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Article

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Interleukin-11 therapy selectively downregulates type I cytokine proinflammatory pathways in psoriasis lesions

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Introduction

Psoriasis is a chronic cutaneous inflammatory disease characterized by hyperplastic regenerative epidermal growth. Numerous cytokine-induced inflammatory molecules are expressed in the epidermis and dermis of defined lesions (1). A lymphocytic infiltrate in psoriasis plaques consists of a mixture of activated $CD4^+$ and $CD8^+$ T cells, with $CD8^+$ cells predominating in lesional epidermis and $CD4^+$ cells in the dermis (2). The therapeutic benefit of immunosuppressive drugs that act on T cells, e.g., cyclosporin A, FK506, anti-CD4 antibody, and IL-2 diphtheria toxin conjugate, suggests that activated T cells are pathogenic effectors of psoriasis (3–5). Furthermore, psoriasis can be reproduced in xenotransplanted human skin by direct injection of activated T cells into the dermis (6).

Consistent with a T-cell mediated inflammatory response, proinflammatory mediators are overexpressed in psoriatic lesions compared with uninvolved skin. Expression of IL-8, IL-2, IFN-γ, IL-6, iNOS, B7.1, and TNF-α are all reported to be elevated in psoriatic tissue (7–10). The pattern of cytokine expression suggests that Th1 T cells may mediate or maintain disease (11). Furthermore, a type I differentiation bias of $CD4^+$ and $CD8^+$ T cells has been detected in circulating lymphocytes in psoriasis patients by individual cell cytokine synthesis profiles (12). A preliminary report also suggests that IL-12, a key cytokine for directing type I T-cell differentiation, is also increased in psoriasis (13). Epidermal responses to T cell–mediated inflammation include (a) increased proliferation marked by elevated expression of the nuclear Ki67 protein; (b) altered differentiation marked by synthesis of keratin 16 (K16); (c) increased synthesis of growth-regulating cytokines or receptors on keratinocytes, e.g., increased levels of KGF and KGF-R; and (d) synthesis of cytokine-induced proteins by keratinocytes including ICAM-1, HLA-DR, and IP-10 (6, 14, 15).

A multifunctional cytokine, rhIL-11, interacts with a variety of hematopoietic and nonhematopoietic cell types (16). It is currently approved for use in the treatment of chemotherapy-induced thrombocytopenia because of its ability to stimulate megakaryopoiesis and thrombopoiesis. In addition to its thrombopoietic

activity, rhIL-11 has demonstrated anti-inflammatory activity in vitro and in vivo. It directly interacts with macrophages to reduce proinflammatory cytokine production including TNF-α, IL-1β, and IL-12 p40 (17, 18). This effect is due to the inhibition of NF-κB nuclear translocation through enhanced expression of the inhibitor of NF-κB, IκB (19). Further studies have identified that rhIL-11 also modulates T-cell function. Studies with $CD4^+$ murine T cells showed that the presence of rhIL-11 blocked Th1 differentiation as indicated by inhibition of IL-12–induced IFN-γ production and enhanced the Th2 response (W. Trepicchio, unpublished observations). Presence of rhIL-11 has no direct effect on human neutrophil function (20).

In multiple animal models of inflammatory disease, treatment with rhIL-11 has ameliorated disease signs. Efficacy in these models has been associated with downregulation of inflammatory parameters. In the HLA-B27 rat model of chronic inflammatory bowel disease, treatment with rhIL-11 reduced gross and histologic colonic lesions (21). Molecular analysis demonstrated that improvement of disease was associated with reduced RNA levels in the colon for proinflammatory cytokines including IFN-γ, IL-1β, TNF-α, and IL-12 p40 (22). Also, rhIL-11 has shown beneficial effects in models of systemic inflammatory responses. Treatment with rhIL-11 reduced proinflammatory cytokine levels such as IFN-γ and TNF-α in a murine model of endotoxemia (17). TNF-α levels were reduced and survival increased in a neutropenic rat model of sepsis and in a murine model of radiation-induced pulmonary injury (23, 24). In a murine model of graft versus host disease (GVHD), treatment with rhIL-11 polarized the T-cell response toward a Th2 response with reduced IFN-γ and increased IL-4 production (25). Taken together, these studies demonstrate that rhIL-11 has immunomodulatory activity for both macrophages and T cells to reduce an inflammatory response.

Because rhIL-11 has demonstrated immunomodulatory activity to regulate both T cell and macrophage function, we have begun an assessment of rhIL-11 therapy for psoriasis. Because of the ease of obtaining skin biopsies, psoriasis is accessible for a comprehensive study of molecular alterations brought about by immune-directed therapy, but to date a detailed analysis of gene expression before and after immunotherapy has not been performed. We have used quantitative RT-PCR and immunohistochemistry to measure the pharmacogenomic and cellular effects of subcutaneous administration of rhIL-11 on psoriatic lesions in an open-label dose-escalation clinical trial. Here we describe the results from 12 patients, identifying multiple inflammatory and epidermal genetic markers of disease. These results indicate that, consistent with its immunomodulatory activity, rhIL-11 treatment can reduce cutaneous inflammation, inflammatory cyto-kine expression, and keratinocyte hyperplasia, as measured by changes in immunohistochemical as well as genetic markers of disease. Furthermore, to our knowledge, this is the first demonstration that administering an exogenous cytokine to humans can selectively reduce production of type I proinflammatory cytokines, thereby modulating a chronic immune reaction.

Methods

Study design and patient entry criteria. Twelve patients with extensive psoriasis (> 10% body surface area affected) were treated with 2.5 or 5.0 mg/kg of rhIL-11 subcutaneously every day for 8 weeks. The dose selection of rhIL-11 was based on the outcome of a clinical trial in patients with Crohn's disease, where biological activity was observed. In addition, 3 patients with similar disease severity were treated with 5 mg/kg per day of cyclosporin A as described previously (3). Upon enrollment, a psoriatic plaque was chosen for weekly assessment of lesion severity. A 6-mm punch biopsy was taken of this lesional skin before rhIL-11 or cyclosporin A treatment and at

Table 1
Histology measures in responding and nonresponding patients

	Epidermal thickness[A]	*P* value[B]	Ki67$^+$ keratinocytes	*P* value	Epidermal CD3$^+$	*P* value	Total CD3$^+$	*P* value	ICAM-1	K16
Responders (*n* = 7)										
Pretreatment	499 ± 69	-	239 ± 62	-	237 ± 55	-	458 ± 63	-	-	-
Week 1	418 ± 23	0.13	197 ± 62	0.11	195 ± 33	0.25	454 ± 107	0.49	↓5/7	u[C]
Week 4	359 ± 59	0.07	146 ± 65	0.09	115 ± 43	0.01	271 ± 112	0.01	↓6/7	↓6/7
Week 8	286 + 28	0.003	81 ± 28	0.01	56 ± 18	0.003	237 ± 81	0.001	↓7/7	↓7/7
Nonresponders (*n* = 5)										
Pretreatment	532 ± 45	-	248 ± 97	-	139 ± 36	-	477 ± 131	-	-	-
Week 1	475 ± 80	0.21	216 ± 86	0.29	100 ± 23	0.46	355 ± 99	0.28	u[C]	u[C]
Week 4	478 ± 42	0.16	138 ± 22	0.16	134 ± 32	0.46	448 ± 84	0.40	↓2/5	↓1/5
Week 8	537 ± 74	0.47	210 ± 50	0.38	133 ± 14	0.45	441 ± 96	0.35	↓1/5	↓1/5

[A]Mean + SE; [B]paired *t* test; [C]u = unchanged.

