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Susceptibility-associated genetic variation at *IL12B* enhances Th1 polarization in psoriasis

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The *IL12B* gene encodes the common p40 subunit of IL-12 and IL-23, cytokines with key roles in Th1 and Th17 biology, respectively, and genetic variation in this region significantly influences risk of psoriasis. Here, we demonstrate that a psoriasis-associated risk haplotype at the *IL12B* locus leads to increased expression of *IL12B* by monocytes and correlated with increased serum levels of IL-12, IFN-γ and the IFN-γ induced chemokine, CXCL10. In contrast, serum IL-23 levels were decreased in risk carriers when compared with non-carriers. We further demonstrate that IL-12 is increased in psoriatic skin and that risk carriers manifest a skewing of the inflammatory network toward stronger IFN-γ responses. Taken together, our data demonstrate that the risk variant in *IL12B* associates with its increased expression and predisposes to stronger Th1 polarization through deviation of the local inflammatory environment toward increased IL-12/IFN-γ at the expense of IL-23/IL-17 responses.

INTRODUCTION

Psoriasis is one of the most common chronic inflammatory diseases affecting approximately 2–3% of Caucasians (1,2), but is found in all populations (1). The most common form, psoriasis vulgaris, is characterized by sharply demarcated red scaly plaques on the elbows, scalp and knees, and approximately 25% of patients go on to develop a debilitating inflammatory arthritis. It has been known for a long time that the predisposition to psoriasis is to a large extent genetic (2–4), and in recent years, considerable progress has been made in identifying these risk factors (4). One of these risk loci is proximal to the *IL12B* gene on chromosome 5q31.1–33.1 (5). In the initial report (5), two single nucleotide polymorphisms (SNPs), which are in linkage disequilibrium, were identified: rs3212227 located in the 3′UTR and rs6887695 located upstream of the *IL12B* gene.

The *IL12B* gene encodes the common p40 subunit of IL-12 (6) and IL-23 (7), key cytokines in Th1 and Th17 differentiation and function (8), and this gene's role in psoriasis pathogenesis is illustrated by the clinical efficacy observed with anti-p40 treatments (9,10). Although no studies have been published on the effect of the rs6887695 SNP, several studies have attempted to address the role of the 3′UTR SNP rs3212227 on IL-12p40 expression and secretion. Overall, the results from these studies have been inconsistent. Thus, whereas some have shown increased IL-12p40 expression (11) and secretion (12,13), others have shown decreased IL-12p70 production for the psoriasis-associated rs3212227 A allele (14).

In this paper, we report that the *IL12B* psoriasis risk haplotype defined by the G allele of rs6887695, and its associated A allele of rs3212227 [haplotype odds ratio of 1.52 (15)], leads to increased expression of the IL12p40 subunit by monocytes, the primary cellular source of IL-12 and IL-23 (16) and that this is further enhanced by IFN-γ stimulation. We also show that affected individuals carrying this haplotype have increased serum IL-12 levels when compared with non-carriers, whereas serum IL-23 levels are decreased. We show that levels of IL-12 and IL-23 protein are increased in

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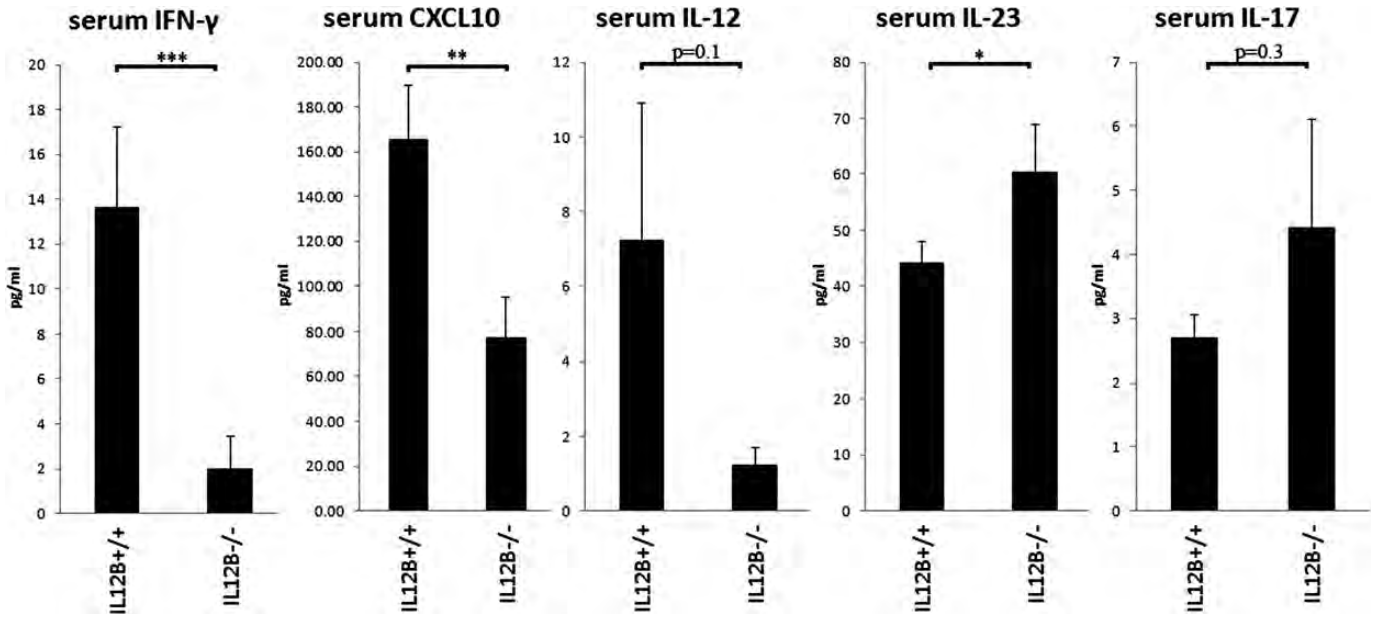

