Autoinflammatory skin disorders are a group of heterogeneous diseases that include diseases such as cryopyrin-associated periodic syndrome (CAPS) and familial Mediterranean fever (FMF). Therapeutic strategies targeting IL-1 cytokines have proved helpful in ameliorating some of these diseases. While inflammasomes are the major regulators of IL-1 cytokines, inflammasome-independent complexes can also process IL-1 cytokines. Herein, we focus on recent advances in our understanding of how IL-1 cytokines, stemming from inflammasome-dependent and -independent pathways are involved in the regulation of skin conditions. Importantly, we discuss several mouse models of skin inflammation generated to help elucidate the basic cellular and molecular effects and modulation of IL-1 in the skin. Such models offer perspectives on how these signaling pathways could be targeted to improve therapeutic approaches in the treatment of these rare and debilitating inflammatory skin disorders.

Skin: Our First Line of Defense
The skin is the first major barrier protecting us against invasion from microbes and pathogens. It is the largest organ composed of two major layers, the epidermis and the dermis (Figure 1). The epidermis comprises a densely packed keratinocyte layer that serves as a physical barrier against pathogens and constant environmental stimuli [1]. The epidermal layer also consists of melanocytes (specialized melanin-producing cells rendering skin color) and resident immune cells, which play an equally important role in maintaining the barrier. Recent studies have shown that our skin epidermis is full of commensal microbiota (see Glossary) required to ensure skin homeostasis [2]. Commensal skin microbiota inhibit the growth of pathogenic microbiota in the skin [3,4] and further regulate immune responses against invading pathogens [5,6]. While the different functional roles of skin microbiota are only beginning to be understood, it is possible (and perhaps likely) that microbiota also play a major role in inflammatory skin disorders [7].

The dermis lying beneath the epidermis is a connective tissue composed of collagen, elastic fibers, and a mixture of extracellular matrix proteins. Unlike the epidermis, the dermal layer is supplied with blood and lymphatic vessels, nerve endings, fibroblasts, and various types of immune cells. The dermis is sterile and free of any commensal microbiota, and is frequently supplied with migrating immune cells which traffic in and out of these sites through lymphatic and blood vessels [8]. In a way, the dermis serves a supporting role to the epidermis by providing mechanical support and immune cells to combat infection and inflammation.

Aberrant cytokine production by both immune and non-immune cells in the skin can promote excess cellular infiltration and cell death, resulting in skin inflammation and disorders. The major cytokines secreted and operating in the skin are members of the interleukin (IL)-1 family.

Trends
The skin harbors specific commensal microbiota with many known roles in modulating tissue homeostasis and controlling immunity. Limiting gut microbiota in murine models of cryopyrin-associated periodic syndrome (CAPS) delays the onset of disease.

More than 175 different sequence variants of the NLRP3 inflammasome have been linked to skin inflammation in CAPS patients. IL-1β-specific blocking antibodies are effective in suppressing CAPS symptoms. Mice carrying similar NLRP3 gain-of-function (GOF) mutations have significantly enhanced our current understanding of CAPS.

GOF mutations in the B30.2 domain of PYRIN in humans result in skin Familial Mediterranean fever (FMF). Transgenic mice expressing chimeric PYRIN (murine PYRIN fused with the human B30.2 domain containing FMF mutations) have facilitated our understanding of FMF.

SHARPIN deficiency (a member of LUBAC) promotes inflammatory skin disease. Aberrant TNF-TNFR signaling as well as NLRP3 inflammasome activation contributes to disease development in SHARPIN-deficient mice.

SHARPIN appears to have cell-type specific roles in regulating inflammasome activation and cell death.

Ptpnsenm mice are used as a model of neutrophilic dermatoses and have revealed a specific role for IL-1α, but not IL-1β, in instigating this inflammatory skin disease. The involvement of RIPK1 suggests that cell death pathways might be critical in this disease.
Dysregulation of IL-1 cytokines has been demonstrated to result in autoinflammatory skin diseases such as cryopyrin-associated periodic syndrome (CAPS) and familial Mediterranean fever (FMF). Consequently, given the success in clinical trials, the US food and drug administration (FDA) approved various IL-1 blockade therapies for the treatment of a wide spectrum of skin diseases constituting CAPS [9]. In this review we examine several rare autoinflammatory skin disorders that are mediated by aberrant IL-1 production and signaling. In doing so, we also address the current knowledge gaps and discuss potential studies that may very likely be important in providing a better understanding of the mechanisms driving these diseases. Finally, we examine the progress that has been made in recent years to elucidate the cellular and molecular pathways governing the IL-1 signaling cascade, and discuss how this knowledge can contribute to the timely development of putative novel therapeutics for these rare autoinflammatory skin disorders.

While this review focuses mostly on the role of IL-1 in autoinflammatory skin diseases, it is important to keep in mind that complex interactions exist between several immune and non-immune cells present within the skin environment (Boxes 1 and 2), driving autoinflammation in the skin. Our current understanding of the etiology of these rare diseases is still at its infancy, and future studies will be necessary to fully examine the individual and interconnected contribution of these specific cell types within the skin in immunity and disease.

**Regulation of IL-1 Cytokines**

The IL-1 family consists of a group of pleiotropic cytokines with central roles in infection, inflammation, and disease [10]. For instance, aberrant IL-1 production has been linked to several inflammatory skin disorders, such as familial cold autoinflammatory syndrome.
Box 1. Major Immune Cells in the Epidermis

Although predominantly populated by keratinocytes, the epidermis also consists of Langerhans cells (LCs), specialized skin-resident dendritic cells that sample the skin environment for potential damage and pathogenic insults. Phenotypically, LCs can be distinguished by the expression of langerin protein in mice, and CD1a in humans [112]. LCs originate from the yolk sac and fetal liver to populate the skin early in development (before birth), suggesting a central role in skin tissue homeostasis [113]. While LCs can help to promote proper immune responses to eliminate invading pathogens by cross-priming of T cells [114], in vivo studies of LC depletion have demonstrated that LCs are dispensable for eliciting immune responses; they appear instead to play a regulatory role in promoting tolerance [115,116]. A newer study with autologous human skin cells has reconciled disparate results of LC function by demonstrating that LCs can promote the proliferation of both T regulatory cells ($T_{reg}$) and tissue-resident memory T cells ($T_{em}$), depending on the context of inflammation [118]. Thus, LCs are central regulators of skin tissue homeostasis and modulate both the proinflammatory and regulatory responses of T cells.

The skin houses 10–20 billion T cells, more than the total number of T cells circulating in the blood [119]. Specifically, sentinel CD8$^+$ $T_{em}$ cells are abundant in the epidermis. CD8$^+$ $T_{em}$ cells are permanent residents of the skin epidermis and do not circulate (discussed in detail elsewhere) [120]. They supervise circulating memory CD8$^+$ T cells when providing protective immunity against localized skin infections [121]. Recent studies have shown that, in addition to directly responding to pathogens, CD8$^+$ $T_{em}$ cells also recruit circulating memory CD8$^+$ T cells to help clear infections in the skin [122].

Although present at very low frequency in the human skin epidermis, T cells of the γδ-origin, commonly known as dendritic epidermal T cells (DETC), are found abundantly in the murine skin epidermis [120]. DETC are important for wound repair processes and tissue homeostasis [123,124]. During viral infection, DETC also produce a wide array of cytokines and chemokines that are important for recruiting both innate and adaptive immune cells to the site of infection [120].

(FCAS), Muckle–Wells syndrome (MWS), and neonatal-onset multisystem inflammatory disease (NOMID). Therapies blocking IL-1 activity (i.e., Anakinra [Kineret®]) have so far proven helpful, ameliorating symptoms associated with these diseases [10]. IL-1 cytokines consist of IL-1α and IL-1β; however, both cytokines have been thought to have similar functions [11]. As such, IL-1β has been regarded as the prototypical IL-1 cytokine, while IL-1α has remained

Box 2. Major Immune Cells in the Dermis

Dermal dendritic cells (DCs) are abundantly present in the skin dermis. Two major subsets of dermal DCs have been identified in mice (CD103$^+$ and CD11b$^+$ DCs) [125] and three major subsets of dermal DCs have been identified in humans (CD14$^+$, CD1a$^+$, and LaNAC$^+$ DCs) [126,127]. During infection and inflammation, dermal DCs migrate rapidly to draining lymph nodes where they play important roles in priming appropriate T cell subsets by producing several cytokines in the dermis to drive Th1 [128], Th2 [129,130], or Th17 [131] responses.