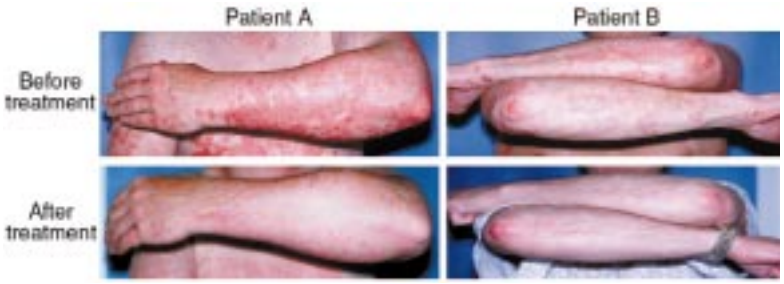

Figure 1
Photographs of psoriatic plaques undergoing resolution in 2 patients (A and B) before and after 8 weeks of treatment with rhIL-11.

weeks 1, 4, and 8 during treatment. In addition, before initiation of treatment, a 6-mm punch biopsy was taken from uninvolved skin at a location of the patient's choosing. Biopsies were equally divided for immunohistochemical analysis and for RNA preparation.

Clinical and histopathological assessment. Global clinical assessment of each patient was based on the Psoriasis Area and Severity Index (PASI) score in each patient before and during therapy as described previously (3). The grading system used to measure local disease activity at the lesion biopsy site was the Psoriasis Severity Index (PSI). This index grades individual psoriasis lesions for scale, erythema, and induration on a 0- to 6-point scale for each parameter (0, absent; 1, trace; 2, mild; 3, mild to moderate; 4, moderate; 5, moderate to severe; and 6, severe). The final score is the sum of the individual parameters for a range of 0–18 (26, 27). Histopathological assessment was performed on one-half of the 6-mm punch biopsy. Cryostat sections (6 μm) were made from frozen biopsies of psoriatic lesions obtained before and during rhIL-11 or cyclosporin A treatment. Sections were reacted with antibodies to CD3, CD8, K16, Ki67, ICAM-1, or HLA-DR and processed for immunohistochemistry as described previously. Computer-assisted image analysis was used to quantify epidermal thickness and the number of $CD3^+$, $CD8^+$, or $Ki67^+$ cells in tissue sections as described (4).

Quantitative RT-PCR. RNA was prepared using the RNAgents Total RNA Isolation System according to the manufacturer protocol (Promega Corp., Madison, Wisconsin, USA) as described previously (19). RNA was treated with 10 units of RQ1 DNase I (Promega) for 30 minutes at 37°C. Samples were extracted with phenol/chloroform, and RNA was precipitated with 0.3 M NaAc and 2 volumes of 100% ethanol. RNA was resuspended in diethylpyrocarbonate-treated (DEPC-treated) sterile water, and the RNA concentration was determined by measuring the optical absorbance at 260 nm. Then, *rTth* DNA Polymerase was used to reverse transcribe and amplify 25 ng of total RNA in a single tube assay using the Perkin Elmer TaqMan EZ RT-PCR kit (Perkin Elmer Applied Biosystems, Foster City, California, USA) with gene-specific sense and antisense primers and a probe fluorescently labeled at the 5′ end with 6-carboxy-fluorscein (6-FAM) (28, 29). Primers and fluorescently labeled probes were generated using Primer Express software (Perkin Elmer) and were synthesized by Perkin Elmer. To avoid amplification of contaminating genomic DNA, primer pairs were selected that crossed intron/exon borders whenever possible. Duplicate samples were reverse transcribed for 30 minutes at 60°C and then subjected to 40 rounds of amplification for 15 seconds at 95°C and 1 minute at 60°C using the ABI Prism 7700 sequence detection system as described by the manufacturer (Perkin Elmer) (28). Sequence-specific amplification was detected as an increased fluorescent signal of 6-FAM during the amplification cycle. Quantitation of gene-specific message levels was based on a comparison of the fluorescent intensity in the unknown mRNA sample to the fluorescent intensity from a standard curve of known mRNA levels. Amplification of the gene for human acidic ribosomal protein (HARP) was performed on all samples tested to control for variations in RNA amounts (30). All genes were subsequently normalized to HARP mRNA levels. Levels of gene-specific messages were graphed as normalized message units as determined from the standard curve. A no-template control was included in each amplification reaction to control for contaminating templates. For valid sample analysis the fluorescent intensity in the no-template control was required to be zero.

Statistical analysis. Quantitative measures of gene expression changes were statistically evaluated using the JMP Statistical Discovery software package (SAS Institute Inc., Cary, North Carolina, USA). Differences between paired sites were analyzed using 2-tailed paired *t* test comparisons. In all comparisons a *P* value less than 0.05 was used to indicate statistical significance.

Results

Clinical and histopathological response to rhIL-11 treatment in psoriatic patients. Twelve patients with psoriasis vulgaris were treated with daily subcutaneous injections of rhIL-11 for 8 weeks. Eleven of 12 patients had reductions in clinical disease severity ranging from 20 to 80%, as judged by the PASI score. Mean reductions in disease severity were statistically significant at all points analyzed from weeks 2 through 8 following rhIL-11 treatment. Figure 1 illustrates 2 patients with marked reductions in erythema, scaling, and induration in psoriatic skin lesions after 8 weeks of treatment with rhIL-11. A 50% drop in the PASI score from 22.6 to 11.7 was observed in patient A following rhIL-11 treatment. Similarly, an 80% reduction in the PASI score from 19.8 to 3.5 was observed in patient B.