Figure 1. Increased levels of IFN-γ- and the IFN-γ-induced chemokine; CXCL10 were observed in serum of affected *IL12B* risk haplotype carriers (rs6887695G/ rs3212227A) when compared with non-carriers. Similarly, IL-12 levels were increased about 6-fold, whereas IL-23 levels were decreased about 1.4-fold. Similar to IL-23, serum IL-17 levels were down about 1.6-fold, although this was not significant ($n = 202$ affected homozygous risk carriers, $n = 17$ affected non-carriers). All values are expressed as mean ± SEM. Statistical significance of two-tailed *t*-test is indicated by one ($P < 0.05$), two ($P < 0.01$) or three ($P < 0.001$) asterisks.

psoriatic lesions and that *IL12B* haplotype carriers have an increased IFN-γ signature in lesional psoriatic skin. These findings suggest that the cytokine environment in psoriatic lesions directs an increase in p40 expression associated with the *IL12B* risk haplotype, toward IL-12 at the cost of IL-23, resulting in amplification of the IFN-γ environment in lesional skin. These data emphasize the pathologic role of the Th1 axis in psoriatic pathogenesis and may have implications in terms of future targeted therapeutics.

RESULTS

The IL12B risk haplotype influences IL-12 and IL-23 serum levels

To determine if the risk-associated haplotype affects serum levels of IL-12 and IL-23 in psoriatic patients, we first addressed whether serum levels of IL-12p70, IL-22 and IL-23 differed between cases and controls. Both IL-12 and IL-23 are heterodimeric cytokines, with IL-12 (IL-12p70) being composed of the p35 and p40 subunits encoded by the genes *IL12B* and *IL12A*, respectively, whereas IL-23 is composed of the p19 (encoded by *IL23A*) and the p40 subunits. IL-12 levels were higher in affected cases when compared with controls, although this did not reach significance. No difference was observed with IL-23 serum levels between cases and controls, whereas IL-22 levels were significantly higher in affected patients ($P < 0.05$) (Supplementary Material, Fig. S1). To address the effect of the psoriasis-associated *IL12B* risk haplotype, as defined by the G allele of rs6887695 and the A allele of rs32112227A (15), on the serum levels of these two cytokines, we compared the serum cytokine levels in affected homozygous risk haplotype carriers against the levels in affected non-carriers. We found IL-12p70 serum levels to be higher in homozygous carriers when compared with non-carriers (6-fold, $P = 0.10$), whereas IL-23 levels were decreased (1.4-fold, $P < 0.05$). We then compared serum levels of IFN-γ and the IFN- γ induced chemokine; CXCL10 between the two groups, finding that IFN-γ was about 6.9-fold higher in homozygous risk carriers when compared with non-carriers ($P < 0.0001$), and CXCL10 was 2.1-fold higher ($P < 0.01$). Consistent with serum IL-23, IL-17A levels were lower in homozygous risk carriers when compared with non-carriers (1.6-fold), although this did not reach statistical significance. IL-22 levels did not differ between the two groups (Supplementary Material, Fig. S2). (Fig. 1)

IFN-γ pre-stimulation enhances IL-12 and IL-23 mRNA expression and secretion

Monocytes and dendritic cells are the primary source of IL-12 and IL-23 (16). To better reproduce the conditions in psoriatic skin, we compared IFN-γ pre-stimulated and non-stimulated monocytes that were stimulated with lipopolysaccharide (LPS) at 0.1 and 1.0 μg/ml for 24 h. Consistent with our previously published findings (17), IFN-γ stimulation promotes increased expression of *IL12B*, *IL12A*, *IL23A* and *IL1B* mRNAs from monocytes in a stimulation-dependent manner (Fig. 2). The fold increase in expression was highest for *IL23A* at the 12 h time point (140-fold, $P < 0.01$, Supplementary Material, Fig. S5), whereas *IL12B* was more strongly

