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Interleukin-36 promotes systemic Type-I IFN responses in severe forms of psoriasis

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Short title: An IL-36/Type I IFN axis in psoriasis

ABSTRACT

Psoriasis is an immune-mediated skin disorder associated with severe systemic co-morbidities. While IL-36 is a key disease driver, the pathogenic role of this cytokine has mainly been investigated in skin. Thus, its effects on systemic immunity and extra-cutaneous disease manifestations remain poorly understood.

To address this issue, we investigated the consequences of excessive IL-36 activity in circulating immune cells. We initially focused our attention on generalised pustular psoriasis (GPP), a clinical variant associated with pervasive up-regulation of IL-36 signalling. By undertaking blood and neutrophil RNA-sequencing, we demonstrated that affected individuals display a prominent Type-I IFN signature, which correlates with abnormal IL-36 activity. We then validated the association between IL-36 de-regulation and Type-I IFN over-expression in patients with severe psoriasis vulgaris (PsV). We also found that the activation of Type-I IFN genes was associated with extra-cutaneous morbidity, in both GPP and PsV. Finally, we undertook mechanistic experiments, demonstrating that IL-36 acts directly on plasmacytoid dendritic cells (pDCs), where it potentiates Toll-like Receptor (TLR)-9 activation and IFN-α production. This effect was mediated by the up-regulation of PLSCR1, a phospholipid scramblase mediating endosomal TLR-9 translocation.

These findings identify an IL-36/Type-I IFN axis contributing to extra-cutaneous inflammation in psoriasis.

Key words:

Generalized pustular psoriasis, psoriasis vulgaris, systemic inflammation, IL-36, Type-I IFN, PLSCR1

Abbreviations: CAPS, cryopyrin associated periodic syndrome; DEG, differentially expressed genes; FC, fold change; GPP, generalised pustular psoriasis; IFN, interferon; IL-36, interleukin-36; IL36R, IL-36 receptor; *IL36RN*: IL-36 receptor antagonist; MAPK, mitogen-activated protein kinases; pDCs, plasmacytoid dendritic cells; *PLSCR1*, Phospholipid Scramblase 1; PsV: psoriasis vulgaris; TLR- 9: Toll-like receptor- 9

INTRODUCTION

Interleukin-36α, -β and -γ (hence IL-36) are group of IL-1 family cytokines that are mainly produced by keratinocytes, monocytes and dendritic cells (Bassoy et al., 2018). IL-36 signalling plays an important role in epithelial immune homeostasis and its de-regulation has been repeatedly implicated in the pathogenesis of psoriasis vulgaris (PsV), a common and chronic, immune-mediated skin disorder (Bassoy et al., 2018).

Numerous studies have shown that IL-36 responses are elevated in PsV skin (Mahil et al., 2017, Quaranta et al., 2014, Swindell et al., 2015) where they stimulate chemokine production and amplify the effects of IL-17 signalling (Mahil et al., 2017). Animal studies have also demonstrated that IL-36 promotes the activation of dendritic cells and the polarization of T lymphocytes into Th17 cells (Tortola et al., 2012). Thus, the mechanisms whereby IL-36 contributes to cutaneous inflammation have been extensively investigated. Its effects on circulating leukocytes, however, remain poorly understood.

We and others have shown that recessive mutations of the IL-36 receptor antagonist (*IL36RN*) are associated with generalised pustular psoriasis (GPP), a disease variant characterized by severe extra-cutaneous symptoms (Marrakchi et al., 2011, Onoufriadis et al., 2011). In fact, GPP patients suffer from flares of skin pustulation that are often accompanied by systemic upset (fever, elevation of acute phase reactants and neutrophilia) (Burden and Kirby, 2016). This suggests that IL-36 signalling is likely to influence immune responses beyond skin.

Extra-cutaneous co-morbidities are also well documented in PsV, as individuals suffering from severe disease are at high risk of psoriatic arthritis, metabolic syndrome and atherosclerosis (Burden and Kirby, 2016, Fang et al., 2016, Shah et al., 2017). It has therefore been proposed that PsV is a systemic disease, manifesting with skin, joint and vascular inflammation (Davidovici et al., 2010, Reich, 2012).

In this context, we hypothesise that abnormal IL-36 signalling has extra-cutaneous effects in both GPP and PsV, driving acute systemic flares in the former and contributing to a state of chronic systemic inflammation in the latter. To explore this model, we integrated the transcription profiling of patient leukocytes with ex-vivo IL-36 stimulations. We show that IL-36 potentiates Toll-like receptor (TLR)-9 activation and enhances the production of Type-I IFN, a cytokine that contributes to systemic immunity, arthritis and atherosclerosis.

