Amelioration of epidermal hyperplasia by TNF inhibition is associated with reduced Th17 responses

Lisa C. Zaba,[1] Irma Cardinale,[1] Patricia Gilleaudeau,[1]
Mary Sullivan-Whalen,[1] Mayte Suárez-Fariñas,[1]
Judilyn Fuentes-Duculan,[1] Inna Novitskaya,[1] Artemis Khatcherian,[1]
Mark J. Bluth,[2] Michelle A. Lowes,[1] and James G. Krueger[1]

[1]Laboratory for Investigative Dermatology, The Rockefeller University, New York, NY 10065
[2]Department of Dermatology, Weill Medical College of Cornell University, New York, NY 10065

Biological agents have dramatically improved treatment options for patients with severe psoriasis. Etanercept (tumor necrosis factor [TNF] receptor–immunoglobulin fusion protein) is an effective treatment for many psoriasis patients, and blockade of TNF is considered to be its primary action. However, in this clinical trial, we show that etanercept has early inhibitory effects on a newly appreciated type of T cells: T helper type 17 (Th17) cells. Etanercept reduced the inflammatory dendritic cell products that drive Th17 cell proliferation (interleukin [IL] 23), as well as Th17 cell products and downstream effector molecules (IL-17, IL-22, CC chemokine ligand 20, and β-defensin 4). In contrast, Th1 cellular products and effector molecules (interferon γ, lymphotoxin α, and myxovirus resistance 1) were reduced late in disease resolution. This study suggests a role for Th17 in addition to Th1 cells in the pathogenesis of psoriasis. Th17 cells may be particularly important in driving epidermal activation in psoriatic plaques, whereas Th1 cells must also be eliminated for final disease resolution.

CORRESPONDENCE
Lisa C. Zaba:
lzaba@rockefeller.edu

Abbreviations used: CCL, CC chemokine ligand; DC-LAMP, DC–lysosomal-associated membrane protein; DEFB4, β-defensin 4; EAE, experimental autoimmune encephalomyelitis; HARP, human acidic ribosomal protein; IBD, inflammatory bowel disease; ICAM, intracellular adhesion molecule; iNOS, inducible NO synthase; K16, keratin 16; LTA, lymphotoxin α; MFI, mean fluorescence intensity; MLR, mixed leukocyte reaction; MoDC, monocyte-derived DC; mRNA, messenger RNA; MX-1, myxovirus resistance 1; PASI, psoriasis area and severity index; TipDC, TNF–iNOS-producing DC.

Psoriasis is a common autoimmune skin disease affecting 1–2% of the population in North America and Europe. Over the years, psoriasis has been considered either a primary disease of keratinocytes or of T cells, with a strong genetic component (1). Until recently, IFN-γ–producing Th1 cells were implicated as the main pathogenic cells (2), as certain T cell–targeted therapies were successful in clearing psoriasis (1), and clonal T cells have been found in psoriatic skin (3). However, we are beginning to appreciate that there may be an important pathogenic contribution from a recently recognized subset of T cells: Th17 cells producing IL-17 and IL-22 (2, 4). In model systems, IL-17 stimulates keratinocyte production of innate inflammatory "danger signals" such as defensins and S100 proteins, as well as IL-8 neutrophil chemokine (5), whereas IL-22 modulates defensins (6) and keratinocyte hyperproliferation (7, 8). Upstream inducers of Th17 cells are still being understood, as most experiments have been performed in mouse model

The online version of this article contains supplemental material.

systems. Mediators may include IL-1, IL-6, and TGF-β, which stimulate the differentiation of naive CD4^{+} T cells into activated memory Th17 cells (9–11), and IL-23, which drives Th17 cell proliferation (12).

Th17 T cells producing IL-17 and IL-22 have been implicated as pathogenic in mouse models of autoimmune diseases such as experimental autoimmune encephalomyelitis (EAE), collagen-induced arthritis, and inflammatory bowel disease (IBD) (13–16). IL-17 knockout mice are resistant to both EAE and collagen-induced arthritis. Also, mice with EAE have increased numbers of Th17 cells but are resistant to disease if immunized against IL-17 (17). The DC product IL-23, a survival factor for Th17 cells, also appears to be necessary for IBD pathogenesis in mice (18). Thus, a model is emerging of autoimmune inflammation that begins with activated APCs producing IL-23, subsequent Th17 cell proliferation and IL-17/IL-22 release, and downstream inflammatory tissue damage.