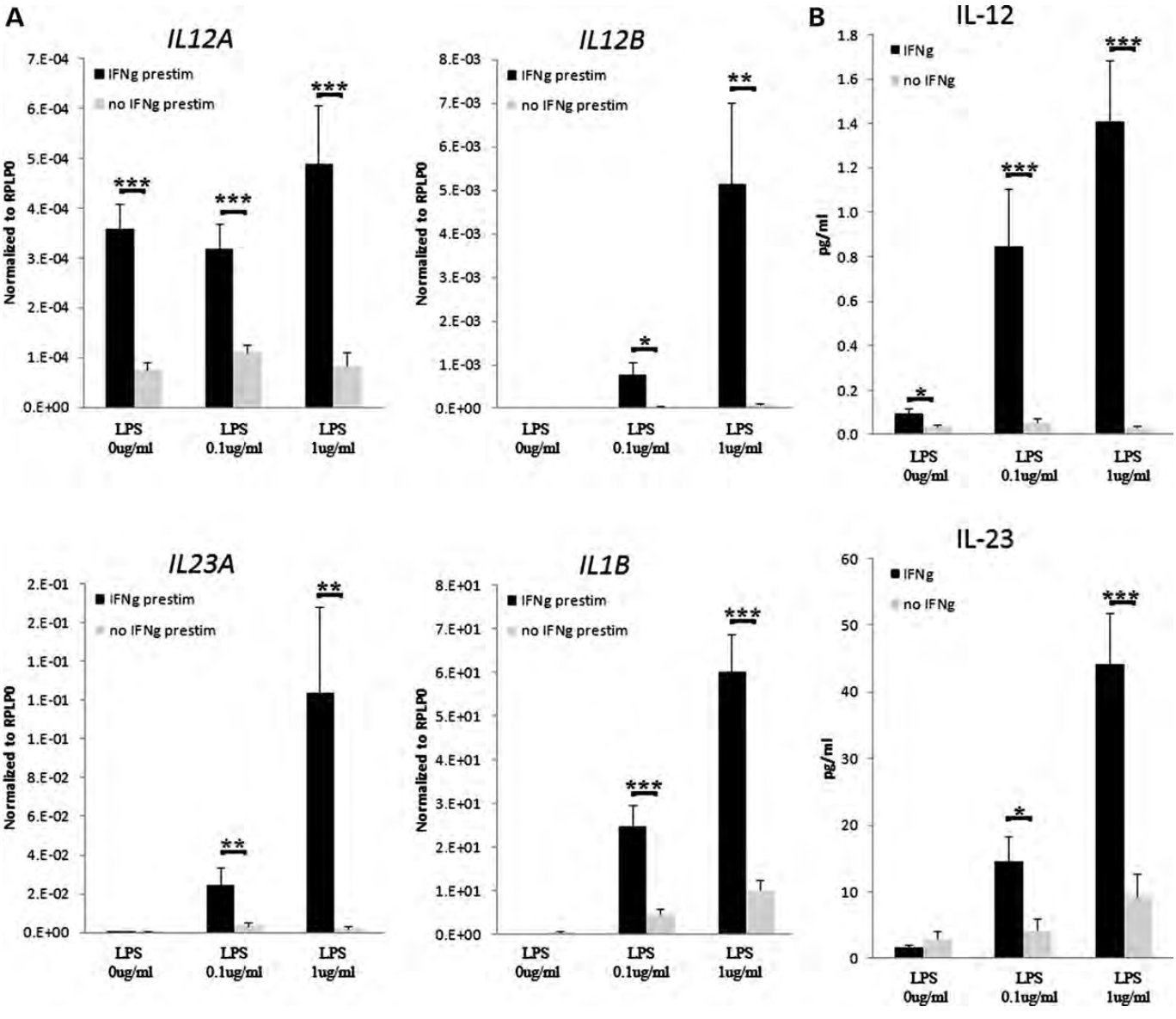

Figure 2. Pre-stimulation of monocytes with IFN-γ (50 ng/ml) leads to increased mRNA expression of *IL12B*, *IL12A*, *IL23A* and *IL1B* after 24 h of LPS stimulation (**A**). Similarly, protein levels of IL-12 and IL-23 were increased at 24 h in monocyte-conditioned medium after IFN-γ pre-stimulation. Gene expression values (mRNA) are shown relative to the housekeeping gene *RPLP0* as mean ± SEM ($n = 20$ for IL-12 and $n = 35$ for IL-23). Statistical significance of two-tailed *t*-test is indicated by one ($P < 0.05$), two ($P < 0.01$) or three ($P < 0.001$) asterisks.

induced at 24 h (98-fold, $P < 0.01$) (Fig. 2A). Similarly, IFN-γ stimulation increased LPS-induced secretion of both IL-12 and IL-23 proteins ($P < 0.001$, Fig. 2B), although the fold change was more pronounced for IL-12 when compared with IL-23 (47-fold versus 5.4-fold at 24 h, 1 ug/ml LPS).

The IL12B risk haplotype leads to increased IL12B mRNA expression in IFN-γ conditioned monocytes

As shown in Figure 3, homozygous carriers of the *IL12B* risk haplotype (rs6887695 G/rs32112227 A) had consistent increases in *IL12B* expression in both IFN-γ primed cultures, when compared with non-risk carriers (12.5-fold, $P < 0.01$; 3.8-fold $P < 0.01$, respectively, at 24 h time point, Fig. 3). *IL12A* had slightly lower expression in unstimulated IFN-γ primed cultures at 24 h (1.6-fold down, $P < 0.05$). No significant differences were observed between risk haplotype carriers and non-carriers in terms of *IL23A* or *IL1B* mRNA expression (Fig. 3). No consistent differences were observed in the secretion of IL-12 and IL-23 proteins in culture supernatants between the two groups.