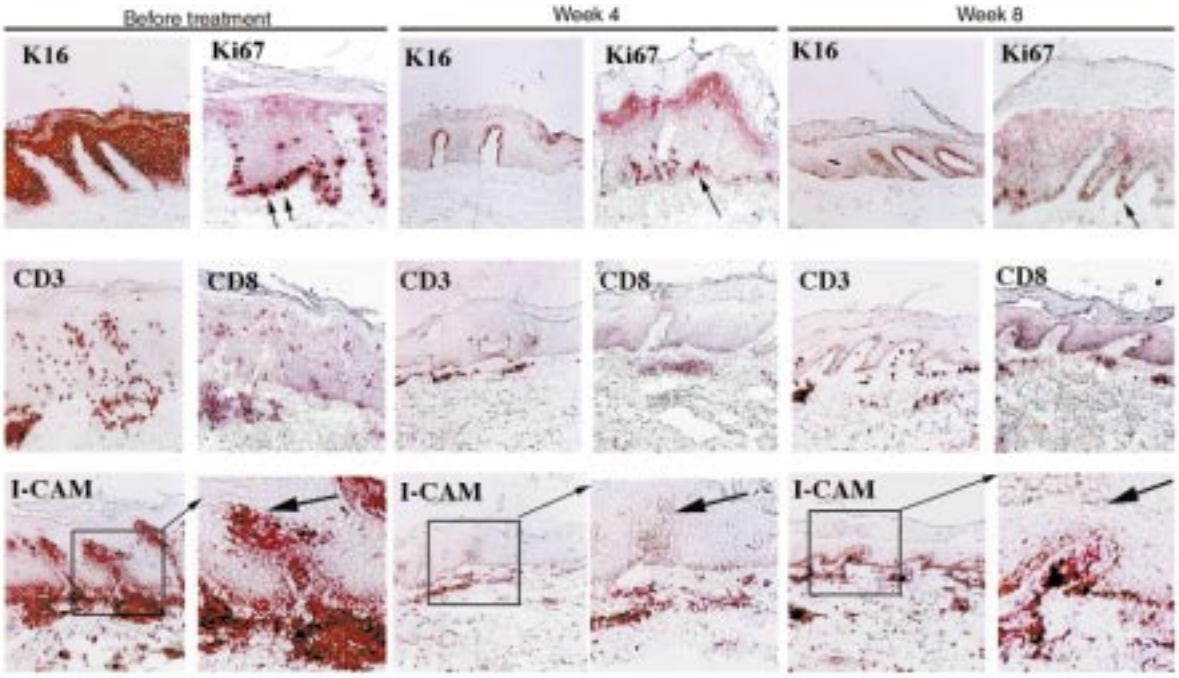

Figure 2
Six-millimeter punch biopsies were obtained from lesional and nonlesional skin of a patient before treatment with rhIL-11 (pretreatment) and at weeks 1, 4, and 8 following daily treatment with 2.5 mg/kg of rhIL-11. Biopsies were equally divided for immunohistochemical analysis and RNA preparation. Frozen biopsies from pretreatment, week 4, and week 8 were sectioned and stained with antibodies to Ki67$^+$, K16, CD3, CD8, and ICAM-1. Enlargements of ICAM-1 staining (boxes) show elimination of its production by epidermal keratinocytes during IL-11 administration (large arrows).

To rigorously quantify disease improvement, serial biopsies from an indicator plaque were used to measure disease-related pathology in each patient during IL-11 administration. Representative histopathology of a responding patient is shown in Figure 2. Based on this analysis (Figure 2 and Table 1), we found that 7/12 patients had remarkable disease reduction as defined by consistent decreases in (a) epidermal hyperplasia (epidermal thickness, number of proliferating [Ki67$^+$] keratinocytes, and expression of K16 in suprabasal keratinocytes); (b) number of T cells in skin lesions; and (c) ICAM-1 production by epidermal keratinocytes. The 7 patients with consistent improvements in each of these histologic parameters at the end of 8 weeks of rhIL-11 treatment were listed as "responders" in Table 1. In contrast, 5 patients had smaller or less-consistent improvements in disease-defining histopathology, and these patients were labeled as "nonresponders" in Table 1.

Responding patients showed marked improvement in several measures of pathological epidermal hyperplasia (epidermal acanthosis, keratinocyte proliferation, and synthesis of keratin) (17). Epidermal thickness was reduced on average by 40%, which represents about a 60% decrease in pathological acanthosis. Keratinocyte proliferation was reduced by a mean of 66% at 8 weeks of treatment. Importantly, synthesis of K16, which is produced only by "regenerative" or hyperplastic keratinocytes, was eliminated in suprabasal keratinocytes in all responding patients (Figure 2 and Table 1). Hence, homeostatic epidermal growth was restored in all 7 responding patients, and quantitative reductions in epidermal thickness and keratinocyte proliferation were statistically significant at 8 weeks (Table 1). Proliferative decreases in epidermal keratinocytes were accompanied by marked reductions in T lymphocytes infiltrating the epidermis by 4 weeks of treatment. Mean reductions in intraepidermal T cells and total lesional T cells averaged 50 to 75% at weeks 4–8 of treatment and were statistically significant ($P <$ 0.01) by 4 weeks of treatment (Table 1). Synthesis of ICAM-1 by epidermal keratinocytes (Figure 2) was eliminated by 8 weeks of treatment in all responders, and, importantly, reduced expression of this adhesion molecule was detected as early as 1 week of treatment with IL-11, at a time when T-cell numbers were not reduced in lesional tissue (Table 1). In contrast, no statistically significant improvements in epidermal growth parameters or T-cell infiltration of lesions were measured in nonresponders (Table 1).

Expression patterns of disease-related genes in lesional and uninvolved skin. The designation of responders and nonresponders was created principally to determine how alterations in expression of a variety of inflammation-associated genes that could potentially be regulated by IL-11 would relate to major changes in cellular features of tissue inflammation or clinical disease activity. For example, reduced production of ICAM-1 by epidermal keratinocytes at 1 week after starting rhIL-11 treatment, but no major changes in the number of T cells in lesional tissue (Table 1), suggests that T cells in skin

lesions produce less IFN-γ or that keratinocyte responsiveness to this cytokine is altered (31). Accordingly, a comprehensive survey of expression patterns of genes involved in the pathogenesis of this disease and the effect of rhIL-11 on this expression pattern was undertaken using a new quantitative RT-PCR technique.