RESULTS

Expression profiling identifies a Type-I IFN signature in GPP and PsV whole-blood samples

We reasoned that GPP would represent an ideal model in which to investigate the systemic effects of IL-36, given the well-established link with *IL36RN* mutations (Marrakchi et al., 2011, Onoufriadis et al., 2011) and enhanced IL-36 activity (Johnston et al., 2016). We therefore undertook whole-blood RNA-sequencing in 9 affected individuals and 7 healthy controls (Supplementary Table S1 a). While the deconvolution of transcription profiles showed that leukocyte frequencies were comparable in cases vs. controls (Supplementary Table S1 b), differential expression analysis identified 111 genes that were over-expressed (fold change≥1.5; FDR< 0.05) in patients (Figure 1 a, Supplementary Table S2 a). As expected, genes that can be induced by IL-36 (*IL1B, PI3, VNN2, TNFAIP6, SERPINB1*) were collectively up-regulated in cases vs. controls (P=0.019) (Figure 1 b). Of note, the analysis of a publicly available PsV dataset (Wang et al., 2014) identified a moderate, but statistically significant, over-expression of the same genes in patient whole-blood (P=0.001) (Figure 1 b), suggesting that IL-36 may have systemic effects in PsV.

To further explore the biological significance of our findings, we mapped the genes up-regulated in GPP to the blood co-expression modules described by Li et al (Li et al., 2014). We found that the over-expressed genes were significantly enriched among modules related to innate immune activation (e.g. *enriched in activated dendritic cells*, FDR<0.005) and antiviral responses (e.g. *type I IFN response*; FDR<0.05) (Figure 1 c). These findings were validated by Ingenuity Pathway Analysis (IPA), which identified *interferon signalling* as the most significantly enriched pathway (FDR<5×10^{-6}) (Figure 1 d). An upstream regulator analysis also highlighted IRF7, STAT1 and STAT3 as the transcriptional activators that are most strongly associated with gene over-expression (FDR<10^{-10} for all) (Figure 1 e, Supplementary Table S2 c). This is of interest since proteins are critical mediators of IFN signal transduction and IFN-α production by pDCs (Honda et al., 2005).

Finally, the analysis of two publicly available datasets (Liu et al., 2012, Rodero et al., 2017) demonstrated a significant overlap (P<10^{-10}) between the genes that are up-regulated in GPP and those that are over-expressed in autoinflammatory syndromes caused by abnormal activation of Type-I IFN

responses (Figure 1 f). Of note, no overlap was found with the up-regulated genes detected in cryopyrin associated periodic syndrome (CAPS), a disease caused by excessive IL-1 activity, which was analysed as a negative control (Supplementary Figure S1). Thus, the presence of a Type-I IFN signature in GPP leukocytes is supported by several lines of evidence.

To further investigate the relevance of these observations we built an interferon score by measuring the aggregate expression of 5 genes (*IFI6, IFIT3, IFITM3, OASL, PLSCR1*), which were up-regulated in the GPP dataset and annotated as Type-I IFN dependent in the interferome database (Rusinova et al., 2013). As expected, the score was elevated in GPP cases, compared to controls. A similar increase was observed in the publicly available PsV dataset (Figure 1 g). Importantly, we found that the interferon score documented in GPP and PsV significantly correlated with the up-regulation of IL-36 related genes ($P<0.01$) (Figure 1 h). Thus, we have shown that systemic Type-I IFN responses are abnormally active in psoriasis, which may be linked to increased IL-36 production.

The Type-I IFN signature is driven by gene up-regulation in neutrophils

The presence of heterogeneous cell populations in whole-blood can complicate the interpretation of transcription profiling experiments. We therefore sought to validate our results through an independent analysis of a single cell type. We focused our attention on neutrophils, as they play a critical role in systemic inflammation and can be activated by Type-I IFN (Zimmermann et al., 2016).

We obtained fresh blood samples from 8 GPP cases and 11 controls (Supplementary Table S1 a). Following neutrophil isolation and RNA-sequencing, we detected 200 up-regulated genes (Figure 2 a, Supplementary Table S2 b). The analysis of transcriptional networks identified *Type-I interferon response* as the most significantly enriched module (FDR<10^{-12}), followed by *innate antiviral response* and *antiviral interferon signature* (FDR<10^{-10}) (Figure 2 b). IPA also demonstrated a marked enrichment of pathways related to interferon signalling (FDR<10^{-11}) (Figure 2 c) and highlighted IRF7 and STAT1 as the most likely drivers of gene up-regulation (FDR<10^{-30}) (Figure 2 d, Supplementary Table S2 d). In keeping with these findings, interferon scores were elevated in GPP cases compared to controls ($P=0.02$) (Figure 2 e). These observations validate the results obtained in whole-blood and suggest that the Type-I IFN signature is driven at least in part, by gene up-regulation in neutrophils.

The Type-I IFN signature can be validated in extended PsV and GPP datasets

We next sought to validate the type I IFN signature through the analysis of further affected individuals. We examined neutrophils obtained from 17 GPP cases (including 8 newly recruited cases) and 16 PsV patients suffering from severe disease (average Psoriasis Area and Severity Index: 17.9). We also analysed two control groups including 9 individuals affected by CAPS and 26 healthy volunteers. Real-time PCR demonstrated that the interferon score was significantly increased in GPP and PsV cases compared to healthy controls ($P<0.005$). Conversely, and in keeping with the specificity of our observations, the scores of CAPS patients were within the normal range defined in unaffected individuals (Figure 3 a).