Dermal resident macrophages are similar to LCs in that they are derived from the yolk sac [132]. These macrophages are able to proliferate and self-renew within the dermal tissue [132]. Functionally, resident macrophages survey the dermis for potential danger signals, phagocytose and kill pathogens, and secrete cytokines and chemokines to initiate appropriate inflammatory responses [1]. In addition to resident macrophages, circulating monocytes can traffic to the dermis from the lymphatics and blood vessels where they support DCs and resident macrophages in surveillance and in transport of antigens to the draining lymph nodes [133].

Primarily, most T cells found in the dermis are CD4$^+$ and include both resident memory CD4$^+$ T cells (CD4$^+$ $T_{em}$) and circulating CD4$^+$ T cells [134]. Although a few CD8$^+$ T cells can be found in the dermis, these tend to be recruited in response to active infection and usually disappear from the dermis with the resolution of the infection [135–137]. Importantly, all major subtypes of CD4$^+$ T cells (Th1, Th2, Th17, and $T_{em}$) have been observed in the dermal layer of the skin [8].

Mast cells are present in the skin and carry out the essential function of providing rapid immunity by releasing preformed inflammatory mediators such as antimicrobial peptides, proteases, and histamine from stores in cytoplasmic granules [138]. Mast cells and their inflammatory mediators further promote innate immunity and play a key role in clearing infections [139]. Importantly, mast cells degranulate in response to bacterial staphylococcal toxin in mouse models of delta toxin-induced dermatitis [140]. Furthermore, such degranulation has been found to be further induced by systemic immunoglobulin IgE and IL-4 molecules, resulting in severe skin inflammation [140].

Of note, the dermis also comprises γδ-T cells and innate lymphoid cells (ILC), and their involvement in various skin conditions is by no means less important (discussed in detail elsewhere) [1]. Moreover, in an inflammatory state, additional immune cells such as neutrophils and basophils can also traffic to the dermis and epidermis where they are also able to promote inflammation [1].
severely understudied. The pleiotropic functions of IL-1α and IL-1β are achieved upon signaling through a common IL-1 receptor, IL-1R, expressed on various target cells. These cytokines are capable of regulating diverse cell processes such as cell proliferation, survival, and migration, depending on environmental conditions.

IL-1 regulation and signaling are essential for cellular homeostatic maintenance. One of the major complexes responsible for the direct regulation of IL-1β and to some extent IL-1α is the inflammasome. The inflammasome is a multiimeric protein complex that forms in the cytoplasm of the cell. The basic components of an inflammasome include an inflammasome (cytoplasmic) sensor, the adaptor apoptosis-associated speck-like protein containing a CARD (ASC) and the protease caspase-1 (Figure 2A). Only a few cytoplasmic sensors have been well established to form an inflammasome complex, and these include Nod-like receptor (NLR) pyrin domain-containing 3 (NLRP3), NLRP1b, and NLRC4 [12]. In addition, non-NLR cytoplasmic sensors such as absent in melanoma 2 (AIM2) and pyrin also assemble an inflammasome [12]. Other NLR sensors such as NLRP2, NLRP6, NLRP7, and NLRP12 have also been proposed to form inflammasomes, although additional studies are needed to confirm these findings [13]. Each of these inflammasome sensors can recognize a specific pathogen-associated molecular pattern (PAMP) or damage-associated molecular pattern (DAMP) derived from pathogens or damaged cells [12]. Once activated, these cytoplasmic sensors recruit ASC, which in turn recruits caspase-1. In this complex, caspase-1 auto-cleavage occurs, resulting in its activation. Active caspase-1 has two major functions: processing of pro-IL-1β into bioactive IL-1β and the induction of pyroptosis, an inflammatory form of cell death [12]. While pro-IL-1α cleaved N-terminal domain of gasdermin-D executes pyroptotic cell death.

Familial cold autoinflammatory syndrome (FCAS): also known as familial cold urticaria (FCU), the syndrome is characterized by an autosomal dominant inherited genetic mutation in the NLRP3 gene. Common symptoms include skin rash, periodic fever, headaches, joint pain, and conjunctivitis, all of which are often triggered by exposure to cold or cooling temperatures.

Familial Mediterranean fever (FMF): a genetically autosomal recessive inherited autoinflammatory skin disease arising from mutations in the MEFV gene which encodes pyrin.

Hepatosplenomegaly: swelling or enlargement of both liver and spleen beyond their normal size.

Inflammasome: mutimeric protein complex consisting of an inflammasome sensor, the adaptor protein ASC, and cysteine protease caspase-1. Its major function is to process pro-IL-1β and pro-IL-18 into their mature forms, and to execute inflammatory cell death termed pyroptosis.

Langerhans cells (LC): specialized tissue-resident dendritic cells residing in the skin epidermis. They play crucial roles in patrolling the skin barrier and maintaining homeostasis.

Leukocytosis: increased numbers of white blood cells in the blood.

Linear ubiquitin assembly complex (LUBAC): composed of the three proteins HOIL-1, HOIP, and Sharpin. LUBAC promotes linear ubiquitination of target proteins to regulate signaling pathways.

Microbiota: general term defining the commensal, symbiotic, and pathogenic microorganisms found within a particular niche of the body such as the gut or skin.

Mixed-lineage kinase-like (MLKL): a pseudokinase that promotes a cell death process termed necroptosis. Once activated by phosphorylation, MLKL intercalates into the cell membrane, disrupting membrane integrity.

Muckle–Wells syndrome (MWS): a rare disorder due to an autosomal dominant mutation in the NLRP3 gene. The recurrent episodes of MWS begin during infancy or childhood and can occur spontaneously or be triggered by cold, heat fatigue, or stress. Common symptoms associated with
can be cleaved by caspase-1 to promote its maturation, the precursor form of IL-1α (which is bioactive) can be passively released from cells during pyroptosis [14,15]. Recently, caspase-1 has been shown to cleave and activate gasdermin-D that ultimately promotes pyroptotic cell death (Box 3) [16,17].

Although the inflammasome is a major complex that promotes caspase-1-dependent processing of inactive pro-IL-1β into mature forms, recent studies have highlighted the inflammasome-independent processing of IL-1β (Figure 2B). For instance, the inflammasome-independent activation of another caspase, caspase-8, has been documented in Toll-like receptor (TLR)-primed macrophages and dendritic cells (DCs) upon engagement of the TNF receptor family member CD95 by its ligand CD95L, drug-induced mitochondrial damage, or drug-induced endoplasmic reticulum (ER) stress [18]. Activated caspase-8 functions as a proenzyme to directly process IL-1β into its bioactive form [18]. In addition to caspase-8, several other proteases such as cathepsins, proteinase-3, elastase, granzyme-A, chymase, matrix metalloproteinase 9 (MMP9), and chymotrypsin have also been demonstrated to cleave pro-IL-1β under various in vitro conditions [11]. However, the physiological relevance of IL-1β processing by non-caspase proteases is not completely understood and warrants further investigation.

**Aberrant IL-1 Responses Drive Autoinflammatory Skin Disorders**

IL-1 cytokines play a major role in several autoinflammatory diseases including skin disorders associated with CAPS and FMF (Box 4). Not surprisingly, mutations affecting inflammasome components (major regulators of IL-1) or IL-1 functions have been associated with a wide spectrum of autoinflammatory diseases that often present with skin inflammation. There are various examples of human genetic studies and mouse models that explore the role of inflammasome-dependent and -independent IL-1 cytokines in promoting skin inflammation.

**Box 3. Gasdermin-D Mediates Pyroptotic Cell Death**

Active caspase-1 processes gasdermin-D into a N-terminal and C-terminal fragment. The active N-terminal domain of gasdermin-D then executes pyroptotic cell death [16,17] via a yet to be described mechanism. Future studies are geared towards investigating how gasdermin-D executes pyroptosis. It remains to be examined whether gasdermin-D directly integrates itself into the membrane in a manner similar to that of mixed-lineage kinase-like (MLKL), a pseudokinase that promotes necroptosis [14], or works in concert with other caspase-1-targeted proteins to promote pyroptosis. Understanding the mechanisms regulating cell death will be important given that cell death plays a major role in mediating inflammatory diseases, autoinflammatory skin disorders included.

**MWS include episodes of skin rash, fever and joint pain, but can also result in hearing loss and kidney dysfunction.**

**Necroptosis**: programmed necrosis is an inflammatory cell death driven by receptor interacting protein kinase 3 (RIPK3) and RIPK1. The effector molecule, MLKL, executes necroptotic cell death.

**Neonatal-onset multisystem inflammatory disease ( NOMID):** severe autosomal dominant autoinflammatory disease resulting from GOF mutations in the NLRP3 gene. NOMID patients present with skin rashes associated with periodic fever from the time of birth. Persistent inflammation in NOMID patients often affects the nervous system, skin, and joints.

