A single intradermal injection of IFN-γ induces an inflammatory state in both non-lesional psoriatic and healthy skin

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Abstract

Psoriasis is a chronic, debilitating, immune-mediated inflammatory skin disease. As IFN-γ is involved in many cellular processes, including activation of dendritic cells (DCs), antigen processing and presentation, cell adhesion and trafficking, and cytokine and chemokine production, IFN-γ-producing Th1 cells were proposed to be integral to the pathogenesis of psoriasis. Recently, IFN-γ was shown to enhance IL-23 and IL-1 production by DCs and subsequently induce Th17 cells, important contributors to the inflammatory cascade in psoriasis lesions. To determine if IFN-γ indeed induces the pathways expressed in psoriasis lesions, a single intradermal injection of IFN-γ was administered to an area of clinically normal, non-lesional skin of psoriasis patients and biopsies were collected 24 hours later. Although there were no visible changes in the skin, IFN-γ induced many molecular and histological features characteristic of psoriasis lesions. IfN-γ increased a number of differentially expressed genes in the skin, including many chemokines concomitant with an influx of T cells and inflammatory DCs. Furthermore, inflammatory DC products TNF, iNOS, IL-23, and TRAIL were present in IFN-γ-treated skin. Thus, IFN-γ, which is significantly elevated in non-lesional skin compared to healthy skin, appears to be a key pathogenic cytokine that can induce many features of the inflammatory cascade of psoriasis.

Introduction

Psoriasis is an inflammatory skin disease affecting approximately 2% of the population of the United States, and is considered one of the most prevalent cell-mediated inflammatory diseases (Lebwohl, 2003). Psoriasis lesions are typically erythematosus scaly plaques, and patients are classified based on the severity of their disease, with a gradient ranging from...
mild to moderate-to-severe based on body surface area (BSA) involved. Psoriatic lesions are characterized by increased infiltration of both activated T cells and dendritic cells (DCs) compared to normal or uninvolved (non-lesional, NL) psoriatic skin (Lowes et al., 2007). Through the use of biological agents and therapeutic treatments, many immune pathways involved in psoriasis are being elucidated (Nogales et al., 2010).

Historically, psoriasis was considered to be a predominantly type-1, IFN-γ-mediated disease, based on the significant IFN-γ genomic signature in lesional skin (Lew et al., 2004; Suarez-Farinas et al., 2010). Flow cytometric analysis revealed overexpression of IFN-γ in both circulating T cells and those isolated from lesional skin (Austin et al., 1999). In recent years, focus has shifted to the role of IL-17 and the contributions of Th17 cells to the pathogenesis of autoimmunity. Although IFN-γ inhibits the production of IL-17 by T cells in vitro (Harrington et al., 2005; Park et al., 2005), Th17 cells cultured with IL-12 are able to produce IFN-γ (Annunziato et al., 2007). The impact of the co-production of IL-17 and IFN-γ has recently been underscored in a murine model of diabetes where Th17 cells become more pathogenic once they acquired the ability to produce IFN-γ (Bending et al., 2009; Martin-Orozco et al., 2009). In human autoimmune disease, Th1 and Th17 cells often coexist (Aggarwal et al., 2003; Annunziato et al., 2007; Langrish et al., 2005; Murphy et al., 2003). Our group has identified a population of T cells that co-produce IFN-γ and IL-17 in psoriasis lesions (Lowes et al., 2008), induced by DCs isolated from the dermis of psoriatic lesions, but not by DCs from healthy skin (Zaba et al., 2009a). This difference in immunostimulatory potential has been attributed, in part to the presence of an additional population of CD11c+CD1c− inflammatory DCs that are found in psoriatic, but not healthy skin (Zaba et al., 2009a). In situ, inflammatory DCs express IL-23 and IL-12, which may potentially drive Th1/Th17 cell responses (Guttman-Yassky et al., 2008; Lowes et al., 2005). However, the initial stimulus for this cascade in situ is currently unknown. Recent studies have implicated IFN-γ as a possible contributor, as it can program myeloid DCs to produce IL-23 and IL-1, promoting IL-17 production by memory T cells (Kryczek et al., 2008). Thus, the contributions of IFN-γ to the pathogenesis of psoriasis are now being reevaluated.

Before the discovery that IFN-γ plays a role in autoimmunity, IFN-γ was used a therapy for several diseases including rheumatoid arthritis, psoriatic arthritis, psoriasis and lepromatous leprosy infections (Cannon et al., 1989; Fierlbeck and Rassner, 1990; Fierlbeck et al., 1990; Morhenn et al., 1987; Veys et al., 1997). Regular cutaneous injections of IFN-γ had some efficacy in the treatment of these diseases; however, a subset of patients developed focal skin reactions at the site of injection (Cannon et al., 1989; Fierlbeck and Rassner, 1990; Fierlbeck et al., 1990; Morhenn et al., 1987; Veys et al., 1997). Injection site reactions (often assessed several weeks after starting IFN-γ) were characterized by induration, erythema, epithelial thickening, increased expression of HLA-DR, and accumulation of T cells and monocytes in the dermis (Barker et al., 1989, 1990; Fierlbeck and Rassner, 1990; Fierlbeck et al., 1990; Morhenn et al., 1987; Nathan et al., 1990; Nathan et al., 1986). These studies suggested that prolonged exposure to IFN-γ might initiate changes in the skin that are consistent with a psoriatic phenotype, and thus IFN-γ may be involved in the initial development of psoriasis lesions.

