TNF Inhibition Rapidly Down-Regulates Multiple Proinflammatory Pathways in Psoriasis Plaques

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The mechanisms of action of marketed TNF-blocking drugs in lesional tissues are still incompletely understood. Because psoriasis plaques are accessible to repeat biopsy, the effect of TNF/lymphotoxin blockade with etanercept (soluble TNFR) was studied in ten psoriasis patients treated for 6 months. Histological response, inflammatory gene expression, and cellular infiltration in psoriasis plaques were evaluated. There was a rapid and complete reduction of IL-1 and IL-8 (immediate/early genes), followed by progressive reductions in many other inflammation-related genes, and finally somewhat slower reductions in infiltrating myeloid cells (CD11c+ cells) and T lymphocytes. The observed decreases in IL-8, IFN-γ-inducible protein-10 (CXCL10), and MIP-3α (CCL20) mRNA expression may account for decreased infiltration of neutrophils, T cells, and dendritic cells (DCs), respectively. DCs may be less activated with therapy, as suggested by decreased IL-23 mRNA and inducible NO synthase mRNA and protein. Decreases in T cell-inflammatory gene expression (IFN-γ, STAT-1, granzyme B) and T cell numbers may be due to a reduction in DC-mediated T cell activation. Thus, etanercept-induced TNF/lymphotoxin blockade may break the potentially self-sustaining cycle of DC activation and maturation, subsequent T cell activation, and cytokine, growth factor, and chemokine production by multiple cell types including lymphocytes, neutrophils, DCs, and keratinocytes. This results in reversal of the epidermal hyperplasia and cutaneous inflammation characteristic of psoriatic plaques.

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Abbreviations used in this paper: DC, dendritic cell; IP-10 (CXCL10), interferon-γ-inducible protein-10; iNOS, inducible nitric oxide synthase; K16, keratin 16; LT, lymphotoxin; MMP, matrix metalloproteinase; MIG, monokine induced by IFN-γ; PASI, Psoriasis Area and Severity Index; RS, response score; HARP, human acidic ribosomal protein.
or both. Etanercept is an ideal agent with which to study the effect of TNF blockade on cellular immune regulation in plaques because its actions are thought to be due to neutralization of TNF and not to depletion of cells bearing cell surface TNF.

Materials and Methods

Patient studies

Adult patients with moderate to severe psoriasis were treated with etanercept monotherapy (25 mg s.c. twice weekly) for 24 wk under a protocol approved by the University of Medicine and Dentistry of New Jersey- Robert Wood Johnson Medical School Institutional Review Board. At the time this study was performed, the 25-mg twice-weekly dose was the only Food and Drug Administration-approved dose of etanercept for adults with psoriasis. Systemic and topical therapies were excluded for 1 mo before dosing, and topical medications were excluded for 2 wk before dosing. Eucerin cream (Beiersdorf) was the standard moisturizer used throughout the study but was not applied before study evaluations. Clinical efficacy was assessed using the Psoriasis Area and Severity Index (PASI) (25). At baseline, biopsies were taken from uninvolved skin and an index psoriasis lesion. Repeat biopsies were taken from the index lesion with which to study the effects of TNF blockade on cellular immune regulation in plaques because its actions are thought to be due to neutralization of TNF and not to depletion of cells bearing cell surface TNF.

Innate Immune Restrictions

Tissue sections were stained with hematoxylin (Fisher) and eosin (Shandon) and with mouse anti-human mAbs to elastase (Dako), K16 (Sigma-Aldrich), ICAM-1 (BD Pharmingen), CD3 (BD Biosciences), and CD11c (BD Pharmingen). A secondary biotin-labeled horse anti-mouse Ab (Vector Laboratories) was amplified with the avidin-biotin complex (Vector Laboratories). 3-Amino-9-ethylcarbazole (Sigma-Aldrich) was the chromogen used. Epidermal thickness measures and cell counts (per mm per field) were determined using computer-assisted image analysis (Image Pro-Plus (Media Cybernetics)). K16 and ICAM-1 protein expression and statistical results until all data were collected.