Of note, medical records showed that GPP patients with high IFN scores were more likely to experience systemic flares than those with low scores (88% vs 33%; $P=0.049$). Likewise, the prevalence of psoriatic arthritis was higher among PsV subjects with high IFN scores (67% vs 18%; $P=0.03$) (Figure 3 b).

Thus, the Type-I IFN signature detected by RNA-sequencing can be validated in independent PsV and GPP samples, where it is associated with extra-cutaneous morbidity.

The IL-36 receptor is expressed on the surface of plasmacytoid dendritic cells

We next hypothesised that IL-36 has a direct effect on Type-I IFN producing cells. To investigate this possibility, we systematically examined the surface expression of the IL-36 receptor (IL36R) in innate immune cells. In keeping with published findings (Foster et al., 2014), we found that IL36R was barely detectable on the surface of healthy neutrophils (Figure 4 a), suggesting that the effects of IL-36 on these cells are mediated by the activation of different immune population(s).

We also showed that $IL36R^+$ cell numbers were low among innate lymphoid cells (Figure 4 b) and in monocytes (Figure 4 c). Higher IL36R levels were observed in myeloid (mDC) and plasmacytoid dendritic cells (pDCs) (Figure 4 d, Supplementary Figure S2), with the largest percentage of $IL36R^+$ cells detected in the pDCs of GPP patients (Figure 4 e). Thus, we have shown that IL-36R is robustly

expressed in pDCs, which are the main producers of IFN-α (a member of the Type-I IFN family) in the immune system.

IL-36 potentiates IFN-α production in response to Toll-like receptor 9 stimulation

Based on the results obtained in the above experiments, we hypothesised that IL-36 potentiates Type-I IFN production by pDCs. To investigate this possibility, we pre-treated PBMCs obtained from healthy donors with IL-36 or vehicle. We then stimulated the cells with CpG-containing DNA (hence CpG), a TLR-9 ligand which induces IFN-α release by pDCs. Finally, we measured the up-regulation of the IFN signature genes as a readout of Type-I IFN production. While CpG increased the expression of most signature genes, its effect was more pronounced in cells that had been pre-incubated with IL-36 ($P<0.05$ for *IFIT3*, *OASL* and *PLSCR1*) (Figure 5 a). This observation was validated by direct measurements of IFN-α production, showing increased cytokine release following IL-36 pre-treatment (Figure 5 b). Finally, flow cytometry documented an increased proportion of $IFN\alpha^+$ pDCs among the cells that had been stimulated with IL-36 and CpG, compared to those that had been exposed to CpG alone (Figure 5 c). Thus, multiple experimental readouts support the notion that IL-36 up-regulates TLR-9 dependent IFN-α release.

IL-36 up-regulates *PLSCR1*, a known TLR-9 transporter

We next sought to define the mechanisms whereby IL-36 enhances cytokine production downstream of TLR-9. A closer inspection of the PBMC stimulation results showed that IL-36 treatment up-regulates *PLSCR1*, even in the absence of CpG. This is of interest, as the gene encodes phospholipid scramblase 1, a protein which regulates TLR-9 trafficking to the endosomal compartment (Talukder et al., 2012). To further explore the link between IL-36 and PLSCR1, we first validated our initial observation in additional donors (Figure 6 a). Next, we demonstrated that IL-36 treatment increases PLSCR1 protein levels in isolated pDCs, showing a direct effect of the cytokine on these cells ($P<0.05$) (Figure 6 b). Finally, we investigated the mechanism whereby IL-36 up-regulates *PLSCR1*. As expected for an IFN signature gene, an analysis of the *PLSCR1* promoter uncovered a STAT1 binding site. Given that IL-36 can signal through mitogen-activated protein kinases (MAPK) (Bassoy et al., 2018), and that there

have been reports of cross-talk between STAT1 and MAPK signalling (Zhang et al., 2004), we reasoned that the latter pathway was likely to be involved. Real-time PCR experiments confirmed this hypothesis, as the SB-203580 MAPK inhibitor abolished the effect of IL-36 on *PLSCR1* expression (Figure 6 c). Thus, we have demonstrated that IL-36 can act directly on pDCs, where it up-regulates PLSCR1, in a MAPK-dependent fashion.

DISCUSSION

While PsV has been historically described as a dermatological condition, the importance of extra-cutaneous co-morbidities is increasingly recognised (Armstrong et al., 2013). Of note, the prevalence of most co-morbid conditions increases with the severity and the duration of the disease (Burden and Kirby, 2016, Egeberg et al., 2017). There is therefore a dose-dependent association between cutaneous and extra-cutaneous inflammation, which suggests a shared systemic pathogenesis. The underlying pathways, however, remain poorly understood.