Punch biopsies of psoriatic plaques and noninvolved skin from the 6 initial patients were obtained and analyzed for gene-expression differences between lesional and nonlesional skin by RT-PCR. Quantitative mRNA levels were determined using an ABI 7700 sequence detection system and all values were normalized to a housekeeping gene, human acidic ribosomal protein (HARP) mRNA, that was coamplified during each PCR run. Based on initial experiments evaluating the reproducibility of the sequence detection system as well as patient-to-patient variability, changes in mRNA expression levels of 2-fold or greater for lesional versus nonlesional skin were deemed to be significant (W. Trepicchio, unpublished observations). Where possible, changes in gene expression were compared with changes in immunohistochemical markers of protein expression (taken from adjacent skin) to further confirm expression data.

A total of 32 potentially disease-associated genes, as well as 3 housekeeping genes, were analyzed. Twenty-four of these 32 genes were found to provide consistent results across all patients and were considered for further analysis in biopsies from rhIL-11–treated patients. Expression levels of many genes known to play or thought to play a role in the disease process, such as proinflammatory markers, leukocyte surface markers, and keratinocyte hyperplasia markers displayed consistently higher expression levels in lesional psoriatic tissue compared with uninvolved (nonlesional) skin (see Figure 3). For example, 2-fold to greater than 10-fold elevations in mRNA levels of the p40 subunit of IL-12, IFN-γ, TNF-α, IL-1β, IL-8, and iNOS were observed in psoriatic lesions compared with nonlesional skin (Figure 3a).

A number of leukocyte markers such as CD4, CD8, CD25, CD80, and IL-12Rβ2 mRNA levels were also elevated from 2- to 5-fold in psoriatic tissue compared with uninvolved skin (Figure 3b). The elevated level of these transcripts correlates with increased numbers of activated $CD3^+$, $CD4^+$, and $CD8^+$ T cells in psoriatic lesions as measured by histologic analysis (Table 1 and Figure 2). In addition, elevated expression levels of genes associated with keratinocyte hyperplasia (K16 and KGF) were observed in lesional skin versus nonlesional skin (Figure 3c). Increased expression of these genes is in agreement with established differences in protein expression of K16 and KGF in psoriatic skin lesions (14, 15).

In contrast to inflammatory mediators, levels of a number of other genetic markers were unchanged. For example, mRNA levels for the Th2 cytokine, IL-4, was not significantly different between lesional and nonlesional skin (Figure 3d). Coupled with the previously

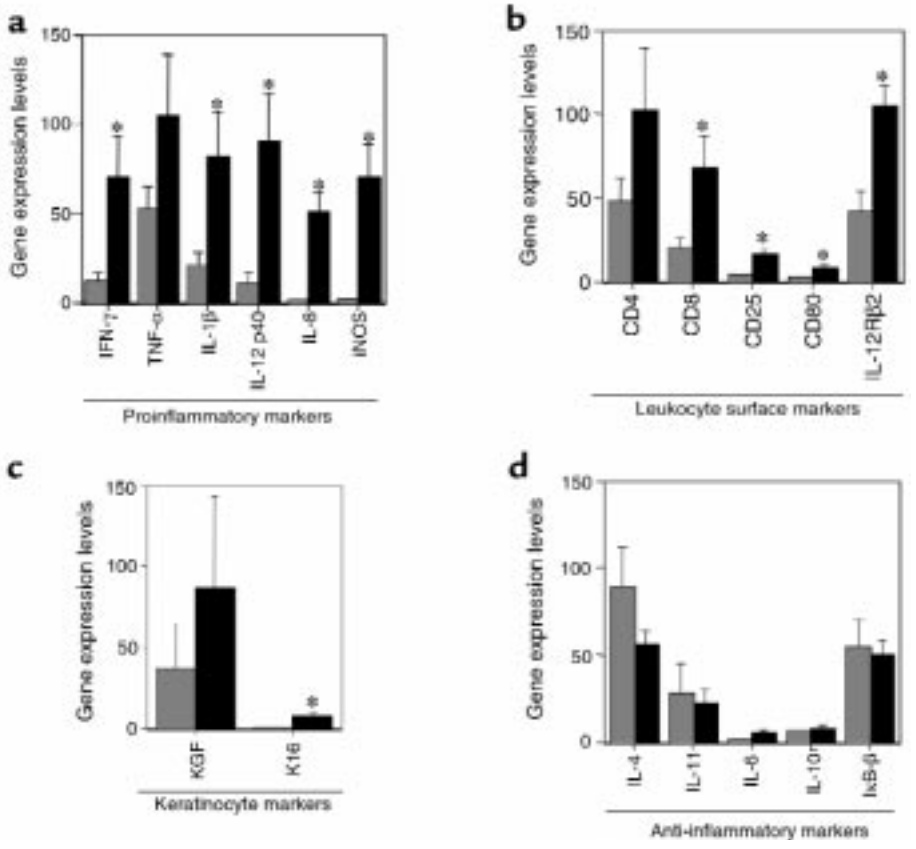

Figure 3
RNA was prepared from nonlesional and lesional skin of 6 untreated psoriasis patients. The mRNA was amplified for the indicated genes by quantitative RT-PCR. Levels of RNA were normalized to HARP. Average expression levels of nonlesional and lesional skin for all 6 patients are presented ± SEM. *Statistically significant differences ($P < 0.05$) between nonlesional and lesional skin; filled box, lesion; shaded box, nonlesion.

Table 2

Gene	Group	Psoriatic lesion	Week 1 IL-11	Week 4 IL-11	Week 8 IL-11
TNF-α	R	2.5 ± 1.3	1.4 ± 0.4	1.3 ± 0.2	1.2 ± 0.2
TNF-α	NR	1.7 ± 0.6	1.6 ± 0.6	2.2 ± 0.9	1.9 ± 0.9
B7.1	R	15.4 ± 8.2	12.5 ± 6.9	7.5 ± 4	7.4 ± 5.4
B7.1	NR	14.9 ± 7.2	9.7 ± 3.1	8.3 ± 1.8	16.9 ± 8
IL-12Rβ1	R	1.8 ± 0.3	2.9 ± 1.0	1.8 ± 0.3	1.8 ± 0.5
IL-12Rβ1	NR	1.4 ± 0.5	0.99 ± 0.3	1.5 ± 0.5	2.1 ± 0.6
IL-12Rβ2	R	5.4 ± 3.3	4.3 ± 0.9	4.1 ± 1.2	2.2 ± 0.5[A]
IL-12Rβ2	NR	3.4 ± 1.0	3.1 ± 0.5	3.4 ± 0.5	6.3 ± 2.9
IL-10	R	1.7 ± 0.4	2.5 ± 1.1	1.6 ± 0.4	2.2 ± 0.6
IL-10	NR	3.1 ± 0.7	3.1 ± 1.3	5.4 ± 1.6	10.1 ± 7.2
IL-11	R	1.3 ± 0.2	1.0 ± 0.2	1.8 ± 0.7	2.8 ± 1.1[A]
IL-11	NR	2.1 ± 0.9	0.9 ± 0.2	2.2 ± 1.3	1.4 ± 0.5

