arachidonic acid into prostaglandin E2, which, in turn, acts directly on the hypothalamus to raise the body temperature set point (108). Fever is associated with the general sickness syndrome additionally involving anorexia, body aches, lethargy, and social withdrawal. Topical application of the antiviral drug imiquimod causes behavioral changes in mice resembling the human sickness syndrome (109, 110). These behavioral changes depend on IL-1 and IL-36α signaling (109). The treatment is also associated with increased IL-1β in the brain (111) and migration of leukocytes into the brain (110). In vitro, IL-36 induces IL-1β and IL-6 production in keratinocytes, monocytes, and DCs (30, 92). IL1RL2 mRNA has been detected in astrocyte and microglial cell cultures (112, 113), and in vivo, it has been observed in the cerebral cortex and the cerebellar vasculature (114). Furthermore, IL-36β and IL-36γ can be produced by neurons, microglial, oligodendrocytes, astrocytes, and neutrophils in vivo (112, 115). Hence, the IL-36 system is present in the brain, and it is plausible that IL-36 can induce fever directly or indirectly, for example, by promoting IL-1 and IL-6 production or inflammation or both. However, this and the mechanisms involved remain to be established.

Role in immunity against viruses

The ability of the IL-36s to promote neutrophil recruitment, production of antimicrobial peptides, and macrophage activation suggests classical immune functions of the IL-36s in defense against bacteria. However, many of the reported cellular and physiological responses described above may also be important for immunity against viruses. For example, enhanced activity of DCs may promote the development of adaptive immune responses such as the production of neutralizing antibodies and activation and expansion of cytotoxic CD8+ cells that are essential for the killing of virus-infected cells. IFN-γ potently inhibits viral replication, and recruitment of neutrophils can guide IFN-γ-producing CD8+ cells to the location of the virus-infected cells (116). Furthermore, fever promotes the recruitment and activation of immune cells (108), including IL-1–induced expansion of lymphocytes (117). Production of antimicrobial peptides provides additional means to inhibit viral replication and modulate antiviral immune responses, for example, by triggering virus aggregation or trapping, viral envelope disruption, and enhanced IFN-β production (118, 119). In agreement with this, an in vivo role for IL-36α was demonstrated in a murine model of influenza pneumonia (120). However, given the apparent redundancy in how the IL-1s and IL-36s activate cells (Fig. 2A), it remains an enigma why humans and mice have IL-18, IL-33, two IL-1s, and three IL-36s. How pathogenic viruses have developed strategies to block IL-1 or IL-18 signaling, or both, is reviewed below. Furthermore, I propose that the IL-36s have been preserved during mammalian evolution because they counteract such immune evasion mechanisms and thereby ensure induction and recruitment of effective immune responses.

Viral Immune Evasion and Potential Counteraction by IL-36

Diverse viruses replicate in human skin or cause skin diseases, for example, poxvirus, human papillomavirus (HPV), HSV-1 and HSV-2, measles virus, and Kaposi’s sarcoma–associated herpesvirus (KSHV). A common theme among these viruses is the initial viral deployment of immune evasion strategies that allow infections to be established. However, in the immune-competent host, these viruses are typically, over time, eventually cleared or prevented from spreading. This suggests the presence of host mechanisms that can overcome virus-induced immune suppression. Further evidence for unique immune properties of the skin comes from studies demonstrating that protective immunity against smallpox induced by the vaccine strain vaccinia is dependent on inoculation of the virus by skin scarification; intramuscular vaccine administration does not provide the same level of protection as scratching the virus into the surface of the skin (121). Viral strategies known to interfere with IL-1 or IL-18 functions are reviewed below. This is followed by a hypothesis for how the IL-36s may overcome these mechanisms due to being insensitive to specific viral inhibitory strategies.

