An Anti-IL-12p40 Antibody Down-Regulates Type 1 Cytokines, Chemokines, and IL-12/IL-23 in Psoriasis

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An Anti-IL-12p40 Antibody Down-Regulates Type 1 Cytokines, Chemokines, and IL-12/IL-23 in Psoriasis

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Psoriasis is characterized by activation of T cells with a type 1 cytokine profile. IL-12 and IL-23 produced by APCs are essential for inducing Th1 effector cells. Promising clinical results of administration of an Ab specific for the p40 subunit of IL-12 and IL-23 (anti-IL-12p40) have been reported recently. This study evaluated histological changes and mRNA expression of relevant cytokines and chemokines in psoriatic skin lesions following a single administration of anti-IL-12p40, using immunohistochemistry and real-time RT-PCR. Expression levels of type 1 cytokine (IFN-γ) and chemokines (IL-8, IFN-γ-inducible protein-10, and MCP-1) were significantly reduced at 2 wk posttreatment. The rapid decrease of these expression levels preceded clinical response and histologic changes. Interestingly, the level of an anti-inflammatory cytokine, IL-10, was also significantly reduced. Significant reductions in TNF-α levels and infiltrating T cells were observed in high responders (improvement in clinical score, ≥75% at 16 wk), but not in low responders. Of importance, the levels of APC cytokines, IL-12p40 and IL-23p19, were significantly decreased in both responder populations, with larger decreases in high responders. In addition, baseline levels of TNF-α significantly correlated with the clinical improvement at 16 wk, suggesting that these levels may predict therapeutic responsiveness to anti-IL-12p40. Thus, in a human Th1-mediated disease, blockade of APC cytokines by anti-IL-12p40 down-regulates expression of type 1 cytokines and chemokines that are downstream of IL-12/IL-23, and also IL-12/IL-23 themselves, with a pattern indicative of coordinated deactivation of APCs and Th1 cells. The Journal of Immunology, 2006, 177: 4917–4926.

Psoriasis vulgaris is a chronic inflammatory skin disease with a complex pathophysiology. The cellular and molecular mechanisms underlying the disease involve interrelationships between hyperplastic epidermal keratinocytes and infiltrating leukocytes, including memory T cells, neutrophils, dendritic cells (DCs), and macrophages (1–3). The clinical efficacy of several interventions targeting T cells, including cyclosporine A (4), Denileukin Diftitox (5), CTLA4Ig (6), and Alefacept (LFA-3 TIP) (7) demonstrates a critical role of T cell activation in psoriasis, specifically, that of memory/effector cells (8). Research using AGR129 mice demonstrated that retained T cells in tissue uninvolved by psoriasis could proliferate, leading eventually to the development of psoriatic plaques (9). Studies demonstrating increased activity of lesional APCs (9, 10), their apposition to psoriatic T cells undergoing activation (2), their reduction during cyclosporine A therapy (11), and the efficacy of intralesional cyclosporine A (12) all attest to the ongoing and critical role of intralesional T cell activation.

Many studies examining the cytokine profile of T cells derived from psoriatic lesions suggest that the type 1 cytokine-producing T cell subset is a prominent component of the lesions (13–17). Psoriatic lesions showed elevated mRNA expression for type 1 cytokines (IFN-γ, IL-2, and TNF-α), compared with lesion-free psoriatic skin and normal skin, without a significant component of type 2 cytokines (IL-4, IL-5, and IL-10) (18). IFN-γ was shown to be an important cytokine involved in the ability of lesional T cell clones to induce psoriatic uninvolved basal keratinocytes to enter the cell cycle (13). However, the presence of IFN-γ does not result that its role is critical; in experimental autoimmune encephalomyelitis, for example, treatment with anti-IFN-γ Abs does not result in a decrease in clinical severity (19, 20). Further implicating the likely involvement of Th1 cells is the fact that inhibition of TNF-α, a cytokine that is produced (although not exclusively) by Th1 cells, results in clinical improvement of psoriasis (21, 22).

IL-12, a heterodimer formed from p40 and p35 subunits, is a key cytokine in Th1 differentiation and proliferation (23–26). Produced by activated APCs (macrophages and DCs, including human epidermal Langerhans cells), IL-12 strongly promotes differentiation of naïve T cells into IFN-γ-producing Th1 cells (25, 27–29). It has been reported that both IL-12p40 mRNA and IL-12p70 heterodimer are increased in psoriatic skin lesions (30). IL-23, a recently discovered member of the IL-12 family, is a disulfide-bridged complex of a unique p19 subunit and the p40 subunit of IL-12 (26, 31). IL-23 possesses similar as well as distinct properties from those of IL-12. IL-23 is particularly efficient at inducing the proliferation of memory Th1 cells (31). IL-23p19 transgenic mice demonstrate multiorgan inflammation, including skin (32). Keratin 14/p40 transgenic mice that produce IL-23, but not IL-12,
demonstrate skin inflammation (33), and unlike IL-12, the expression of IL-23 is critical for experimental autoimmune encephalomylitis (34). Target organ expression of IL-23, consistent with its production by macrophages and microglia, is thought to be critical for its role (35), and importantly, IL-23p19 mRNA is increased in psoriatic skin (36).