Immunofluorescence

Probes

Frozen lesional tissue sections from psoriasis patients (n = 4) were fixed in acetone and treated with 10% normal horse serum. For CD11c and iNOS colocalization experiments, sections were initially incubated overnight with purified CD11c (BD Pharmingen) (1/100) and then secondary Ab Alexa fluoro 488 goat anti-mouse IgG1 (Molecular Probes) for 30 min (1/250). iNOS (R&D Systems) conjugated with Alexa fluor 546 (Molecular Probes) was then incubated with these CD11c-stained slides for 2 h (1/500). Images were acquired using appropriate filters of a Zeiss Axioplan 2 microscope with Plan Apochromat 20 ×0.7NA lens and a Hamamatsu Photonics model C4742 “Orca” ER-cooled charge-coupled device camera, controlled by Universal Imaging MetaVue software.

Statistical analysis

Cell counts and gene expression changes were computed by Student’s t test, and significance was accepted as p < 0.05. U statistics (U score) were developed to rank patient responses by combining percent change in epidermal thickness and the presence or absence of K16 immunostaining into a response score (RS), as previously published (26, 27). This mathematical model describes psoriasis disease activity across the range of complete disease resolution to highly active disease present and stratifies patient responses from best (lowest score) to worst (highest score). Changes in inflammatory cells or genes, either singly or in combinations, were ranked by a similar U score stratification method, and comparison of multiples of up to three genes at a time was designated a pathway score. We ordered changes in individual patients by U scores, because nonparametric statistical methods make the fewest assumptions about quantitative cause/effect relationships. Correlations between the RS and lesional cell counts, gene expression, or the pathway score were then determined, and the r score is indicated for the given comparisons.

Results

Clinical and histological responses

In this study of 10 patients with moderate to severe psoriasis vulgaris, etanercept decreased the PASI by a mean of 29% (range, 5–80% decrease) after 1 month treatment (p < 0.02), and 57% (range, 24–94% decrease) after 3 mo of treatment (p < 0.01). At 3 mo of treatment, 6 of 10 patients attained a PASI 50 response, whereas at 6 mo of treatment 6 of 10 patients attained a PASI 75 response. The time course and extent of improvement seen in patients in this trial are thus similar to outcomes seen in larger, blinded clinical trials (22, 24).

The effects of etanercept on disease histopathology, expression of K16 (innate immunechemistry and quantitative mRNA measures), and ICAM-1 expression in epidermal keratinocytes are illustrated in Fig. 1. In general, progressive reversal of epidermal acanthosis and psoriasiform rete elongation was seen during 6 mo of treatment, although near maximal improvement was noted in a few cases after 3 mo of treatment. After 6 mo of treatment, thinning of the epidermis and normalization of keratinocyte differentiation occurred in 9 of 10 subjects, and absence of K16 in suprabasal keratinocytes was noted in 8 of 10 cases. Likewise, expression of ICAM-1 by epidermal keratinocytes was eliminated in 8 of 10 cases after 6 mo of treatment. We consider that histological remission of psoriasis was attained in these 8 patients. The progression in disease improvement during 6 mo of treatment can be appreciated from the overall histopathology and from quantitative measures of epidermal thickness and K16 mRNA in biopsy specimens (Fig. 1).

Etanercept decreases myeloid DCs and T cells in plaques

CD11c is a marker for a group of DCs with very large increases in the epidermis and dermis of psoriasis lesions (26, 28, 29). Both
CD11c+ DCs and CD3+ T cells were highly increased in pretreatment plaques compared with uninvolved skin (Fig. 2). Etanercept treatment resulted in progressive decreases in total CD3+ and CD11c+ cell populations (Fig. 2) as well as pathological epidermal thickness (Fig. 1). However, for both DCs and T cells, decreases in intraepidermal cell numbers were more marked than in dermal cell numbers. For example, the mean decrease in epidermal CD11c+ cells was 95% at 6 mo vs 60% for dermal CD11c+ cells. Additionally, the decrease in intraepidermal DCs was greater than that of intraepidermal T cells (95% vs 74%, respectively).