Most studies of Th17 cells have been performed in mouse models or in vitro. However,

there are some human data also supporting a similar model of Th17 cell–mediated autoimmune inflammation. Patients with IBD have elevated IL-17 and IL-22 in affected colonic tissue and serum, depending on disease activity and severity (19–21), and patients with rheumatoid arthritis have elevated IL-17 and IL-22 protein in synovial fluid (22, 23). In psoriasis patients, IL-17 messenger RNA (mRNA) has been demonstrated within lesions (24), but protein levels are not increased in the serum (25). IL-22 protein is increased in psoriatic serum compared with normal, and mRNA is increased in lesional tissue (6). High levels of IL-23 have also been detected in psoriasis lesions (26) and are strongly diminished by effective therapies for psoriasis (27).

Biological treatments provide researchers with tools to directly target components of the immune system and begin to dissect molecular circuitry and pathogenic pathways. Treatment of psoriasis patients with etanercept, a TNFR-Ig fusion protein, presents an opportunity to further understand the effects of blocking TNF at molecular and cellular levels. The comparative modulation of Th17 versus Th1 cell activation in psoriasis within the context of a therapeutic trial has not been previously reported. We found that psoriasis disease improvement correlated with the rapid down-modulation of DC and Th17 cell products and downstream effector molecules, and the final disease resolution correlated with the late down-modulation of Th1 cells.

RESULTS
Clinical and histological responses
In this study, 20 patients were given 50 mg etanercept biweekly for 12 wk. Psoriasis area and severity index (PASI) was decreased by a mean of 36% (range = 9–67%) after 4 wk of treatment and 69% (range = 33–96%) after 12 wk of treatment (Fig. 1 A). The time course and extent of improvement with biweekly etanercept treatment in this trial were similar to outcomes seen in larger, double-blind clinical trials (28, 29).

The effects of etanercept on disease histopathology, epidermal thickness, expression of keratin 16 (K16; immunohistochemistry and quantitative mRNA measures), and Ki67 cell counts are illustrated in Fig. 1 (A and B). After 12 wk of treatment, epidermal thinning and normalization of keratinocyte differentiation occurred in 16 out of 20 patients, who we considered to be histological responders (30). The data presented are from the 16 histological responders to study immunologic response within the target lesion.

The mean PASI score for histological responders was 7.1 (range = 0.6–22; SEM = 1.4), with a mean percent clearance of 74.5 (range = 38.9–97.5; SEM = 4.9; Fig. 1 A). Mean epidermal thickness was significantly reduced by week 1 compared with baseline lesional skin (P < 0.05). K16 mRNA levels (a measure of epidermal regenerative activation) and Ki67 cell numbers per millimeter (a measure of keratinocyte proliferation) were also significantly reduced by week 1 (P < 0.001 and 0.01, respectively). Representative hematoxylin and eosin, K16, and Ki67 immunostainings for a responding patient are shown (Fig. 1 B). Thus, keratinocyte acanthosis,

differentiation, and proliferation were all rapidly down-modulated at week 1 of treatment.

Inflammatory infiltrate in psoriasis skin was reduced with etanercept treatment
Nonlesional skin contained relatively low numbers of CD11c^{+} myeloid DCs, CD3^{+} T cells, and CD163^{+} macrophages (Fig. 1 C). In psoriasis plaques, inflammatory cell numbers were increased two to four times above normal. Little or no change in inflammatory cell infiltrate was seen by week 1 of etanercept treatment. By week 2, cell numbers began to decrease but did not approximate baseline values until week 12. At week 12, CD11c, CD3, and CD163 cell counts were not significantly different from nonlesional values. Representative immunohistochemistry for CD11c, CD3, and CD163 antigens at each biopsy time point is shown in Fig. 1 D. Therefore, decreased dermal inflammatory infiltrate with etanercept treatment lagged behind decreased keratinocyte thickness.

