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Targeting IL-1 controls refractory Pityriasis rubra pilaris

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Abstract

Pityriasis rubra pilaris (PRP) is a rare inflammatory skin disease which lacks efficacious standard-of-care treatments. Molecular studies of skin lesions revealed that IL-1β is central to the pathogenesis of PRP. Treatment of three patients with the IL-1-targeting biologics anakinra and canakinumab resulted in rapid clinical improvement and reversal of the PRP-associated molecular signature. We identified an NF-κB-mediated IL-1β-CCL20 axis central to the inflammatory response in PRP. Our results reveal the central role of IL-1β signaling in the pathogenesis of PRP and highlights its prominence as a therapeutic target.

Summary

Pityriasis rubra pilaris (PRP) is a rare inflammatory skin disease which lacks efficacious standard-of-care treatments. Molecular studies of skin lesions revealed that IL-1β is central to the pathogenesis of PRP. Treatment of three patients with the IL-1-targeting biologics anakinra and canakinumab resulted in rapid clinical improvement and reversal of the PRP-associated molecular signature. We identified an NF-κB-mediated IL-1β-CCL20 axis central to the inflammatory response in PRP. Our results reveal the central role of IL-1β signaling in the pathogenesis of PRP and highlights its prominence as a therapeutic target.

Introduction

Pityriasis rubra pilaris (PRP) is a rare inflammatory skin condition that presents unique features including salmon-colored erythema, waxy palmoplantar keratoderma, folliculocentric papules and traits of psoriasis and atopic dermatitis (AD). Although the IL-23/Th17 axis has been proposed to be involved, the pathogenesis of PRP is not well-understood[1]. Treatments with TNF-, IL-23- and IL-17-inhibitors, have shown limited efficacy, while phototherapy may worsen manifestations[2,3]. Response rates of improvement by 75% in the Psoriasis Area and Severity Index (PASI75) have been reported in 50% of patients treated with IL-17 inhibitors[4,5]. The low response rate to therapy including biologics, underscores our limited understanding of the disease and the need for novel effective therapeutic strategies.

We sought to unravel the mechanisms behind PRP through a molecularly-driven precision medicine approach. Three patients who had failed prior treatments showed rapid and significant clinical improvement when administered the IL-1-receptor antagonist anakinra or the IL-1β inhibitor canakinumab. With molecular profiling, we demonstrate a distinct role for IL-1β in the pathogenesis of adult-type PRP.

For methods see, **Supplementary Appendix**.

Results

The PRP skin transcriptome differs from other immune-mediated inflammatory diseases (IMIDs)

To differentiate PRP from other related IMIDs, we performed a targeted transcriptomic assessment of lesional skin from patients with adult-type PRP (active and post-PRP), psoriasis, AD, and healthy controls (HC) (Fig. 1A, **Table S1**). Analysis of differentially expressed genes (DEG) revealed a distinct inflammatory pattern associated to PRP (Fig. 1B, **Figure S1-2**) with significantly increased expression of genes encoding the cytokines, *IL1A*, *IL1B*, *IL23A*, *IL17A/F*, *TNF*, chemokines *CCL18*, *CCL20*, and defensin/alarmins *S100A8/9*, and *DEFB4A* (Fig. 1C). The transcriptional signature for PRP included *CCL20*, consistent with previous reports[6], and *IL1B* as the predominant DEGs. Their gene expression correlated significantly with clinical severity of PRP measured by PASI ($p = 0.0018$ and > 0.0001 for IL1B and CCL20, respectively), indicating a pathogenic relevance (Fig. 1D). In summary, PRP embodies a unique transcriptional profile compared with related IMIDs (Fig. 1E), thereby highlighting its distinct pathophysiology.

IL-1β is a key regulator of PRP and linked to keratinization

To confirm our findings, we then constructed a regulatory network of PRP, further dissecting its molecular signature. We analyzed publicly available data of PRP patients with paired lesional vs. non-lesional skin biopsies[5]. Ingenuity Pathway Analysis (IPA) identified *IL1B* as the second strongest predictor for upstream regulation of the PRP transcriptomic signature (Fig. 1F). A subsequent gene-gene co-expression analysis identified ten co-expressed gene modules (Fig. 1G, **S3A**) where *IL1B*-containing module 1 strikingly overlapped with the PRP signature and demonstrated the highest percentage of significant PRP-DEGs amongst all modules (**Figure S3B**). Furthermore, module 1 genes exhibited a notable log-fold increase in all genes that were shared with PRP DEGs (**Figure S3C**), and their enrichment/predominance in PRP was confirmed by gene set enrichment analysis (GSEA) (Fig. 1G, **S3D**).