RNA was prepared from nonlesional and lesional skin of 12 psoriasis patients before and at weeks 1, 4, and 8 following daily administration of rhIL-11. Twenty-five nanograms of RNA was amplified for the indicated genes. Levels of mRNA were normalized to the housekeeping gene, *HARP*. Fold changes in mRNA levels between nonlesional and lesional skin before and after rhIL-11 treatment were calculated for each gene. Average fold changes over nonlesional skin ± SEM for R and NR patients are indicated at various time points following rhIL-11 administration. R, responding; NR, nonresponding. [A]Statistically significant differences between lesional skin before and after rhIL-11-treatment ($P < 0.05$).

demonstrated 10-fold elevation in IFN-γ production, these results indicate that the ratio of IL-4 to IFN-γ mRNA is altered in lesional skin and suggests a dominant Th1 T-cell response is found in the psoriatic tissue. Anti-inflammatory cytokine IL-10, IL-11, and IL-6 mRNA was found at comparable levels in uninvolved and lesional skin (Figure 3d). In addition, mRNA levels for IκB-β, the inhibitor of the proinflammatory transcriptional inducer, NF-κB, were not changed between nonlesional and lesional skin (Figure 3d). An NK cell marker, CD56; a Langerhans/dendritic cell marker, CD1a; and a T-cell costimulatory molecule, CD86, were present in all samples but were not differentially expressed in disease tissue (data not shown).

Several genes did not demonstrate consistent changes in all patients examined and were not evaluated further. A number of T-cell signal transducers, such as NFAT-1, STAT-1, and STAT-4; cell surface markers, such as CD40, CD122, and ICAM-1; the cell cycle and apoptosis regulator, p53; and the inflammatory cytokine, IL-18, were not differentially expressed in a consistent pattern across all patients (data not shown). These results could be due to the small patient sample size or a lack of transcriptional regulation of these genes. For example, the STAT genes are primarily activated by posttranslational phosphorylation events (reviewed in refs. 32, 33).

Pharmacogenomic analysis of skin lesions of rhIL-11 treated patients. From the panel of disease-related genes identified above, we sought to identify genetic markers of psoriatic pathology that change following rhIL-11 treatment. These markers would then be correlated with patient responsiveness as determined by histopathological criteria. Total RNA was prepared from one-half of the biopsy that was taken for im-munohistochemical study and analyzed by quantitative RT-PCR. Comparisons were made in the levels of mRNA between normal skin and psoriatic lesions before and during treatment with rhIL-11. Levels of expression of 14 disease-associated genes were analyzed following patient treatment with rhIL-11 and are presented below. Consistent effects of rhIL-11 on expression levels of another 4 genes, CD86, CD56, CD1a, and KGF, were not observed and data are not presented.

Data showing key disease-related genes from a representative responding patient are presented in Figure 4. The levels of TNF-α, IFN-γ, IL-12 p40, IL-8, and iNOS mRNA in the lesional tissue decreased as early as 1 week following rhIL-11 treatment. This observed effect of rhIL-11 on gene expression preceded any significant clinical changes. At the end of 8 weeks of treatment, these inflammatory markers were significantly reduced compared with pretreatment levels (Figure 4). CD8 mRNA levels also decreased following rhIL-11 treatment, and this result correlated with decreased $CD8^+$ cells detected by immunohistochemistry. Levels of IL-4 mRNA increased slightly after rhIL-11 treatment (Figure 4). As a percentage of total T cells present in the lesion (as measured by CD3 staining and CD4 or CD8 expression) IL-4 mRNA levels actually increased following rhIL-11 treatment. Finally, levels of K16 mRNA, which were elevated in lesional versus uninvolved skin, were significantly reduced following rhIL-11 treatment. This change in K16 levels also correlated with changes in immunohistochemical staining following rhIL-11 treatment (Figure 2). These changes in gene expression also correlated with an improvement in the lesion severity score from 8 to 0 in this patient over the 8-week treatment period. In contrast to the responding patients, 5 patients designated as nonresponders by histologic analysis failed to show consistent and sustained reductions in proinflammatory gene transcripts (data summarized in Figure 5). This lack of a response at the genetic level also correlated with the absence of histologic improvement (Table 1).

A summary of gene-expression profiles for key genes in all responders and nonresponders before and during rhIL-11 treatment is presented in Figure 5 and Table 2. Data are represented as the average fold change of lesional skin over nonlesional skin so that a comparison across all patients can be made. Selective downregulation of iNOS, IFN-γ, IL-8, IL-12 p40, CD8, and K16 genes occurred in 7 responding patients during rhIL-11 treatment (Figure 5). Importantly, reduced production of mRNA for proinflammatory cytokines such as IFN-γ, IL-12 p40, and TNF-α was detected as early as 1 week after starting IL-11 treatment, a time point when no significant overall reductions in T lymphocytes in skin lesions had occurred (compare Table 1 with Figure 5 and Table 2). Statistically significant reductions in iNOS, IFN-γ, IL-8, IL-12 p40, K16, CD80, and IL-12Rβ2 mRNAs were measured after 4–8 weeks of treatment with rhIL-11. In contrast, levels of IL-4 and IL-10 mRNA remained constant over the course of treatment in responding patients. Of particular interest, levels of IL-11 mRNA actually increased in responding patients (Table 2).

These data suggest that rhIL-11 decreases production of proinflammatory molecules by T cells and other leukocytes soon after initiation of treatment, whereas continued administration of rhIL-11 for several weeks also decreases T-cell trafficking into psoriatic skin lesions. Progressive reductions in epidermal hyperplasia, as well as decreased synthesis of ICAM-1 and K16 by keratinocytes, during 8 weeks of treatment with IL-11 are probably best explained by the combination of reduced levels of proinflammatory cytokines in skin and the presence of fewer T cells, especially those within the epidermis. After 4–8 weeks of treatment, there is clearly an altered balance of type 1 versus type 2 T cells within skin lesions, as judged by a change in the IFN-γ/IL-4 ratio. Although this change could reflect outward trafficking of type 1 T cells, we noted a decrease in peripheral T cells producing IFN-γ and an increase in cells producing IL-4 by intracellular staining in a number of patients during rhIL-11 treatment (data not shown). Hence, cytokine mRNA expression profiles may also indicate an effect of rhIL-11 on differentiation of type 1 versus type 2 T cells.