IL-12 is up-regulated in psoriatic skin

To assess whether the levels of transcripts encoding the subunits of IL-12 and IL-23 are altered in psoriatic skin, we performed quantitative real time-polymerase chain reaction (QRT-PCR) on RNA isolated from healthy control,

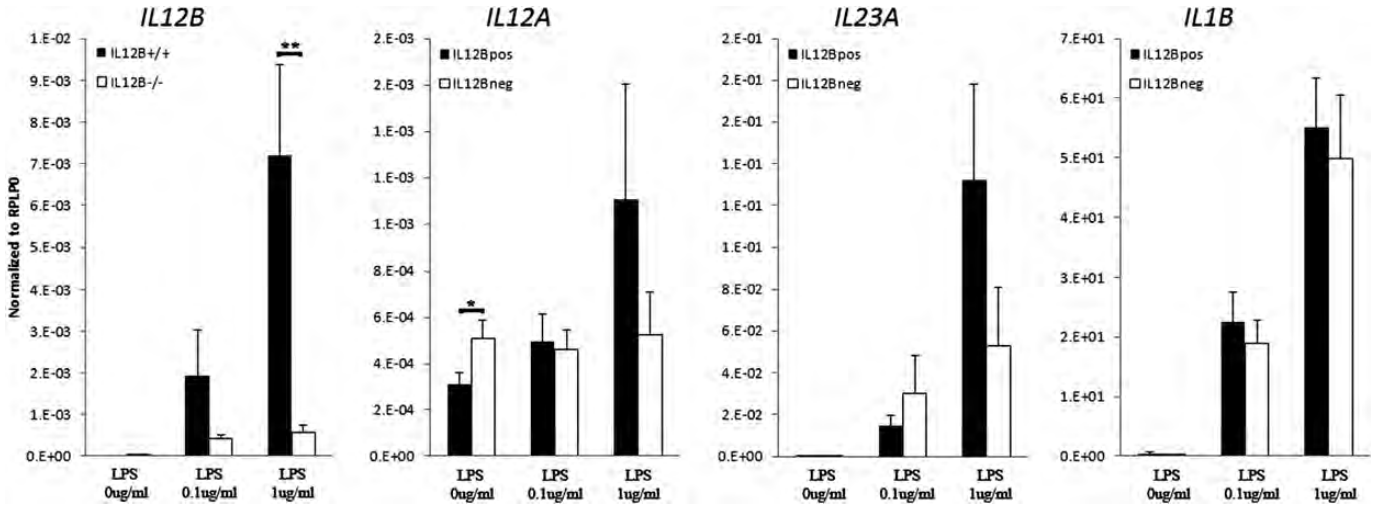

Figure 3. Homozygous *IL12B* risk haplotype (rs6887695G/rs3212227A) carriers had on average 12.5-fold higher *IL12B* expression after 24 h stimulation when compared with homozygous non-carriers. IL12A had slightly decreased expression in unstimulated cultures. In contrast, there were no consistent changes observed in the expression of either *IL23A* or *IL1B* between the two groups. All values are expressed relative to the housekeeping gene RPLP0 as mean ± SEM ($n = 29$ homozygote carriers and 15 non-risk carriers). Statistical significance of two-tailed *t*-test is indicated by one ($P < 0.05$) or two 9 $P < 0.01$) asterisks.

uninvolved and lesional psoriatic skin. Consistent with previous findings (16), increased expression of *IL12B* and *IL23A* mRNA was observed in lesional skin (6.7-fold and 4.9-fold, respectively, $P < 0.01$), whereas *IL12A* mRNA levels were down-regulated (3.1-fold, $P < 0.05$) when compared with uninvolved skin (Fig. 4). To confirm this at the protein level, skin lysates from healthy control, uninvolved and lesional psoriatic skin were obtained, and soluble IL-12 and IL-12p40 were measured by multiplex immunoassays. IL-12p70 levels were similar between control and non-lesional skin, but were approximately 4-fold higher in lesional skin ($P < 0.01$, Fig. 4). Likewise, protein levels of the IL-12p40 subunit were increased by about 4-fold in lesional when compared with non-lesional skin ($P < 0.01$) (Fig. 4). IL-12p40 was approximately five to six times more abundant than IL-12p70 ($P < 0.01$, Fig. 4) per milligram total protein tissue lysate.

Enrichment of IFN-γ induced genes in lesional skin of IL12B risk allele carriers

To ask whether the *IL12B* risk allele would lead to shifts in the cytokine environment in psoriatic skin, we assessed genome-wide expression profiles of lesional skin samples from 53 psoriasis patients (18). We identified 356 genes for which expression was significantly elevated in the lesional skin of patients in proportion to *IL12B* risk allele carriage (number of rs6887695 G alleles + number of rs3212227 A alleles, $n = 53$; $P < 0.05$) (Fig. 5). Of the 30 genes showing the highest fold change in lesional skin from *IL12B* risk allele positive individuals, several included known cytokine-responsive genes, including *CXCL9* and *CXCL10* (Fig. 5A and B). We, therefore, evaluated whether there was a significant tendency for genes elevated in lesional skin from *IL12B* risk carriers to also be induced by the cytokines IFN-γ, IL-17, TNF-α and IL-22 in cultured keratinocytes (Fig. 5C). Our analysis revealed that the top-ranked genes showing a trend toward elevated expression in lesional skin of *IL12B* risk carriers overlapped significantly with genes most strongly induced by IFN-γ in cultured keratinocytes ($P < 0.001$; Fig. 5A and C). In contrast, genes induced by IL-17 and IL-22 had significantly decreased overlap ($P < 0.001$; Fig. 5A and C), and a modest decrease in overlap was observed for TNF-α induced genes ($P = 0.028$). These analyses show that genes with elevated expression in lesional skin of *IL12B* risk carriers are significantly more likely to be induced by IFN-γ, but not by IL-17 or IL-22. This pattern was manifested by a number of *IL12B* risk-associated genes, each of which was induced by IFN-γ in cultured keratinocytes, despite weak or significant repression of the same genes by IL-17, TNF-α or IL-22 (Fig. 5A and C). These results are consistent with deviation of the inflammatory network toward IFN-γ and Th1 responses at the cost of IL-17 and IL-22 in *IL12B* risk allele carriers (Supplementary Material, Fig. S3 and S4).