To describe overall improvement in psoriasis at the various analysis time points, we used a new multivariate tool that generates a RS by integrating epidermal thickness, K16 mRNA levels, and K16 protein in suprabasal keratinocytes (27). This mathematical model describes psoriasis disease activity across the range of complete disease resolution to highly active disease present, and stratifies patient responses from best (lowest score) to worst (highest score). The RS, which is a multivariate U score, can then be used to establish the extent to which a given degree of improvement is related to modulation of inflammatory cell types or inflammatory gene products in that biopsy. Overall, we found that reversal of epidermal hyperplasia (quantified as the RS) was more highly correlated with reductions in CD11c+ cells than T cells, especially for infiltration of the epidermis by these cell subsets (Fig. 3). Taken together, these observations suggest that inflammatory leukocytes may act locally in the epidermis to produce the psoriatic phenotype and that DCs may play a larger role than previously suspected.

**Effects on expression of inflammation-related genes**

Table I lists numerous inflammation-related gene products that are increased in psoriasis lesions and that were assessed by real time RT-PCR during etanercept-induced disease improvement (30–35). These products include early genes induced by TNF in model systems (IL-1β, IL-8, MIP-3α, IL-6), type 1 pathway genes broadly related to IFN-regulated responses (IL-23, STAT-1, MIG, iNOS, IP-10, IL-8) (36, 37), and genes typically activated in myeloid cells (iNOS, IL-19, MMP-12, IL-23, CD83) vs lymphocytes (granzyme B, IFN-γ). In previous work, we have proposed this type 1 pathway of gene activation, which may control pathogenic inflammation in psoriasis lesions. According to this model, several upstream cytokines (including IL-23, IFN-γ, TNF) induce expression of end stage inflammatory genes such as iNOS or IL-8 (36). These gene groups are not mutually exclusive, e.g.: IL-8 is an early response gene but can also be a secondary product of the type 1 pathway; iNOS is produced by myeloid DCs but is also a product of the type 1 pathway.

Fig. 4 illustrates expression of each of these gene products (normalized to HARP) in uninvolved skin vs psoriatic skin lesions at baseline and after 1, 3, and 6 mo of etanercept treatment. Two basic patterns of gene regulation by etanercept are seen. IL-1 and
IL-8, which are considered to be immediate response genes to induction by TNF, are strongly suppressed at all analysis time points, and the suppression appears to be maximal by 1 mo of treatment. In contrast, most other gene products show more gradual reductions and, in general, they are most strongly suppressed after 6 mo of continuous etanercept treatment. At the 1-mo time point, the degree to which late genes were suppressed by etanercept was highly variable in different patients, but the suppression was much more consistent at later time points (the \( p \) values reflect this response trend).