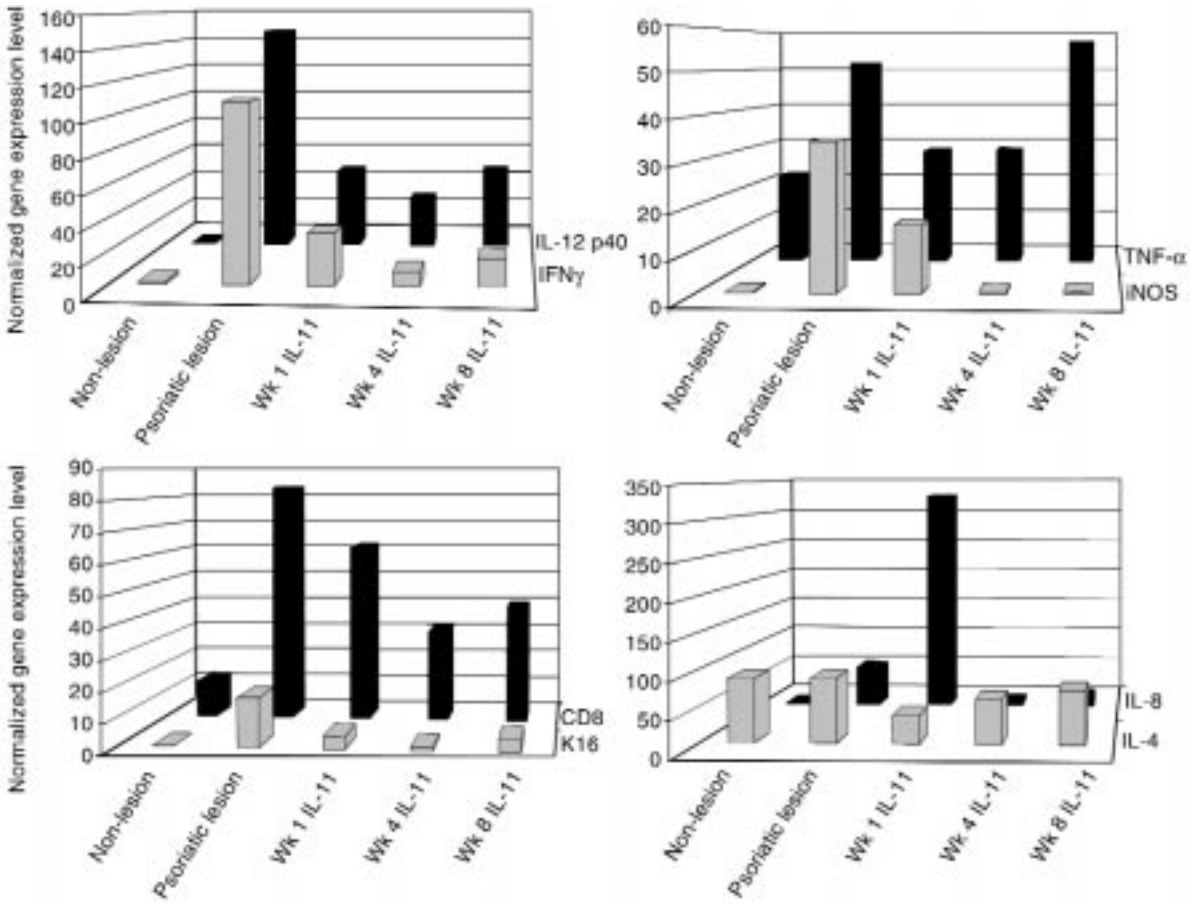

Figure 4
Six-millimeter punch biopsies were obtained from lesional and nonlesional skin of a responding patient before treatment with rhIL-11 (pretreatment) and at weeks 1, 4, and 8 following daily treatment with 2.5 mg/kg of rhIL-11. Biopsies were equally divided for immunohistochemical analysis and RNA preparation. RNA was prepared from nonlesional skin and lesional skin from pretreatment, week-1, week-4, and week-8 biopsies. The mRNA for IL-12 p40, IFN-γ, TNF-α, iNOS, CD8, IL-8, IL-4, and K16 were amplified by quantitative RT-PCR. Levels of mRNA were normalized to HARP to control for variations in starting RNA amounts.

Table 3

	IFN-γ			iNOS			IL-8			K16			IL-4		
Patient no.	Lesion	Week 1	Week 4	Lesion	Week 1	Week 4	Lesion	Week 1	Week 4	Lesion	Week 1	Week 4	Lesion	Week 1	Week 4
1 NR	3.1	2.0	3.7	107	96	174	374	353	102	109	65	75	0.4	0.3	0.23
2 R	6.0	1.9	1.4	111	8.8	1.5	90	2.0	1.3	95	2.7	1.2	0.63	0.81	1.3
3 R	12.3	6.8	1.4	320	14	0.8	363	5	1.7	94	24.5	1.6	0.3	0.5	0.7

RNA was prepared from nonlesional and lesional skin of 3 psoriasis patients before and following daily administration of 5mg/kg of cyclosporin A. Patients were characterized as R or NR to cyclosporin A treatment based on clinical and histological scores. Twenty-five nanograms of RNA was amplified for the indicated genes. Levels of mRNA were normalized to the housekeeping gene, *HARP*. The level of RNA present in nonlesional skin was arbitrarily set to a value of 1. Fold changes in mRNA levels between nonlesional and lesional skin before and after 1 and 4 weeks of cyclosporin A treatment were calculated for each gene. R, responding; NR, nonresponding.

To compare the effects of rhIL-11 to another immunomodulatory drug, gene-expression analysis of psoriatic lesions before and during cyclosporin A treatment was also performed. Lesions from 3 cyclosporin A–treated patients were analyzed before and at 1 and 4 weeks of therapy. On the basis of clinical and histological analysis, 2 patients were considered responding to therapy and 1 was considered nonresponding (data not shown). Similar to rhIL-11 treatment, patients responding to cyclosporin A treatment demonstrated significant reduction in levels of IFN-γ, iNOS, IL-8, and K16, stable expression of CD8 and elevated levels of IL-4 mRNA in resolving lesions compared with pretreatment levels (Table 3). These changes were not observed in the nonresponding patient (Table 3).

A comparison of gene-expression levels in lesional tissue of responding versus nonresponding populations before rhIL-11 treatment allows a retrospective analysis of differences in the patient population that may explain differential responsiveness to therapy. For example, differential patient responsiveness to rhIL-11 could be due to low-level expression of the IL-11 receptor measured in nonresponding patients On average, lower levels of IL-11R α chain mRNA was observed in nonresponding patients, but this difference was not statistically significant. In addition, lower iNOS and IL-8 mRNA levels and higher IL-12 p40 and K16 levels are observed at baseline in the lesional skin of responding patients versus the lesional skin of nonresponding patients, but only K16 and IL-8 differences were statistically significant. Further analysis of this patient population using broader gene expression arrays is in progress to identify patterns of expression correlating with responsiveness to rhIL-11.