DISCUSSION

The involvement of IFN-γ in psoriasis pathogenesis is widely appreciated (19–21), and for a long time, psoriasis was believed to be primarily a Th1, IFN-γ-mediated disease based on the significant IFN-γ expression in psoriatic skin (20) and Th1 dominance of circulating and lesional T-cells (22). The key cytokine that polarizes T-cells toward the Th1 phenotype is IL-12 (8)that is composed of the p40 and p35 protein subunits encoded by the *IL12B* and *IL12A* genes, respectively. Both chains of the IL-12 receptor, IL-12Rβ1 and IL-12Rβ2, are up-regulated in psoriasis (23), suggesting that this cytokine may have an active role in the disease pathogenesis. However, as *IL12A* mRNA expression is generally down-regulated in psoriatic lesions (16) (Fig. 4A), whereas *IL12B* and *IL23A* mRNAs are increased, this has been interpreted as IL-12 playing a more limited role in psoriasis when compared with IL-23 (16).

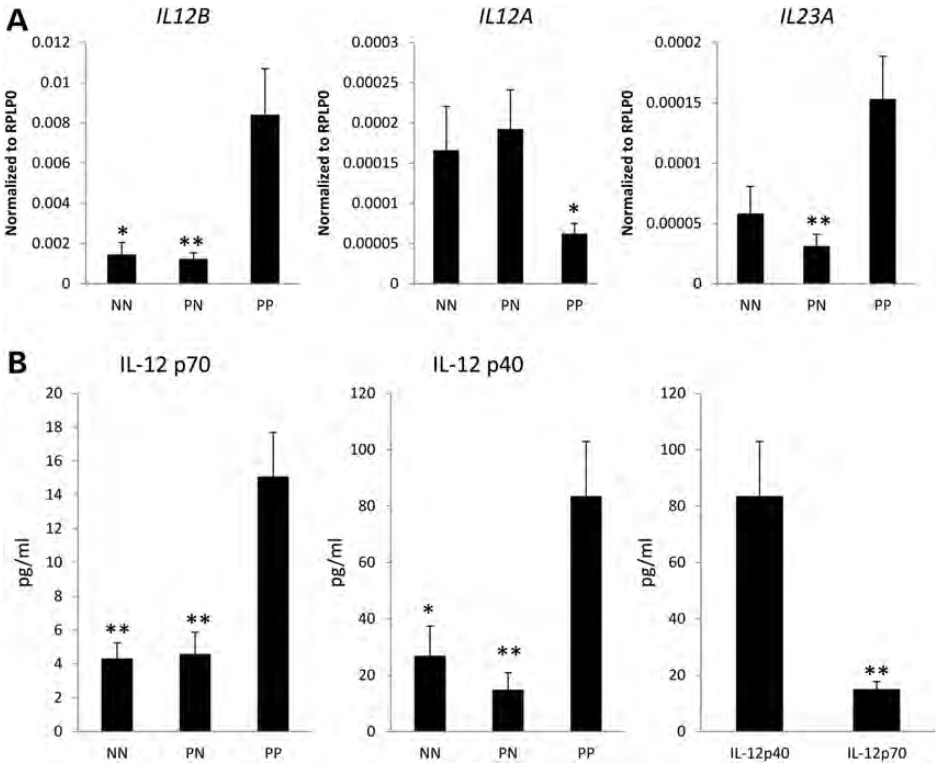

Figure 4. Expression of *IL12B* and *IL23A* mRNA were increased in lesional psoriatic skin, whereas *IL12A* expression was decreased (**A**) ($n = 7–10$). Measurements of IL-12 (IL-12p70) and IL-12p40 from tissue lysates obtained from control (NN), uninvolved (PN) and lesional psoriatic skin (PP) ($n = 9–13$) revealed significantly increased protein levels of both IL-12p70 and IL-12p40 in lesional psoriatic skin (**B**). All mRNA values are expressed relative to the housekeeping gene *RPLP0* as mean ± SEM. Protein values are expressed relative to total protein tissue lysate (1 mg/ml) as mean ± SEM. Statistical significance of two-tailed *t*-test indicated by one ($P < 0.05$) or two ($P < 0.01$) asterisks.

The role of IL-23 appears to be primarily through its effect on Th17 responses. In contrast to the direct effect of IL-12 on Th1 differentiation (24), IL-23 does not act directly on naïve T cells to induce Th17 differentiation (25,26), but instead up-regulates IL-17 production and promotes survival and expansion of activated Th17 cells. These cells have been shown to be highly pathogenic and essential for the establishment of autoimmune inflammation in mouse models (27). Likewise, effective management of psoriasis is linked to suppression of IL-17 signaling (28,29), and genetic findings from genome-wide association studies have implicated genes that are more strongly involved in IL-23/IL-17 signaling than those for IL-12 (5,30). Likewise, in a xenograft system, where uninvolved skin spontaneously transforms into psoriatic lesions, blockade of IL-23 prevents the development of psoriasis (31).