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To address the role of IFN-γ in the induction of the inflammatory cascade in psoriasis and to identify the early underlying changes that occur before the development of a visible lesion, we analyzed responses to a single intradermal injection of IFN-γ at an early 24 hour time-point in psoriasis patients with mild disease (<10% BSA) and healthy volunteers. In psoriasis patients, IFN-γ injections were administered to an area of NL skin, which allowed the early genomic and histological IFN-γ responses to be determined. Although there were no visible changes in the skin, IFN-γ induced a genomic signature and cellular infiltration pattern that was similar to lesional psoriatic skin. Many inflammatory cytokines and chemokines were upregulated concurrent with increases in multiple dermal cell populations, including CD3+ T cells and CD11c+ DCs. Notably, inflammatory DCs (CD11c+CD1c−) were increased in the skin after IFN-γ injection, in addition to enhanced expression of their inflammatory products, TNF, iNOS, IL-23p19, and TRAIL. Furthermore, Ingenuity Pathway Analysis (IPA) identified pathways involved in lymphoid tissue development that were enriched in the transcriptome of the skin after IFN-γ injection. IFN-γ induced the lymphoid organizing chemokine, CCL19, and associated organization of T cell and DC clusters, suggesting that IFN-γ may induce lymphoid tissue neogenesis before the development of visible lesions. Thus, this in vivo study suggests that IFN-γ can prime an inflammatory environment in the skin that has many features of psoriasis.

Results

A single intradermal injection of IFN-γ was biologically active in the skin

In order to confirm that the injected IFN-γ was biologically active, we assessed human leukocyte antigen-DR (HLA-DR), a protein known to be upregulated by IFN-γ after intradermal injection (Nathan et al., 1990; Nathan et al., 1986). There were increased HLA-DR+ cells in both normal and NL skin after injection of IFN-γ, but not the placebo control, indicating the IFN-γ was functional (Fig S1). Clinically, a single injection of IFN-γ did not induce the visible development of a psoriasis-like lesion at the site of injection, as had been seen after a month of repeated IFN-γ injections (Fierlbeck and Rassner, 1990; Fierlbeck et al., 1990). Histologically, epidermal thickness was not altered by IFN-γ injection in either group (Fig S2a), and expression of keratin 16 (K16), a measure of altered keratinocyte differentiation, was not induced (Fig S2b–c). However, there were dramatic genomic and cellular changes induced by IFN-γ in both groups.

IFN-γ induced genomic changes in the skin consistent with a psoriatic phenotype

Microarray analysis was performed to compare the IFN-γ response in healthy and psoriatic skin using fold change (FCH) >2 and false discovery rate (FDR) <0.01. The “IFN-γ response” was defined as the differentially expressed genes (DEGs) in IFN-γ-treated skin compared to placebo for psoriasis patients and healthy individuals. Using this definition, 706 unique DEGs (964 probe-sets) were upregulated and 547 DEGs (708 probe-sets) were downregulated in healthy IFN-γ-treated skin. There were 775 upregulated DEGs (1055 probe-sets) and 719 downregulated DEGs (900 probe-sets) in IFN-γ-treated psoriatic skin (Table S1). Although the IFN-γ response (FCH) of many genes was higher in psoriasis than healthy skin, these differences were not statistically significant after correcting for multiple hypotheses with the Benjamini-Hochberg procedure. Additionally, there was very strong...

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correlation in the response to IFN-γ in both groups (r=0.96; Fig. S3), further supporting that there was no difference in IFN-γ-induced gene expression between healthy controls and psoriasis patients. Therefore, hereafter, we present data mostly from psoriatic NL skin.

IFN-γ induced the expression of numerous genes in the skin that are known to contribute to inflammation and that have been implicated in the pathogenesis of psoriasis. A selected list of inflammatory DEGs that are upregulated in psoriasis (Suarez-Farinas et al., 2010), as well as after IFN-γ injection, is presented in Table 1. This list included cytokines and chemokines, as well as other inflammatory products. Expression of select known IFNγ-induced genes (CXCL9, CXCL10, CCL2, and CX3CL1) was verified by real-time PCR (Fig. 1). Additional DEGs were confirmed by RT-PCR, and the IFN-γ response measured by RT-PCR was strongly correlated with the microarray data (Fig. S4). Furthermore, using gene set enrichment analysis (GSEA), four published psoriasis gene sets (Suarez-Farinas et al., 2010) correlated with the IFN-γ response in psoriasis patients (Table S2), indicating that IFN-γ can induce many genes that are present in psoriasis. Ingenuity Pathway Analysis (IPA) was also used to identify the statistically significant biological functions represented by these IFN-γ-induced DEGs (Table S2). The top functions represented by the upregulated DEGs after IFN-γ injection were consistent with pathways involved in psoriasis, including inflammatory response, inflammatory disease, cellular movement and development, immune cell trafficking, cell-mediated immune responses. Thus, the IFN-γ response in skin reflects an inflammatory molecular phenotype.

**IFN-γ induced T cells and inflammatory myeloid DCs infiltration into the skin**

Given the upregulation of many chemokines by IFN-γ, we evaluated the cellular infiltrates induced by IFN-γ in the skin. Previous studies have found an increased number of T cells in the skin 3 days after repeated IFN-γ injections (Nathan et al., 1990; Nathan et al., 1986). Here, we showed that even after only 24 hours post-IFN-γ injection, there were significantly increased CD3+ T cells in skin (Fig. S5).

Dendritic cells and their inflammatory products have also been implicated in the pathogenesis of psoriasis. Thus, we sought to determine if IFN-γ induced infiltration of DCs into skin. We have previously classified CD11c+CD1c+ DCs in normal skin as “resident” DCs. In psoriatic lesional skin, an additional population of inflammatory DCs, which express CD11c, but not CD1c, are present (Zaba et al., 2009a). IFN-γ induced greater numbers of CD11c+ DCs in the skin, while CD1c+ DCs remained constant throughout all samples analyzed (Fig. 2a–b). As there is no exclusive marker of inflammatory DCs, an approximation of their numbers can be calculated by subtracting the number of CD1c+ DCs from the number CD11c+ cells (Johnson-Huang et al., 2010). Fig. 2c shows significantly increased numbers of CD11c+CD1c− inflammatory DCs in both normal and non-lesional skin only after IFN-γ-injection. To further analyze these cells, we performed two-color immunofluorescence with antibodies against CD11c and CD1c. While the majority of CD11c+ cells in the placebo treated skin co-expressed CD1c (yellow cells), a large proportion of CD11c+ cells in IFN-γ-treated skin were CD1c negative (Fig. 2d), confirming the presence of inflammatory DCs. We have previously generated a list of DEGs specific for psoriatic inflammatory CD1c− DCs (Zaba et al., 2010). Using GSEA, the inflammatory...
CD1c− DC DEGs were highly enriched in the IFN-γ response genes (Table S2; ES=0.38, NES=1.8, p<0.0001, for both groups). Thus, IFN-γ can induce the accumulation of inflammatory DCs, both at the cellular and genomic levels, even in normal skin where they are typically absent.