Discussion

Psoriasis is one of the most common immune-mediated diseases in humans, affecting 2 to 3% of the population. It is a complex inflammatory disease in which several distinct types of leukocytes, cytokines, and keratinocyte growth/differentiation abnormalities are aberrantly regulated in skin lesions. In this study we report results from a small group of patients with severe psoriasis who were treated with daily subcutaneous injections of rhIL-11 for 2 months in an initial safety study. Of the 12 patients treated with low doses of rhIL-11, 7 showed marked improvement of psoriatic skin lesions following rhIL-11 administration. Effects of IL-11 on disease-related inflammation was assessed by standard clinical scoring of target lesions and quantitative disease measurement by histopathology. Responding patients showed clear reductions in clinical PASI scores as well as reductions in pathological epidermal hyperplasia ($Ki67^+$ and $K16^+$ keratinocytes), reduced expression of ICAM-1 on epidermal keratinocytes, and reduced numbers of infiltrating T lymphocytes in skin lesions.

A detailed analysis of gene expression before and after immunotherapy has not been performed in psoriasis patients. In this study, using quantitative RT-PCR, altered expression of many genes associated with an inflammatory response and T-cell activation were identified in psoriatic tissue. The expression of these genes is consistent with the pathology of psoriasis and indicates that gene-expression profiling has the potential to identify dysregulated genes in complex disease tissue. Furthermore, modifications in this expression profile correlated with clinical improvement of lesions following treatment with rhIL-11 and cyclosporin A.

Consistent with the involvement of T cells in this disease, we have detected increased mRNA levels in psoriatic tissue for IL-12, IFN-γ, CD80, and the β2 subunit of the IL-12 receptor complex (34–36). IL-12 stimulates the differentiation of naïve T cells to the Th1 lineage resulting in the production of IFN-γ (37). Elevated IL-12 production has also been implicated in a number of inflammatory disease models including colitis, sepsis, and multiple sclerosis (38–40). Increased expression of these genes in psoriatic lesions is consistent with the hypothesis that the disease is Th1 T-cell mediated and implicates a role for IL-12, IFN-γ, CD80, and for the first time the IL-12Rβ2 gene in the pathogenesis of psoriasis.

Patient responsiveness to IL-11 therapy has allowed a detailed analysis of the mechanism through which rhIL-11 ameliorates psoriasis. A primary component of this mechanism may be through a reduction in the activation of Th1 lymphocytes. In numerous animal models and in vitro systems, rhIL-11 has shown im-munomodulatory activity on both T cells and macro-phages to

suppress immune-mediated tissue damage (17, 19, 22, 23, 25). It has been shown that rhIL-11 blocks IL-12 expression at the transcriptional level in macrophages through the inhibition of NF-κB nuclear translocation (18, 19). Inhibition of IL-12 expression would suppress IFN-γ production and Th1 differentiation. In addition to effects on macrophages, rhIL-11 directly blocks IL-12–induced Th1 differentiation and IFN-γ production and enhances Th2 differentiation (W. Trepicchio, unpublished observations).This finding is consistent with an in vivo model of GVHD, whereby administration of rhIL-11 polarized T cells to a Th2 response and increased IL-4 secretion (25). In the responding patients in our study, the mRNA levels of IL-12 and IFN-γ were reduced in the target lesion. Furthermore, IL-12Rβ2 and CD80 levels were significantly reduced in responding patients over the course of rhIL-11 treatment. This is consistent with rhIL-11 inhibition of the Th1 phenotype. The observation that rhIL-11 treatment can reduce expression levels of the IL-12Rβ2 chain is a novel finding for an anti-inflammatory molecule.

Although intraepidermal T lymphocytes were reduced by 75%, on average, following 8 weeks of therapy in patients responding to rhIL-11 treatment, reduced expression of IFN-γ mRNA was often observed as early as 1 week of treatment, when little change in overall T-cell numbers was measured. Given that mRNA levels for IL-4 were slightly increased and levels of CD80 IL-10 and IL-12Rβ1 were not decreased in resolving psoriasis lesions, it is unlikely that changes in cytokine expression in skin lesions are only due to effects of IL-11 on T-cell trafficking. Instead, the overall data suggest that rhIL-11 treatment deviated the cytokine environment toward a type II response, but this alteration was characterized more by a reduction in IFN-γ levels than large increases in IL-4 or IL-10. Taken together, these data indicate that rhIL-11 treatment may ameliorate psoriasis through direct and indirect effects on T cells to change the cytokine milieu to a Th2-type response.

Overall, the effects of IL-11 on cytokine expression are different from those of another immunomodulatory agent, FK506, which is efficacious in the treatment of psoriasis. This agent reduces T-cell maturation and hence production of both Th1 and Th2 cytokines (41–43). Interestingly, in this study cyclosporin A treatment was found to preferentially affect Th1 cytokine production. The effects of rhIL-11 are also different from IL-10 in that production of type II cytokines is