The relationship between individual gene expression at 1 mo of etanercept treatment and psoriatic disease phenotype (as measured by the RS) is demonstrated in Table I (\( r \) score). This time point was chosen because this is when the largest range of responses were evident. Psoriasis disease improvement at 1 mo was highly related to IL-1, MMP-12, and several type 1 pathway products (STAT-1, IL-23, MIG, IL-8, and iNOS). However, changes in gene expression should be considered in the context of reduced numbers of infiltrating leukocytes during the analysis period. The correlations between disease improvement and iNOS mRNA levels (\( r = 0.83; \) Table I) and CD11c\(^+\) DCs (\( r = 0.70; \) Fig. 3) are interrelated events, because iNOS production is restricted to this cell subset (see analysis below). Similarly, reductions in T cells in tissue could explain part of the reduction in gene expression linked to T cells, e.g., IFN-\( \gamma \) or granzyme B mRNAs. Additionally, total plaque neutrophil counts decreased from a mean of 485 \pm 368 in pretreatment plaques to 170 \pm 343 (\( p = 0.03 \)) after only 1 mo of etanercept treatment (as quantitated by neutrophil elastase). Reductions in total neutrophil counts correlated with decreased IL-8 gene expression (data not shown). Even so, the suppression of lineage-associated inflammatory genes occurs more quickly and to a larger extent than overall reductions in associated leukocyte subsets. The observed decreases in IL-8, IP-10, and MIP-3\( \alpha \) mRNA expression may account for decreased infiltration of neutrophils, T cells, and DCs, respectively.

Because inflammatory gene sets may be additive or interactive for producing pathogenic inflammation, we also used a novel method to assess which combination of genes was most highly
correlated with the RS at different analysis time points (27). At 1 mo, there was a high correlation ($r = 0.91, p < 0.001$) between the combined expression of IL-1, STAT-1, and IL-23/IL-12 p40 mRNAs, as illustrated in the bottom right panel of Fig. 4. However, the final degree of disease improvement seen at 6 mo was associated with a different set of inflammatory gene products, with the combined expression of IFN-$\gamma$, granzyme B, and IL-19 mRNAs most correlated ($r = 0.93$) with the RS (data not shown). Thus, the disease-resolution response to etanercept is a rapid and complete reduction in IL-1 and IL-8 (immediate/early genes), followed by progressive reductions in many other inflammation-related genes, and finally somewhat slower reductions in infiltrating myeloid cells and T lymphocytes.

**Etanercept causes a decrease in iNOS protein in DCs**

In general, expression of inflammatory genes was decreased to a greater extent than infiltrating DCs or T cells. This was particularly true for the earlier time points and suggests that etanercept reduces expression of inflammatory genes produced by infiltrating leukocytes, rather than just reducing trafficking of leukocytes and constitutive gene products. Recently, we have determined that iNOS-producing myeloid (CD11c$^+$) DCs are tremendously increased in psoriasis lesions (29). Because iNOS mRNA is jointly regulated by NF$\kappa$B and STAT-1 (TNF- and IFN-induced transcription factors, respectively), we examined the production of iNOS protein in CD11c$^+$ cells in lesions using two-color immunofluorescence microscopy (Fig. 5).

Table I. Functional significance of genes evaluated during etanercept treatment of psoriasis patients, and correlation of mRNA level and RS at 1 mo