**IFN-γ induced products of inflammatory myeloid DCs in the skin**

Inflammatory DCs in psoriasis have been previously shown to produce inflammatory mediators, including TNF, iNOS, IL-12/23p40 and IL-23p19 and express TNFSF10/TRAIL, a putative, new marker of inflammatory DCs (Guttman-Yassky et al., 2008; Lowes et al., 2005; Zaba et al., 2007; Zaba et al., 2010). In order to determine if the DCs induced by IFN-γ could be functioning as inflammatory DCs, we assessed the presence of these inflammatory products. Gene expression of TNFSF10/TRAIL is elevated by IFN-γ (Table 1) and similarly, immunofluorescent labeling identified many CD11c+ cells in the skin co-express TRAIL+ after IFN-γ injection (Fig. 3a). In comparison to baseline and placebo controls, which had minimal TNF and iNOS staining, co-expression of these mediators with CD11c+ DCs was increased in IFN-γ-treated skin (Fig. 3b–c). Additionally, using RT-PCR, we detected a significant increase in IL-23p19 mRNA expression in the skin after IFN-γ injection, and a trend towards increased IL-12p35 and IL-12/23p40 (Fig. 3d). These data are in line with a recent study that described a role of IFN-γ in priming DCs ex vivo to produce IL-23 (Kryczek et al., 2008). This in vitro priming stimulated IL-17 production by T cells, and thus we assessed expression of IL17A mRNA in the skin after IFN-γ injection by RT-PCR. Despite increased levels of IL-23 after IFN-γ injection, we did not find mRNA expression of IL17, or two IL-17-regulated genes CCL20 and DEFB4, in skin (data not shown). As biopsies were taken only 24 hours after injection of IFN-γ, there may not have been sufficient time to induce a Th17 cell response in NL skin. Taken together, these findings further suggest that IFN-γ primes an inflammatory environment.

**Molecules involved in ectopic lymphoid neogenesis were induced by IFN-γ**

During chronic inflammation, ectopic lymphoid tissue develops in peripheral non-lymphoid organs (Aloisi and Pujol-Borrell, 2006). Previously, based on analysis of upregulated DEGs, the existence of these structures within psoriasis LS skin has been proposed (Lew et al., 2004). Additionally, organized clusters of T cells and DCs are often seen in LS skin (Lowes et al., 2007). IPA biological function pathways of lymphoid tissue structure and development, hematological system development and function, tissue morphology, and hematopoiesis were significantly represented by the IFN-γ-induced genes (Table S2). Genes involved in ectopic lymphoid neogenesis were further investigated by RT-PCR and protein staining. Lymphotoxin-β (LTb), which is critical in development of lymphoid structures (Banks et al., 1995; De Togni et al., 1994; Futterer et al., 1998), was elevated by IFN-γ (Fig. 4a). Lymphoid tissue organizing chemokines, CXCL12/SDF-1, CXCL13, and CCL21 were also assessed, but were unchanged by IFN-γ (Fig. S4). IFN-γ did, however, increase expression of CXCR4, the receptor for CXCL12/SDF-1, as well as regulator of G protein signaling 16 (RGS16), which is involved in retention of CXCR4+ cells in sites of inflammation (Hsu et al., 2008). CCL19, an important factor involved in organization of T cells and DCs (Fukuyama et al., 2006), was significantly increased by IFN-γ (Fig. 4a).
Consistent with CCL19 upregulation, CD11c^+ DCs and CD3^+ T cells in IFN-γ injected skin were organized in clusters reminiscent of T cell zones of lymph nodes (Fig. 4b). The development of lymphoid tissue is also associated with high endothelial venules (HEVs) marked by peripheral node addressin (PNAd), and IFN-γ enhanced expression of PNAd in the skin (Fig. 4c). Finally, at both the mRNA (Table 1) and protein (Fig. 4d) levels, IFN-γ induced expression of ICAM-1 and VCAM-1, adhesion molecules found in secondary and tertiary lymphoid organs. Taken together, these data suggest that IFN-γ may indeed induce some facets of ectopic lymphoid neogenesis in the skin.

**IFN-γ as a biomarker of psoriasis disease potential and/or severity**

The molecular and histological responses to IFN-γ injection were similar between healthy and psoriatic skin. This finding suggested that the difference between psoriatic patients and controls might be upstream of the “response” to IFN-γ, potentially in the induction of IFN-γ, with psoriasis patients having a lower threshold for IFN-γ production than healthy controls. To address this question, IFN-γ mRNA expression was compared across NL and LS skin of patients with varying severities of psoriasis, from mild psoriasis (this study) to moderate-to-severe psoriasis, published previously (Johnson-Huang et al., 2010). The lowest level of IFN-γ expression was found in healthy skin, with an increasing gradient of IFN-γ mRNA expression as disease severity worsened. IFN-γ expression was significantly increased in NL skin from moderate-to-severe psoriasis patients compared to mild NL skin, and was highest in LS psoriatic skin (Fig. 5a). Moreover, an intradermal injection of IFN-γ into mild NL skin induced IFN-γ mRNA at a level between NL and LS skin of moderate-to-severe patients.

To evaluate if the level of IFN-γ mRNA in the skin correlated with a downstream genomic effect, we analyzed two lists of DEGs from comparison of NL versus healthy skin by IPA. The IFN signaling pathway was significantly represented (p=0.03) by DEGs in moderate-to-severe NL skin (Zaba et al., 2009b), but not by DEGs in mild NL skin compared to healthy skin. For comparison, the IFN signaling pathway was also significantly represented by the DEGs in IFN-γ-treated NL skin (p<10^-9). The range of IFN-γ expression and activation of downstream signaling molecules based on disease severity, suggests that IFN-γ may be a useful biomarker of psoriasis disease potential.

