Paired Transcriptomic and Proteomic Analysis Implicates IL-1β in the Pathogenesis of Papulopustular Rosacea Explants

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Papulopustular rosacea (PPR) is a chronic inflammatory skin disease with limited treatment options. Although multiple pathways have been described to be upregulated in PPR, a mechanistic understanding of the key drivers and interaction between pathways in PPR pathology is lacking. In this study, we utilized PPR skin biopsy explants to integrate both differentially expressed genes and differentially expressed proteins in paired nonlesional and lesional PPR tissue (n = 5 patients). The results of this study identified 92 differentially expressed genes and 20 differentially expressed proteins between paired PPR lesional and nonlesional explants. MAPK and TNF signaling pathways were the most significantly upregulated pathways in PPR lesional tissue and aligned with differently expressed proteins identified in this study. Both MAPK and TNF signaling pathways highlighted IL-1β as a potential central mediator for PPR pathogenesis. In support of this, stimulation of nonlesional explants with IL-1β resulted in transcriptomic and proteomic profiles similar to those of lesional PPR. In this integrative transcriptomic and quantitative protein analysis, we identified several inflammatory genes, proteins, and pathways, which may be contributing to PPR, as well as highlighted a potential role of IL-1β in driving inflammation in PPR.


INTRODUCTION

Papulopustular rosacea (PPR) is a chronic inflammatory skin disease characterized by flushing, telangiectasia, papules and pustules, and sensitive skin. As its clinical presentation may imply, recent studies have suggested that dysregulation in immune, nervous, and vasculature systems all contribute to the pathogenesis of PPR. Despite our increasing understanding of the disease, the management of PPR remains difficult because the treatment options are limited.

The characterization of the immune response (expression of various cytokines and chemokines) in PPR has been classically performed with expression studies focused on specific genes (e.g., through quantitative RT-PCR) or with qualitative and/or descriptive interrogation of proteins in tissue (e.g., immunohistochemistry). There are few unbiased searches of differentially expressed genes (DEGs) in PPR, and there has been no comprehensive analysis of both quantitative mRNA and quantitative protein analysis of cytokines and/or chemokines in paired nonlesional skin (NLS) and lesional PPR skin to date. Cytokines that have been identified in previous gene expression studies of PPR include IL-1β (Buhl et al., 2015; Dajnoki et al., 2017; Shih et al., 2020), IL-6 (Buhl et al., 2015; Dajnoki et al., 2017; Shih et al., 2020), and IL-17 (Buhl et al., 2015; Dajnoki et al., 2017; Shih et al., 2020). Members of the TNF-α pathway (Buhl et al., 2015; Gerber et al., 2011) and the IFN-γ pathway (Buhl et al., 2015; Dajnoki et al., 2017) have also been associated with PPR in previous studies. Numerous chemokines and chemokine receptors that are involved in the activation and trafficking of T helper (Th1, Th2, Th17, monocytes, macrophages, and neutrophils have also been identified in previous gene expression studies of PPR (Buhl et al., 2015; Dajnoki et al., 2017; Gerber et al., 2011; Muto et al., 2014). These data all point to a heterogeneous inflammation occurring in PPR but with little understanding of what are the key drivers of the disease. Many of these cytokines and chemokines could become potential targets for the treatment of PPR and are worth to be validated at the protein level with quantitative approaches. A detailed understanding of the interplay of transcriptomic and proteomic data from PPR biopsies might also help elucidate the key pathogenic mechanisms in PPR.