Early work in murine models indicates that administration of Ab specific for the IL-12p40 subunit (anti-IL-12p40) can prevent and also ameliorate autoimmune-like conditions (37–44). Anti-IL-12p40 abolished the development of psoriasis in mice, even when administered after transfer of the T cell subset that induced the psoriasis-like skin disorder, and led to the down-regulation of IFN-γ and TNF-α (44). This is an important finding that suggests that anti-IL-12p40 may be a valuable treatment strategy against psoriasis, as well as a good tool in determining whether activated T cells critical for psoriasis are indeed Th1 cells and IL-12/IL-23 dependent.

Anti-IL-12p40 used in our study is a human IgG1 κ mAb that binds to the p40 subunit of human IL-12 and IL-23 and prevents its interaction with IL-12Rβ1. This first-in-human study of the administration of anti-IL-12p40 in patients with moderate-to-severe psoriasis vulgaris, showed remarkable clinical responses (45). Safety results showed that anti-IL-12p40 was well tolerated, and only transient variable decreases in CD4+ T cells and CD16+CD56+ NK cells were noted in some patients (45).

The study presented here was intended to evaluate histology and cytokine expression in psoriatic skin, before and after anti-IL-12p40 administration, and assess the association with clinical response. The objectives were 2-fold: first, to evaluate the early effect of anti-IL-12p40 administration on cytokine and chemokine expression in an inflammatory pathway; and second, to elucidate the molecular mechanism of psoriasis and reveal the mechanism of action for anti-IL-12p40 therapy in psoriasis.

Materials and Methods

Study protocol and patient eligibility

Patients with moderate-to-severe plaque psoriasis, diagnosed at least 6 mo before screening, involving at least 3% body surface area with at least two plaques located on either the trunk or extremities, were enrolled in the first-in-human, phase 1, open-label, single administration, dose-escalating study of anti-IL-12p40. Details of the study design as well as the safety, pharmacokinetic, and clinical response results are reported elsewhere (45). Each patient was assigned to one of four dose groups and received a single i.v. infusion of 0.1 mg/kg (n = 4), 0.3 mg/kg (n = 4), 1.0 mg/kg (n = 5), or 5.0 mg/kg (n = 5) of anti-IL-12p40, administered over a minimum of 120 min.

The study was conducted in conformance with the regulations established for the Protection of Human Subjects (21 Code of Federal Regulations, Part 50) and Institutional Review Boards (21 Code of Federal Regulations, Part 56), in accordance with Good Clinical Practices, and in compliance with local regulations. Before any study-specific procedures were performed, all patients provided written informed consent.

Clinical response assessments

Clinical response was evaluated using the Psoriasis Area and Severity Index (PASI) score (46). The PASI combines scores for the degree of lesional erythema, induration, and desquamation, and the percentage of body surface area affected. PASI scores were assessed at baseline, 72 h, and at 1, 2, 4, 8, 12, and 16 wk after administration of anti-IL-12p40.

Skin biopsy

A psoriatic lesion, located on the trunk or extremities, was identified by the investigator to be the target site for biopsy collection. Punch biopsies (6 mm) were obtained from the identified target lesions 24 h before anti-IL-12p40 administration (baseline) and again at intervals of 48 h and 2 wk after anti-IL-12p40 administration. The biopsy samples were flash frozen in liquid nitrogen and stored at −80°C before sample processing.

Immunohistochemical staining

Frozen 5-μm sections from one-half of skin biopsies were fixed in acetone. Sections were pretreated with PBS containing 0.3% hydrogen peroxide to block endogenous peroxidase activity, and then blocked with 10% normal goat serum. Sections were incubated with mouse anti-human CD3 Ab or mouse anti-human CD11c (IgG2, DakoCytomation) for 1 h at room temperature. After washing, sections were exposed to a goat anti-mouse biotinylated secondary Ab (DakoCytomation) for 30 min at room temperature. After subsequent washing, sections were exposed to avidin/HRP (DakoCytomation) for 30 min, washed, and developed with diaminobenzidine followed by copper sulfate. Sections were then incubated with methyl green or hematoxylin for counterstain. A negative control was established using a matched isotype primary Ab and developed as described above. Frozen sections were also stained using H&E for assessment of epidermal thickness. Electronic images were acquired using a Zeiss Axioshot microscope and an Axiocam HRC-cooled charge-coupled device camera, controlled by Axiovision software, version 4.4. Epidermal thickness measurement and positive cell counts were performed using computer-assisted image analysis. Staining and measurements were performed on 12 of 18 patients, because sufficient quantity of tissues at multiple time points was only available for these patients.