Gene-Set over representation analysis of the module 1 exposed connections with immune and inflammatory signals, including *Interleukin-1 Receptor Binding*, and with keratinocyte differentiation (Fig. 1H, **S3E**). Module 1 was subsequently dissected into 8 functional clusters (**Figure S3F, S4A**) using STRING functional network analysis (protein-protein interaction, PPI). The two primary subclusters relate to keratinization (orange) and IL1/inflammation (blue, **Fig. 1I**). The IL1/inflammation cluster includes 8 genes from the IL-1 family: IL1A, IL1B, IL36A, IL36G, IL36RN, IL1RN, IL1F10, and IL36B, as well as CCL20, IL23A, CARD14, and S100A8. Both subclusters exhibit strong interaction with each other. Enrichment of each module confirmed these annotations (over representation analysis, **Figure S4B-C**), which contained a substantial number of genes overexpressed in PRP (**Figure S4D-E**). This analysis provides strong evidence that the immune pathways involving IL-1 are closely intertwined with keratinization mechanisms in PRP. These findings advocate the application of IL-1-targeting biologics in the management of PRP.

Treatment of PRP with IL-1 inhibitors rapidly ameliorates disease

To validate the potential of IL-1β blockade for PRP treatment, we treated three therapy-refractory PRP patients (two males, ages 53 and 59 and one female, age 53) with the IL-1 receptor antagonist anakinra

(Fig. 2A-B, **Figure S5A-D, Table S2**).

Patient 1 responded well initially to a standard anakinra dose of 100 mg/day. Skin severity improved by 50% PASI (PASI50) at week 2 in patients 1 and 2 and at week 3 in patient 3 (Fig. 2C). When the disease worsened at week 6 in patient 1, the dose was doubled to 200 mg/day. The same dose regimen was administered to patients 2 and 3. PASI75 was reached by week 8 in patients 1 (PASI 11.4 to 2.6, ΔPASI 77%) and 2 (PASI 21.4 to 5.7, ΔPASI 73%) and by week 12 in patient 3 (PASI 34.2 to 9.4, ΔPASI 73%). All patients tolerated the treatment well (**Supplementary Appendix**) and reduced the concomitant use of topical steroids.

After week 12, patients 1 and 2 stopped treatment due to lack of cost coverage through health insurance. Patient 2 continued to improve further and reached complete resolution without any other treatment. Patient 1 was subsequently switched to biologics targeting TNF, IL-23, or IL-17, none of which adequately controlled the disease (**Table S2**). However, when switched to canakinumab (anti-IL-1β), the patient improved significantly within 8 weeks (ΔPASI 85%) (Fig. 2D). Patient 3 continued treatment with anakinra. From baseline to week 8, itch severity significantly reduced in patients 1–2, whereas patient 3 showed no symptoms (**Supplementary Appendix**).

The clinical improvement in all patients was confirmed histologically (Fig. 2E-F, **S5E-F**) including normalization of IL-1β expression at week 8, reaching similar levels to those in non-lesional tissue (Fig. 2G-H, **S5G-H**).

We report here a rapid and successful response to the IL-1 antagonists anakinra and canakinumab in 3 PRP patients, underscoring the importance of IL-1β in PRP disease pathogenesis.

Anakinra reverses the PRP transcriptional signature in patients with PRP

We sought to dissect the mechanisms involved in the development of PRP by exploring the transcriptional signature following treatment and confirm IL-1β as a potential target. All top 5 positive upstream regulators in PRP (Fig. 1F) were significantly downregulated upon IL-1β-targeting treatment (Fig. 3A). Overall, predicted activation scores of IPA disease, pathways, and upstream regulators annotations exhibited significant negative correlation between PRP and the *treatment signal* (Figs. 3B, **S6A-B**), which is defined as the DEGs from analysis of lesional samples after vs. before treatment. Positive DEGs in PRP showed significant negative enrichment in the *treatment signal* (Fig. 3C**)**. IPA mechanistic network analysis further showed that gene regulation arising from *IL1B* could itself activate the regulation of *TNF, IFNG, IL6* and *TGFB1*, with downstream activation of *NFkB* and *STAT* (Fig. 3D), which was reversed with treatment (Fig. 3E). The interaction of these cytokines is also supported by the IPA summary network of PRP and *treatment* DE analysis, showing a central role of IL-1β (**Figure S6C-D**). Furthermore, GSEA analysis of selected pathways of interest, such as *TNF-alpha Signaling via NF-kB*, showed positive enrichment with PRP and negative enrichment following IL-1 antagonist treatment (Fig. 3F). In summary, these results highlight a reversion of PRP transcriptional signals and NF-kB inhibition upon anakinra treatment (Fig. 3G).