One cytokine that has been suggested to play a role in PPR is IL-1β. Several groups have described increased expression of IL-1β mRNA in PPR skin biopsies compared with that of healthy controls (Buhl et al., 2015; Casas et al., 2012; Dajnoki et al., 2017; Shih et al., 2020). In addition, IL-1β protein is elevated in the peripheral blood of patients with PPR (Falay Gur et al., 2018). UVB radiation, a known trigger
of PPR, has been found to induce IL-1β release from keratinocytes, which has been postulated as a pathogenic mechanism in rosacea (Salzer et al., 2014). Furthermore, IL-1β is associated with known rosacea comorbidities such as Alzheimer’s disease (Schjeide et al., 2009; Walker et al., 2006), anxiety or depression (Kovacs et al., 2016), cardiovascular diseases (Chen et al., 2016; Stegger et al., 2012), multiple sclerosis (Mirowska-Guzel et al., 2011; Paré et al., 2018; Romme Christensen et al., 2012), rheumatoid arthritis (Addobbi et al., 2018; Jahid et al., 2018), and type 1 diabetes mellitus (Kaizer et al., 2007). These findings suggest that IL-1β may be a major driver of PPR; however, more mechanistic studies demonstrating the role of IL-1β in rosacea pathogenesis are lacking.

Because there are no preclinical animal models of PPR, human skin explants provide an opportunity to interrogate the pathogenesis of this inflammatory skin disease. In this study, we utilized a human skin explant model to investigate two specific aims. First, we utilized the skin explant model to obtain both RNA for RNA sequencing (RNAseq) and explant conditioned cell media for proteomic analysis between paired PPR skin and NLS. Second, we stimulated an NLS explant with IL-1β and compared the induced transcriptome and proteome with the PPR LS explant to evaluate the possible contribution of IL-1β to the PPR inflammatory signature. This paired integrative transcriptomic and proteomic study of ex vivo PPR biopsy explants provides an opportunity to advance the understanding of PPR pathogenesis and evaluate the possible contribution of IL-1β in driving this disease.

**RESULTS**

**DEGs and differentially expressed proteins in PPR skin versus NLS explants**

In total, five subjects with PPR were enrolled, and the demographic characteristics of the study subjects are shown in Supplementary Table S1. A diagram of the PPR biopsy explant experimental design is shown in Figure 1a. Biopsies from PPR lesional skin (LS) and adjacent NLS were cultured ex vivo for 24 hours as indicated and then subjected to RNAseq of the tissue and quantitative proteomic analysis of the conditioned culture medium.
Slightly altered genes were identified in PPR skin versus NLS each sample. Among 35,681 unique transcripts, 92 significantly upregulated genes and 5 downregulated genes were confirmed by quantitative RT-PCR (Supplementary Figure S1). The raw RNAseq of the tissue led to an average of 17,045,022 mapped reads (range = 7,220,871–32,701,017 reads) out of 20,853,715 total reads (range = 8,668,865–40,760,939) for each sample. Among 35,681 unique transcripts, 92 significantly altered genes were identified in PPR skin versus NLS using a cutoff point of \( P \)-value < 0.01, adjusted for false discovery rate (adjusted \( P \)-value \( \leq 0.01 \)), with 87 upregulated genes and 5 downregulated genes (Supplementary Table S2a and b). A heatmap of these DEGs is shown in Figure 1b. Selected DEGs were confirmed by quantitative RT-PCR (Supplementary Figure S1). The raw RNAseq data have been deposited in the National Center for Biotechnology Information Gene Expression Omnibus (accession number GSE155141).

The PPR skin and NLS explants conditioned culture media were also subjected to two multiplex proteomic methods (OLINK, Olink Proteomics, Watertown, MA and Meso Scale Diagnostics, Rockville, MD) to quantify over a 100 inflammatory proteins \( n = 4 \), patient 1 media samples were not available). Of the 92 different proteins in the OLINK Inflammation Panel, 16 different proteins were found to have statistically significant differences between PPR skin and NLS explants \( (P_{\text{adj}} < 0.05) \) (Supplementary Table S3a). Of 30 inflammatory proteins assessed in the MSD Human Cytokine panel, five different proteins were found to have statistically significant differences between PPR skin and NLS explants \( (P_{\text{adj}} < 0.05) \) (Supplementary Table S3b). A heatmap of these differentially expressed proteins (DEPs) is shown in Figure 1c.