Quantitative RT-PCR

Total RNA was extracted from one-half of a punch biopsy specimen, and the other half was retained for immunohistochemistry analysis. Total RNA was obtained using a RNeasy mini-kit (Qiagen) following tissue pulverization in a Mikrodisembrator. RNA was reverse transcribed using 200 U of Moloney-murine leukemia virus reverse transcriptase (Invitrogen Life Technologies) for 60 min at 37°C in the presence of 50 nm Tris-HCl (pH 8.3), 75 mM KCl, 3 mM MgCl2, 10 mM DTT, 100 ng of random hexamer primers (Invitrogen Life Technologies), 0.5 mM dNTPs, and 40 U of recombinant RNase inhibitor (Fisher). For every reaction set, some RNA samples were performed without Moloney-murine leukemia virus reverse transcriptase to provide a negative control in subsequent PCR. cDNA was amplified in the presence of gene-specific primers and probes (FAM reporter for specific cytokine and VIC reporter for 18S rRNA) and TaqMan universal master mix (PE Applied Biosystems) in a 96-well microtiter plate format on an ABI PRISM 7700 Sequence Detection System (PE Applied Biosystems). Each PCR was performed in triplicate, using the following conditions: 2 min at 50°C and 10 min at 95°C followed by 40 cycles of 15 s at 95°C and 1 min at 60°C. Ten-fold serial dilutions of cDNA plasmid for each target gene that contained known copy numbers were amplified with the samples during the same PCR. The absolute copy numbers of IL-12p40, IL-12p35, IL-23p19, IL-8, IL-10, IL-18, RANTES (CCL5), MCP-1 (CCL2), IFN-γ, TNF-α, and IFN-γ-inducible protein-10 (IP-10; CXCL10) were calculated against input copy numbers of plasmid standards for each target gene, and then normalized to the copy numbers of housekeeping gene 18S rRNA to control the variations in starting RNA amounts. For CD2 analysis, serial dilutions of a cDNA sample were used as relative standards, and relative quantification was made for each sample.

Statistical analysis

A two-tailed paired t test was used to evaluate the change from baseline in histology and mRNA expression at indicated time points. Pearson correlation analysis was used to determine the significance of correlation between baseline cytokine expression and percent improvement in PASI for TNF-α analysis. Partial correlation to remove the effect of dose was calculated by first-fit regression models of both variables on dose, and the Pearson correlation of the residuals of the two models was then calculated. Pearson correlation for baseline cytokine and percent change in cytokine correlation was also used.

Results

Clinical response to anti-IL-12p40 treatment

Eighteen patients with moderate-to-severe psoriasis vulgaris received a single i.v. administration of 0.1, 0.3, 1.0, or 5.0 mg/kg of anti-IL-12p40 according to dose group assignment. The demographics, baseline disease characteristics, safety, and pharmacokinetic results, and detailed analysis of clinical response of these patients were previously reported (45). A clinical response profile, as measured by percent improvement from baseline in PASI, for the 18 individual patients in the four dose groups is shown in Fig. 1. Clinical improvement was evaluated at 72 h and at 1, 2, 4, 8, 12,
Effects of anti-IL-12p40 treatment on cutaneous cytokine and chemokine mRNA expression

Expression levels of 11 genes known or thought to play a role in the pathogenesis of psoriasis were analyzed by real-time PCR before and after anti-IL-12p40 administration.

First, the effect of anti-IL-12p40 administration on mRNA expression of each gene across all patients was assessed. Expression levels of a type 1 cytokine, IFN-γ, across all patients were significantly reduced in biopsies taken 2 wk after anti-IL-12p40 administration compared with baseline ($p = 0.00001$) (Fig. 3A). Individually, 17 of the 18 patients had reduced levels of IFN-γ at this time point. Reduction in expression levels of a chemokine, IL-8,
was also significant at 2 wk (p < 0.05), with 17 of the 18 patients having reduced IL-8 levels (Fig. 3B). It is noted that individual expression levels of IL-8 at baseline were highly variable across the patient population, with up to a 504-fold difference between patients having the highest and lowest levels. Among other type 1 cytokines and chemokines, the expression levels of TNF-α, IP-10, and MCP-1 across all patients were also significantly reduced at 2 wk (p < 0.05, p = 0.001, p = 0.002, respectively; data not shown). Expression levels of another inflammatory cytokine, IL-18, did not show any significant changes at 2 wk (data not shown).

For APC cytokines, expression levels of both IL-12p40 and IL-23p19 were significantly reduced at 2 wk (p < 0.007 and p = 0.0001, respectively) (Fig. 3, C and D). Individually, 16 of the 18 patients had reduced levels of IL-12p40, and 17 of the 18 patients had reduced levels of IL-23p19. One patient who exhibited an increased level of IL-12p40 at 2 wk compared with baseline also showed a slight elevation in several other cytokines including IL-23p19 at 2 wk. In fact, this patient showed transient increases in the levels of these cytokines at 48 h, so the levels at 2 wk were still lower compared with those at 48 h. In contrast to what was seen for IL-12p40 and IL-23p19, the expression levels of IL-12p35 across all patients were significantly increased at 2 wk (p < 0.01) (Fig. 3E). Note that the copy number for IL-12p35 subunit was the lowest amplified and thus subject to experimental variation. Interestingly, expression levels of an anti-inflammatory cytokine, IL-10, was significantly reduced across all patients at 2 wk (p < 0.00001) (Fig. 3F), with 17 of the 18 patients having reduced IL-10 levels. These data indicate that administration of anti-IL-12p40 effectively down-regulates the expression of cytokines and chemokines associated with activation of Th1 cells and APCs.