IL-1β drives PRP-specific signature in keratinocytes *in vitro*

Next, we assessed the impact of IL-1β signaling and activation in keratinocytes. DE analysis of IL-1β-stimulated keratinocytes showed a significant upregulation of genes encoding CCL20, IL-1β, TNF, IL-23A, IL-36γ, *NFKB1*, as well as enrichment in pathways of inflammation, *IL-1*, and *NF-kB* (**Figure S7A-C**). DEGs in IL-1β stimulated keratinocytes were enriched in PRP which showed similar activated pathways. Many significantly overexpressed genes overlapped between IL-1β-stimulated keratinocytes and lesional PRP, with a strong enrichment of *NFkB*-related signals (**Figure S7D-G**). The *in vitro* data overlapping with the PRP molecular signature demonstrates the central role of IL-1β and hints at the involvement of previously described downstream players such as such as CCL20, TNF, IL-23A, IL-36γ, DEFB4A, IL-17C, CXCL8 and NOD2.

The strongest upregulated chemokine in the transcriptome analysis was CCL20 (Fig. 1B). TNF and IL-1β were also the strongest inducers of *CCL20* in keratinocytes *in vitro* (**Figure S8A-B)** and inhibition of IL-1 signaling with anakinra decreased *CCL20* expression (**Figure S5I**), suggesting a major role of an IL-1β-CCL20 axis in disease pathogenesis.

Additionally, our analysis identifies NOD2 and CARD14 as the most prominent CARD proteins in active and lesional PRP. These proteins signal via NF-κB and display a strong correlation with IL1B (**Figure S8**; detailed in Supplementary Material), which underscores the role of CARD14 and NOD2 in keratinocyte interactions within PRP-affected skin. Moreover, Caspase-1 expression is diminished in PRP lesional skin during anakinra treatment (**Figure S5J**; detailed in Supplementary Material).

Ultimately, the overlap of the gene signature in active PRP with IL-1β-stimulated keratinocytes suggests a crucial role of keratinocytes and IL-1β in triggering major inflammatory reactions, with NF-kB being a key factor involved, all of which is central to PRP pathogenesis.

Discussion

Our findings unequivocally establish the IL-1 pathway and IL-1β as pivotal upstream regulators in PRP pathogenesis, providing an important proof-of-principle of a molecularly-guided precision medicine approach in a rare disorder.

Treatment with IL-1 antagonists resulted in profound clinical improvement and a reversal of the PRP transcriptional signature. Our work not only enhances the understanding of the mechanisms underlying PRP pathogenesis but also highlights the central role of the IL-1β pathway in its progression.

The rare subtype of inherited PRP[7] harbors *CARD14* gain-of-function (GOF) mutations leading to NF-κB signaling, upregulating genes such as *CCL20* and *IL1B* in the epidermis[7], which has been confirmed in cell lines[8] and mouse models[9,10]. Additionally, *CARD14* overexpression has been reported in PRP patients without *CARD14* mutations[11]. This suggests that both inherited and sporadic PRP share similar mechanisms, where overexpressed CARD14 activates NF-κB, leading to the expression of pro-

inflammatory genes such as *CCL20* and *IL1B*. Analysis of a *CARD14* GOF dataset in mice[10] revealed similar enrichments and molecular patterns to those observed in our studies (**Figure S9A-B**). These results underline the classification of inherited PRP as an autoinflammatory keratinization disease and suggest the same for sporadic adult-type PRP.

Moreover, we observed that *NOD2* is overexpressed in active and lesional PRP, which has also been described in *CARD14* GOF mice[12], and correlates with *IL1B* expression. As NOD2 is a cytoplasmic pattern recognition receptor for bacterial peptidoglycan motifs and activates the NF-κB pathway[13], this supports the hypothesis that microbial response contributes to PRP pathogenesis.

Our clinical and molecular data also underlines that IL-1β signaling is necessary for the sustained NF-κB activation in PRP, as confirmed with *in vitro* IL-1β-stimulated keratinocytes. Of note, CCL20 signaling, downstream of IL-1β signaling, plays a key role in the recruitment of Th17 cells[14]. We also hypothesize that the reason for the partial response seen with IL-17 inhibitors could be on the one hand the upstream regulation of IL-1β by bypassing the Th17 axis and on the other hand the possibility that IL-1β induces Th17 cells and therefore an IL-1b-Th17 feedback loop to trigger PRP further[15].