Etanercept rapidly down-modulated Th17 cell products and had a delayed effect on Th1 and Th2 cell products
IL-17 and IL-22, the hallmark cytokines of Th17 cells, were rapidly down-modulated in histologic responders by weeks 1 (P = 0.05) and 2 (P = 0.05) of etanercept treatment, respectively (Fig. 2 A). Variability in IL-17 expression at weeks 2 and 4 resulted in p-values that approached significance (P = 0.056 and 0.057, respectively). In contrast, IFN-γ, the hallmark cytokine of Th1 cell response, was not down-modulated until week 12 (P < 0.01; Fig. 2 B). Lymphotoxin α (LTA)–1, another Th1 response cytokine, was also down-modulated at week 12 (P < 0.05; Fig. 2 C).

To assess the biological significance of early Th17 cytokine down-modulation and late Th1 cytokine down-modulation with etanercept treatment, we used multivariate U-statistics to correlate a "Th17 score" (a composite of IL-17 and IL-22 mRNA expression values) or "Th1 score" (a composite of IFN-γ and LTA-1 expression values) and correlated them with an histological disease improvement "response score" (epidermal thickness, K16 expression, and Ki67 counts; Fig. 2 C). There was a strong correlation between Th17 cytokines and the epidermal response score (R = 0.89; P = 3.7 × 10⁻⁶) and less so between Th1 cytokines and the epidermal response score (R = 0.48; P = 0.055). We further confirmed the biological significance of early Th17 cell down-modulation by measuring genes regulated by IL-17, CC chemokine ligand (CCL) 20, and β-defensin 4 (DEFB4; Fig. 2 D). CCL20 and DEFB4 were both down-modulated by week 1 of etanercept treatment (P = 0.01 and 0.05, respectively) and were consistently suppressed at all weeks of treatment. In contrast, an IFN-γ–regulated gene, myxovirus resistance 1 (MX-1), was not significantly reduced until week 4 (P = 0.05) and even more strongly suppressed by week 12 (P < 0.001; Fig. 2 E). Also of interest was IL-4, the defining cytokine of the Th2 cell, which was up-regulated at week 12 (P = 0.09; Fig. 2 F).