Here, we demonstrated that IL-36 signalling is enhanced in the leukocytes of PsV patients, where abnormal IL-36 activity correlates with Type-I IFN over-expression. While many of the genes that are induced by IL-36 are also up-regulated by IL-1, this set of shared targets does not include mediators of Type-I IFN production (Swindell et al., 2018). Accordingly, we found that IFN signature genes are not over-expressed in CAPS, a condition caused by excessive IL-1 signalling. Thus, IL-1 is unlikely to play a significant role in promoting Type-I IFN responses in psoriasis.

Several studies have found that Type-I IFN is a mediator of vascular inflammation, which promotes the recruitment of leukocytes to atherosclerotic plaques (Goossens et al., 2010, Niessner et al., 2007). Experiments carried out in animal models have also shown that TLR-9 dependent Type-I IFN production is a key driver of systemic autoimmunity (Di Domizio et al., 2012).

In keeping with these observations, signatures of excessive Type-I IFN activity have been documented in various diseases presenting with prominent systemic involvement. One notable example is systemic lupus erythematosus (SLE), a disorder associated with skin and joint inflammation, accelerated atherosclerosis and up-regulation of genes such as *IFI6* and *OASL* (El-Sherbiny et al., 2018). Of interest, three independent studies have reported that IL-36 serum levels correlate with disease activity in SLE

(Chu et al., 2015, Ismail et al., 2018, Mai et al., 2018), which further reinforces the link between IL-36 and Type-I IFN. Our work adds to these observations and provides mechanistic insights into the underlying pathways.

Our computational and experimental results implicate pDCs as the most likely mediators of IL-36 activity. First, we identified IRF7 as one of the most significant drivers of differential gene expression in GPP. Second, we demonstrated that IL-36R levels are highest in pDCs, especially among GPP patients. Of note, it has long been established that pDCs accumulate within psoriatic skin lesions, where they contribute to early disease processes alongside slanDC (Hansel et al., 2011, Nestle et al., 2005). It has also been reported that IL36R is abundantly expressed in various classes of skin-resident DC (Dietrich et al., 2016). Thus, it is tempting to speculate that IL-36 mediated pDC activation may also have a pathogenic role in skin.

Our results show that the effects of IL-36 on pDCs are mediated at least in part by PLSCR1 up-regulation. Interestingly, *PLSCR1* siRNA knockout inhibits Type I IFN production by human pDCs (Talukder et al., 2012), so it is reasonable to hypothesise that an increase in gene expression would have the opposite effect. While the PLSCR1 induction observed in our IL-36 stimulation experiments was modest (1.5-2.0 fold), it might be sufficient to activate a feed-forward loop whereby up-regulated PLSCR1 promotes the production of Type-I IFN, which in turn induces further *PLSCR1* transcription. In fact, self-amplifying loops are a key feature of Type-I IFN signalling, as they are required for robust antiviral responses (Hall and Rosen, 2010).

We cannot exclude the possibility that additional IL-36 responsive genes or cell types may also contribute to the up-regulation of Type-I IFN. However, we have found that IL-36 does not affect the expression of *TLR9* or that of key downstream genes (*IRF1*, *IRF3*, *IRF7*; data not shown). We have also observed that genes driving other antiviral pathways (*DDX58*/RIG-I, *IFIH1*/MDA5, *TMEM173*/STING) are not up-regulated in PsV or GPP whole-blood.

While our pDC stimulations were carried out with a synthetic TLR-9 agonist, the identity of the agents that cause IFN-α production in patients remains to be determined. In lesional skin, pDCs are activated by self-nucleic acids released by apoptotic keratinocytes and bound to the LL-37 antimicrobial peptide (Lande et al., 2007). Our transcriptomic data, however, suggests that this mechanism is unlikely to be

relevant at the systemic level. While *CAMP* (the gene encoding LL-37) was up-regulated in psoriatic skin, it was not over-expressed in GPP or PsV whole-blood. Moreover, there was no correlation between *CAMP* whole-blood expression and the up-regulation of Type-I IFN genes ($r<0.1$). Thus, the agents that activate the circulating pDC of psoriatic patients may be different from those that are present in skin.

In conclusion, we have identified an IL-36/TLR-9 axis which up-regulates systemic Type-I IFN production in psoriasis (Figure 6 d). In GPP patients, the effects of IL-36 signalling are amplified by inherited *IL36RN* mutations, a phenomenon which is likely to account for the severe nature of systemic flares. In PsV, the Th17-dependent up-regulation of IL-36 cytokines is associated with a less pronounced transcriptional signature and with signs of chronic systemic inflammation.

Given that IL-36 is down-regulated by IL-17 inhibitors such as secukinumab (Kolbinger et al., 2017), it is possible that treatment of psoriasis with IL-17 antagonists might also modulate Type-I IFN production. Of note, the effects of direct IL-36 inhibition are currently being investigated in clinical trials, with promising results obtained in a Phase I study (Bachelez, 2018). In this context, our work suggests that IL-36 antagonists have the potential to improve systemic Type I IFN up-regulation and extra-cutaneous manifestations of psoriasis.