Preventing production and extracellular release of active cytokines

Many viruses that directly or indirectly affect epithelial cells, such as keratinocytes in the skin, interfere with production of active IL-1 or IL-18, or both (Fig. 4A). Some mechanisms target the initial production of the precursors pro–IL-1β and pro–IL-18, whereas others interfere with the inflammasomes the trigger cleavage of the pro forms or with the subsequent secretion of the mature cytokines. HPV and KSHV are examples of viruses that reduce the production of pro–IL-1β and pro–IL-18. The HPV16 E6 oncogene promotes degradation of pro–IL-1β through a proteasome-dependent pathway involving the host ubiquitin ligase E6-AP and p53 (Fig. 4A) (122). KSHV reduces IL18 mRNA abundance by expressing a noncoding polyadenylated nuclear RNA (PAN RNA) that diverts interferon regulatory factor 4 (IRF4) away from gene promoters (Fig. 4A) (123).

Viral inhibition of the inflammasome takes many forms. KSHV targets it through the viral protein open reading frame-63 (Orf63) (124). Orf63 is a homolog of the inflammasome component NLR family pyrin domain--containing 1 (NLRP1) and, as such, disrupts assembly of a functional inflammasome through interactions with the NLR sensors (Fig. 3A) NLRP1, NLRP3, and NOD2 (Fig. 4A). Because caspase-1 is not activated in KSHV-infected cells, IL-1β and IL-18 remain in their inactive, intracellular pro forms (Fig. 4A) (124). Related mechanisms have been identified in pox and measles viruses: The vaccinia Bcl-2 homolog F1L and the measles V protein inhibit NLRP1 and NLRP3, respectively (Fig. 4A) (125, 126). Poxviruses further prevent activation of IL-1β and IL-18 by producing the virus-encoded serine protease inhibitor-2 (SPI-2), which inhibits caspase-1 (Fig. 4A) and caspase-8 (127, 128). HSV-1 also seems to take multiple approaches to ensure that active IL-1β, and possibly IL-18, is not produced by infected cells. The HSV-1 infected-cell polypeptide 0 (ICP0) protein promotes degradation of the ALR sensor (Fig. 3A) IFN-γ-inducible protein-16 (IFI16; Fig. 4A), thus preventing activation of the inflammasome (129, 130). In addition, the viral-encoded enzyme UL37 (unique long region 37) deamidates two asparagine residues in RIG-I that are critical for the ability of RIG-I to bind to double-stranded RNA (131). Consequently, this RLR-type sensor (Fig. 3A) is not activated, and production of antiviral IFN-β is inhibited (131). Although whether or not HSV-1 UL37 has an effect upon IL-1β and IL-18 activation was not examined in this study (131), the viral immune evasion strategy that was identified is likely to also affect these proinflammatory cytokines due to the known role of RIG-I in inducing their inflammasome-dependent processing (132). Finally, IL-1β that has been processed is retained within HSV-1–infected cells (Fig. 4A) through an incompletely understood mechanism (129, 133). Given the similarities between IL-1β and IL-18 activation (Fig. 3A), IL-18 may be inhibited in a similar manner (Fig. 4A).
Despite the evasion mechanisms deployed by HSV-1, IL-1α is released from HSV-1–infected keratinocytes in vitro in a manner that is independent of caspase-1 and in vivo promotes inflammation at infected sites in the skin (97). Hence, IL-1α has been proposed as an evolutionary adaptation of the host to counteract the anti-inflammatory activities of HSV-1 (Fig. 4B) to ensure initiation of appropriate immune responses (97). It is conceivable that IL-33 can act in a similar manner. Although it remains to be determined whether the IL-36s are proteolytically processed in vivo, the likelihood that they can act as full-length proteins (20–24, 78) or may be activated by proteases other than caspase-1 (73, 75, 76) opens the possibility that the IL-36s are not affected by the viral immune evasion mechanisms that block the activation of the inflammasome (Fig. 4). Furthermore, the distinct nucleotide and protein sequences of the IL-36s may render these cytokines resistant to targeting by HPV16 E6 and KSHV PAN RNA, so they may still be produced and secreted by infected cells (Fig. 4B). Hence, in combination with IL-1α, the IL-36s may promote immunity against these pathogens to clear or control the infections over time. Although this review is focused on skin immunity, it is noteworthy and in agreement with this hypothesis that IL-36α plays an essential role in initiating immune responses against influenza (120). The influenza protein NS1 (nonstructural protein 1) blocks NLRP3 function (134, 135), similar to inflammasome inhibition by other viruses, as described above.