**Nephrotic syndrome:** results from various diseases that directly affect kidney function. Some symptoms include protein in the urine, low protein levels in the blood, elevated cholesterol, and inflammation.

**Neutrophilic dermatoses:** rare genetically inherited autoinflammatory disorders that include a spectrum of diseases such as Sweet’s syndrome, Pyoderma gangrenosum, and subcorneal pustular dermatitis. Common features of these diseases include skin lesions dominated by neutrophils.

**Nod-like receptor (NLR) pyrin domain-containing 3 (NLRP3):** a cytoplasmic sensor that assembles an inflammasome. NLRP3 is a global sensor of pathogen- and damage-associated molecular patterns.

**Pyogenic arthritis, pyoderma gangrenosum, and acne (PAPA) syndrome:** a rare genetic autoinflammatory disorder. Missense mutations in PSTPIP1 gene have been associated with PAPA syndrome.

**Proline-serine-threonine phosphatase interacting protein 1 (PSTPIP1):** also known as CO2BP1, a protein associated with PAPA syndrome. It functions by interacting with several proteins involved in cytoskeletal organization and inflammation.

**Ptprots<sup>−/−</sup>:** Y208N mutation in the Ptpn6 gene (encoding protein tyrosine phosphatase SHP-1) resulting in spontaneous inflammation (spin) in mice. Ptpn6<sup>−/−</sup> mice develop footpad skin inflammation consisting of neutrophil infiltrates, a hallmark of...
and disease. We focus here on CAPS and FMF as inflammasome-dependent IL-1 diseases. **Shank-associated RH domain interacting protein** (Sharin) deficiency-associated skin inflammation will also be discussed as an example of a disease implicating both inflammasome-dependent and -independent IL-1 cell death pathways in disease progression. Finally, with regard to inflammasome-independent IL-1 signaling and skin inflammation, we describe **neutrophilic dermatoses** as modeled in *Pttn6shpl* mice.

**Cryopyrin-Associated Periodic Syndrome (CAPS)**

Inflammatory diseases such as familial cold autoinflammatory syndrome (FCAS), Muckle–Wells syndrome (MWS), and neonatal-onset multisystem inflammatory disease (NOMID) are characterized by well-defined clinical manifestations, particularly rash and joint involvement, with high disease activity [19]. These rare diseases are associated with various grades of skin inflammation and rashes. In terms of phenotype and the overall disease severity, FCAS displays the mildest symptoms followed by MWS [19]. NOMID patients, also known as CINCA patients (chronic, infantile, neurological, cutaneous, and articular syndrome), present with the most severe disease affecting multiple organs, including skin rashes within the first 6 weeks of life, bony overgrowth in knees, central nervous system manifestations (aseptic meningitis, cerebral atrophy), hepatosplenomegaly, and leukocytosis [20]. These three rare diseases were thought to be etiologically distinct until studies in the early 2000s proved that all three diseases were caused by mutations in the gene encoding the cytoplasmic sensor, *NLRP3* (previously named cryopyrin or NALP3) [21–24]. Mutations in *NLRP3* were associated with exacerbated IL-1β production in all 3 diseases.

This spectrum of rare diseases is now categorized under CAPS to unify different autoinflammatory diseases that result from mutations in *NLRP3*, exhibit increased IL-1β production, and are completely independent of B and T lymphocytes. While the reported disease incidence of CAPS is very rare (1–2 cases/1 million people in the USA), the true incidence of CAPS is most certainly higher because these diseases are frequently misdiagnosed due to a lack of understanding of the etiology of these diseases [19]. More than 175 different sequence variants of *NLRP3*—mostly in exon 3 [encoding the nucleotide oligomerization domain (NOD) of NLRP3] have been associated with a CAPS phenotype [25] (Infevers: an online database for autoinflammatory mutations; http://fmf.igh.cnrs.fr/ISSAID/infevers). Of these, more than 90 heterozygous *NLRP3* mutations have been associated with a CAPS phenotype [19]. Noteworthy, most *NLRP3* mutations are heterozygous with mutations found on only one allele, and are inherited in an autosomal dominant manner [22].

**Current Treatment Options for CAPS Patients**

IL-1 blockade therapy has proven successful in most CAPS patients, demonstrating an important role for IL-1 cytokines in the pathogenesis of the disease. The first line of evidence for IL-1 involvement came from studies utilizing an IL-1 receptor (IL-1R) antagonist, Anakinra, which blocks IL-1 signaling by binding to IL-1R [10]. FCAS patients with L353P mutations in the NLRP3 protein develop a skin rash following cold exposure [26]. However, FCAS patients treated with Anakinra, 24 h or 1 h before cold challenge did not develop the typical skin rashes and remained protected for 24–48 h after treatment [26]. In addition, two MWS patients harboring R260W mutations in NLRP3 were treated daily with 100 mg Anakinra and showed a remarkable improvement in disease symptoms. These patients responded rapidly; inflammatory symptoms were blunted within hours of treatment and remained sustained after 6 months of treatment (study endpoint) [27]. Further support for these studies was provided by the successful treatment of three additional MWS patients harboring V200M mutations in NLRP3 [28].

Similarly to the first study, these patients also responded within 4 h of Anakinra treatment and continued to show remarkable progress and disease remission until the conclusion of the study, 3 months later [28]. Finally, 18 NOMID patients (12 with NLRP3 mutations) treated with 1–2 g skin lesions associated with neutrophilic dermatoses.


**Pyrin domain-only protein 1** (POP1): protein encoded in humans but not in mice. POP1 sequesters ASC to negatively regulate inflammasome activation.

**Pyoderma gangrenosum**: rare autoinflammatory condition of unknown etiopathology characterized by large painful skin lesions.

**Pyroptosis**: inflammatory cell death mediated by caspase-1. Usually observed during inflammasome activation following activation of caspase-1. Caspase-1 executes pyroptotic cell death.

**RAG-deficient mice**: mice deficient in recombination activation gene (RAG) enzymes that mediate the recombination of genes encoding immunoglobulins and T cell receptors in B and T cells, respectively. The absence of RAG results in deficiency of mature B and T cells.

**Anakinra (interleukin-1 Trap, Arcalyst®)**: a dimeric fusion protein composed of IL-1R1 and IL-1RACP fused together with the Fc region of human IgG1. Anakinra sequesters IL-1 cytokines.

**Shank-associated RH domain interacting protein** (Sharin): member of the LUBAC. Sharpin deficiency in mice results in spontaneous skin inflammation as early as 3–4 weeks of age and severe autoinflammation.

**Spondylitis**: form of arthritis that results in inflammation of the spine joints.

**Subcutaneous pustular dermatoses**: also known as Shneden–Wilkinson disease, a rare autoinflammatory disease characterized by the appearance of benign chronic relapsing pustules in the skin.

**Sweet’s syndrome**: rare autoinflammatory skin disorder characterized by fever and painful skin lesions.

**γδ T cells**: these cells present the alternative γδ T cell receptor and are involved in innate immunity. While present in very low numbers, γδ-T
Anakinra/kg body weight showed similar positive effects, which included a rapid disappearance of skin rashes and a continued improvement of disease-associated symptoms [29].

Importantly, while Anakinra treatment has shown great promise in the treatment of patients across the broad spectrum of CAPS diseases, it also targets both IL-1α and IL-1β signaling. Furthermore, the therapeutic regimen of this treatment is somewhat impractical because the short half-life of Anakinra requires daily injections to be effective. Rilonacept (interleukin-1 Trap) is a soluble fusion protein consisting of human IL-1R bound to the Fc region of human IgG1. Rilonacept (Arcalyst®, Regeneron) exhibits a slightly longer half-life than Anakinra, such that even weekly treatment is efficacious [30]. In one study, 47 CAPS patients (FCAS and MWS) were treated with placebo or a weekly dose of Rilonacept in a randomized double-blind placebo-controlled study [31]. Rilonacept provided marked and lasting improvement in the disease severity associated with CAPS, although continued Rilonacept therapy was required for sustained improvement of disease symptoms [31].

More recent human studies have used Canakinumab (Ilaris®, Novartis), a human anti-IL-1β antibody specifically targeting IL-1β [32] to treat CAPS. Unlike Anakinra (daily dose) and Rilonacept (weekly), Canakinumab has a longer half-life and does not require frequent administration [33]. Importantly, it is specific to IL-1β and does not affect IL-1α [34]. In a randomized, double-blind, placebo-controlled study, 35 CAPS patients were treated with 150 mg of Canakinumab subcutaneously [32]. Of the 35 patients, 34 exhibited a complete response (as determined by minimal or no rash and reduced overall disease activity) to Canakinumab at up to 8 weeks. In part two of the study at the end of 8 weeks, responding patients were randomly divided into placebo or treatment groups, where treatment groups received 150 mg of Canakinumab every 8 weeks for up to 24 weeks, at which point the experiment ended. Patients who continued on Canakinumab therapy presented complete remission of the disease, while patients on placebo experienced disease flares with a median of 100 days from the end of Canakinumab treatment. In part three of the study, 29 remaining patients (placebo and treatment group) received Canakinumab treatment (150 mg every 8 weeks) for an additional 16 weeks. At the end of the study, 28 of 29 patients receiving treatment demonstrated remarkable improvement and remission of CAPS symptoms [32].