Taken together, these findings have resulted in the focus being shifted toward IL-17 and a Th17-centric model of psoriasis (32). However, the relationship between IFN-γ and IL-17 is complex. Th1 and Th17 cells are often co-localized in pathologic environments (33,34), and Th1 cells are reported to inhibit Th17 development through IFN-γ (26,35,36). Moreover, IFN-γ can act on resident dendritic cells to promote the induction and expansion of Th17 cells as well as their recruitment, through expression and production of CCL20 (17). In addition, Th17 cells demonstrate considerable plasticity, and IFN-γ and IL-12 have been shown to synergize to convert Th17 cells into Th17-Th1 cells (33,37). These Th17-Th1 cells may have a more important pathologic role as recent data suggest that Th17 cells become pathogenic only after switching to a Th1 phenotype (38,39).

Both IFN-γ- and IL-17-secreting cells are found in lesional psoriatic skin, with IFN-γ positive cells being about nine times more frequent than those producing IL-17(34). Psoriatic dendritic cells are able to induce a population of activated T cells that simultaneously produce IL-17 and IFN-γ, which is not seen with dendritic cells from normal skin(40), suggesting that psoriasis is a mixed Th1 and Th17 inflammatory disease. Interestingly, a single intradermal injection of IFN-γ can promote development of an inflammatory environment that, in some respects, parallels that observed in psoriatic lesions (41). Likewise, IFN-γ expression has been shown to be increased in uninvolved skin (17,41,42), and becomes increasingly prominent as psoriasis becomes more severe (41). This suggests that psoriasis patients have greater propensity to produce IFN-γ when compared with healthy controls (41) and that IFN-γ may have a crucial role in psoriatic pathogenesis (41).

Our data are consistent with a model in which increased expression of p40 (*IL12B*) leads to increased production of IL-12, resulting in increased serum levels of IFN-γ and amplification of the IFN-γ signature in lesional psoriatic skin (Fig. 6). No clinical trials are available on the effect of neutralization of IFN-γ in psoriasis, but the effect of