Selected DEGs and DEPs in PPR skin explant versus NLS explant are shown in Figure 2. Notable inflammatory-related DEGs (Figure 2a) included IL-1B \( (P_{\text{adj}} = 0.008) \) and members in TNF pathways, specifically FOS \( (P_{\text{adj}} = 0.005) \), JUN \( (P_{\text{adj}} = 0.001) \), and NFKBIA \( (P_{\text{adj}} = 0.003) \). In addition, there were several upregulated DEGs involved in the recruitment of immune cells, such as the chemokine CXCL5 \( (P_{\text{adj}} = 4.41 \times 10^{-4}) \), and adhesion molecules on endothelial cells, such as ICAM1 \( (P_{\text{adj}} = 0.002) \) and SELE \( (P_{\text{adj}} = 0.006) \), which regulate the tethering and rolling of neutrophils and monocytes (Morikis et al., 2017). TRPV3 was also significantly upregulated in the PPR skin explants \( (P_{\text{adj}} = 0.002) \), which may link to the heat and/or spice sensitivity of the patients and has also been previously shown to be upregulated in rosacea (Sulk et al., 2012).

Evaluation of the DEPs demonstrated a complex innate and adaptive inflammatory milieu in PPR skin explant versus NLS explant conditioned culture medium (Figure 2b and Supplementary Tables S3a and S2b). Several proteins in the gp130-utilizing cytokine family were significantly upregulated, such as IL-6 (1.6-fold, \( P_{\text{adj}} = 0.022 \)), LIF (2.4-fold, \( P_{\text{adj}} = 0.011 \)), and oncostatin M (OSM) (5-fold, \( P_{\text{adj}} = 0.005 \)). These three cytokines play key roles in stromal–immune cell crosstalk (West, 2019). Consistent with the RNAseq data, CXCL5 protein was significantly increased over 11-fold in the conditioned culture medium collected from PPR skin explants versus that collected from NLS explants \( (P_{\text{adj}} = 0.029) \). Although a specific role of this chemokine has not been
Previously described in PPR, CXCL5 has been associated with angiogenesis (Strieter et al., 2005) and UVB-induced sensitive skin (Dawes et al., 2011). Interestingly, angiogenesis might lead to telangiectasia, one of the major features of rosacea, and UV light is one of the common external triggers for rosacea.

Several other chemokines were also upregulated at the protein level in PPR skin versus NLS, including CCL20 (8.3-fold, \( P_{\text{adj}} = 0.003 \)), CCL8 (3.7-fold, \( P_{\text{adj}} = 0.014 \)), and CCL3 (9.8-fold, \( P_{\text{adj}} = 0.041 \)). Furthermore, we identified a complex adaptive inflammatory milieu in PPR, with statistically significant increases in both Th1-associated proteins (IFN-\( \gamma \) [3.2-fold, \( P_{\text{adj}} = 0.042 \)], IL-12p70 [3.4-fold, \( P_{\text{adj}} = 0.033 \]), PD-L1 [1.7-fold, \( P_{\text{adj}} = 0.043 \]), CXCL10 [14.3-fold, \( P_{\text{adj}} = 0.019 \]) and Th2-associated proteins (IL-13 and IL-5).

Kyoto Encyclopedia of Genes and Genomes pathway analysis of RNAseq data highlights MAPK and TNF signaling as differentially expressed biologic pathways in PPR skin explants

To determine whether there were common biologic pathways within this set of DEGs from the PPR skin versus NLS biopsies, the data were interrogated for biologic themes by the Kyoto Encyclopedia of Genes and Genomes pathway analysis. The pathway terms that were most significantly altered in PPR skin explants are shown, along with the genes comprising each term (Figure 3a). The most significant pathways include the MAPK signaling pathway (11 DEGs) (\( P_{\text{adj}} = 0.001 \)) and the TNF signaling pathway (6 DEGs) (\( P_{\text{adj}} = 0.042 \)).