The successful results from IL-1 blockade clinical trials in CAPS patients have prompted the FDA to approve the use of IL-1 blockade therapy for the treatment of CAPS. As such, Rilonacept was the first drug approved by the FDA in 2008 for FCAS and MWS patients. The successful trial of Canakinumab also resulted in its recent FDA approval in 2009 for FCAS and MWS patients. Clinical trials to test the efficacy of Canakinumab in the treatment of NOMID patients are still ongoing; however, the FDA approved in 2013 the use of Anakinra for treating NOMID patients.

Mouse Models of CAPS

Despite the overwhelming success of IL-1 blockade therapy in the treatment of CAPS patients as early as 2003 [27], the molecular mechanisms triggering aberrant production of IL-1 cytokines remained elusive until recently. With the discovery of the inflammasome as the major regulator of IL-1 cytokines in 2002 [35], subsequent seminal discoveries have revealed the NLRP3 inflammasome as a crucial complex that processes IL-1β and induces pyroptotic cell death in mice [36–39]. Thus, it can be posited that NLRP3 CAPS-associated mutations result in inflammasome complex assembly, ultimately leading to unregulated IL-1β production and CAPS disease.

The recent development of mouse models has significantly advanced our understanding of the molecular mechanisms, pathogenesis, and etiology of CAPS. Two different groups generated knock-in (KI) mice expressing NLRP3 mutations associated with CAPS in humans [40,41]. Similarly to human CAPS patients, these NLRP3 mutations were heterozygously expressed in cells are abundant in skin and mucosal surfaces.

T helper cells (Th): upon stimulation can differentiate into different T cell lineages as defined by their specific transcription profiles and ability to produce cytokines. Th1 cells are regulated by transcription factor T-bet and produce IFN-γ. Th2 cells are regulated by transcription factor GATA3 and produce IL-4. Th17 cells are regulated by transcription factor RORγt and produce IL-17.

Toll-like receptors (TLRs): membrane-bound germline-encoded pathogen recognition receptors located in the plasma membrane and endosome membrane. TLRs sense conserved molecular patterns that are present in pathogens, and initiate signaling pathways to induce inflammation and clear infection.

Tissue-resident memory T cells (T<sub>RM</sub>): T cells residing within a specific tissue and that do not traffic through the blood and vasculature. CD69<sup>T<sub>RM</sub></sup> cells are sentinel cells in the epidermis and provide protection against several pathogens that are encountered on a daily basis.

T regulatory cells (T<sub>reg</sub>): a subset of T cells that have regulatory/suppressive properties. T<sub>reg</sub> cells express the transcription factor FOXP3 and secrete regulatory cytokines such as IL-10 and TGF-β.

Wdr41<sup>kd</sup> mice: carry a hypomorphic mutation in the Wdr41 gene (kd/kd). They present macrothrombocytopenia and autoinflammatory disease (including skin inflammation). WDR1 protein is involved in the disassembly of actin filaments.
mice. Hoffman and colleagues generated the NLRP3 A350V (MWS-associated) mutation and the L351P (FCAS-associated) mutation, noted as \( \text{Nlrp}3^{A350V} \) and \( \text{Nlrp}3^{L351P} \), respectively [41]. In an independent study, Strober and colleagues generated a mouse model of MWS by generating gene-targeted mice bearing the R258W mutation in NLRP3 (\( \text{Nlrp}3^{R258W} \)) [40].

Bone marrow-derived macrophages (BMDM) obtained from \( \text{Nlrp}3^{A350V} \), \( \text{Nlrp}3^{L351P} \), and \( \text{Nlrp}3^{R258W} \) mice produce exacerbated IL-1\( \beta \) in response to priming signal alone (TLR priming) without the need for a second activation signal [40,41] (Figure 3A). By contrast, wild-type (WT) BMDM require two signals for NLRP3 inflammasome activation and production of IL-1\( \beta \) [40,41]. The first signal, referred to as priming, occurs through TLRs (recognition of PAMPs) and stimulates the expression of pro-IL-1\( \beta \) and inflammasome components. The second signal constitutes the recognition a wide variety of danger signals in the cytoplasm by NLRP3 [42]. Similarly, monocytes derived from CAPS patients also produce excess IL-1\( \beta \) \textit{in vitro} in response to TLR stimulation alone, without the need for a second activation signal [36,41].

Potassium efflux has been identified as a common pathway in the activation of the NLRP3 inflammasome in response to a wide array of stimuli [43–45]. Although exogenous potassium has been found to inhibit IL-1\( \beta \) production completely in the presence of NLRP3 inflammasome activation signals in WT BMDM, it is unable to inhibit IL-1\( \beta \) production from \( \text{Nlrp}3^{R258W} \) BMDM [40]. This suggests that mutated NLRP3 is not regulated by potassium efflux. Nevertheless, several post-translational modifications of NLRP3, including deubiquitination [46,47] and phosphorylation [48], have been shown to be important for its activation. Whether mutations associated with CAPS affect these NLRP3 modifications to promote the constitutive activation of NLRP3 is not yet known, and future studies should investigate these possibilities.

Figure 3. Mouse Models of Autoinflammatory Skin Diseases Triggered by Inflammasomes. (A) Cryopyrin-associated periodic syndrome (CAPS): mouse models of CAPS were generated by mutating murine \( \text{Nlrp}3 \) with the same gain-of-function mutations (\( \text{R258W, A350V, and L351P} \)) reported in humans. Unlike wild-type (WT) NLRP3, these mutant NLRP3 proteins form spontaneous inflammasome complexes in response to TLR priming signals and produce enhanced levels of IL-1\( \beta \) cytokines. Mice expressing these point mutations develop skin inflammation and disease similar to CAPS in humans. Overexpression of pyrin domain-only protein 1 (POP1) sequesters ASC and inhibits aberrant inflammasome activation and IL-1\( \beta \) production. Familial Mediterranean fever (FMF) mice expressing a chimeric pyrin protein containing a mutant form of the human B30.2 domain (M680I, M690V, and V726A mutations) model FMF in humans. These chimeric pyrin-expressing mice develop spontaneous autoinflammation and skin lesions mediated by assembly of the pyrin inflammasome. Based on the ability of POP1 to sequester ASC, we propose that POP1 overexpression can also reverse FMF disease in these mice. (B) Chronic proliferative dermatitis mutation (cpdm) in Sharpin mice (\( \text{Sharpw}^{cpdm} \)) results in Sharpin deficiency, which leads to spontaneous inflammation and severe dermatitis. Sharpin is centrally required in macrophages for NF-\( \kappa \)B and ERK signaling and for promoting NLRP3 inflammasome activation. By contrast, Sharpin deficiency promotes NLRP3 inflammasome activation and IL-1\( \beta \) production in inflamed skin (keratinocytes). Sharpin also inhibits aberrant cell death downstream of TNF signaling (FADD-caspase-8 promotes apoptosis; RIPK1-RIPK3 promotes necroptosis) to control skin inflammation in keratinocytes.
Embryonic- or myeloid-specific expression of A350V and L351P has resulted in neonatal lethality of mouse pups [41]. Nlrp3<sup>A350V</sup> mice demonstrated an inflammatory phenotype on postnatal day 1, developing skin abscesses by day 4 and succumbing between days 2 and 14. Analysis of surviving Nlrp3<sup>A350V</sup> pups aged 6–8 days revealed a profound increase in inflammatory cytokines in the serum and skin, including IL-1β [41]. Nlrp3<sup>L351P</sup> mice were either stillborn or died on postnatal day 1. Such differences in A350V and L351P mutant phenotypes suggest that specific mutations can differentially affect NLRP3 protein function, in accordance with the broad spectrum of diseases encompassing CAPS patients. These mutations represent a specific gain-of-function (GOF) in the NLRP3 gene. Indeed, IL-1R deficiency in Nlrp3<sup>A350V</sup> mice completely rescues from death, whereas only approximately 20% of Nlrp3<sup>L351P</sup> mice survive when also deficient in IL-1R [41]. These studies demonstrate that IL-1-independent pathways are also involved in the pathogenesis of CAPS in the case of Nlrp3<sup>L351P</sup> mice. Of note, given that both IL-18 and IL-1β are increased in Nlrp3<sup>L351P</sup> mice, it would be of interest to examine the potential role of IL-18 in the pathogenesis of CAPS in these mice. One would predict that double deficiency of both IL-1β and IL-18 should completely rescue the morbidity and mortality observed in these mice.