Other inflammatory cytokines rapidly down-modulated with etanercept were IL-1β (week 1, P < 0.01), IL-6 (week 2,
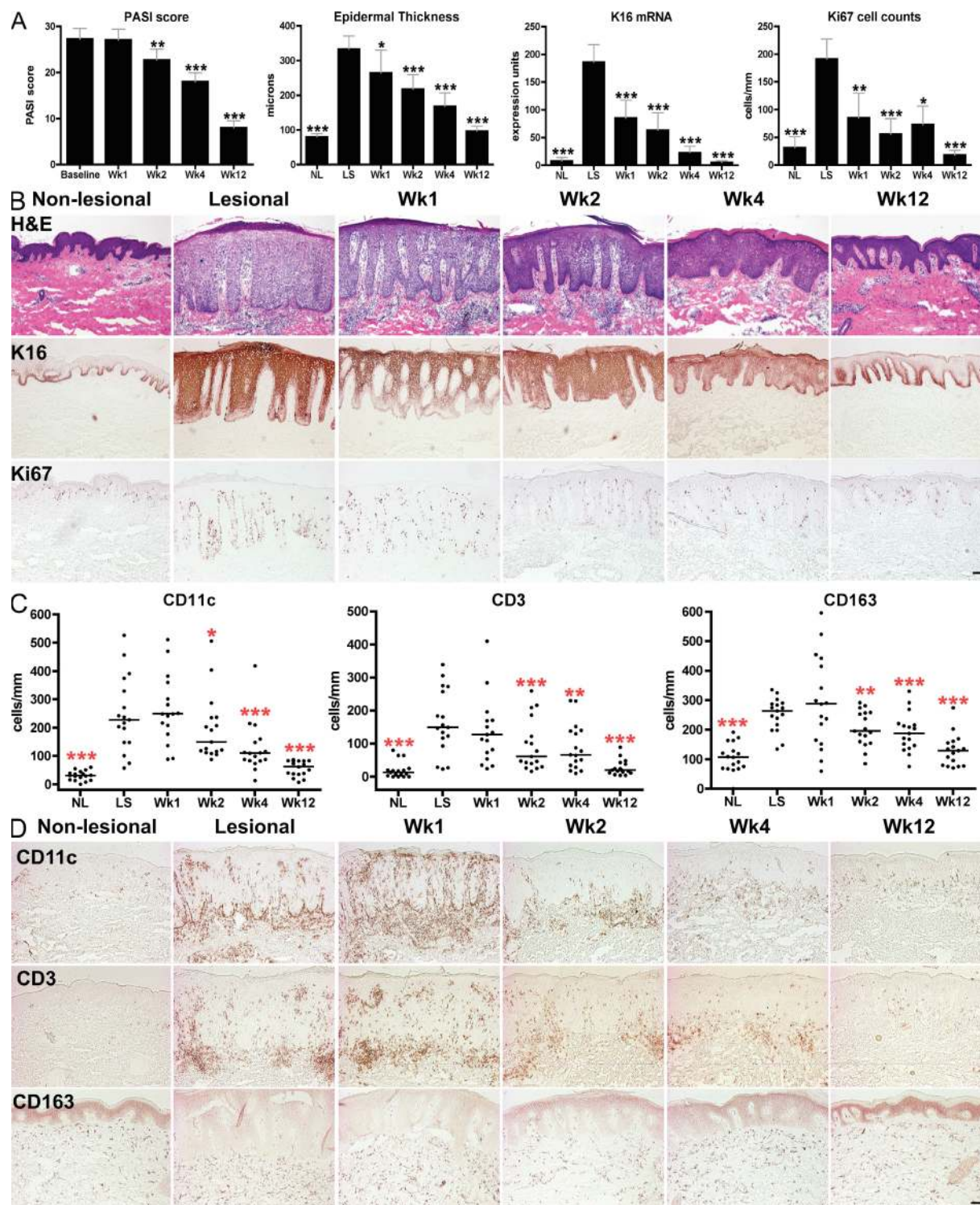
Figure 1. Clinical and histological resolution of psoriasis with etanercept treatment. (A) Mean PASI scores, epidermal thickness, K16 mRNA expression, and Ki67 cell counts in histological responders ($n = 16$) during treatment with etanercept. Clinical response was measured at baseline and weeks 1, 2, 4, and 12; biopsies were evaluated in nonlesional skin (NL), lesional skin (LS), and in the lesional index plaque at weeks 1, 2, 4, and 12. Error bars represent the mean $\pm$ SEM. Baseline lesional values were compared with other time points. *, $P < 0.05$; **, $P < 0.01$; ***, $P < 0.001$. (B) Histology and immunohistochemistry showing hematoxylin and eosin (H&E), K16, and Ki67 expression during treatment. Bar, 100 μm. (C) CD11c+ myeloid DCs, CD3+ T cells, and CD163+ macrophages per millimeter in nonlesional skin (NL), lesional skin (LS), and in the lesional index plaque at weeks 1, 2, 4, and 12. Horizontal bars represent the mean. Baseline lesional values were compared with other time points. *, $P < 0.05$; **, $P < 0.01$; ***, $P < 0.001$. Ki67, CD11c, and CD3 baseline lesional cell counts have been previously reported (reference 50). (D) Immunohistochemistry showing CD11c, CD3, and CD163 expression during treatment. Bar, 100 μm.
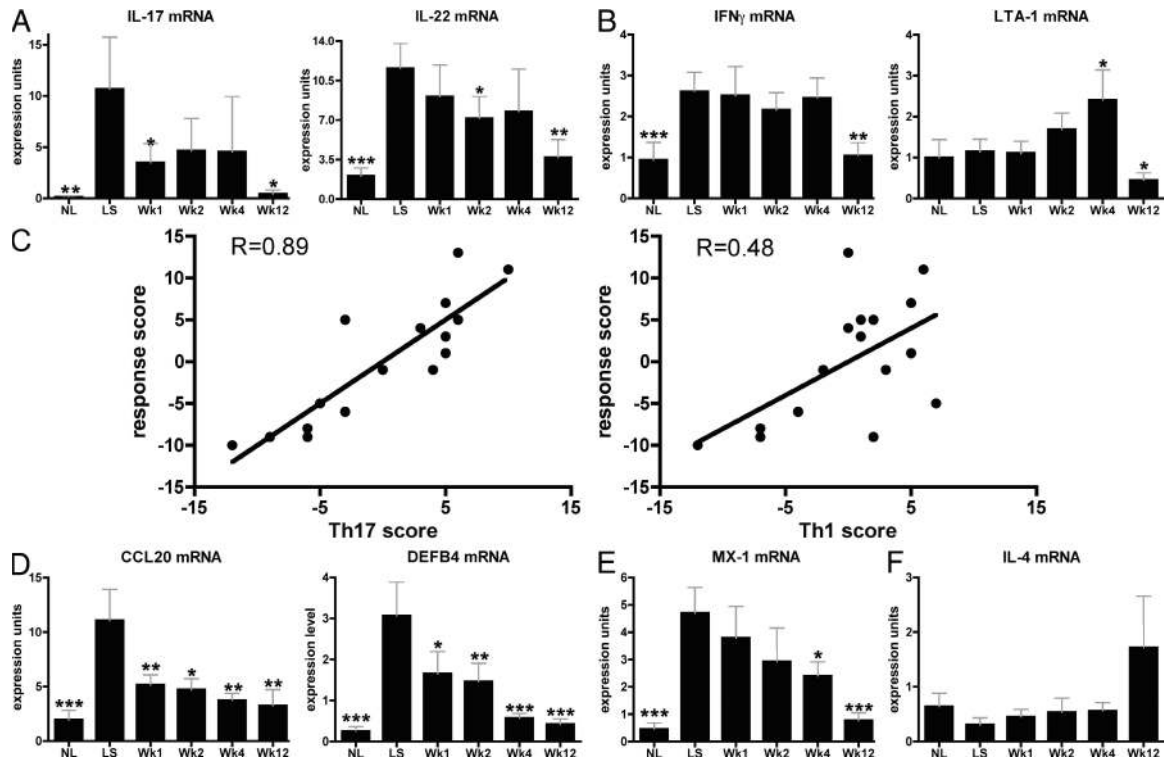
Figure 2. Th17 cell products and downstream mediators are rapidly down–modulated with etanercept treatment compared with Th1 and Th2 cell products. mRNA expression normalized to HARP for (A) Th17 cell products IL-17 and IL-22 and (B) Th1 cell products IFN-γ and LTA-1. Error bars represent the mean ± SEM. (C) Multivariate U-statistics correlating the change in Th17 or Th1 cell products with histological response (epidermal thickness, K16, and Ki67) over time. (D) Downstream effectors of Th17 cells, CCL20, and DEFB4. (E) MX-1, downstream effector of Th1 cells. (F) Th2 cell product IL-4. All mRNA was evaluated in nonlesional skin (NL), lesional skin (LS), and in the lesional index plaque at weeks 1, 2, 4, and 12. Error bars represent the mean ± SEM. Baseline lesional values were compared with other time points. *, P < 0.05; **, P < 0.01; ***, P < 0.001.