METHODS

Human subjects

The study was performed according to the principles of the Declaration of Helsinki. Patients were ascertained at St John's Institute of Dermatology and Royal Free Hospital (London, UK), Glasgow Western Infirmary (Glasgow, UK), Salford Royal Foundation Trust (Manchester, UK) and Hospital Sultanah Aminah (Johor Bahru, Malaysia). The study was approved by the ethics committees of participating institutions and written informed consent was obtained from all participants.

Nine unrelated GPP patients and 7 healthy controls were recruited for whole-blood RNA-sequencing, while neutrophil RNA-sequencing was carried out in 8 GPP patients and 11 healthy controls. Five controls and six cases were common to both studies (Supplementary Table S1 a). For the validation of neutrophil RNA-sequencing results, fresh blood was obtained from 17 GPP, 26 control, 9 CAPS and 17 PsV individuals (Supplementary Table S3). All PsV patients suffered from moderate-to-severe

disease (Psoriasis Area Severity Index >10) and were recruited from the same centre (Severe psoriasis service, St John's Institute of Dermatology). Patients presenting with joint pain were referred to an expert rheumatologist, who diagnosed PsA, when applicable. The *IL36RN* gene was screened in all GPP cases and mutations were identified in 4 individuals (Supplementary Table S1 a).

RNA sequencing data analysis

The raw sequence data generated in house and that retrieved from public repositories (Supplementary Table S4) were processed with the same computational pipeline (described in supplementary methods), in order to standardise the data analysis process. Genes were considered up-regulated if the fold change exceeded 1.5 (FDR<0.05). When RNA-sequencing and microarray data were compared, the analysis focused on the 100 genes that were most significantly up-regulated in each sample, in order to account for the different sensitivity of the two platforms.

Genes up-regulated in GPP were used as input for pathway and upstream regulator enrichment analyses (IPA, Qiagen). STAT1- STAT3- and IRF7-centered networks were visualised with the igraph v1.0.1 R Package.

The transcriptional modules that were active in our datasets were selected from the library published by Li et al (Li et al., 2014). The enrichment_test function was then applied to the lists of up-regulated genes.

The interferon score was built using the five Type-I IFN dependent genes that were most up-regulated in GPP whole-blood (*PLSCR1, OALS, IFI6, IFIT3, IFITM3)*. As IL-36 dependent genes have not been systematically characterised in leukocytes, the IL-36 score was based on the analysis of five genes which were strongly induced by IL-36 in keratinocytes (Mahil et al., 2017) and robustly expressed in whole-blood (*IL1B, PI3, VNN2, TNFAIP6, SERPINB1)*. Both scores were derived by normalising RPKM values to a calibrator sample and then computing the median expression of the five genes.

Statistics

Differences between patient and control cytokine scores were assessed using an unpaired t-test or one-way ANOVA, as appropriate. To account for donor variability in cytokine responses, IL-36/CpG

stimulations were analysed with non-parametric methods (Wilcoxon signed-rank test for comparisons between two groups and Friedman's test for comparison between three groups), as these do not assume equal variance among samples. The correlation between cytokine scores was calculated using Spearman method. The significance of overlaps observed in Venn diagrams was computed with a hyper-geometric test and confirmed by bootstrap analysis. Fisher's exact test was used to compare the clinical features of patients with high and low IFN scores.

CREDIT STATEMENT: MC: data curation, formal analysis, investigation, visualization; MV: formal analysis, investigation, validation, visualization; MR: investigation; SKM, SEC, ADB, HSY, IMC, HJL: resources; GL: supervision; CHS: resources, writing-review & editing; FDC: supervision, writing-review & editing; JNB: funding acquisition, resources, writing-review & editing; FC: conceptualization, funding acquisition, project administration, supervision, writing-original draft.

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DATA AVAILABILITY

The RNA-sequencing data generated in this study are available through the Gene Expression Omnibus (identifier: GSE123787).

REFERENCES

Armstrong EJ, Harskamp CT, Armstrong AW. Psoriasis and major adverse cardiovascular events: a systematic review and meta-analysis of observational studies. Journal of the American Heart Association 2013;2(2):e000062.

Bachelez H. Efficacy and safety of BI 655130, an anti-interleukin-36 receptor antibody, in patients with acute generalised pustular psoriasis. 27th European Academy of Dermatology and Venereology (EADV) Congress Paris2018.

Bassoy EY, Towne JE, Gabay C. Regulation and function of interleukin-36 cytokines. Immunological reviews 2018;281(1):169-78.

Burden AD, Kirby B. Psoriasis and related disorders. In: Griffiths CEM, Barker JN, Bleiker T, Chalmers RJ, Creamer D, editors. Rook's Textbook of Dermatology. Chichester: Wiley-Blackwell; 2016.