**Extracellular neutralization of cytokines by viral proteins**

Perhaps not surprising, several viruses deploy direct or indirect decoy mechanisms to neutralize the activities of IL-1 or IL-18 (Fig. 5A). The poxvirus proteins B15R (vaccinia virus) and vIL-1βR (ectromelia virus) exhibit about 30% amino acid sequence identity with IL-1R2 (Fig. 2B). Both of these viral proteins bind to IL-1β (Fig. 5A) but not to IL-1α (Fig. 5B) (136–138). These inhibitory mechanisms modulate lymphocyte proliferation in vitro and the inflammatory response and virulence in vivo (136–138). Poxviruses, including the molluscum contagiosum virus, encode the IL-18BP homologs MC54L (molluscum contagiosum) and C12L (other poxviruses) (37, 139, 140). These proteins bind directly to human and mouse IL-18 (Fig. 5A) and thereby block IL-18–induced IFN-γ production by T cells in vitro (37, 139, 140). A more indirect mechanism is used by HPV. The HPV oncogene product E7 increases gene

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**Fig. 4. Viruses inhibit IL-1β and IL-18 production transcriptionally and posttranslationally, possibly without affecting the release of IL-1α, IL-33, or IL-36.** (A) The viral proteins KSHV Orf63, vaccinia F1L, measles V, and HSV-1 UL37 dock with the NLR sensors NLRP1, NLRP3, NOD2, or RIG-I, as indicated, and prevent the assembly of functional inflammasomes. Consequently, caspase-1 is not activated, and pro–IL-1β and pro–IL-18 remain in their inactive, unprocessed states. The poxvirus protein SPI-2 inhibits caspase-1 directly. The HSV-1 protein ICP0 binds to and promotes degradation of the nuclear sensor IFI16. The KSHV PAN RNA binds IRF4 and reduces IL18 mRNA expression. HPV16 E6 protein promotes the degradation of pro–IL-1β by interacting with the host ubiquitin ligase E6-AP and p53. Through an unknown mechanism, HSV-1 retains mature IL-1β, and presumably IL-18, within infected cells. (B) IL-1α, IL-33, IL-36α, IL-36β, and IL-36γ may be released by dying cells and through an active mechanism that has yet to be identified and is independent of the inflammasomes. Thus, release of these cytokines may be unaffected by the viral proteins that inhibit the production or release of IL-1β and IL-18: KSHV Orf63, measles V, vaccinia F1L, pox SPI-2, HSV-1 ICP0, and HSV-1 UL37. The IL-1α and IL-36 mRNAs and proteins may also be resistant to the activities of the KSHV PAN RNA and the HPV16 E6 protein.
Viral interference with intracellular signaling

Engagement of the heteromeric receptor complexes IL-1R1–IL-1RAP by IL-1 and IL-1R1–IL-1RAP by IL-18 triggers the recruitment of the adaptor protein Myd88 and the IRAKs (Fig. 2A), including IRAK2 (14, 50), to the cytoplasmic tails of the receptor complex. Around the same time that the IL-36s were discovered, it was noted that two vaccinia proteins, A46R and A52R, share sequence homology with the intracellular domains of IL-1R1 and the TLRs (142). Both vaccinia proteins were shown to reduce IL-1 signaling in vitro, with A52R being the more potent inhibitor (142). A52R mediates this inhibitory activity by associating with IRAK2 (143), an interaction that prevents recruitment of IRAK2 to the IL-1–IL-1R1–IL-1RAP complex (Fig. 6A). In a related manner, A46R blocks signaling by associating with MyD88 and the related adaptor proteins TRAF (TNF receptor–associated factor), Mal, and TRAM [TRIF (Toll/interleukin-1 receptor domain–containing adapter-inducing IFN-β)–related adaptor molecule] used by some TLRs (Fig. 6A) (144).