We confirmed IL-1β expression in keratinocytes of lesional PRP tissue. This aligns with the strong interaction between IL-1 family genes and keratinocyte function shown by STRING molecular analysis, underscoring a keratinocyte-driven inflammation. As we observed increased caspase-1 expression and CCL20 production in active PRP, and since UV-light activates the inflammasome and IL-1β production via caspase-1[16] and CCL20 in human keratinocytes (**Figure S8A-B**), this could explain the photosensitivity observed in PRP.

Importantly, we noticed a rapid clinical improvement within 2–3 weeks of IL-1 antagonist therapy in our 3 PRP patients, which is faster than usually observed with biologics in other IMIDs such as psoriasis and AD. The IL-23p19 inhibitor tildrakizumab leads to a PASI50 response in 50% of PRP patients after at least 4 weeks[17], whereas in innate-driven autoinflammatory diseases such as generalized pustular psoriasis, the IL-36-receptor inhibitor spesolimab clears more than 50% of patients already by one week[18]. Therefore, our findings are further supporting the classification of PRP as an autoinflammatory keratinization disease.

Limitations of this study are the retrospective character with a small sample size and limited amount of tissue samples at critical time-points, such as weeks 0 and 8, for performing more extensive mechanistic studies.

In summary, our study provides a proof-of-principle molecularly-guided precision medicine approach establishing the IL-1 pathway, and in particular IL-1β, as a therapeutic target in PRP. We demonstrate that PRP can be distinguished from psoriasis and AD by molecular profiling, and we present an IL-1-driven regulatory network of PRP. The quick and reproducible improvement in three patients, treated with IL-1/IL-1β antagonists, supports the predicted driver function of IL-1β and, therefore, its position upstream of the

inflammatory pathway. Additionally, we propose a mechanism involving NF-κB-mediated IL-1β-CCL20 signaling in PRP, which includes activation of *CARD14* and *NOD2*. Our findings define PRP as an autoinflammatory keratinization disease, suggesting a novel therapeutic approach. While further clinical studies are essential, our findings support the use of IL-1β antagonists in PRP.

Declarations

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The authors declare no conflict of interest related to this work.

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Figures

Figure 1

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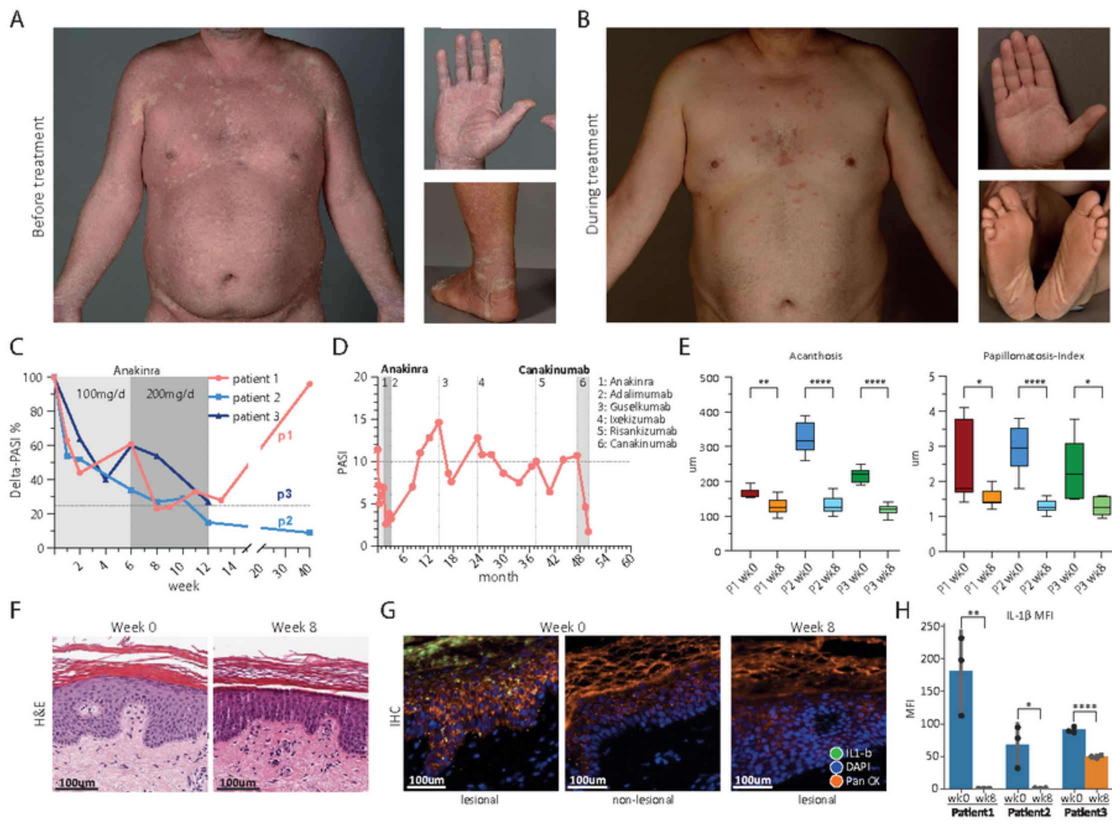