Chu M, Wong CK, Cai Z, Dong J, Jiao D, Kam NW, et al. Elevated Expression and Pro-Inflammatory Activity of IL-36 in Patients with Systemic Lupus Erythematosus. Molecules 2015;20(10):19588-604.

Davidovici BB, Sattar N, Prinz J, Puig L, Emery P, Barker JN, et al. Psoriasis and systemic inflammatory diseases: potential mechanistic links between skin disease and co-morbid conditions. J Invest Dermatol 2010;130(7):1785-96.

Di Domizio J, Dorta-Estremera S, Gagea M, Ganguly D, Meller S, Li P, et al. Nucleic acid-containing amyloid fibrils potently induce type I interferon and stimulate systemic autoimmunity. Proc Natl Acad Sci U S A 2012;109(36):14550-5.

Dietrich D, Martin P, Flacher V, Sun Y, Jarrossay D, Brembilla N, et al. Interleukin-36 potently stimulates human M2 macrophages, Langerhans cells and keratinocytes to produce pro-inflammatory cytokines. Cytokine 2016;84:88-98.

Egeberg A, Skov L, Joshi AA, Mallbris L, Gislason GH, Wu JJ, et al. The relationship between duration of psoriasis, vascular inflammation, and cardiovascular events. J Am Acad Dermatol 2017;77(4):650-6 e3.

El-Sherbiny YM, Psarras A, Md Yusof MY, Hensor EMA, Tooze R, Doody G, et al. A novel two-score system for interferon status segregates autoimmune diseases and correlates with clinical features. Scientific reports 2018;8(1):5793.

Fang N, Jiang M, Fan Y. Association Between Psoriasis and Subclinical Atherosclerosis: A Meta-Analysis. Medicine 2016;95(20):e3576.

Foster AM, Baliwag J, Chen CS, Guzman AM, Stoll SW, Gudjonsson JE, et al. IL-36 promotes myeloid cell infiltration, activation, and inflammatory activity in skin. J Immunol 2014;192(12):6053-61.

Goossens P, Gijbels MJ, Zernecke A, Eijgelaar W, Vergouwe MN, van der Made I, et al. Myeloid type I interferon signaling promotes atherosclerosis by stimulating macrophage recruitment to lesions. Cell Metab 2010;12(2):142-53.

Hall JC, Rosen A. Type I interferons: crucial participants in disease amplification in autoimmunity. Nat Rev Rheumatol 2010;6(1):40-9.

Hansel A, Gunther C, Ingwersen J, Starke J, Schmitz M, Bachmann M, et al. Human slan (6-sulfo LacNAc) dendritic cells are inflammatory dermal dendritic cells in psoriasis and drive strong TH17/TH1 T-cell responses. The Journal of allergy and clinical immunology 2011;127(3):787-94 e1-9.

Honda K, Yanai H, Negishi H, Asagiri M, Sato M, Mizutani T, et al. IRF-7 is the master regulator of type-I interferon-dependent immune responses. Nature 2005;434(7034):772-7.

Ismail SM, Abd EMK, Mohamed MS. Serum Levels of Pentraxin3 and Interlukin36 in Patients with Systemic Lupus and their Relation to Disease Activity. The Egyptian journal of immunology 2018;25(1):81-91.

Johnston A, Xing X, Wolterink L, Barnes DH, Yin Z, Reingold L, et al. IL-1 and IL-36 are dominant cytokines in generalized pustular psoriasis. The Journal of allergy and clinical immunology 2016.

Kolbinger F, Loesche C, Valentin MA, Jiang X, Cheng Y, Jarvis P, et al. beta-Defensin 2 is a responsive biomarker of IL-17A-driven skin pathology in patients with psoriasis. The Journal of allergy and clinical immunology 2017;139(3):923-32 e8.

Lande R, Gregorio J, Facchinetti V, Chatterjee B, Wang YH, Homey B, et al. Plasmacytoid dendritic cells sense self-DNA coupled with antimicrobial peptide. Nature 2007;449(7162):564-9.

Li S, Rouphael N, Duraisingham S, Romero-Steiner S, Presnell S, Davis C, et al. Molecular signatures of antibody responses derived from a systems biology study of five human vaccines. Nat Immunol 2014;15(2):195-204.

Liu Y, Ramot Y, Torrelo A, Paller AS, Si N, Babay S, et al. Mutations in proteasome subunit beta type 8 cause chronic atypical neutrophilic dermatosis with lipodystrophy and elevated temperature with evidence of genetic and phenotypic heterogeneity. Arthritis Rheum 2012;64(3):895-907.

Mahil SK, Catapano M, Di Meglio P, Dand N, Ahlfors H, Carr IM, et al. An analysis of IL-36 signature genes and individuals with IL1RL2 knockout mutations validates IL-36 as a psoriasis therapeutic target Science translational medicine 2017;9:eaan2514.

Mai SZ, Li CJ, Xie XY, Xiong H, Xu M, Zeng FQ, et al. Increased serum IL-36alpha and IL-36gamma levels in patients with systemic lupus erythematosus: Association with disease activity and arthritis. International immunopharmacology 2018;58:103-8.