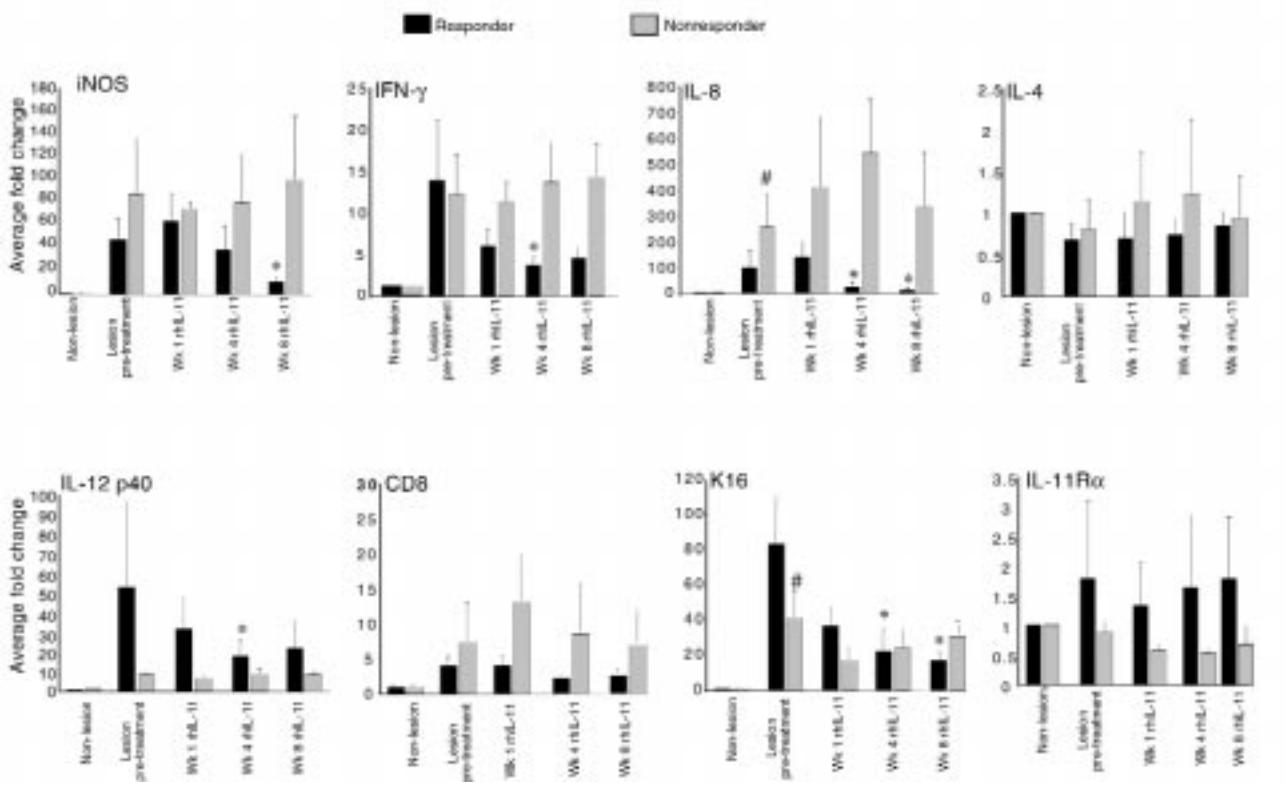

Figure 5
RNA was prepared from nonlesional skin biopsies and lesional skin biopsies of 12 patients before treatment (pretreatment) and at week 1, week 4, and week 8 following daily treatment with 2.5 or 5.0 mg/kg of rhIL-11. Quantitative RT-PCR was performed on individual samples for the indicated genes. Gene expression levels were normalized to HARP. Levels of gene expression observed in the nonlesional skin of each patient were arbitrarily set to equal 1, and the fold change in expression in lesional skin before and after treatment with rhIL-11 over nonlesional skin was calculated. Average fold change for rhIL-11–responding (n = 7) and rhIL-11–nonresponding (n = 5) patients was calculated. Data are presented as the average fold change over nonlesional skin ± SEM. *Statistically significant differences between pretreatment and rhIL-11 treatment ($P < 0.05$); #statistically significant differences between responder and nonresponder pretreatment lesions ($P < 0.05$).

increased by IL-10, but IFN-γ production is undiminished (44). Lesions of patients treated with IL-10 have been reported to contain elevated levels of IL-10 mRNA (44), but no increase of IL-10 mRNA was detected in the rhIL-11 treated patients. Hence, there is no evidence that disease improvements produced by rhIL-11 in our psoriatic patients are mediated indirectly via increased production of IL-10. Interestingly, responding patients demonstrated a statistically significant increase in the levels of endogenous IL-11 mRNA following 8 weeks of rhIL-11 treatment. We have previously reported that IFN-γ inhibits IL-11 production from macrophages (19). Therefore, the inhibition of IFN-γ production by rhIL-11 in responding patients may actually result in localized induction of endogenous IL-11 that may further enhance the healing process.

In addition to effects of rhIL-11 on lymphocyte marker mRNA levels, changes in expression of other disease-related genes that encode cytokines, inflammatory mediators, and keratinocyte-response proteins were detected, such as IL-8, iNOS, TNF-α, and K16. Previous studies have reported that IL-8 and its receptor are overexpressed in psoriatic skin (45, 46). IL-8 is a chemotactic factor for neutrophils, and, along with IFN-γ, IL-8 has been shown to induce HLA-DR expression in keratinocytes (47). Whereas under normal circumstances keratinocytes do not express HLA-DR, its expression is observed in several skin disorders (48). Aberrant expression of HLA-DR on keratinocytes may result in antigen presentation leading to induction of a T cell response. In responding patients, rhIL-11 treatment resulted in a clear reduction in IL-8 and IFN-γ mRNA levels. Consistent with the role of IL-8 and IFN-γ in inducing HLA-DR expression, HLA-DR expression was also decreased in the lesions of these responding patients as detected by immunohistochemistry (data not shown).

IL-8 and IFN-γ, in combination, induce another proinflammatory mediator, iNOS. Nitric oxide production by iNOS has been associated with inflammatory tissue injury in animal models and human disease (49). As described previously (50), we observed elevated iNOS expression in psoriatic lesions. Treatment with rhIL-11 decreased these levels to that observed in noninvolved skin. Nitric oxide production by the Langerhans cells and keratinocytes may play a role in psoriasis to stimulate keratinocyte proliferation and recruitment of T cells (51); rhIL-11 has been shown to inhibit nitric oxide production from LPS-stimulated macrophages (17) and in an in vivo rabbit endotoxemia model (52). Because Langerhans cells are specialized epidermal macrophages, rhIL-11 may have direct effects on the modulation of nitric oxide production from Langerhans cells and/or through the downregulation of proinflammatory cytokines such as IL-8, TNF-α, and IFN-γ, which induce iNOS expression.

A utility of gene-expression profiling is the potential ability to predict patient responsiveness to a new agent. For example, the current data indicate that lower iNOS and IL-8 mRNA levels and higher IL-11Rα, IL-12 p40, and K16 levels are observed in the lesional skin of responding patients versus the lesional skin of nonresponding patients. Genetic screening of responding and nonresponding patients using high-density arrays is currently underway to determine whether differential expression of other genes (or gene combinations) in individual patients can potentially predict responsiveness to rhIL-11 or other therapeutic agents. Clearly, this potential will need to be tested in future clinical trials.

In conclusion, this pharmacogenomic approach has elucidated a potential mechanism of rhIL-11 activity in psoriasis patients and has identified several surrogate markers of disease activity that may be more sensitive than histologic or clinical evaluation can measure. Of particular note, many changes in gene expression following rhIL-11 treatment preceded histologic or clinical measures of disease activity and were well correlated with subsequent cellular changes in skin lesions, thus might provide the earliest indicators of eventual clinical response to this agent or other immune modifiers. These are the first data in humans that a chronic type I–predominant inflammatory disease can be counterregulated by any immune-modifying agent such as rhIL-11.

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