The role for NLRP3 inflammasome involvement in CAPS pathogenesis has been confirmed by complete rescue of disease pathology in both Nlrp3<sup>A350V</sup> and Nlrp3<sup>L351P</sup> mice deficient in the protein ASC [41]. Collectively, these models have provided substantial evidence for abundant NLRP3 inflammasome activation in the progression of CAPS and have proved useful in the identification of putative novel therapeutic targets for CAPS treatment. Indeed, a recent study by Almeida et al. has demonstrated that pyrin domain-only protein 1 (POP1) can inhibit NLRP3 inflammasome assembly [49]. POP1 deletion by shRNA in human monocytes increased IL-1β production in response to lipopolysaccharide (LPS) priming <i>in vitro</i>. Mechanistically, POP1 directly sequestered ASC (inflammasome adaptor), preventing inflammasome assembly. To further study the role of POP1 (present only in humans), transgenic mice expressing POP1 specifically in macrophages (CD68–POP1 mice) were generated [49]. As expected, these mice exhibit reduced inflammasome activation and subsequent IL-1β production. Importantly, the inflammatory symptoms observed in Nlrp3<sup>A350V</sup> mice, including neonatal lethality, morbidity, and skin inflammation, are significantly rescued by breeding these mice to CD68–POP1 mice. The rescue of the CAPS phenotype in Nlrp3<sup>A350V</sup> × CD68–POP1 mice correlates with reduced secretion of IL-1β [49]. Because POP1 can sequester ASC, it will be important to dissect whether POP1 can be a universal therapeutic target for the treatment of all inflammasome-associated disorders.

Of the three CAPS models generated, Nlrp3<sup>R258W</sup> mice have the mildest phenotype [40,41]. Nlrp3<sup>R258W</sup> mice develop skin and ear inflammation resembling dermatitis and hair loss at 8 to 16 weeks of age [40]. Unlike the neonatal lethality associated with A350V and L351P mutations, only some of the mice harboring the R258W-mutation perish after birth [40,41]. Full necropsy of Nlrp3<sup>R258W</sup> mice revealed enlarged spleens and lymph nodes as well as hepatomegaly, consistent with hallmarks of systemic autoinflammation [40]. While rescue experiments with Nlrp3<sup>R258W</sup> mice (crossing the mice to Asc<sup>−/−</sup> or Il1r<sup>−/−</sup> mice) have not been performed, one would expect that ASC or IL-1R deficiency might ameliorate the disease phenotypes of these mice. Although these studies have demonstrated that exacerbated IL-1 could promote increased production of IL-17 cytokines and type 17 T helper cell (Th17) responses [40,41], these cytokines are probably dispensable for disease pathogenesis because RAG deficiency does not rescue the disease [41]. T cells are the major sources of Th17 cells and IL-17 cytokines, and RAG-deficient mice lack mature T and B cells. It would be interesting to determine whether these GOF mutations in NLRP3 are required in hematopoietic and/or radioresistant compartments.
Given the mild phenotype of \( \text{Nlrp}^{3 \text{R258W}} \) mice, these mice have been extremely useful in understanding the cellular mechanisms involved in the pathogenesis of CAPS. For example, a recent study by Nakamura et al demonstrated that mast cells play a crucial role in driving skin inflammation and CAPS-associated morbidity in \( \text{Nlrp}^{3 \text{R258W}} \) mice [50]. \( \text{Nlrp}^{3 \text{R258W}} \) mice bred with mice deficient in mast cells (\( \text{Kit}^{\text{--}} \) mice) are protected from skin inflammation and overall CAPS morbidity. Interestingly, microbiota depletion from \( \text{Nlrp}^{3 \text{R258W}} \) mice also provides protection. The protection due to mast cells or microbiota depletion in these mice correlates with reduced IL-1\( \beta \) production. Mechanistically, mast cells derived from \( \text{Nlrp}^{3 \text{R258W}} \) mice produce elevated levels of IL-1\( \beta \) in response to TLR activation or TNF stimulation, essential for the induction of CAPS [50]. Hence, this study has suggested that mast cell depletion could be beneficial in ameliorating CAPS, further identifying the gut microbiota as an important component that could be potentially modulated in the treatment of CAPS.

Our understanding of the complex cellular and molecular mechanisms involved in the disease progression of CAPS is only beginning to unfold. The three mouse models discussed here will undoubtedly be helpful in further uncovering detailed molecular mechanisms. In the context of autoinflammatory skin disease, future studies will need to investigate specific cell types involved which produce inflammatory cytokines and respond to IL-1 signals. In addition, further insight into the crosstalk between immune cells, epidermal cells, and the microbiota driving autoinflammation will be required.

**Familial Mediterranean Fever (FMF)**

FMF is one of the best-characterized hereditary autoinflammatory disorders. FMF is a disease that is prevalent in the Eastern Mediterranean population affecting mainly people of Armenian, Turkish, Arabic, or Jewish descent [51]. Common symptoms include episodes of generalized fever and inflammation with a characteristic skin rash usually confined to the lower extremities [52,53]. Two independent groups in 1997 identified the human \( \text{MEFV} \) (Mediterranean fever) gene on chromosome 16 as the gene responsible for FMF [54,55]. It is now known that autosomal recessive (AR) mutations in \( \text{MEFV} \) cause FMF, although heterozygous \( \text{MEFV} \) mutations have also been reported to cause FMF in rare cases [56,57]. According to Infevers, more than 314 sequence variants of \( \text{MEFV} \) have been reported in FMF patients (http://fmf.igh.cnrs.fr/SSAID/infevers/) [25,26]. \( \text{MEFV} \) encodes a 781 amino acid protein called pyrin (for its association to fever, also known as marenosrin and TRIM20) [54,55]. Human pyrin consists of an N-terminal pyrin domain, a central B-box and coiled-coil domain, and a C-terminal B30.2/SPRY domain [51]. Most recessive mutations associated with FMF are congregated in the B30.2 domain, suggesting that it may harbor important functions. Interestingly, the C-terminal B30.2/SPRY domain is absent in murine pyrin [58].

**Pyrin-Associated FMF-Independent Diseases**

In addition to FMF, recently published study by Masters et al. has found a S242R mutation in the gene encoding pyrin (\( \text{MEFV} \)) that results in an autoimmune skin disease termed **pyrin-associated autoinflammation with neutrophilic dermatosis** (PAAND) [59]. These are dominantly inherited autoinflammatory diseases that often present with neutrophilic dermatoses and recurrent episodes of fever. The S242R mutation results in pyrin GOF that aberrantly activates inflammasome and IL-1\( \beta \) production [59]. More importantly, the clinical symptom in one patient was completely reversed by IL-1 blockade with Anakinra [59].

The autoinflammatory skin disease known as **pyogenic arthritis, pyoderma gangrenosum, and acne (PAPA) syndrome**, which bears clinical similarity to FMF, is also caused by aberrant production of IL-1 [60]. While the exact molecular mechanisms are not completely understood, PAPA syndrome is associated with two missense mutations (A230T and E250Q) in the gene encoding **proline-serine-threonine phosphatase interacting protein 1** (\( \text{PSTPIP1} \)) [61].
Interestingly, PSTPIP1 can directly bind to pyrin, and mutated versions of PSTPIP2 (A230T and E250Q) strongly associate with pyrin, suggesting a potential role for pyrin in promoting IL-1 production in PAPA syndrome [62].

These studies demonstrate that pyrin, although known for its association with FMF, can also modulate other autoinflammatory skin disorders.

Treatment Options for FMF Patients

For a long time, colchicine has been the drug of choice to treat symptoms associated with FMF. The administration of 1–3 mg colchicine/day is the preferred dose, demonstrating extended remissions of episodic inflammation and fever in FMF, but also preventing amyloidosis in the majority of FMF patients [63,64]. While the exact mechanisms of how colchicine provides relief in FMF patients have not been completely elucidated, it is well known that colchicine affects leukocyte migration, signal transduction, and gene expression [65].