The directionality of gene members in the MAPK and TNF signaling pathways were assessed using our dataset. The 20 most enriched genes in each term are shown by the heatmaps.
several of the DEPs identified in this study, including TGFβ (Shih et al., 2020) as well as in the cultured biopsies (Shih et al., 2020) as the genes encoding the 20 DEPs identified in the conditioned culture media and the DEGs in both the MAPK and TNF signaling pathways. Of note, IL-1β was found in both the MAPK signaling pathway and the TNF signaling pathway.

Comparison of our transcriptomic results with those from a previous study consisting of skin biopsies without tissue culture (an independent group of PPR patients, not the patients with PPR in this study) (Shih et al., 2020) further supported our findings. Both the TNF and MAPK signaling pathways were upregulated in uncultured, directly analyzed PPR LS versus NLS skin (Shih et al., 2020) (Supplementary Figure S4). Importantly, IL-1β was highly upregulated in uncultured biopsies (Shih et al., 2020) as well as in the cultured PPR LS explants in this study.

**Network analysis of genes and proteins altered in PPR highlights IL-1β as a central player**

Because the TNF and MAPK signaling pathways are the most enriched pathways identified in the DEGs from PPR skin explants using the Kyoto Encyclopedia of Genes and Genomes pathway analysis, we next assessed whether there were pathway connections with the DEPs previously identified as well. To do so, the 6 DEGs from the TNF signaling pathway or the 11 DEGs from the MAPK signaling pathway plus the genes encoding the 20 DEPs were probed by GeneMANIA Network analysis. The results of the combined DEGs (blue dots) and DEPs (pink dots) analysis are shown in Figure 4.

The predicted network demonstrated several physical interactions and shared pathways between the DEGs and the genes encoding the DEPs, supporting significant crosstalk between them. For example, gp130-utilizing cytokines (such as IL-6, LIF, and OSM) engage MAPK for signaling, and thus, the increase in protein expression of these cytokines may partially explain the highly significant enrichment of the MAPK signaling pathway in the PPR skin explants. Similarly, several of the DEPs in PPR skin explants are found within the TNF signaling pathway network, including CXCL5, IL-6, CCL20, and LIF.

The GeneMANIA Network analysis also provided predicted interactions (gray dots) on the basis of the input DEGs and DEPs. Although these predicted genes and proteins did not quite reach statistical significance, many of these predicted inflammatory genes and proteins were indeed also upregulated in the PPR lesional tissue, including CCL4, CCL7, CXCL8 (IL-8), and CXCL9 (Supplementary Figure S5). Finally, in both the MAPK and TNF signaling pathways, network analysis placed IL-1β centrally among the DEGs and DEPs, suggesting that this cytokine might play a key role in driving PPR pathology.

**Stimulation of NLS explants with IL-1β induces a papulopustular-like transcriptomic and proteomic profile**

Because the characterization of PPR skin explant versus NLS explant identified IL-1β as a potential central mediator to PPR pathology, we inquired whether the exposure of an NLS biopsy to exogenous IL-1β might result in a resemblance to PPR...
tissue. The experimental design is shown in Figure 5a. In brief, the NLS biopsy from a patient with PPR was exposed to 50 ng/ml of recombinant human IL-1β for 24 hours. After the 24-hour culture, the tissue was preserved for RNA isolation and subsequent RNAseq, and the conditioned cell medium was utilized for quantiative proteomics. A heatmap of (b) DEGs and (c) differently expressed inflammatory proteins are shown. Each row corresponds to a single gene or protein. Clustering on the x-axis is by Euclidean distance. Yellow signifies increased mean gene and/or protein expression, and blue indicates reduced mean gene and/or protein expression. DEG, differentially expressed gene; NLS, nonlesional skin; PPR, papulopustular rosacea; rhIL-1β, recombinant human IL-1β; RNAseq, RNA sequencing.