Next, patients’ mRNA expression levels were examined with consideration to subsequent clinical responses to the anti-IL-12p40 therapy. Expression levels of IFN-γ were significantly reduced at 2 wk after anti-IL-12p40 administration compared with baseline in both high responders and low responders with 57 and 61% reduction on average, respectively (p < 0.001 and p = 0.004, respectively) (Fig. 4A). In high responders, the levels of TNF-α were significantly reduced at 2 wk (p < 0.05), whereas the reduction in

**FIGURE 3.** Cytokine mRNA expression before and after anti-IL-12p40 treatment. RNA was prepared from the one-half of punch biopsies obtained from the psoriatic skin lesions at baseline and 2 wk after anti-IL-12p40 administration. Quantitative RT-PCR was performed for the indicated genes. Gene expression levels were normalized to 18S rRNA. The expression levels of each cytokine for all the patients (n = 18) at baseline and 2 wk posttreatment are presented. The mean ± SE of all patients is indicated by the diamond symbol (♦). ∗, p < 0.05; ∗∗, p < 0.01; statistically significant differences between baseline and 2 wk posttreatment.

### Table I. Quantitation of epidermal thickness, CD3-, and CD11c-positive cells

<table>
<thead>
<tr>
<th>Epidermal Thickness</th>
<th>Epidermal CD3+ Cells</th>
<th>Total CD3+ Cells</th>
<th>Total CD11c+ Cells</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>High responders</strong> (n = 9)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Baseline</td>
<td>304.9 ± 17.8</td>
<td>65.5 ± 7.9</td>
<td>161.4 ± 23.0</td>
</tr>
<tr>
<td>Week 2</td>
<td>290.8 ± 25.4</td>
<td>48.5 ± 5.6</td>
<td>122.2 ± 12.3</td>
</tr>
<tr>
<td><strong>Low responders</strong> (n = 3)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Baseline</td>
<td>362.4 ± 71.7</td>
<td>58.1 ± 18.5</td>
<td>118.3 ± 14.8</td>
</tr>
<tr>
<td>Week 2</td>
<td>252.0 ± 43.1</td>
<td>43.9 ± 8.7</td>
<td>115.7 ± 0.9</td>
</tr>
</tbody>
</table>

* Values of epidermal thickness (in micrometers).
† Paired t test.
‡ Number of indicated cells in epidermis (epidermal) or epidermis and dermis (total) per low power field (×10).
§ Mean ± SE.
low responders was not significant (Fig. 4B). At 48 h, there was a rapid decrease of IL-8 expression levels only in high responders (Fig. 4C), although the decrease was not statistically significant. Eight of 12 high responders showed such large decreases at 48 h. The expression levels of chemokines, IP-10 and MCP-1, significantly decreased from baseline in both high responders and low responders at 2 wk (all values of \( p < 0.05 \)) (Fig. 4, D and F). Reductions from baseline in RANTES levels were also observed for both responder groups at 2 wk; however, the decreases were not statistically significant (Fig. 4E). Expression levels of IL-18 were almost constant and did not show significant changes in either responder group at 2 wk (Fig. 4G). For APC cytokines, both high responders and low responders exhibited significant decreases in the levels of both subunits, IL-12p40 (\( p = 0.03 \) and \( p = 0.001 \), respectively) (Fig. 5A) and IL-23p19 (\( p = 0.002 \) and \( p = 0.007 \), respectively) (Fig. 5B). The decreases at 2 wk from baseline in IL-12p40 and IL-23p19 levels were larger in high responders than in low responders, mainly due to higher baseline levels in high responders (Fig. 5, A and B). Expression levels of IL-12p35 trended upward at 2 wk in both high responders and low responders, although neither of them achieved a change from baseline level that reached statistical significance (Fig. 5C). IL-10 levels were significantly reduced at 2 wk in both high responders and low responders (\( p = 0.0001 \) and \( p < 0.05 \), respectively) (Fig. 5D). Thus, the major difference between high responders and low responders is a significant reduction in TNF-\( \alpha \) levels at 2 wk and greater magnitude of decrease in the levels of IL-12p40 and IL-23p19 at 2 wk in high responders.

**Correlation among the changes of cytokine and chemokine expression following anti-IL-12p40 administration**

Correlation among percent change at 2 wk from baseline values of cytokines and chemokines across all patients is presented in Table II. We found that the extent of reduction in IFN-\( \gamma \) mRNA expression strongly correlated with the reductions in mRNA expression of IP-10, IL-23p19, IL-12p40, and IL-8 (all values of \( p < 0.01 \)). Changes in IL-23p19 significantly correlated with changes in IL-8, IL-10, and the other subunit of IL-23, IL-12p40 (all values of \( p < 0.01 \)), in addition to the correlation with IFN-\( \gamma \). The strongest correlation observed was between the changes of IL-12p40 and IL-8 (\( r = 0.93, p < 0.01 \)). In addition to those discussed above, Table II lists the strength of correlation observed among the changes in expression of cytokines and chemokines associated with a type 1 response.