<table>
<thead>
<tr>
<th>Gene</th>
<th>Functional Significance</th>
<th>Correlation ($r$) with RS at 1 mo</th>
</tr>
</thead>
<tbody>
<tr>
<td>Early TNF-induced genes</td>
<td></td>
<td></td>
</tr>
<tr>
<td>IL-1$\beta$</td>
<td>Inflammatory cytokine</td>
<td>0.70</td>
</tr>
<tr>
<td>IL-8</td>
<td>Inflammatory cytokine for neutrophils, also product of type 1 pathway</td>
<td>0.77</td>
</tr>
<tr>
<td>MIP-3$\alpha$ (CCL20)</td>
<td>Most potent chemokine for DC attraction, inflammatory cytokine, also may be produced by IL-1 stimulation</td>
<td>0.65</td>
</tr>
<tr>
<td>IL-6</td>
<td>Inflammatory cytokine</td>
<td>0.06</td>
</tr>
<tr>
<td>Type 1 pathway genes (induced or regulated by IFN-$\gamma$)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>IL-23</td>
<td>Proximal type 1 pathway gene, induces IFN-$\gamma$</td>
<td>0.72</td>
</tr>
<tr>
<td>STAT-1</td>
<td>Transcription factor induced by IFN-$\gamma$, central factor of type 1 pathway</td>
<td>0.69</td>
</tr>
<tr>
<td>MIG</td>
<td>Chemokine regulated mainly by IFN-$\gamma$, inflammatory cytokine</td>
<td>0.78</td>
</tr>
<tr>
<td>iNOS</td>
<td>Coregulated by TNF/IFN-$\gamma$, produces NO, inflammatory mediator</td>
<td>0.83</td>
</tr>
<tr>
<td>IP-10 (CXCL10)</td>
<td>Chemokine regulated by TNF/IFN-$\gamma$, chemotaxis T cells, inflammatory cytokine</td>
<td>0.79</td>
</tr>
<tr>
<td>IL-8</td>
<td>Type 1 pathway product, chemokine for neutrophils</td>
<td>0.77</td>
</tr>
<tr>
<td>Myeloid genes</td>
<td></td>
<td></td>
</tr>
<tr>
<td>iNOS</td>
<td>Produces NO, inflammatory mediator</td>
<td>0.83</td>
</tr>
<tr>
<td>IL-19</td>
<td>Product of activated monocytes/myeloid cells, inflammatory cytokine</td>
<td>0.72</td>
</tr>
<tr>
<td>MMP-12</td>
<td>Product activated macrophages/myeloid cells, metalloelastase</td>
<td>0.74</td>
</tr>
<tr>
<td>IL-23p19</td>
<td>Defining IL-23 subunit, inducer of IFN-$\gamma$, inflammatory cytokine, proximal type 1 pathway gene</td>
<td>0.72</td>
</tr>
<tr>
<td>IL-23p40</td>
<td>Shared subunit with IL-12 and IL-23, inflammatory cytokine</td>
<td>0.14</td>
</tr>
<tr>
<td>CD83</td>
<td>Marker of mature/activated DCs</td>
<td>0.31</td>
</tr>
<tr>
<td>Lymphocyte genes</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Granzyme B</td>
<td>Product of activated CD8$^+$ T cells, inflammatory mediator</td>
<td>0.86</td>
</tr>
<tr>
<td>IFN-$\gamma$</td>
<td>Product of stimulated type 1 T cell, proximal type 1 pathway gene</td>
<td>0.20</td>
</tr>
</tbody>
</table>
consistently observed that before etanercept treatment, there were abundant CD11c<sup>+</sup>/iNOS<sup>+</sup> cells. However, in etanercept-treated biopsies, there were numerous CD11c<sup>+</sup> cells with little or no detectable iNOS protein expression. Because iNOS expression by DCs can be viewed as a TNF-mediated activation-related response, we also studied expression of other activation or maturation-related DC products. The IL-23p19 mRNA, which is induced in monocyte-derived DCs in response to a

FIGURE 4. Reduced inflammatory gene expression in response to etanercept treatment. Results of RT-PCR for a panel of genes increased in psoriasis and the effect of etanercept treatment on their expression (normalized to HARP) at baseline nonlesional (BL NL), baseline lesional (BL LS), and after 1, 3, and 6 mo of treatment. Comparison of results in treatment groups to lesional results, p value indicated. Bottom right panel indicates the correlation at 1 mo between RS and pathway score (comprising change in expression of a combination of IL-1, STAT-1, IL-12p40), with r value indicated.
TNF-containing maturation stimulus, was significantly down-regulated in 3- and 6-mo biopsies (Fig. 4). Another marker of mature/activated DCs is CD83. We observed virtual elimination of CD83 immunoreactivity in several etanercept-treated cases (data not shown) and in agreement with reduced CD83 mRNA expression by 3 mo (Fig. 4). Limited availability of biopsy sections prevented quantitative analysis of CD83 expression in etanercept-treated lesions from all patients; thus a formal correlation between response and CD83 protein expression could not be attained.