We also compared cell counts across the range of disease states, and showed that CD3^+ T cells and CD11c^+CD1c^- inflammatory DC followed a similar pattern, with an increase in cells as disease severity worsened (Fig. 5b). The patterns of increasing cellularity and IFN-γ mRNA expression with psoriasis disease progression were associated with an enrichment of psoriasis genes in the IFN-γ response DEGs (by GSEA). Thus, we sought to determine how the IFN-γ response genes compared to moderate-to-severe NL and LS psoriasis. Using unsupervised hierarchical clustering, we found that there were two main clusters of samples: 1) healthy skin samples clustered with NL skin and 2) IFN-γ-treated skin clustered with LS psoriasis skin (Fig. S6). Taken together, these data suggest that injection of IFN-γ may induce an inflammatory state with some features of LS psoriatic skin.
Discussion

Factors that induce the progression of psoriatic NL skin into LS skin and why some patients have more severe disease than others are still unclear. Here, we have analyzed the molecular and cellular changes that occur in the skin in response to IFN-γ, and found that a single intradermal injection of IFN-γ induced an inflammatory environment in both healthy and psoriatic skin. We showed upregulation of many chemokines and inflammatory cytokines, as well as the influx of inflammatory cells, most notably T cells and inflammatory DCs. Additionally, IFN-γ induced features of lymphoid tissue, including organization of T cell and DC clusters. At the genomic level, psoriasis gene sets (DEGs in LS skin compared to NL) (Suarez-Farinas et al., 2010) were highly enriched in the IFN-γ response genes, indicating a significant degree of overlap of these genes. Thus, the inflammatory response induced by IFN-γ in the skin resembled some of the changes that occur in psoriasis lesions.

The observation that the response to IFN-γ was similar in both NL psoriatic skin and healthy skin indicates that the pathogenic etiology in psoriasis is not in the “response” to IFN-γ per se, but may be further upstream. Conceivably, the threshold to produce IFN-γ may be different between healthy controls and psoriasis patients. Consistently, elevated IFN-γ expression has been found in NL psoriatic skin compared to healthy skin (Guttman-Yassky et al., 2008; Kryczek et al., 2008; Uyemura et al., 1993). Here, we also show that IFN-γ mRNA expression increased, even in NL skin, as psoriasis became more severe, suggesting that psoriasis patients have a greater propensity to produce IFN-γ than healthy controls. The increase in IFN-γ in psoriasis patients has been linked to polymorphisms in the IL4 and IL13 genes resulting in hypo-function of these IFN-γ-inhibitory cytokines (Elder, 2009).

Which cells contribute to the elevated expression of IFN-γ in NL compared to healthy skin is not known. Slightly increased numbers of activated CD4+ and CD8+ T cells have been found in NL skin (Baker et al., 1984; Placek et al., 1988), which may account for augmented IFN-γ. Here, we also show that as psoriasis progresses, the numbers of CD3+ T cells in NL skin increase, with the highest numbers being in LS skin (Fig. 5b), consistent with previous reports (Bos et al., 1983; Krueger, 2002). Additional cell types may also be contributing to the elevated IFN-γ. A significant proportion of mast cells in NL skin showed positive IFN-γ staining (Ackermann et al., 1999). Neutrophils have also been implicated as a source of IFN-γ (Ethuin et al., 2004), although they are rare in NL skin. Additionally, keratinocytes in NL skin express CD1d, which can stimulate CD161+ NK-T cells to produce large amounts of IFN-γ (Bonish et al., 2000). In another genomic study comparing NL skin to healthy skin, Gudjonsson, et al., showed that innate defense genes are elevated in NL skin, raising the possibility that psoriatic NL skin may be “pre-activated” (Gudjonsson et al., 2009). Despite these changes, not all NL skin evolves into LS skin, indicating that a secondary trigger, perhaps coupled with the presence of factors like IFN-γ, may induce lesion formation in psoriatic skin, but not healthy individuals which lack IFN-γ.

Previous studies using a skin organ culture system where NL psoriatic skin was cultured in vitro with IFN-γ showed that the epidermal changes induced by IFN-γ were partially mediated by IL-1β (Wei et al., 1999). In this study, although there was abundant DC infiltration and cytokine production (including IL-1β and IL-23), with concomitant T cell...
infiltration after IFN-γ injection, these changes were insufficient to induce full plaque development, possibly because there was no Th17 cell cytokine production. The fact that the epidermis was not altered by this regime of IFN-γ is consistent with lack of IL-17. The dose of IFN-γ may be insufficient for the induction of T cell responses or the early 24 hour time-point may not have allowed enough time to induce T cell activation and polarization and if biopsies were taken at later time-points, the T cell phenotype may have been altered. Along these lines, previous reports found that epidermal changes and visible lesions resulted after a month of continuous IFN-γ injections (Barker et al., 1989, 1990; Fierlbeck and Rassner, 1990; Fierlbeck et al., 1990; Morhenn et al., 1987; Nathan et al., 1990; Nathan et al., 1986), although the induction of IL-17 and IL-22 was not assessed in these studies.

In conclusion, the present study strengthens the hypothesis that IFN-γ is a crucial mediator of skin inflammation. Although, this study examines the effect of IFN-γ only in healthy and psoriasis patients, it may provide a framework for evaluating other diseases where IFN-γ is considered to play a major pathogenic role (Dardalhon et al., 2008).