However, approximately 10% of FMF patients do not respond to colchicine and require alternative therapy [66]. TNF and IL-1 blockade therapies have proved helpful in FMF patients who are resistant to colchicine therapy. Anti-TNF treatment in 10 FMF patients non-responsive to colchicine significantly reduced disease episodes, with seven patients showing complete remission of episodes and FMF-associated factors [67]. Similar positive effects of anti-TNF therapy have been observed in FMF patients with amyloidosis and spondylitis [68,69]. In a recent study, 13 FMF patients received IL-1 blockade (seven because of colchicine resistance, and six because of FMF-related amyloidosis) [70]. These patients received either Anakinra (1 mg/kg/day) or Canakinumab (2–4 mg/kg/6–8 weeks). All colchicine-resistant patients receiving IL-1 blockade therapy showed remarkable progress and alleviation of FMF attacks and episodes [70]. Of the six patients with amyloidosis, one patient presented nephrotic syndrome, two patients had chronic kidney disease, and three patients had a history of renal transplantation. The patient with nephrotic syndrome presented no attacks and showed complete remission after 1 year of treatment with Anakinra [70]. Two patients with chronic kidney disease on Anakinra showed no FMF-associated attacks or amyloidosis and had an improved lifestyle; however, the complications associated with kidney disease persisted. All three patients with renal transplantation demonstrated improved lifestyle with Anakinra treatment and complete remission of FMF attacks [70]. These studies provide solid evidence of the positive effects of IL-1 blockade therapy in alleviating FMF-associated symptoms and complications. Nonetheless, randomized, double-blind, placebo-controlled studies examining the safety and efficacy of IL-1 blockade therapy on FMF patients (especially those resistant to colchicine therapy) will still be necessary to fully validate these results. Indeed, a combination of colchicine and IL-1 blockade therapy could potentially result in synergistic beneficial effects in FMF patients, and should be further investigated.

Mouse Models of FMF

Pyrin was originally suggested to compete with caspase-1 in binding ASC and thus inhibit activation of the inflammasome [71]. It was generally accepted that recessive mutations in pyrin found in FMF patients resulted in loss of protein function. To understand the molecular mechanisms of this pathway, a mouse bearing a truncated pyrin protein (Mefv<sup>truncated</sup>) was generated, and it was assumed that this mutant could recapitulate the mutant pyrin from FMF patients [71]. While Mefv<sup>truncated</sup> mice do not display any spontaneous autoinflammatory disease, they are hyper-responsive to LPS treatment in vivo [71]. Furthermore, activation of caspase-1 and IL-1β production in response to inflammasome activation is dramatically increased in Mefv<sup>truncated</sup> mice, suggesting a regulatory role for pyrin in inhibiting aberrant inflammasome activation [71]. It is now understood that pyrin actually forms an inflammasome by recruiting ASC and caspase-1 [72,73]. Thus, an alternative explanation of the results above
could be that truncated pyrin proteins are able to directly recruit ASC and caspase-1 to assemble the inflammasome and promote IL-1β production.

Located in exon 10 of murine ME芙, M694V, M694I, V726A, and M680I are the most frequent missense mutations in pyrin [74]. In addition, these mutations are all located within the B30.2 domain of the pyrin protein [74]. To better understand the functions of FMF-associated mutations and how they contribute to the induction of FMF-associated pathology, KI mice bearing a pyrin protein fused to the human B30.2 domain and carrying common FMF mutations (M680I, M694V, and V726A) have been generated (M芙M680I, M芙M694V, and M芙V726A respectively) [75] (Figure 3A). All homozygous KI mice developed autoinflammatory disease, with M芙V726A showing the most severe phenotype [75]. M芙V726A mice are runted and develop scaly skin as early as 1 week after birth, with some mice succumbing to overt inflammation at around 2–3 weeks [75]. Similarly to CAPS, the FMF pathology in M芙V726A mice is not dependent on adaptive immunity because RAG deficiency does not rescue the phenotype [75]. In addition, the inflammation and disease phenotype of M芙V726A mice could be completely rescued by ASC and IL-1R, but not NLRP3, deficiencies, suggesting a NLRP3-independent inflammasome role in this pathology [75]. To determine whether the V726A mutation resulted in GOF, mice with hemizygous expression of V726A (M芙V726A+/−) were generated. These mice express a single copy of the V726A mutant pyrin in the absence of endogenous pyrin. Interestingly, M芙V726A+/− mice do not develop any spontaneous inflammatory disease, suggesting a dose-dependent effect of mutant V726A pyrin. Together, these data indicate that mutant pyrin expression is not a loss-of-function (LOF) but a GOF mutation. These statements are further supported by the lack of spontaneous FMF disease in M芙−/− mice which do not express any pyrin [75].

Although it had not been known which inflammasomes are involved in spontaneous caspase-1 activation and IL-1β production in M芙 KI mice, recent studies by Garvilin et al. and Xu et al. have imparted much knowledge on this matter [72,73]. For instance, it has been demonstrated that pyrin assembles an inflammasome complex in response to RHO-modifying toxins produced by various bacterial species such as Clostridium difficile, Vibrio parahemolyticus, Histophilus somni, Clostridium botulinum, and Burkholderia cenocepacia [72,73]. Mechanistically, these toxins induce RHO modifications that are sensed by pyrin, leading to inflammasome assembly [72]. In this sense, pyrin is a universal sensor of global cellular changes that can modify RHO [72]. Further identifying pyrin as an inflammasome, Wdr1fl/flrd mice have been shown to present an auto-inflammatory skin disease characterized by myeloid infiltrates [76]. The mouse phenotype can only be rescued by pyrin, ASC, and caspase-1 deficiencies, but not by NLRP3, NLRC4, NLRP1, or AIM2 deficiencies (the four major inflammasome sensors) [76].

Based on these findings suggesting a role for pyrin as a novel inflammasome sensor, we can hypothesize that the FMF-associated GOF mutations in pyrin result in the spontaneous formation of the pyrin inflammasome, subsequent caspase-1 activation, and release of IL-1 cytokines. These mouse models expressing chimeric pyrin protein with the human mutant B30.2 domain have thus provided solid insight into FMF disease and aided in the discovery of the pyrin inflammasome. Future studies examining the contribution of specific cell types in the progression of these diseases through GOF Pyrin mutant mice (use of chimeras or conditional knockout mice) might also impart much needed knowledge on the cellular mechanisms involved.

Sharpin Deficiency-Associated Autoinflammation and Skin Disease

A spontaneous mutation was found to cause chronic proliferative dermatitis (cpdm) in C57BL/6 mouse [77]. The gene responsible for this cpdm phenotype was later identified as Shank-associated RH domain interacting protein (Sharpin) (Sharpin<sup>cpdm</sup>) [78]. Sharpin<sup>cpdm</sup> mice have a single base-pair deletion in exon 1 resulting in early termination of the transcript that leads to
complete absence of Sharpin protein [78]. In this regard, Sharpin<sup>cpdm</sup> mice mimic Sharpin-deficient mice. Phenotypically, Sharpin<sup>cpdm</sup> mice are runted and develop spontaneous systemic inflammation with apparent inflammation of the skin that is observed as early as 3–4 weeks of age [78]. Recently, the function of Sharpin was defined and the protein has been identified as a member of the linear ubiquitin assembly chain complex (LUBAC), which also consists of HOIL-1 and HOIP [79–81]. LUBAC ubiquitinates target proteins through methylion-to-glycine linkage of ubiquitin, resulting in a linear ubiquitin chain that is important for the activation of NF-κB and ERK signaling and for the prevention of apoptosis [82].

In line with the important role of Sharpin in activating NF-κB and MAPK signaling pathways, activation of the NLRP3 inflammasome is completely abrogated in Sharpin<sup>cpdm</sup> macrophages and DCs [83] (Figure 3B). Studies in HOIL-1-deficient murine macrophages have demonstrated a similar phenotype, with loss of NLRP3 inflammasome activation, suggesting a common role for LUBAC in the activation of the NLRP3 inflammasome [84]. This study proposed that LUBAC mediates linear ubiquitination of ASC, which is required for optimal NLRP3 inflammasome activation in mice [84]. LUBAC components have also been implicated in NF-κB and ERK signaling in mice, which could influence the essential priming signal required for upregulation of NLRP3 and pro-IL-1β, thus acting as a regulator of NLRP3 inflammasome activation [83].

Despite the requirement for Sharpin in the activation of the NLRP3 inflammasome and subsequent IL-1β production in myeloid cells [83], a longitudinal transcriptome pathway analysis of skin samples from Sharpin<sup>cpdm</sup> mice has shown significantly increased gene expression of IL-1 family cytokines [85]. In support of these data, Sharpin<sup>cpdm</sup> mice lacking IL-1 receptor accessory protein (IL-1RAcpl) present reduced skin inflammation and disease severity [85]. Similarly, IL-1R deficiency also delays dermatitis and onset of disease in Sharpin<sup>cpdm</sup> mice [86]. A recent study reported that skin tissues of Sharpin<sup>cpdm</sup> mice exhibited dramatically increased levels of the inflammasome-regulated cytokines IL-1β and IL-18 cytokines [87]. Western blot analysis revealed that diseased skin samples also have increased caspase-1 activation, directly implicating the inflammasome in the regulation of these cytokines as well as in skin inflammation in Sharpin<sup>cpdm</sup>. Furthermore, genetic ablation of either NLRP3 or caspase-1 in Sharpin<sup>cpdm</sup> mice has been found to reduce IL-1β and IL-18 cytokines to levels similar to that of WT and, importantly, delaying the disease phenotype [87]. Thus, Sharpin could have cell-specific roles, where Sharpin might be promoting NLRP3 inflammasome activation in myeloid cells, while inhibiting NLRP3 inflammasome activation in skin cells. Future studies investigating the role of Sharpin in the regulation of the NLRP3 inflammasome in different cell types ex vivo will be important in clarifying its cell type-specific roles.