**Baseline cytokine level correlation**

We next examined the potential relationships between baseline cytokine mRNA expression levels across all patients. As shown in Table III, correlation was observed among a number of baseline cytokine levels. Baseline levels of IFN-\( \gamma \) and IP-10 demonstrated the strongest correlation among all the cytokines examined (\( r = 0.80, p < 0.01 \)). IFN-\( \gamma \) and IP-10 each also demonstrated statistically significant correlation with 7 of 10 other cytokines. For example, IFN-\( \gamma \) correlated with IP-10, IL-23p19, TNF-\( \alpha \), RANTES, and IL-10 (\( p < 0.01 \)) and with less strength of correlation with IL-12p40 and MCP-1 (\( p < 0.05 \)). Baseline IL-23p19 levels significantly correlated with baseline levels of the other subunit, IL-12p40 (\( p < 0.01 \)). Baseline TNF-\( \alpha \) mRNA levels had a distinctive profile, correlating not only with levels of IFN-\( \gamma \), but also with those of RANTES (\( p < 0.01 \)), and less strongly with IL-10 and IL-23p19 (\( p < 0.05 \)). In contrast, IL-18 and IL-12p35 baseline levels did not correlate significantly with those of any of other cytokines examined.

**Correlation of TNF-\( \alpha \) with the efficacy of anti-IL-12p40 treatment**

A comparison of gene expression levels of high responders vs low responders before treatment allows a retrospective analysis of differences in the patient population that may explain differential responsiveness to the treatment. The mRNA expression levels of TNF-\( \alpha \) at baseline in high responders were significantly higher than those in low responders (\( p = 0.02 \)) (Fig. 6A). Across all
patients, the baseline mRNA expression levels of TNF-α (γ) significantly correlated with the percent improvement in PASI at 16 wk (x) posttreatment (rxy = 0.56, p = 0.016) (Fig. 6B). This result was further confirmed by calculating the partial correlation between these two variables with effect of dose removed (rxydose = 0.65, p = 0.0032). No other cytokine showed a correlation between baseline mRNA levels and PASI improvement. These results suggest that the baseline TNF-α mRNA level may be predictive of the responsiveness to anti-IL-12p40 treatment.

Discussion
This study of anti-IL-12p40 therapy in patients with psoriasis demonstrated potent and rapid effects on cytokine and chemokine expression in psoriatic skin. IL-12 and IL-23 with their distinct p35 and p19 subunits and shared IL-12p40 subunit have overlapping yet distinct functions in type 1 response and both cytokines are targeted by this anti-p40 Ab. Previous reports indicate that anti-IL-12p40 ameliorates a psoriasis-like skin disorder in rodent models (44). However, this is the first report to demonstrate that anti-IL-12p40 administration to psoriasis patients down-regulates type 1 cytokines, chemokines and also IL-12/IL-23 themselves in lesional skin.

It has been shown that IFN-γ mRNA expression is elevated in psoriatic skin lesions and the lesions are infiltrated by numerous IFN-γ-producing T cells (13, 15, 17). In this study, the levels of IFN-γ expression in the lesional skin was reduced in almost all patients, as early as 2 wk after anti-IL-12p40 administration (Fig. 3A). This result directly indicates that anti-IL-12p40 worked in vivo and suppressed type 1 response. In addition, this effect immediately preceded a noticeable clinical improvement. IFN-γ expression was reduced ~60%, whereas PASI was only improved ~30% in high responders at 2 wk. Cytokine mRNA change preceded histologic changes, because statistically significant decreases in epidermal thickness and the number of CD11c+ DCs were not yet observed. A decrease in the number of T cells in the lesion, although statistically significant in high responders, was not robust (~17%) at this time point. Therefore, it is unlikely that reduced IFN-γ mRNA levels are due to decreased infiltration of T cells at this early time point. It has been reported that psoriatic lesions, which improved by other treatments, began to show large and significant decreases in epidermal thickness and lesional T cells and DCs at 4–6 wk after treatment (47–49). Therefore, we presume that all of these markers of histologic changes become more evident at time points later than 2 wk. This finding is consistent with our prior observations that induction of the basal keratinocyte cell cycle in psoriatic skin is critically dependent upon IFN-γ (13), and with the report that acanthosis is a murine model of psoriasis is IFN-γ dependent (44). Thus, cytokine gene expression level is a sensitive marker for anti-IL-12p40 therapy at this early time point.