Materials and Methods

Study design and skin biopsies

We conducted a research study under a Rockefeller University Institutional Review Board-approved protocol in which healthy volunteers and patients with mild psoriasis (n=10/group) received single intradermal injections of IFN-γ (Actimmune, 1×10^6 IU) and saline (placebo) in areas of skin that were clinically normal (NL skin). Saline was used as a control as the inactive components of the Actimmune solution were not available. After 24 hours, biopsies of both sites were taken; biopsies at baseline were also taken prior to injections. Each biopsy was cut in two: half was stored in OCT for cryosections, and half snap-frozen in liquid nitrogen for RNA extractions. Written informed consent was obtained and the study was performed in adherence with the Declaration of Helsinki Principles. NL and LS tissue biopsies (n=9 pairs) from moderate-to-severe psoriasis patients were obtained under a Rockefeller University Institutional Review Board-approved protocol (NCT ID: 00220025), which has been previously published (Johnson-Huang et al., 2010).

Immuo-staining

Skin sections were stained for immunohistochemistry and immunofluorescence as previously described (Fuentes-Duculan et al., 2010). All antibodies used for staining are listed in Table S3.

mRNA extraction and real-time PCR

RNA extraction and real-time PCR using Taqman gene expression assays (Table S4) were performed as previously described (Chamian et al., 2005). Custom primers for IFN-γ and IL-12/23p40 were generated as previously described (Chamian et al., 2005). Data normalized to hARP housekeeping gene were quantified by software provided with Applied Biosystems PRISM 7700 (Sequence Detection Systems, version 1.7). Normalized PCR data were log2 transformed before statistical analysis.
**Microarray hybridization**

Human Genome U133 2.0 arrays (Affymetrix Inc, Santa Clara, CA) were used. See Supplemental Materials and Methods for details.

**Statistical analysis**

For comparison of RT-PCR data and cell counts from IFN-γ-injected skin versus respective placebo-treated skin, a Wilcoxon matched pairs test was used. A Mann-Whitney U test was used to compare unpaired moderate-to-severe NL and LS to IFN-γ or placebo-treated skin. A p-value less than 0.05 was considered significant.

**Supplementary Material**

Refer to Web version on PubMed Central for supplementary material.

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**Abbreviations**

| Abbreviation | Description |
|--------------|-------------|
| DC           | Dendritic cell |
| BSA          | body surface area |
| NL           | non-lesional skin |
| LS           | lesional skin |
| FCH          | fold change |
| FDR          | false discovery rate |
| RT-PCR       | real-time polymerase chain reaction |
| IPA          | Ingenuity Pathway Analysis |
| HLA-DR       | human leukocyte antigen-DR |
| ICAM-1       | intracellular adhesion molecule-1 |
| K16          | keratin 16 |
| DEGs         | differentially expressed genes |
| GSEA         | gene set enrichment analysis |
| HEVs         | high endothelial venules |
| PNAd         | peripheral node addressin |
| LTb          | lymphotoxin-β |
| RGS16        | regulator of G protein signaling 16 |
| TRAIL        | TNF-related apoptosis-inducing ligand |
References

Ackermann L, Harvima IT, Pelkonen J, Ritamäki-Salo V, Naukkarinen A, Harvima RJ, et al. Mast cells in psoriatic skin are strongly positive for interferon-gamma. Br J Dermatol. 1999; 140:624–33. [PubMed: 10233311]

Aggarwal S, Ghilardi N, Xie MH, de Sauvage FJ, Gurney AL. Interleukin-23 promotes a distinct CD4 T cell activation state characterized by the production of interleukin-17. J Biol Chem. 2003; 278:1910–4. [PubMed: 12417590]

Aloisi F, Pujol-Borrell R. Lymphoid neogenesis in chronic inflammatory diseases. Nat Rev Immunol. 2006; 6:205–17. [PubMed: 16498451]

Annunziato F, Cosmi L, Santarlasci V, Maggi L, Liotta F, Mazingh B, et al. Phenotypic and functional features of human Th17 cells. J Exp Med. 2007; 204:1849–61. [PubMed: 17635957]

Austin LM, Ozawa M, Kikuchi T, Walters IB, Krueger J, et al. The majority of epidermal T cells in Psoriasis vulgaris lesions can produce type 1 cytokines, interferon-gamma, interleukin-2, and tumor necrosis factor-alpha, defining TC1 (cytotoxic T lymphocyte) and TH1 effector populations: a type 1 differentiation bias is also measured in circulating blood T cells in psoriatic patients. J Invest Dermatol. 1999; 113:752–9. [PubMed: 10571730]

Baker BS, Swain AF, Fry L, Valdimarsson H. Epidermal T lymphocytes and HLA-DR expression in psoriasis. Br J Dermatol. 1984; 110:555–64. [PubMed: 6232938]

Banks TA, Rouse BT, Kerley MK, Blair PJ, Godfrey VL, Kuklin NA, et al. Lymphotoxin-alpha-deficient mice. Effects on secondary lymphoid organ development and humoral immune responsiveness. J Immunol. 1995; 155:1685–93. [PubMed: 7636227]

Barker J, Allen MH, MacDonald DM. The effect of in vivo interferon-gamma on the distribution of LFA-1 and ICAM-1 in normal human skin. J Invest Dermatol. 1989; 93:439–42. [PubMed: 2476508]

Barker J, Allen MH, MacDonald DM. Alterations induced in normal human skin by in vivo interferon-gamma. Br J Dermatol. 1990; 122:451–8. [PubMed: 2140046]

Bender D, De La Pena H, Veldhoen M, Phillips JM, Uyttenhove C, Stockinger B, et al. Highly purified Th17 cells from BDC2.5NOD mice convert into Th1-like cells in NOD/SCID recipient mice. J Clin Invest. 2009; 119:565–72. [PubMed: 19188681]

Bonish B, Jullien D, Dutronc Y, Huang BB, Modlin R, Spada FM, et al. Overexpression of CD1d by keratinocytes in psoriasis and CD1d-dependent IFN-gamma production by NK-T cells. J Immunol. 2000; 165:4076–85. [PubMed: 11034419]

Bos JD, Hulsebosch HJ, Krieg SR, Bakker PM, Cormane RH. Immunocompetent cells in psoriasis. In situ immunophenotyping by monoclonal antibodies. Arch Dermatol Res. 1983; 275:181–9. [PubMed: 6604503]