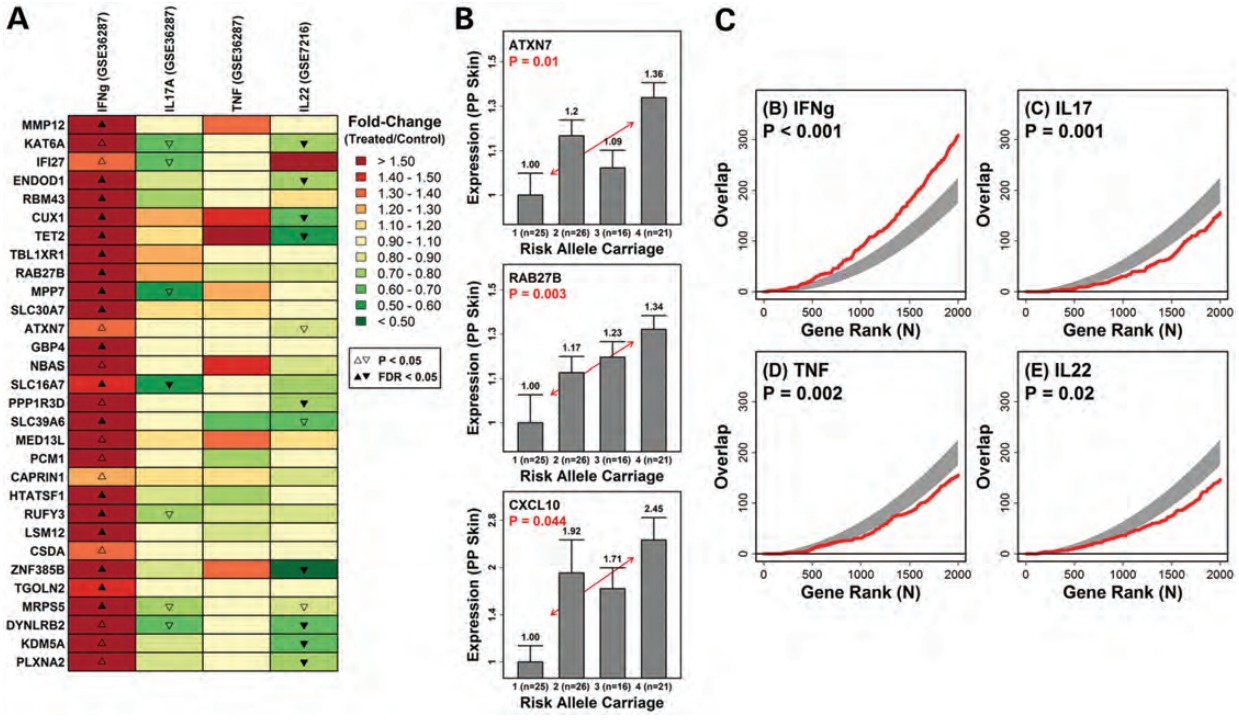

Figure 5. We identified 356 genes for which expression was significantly elevated in the lesional skin of patients in proportion to *IL12B* risk allele carriage (0–4 risk alleles, including rs6887695 G allele and rs3212227 A allele; $n = 53$ patients; $P < 0.05$). The heat map shows 30 genes with increased expression in lesional skin from individuals with more *IL12B* risk alleles (rs6887695 and rs3212227, $P < 0.035$) and the expression responses of these genes to IFN-γ, IL-17, TNF-α and IL-22 in cultured keratinocytes (**A**). Expression levels of three representative genes demonstrate a dose-dependent increase in the expression levels in psoriatic skin based on risk allele carriage. The red line denotes the least squares regression estimate with a positive slope in each case. (**B**) Furthermore, all genes were ranked according to their level of positive association with *IL12B* risk carriage (rs6887695 and rs3212227), and this list was compared for overlap with gene lists ranked by their level of induction by IFN-γ, IL-17, TNF-α or IL-22 in keratinocytes (**C**). In each box, the red line shows the level of overlap among the top *n* genes taken from each list, whereas the gray region outlines the central 95% of the null distribution under a random sampling model (hypergeometric distribution). Larger than expected overlap (for given *n*) is present, if the red line lies above the gray region, whereas lower than expected overlap (for given *n*) is present, if the red line lies below the gray region. *P*-values are based upon the overlap between the top 1000 genes from each gene list.

treatments neutralizing IL-17 is unmistakable and associated with a very strong clinical response with a good proportion of patients achieving clinical remission while on treatment (43,44). Although our data cannot determine whether the pathogenic effect of the *IL12B* risk haplotype is directly mediated by Th1 cells, or through accelerated survival and expansion of Th17 cells to a more pathogenic Th1 phenotype, it is tempting to speculate that the role of the *IL12B* risk allele is to prime the skin for induction of the psoriatic process through increased expression of IFN-γ that may also amplify the inflammatory response in concert with IL-17. Taken together, our data provide insight into how genetic variation at the *IL12B* locus influences the inflammatory network in psoriasis, reemphasizing the importance of the Th1 axis with implications for the development of targeted therapeutics.

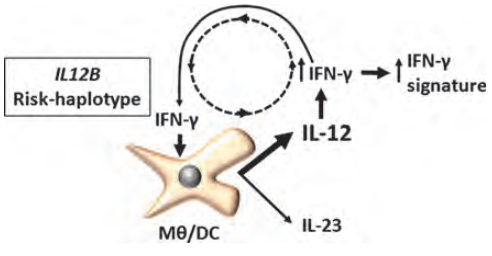

Figure 6. Proposed model of the pathologic role of the *IL12B* risk locus in psoriasis. Under normal circumstances, there is a balance between IL-12 and IL-23 production by monocytes. When monocytes/dendritic cells are exposed to IFN-γ, there is a shift toward increased IL-12 production, although there is concomitant, but less, increase in IL-23 production. In psoriatic lesions, which are rich in IFN-γ, the increase in the expression of *IL12B* mRNA as a function of the *IL12B* risk haplotype is shifted toward IL-12, leading to an even greater increase in IFN-γ that locks the mechanism in a positive feedback cycle favoring IL-12 and resulting in progressive increase in the strength of the IFN-γ signature in inflamed psoriatic skin.

MATERIALS AND METHODS

Study population

Forty-nine individuals were identified and recruited from our extended genetic cohort, and these were evenly distributed between cases and controls (25 healthy individuals versus 24 psoriatics and between risk allele carriers versus non-carriers). Thirty-three were homozygous carriers and 16 were non-carriers of the risk haplotype in the *IL12B* gene (rs6887695 G allele and rs3212227 A allele). Typing was performed as described in Nair *et al.* (15). In addition, 11 healthy controls and 13 psoriatic patients were recruited for skin biopsies (6 mm) of normal, uninvolved and lesional psoriatic skin.

Informed consent was obtained from all subjects, under protocols approved by the Institutional Review Board of the University of Michigan. This study was conducted in compliance with good clinical practice and according to the Declaration of Helsinki Principle.

Microarrays

The subjects enrolled for gene expression analysis have been previously described in accord with a protocol approved by the Institutional Review Board of the University of Michigan. Shortly, it involved 58 subjects with untreated chronic plaque psoriasis with all samples run on HU133 Plus 2.0 arrays (Affymetrix, Foster City, CA, USA) as described (45). Patients were typed for risk allele polymorphisms in the *IL12B* gene as described (15). The raw data from the psoriatic microarrays were processed using the Robust Multichip Average method (46) and adjusted to account for gender and batch effect (patient samples only). The raw and normalized expression data are available from the Gene Expression Omnibus database (accession GSE13355).

Keratinocyte cultures

Normal human keratinocyte (NHK) cultures were established from sun-protected adult human skin as described (47) from three separate donors. Keratinocytes were grown in serum-free medium optimized for high-density keratinocyte growth (Medium 154; Invitrogen/Cascade Biologics, Portland, OR, USA). NHKs were used for experiments in the second or third passage. All cells were plated at 5000 cells/cm^2 and maintained to 4 day post-confluency. Cultures were then starved of growth factors in unsupplemented medium M154 for 24 h before cytokine stimulation. NHKs were stimulated with TNF-α (10 ng/ml), IFN-γ (50 ng/ml) and IL-17(10 ng/ml) (R&D Systems, Minneapolis, MN, USA) or unstimulated control. Experiments were carried out under low-calcium (0.1 mM) conditions. Cells were harvested after 24 h, and RNA was isolated and extracted using RNeasy columns (Qiagen, Chatsworth, CA). RNA quality was checked using Agilent 2100 Bioanalyzer (Agilent Technologies, Santa Clara, CA, USA) before running on Affymetrix HU133 Plus 2.0 arrays (Affymetrix, Santa Clara, CA, USA).