Several chemokines have been reported to be up-regulated in psoriatic skin lesions (50–52). IP-10, produced by keratinocytes with the stimulation of IFN-γ, attracts CXCR3+ T cells into the inflamed skin. RANTES induces migration and activation of CCR5+ T cells. MCP-1 stimulates migration and activation of monocytes and CCR2+ T cells. Two weeks after anti-IL-12p40 administration, the expression levels of all three of these chemokines were reduced, with significant reductions for IP-10 and MCP-1 noted (Fig. 4, D–F). Because both CXCR3 and CCR5 are associated with Th1 cells, and CCR2 is associated with both Th1 and Th2 cells, reductions in these chemokines lead to less activation of Th1 cells and monocytes, which may result ultimately in decreased recruitment of these cells into psoriatic skin lesions. IL-8, produced by keratinocytes, is a main chemoattractant for neutrophils into the epidermis. The baseline IL-8 expression levels we observed were highly variable among patients (Fig. 3B), and did not correlate with baseline PASI scores (data not shown). Although the reason for such variability in IL-8 expression has not been clarified, it is notable that expression of IL-8 was reduced in almost all of our patients at 2 wk following anti-IL-12p40 therapy (Fig. 3B). In addition, in high responders, large decreases in IL-8 levels were observed at 48 h (Fig. 4C), which suggests rapid down-regulation of the level of keratinocyte activation.

It has been reported that IL-12 and IL-23 are up-regulated in psoriatic skin (30, 36). These two cytokines are produced by APCs. Thus, it is likely that anti-IL-12p40 effectively blocked the action of intracutaneous IL-12 and IL-23, from lesional APCs on lesional Th1 cells. According to recent reports, CD11c+ DCs are greatly increased in psoriatic skin compared with uninvolved skin, and the number of CD11c+ cells exceeds the number of CD3+ T cells (53). In patients treated with efalizumab (anti-CD11a), CD11c+ cells in psoriatic skin significantly decreased at 2 wk posttreatment, probably because efalizumab directly inhibits the migration of these cells (53). In our study, as shown in Table I, the number of CD11c+ cells in both responder groups decreased, but this decrease was not yet statistically significant at 2 wk posttreatment. Therefore, the changes in cytokine mRNA levels relevant to these cells likely predict initiation of cellular changes. Of particular interest is that 2 wk after anti-IL-12p40 administration, the expression levels of IL-12p40 mRNA themselves were decreased in almost all patients (Fig. 3C), with larger decreases in high responders (Fig. 5A). There are several possible explanations for this observation. First, IL-12-producing APCs themselves have IL-12 receptors (26), so neutralizing IL-12p40 could have inhibited a positive-feedback loop for IL-12. Second, because IFN-γ enhances transcription of IL-12p40 and p35 (54), reduced IFN-γ would result in less stimulation for IL-12. Third, a decrease of cell-cell
Table II. Correlation among the changes of biomarkers at 2 wk

<table>
<thead>
<tr>
<th>Biomarker</th>
<th>IFN-γ</th>
<th>IP-10</th>
<th>IL-23p19</th>
<th>IL-12p40</th>
<th>IL-8</th>
<th>TNF-α</th>
<th>MCP-1</th>
<th>RANTES</th>
<th>IL-10</th>
<th>IL-12p35</th>
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<tbody>
<tr>
<td>IFN-γ</td>
<td>0.83**</td>
<td>0.77**</td>
<td>0.81**</td>
<td>0.82**</td>
<td>0.45</td>
<td>0.47</td>
<td>0.40</td>
<td>0.47</td>
<td>0.47*</td>
<td>−0.10</td>
<td></td>
</tr>
<tr>
<td>IP-10</td>
<td>0.83**</td>
<td>0.54*</td>
<td>0.71**</td>
<td>0.68**</td>
<td>0.48*</td>
<td>0.59**</td>
<td>0.29</td>
<td>0.21</td>
<td>0.47*</td>
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<tr>
<td>IL-23p19</td>
<td>0.77**</td>
<td>0.54*</td>
<td>0.75**</td>
<td>0.79**</td>
<td>0.43</td>
<td>0.29</td>
<td>0.54*</td>
<td>0.62**</td>
<td>0.15</td>
<td>−0.16</td>
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<tr>
<td>IL-12p40</td>
<td>0.81**</td>
<td>0.71**</td>
<td>0.75**</td>
<td>0.93**</td>
<td>0.50*</td>
<td>0.36</td>
<td>0.28</td>
<td>0.25</td>
<td>0.21</td>
<td>−0.13</td>
<td></td>
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<tr>
<td>IL-8</td>
<td>0.82**</td>
<td>0.68**</td>
<td>0.79**</td>
<td>0.93**</td>
<td>0.54*</td>
<td>0.37</td>
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<td></td>
</tr>
<tr>
<td>TNF-α</td>
<td>0.45</td>
<td>0.48*</td>
<td>0.50*</td>
<td>0.54*</td>
<td>0.61*</td>
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Pearson correlation coefficients among changes of cytokine expression levels at 2 wk (relative percentage change from baseline, n = 18). **, p < 0.01; *, p < 0.05.