Marrakchi S, Guigue P, Renshaw BR, Puel A, Pei XY, Fraitag S, et al. Interleukin-36-receptor antagonist deficiency and generalized pustular psoriasis. N Engl J Med 2011;365(7):620-8.

Nestle FO, Conrad C, Tun-Kyi A, Homey B, Gombert M, Boyman O, et al. Plasmacytoid predendritic cells initiate psoriasis through interferon-alpha production. The Journal of experimental medicine 2005;202(1):135-43.

Niessner A, Shin MS, Pryshchep O, Goronzy JJ, Chaikof EL, Weyand CM. Synergistic proinflammatory effects of the antiviral cytokine interferon-alpha and Toll-like receptor 4 ligands in the atherosclerotic plaque. Circulation 2007;116(18):2043-52.

Onoufriadis A, Simpson MA, Pink AE, Di Meglio P, Smith CH, Pullabhatla V, et al. Mutations in IL36RN/IL1F5 are associated with the severe episodic inflammatory skin disease known as generalized pustular psoriasis. Am J Hum Genet 2011;89(3):432-7.

Quaranta M, Knapp B, Garzorz N, Mattii M, Pullabhatla V, Pennino D, et al. Intraindividual genome expression analysis reveals a specific molecular signature of psoriasis and eczema. Science translational medicine 2014;6(244):244ra90.

Reich K. The concept of psoriasis as a systemic inflammation: implications for disease management. Journal of the European Academy of Dermatology and Venereology : JEADV 2012;26 Suppl 2:3-11.

Rodero MP, Tesser A, Bartok E, Rice GI, Della Mina E, Depp M, et al. Type I interferon-mediated autoinflammation due to DNase II deficiency. Nature communications 2017;8(1):2176.

Rusinova I, Forster S, Yu S, Kannan A, Masse M, Cumming H, et al. Interferome v2.0: an updated database of annotated interferon-regulated genes. Nucleic acids research 2013;41(Database issue):D1040-6.

Shah K, Paris M, Mellars L, Changolkar A, Mease PJ. Real-world burden of comorbidities in US patients with psoriatic arthritis. RMD open 2017;3(2):e000588.

Swindell WR, Beamer MA, Sarkar MK, Loftus S, Fullmer J, Xing X, et al. RNA-Seq Analysis of IL-1B and IL-36 Responses in Epidermal Keratinocytes Identifies a Shared MyD88-Dependent Gene Signature. Frontiers in immunology 2018;9:80.

Swindell WR, Remmer HA, Sarkar MK, Xing X, Barnes DH, Wolterink L, et al. Proteogenomic analysis of psoriasis reveals discordant and concordant changes in mRNA and protein abundance. Genome medicine 2015;7(1):86.

Talukder AH, Bao M, Kim TW, Facchinetti V, Hanabuchi S, Bover L, et al. Phospholipid scramblase 1 regulates Toll-like receptor 9-mediated type I interferon production in plasmacytoid dendritic cells. Cell research 2012;22(7):1129-39.

Tortola L, Rosenwald E, Abel B, Blumberg H, Schafer M, Coyle AJ, et al. Psoriasiform dermatitis is driven by IL-36-mediated DC-keratinocyte crosstalk. The Journal of clinical investigation 2012;122(11):3965-76.

Wang CQF, Suarez-Farinas M, Nograles KE, Mimoso CA, Shrom D, Dow ER, et al. IL-17 induces inflammation-associated gene products in blood monocytes, and treatment with ixekizumab reduces their expression in psoriasis patient blood. J Invest Dermatol 2014;134(12):2990-3.

Zhang Y, Cho YY, Petersen BL, Zhu F, Dong Z. Evidence of STAT1 phosphorylation modulated by MAPKs, MEK1 and MSK1. Carcinogenesis 2004;25(7):1165-75.

Zimmermann M, Arruda-Silva F, Bianchetto-Aguilera F, Finotti G, Calzetti F, Scapini P, et al. IFNalpha enhances the production of IL-6 by human neutrophils activated via TLR8. Scientific reports 2016;6:19674.

FIGURE LEGENDS

Figure 1. Transcription profiling of GPP and PsV whole-blood uncovers a Type-I IFN signature that correlates with IL-36 activity. (**a**) Identification of genes that are differentially expressed in GPP. Horizontal and vertical lines represent significance and fold change thresholds, respectively. The genes underlying the IFN score are in red. (**b**) Higher expression of IL-36 dependent genes in whole-blood of GPP and PsV patients, compared to controls (CTR). (**c**) Transcriptional modules enriched among genes up-regulated in GPP. The FDR for each module is reported, with the underlying up-regulated genes shown as grey cells. (**d**) Enriched pathways detected among genes over-expressed in GPP. (**e**) Key transcriptional factors driving gene over-expression in GPP. (**f**) Overlap between the genes that are up-regulated in GPP and IFNpathies. (**g**) Elevated IFN score in whole-blood samples of GPP and PsV patients, compared to controls (CTR). (**h**) IL-36 and IFN scores are significantly correlated, in both GPP and PsV patients. Dashed regression lines are plotted with 95% confidence intervals (grey areas). The data in (b) and (g) are presented as mean +/- SD; **P*<0.05, ***P*<0.01 (unpaired t-test).