Cannon GW, Pincus SH, Emkey RD, Denes A, Cohen SA, Wolfe F, et al. Double-blind trial of recombinant gamma-interferon versus placebo in the treatment of rheumatoid arthritis. Arthritis Rheum. 1989; 32:964–73. [PubMed: 25042610]

Chamian F, Lowes MA, Lin SL, Lee E, Kikuchi T, Gilleaudeau P, et al. Alefacept reduces infiltrating T cells, activated dendritic cells, and inflammatory genes in psoriasis vulgaris. Proc Natl Acad Sci U S A. 2005; 102:2075–80. [PubMed: 15671179]

Dardalhon V, Korn T, Kuchroo VK, Anderson AC. Role of Th1 and Th17 cells in organ-specific autoimmunity. J Autoimmun. 2008; 31:252–6. [PubMed: 18502610]

De Togni P, Goellner J, Ruddle NH, Streeter PR, Fick A, Mariathasan S, et al. Abnormal development of peripheral lymphoid organs in mice deficient in lymphotoxin. Science. 1994; 264:703–7. [PubMed: 8171322]

Elder JT. Genome-wide association scan yields new insights into the immunopathogenesis of psoriasis. Genes and immunity. 2009; 10:201–9. [PubMed: 19262574]

Etherum F, Gerard B, Benna JE, Boutten A, Gougerot-Pocidalo MA, Jacob L, et al. Human neutrophils produce interferon gamma upon stimulation by interleukin-12. Laboratory investigation; a journal of technical methods and pathology. 2004; 84:1363–71.

Fierlbeck G, Rassner G. Treatment of psoriasis and psoriatic arthritis with interferon gamma. J Invest Dermatol. 1990; 95:138S–41S. [PubMed: 2124243]
Fierlbeck G, Rassner G, Muller C. Psoriasis induced at the injection site of recombinant interferon gamma. Results of immunohistologic investigations. Arch Dermatol. 1990; 126:351–5. [PubMed: 2106838]

Fuentes-Duculan J, Suarez-Farinas M, Zaba LC, Nograles KE, Pierson KC, Mitsui H, et al. A subpopulation of CD163-positive macrophages is classically activated in psoriasis. J Invest Dermatol. 2010; 130:2412–22. [PubMed: 20555352]

Fukuyama S, Nagatake T, Kim DY, Takamura K, Park EJ, Kaiso T, et al. Cutting edge: Uniqueness of lymphoid chemokine requirement for the initiation and maturation of nasopharynx-associated lymphoid tissue organogenesis. J Immunol. 2006; 177:4276–80. [PubMed: 16982861]

Futterer A, Mink K, Luz A, Kosco-Vilbois MH, Pfeffer K. The lymphotixin beta receptor controls organogenesis and affinity maturation in peripheral lymphoid tissues. Immunity. 1998; 9:59–70. [PubMed: 9697836]

Gudjonsson JE, Ding J, Li X, Nair RP, Tejasvi T, Qin ZS, et al. Global gene expression analysis reveals evidence for decreased lipid biosynthesis and increased innate immunity in uninvolved psoriatic skin. J Invest Dermatol. 2009; 129:2795–804. [PubMed: 19571819]

Guttman-Yassky E, Lowes MA, Fuentes-Duculan J, Zaba LC, Cardinale I, Nograles KE, et al. Low expression of the IL-23/Th17 pathway in atopic dermatitis compared to psoriasis. J Immunol. 2008; 181:7420–7. [PubMed: 18981165]

Harrington LE, Hatton RD, Mangan PR, Turner H, Murphy TL, Murphy KM, et al. Interleukin 17-producing CD4+ effector T cells develop via a lineage distinct from the T helper type 1 and 2 lineages. Nat Immunol. 2005; 6:1123–32. [PubMed: 16200070]

Hsu HC, Yang P, Wang J, Wu Q, Myers R, Chen J, et al. Interleukin 17-producing T helper cells and interleukin 17 orchestrate autoreactive germinal center development in autoimmune BXD2 mice. Nat Immunol. 2008; 9:166–75. [PubMed: 18157131]

Johnson-Huang LM, Suarez-Fariñas M, Sullivan-Whalen M, Gilleaudeau P, Krueger JG, Lowes MA. Effective narrow-band UVB radiation therapy suppresses the IL-23/IL-17 axis in normalized psoriasis plaques. J Invest Dermatol. 2010; 130:2654–63. [PubMed: 20553551]

Krueger JG. The immunologic basis for the treatment of psoriasis with new biologic agents. J Am Acad Dermatol. 2002; 46:1–23, quiz -6. [PubMed: 11756941]

Kryczek I, Bruce AT, Gudjonsson JE, Johnston A, Aphale A, Vatan L, et al. Induction of IL-17+ T cell trafficking and development by IFN-gamma: mechanism and pathological relevance in psoriasis. J Immunol. 2008; 181:4733–41. [PubMed: 18802076]

Langrish CL, Chen Y, Blumenschein WM, Mattson J, Basham B, Sedgwick JD, et al. IL-23 drives a pathogenic T cell population that induces autoimmune inflammation. J Exp Med. 2005; 201:233–40. [PubMed: 15657292]

Lebwohl M. Psoriasis. Lancet. 2003; 361:1197–204. [PubMed: 12686053]

LeW W, Bowcock AM, Krueger JG. Psoriasis vulgaris: cutaneous lymphoid tissue supports T-cell activation and ‘Type 1’ inflammatory gene expression. Trends Immunol. 2004; 25:295–305. [PubMed: 15145319]

Lowes M, Bowcock AM, Krueger J. Pathogenesis and therapy of psoriasis. Nature. 2007; 445:866–73. [PubMed: 17314973]

Lowes MA, Chaimian F, Abello MV, Fuentes-Duculan J, Lin SL, Nussbaum R, et al. Increase in TNF-alpha and inducible nitric oxide synthase-expressing dendritic cells in psoriasis and reduction with efalizumab (anti-CD11a). Proc Natl Acad Sci U S A. 2005; 102:19057–62. [PubMed: 16380428]