Although NLRP3 inflammasome and IL-1 signaling deficiencies significantly reduce skin inflammation and delay the onset of skin disease symptoms [85–87], Sharpin<sup>cpdm</sup> inflammasome-component deficient mice still develop a full spectrum of disease, suggesting that the IL-1 signaling pathway may play only a minor role in disease progression.

When examining another signaling pathway, it has been noted that Sharpin<sup>cpdm</sup> cells (mouse embryonic fibroblasts) are highly sensitive to TNF-induced cell death [80,81]. In addition, increased keratinocyte cell death has been observed in the epidermis of Sharpin<sup>cpdm</sup> mice [88]. To test the potential role of TNF, Sharpin<sup>cpdm</sup> mice were bred with TNF-deficient mice. Sharpin<sup>cpdm</sup> × Tnf<sup>−/−</sup> mice are completely protected from skin inflammation and onset of disease, establishing a crucial role for the TNF signaling pathway in disease progression [81]. TNF signaling through TNF-R1, but not TNF-R2, provokes dermatitis and disease symptoms in Sharpin<sup>cpdm</sup> mice [86,89]. TNF signaling through TNF-R activates RIPK1 and RIPK3 to induce necroptosis, or FADD and caspase-8 to induce apoptosis. Sharpin<sup>cpdm</sup> mice deficient in RIPK1 kinase activity (Sharpin<sup>cpdm</sup> × Ripk1<sup>K129Δ</sup>) are completely protected from skin inflammation and
disease onset, suggesting a major role for necroptotic cell death in disease progression [90]. Because necrotic cell death activates the NLRP3 inflammasome [91], it might represent a possible mechanism by which the NLRP3 inflammasome is constitutively activated in the skin of Sharpin<sup>cpdm</sup> mice. The observed inflammatory effects of necrotic cell death might be further supported by findings showing that there is a delayed disease onset and milder dermatitis in Sharpin<sup>cpdm</sup> mice deficient in RIPK3 (a major regulator of necroptosis) [89]. Sharpin<sup>cpdm</sup> mice lacking FADD or caspase-8 are almost fully protected from the onset of inflammatory disease and dermatitis, demonstrating the crucial role of Sharpin in regulating apoptosis [86,89]. Together, these studies show that Sharpin is a crucial regulator of both apoptotic and necroptotic cell death pathways in epidermal cells. Moreover, these findings have been further strengthened by the demonstration that Sharpin<sup>cpdm</sup> mice lacking TNFR1 specifically in epidermal cells (Sharpin<sup>cpdm</sup> × Tnfr<sup>1<sub>fl/fo</sub></sup> × K14<sup>Cre</sup> mice) are completely protected from disease [89].

There are several open questions that remain unanswered in the Sharpin<sup>cpdm</sup> model of dermatitis. Whether Sharpin regulates cell death in immune cells is not known. Moreover, the contribution of IL-1α versus IL-1β, the role of necroptotic cell death in NLRP3 activation, and the role for Sharpin in immune cells (specifically adaptive immune cells) in disease progression are not fully understood. Of note, Sharpin<sup>cpdm</sup> × Rag<sup>−/−</sup> mice (deficient in both B and T cells) develop dermatitis similarly to Sharpin<sup>cpdm</sup> mice; however, disease onset is delayed [86]. This suggests that, while adaptive immune cells including B and T cells are not required for instigation of disease, they do play minor roles in the process to accelerate the progression of disease.

Although mutations in Sharpin have not been identified in human patients so far, Sharpin<sup>cpdm</sup> mice have proved very helpful in understanding the basic biology of how several immune components are involved in the progression of an autoinflammatory skin disease resembling dermatitis. It is possible that mutations in human SHARPIN have lethal consequences because Sharpin plays such an important role in various signaling as well as cell death pathways. Regardless, these studies suggest that inhibition of cell death pathways could prove beneficial in the treatment of patients suffering from dermatitis.

Neutrophilic Dermatoses

Neutrophilic dermatoses are genetically inherited autoimmune skin disorders characterized by neutrophilic skin lesions. The umbrella term neutrophilic dermatoses describes a spectrum of skin disorders, including Sweet’s syndrome, pyoderma gangrenosum, and subcorneal pustular dermatosis, all of which have varying degrees of disease severity. Current treatment strategies for these disorders are limited to the use of strong non-specific immunosuppressants, which have obvious drawbacks including a high risk for infection. Lack of mouse models in the past has been one of the major limitations in understanding the etiology and pathology of neutrophilic dermatoses.

Mice with a Y208N missense mutation in the Ptpn6 gene (Ptpn6<sup>cpdm</sup> mice) exhibit spontaneous skin lesions that are infiltrated with neutrophils and closely resemble neutrophilic dermatoses in humans [92,93]. Furthermore, PTPN6 gene analyses from patients with neutrophilic dermatoses such as Sweet’s syndrome and pyoderma gangrenosum have revealed PTPN6 splice variants or heterozygous mutations in affected individuals [94]. Thus, Ptpn6<sup>cpdm</sup> mice are used as a mouse model to understand the disease etiology of neutrophilic dermatoses.

Ptpn6 encodes a Src homology region 2 (SH2) domain-containing phosphatase-1 (SHP-1) protein, known for its role as a negative regulator of signal transduction in a variety of immune cell types [35]. Defects in SHP-1 activity correlate with increased risk of psoriatic arthritis, multiple sclerosis, and leukemia in humans [96–99]. Several mouse models presenting similar defects in
SHP-1 have been reported, and these show similar autoinflammatory manifestations to those observed in human patients [100–104].

Characterization of Ptpn6<sup>spin</sup> Mice Reveals Molecular Mechanisms of Neutrophilic Dermatoses

Ptpn6<sup>spin</sup> mice have ~70% reduced SHP-1 phosphatase activity and, as a result, present a mild phenotype (compared to SHP1-deficient mice) with no disease-associated mortality [93]. Most Ptpn6<sup>spin</sup> mice develop spontaneous footpad swelling at 8–16 weeks of age [93]. Histopathological analysis of footpad sections has revealed neutrophilia, intraepidermal pustules, and cutaneous tissue damage, all characteristics of neutrophilic dermatoses [93]. Interestingly, Ptpn6<sup>spin</sup> disease symptoms can be completely rescued by IL-1R and MyD88 deficiency, implicating IL-1R-MyD88 signaling in the pathogenesis of neutrophilic dermatoses in these mice [104].

NLRP3 inflammasomes are key regulators of IL-1 cytokines, but Ptpn6<sup>spin</sup> mice bred with Nlrc3<sup>−/−</sup>, Asc<sup>−/−</sup>, or Casp1<sup>−/−</sup> mice are not protected from footpad inflammation, suggesting a NLRP3- and inflammasome-independent role of IL-1 signaling in provoking cutaneous disease in Ptpn6<sup>spin</sup> mice [93]. Moreover, disease induction in Ptpn6<sup>spin</sup> mice is mediated by IL-1α, but not by IL-1β [93]. Disease progression in Ptpn6<sup>spin</sup> × Il1a<sup>−/−</sup> mice is also completely ablated, and analysis of footpad tissues and blood shows completely normal histology and neutrophil counts. These results are highly interesting in that IL-1-mediated disease in Ptpn6<sup>spin</sup> mice is independent of inflammasome activation and is mediated by the relatively understudied cytokine IL-1α. Transgenic overexpression of IL-1α by epidermal cells causes an inflammatory skin disease in mice, further supporting the pathogenic role of excess IL-1α in skin inflammation [105].

IL-1α exists in three different biologically active forms (precursor, propiece, and mature) [106]. IL-1α also contains a nuclear localization signal allowing it to translocate to the nucleus and regulate gene expression [106]. How IL-1α promotes inflammatory disease in Ptpn6<sup>spin</sup> mice is an area of debate, and innovative studies will be necessary to delineate these IL-1α functions.