Figure 2

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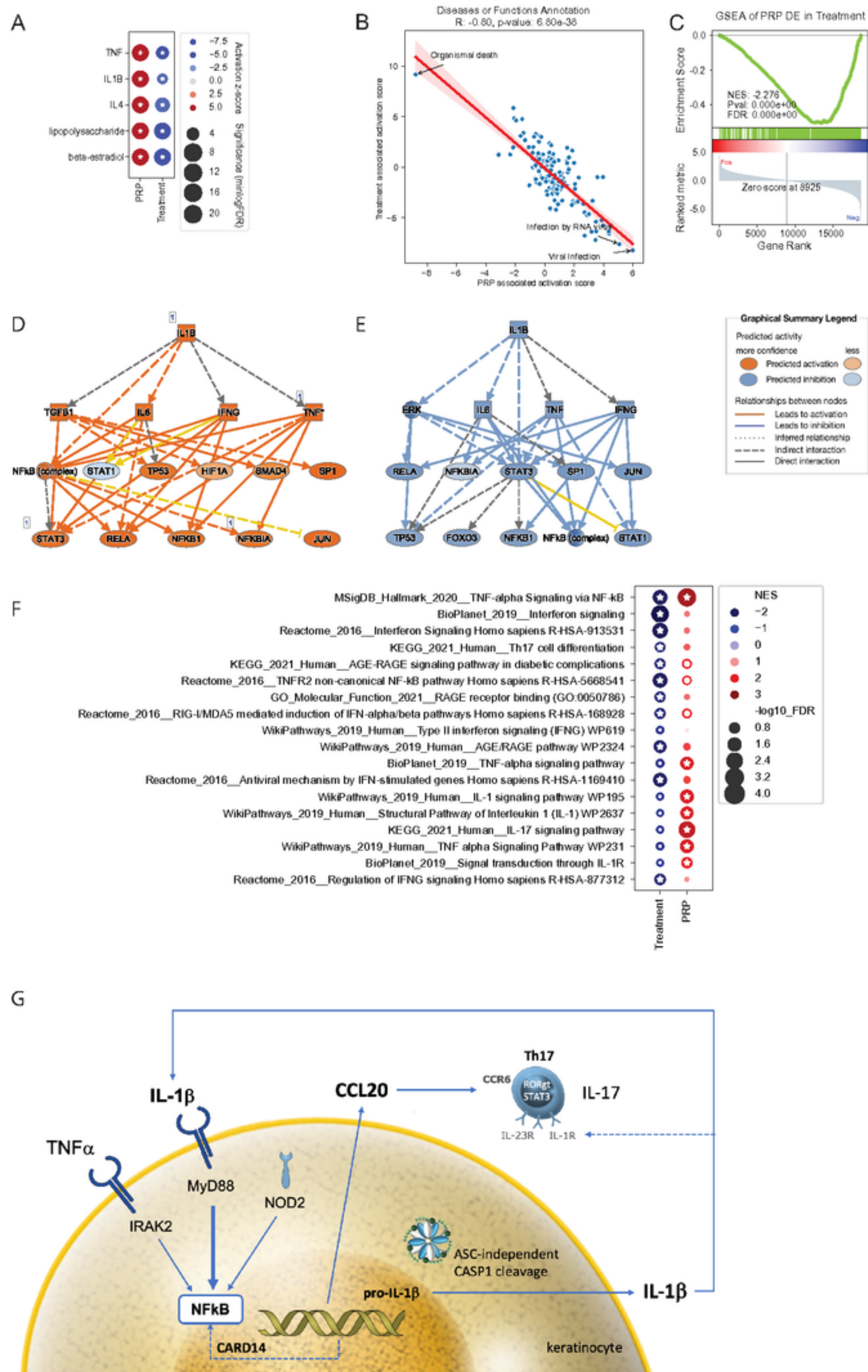

Figure 3

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