Lowes MA, Kikuchi T, Fuentes-Duculan J, Cardinale I, Zaba LC, Haider AS, et al. Psoriasis vulgaris lesions contain discrete populations of Th1 and Th17 T cells. J Invest Dermatol. 2008; 128:1207–11. [PubMed: 18200064]

Martin-Orozco N, Chung Y, Chang SH, Wang YH, Dong C. Th17 cells promote pancreatic inflammation but only induce diabetes efficiently in lymphopenic hosts after conversion into Th1 cells. Eur J Immunol. 2009; 39:216–24. [PubMed: 19130584]

Morhenn VB, Pregerson-Rodan K, Mullen RH, Wood GS, Nickoloff BJ, Sherwin SA, et al. Use of recombinant interferon gamma administered intramuscularly for the treatment of psoriasis. Arch Dermatol. 1987; 123:1633–7. [PubMed: 3120650]
Murphy CA, Langrish CL, Chen Y, Blumenschein W, McClanahan T, Kastelein RA, et al. Divergent pro- and antiinflammatory roles for IL-23 and IL-12 in joint autoimmune inflammation. J Exp Med. 2003; 198:1951–7. [PubMed: 14662908]

Nathan C, Squires K, Griffo W, Levis W, Varghese M, Job CK, et al. Widespread intradermal accumulation of mononuclear leukocytes in lepromatous leprosy patients treated systemically with recombinant interferon gamma. J Exp Med. 1990; 172:1509–12. [PubMed: 2121891]

Nathan CF, Kaplan G, Levis WR, Nusrat A, Wittner MD, Sherwin SA, et al. Local and systemic effects of intradermal recombinant interferon-gamma in patients with lepromatous leprosy. N Engl J Med. 1986; 315:6–15. [PubMed: 3086725]

Nogales KE, Davidovic B, Krueger JG. New insights in the immunologic basis of psoriasis. Semin Cutan Med Surg. 2010; 29:3–9. [PubMed: 20430301]

Park H, Li Z, Yang XO, Chang SH, Nurieva R, Wang YH, et al. A distinct lineage of CD4 T cells regulates tissue inflammation by producing interleukin 17. Nat Immunol. 2005; 6:1133–41. [PubMed: 16200068]

Placek W, Haftek M, Thivolet J. Sequence of changes in psoriatic epidermis. Immunocompetent cell redistribution precedes altered expression of keratinocyte differentiation markers. Acta dermato-venereologica. 1988; 68:369–77. [PubMed: 2461018]

Suarez-Farinas M, Lowes MA, Zaba LC, Krueger JG. Evaluation of the psoriasis transcriptome across different studies by gene set enrichment analysis (GSEA). PLoS One. 2010; 5:e10247. [PubMed: 20422035]

Uyemura K, Yamamura M, Fivenson DF, Modlin RL, Nickoloff BJ. The cytokine network in lesional and lesion-free psoriatic skin is characterized by a T-helper type 1 cell-mediated response. J Invest Dermatol. 1993; 101:701–5. [PubMed: 7693825]

Veys EM, Menkes CJ, Emery P. A randomized, double-blind study comparing twenty-four-week treatment with recombinant interferon-gamma versus placebo in the treatment of rheumatoid arthritis. Arthritis Rheum. 1997; 40:42–8. [PubMed: 9008601]

Wei L, Debets R, Hegmans JJ, Benner R, Preus EP. IL-1 beta and IFN-gamma induce the regenerative epidermal phenotype of psoriasis in the transwell skin organ culture system. IFN-gamma up-regulates the expression of keratin 17 and keratinocyte transglutaminase via endogenous IL-1 production. The Journal of pathology. 1999; 187:358–64. [PubMed: 10398092]

Zaba L, Cardinale I, Gilleaudeau P, Sullivan-Whalen M, Suárez-Fariñas M, Fuentes-Duculan J, et al. Amelioration of epidermal hyperplasia by TNF inhibition is associated with reduced Th17 responses. J Exp Med. 2007; 204:3183–94. [PubMed: 18039949]

Zaba LC, Fuentes-Duculan J, Eungdamrong NJ, Abello MV, Novitskaya I, Pierson KC, et al. Psoriasis is characterized by accumulation of immunostimulatory and Th1/Th17 cell-polarizing myeloid dendritic cells. J Invest Dermatol. 2009a; 129:79–88. [PubMed: 18633443]

Zaba LC, Fuentes-Duculan J, Eungdamrong NJ, Johnson-Huang LM, Nogales KE, White TR, et al. Identification of TNF-related apoptosis-inducing ligand and other molecules that distinguish inflammatory from resident dendritic cells in patients with psoriasis. J Allergy Clin Immunol. 2010; 125:1261–8. e9. [PubMed: 20471070]

Zaba LC, Suarez-Farinas M, Fuentes-Duculan J, Nogales KE, Gutman-Yassky E, Cardinale I, et al. Effective treatment of psoriasis with etanercept is linked to suppression of IL-17 signaling, not immediate response TNF genes. J Allergy Clin Immunol. 2009b; 124:1022–10. e1–395. [PubMed: 19895991]