Discussion

Past studies of therapeutic mechanism(s) with TNF antagonists have been conducted in patients with rheumatoid arthritis and psoriatic arthritis. Several studies support the general view that TNF antagonists reduce expression of vascular adhesion molecules (selectins, VCAM, ICAM-1) with attendant reductions in trafficking of leukocytes to inflamed synovium. However, much of this analysis has been focused on circulating cytokines, soluble fragments of cell adhesion molecules, and properties of peripheral leukocytes, because access to the affected tissue is difficult (38–46). Analyses conducted on inflamed synovium have shown reduced infiltration by CD68+ myeloid cells (most likely tissue macrophages), but reductions in infiltrating T cells have been measured less consistently (47, 48). Reduced expression of mRNA for several cytokines that regulate angiogenesis has been measured in synovial tissue after infiximab treatment (48), but comparable data for inflammatory cytokines do not exist and a time course of cellular/molecular inflammatory events is difficult to construct.

In contrast, the accessibility of diseased skin tissue in psoriasis has made it possible to study sequential alterations in inflammatory leukocytes and numerous inflammation-associated gene products during etanercept treatment. Although it may be predictable that inflammatory genes and infiltrating cells are decreased by this therapy, it is important to perform a detailed characterization and time course of these events to develop insights into such issues as why patients respond, the molecular and cellular mechanisms of action, and developing new applications for biological therapies.

We have developed methods to quantify the extent of disease burden via cellular and genomic measures of epidermal hyperplasia (the response score or RS) and methods to quantify infiltrating leukocytes and numerous inflammation-associated genes within a single biopsy specimen. Our analysis of the therapeutic mechanism of etanercept has also been greatly aided by a series of recent genomic studies of psoriasis vulgaris that point to a series of inflammatory genes (type 1 genes), which can act in a sequential manner or a cascade to produce numerous cellular features of this disease (36, 49). The view of therapeutic action of etanercept obtained in this study is much more detailed than any previous study of a TNF inhibitor in human disease, and it is conceptually different from most models of TNF inhibition in arthritis.

The genomic approach to study inflammatory gene expression after etanercept treatment is based on the idea that genes induced by TNF or LT will be suppressed by cytokine blockade. However, expression of TNF or LT mRNA in producing cells may not be affected by blocking the protein product, because they are likely to be regulated by other stimuli. Hence, we have not concentrated on these gene products (TNF and LT) in the downstream analysis of inflammatory gene expression.

In this study, the most consistent cellular effects of etanercept were 1) reductions in keratinocyte hyperplasia (reflected in epidermal thickness measures, K16 expression, and the integrated RS) and 2) reductions in CD11c+ myeloid cells, which are best classified as myeloid DCs (26). Virtually all CD11c+ DCs synthesize iNOS at high levels, and we found that reduced expression of iNOS mRNA as a single gene product was highly correlated with etanercept-induced therapeutic improvement (Table 1), whereas expression of iNOS protein was clearly reduced in CD11c+ cells (Fig. 5). The enzyme iNOS produces NO from arginine and NO is a molecule with both cell-damaging properties and the ability to dilate small blood vessels. In psoriasis, NO may be a trigger for keratinocyte hyperplasia and a factor accounting for skin erythema, through its vasoactive effects (50, 51). Up-regulation of iNOS mRNA in DCs may also be a general sentinel of activation occurring in this cell type. Past studies in mice have found critical roles of TNF and LT in the generation of DCs (52–55), so a central effect of TNF/LT blockade in psoriatic lesions may be related to blocked in situ differentiation of these cells from circulating or resident precursors. The reduced expression of IL-23, previously traced to myeloid DCs in psoriasis lesions (56) and reduced expression of CD83 (a marker of mature DCs in humans), also suggest a major impact of etanercept on DCs in psoriasis. Changes in T cell infiltrates and reduced expression of IFN-γ and granzyme B may all be secondary to reduced stimulation of T cells in situ by activated DCs. We note, however, that reduced synthesis of IFN-γ by T cells might also be directly attributed to TNF inhibition (57). Gradual reductions in T cells and DCs in psoriasis...
TNF BLOCKADE INHIBITS PROINFLAMMATORY PATHWAYS IN PSORIASIS