Monocyte cultures

Monocytes were isolated from peripheral blood mononuclear cells by negative selection beads (Miltenyi Biotec, Auburn, CA, USA). Purity of cells was evaluated and found to be >75%. Monocytes were cultured for 72 h in Roswell Park Memorial Institute containing 10% fetal calf serum supplemented with IL-6 (5 ng/ml) (R&D Systems), IL-10 (5 ng/ml) (R&D Systems) and M-CSF (10 ng/ml) (R&D Systems) with or without IFN-γ (50 ng/ml) (R&D Systems). The monocytes were then stimulated with varying doses of LPS (0, 0.1 and 1 ng/ml) for 0, 12 and 24 h. RNA and supernatants were harvested at those time points for ELISA and multiplex assays for IL-12 (IL12p35, eBioscience, San Diego, CA, USA), IL-23 (IL23p19, Milliplex^{TM} Map Kit Human Cytokine Panel, Millipore) and IL-1B (Fluorokine MAP human IL-1β, R&D Systems).

Tissue processing

Normal and uninvolved skin biopsies (6 mm) were obtained from sun-protected skin of healthy individuals, whereas lesional skin was obtained from active psoriatic lesions. Skin was anesthetized with lidocaine with epinephrine (1:10 000), and biopsies were snap frozen in liquid nitrogen and stored at −80°C until processing. For protein quantification, biopsies were pulverized and dissolved in complete radioimmunoprecipitation buffer for protein quantitation. Tissue lysates for protein quantitation were normalized to 1 mg/ml of total protein before analysis. For total RNA extraction, biopsies were pulverized, and RNA extraction was performed using RNAeasy kit (Quiagen) using glass beads (Biospec Products, Bartlesville, OK, USA) for homogenization. RNA quantity and quality were measured as described below.

QRT-PCRs

RNA was isolated from stimulated monocytes and extracted using RNeasy columns (Qiagen). RNA quantity and quality were measured on an Agilent 2100 Bioanalyzer (Agilent Technologies), and only samples yielding intact 18S and 28S ribosomal RNA profiles were used. Reversed transcription was performed using High Capacity cDNA Transcription kit (Applied Biosystems Inc., Foster City, CA, USA) as previously described. Transcripts were quantified using a 7990HT Fast Real-Time PCR system (Applied Biosystems) using Taqman primers sets purchased from Applied Biosystems (*IL12A* Hs00168408_m1, *IL12B* Hs01011510_m1, *IL23A* Hs00372324_m1, *RPLP0* Hs99999902_m1 and *IL1B* Hs00174097_m1). All values were normalized to the expression of the housekeeping gene ribosomal protein, large, P0 (RPLP0).

Serum ELISA

Frozen serum was obtained from archived samples from 227 affected and 55 healthy individuals. ELISA was performed for IL-12 (IL12p35, eBioscience, San Diego, CA, USA), IL-22 (IL-22 DuoSet, R&D Systems), CXCL10 (CXCL10 DuoSet, R&D Systems), IL-23 (IL23p19, MilliplexTM Map Kit Human Cytokine Panel, Millipore, Billerica, MA, USA), IL-17 (IL-17, eBioscience) and IFN-γ (Quantikine, R&D Systems).

Statistical analysis

Data were tested for normality using the Kolmogorov–Smirnov test, and statistical significance was calculated using Student's *t*-test or Mann–Whitney test as appropriate using GraphPad Prism version 5.00 for Windows (GraphPad Software, San Diego, CA, USA). Genes associated with *IL12B* risk allele carriage in lesional skin were identified using least squares regression. For each probe set, log2-transformed expression was treated as a continuous response variable, and total risk allele carriage was treated as the predictor variable ($n = 53$ patients). Total risk allele carriage

was calculated by pooling the number of risk alleles across two IL12B SNPs (i.e. number of rs6887695 g alleles + number of rs3212227 A alleles). Among the 53 subjects, 4 (8%) carried 1 risk allele, 12 (23%) carried 2, 16 (30%) carried 3 and 21 (40%) carried 4. Overlap between genes positively associated with *IL12B* risk allele carriage and genes induced by cytokines *in vitro* was assessed by the comparison of ranked gene lists (48). For each analysis, we considered only genes positively associated with *IL12B* risk allele carriage (slope >0) and ranked these genes according to the *P*-value obtained from the least squares regression analysis. This list was evaluated for overlap with genes ordered by their level of induction by IFN-γ, IL-17, TNF or IL-22 in cultured keratinocytes (Gene Expression Omnibus accessions GSE7216 and GSE36287). For each cytokine, only induced genes were considered (treated/control fold-change >1), and genes were ranked according to *P*-values. These *P*-values were generated from two-sample comparisons (i.e. treated versus control) and Bayesian linear modeling methods, as implemented in the Limma package developed for the R statistical software package (49).

SUPPLEMENTARY MATERIAL

Supplementary Material is available at *HMG* online.

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