IL-1α is passively released during necrotic cell death and is implicated in wound-healing responses [107,108]. Microabrasion injury of the footpads of pre-diseased Ptpn6<sup>spin</sup> mice accelerates disease progression and the disease manifestations are more severe, exhibiting pustular dermatitis and edema. Interestingly, microabraded Ptpn6<sup>spin</sup> × Il1a<sup>−/−</sup> mice remain protected, further confirming the crucial role of IL-1α in footpad inflammation. Bone marrow chimera studies (Ptpn6<sup>spin</sup> >> wild-type; donor >> recipient) have demonstrated that bone marrow cells harboring a SHP-1 mutation instigate disease development [93].

Because microabrasion can accelerate and exacerbate disease in Ptpn6<sup>spin</sup> mice, it has been proposed that necroptotic cell death in the footpad releases IL-1α to promote disease. RIPK1 acts upstream of RIPK3 to promote necroptosis; however, Ptpn6<sup>spin</sup> × Ripk3<sup>−/−</sup> mice have been found to develop disease similarly to Ptpn6<sup>spin</sup> mice. Interestingly, Nec1s treatment (which inhibits RIPK1 kinase activity) in Ptpn6<sup>spin</sup> mice was reported to inhibit the inflammatory phenotype, suggesting a RIPK3-independent role for RIPK1. Because RIPK1-deficient mice are perinatally lethal [109], fetal liver chimeras with Ptpn6<sup>spin</sup> × Ripk1<sup>−/−</sup> embryos have been generated. While Ptpn6<sup>spin</sup> >> WT mice chimeras have been found to develop footpad inflammation, Ptpn6<sup>spin</sup> × Ripk1<sup>−/−</sup> >> WT mice chimeras remain protected [93]. Consequently, these studies have demonstrated that the RIPK1–IL-1α signaling axis can promote inflammatory footpad disease in Ptpn6<sup>spin</sup> mice.

Ptpn6<sup>spin</sup> mice have proved extremely useful in delineating the potential signaling pathways involved in neutrophilic dermatoses, but have also allowed us to further understand IL-1α...
cytokine biology, which remains significantly obscure (Figure 4). Unlike CAPS and FMF, IL-1-mediated disease progression in murine models of neutrophilic dermatoses is independent of inflammasome activity. Instead, this type of skin disease is mediated by RIPK1, independently of RIPK3, and possibly through a novel cell death pathway. Future genetic studies in mice may help to elucidate the role of some of these signaling components in disease progression. In light of recent studies demonstrating redundant roles for caspase-1 and caspase-8 function in promoting IL-1β and instigating autoinflammatory disease in a mouse model of osteomyelitis [110,111], it is possible that, in Ptpn6spin mice, these caspases could be playing a similar redundant role. For instance, Ptpn6spin mice deficient in both caspase-1 and caspase-8 might uncover the redundant roles of these caspases in provoking disease. Furthermore, it will be interesting to discern whether the RIPK1 scaffolding function versus its kinase activity are implicated in pathogenesis. Does mixed-lineage kinase-like (MLKL) activity play a role? One might propose that RIPK1 kinase activity is important in disease induction based on the described data with Nec1s-mediated RIPK1 inhibition [93]. Moreover, the cellular compartments responsible for IL-1α production and responding to IL-1α are not yet known. From a therapeutic perspective, these studies highlight the importance of further differentiating the biologic function of IL-1α versus IL-1β because these two IL-1 cytokines can have completely different functions depending on the nature of the disease. Specific blockade of IL-1α in patients with neutrophilic dermatoses could indeed provide much needed targeted therapy for these rare disorders.

Concluding Remarks
A common theme among genetically inherited autoinflammatory skin disorders is the pathogenic role of IL-1 cytokines in promoting disease phenotypes. However, several specific questions still remain pertaining to each disease described here (see Outstanding Questions and Box 4). Nevertheless, most of the pathology of these diseases has been directly attributed to IL-1β without excluding the possible contribution of IL-1α and cell death pathways (which might also contribute significantly to the disease). Although IL-1β-specific treatment in the clinic (with Canakinumab) has proven efficacious in some disease settings such as CAPS, it is not a

Outstanding Questions
What are the mechanisms of autoactivation of the inflammasome complex by GOF mutations in NLRP3? Mice expressing mutant NLRP3 (point mutations reported for CAPS) demonstrate excessive IL-1β production resulting in CAPS-like pathology. Activation of NLRP3 can be regulated by post-transcriptional modifications such as deubiquitination and phosphorylation. Whether these key secondary modifications are different in mutant NLRP3 is not known.

How do FMF-associated mutations in the human B30.2 domain of pyrin promote disease? Whether FMF-associated mutations in the B30.2 domain disrupt or hyperactivate pyrin is still open to debate. The discovery of the pyrin inflammasome and mouse models of either deletion of pyrin, or transgenic expression of pyrin fused to the human B30.2 domain (carrying point mutations), suggests that GOF mutations drive FMF. Murine pyrin does not contain B30.2 domains, and innovative experimental designs will be necessary to address the functions of the B30.2 domain and associated FMF mutations.

How does Sharpin deficiency activate the inflammasome in keratinocytes? Sharpin is required for NLRP3 inflammasome activation in myeloid cells including macrophages and DCs. By contrast, skin inflammation in mice lacking Sharpin is delayed by NLRP3, ASC, caspase-1, and IL-1R deficiency, suggesting that Sharpin negatively regulates NLRP3 inflammasome in a cell-type specific manner. Examining whether aberrant TNF–RIPK1 signaling due to the loss of Sharpin promotes NLRP3 inflammasome activation may be informative. If RIPK1 signaling promotes necroptosis, releasing DAMPs to neighboring cells, then NLRP3 could be activated in a paracrine manner.

Do one or both IL-1 cytokines contribute to autoinflammatory skin disease in Sharpin−/− mice? Although inflammasome and IL-1R signaling play minor roles in Sharpin−/− mice skin disease, the development of effective skin therapies will require accurate evaluation of when and where IL-1α or IL-1β contribute to an inflammasome-dependent phenotype in skin disease.
suitable treatment for other similar skin disorders (as shown in a mouse model of neutrophilic dermatoses, which are driven specifically by IL-1x). Thus, it is crucial to distinguish the exact functions and contributions of these two closely related IL-1 cytokines in the etiology of inflammatory disease where IL-1 cytokines are implicated. Moreover, several considerations need to be taken into account when designing a specific drug target for the treatment of these rare autoinflammatory disorders in the future. First, there must be a solid understanding of how the targeted signaling pathways are regulated within the immune system to generate an effective therapeutic. Second, a single drug is unlikely to be effective for the treatment of these complex autoinflammatory disorders, and future therapeutics should look into targeting multiple specific pathways. Third, a push for the generation of small-molecule inhibitors should be an important focus because these can be produced at a lower cost than monoclonal antibodies. Fourth, dietary considerations should be included because changes in diet can considerably affect the microbiome of a patient, and consequently the disease outcome. Finally and most importantly, these treatment options should not completely compromise the immune system of the patient. With recent advances in science and technology and a continued burst of research to understand these inherited diseases, we should be able to comprehensively grasp the complex nature of these diseases in the near future.

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How does RIPK1 promote skin disease in Ptpn6spin mice? RIPK1 has two major functions; its kinase activity, which regulates necroptosis, and its scaffolding function, which regulates signaling pathways. Whether RIPK1 promotes skin disease in Ptpn6spin mice via cell death, signaling, or both is not clear.

What species of IL-1x promotes disease in Ptpn6spin mice? IL-1x, which exists in three forms, is a cytokine containing a nuclear localization signal, and is able to translocate to the nucleus to regulate the transcription of target genes. Which IL-1x species are crucial for inducing inflammatory skin disease in Ptpn6spin mice? How does this relate to human skin inflammation?

Do IL-1α and IL-1β signaling pathways promote distinct diseases? Both IL-1α and IL-1β signal through the common IL-1R and are thought to promote similar activation of NF-kB and MAPK signaling pathways. Mouse models of autoinflammatory disease have suggested that IL-1β and IL-1x can have distinct and specific functions, especially in the outcome of disease (e.g., IL-1x, but not IL-1β, drives neutrophilic dermatoses). It could be posited that IL-1 cytokines exert differential functions depending on cell and tissue type, genetics, and inflammatory setting.

Are there redundant roles for caspase-8 and caspase-1 in autoinflammatory skin disease? Recent studies have shown that caspase-8 can cleave pro-IL-1β and function independently of inflammasomes. In a mouse model of osteomyelitis, both caspase-1 and caspase-8 play redundant roles in promoting IL-1β, instigating disease. Whether a similar redundancy between caspase-1 and caspase-8 occurs in Ptpn6spin mice and results in skin inflammation needs to be determined.

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