Although the absence of A52R in a vaccinia mutant strain does not affect in vitro replication of the virus, the mutant strain causes milder disease in mice than does the wild-type strain, suggesting a role of A52R in virulence in vivo (143). A46R-deficient virus also causes less severe disease in mice, an outcome associated with enhanced early recruitment of leukocytes to infected lungs (144). More recently, a third vaccinia-encoded inhibitor of IL-1 signaling, K7, was identified (145). This protein, like A52R, interacts with IRAK2 (Fig. 6A) (145).

Despite the immune evasion mechanisms mediated by A46R, A52R, and K7, vaccinia induces potent immune responses that are protective against variola (smallpox) and ectromelia (mousepox) (146). The above described mechanistic studies of how vaccinia proteins interfere with intracellular IL-1 signaling were performed using overexpression of proteins rather than intact virus (142–145). However, during an actual infection, it takes from a few to several hours for K7 and A46R, respectively, to accumulate (144, 145) sufficiently to inhibit immune signaling. Thus, IL-1 family signaling through MyD88 may proceed unhindered during the early stages of infection (Fig. 6B). Furthermore, because IRAK1 is not inhibited by vaccinia A52R (143), it is conceivable that this kinase may provide an additional means to overcome vaccinia immune evasion (Fig. 6B). Vaccinia-infected cells likely still release functional IL-1α, IL-33, IL-36α, IL-36β, and IL-36γ, as described above and as illustrated in Figs. 4B and 5B. These cytokines may, despite the vaccinia A46R, A52R, and K7 proteins, activate neighboring uninfected cells, which, in turn, can initiate inflammatory responses (Fig. 6B).

Conclusions

The IL-36s have gained a lot of attention in recent years; however, our understanding of their physiological function(s) remains at best limited. Several lines of evidence, primarily from cell culture studies, suggest an
important role in immunity. Although, for example, IFN-γ abundance has been shown to be affected when IL-36 signaling is prevented during in vitro infections with fungi and mycobacteria (87, 88, 103), an effect of this upon infection outcomes in vivo has not been detected (107). Given the overlapping signaling mechanisms among IL-1 family members (Fig. 2A), this outcome may not be surprising if the functional consequence of these redundancies is, at least in part, to overcome the immune evasion strategies of pathogens. Further studies will be required to determine whether this hypothesis holds true. Experiments carefully designed to consider both cytokine cooperation and microbial (bacteria, fungi, and viruses) immune evasion strategies may be better able to document in vivo functions of the IL-36s. In addition, innate immune functions of the IL-36s may also be more readily detected in mice deficient in adaptive immunity. It is also very likely that during evolution, members of the IL-1 family, including the IL-36s, have acquired additional physiological functions. Future studies are expected to bring exciting discoveries related to IL-36 functions. Such new findings could lead to improved vaccine approaches or treatments for infectious and immune-related diseases.

REFERENCES AND NOTES


48. J. E. Towne, K. E. Garka, B. R. Brenchaw, G. D. Virca, J. E. Sims, Interleukin (IL)-1F6, IL-1F8, and IL-1F9 signal through IL-1Rrp2 and IL1RAPc to activate the pathway leading to NF-κB and MAPKs. *J. Biol. Chem.* 279, 13677–13688 (2004).


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Interleukin-1 in the pathogenesis and treatment of inflammatory diseases.


responses by enhancing proliferation and Th1 polarization of naive CD4+ T cells.

104. R. Penha, J. Higgins, S. Mutamba, P. Barrow, Y. Mahida, N. Foster, IL-36 receptor is expressed by human blood and intestinal T lymphocytes and is dose-dependently activated via IL-36γ and induces CD4+ lymphocyte proliferation. Cytokine 85, 18–25 (2016).


114. K. Lim, Y.-M. Hyun, K. Lambert-Emo, T. Capece, S. Bae, R. Miller, D. J. Topham, M. Kim, Interleukin-36α, IL-1Rrp2 expression and IL-1F9 (IL-1H1) actions in brain cells. J. Neuroinflammation 13, 75 (2016).


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Interleukin-36 cytokines may overcome microbial immune evasion strategies that inhibit interleukin-1 family signaling
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