Figure 5. IL-1β stimulation of nonlesional tissue compared with PPR skin and NLS. (a) Schematic of experimental design. NLS biopsies cultured ex vivo were stimulated with 50 ng/ml of rhIL-1β for 24 hours and compared with PPR (rosacea) tissue also cultured for 24 hours ex vivo. (b, c) The biopsy tissue was utilized for RNAseq, and the conditioned cell medium was utilized for quantitative proteomics. A heatmap of (b) DEGs and (c) differently expressed inflammatory proteins are shown. Each row corresponds to a single gene or protein. Clustering on the x-axis is by Euclidean distance. Yellow signifies increased mean gene and/or protein expression, and blue indicates reduced mean gene and/or protein expression. DEG, differentially expressed gene; NLS, nonlesional skin; PPR, papulopustular rosacea; rhIL-1β, recombinant human IL-1β; RNAseq, RNA sequencing.

DISCUSSION
PPR is a chronic inflammatory skin disease resulting from an over-reactive immune response, altered vasculature, and skin sensitivity. However, beyond this triad of culprits, the details and drivers of PPR pathology are not well-understood. Although several studies have characterized specific mRNA expression by quantitative RT-PCR or microarray, to our knowledge, there have not been any previously reported paired, quantitative transcriptomic and proteomic evaluation of PPR skin and NLS tissue. Through this integrative transcriptomic and quantitative protein analysis, we identified the upregulation of IL-1β, MAPK, and TNF pathways in PPR skin explants and elevation of multiple inflammatory proteins such as LIF, OSM, IFN-γ, IL-5, IL-6, IL-12p70, IL-13, and chemokines, including CCL3, CCL8, CCL13, CCL20, CXCL5, CXCL10 in PPR conditioned culture medium.

The findings in our study are in alignment with many other previous studies. Upregulated genes found in our PPR skin explants, which have also been found by other groups from directly analyzed PPR biopsies, include TNFAIP2 (Sbidian et al., 2017; Shih et al., 2020), TRPV3 (Sulk et al., 2012), IL-1B (Shih et al., 2020), CXCL5, and ICAM1 (Buhl et al., 2015). In addition, the DEPs found in our PPR skin explant
conditioned culture medium highly corroborate previous transcriptomic analysis by others (Buhl et al., 2015; Dajnoki et al., 2017; Shih et al., 2020), including the upregulation of IFN-γ, IL-6, CCL20, CCL8, CCL3, and CXCL5, now providing quantitative proteomic confirmation of these previous results. The alignment of our transcriptomic and proteomic data with work by others highlights this model as a useful way to obtain samples for both RNA and protein analysis.

In addition, we identified the upregulation of IL-5 and IL-13 in PPR skin conditioned culture medium. IL-5 and IL-13 are type 2 cytokines that play important roles in the pathophysiology of allergic diseases. Although the elevation of these cytokines in PPR has not been previously reported, PPR has been associated with airborne allergies (Rainer et al., 2015). A recent publication identified IL-4L1 and IL-4R as DEGs in PPR LS tissue compared with NLS, suggesting that the type 2 axis may play a role in PPR pathology (Shih et al., 2020). TRPV3 was also found by us and others to be upregulated in PPR LS tissue; TRPV3 overactivation has been linked to Th2-mediated inflammation and itch, key hallmarks of atopic dermatitis-like inflammation (Wang and Wang, 2017; Yoshioka et al., 2009). We identified this Th2 pathway upregulation in conjunction with a Th1 signature (e.g., IL-12p70, IFN-γ, and CXCL10). Although these two pathways antagonize each other, coexpression of Th1 and Th2 signatures has been noted in other dermatological diseases such as chronic atopic dermatitis (Moyle et al., 2019). Only through more targeted anti-inflammatory treatments will the role of Th1 versus Th2 (or another immune axis) be elucidated for PPR.