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Fig. 1. IFN-γ injection induces genomic expression of known IFN-γ-regulated chemokines mRNA expression levels normalized to hARP for the T cell (CXCL9 and CXCL10) and DC (CCL2 and CX3CL1) chemokines in baseline, placebo and IFN-γ-injected healthy (black bars, n=10) and psoriatic skin (white bars, n=10). Error bars represent the mean ± SEM. IFN-γ-injected skin was compared to placebo. (**) P<0.01.
Fig. 2. IFN-γ injection induces dermal inflammatory myeloid DCs
Representative immunohistochemistry of (a) CD11c+ and (b) CD1c+ cells in psoriasis skin.
(c) Quantification of CD11c+, CD1c+, and inflammatory DCs (CD11c+ minus CD1c+ cells) in healthy (black circles) and psoriasis skin (white circles). Each circle represents a different patient. IFN-γ-injected skin is compared to placebo. (***) P<0.001. (d) Two-color immunofluorescence of CD1c+ DCs (red) and CD11c+ DCs (green). The white line delineates the dermal epidermal junction. Scale bar = 100μm.
Fig. 3. IFN-γ injection induces expression of known products of inflammatory myeloid DCs (a–c) Two-color immunofluorescence of CD11c+ DCs with inflammatory products: (a) TRAIL, (b) TNF, and (c) iNOS in baseline, placebo and IFN-γ-injected psoriasis skin. The white line delineates the dermal epidermal junction. Scale bar = 100μm. (d) mRNA expression levels normalized to hARP for additional DC cytokines in healthy (black bars, n=10) and psoriatic skin (white bars, n=10). Error bars represent the mean ± SEM. IFN-γ-injected skin is compared to placebo. (*) P<0.05, (**) P<0.01.
Fig. 4. IFN-γ injection induces lymphoid-like structures and expression of known lymphoid genes and proteins
(a) mRNA expression levels normalized to hARP for the lymphoid genes in healthy (black bars, n=10) and psoriatic skin (white bars, n=10). Error bars represent the mean ± SEM. IFN-γ-injected skin is compared to placebo. (**) P<0.01, (***) P<0.001. (b) Two-color immunofluorescence of CD11c+ DCs (red) and CD3+ T cells (green), in psoriasis skin. White arrows denote DC/T cell clusters. The white line delineates the dermal epidermal junction. (c–d) Representative immunohistochemistry of (c) peripheral node addressin (PNAd) and (d) ICAM-1 in psoriasis skin. Scale bar = 100μm.
Fig. 5. The gradient of IFN-γ mRNA expression parallels T cell and DC infiltration as psoriasis becomes more severe

(a) IFN-γ mRNA expression in healthy (n=10), NL skin from mild (n=10, BSA<10%) versus moderate-to-severe (n=9, BSA>10%) psoriasis patients, IFN-γ-injected healthy (n=10) or mild NL psoriasis (n=10) skin and psoriasis LS (n=9) skin. Error bars represent the mean ± SEM. (**) P<0.01. (***) P<0.001. (b) Quantification of CD3+ T cells (blue bars) and CD11c+CD1c− inflammatory DCs (red bars) counts per mm of skin in patients in (a).
### Table 1

Microarray fold change values of select inflammatory genes upregulated in skin after IFN-\(\gamma\) injection compared to placebo.

| Symbol | Description | IFN-\(\gamma\) Response (Fold change)* |
|--------|-------------|-------------------------------------|
|        |             | Psoriasis | Healthy                |
| CXCL11 | chemokine (C-X-C motif) ligand 11          | 161.89     | 57.09                  |
| CXCL9  | chemokine (C-X-C motif) ligand 9          | 128.78     | 29.87                  |
| CCL8   | chemokine (C-C motif) ligand 8            | 126.85     | 41.36                  |
| CXCL10 | chemokine (C-X-C motif) ligand 10         | 71.38      | 25.86                  |
| CCR1   | chemokine (C-C motif) receptor 1          | 23.10      | 7.78                   |
| CX3CL1 | chemokine (C-X3-C motif) ligand 1         | 19.81      | 12.18                  |
| CCL2   | chemokine (C-C motif) ligand 2            | 12.00      | 7.09                   |
| CCR2   | chemokine (C-C motif) receptor 2          | 9.54       | 4.23                   |
| CCL5   | chemokine (C-C motif) ligand 5            | 8.16       | 5.47                   |
| CCL13  | chemokine (C-C motif) ligand 13           | 5.58       | 3.66                   |
| CXCR4  | chemokine (C-X-C motif) receptor 4        | 5.27       | 1.40                   |
| CCL7   | chemokine (C-C motif) ligand 7            | 4.09       | 2.00                   |
| CCR5   | chemokine (C-C motif) receptor 5          | 3.45       | 2.37                   |
| CCL4   | chemokine (C-C motif) ligand 4            | 3.43       | 1.43                   |
| CCL19  | chemokine (C-C motif) ligand 19           | 2.55       | 3.78                   |
| IL12RB2| interleukin 12 receptor, beta 2           | 37.54      | 14.41                  |
| STAT1  | signal transducer and activator of transcription 1 | 9.02 | 4.07                   |
| TNF    | tumor necrosis factor                     | 7.09       | 3.09                   |
| LTb    | lymphotoxin beta (TNF superfamily, member 3) | 4.24 | 4.15                   |
| IL6    | interleukin 6                             | 4.13       | 1.66                   |
| IL7R   | interleukin 7 receptor                     | 3.26       | 2.9                    |
| IL2RG  | interleukin 2 receptor, gamma             | 4.76       | 2.3                    |
| IL1B   | interleukin 1, beta                       | 2.14       | 1.61                   |
| S100A9 | S100 calcium binding protein A9            | 11.96      | 10.92                  |
| TLR2   | toll-like receptor 2                       | 9.89       | 5.49                   |
| TLR1   | toll-like receptor 1                       | 8.50       | 4.85                   |
| TNFSF10| TNF superfamily, member 10 (TRAIL)        | 5.73       | 4.35                   |
| S100A8 | S100 calcium binding protein A8            | 4.80       | 5.02                   |
| S100A12| S100 calcium binding protein A12          | 4.08       | 2.27                   |
| S100A7 | S100 calcium binding protein A7            | 3.47       | 5.23                   |
| ICAM1  | intracellular adhesion molecule 1          | 18.61      | 8.68                   |
| VCAM1  | vascular cell adhesion molecule 1          | 6.31       | 5.16                   |

Color code: blue=chemokines, yellow = cytokines, orange = inflammatory molecules, green = adhesion molecules.

*Although the IFN-\(\gamma\) response of many genes was higher in psoriasis than healthy skin, these differences were not statistically significant (FCH range= 0.25–3.63, FDR=0.999 for all genes).