interaction (such as CD40 expressed on APC with CD40L on activated T cells) caused by reduced activation of infiltrating T cells could result in decreased production of IL-12. Of additional importance is that the expression levels of IL-23p19 were also reduced after anti-IL-12p40 administration (Fig. 3D), with a lower decrease in high responders (Fig. 5B). Although the regulation of the production of this novel cytokine has not been as well studied as that of IL-12, similar reasons may account for this observation. The coordinate reduction of IL-23p19 and p40 represents a profound effect on IL-23 production, which may in turn reduce the generation of pathogenic memory/effector T cells. It is tempting to speculate that pathogenic T cell reduction could result in disease whose expression is clinically subthreshold. In an environment where functional regulatory T cells in psoriatic lesions are dysfunctional and/or numerically insufficient (55), a treatment targeting the pathogenic memory/effector T cells may result in a rebalancing of the immune system such that regulatory T cells could effectively restrain the remaining pathogenic memory/effector T cell population. Additionally, the regulation of IL-17, which has been reported to play a role in psoriasis, may also be critical to the outcome of anti-IL-12p40 therapy, given the proposed role of IL-23 to promote IL-17 production (56). Interestingly, concepts have been raised in recent reports suggesting a potential key role for IL-18, a member of the IL-1 family that is constitutively expressed in the skin, acts in synergy with IL-12 to induce IFN-γ (67). Of note, IL-18 can induce a type 2 response in the absence of functional regulatory T cells in psoriatic skin lesions (71). TNF-α is produced by various types of cells, including Th1 cells, mast cells, macrophages, DCs, and keratinocytes. TNF-α increases DC migration, promotes T cell activation and migration, increases keratinocyte proliferation, and leads to increased levels of NF-κB in almost all cell types. It has decreased, and this was true for both high responders and low responders (Fig. 5D). These data fit with the inconclusive IL-10 clinical trials and suggest that the improvement of psoriasis by anti-IL-12p40 is not mediated by either an absolute or a relative increase of the anti-inflammatory cytokine IL-10. Given the observed reduction of IL-10, there is no evidence that anti-IL-12p40 administration induces a shift from a type 1 to a type 2 immune response. Although Th2 cells can produce IL-10, untreated psoriatic skin lesions have very few Th2 cells. Although keratinocytes can produce IL-10, they are less secretory for IL-10 than skin macrophages (64). Therefore, it is likely that IL-10 reduction posttreatment is indicative of reduced macrophage activation, as would be suggested by the concomitant reduction in IL-12p40, IL-23p19, MCP-1, and TNF-α. One proposed IL-12-mediated anti-inflammatory mechanism is inhibition of IL-12 production via blocked IL-12 gene transcription (65, 66). A possible explanation for IL-10 reduction is that suppressed production of IL-12 and other cytokines (including macrophage activators such as IFN-γ) by anti-IL-12p40 eliminates the positive-feedback signals for IL-10 usually induced for cessation of the inflammatory response.

IL-18, a member of the IL-1 family that is constitutively expressed in the skin, acts in synergy with IL-12 to induce IFN-γ (67). Of note, IL-18 can induce a type 2 response in the absence of IL-12; however, our patients did not demonstrate the increase in IL-10 that would be expected if a type 2 response were generated. IL-18 is overexpressed in psoriatic lesional skin, suggesting that it plays a role in the pathogenesis of psoriasis (68). The observation that no reduction in IL-18 expression at 2 wk after anti-IL-12p40 administration even in patients demonstrating rapid responses in other parameters (Fig. 4G) suggests that anti-IL-12p40 therapy does not affect the expression levels of IL-18 at least at an early time point.

In inflammation, cytokines and chemokines function in a cascade of reactions. IFN-γ and TNF-α stimulate the production of chemokines by various cell types (52, 69, 70). Therefore, it is possible that reductions of IFN-γ and TNF-α by anti-IL-12p40 could lead to a reduction in chemokines, resulting in decreased activation and migration of T cells and other leukocytes, followed in turn by a further decrease in IFN-γ and TNF-α. Indeed, strong correlation among the extent of reduction of several cytokines and chemokines were observed at 2 wk following anti-IL-12p40 administration (Table II).