The combined results of DEGs and DEPs analysis identified TNF and MAPK as the most significantly upregulated pathways in PPR skin explants. The upregulation of these pathways could be attributed to the enhanced expression of multiple inflammatory proteins identified in this study, including IL-1β, OSM, LIF, and IL-6. Interestingly, both MAPK and TNF signaling pathways have also been associated with ocular rosacea (Wladis et al., 2019, 2017), suggesting a shared mechanism between PPR and ocular rosacea. Furthermore, the TNF signaling pathway can also be triggered by the activation of toll-like receptor 2 with Demodex mites, a known external stimulus of rosacea (Lacey et al., 2018), and toll-like receptor upregulation has been previously described for rosacea (Buhl et al., 2015).
similar levels found in PPR skin explants. Although exogenous stimulation of NLS tissue does not capture the likely influx of immune cells in actual PPR, this finding does suggest that innate inflammation (such as IL-1β signaling) can elicit a transcriptomic and proteomic signature in NLS tissue–resident cells similar to those of PPR. This suggests that IL-1β may be a key driver in the transition of NLS skin to PPR skin.

In conclusion, this study has characterized the immune response in NLS explant versus PPR skin explant at both the transcriptomic and proteomic levels. The PPR skin explant model may also be applied to future studies on the development and evaluation of novel PPR treatments. These findings demonstrate that IL-1β, through possible involvement with both the MAPK and TNF signaling pathways, upregulates several genes and proteins associated with PPR LS. This suggests that IL-1β might be a critical mediator in the inflammation occurring in PPR.

**MATERIALS AND METHODS**

**Study subjects**

With Institutional Review Board approval and written informed consent, five participants with mild to moderate PPR were enrolled. Inclusion criteria included age ≥18 years and PPR diagnosed by at least one board-certified dermatologist. Exclusion criteria were pregnancy, lactating, use of topical treatments on the face within 2 weeks or systemic treatments within 4 weeks of enrollment, laser treatment or chemical peels to the face, history of abnormal scarring, use of oral antibiotics, patients on immunosuppressive therapy, and any skin or medical conditions that, in the judgment of the investigator, could obscure the results of the study.

**Tissue collection and culture conditions**

Biopsies from lesional sites and adjacent nonlesional sites were obtained by standard 4-mm Keyes punch technique from subjects with PPR. All samples were bisected and cultured at 37 °C in 5% carbon dioxide for 24 hours in 1 ml of cornification media (DMEM/F12 [Cat#D6421, Sigma-Aldrich, St. Louis, MO], 90 mM Adenine, 0.94 M calcium chloride, 10 nM Triiodothyronine, 1×-ITS [Cat#51500056, Gibco, New York, NY], 100× Antibiotic/Antimycotic [Cat#15240062, Gibco], 2% heat-inactivated fetal bovine serum [Cat#10082139, Gibco], 1× Glutamax [Cat#35050061, Gibco], 0.01 mg/ml Gentamicin [Cat#15750060, Gibco] in one well of a 24-well plate (Cat#10062-896, VWR International, Radnor, PA). For one half of the NLS biopsy, 50 ng/ml of IL-1β (Cat #BMS331, eBiosciences, San Diego, CA) was used to stimulate the explant for 24 hours. After 24 hours, the conditioned cell media from all samples were collected and stored at −80 °C until analysis. The biopsies were preserved in RNAlater (Cat# AM7022, Ambion, Austin, TX) for RNAseq. The concentration of IL-1β used and the selection of the 24-hour time point to assess changes in both RNA and protein expression after IL-1β stimulation was based on previous studies (including the transcriptomic work by Swindell et al. [2018]) and our own validation work for assaying proteins in conditioned cell media (available at https://data.mendeley.com/datasets/g3s7x5j49f/1). For abdominoplasty studies (performed as a separate study from this work from donor skin without dermatological disease), 8-mm biopsies were cultured using a transwell-mediated air–liquid interface with the same media described above. The biopsy was stimulated with 10 ng/ml of IL-1β, and conditioned cell media was assayed for induction of inflammatory proteins (MSD panel) after 48 hours of culturing. Data are available at https://data.mendeley.com/datasets/g3s7x5j49f/1.

