Neutrophilic dermatoses are a spectrum of autoinflammatory skin disorders that are characterized by extensive infiltration of neutrophils into the epidermis and dermis. The underlining biological pathways that are responsible for this heterogeneous group of cutaneous diseases have remained elusive. However, recent work from our laboratory and other groups has shown that missense mutations in \( \text{Ptpn6} \), which encodes the non-receptor protein tyrosine phosphatase Src homology region 2 (SH2) domain-containing phosphatase-1 (SHP-1), results in a skin disease with many of the major histopathological and clinical features that encompass neutrophilic dermatoses in humans. In particular, we found that loss-of-function mutation in \( \text{Ptpn6} \) results in unremitting footpad swelling, suppurative inflammation, and neutrophilia. Dysregulated wound healing responses were discovered to contribute to chronic inflammatory skin disease in SHP-1 defective mice, and genetic abrogation of interleukin-1 receptor (IL-1R) protected mice from cutaneous inflammation, suggesting that IL-1-mediated events potentiate disease. Surprisingly, inflammasome activation and IL-1\( \beta \)-mediated events were dispensable for \( \text{Ptpn6}^{+/+} \)-mediated footpad disease. Instead, RIP1-mediated regulation of IL-1\( \alpha \) was identified to be the major driver of inflammation and tissue damage.

Introduction

Neutrophilic dermatoses are a heterogeneous group of autoinflammatory skin disorders that include Sweet syndrome, pyoderma gangrenosum, and subcorneal pustular dermatosis.\(^1\) Neutrophilia and cutaneous inflammatory lesions that are dominated by neutrophils are defining clinical features of all neutrophilic dermatoses. Afflicted individuals develop erythematous skin lesions (papules, nodules, and plaques) that are both painful and unsightly. Current strategies to treat inflammatory flare-ups include the prescription of strong immunosuppressive regimens such as systemic administration of corticosteroids. Although such therapies have proven efficacious in the control of immediate inflammation, numerous drawbacks exist. These include the inability to prevent future disease and increased susceptibility to infection that results from global immunosuppression. Complete characterization of neutrophilic dermatosis etiology has been hampered by the lack of an experimental model system to elucidate critical biological players. However, our recent findings and work from other groups demonstrates that missense mutations in the gene that encodes SHP-1 (\( \text{Ptpn6} \)) promotes the development of an inflammatory skin disease in mice that closely resembles neutrophilic dermatosis in humans.\(^4,5\) SHP-1 is a protein tyrosine phosphatase that has been described to function as a negative regulator of signal transduction in a variety of cell types.\(^6\) Alterations in SHP-1 activity have been linked to multiple human diseases including leukemia, psoriatic arthritis, and multiple sclerosis.\(^11,15\) In addition, pivotal roles for SHP-1 in the regulation of autoinflammatory disease came from the
discovery that loss-of-function mutations in *Ptpn6* provoke severe granulocytic skin lesions and pneumonitis in mice.\(^1\)–\(^19\) Persistent cutaneous inflammation in SHP-1 deficient mice results in a moth-eaten phenotype that is characterized by hair loss and runted appearance. Elucidation of the overarching biological role of SHP-1 in autoimmune inflammatory disease pathogenesis has been hindered by the fact that SHP-1 null mice are immunodeficient at birth and succumb to a devastating pneumonitis and glomerulonephritis by 2–9 wk of age.

Recently, a new strain of SHP-1 mutant mice that carry a Y208N amino acid substitution in the C-terminal SH2 domain of SHP-1 (referred to as *Ptpn6*\(^{\text{spin}}\) mice) was generated.\(^20\) Mice homozygous for this mutant allele develop a less aggressive disease phenotype, which is attributed to 70–80\% reductions in SHP-1 phosphatase activity. Importantly, *Ptpn6*\(^{\text{spin}}\) mutation provokes the development of a spontaneous chronic inflammatory disorder at 8–16 wk of age that is characterized by persistent footpad swelling and suppurative inflammation. Characterization of disease pathogenesis revealed that *Ptpn6*\(^{\text{spin}}\) mice exhibit many of the prominent clinical and histopathological hallmarks that define neutrophilic dermatoses.\(^4\) These include neutrophilia, the formation of intraepidermal pustules and cutaneous tissue damage that is associated with marked infiltration of neutrophils. Interestingly, splice variants and coding mutations in *Ptpn6* are closely associated with multiple neutrophilic dermatoses including Sweet syndrome and pyoderma gangrenosum in humans.\(^21\) This association between mutations in *Ptpn6* and clinic cases of neutrophilic dermatoses highlights the value of *Ptpn6* mutant mice in the study of physiological autoinflammatory skin disorders. Indeed, utilization of SHP-1 deficient mice has significantly aided in the elucidation of the cellular and molecular players that contribute to tissue damage and inflammation in neutrophilic dermatosis and other autoinflammatory skin disorders.\(^5,\)\(^22\)