TNF-α, a type 1 and inflammatory cytokine, is increased in psoriatic skin lesions (71). TNF-α is produced by various types of cells, including Th1 cells, mast cells, macrophages, DCs, and keratinocytes. TNF-α increases DC migration, promotes T cell activation and migration, increases keratinocyte proliferation, and leads to increased levels of NF-κB in almost all cell types.
been reported that TNF-α mRNA levels were higher in psoriatic lesions compared with nonlesional skin and they decreased in responders after treatment (47). Because the origin of TNF-α is not only Th1 cells, the production of TNF-α is not necessarily IL-12/IL-23 dependent. An important finding is that blockade of IL-12p40 down-regulated TNF-α expression in high responders (Fig. 4B), which is consistent with the recent clinical results that anti-TNF-α therapy is effective in the treatment of psoriasis (21, 22). The significance of baseline TNF-α expression levels in psoriasis patients is unknown. Baseline TNF-α levels do not seem to reflect the severity of psoriasis, because they did not correlate with baseline PASI scores in our study (data not shown). With a retrospective analysis, we found that, across all patients, baseline TNF-α levels correlated significantly with the percent improvement in PASI at 16 wk (Fig. 6). In each of the two lower dosage groups, the higher the patient baseline TNF-α level, the higher the observed percent PASI improvement at 16 wk (data not shown). In the two higher dose groups, this correlation was not absolute; this may be because the high degree of improvement obscured the correlation (indeed, only one patient in the higher dose groups failed to achieve ≥75% PASI improvement). It is interesting to examine the number of positive responders to treatment within our subjects’ test-positive group. If we consider that TNF-α copy number of 100 or greater is the test-positive group, then 11 of 18 patients meet this criterion. Of the 11 patients, 9 patients achieved ≥75% PASI improvement (82% of the test-positives were also PASI improved). Of the 7 patients whose TNF-α copy number is <100, 3 patients achieved ≥75% PASI improvement (only 43% of the test-negatives were PASI improved). As previously reported, high baseline PASI scores could explain poor improvement of some low responders, but not all (45). Our results suggest that the baseline TNF-α level might be a predictor for therapeutic responsiveness to anti-IL-12p40 in psoriasis, potentially identifying a patient subset. It has been reported that some psoriatic patients have TNF-α 238A allele and that their transcription and production of TNF-α is low (72). Alternatively, the baseline TNF-α level in each patient might reflect an immunologically responsive state at that time point. It is also possible that the TNF-α mRNA levels are not reflective of protein levels in the tissue. It is possible that TNF-α protein levels have a close relationship to the degree of inflammation (e.g., PASI score), and mRNA levels are indicative of a likely responder to the

Table III. Correlation among biomarkers at baseline values

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<tr>
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Pearson correlation coefficients among cytokine expression levels at baseline (n = 18). **, p < 0.01; *, p < 0.05.

FIGURE 6. Baseline TNF-α mRNA expression levels are involved in the efficacy of anti-IL-12p40 treatment. A, Baseline TNF-α expression levels in high responders and those in low responders are presented. Bars, Mean values. *, p < 0.05; statistically significant differences between two patient groups. B, Correlation between the expression levels of TNF-α mRNA at baseline and the percent improvement in PASI at 16 wk posttreatment. r = 0.56; p = 0.016.
therapy. Posttranscriptional regulation and posttranslational processing are important in TNF-α protein production (73, 74) and mRNA levels may also reflect increased half-life of mRNA. Because our study has only a limited number of patients, and TNF-α mRNA and protein processing is quite complicated, a more highly powered study specifically designed to address TNF-α levels and clinical improvement will be necessary to validate these observations.

Among new antipsoriatic reagents, anti-IL-12p40 therapy achieved high efficacy according to the data we currently have (7, 21, 22, 45, 47, 48). Our findings in this study have revealed the mechanism of action for anti-IL-12p40 therapy. Steps in a cutaneous type 1 response consist of APC activation, T cell activation, differentiation and proliferation of Th1 cells, migration of activated memory Th1 cells to skin, and subsequent production of inflammatory cytokines and chemokines. APC cytokines, IL-12/IL-23, are fairly “upstream” in the inflammatory cascade. Our study demonstrated that blockade of IL-12p40 down-regulated type 1 cytokines and chemokines that are downstream of IL-12/IL-23. If IL-12/IL-23 were continuously produced by activated APCs, the effect of neutralizing secreted IL-12/IL-23 would not last. However, we also demonstrated that the blockade of IL-12p40 down-regulated mRNA for IL-12/IL-23 themselves, with larger decreases in high responders, suggesting a single administration of anti-IL-12p40 leads to a long-lasting effect, likely breaking the APC-Th1 cell self-sustaining cycle proposed in psoriasis. These findings also suggest that activation of APCs, more upstream of targeted cytokines in the cascade, may be suppressed by this therapy. It is desirable to selectively target the type 1 response rather than global immune suppression for the treatment of Th1-mediated diseases. It has been recently reported that anti-IL-12p40 therapy was also effective in patients with active Crohn’s disease and induced remission (75). This therapy may also be beneficial to patients with other Th1-mediated inflammatory diseases. In conclusion, this study indicates that the administration of anti-IL-12p40 rapidly down-regulates mRNA expression of type 1 cytokines and chemokines that are downstream of APC cytokines, and APC cytokines themselves in psoriatic skin lesions. Interestingly, an anti-inflammatory cytokine, IL-10, is also down-regulated. High responders, but not low responders, have significant reductions in TNF-α levels and infiltrating T cells, and larger decreases in the levels of IL-12p40 and IL-23p19. A retrospective analysis suggests that baseline expression level of TNF-α may be a predictor of patient therapeutic responsiveness to anti-IL-12p40 therapy. It is proposed that a cyclic interaction between APCs and Th1 cells mediated by IL-12/IL-23 and type 1 cytokines results in the maintenance of chronic inflammation in psoriasis. The observations from this study strongly suggest that blockade of IL-12p40 is an effective treatment strategy for psoriasis acting through control of type 1 cytokine cascade and IL-12/IL-23 production. This study also provides further evidence for the hypothesis that APC-Th1 cell interaction is indeed a critical element of psoriasis.

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