**RNA library preparation and sequencing**

Total RNA from each biopsy sample was isolated with Trizol reagent (Thermo Fisher Scientific, Waltham, MA). RNA libraries were constructed using TruSeq Stranded Total RNA Library Prep Kit with Ribose-Zero (Illumina, San Diego, CA). HiSeq 4000 sequencing system (Illumina) led to 9–41 million of ×2, 75 base pair, paired-end reads per sample. The sequence data were mapped to human reference genome (hg38) using STAR, version 2.5.2b (Dobin et al., 2013). Read pairs aligning concordantly with the genome were counted according to the GENCODE annotation (version 27) using HTseq (version 0.6.0) (Anders et al., 2015).

**Differential gene expression analysis**

Genes with differential expression were called using the DESeq2 package (Love et al., 2014), comparing paired vehicle-treated PPR samples with NLS samples from the same individual and NLS with IL-1β stimulated NLS from the same individual. P-values were adjusted for false discovery rates and reported as \( P_{adj} \). Significantly DEGs and DEPs were normalized by mean and ordered by Euclidean distance, with results displayed in heat maps. For direct comparison with proteomic data, the FDR was readjusted for the 100 genes, which corresponded to the 100 protein products found in both proteomic panels. For comparison of results with uncultured PPR biopsies, a previously published dataset (Shih et al., 2020) was utilized (available at https://data.mendeley.com/datasets/4v3zh2nd972).

**Pathway and/or network analysis**

Significant biologic themes altered in PPR skin explants were identified using DEGs meeting the significance threshold of \( P_{adj} < 0.01 \) through the Kyoto Encyclopedia of Genes and Genomes pathway analysis using the Database for Annotation, Visualization, and Integrated Discovery pathway viewer (Huang et al., 2009). The network consisted of DEGs, and genes encoding DEPs were predicted by GeneMANIA (accessed on 24 April 2019 at http://genemania.org) (Montojo et al., 2010) using a query-dependent, automatically selected weighting method.

**Quantitative proteomic analysis**

A total of 40 μl of conditioned cell media was sent for quantitative proteomic analysis of 92 inflammatory proteins with Proximity Extension Assay (Olink INFLAMMATION, Olink proteomics). Another 150 μl of the conditioned cell media was used to evaluate the expression of 30 different inflammatory proteins in the V-plex Human Cytokine 30-Plex kit (K15054G, MesoScale Diagnostics, Rockville, MD). The assay was conducted per manufacturer’s instructions, and each sample was assayed as a technical duplicate. Media samples from subject 1 were not included owing to a technical issue (unclear labeling). P-values were determined by two-sided paired t-tests and adjusted for false discovery rates using the Benjamini–Hochberg method.

**Data availability statement**

Datasets related to this article can be found at Harden (2020), PPR IL1b manuscript data set, available at https://data.mendeley.com/datasets/g3s7x5j49f/1. The raw RNA sequencing data have been deposited in the National Center for Biotechnology Information Gene Expression Omnibus (accession number GSE155141).
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CONFLICT OF INTEREST
ALSC has been an advisory board member for Galderma, Merck, Regeneron, Jouance, and Pellepharm. She has also been a clinical investigator for studies funded by Merck, Regeneron, Pellepharm, Senhenta, Genentech, and Novartis, which are not relevant to the current study. She has received funding for the present study from Dermira. JLH, DR, and HH were employees of Dermira at the time of this study.

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AUTHOR CONTRIBUTIONS
Conceptualization: JLH; Data Curation: JLH, YHS, JR, RL; Formal Analysis: JLH, YHS, JR; Funding Acquisition: HH; Investigation: JLH, YHS, DR; Methodology: JLH; Project Administration: JLH, YHS, DR, HH, ALSC; Resources: JLH, YHS, JR, RL, HH, ALSC; Software: JLH, RR; Supervision: HH, ALSC; Validation: JLH, YHS; Visualization: JLH, YHS, ALSC; Writing—Original Draft Preparation: JLH, YHS; Writing—Review and Editing: JLH, YHS, DR, HH, ALSC.

SUPPLEMENTARY MATERIAL
Supplementary material is linked to the online version of the paper at www.jidonline.org, and at https://doi.org/10.1016/j.jid.2020.08.013.

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