**IL-1 in Neutrophilic Dermatosis**

The IL-1 family cytokines IL-1\(\alpha\) and IL-1\(\beta\) have emerged as principal mediators involved in both the initiation and perpetuation of multiple inflammatory diseases.\(^23,\)\(^24\) Both IL-1\(\alpha\) and IL-1\(\beta\) are potent immunological mediators that trigger proinflammatory signaling through the engagement of the IL-1 receptor (IL-1R). IL-1\(\beta\) is generated in an inactive pro-form that requires cleavage to elicit its biological activity and secretion. The most well characterized mechanism for IL-1\(\beta\) processing is via activated caspase-1 in the inflammasome complex; although additional inflammasome-independent cleavage pathways have recently been
Inflammasomes are multi-protein complexes that consist of a sensor molecule such as a NOD-like receptor (NLR), the adaptor protein ASC, and caspase-1. The recognition of pathogen- or danger-associated molecular patterns (PAMPs and DAMPs, respectively) by NLRs promotes the recruitment of ASC and caspase-1 into the inflammasome complex, which is required to correctly orient caspase-1 for auto-cleavage and activation. Importantly, recent studies have identified pivotal roles for inflammasome-derived IL-1β in a variety of infectious, metabolic, autoimmune, and inflammatory diseases. In comparison to IL-1β, our understanding of the roles and regulation of IL-1α in disease is severely limited. IL-1α exists in three biological active forms (precursor, propiece, and mature form), however their discrete contributions to inflammation and the molecular pathways that promote their production remain to be formally elucidated. Aberrant forms of cell death, such as necrosis, that result from cellular stress, trauma, and hypoxia are believed to provoke the passive release of IL-1α. Furthermore, IL-1α has been implicated in wound healing responses and sterile inflammation.

Previous work demonstrated that IL-1R is required for the development of persistent cutaneous inflammation in Ptpn6mutant mice, which suggests that dysregulated IL-1 signaling directly contributes to disease pathogenesis. In our studies, we set out to characterize the upstream biological events and players that are responsible for neutrophilic dermatosis-related disease in Ptpn6mutant mice. We initially hypothesized that unchecked regulation of NLRP3/inflammasome-induced IL-1β production was responsible for autoinflammatory skin disease. This was based on emerging data suggesting pivotal roles for inflammasome activation in multiple IL-1-mediated inflammatory disorders. Surprisingly, genetic deletion of critical inflammasome components (NLRP3 and caspase-1) did not provide any protection against Ptpn6-associated footpad inflammation. In addition, targeted ablation of IL-1β also did not rescue disease in this model. Rather, IL-1α was discovered to be the major driver of autoinflammation and skin damage in Ptpn6mutant mice. Genetic abrogation of IL-1α in Ptpn6mutant mice was found to prevent disease progression by limiting the numbers of circulating neutrophils and dampening downstream proinflammatory cytokine production (Fig. 1). These findings help to delineate unique roles for IL-1α in autoinflammatory disease that are distinct from inflammasomes and IL-1β.

IL-1α is an alarmin molecule that orchestrates beneficial wound healing responses following injury, hypoxia, and other inducers of tissue necrosis. However, unchecked IL-1α release can provoke inflammation and tissue destruction, and thus dysregulated IL-1α secretion is believed to play deleterious roles in multiple sterile inflammatory disorders including stroke and atherosclerosis. To elucidate whether aberrant wound healing responses were responsible for driving neutrophil-associated inflammatory skin disease in Ptpn6mutant mice, we induced microabrasion injuries on the plantar surfaces of wild-type and asymptomatic Ptpn6mutant mice. Loss-of-function mutation in Ptpn6 caused exacerbated proinflammatory cytokine production following cutaneous tissue damage. Moreover, Ptpn6mutant mice were unable to resolve the inflammation that was brought upon by microabrasion-induced trauma and as a result developed an aggravated state of footpad inflammation that was characterized by severe purulent dermatosis and edema. Interestingly, genetic abrogation of IL-1α protected against excessive proinflammatory cytokine production and conferred normal wound healing responses in Ptpn6mutant mice. Collectively, these results identify SHP-1 and IL-1α as central regulators of wound-induced inflammation. Furthermore, they suggest that dysregulated wound healing responses may be involved in the pathogenesis of neutrophilic dermatoses.

To identify whether defective SHP-1 signaling in hematopoietic cells or radioresistant skin cells is required to promote persistent autoinflammatory skin disease in Ptpn6mutant mice, reciprocal bone marrow chimera studies were conducted. Confining Ptpn6mutation only to radioresistant cells by transplanting wild-type bone marrow cells into irradiated Ptpn6mutant mice provided complete protection from skin disease. In contrast, loss-of-function mutation in Ptpn6 in the bone marrow compartment alone was sufficient to induce chronic autoinflammatory skin disease. This suggests that impaired SHP-1 function specifically in bone marrow-derived immune cells drives this IL-1α-mediated cutaneous disorder.