Figure 2. Transcription profiling of GPP neutrophils confirms the presence of a Type-I IFN signature (**a**) Identification of genes that are differentially expressed in GPP. Horizontal and vertical lines represent significance and fold change thresholds, respectively. (**b**) Transcriptional modules enriched among the genes that are up-regulated in GPP. The FDR for each module is reported, with the underlying up-regulated genes shown as grey cells. (**c**) Enriched pathways detected among the genes that are up-regulated in GPP. IFN-related pathways are highlighted in bold (**d**) Upstream regulator analysis showing that IRF7 and STAT1 drive the up-regulation of numerous genes that are over-expressed in GPP. (**e**) Elevated IFN score in the neutrophils of GPP patients, compared to controls. The data are presented as mean +/- SD; **P*<0.05 (unpaired t-test).

Figure 3. Validation of the Type-I IFN signature in extended datasets (**a**) Elevated IFN score in the neutrophils of GPP and PsV patients, compared to healthy individuals. CAPS cases were analysed as negative controls. The data are presented as mean +/- standard deviation; ***P*<0.01 and ****P*<0.001 (one-way ANOVA followed by Dunnett's post-test). (b) Left: systemic flares are more prevalent in

GPP patients with high interferon scores (n=8) compared to those with low interferon scores (n=9). Right: psoriatic arthritis (PsA) is more prevalent in PsV patients with high interferon scores (n=6) compared to those with low interferon scores (n=11). In both groups, the cut-off between high and low scores was defined as the median +2SD of the values observed in healthy controls. *P<0.05 (Fisher's exact test).

Figure 4. The IL-36 receptor is preferentially expressed by pDCs. (**a-e**) Representative flow cytometry plots showing IL36R surface expression, compared to fluorescence minus one (FMO) controls. (**a**) neutrophils (gated as $CD14^+$, $CD15^+$, $CD16^+$ cells); (**b**) innate lymphoid cells ($lineage^-$ ($CD3^-$, $CD4^-$, $CD19^-$, $CD20^-$, $CD56^-$), $CD127^+$); (**c**) monocytes ($CD3^-$, $CD20^-$, $CD19^-$, $CD56^-$) separated into classical ($CD16^-$, $CD14^{high}$), intermediate ($CD16^+$, $CD14^+$) and pro-inflammatory ($CD16^{high}$, $CD14^-$) populations; (**d**) pDCs ($lineage^-$, $HLADR^+$, $CD123^+$, $CD11c^-$) and mDCs ($lineage^-$, $HLADR^+$, $CD123^-$, $CD11c^+$). (**e**) Histogram showing the percentage $IL36R^+$ cells in each leukocyte population. Data were obtained in at least 3 GPP cases and 3 sex-matched controls. Results are presented as mean +/- SEM. No significant differences were observed between GPP cases and healthy donors.

Figure 5. IL-36 enhances the production of IFN-α downstream of Toll-like receptor 9. (**a**) PBMCs were stimulated with CpG for 6h, in the presence or absence of IL-36 pre-treatment (6h). The expression of interferon signature genes was measured by real-time PCR. Data represent the mean +/- SEM of results obtained in three independent donors, each stimulated in triplicate. (**b**) Following PBMC stimulation, IFN-α production was measured by ELISA. Data represent the mean +/- SEM of results obtained in two independent donors, each stimulated in triplicate. (**c**) Following PBMC stimulation, the percentage of $IFN\alpha^+$ pDCs was determined by flow cytometry. A representative set of plots is shown (left), together with the data obtained in 3 independent healthy donors (right). *P<0.05; **P<0.01 (Friedman's test, with Dunn's post-test).

Figure 6. IL-36 up-regulates PLSCR1 (**a**) Following treatment of PBMCs with IL-36, *PLSCR1* expression was measured by real-time PCR. (**b**) Following IL-36 treatment of pDCs, PLSCR1 mean fluorescence intensity (MFI) was measured by flow-cytometry, in gated PSLCR1^{+} pDCs. A representative histogram is shown on the left. (**c**) Following pre-treatment with SB203580 (MAPKi), PBMCs where stimulated with IL-36. *PLSCR1* expression was then determined by real-time PCR. (**d**) Proposed pathogenic model. Interleukin-36 produced by mDC up-regulates PLSCR1 in pDCs, potentiating TLR-9 dependent IFN-α release. IFN-α induces further *PLSCR1* transcription, thus propagating an inflammatory feed-forward loop. All data are shown as mean +/- SEM of results obtained in at least 3 donors, each stimulated in triplicate. **P*<0.05 (Wilcoxon signed-rank test (a, b) and Friedman's test with Dunn's post-test (c))