skin lesions may also be produced by reductions in IP-10 and MIP-3α, respectively (30, 58). Rapid reductions in neutrophils may be produced by reductions in IL-8 (59). ICAM-1 expression by dermal blood vessels did not appear to be reduced by etanercept (data not shown). Accordingly, a reduced chemokine stimulus for cell migration into skin seems more likely as a mechanism to explain decreased migration of leukocytes into the dermis. In contrast, ICAM-1 expression by keratinocytes is down-regulated during etanercept treatment; thus, migration of leukocytes from the dermis into the epidermis could be blocked by direct modulation of adhesion molecules (60, 61).

The direct activation of epidermal keratinocytes by TNF or LT may also be an important pathogenic mechanism in psoriasis. A recent study using gene arrays has identified >70 genes that are up-regulated in human keratinocytes after TNF exposure (1). A large number of TNF-induced genes encode chemokines, cytokines, and other proinflammatory molecules that would be expected to enhance leukocyte recruitment into the skin and subsequent cellular activation. IL-1β, IL-8, and IP-10 were identified as TNF-regulated products in keratinocytes using gene arrays, and all of these genes were strongly suppressed in psoriasis lesions during etanercept treatment. ICAM-1 mRNA was induced in keratinocytes after TNF treatment, and our data show decreased expression of ICAM-1 protein in keratinocytes after etanercept treatment. TNF also induces cytokines that stimulate proliferation of resident cells in the skin. TGF-α, nerve growth factor, endothelial cell growth factor, and vascular endothelial cell growth factor are increased in keratinocytes after TNF treatment. These factors were not studied in cutaneous lesions during etanercept treatment, but several angiogenic cytokines are decreased in synovium from psoriatic arthritis after TNF blockade. Accordingly, it seems likely that etanercept exerts major therapeutic actions in psoriasis skin lesions by down-regulating expression of proinflammatory and proproliferative genes that are induced in keratinocytes (as well as other skin-resident cell types) by local synthesis of TNF.

The inflammatory response to TNF could be self-sustaining, because activated DCs are a major source of TNF in psoriasis lesions (18) and because TNF mRNA is induced in keratinocytes after TNF exposure (1). Treatment with etanercept could provide the means to break this self-amplifying inflammatory cascade, but etanercept produces relatively slow down-regulation of some proinflammatory genes in psoriasis lesions. The progressive down-regulation of genes like IP-10 or iNOS during 6 mo of treatment with etanercept contrasts sharply with the rapid induction of these genes in cultured cells after treatment with TNF (1) and also with rapid cellular responses to TNF in sepsis. The most likely explanation for this apparent discrepancy is that proinflammatory genes like IP-10 and iNOS are controlled by multiple transcription factors induced by different classes of inflammatory cytokines (62). Hence, in chronic inflammatory diseases, TNF might broadly tune levels of proinflammatory gene expression, rather than act as a strict on/off switch for inflammatory responses. The expression of TNF and/or LT in cutaneous inflammatory sites may also promote in situ activation/mutation of myeloid DCs, such that therapeutic inhibition of these cytokines gradually collapses organized T/DC infiltrates that sustain inflammation in the skin (36). Accordingly, TNF/LT cytokines can effectively bridge innate and acquired immune responses by inducing early inflammatory genes and also supporting the development of other cellular and cytokine networks that lead to long-range enhancement of T cell-mediated responses.

Disclosures

Three of the authors are connected with Amgen: A. B. Gottlieb is an investigator, consultant, and speaker; J. G. Krueger receives research funding; and M. Magliocco has partial research funding and a Clinical Immunology Fellowship.

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