**RIP1 Controls IL-1α Driven Autoinflammatory Skin Disease**

The molecular pathways that contribute to IL-1α secretion remain poorly defined. The prevailing paradigm is that IL-1α is passively released following cellular catastrophe and necrosis. Until recently, it was thought that necrosis was an unprogrammed form of cell death that follows devastating and rapid cellular demise. However in the last few years, significant progress has been made in the identification of signaling molecules that coordinate necrosis. For instance, RIP3 downstream of RIP1 activation has emerged as a critical mediator of one form of necrosis referred to as necroptosis. In these studies, it was shown that the RIP1 kinase inhibitor necrostatin-1 (Nec1) could block RIP3-driven necroptosis. To elucidate whether RIP3-induced necroptosis contributes to IL-1α release and Ptpn6-mediated autoinflammatory skin disease, wild-type, and Ptpn6mutant mice were treated with Nec1 and then subjected to microabrasion trauma on their footpads. Inhibition of RIP1 with Nec1 protected against excessive proinflammatory cytokine production in Ptpn6mutant mice. Genetic deletion of RIP1 in fetal liver chimera mice also rescued Ptpn6-induced autoinflammatory skin disease, confirming a critical downstream role for RIP1 in disease progression. Surprisingly, targeting deletion of RIP3 did not provide protection, and Ptpn6xRip3mutant mice developed severe neutrophilic dermatosis, suggesting that RIP1 controls autoinflammatory skin disease independently of its role in RIP3-driven necroptosis. Rather, we found that RIP1-mediated regulation of...
inflammatory NFκB signaling contributes to inflammatory syndrome in Ptpn6<sup>−/−</sup> mutant mice.

**Future Perspectives and Conclusions**

Although our recent findings and work by other groups have firmly established that dysregulated immune responses are responsible for driving neutrophilic dermatoses, numerous important questions still remain. Identification of the pathogenic role of unchecked IL-1α-mediated signaling in cutaneous autoinflammation has provided critical insight into the etiology of neutrophilic dermatoses. IL-1α exists in 3 biologically active forms (precursor, propiece, and mature form), however which species trigger inflammatory skin disease still remains to be formally elucidated. Furthermore, whether IL-1α is released passively as a result of cellular catastrophe or requires specific cleavage by a protease to potentiate cutaneous pathology is still unresolved. Our understanding of IL-1α biology in comparison to IL-1β is severely limited, and we have only recently begun to dissect the discrete roles of IL-1α in disease pathogenesis. Thus, additional investigation is greatly needed to further advance this field. Unique IL-1α-dependent functions have recently been described in multiple disease models including atherosclerosis, DNA damage-induced senescence and tumorigenesis, and Legionella pneumophila infection, and inflammation associated with necrotic cell death. However the discrete roles of IL-1α and IL-1β in the etiology of most IL-1R-mediated diseases still remain to be defined.

Blockade of IL-1 signaling with the IL-1R antagonist Anakinra has proven effective in the treatment of various human disorders including cryopyrin-associated periodic syndromes (CAPS) and type 2 diabetes. However, such treatment strategies have significant downsides, including high costs and serious side effects that are associated with aggressive immunosuppression. Improved therapeutics that only target the specific pathogenic IL-1 molecules, while sparing other potentially beneficial IL-1-mediated functions (e.g., protective anti-pathogen and -tumor responses), are needed. Our work highlighting a critical role for RIP1-dependent IL-1α secretion in autoinflammatory skin disease suggests that therapeutic neutralization of IL-1α may prove beneficial for treating autoinflammatory diseases, while not interfering with IL-1β-mediated responses. In addition, we provide in vivo evidence that the RIP1 kinase inhibitor necrostatin-1 (Nec1) can be utilized to limit IL-1α-mediated inflammation and ameliorate exacerbated wound healing responses. It is possible that Nec1 can also be exploited to treat additional IL-1α-driven disorders, thus in the future it will be important to formally test this attractive treatment avenue.

Currently, the roles of RIP1 in inflammatory disease progression also remain poorly defined. The perinatal lethality that is associated with RIP1 deficiency in mice has severely hampered our ability to fully uncover the physiological functions of RIP1 in the regulation of inflammation and immune responses. In vitro studies, however, have positioned RIP1 as a master regulator of various cellular events that have been associated with inflammatory and autoimmune disease. For instance, RIP1 has been described to centrally function in the control of NFκB-mediated inflammatory signaling, apoptosis, and RIP3-associated necroptosis. Thus, it is conceivable that RIP1 is an important in vivo regulator of autoinflammation. Our discovery that RIP1 is essential for the development of persistent inflammatory skin disease in Ptpn6<sup>−/−</sup> mutant mice provides direct evidence that RIP1 can contribute to inflammatory disease pathogenesis. Interestingly, we found that RIP1 controls inflammation through the regulation of NFκB activation and not through its role in RIP3-mediated necroptosis. Future studies that investigate the ability of RIP1 to influence immune responses and inflationation in other disease models are needed to illuminate the physiological functions of RIP1. Utilization of RIP1 deficient fetal liver chimeras and conditional RIP1 deletion mice will facilitate such studies and aid in the characterization of the SHP-1/RIP1/IL-1α inflammatory axis.

Disclosure of Potential Conflicts of Interest

No potential conflict of interest was disclosed.

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