P < 0.05), and IL-8 (week 1, P < 0.01), findings that were previously reported by our group at 1 mo, the earliest time point of that study (30). In contrast, TGF-β was not significantly altered with treatment (Fig. S1, available at http://www.jem.org/cgi/content/full/jem.20071094/DC1). In summary, although Th17 cell products and downstream effector molecules regulating keratinocyte hyperplasia are modulated rapidly during the course of etanercept treatment, Th1 and Th2 cell products are modulated late, months after the disease has significantly improved.

Products of TNF–inducible NO synthase (iNOS)–producing DCs (TipDCs) were rapidly down–modulated with etanercept treatment

We have previously described the TipDC as a major pathogenic cell in psoriasis (27). Using RT-PCR and double-label immunofluorescence, we show that TipDC products were rapidly down-modulated with etanercept treatment (Fig. 3 A). iNOS mRNA was significantly decreased by week 2 (P < 0.05), IL-20 mRNA was decreased by week 1 (P < 0.05), and both IL-23 subunits (p19 and p40) were reduced by weeks 1 and 2 (P = 0.06 and P < 0.05, respectively). In contrast, transcription of the IL-12 p35 subunit was not modulated by etanercept.

We confirmed that IL-20 was primarily a product of CD11c⁺ myeloid DCs in untreated psoriasis using double-

label immunofluorescence showing >90% colocalization (yellow color) of IL-20 antigen with CD11c antigen in baseline lesional sections (Fig. 3 B). IL-20⁺CD11c⁺ cells were clustered in elongated dermal papillae, where there is an extensive vascular supply, and a few cells invaded the epidermis. At week 2 of etanercept treatment, <10% of CD11c⁺ cells produced IL-20, and by week 12 no visible overlap was apparent. Similarly, IL-23 p40 subunit was produced by 100% of CD11c⁺ cells in psoriasis lesional skin but was not detected at weeks 2 and 12 of etanercept treatment (Fig. 3 C).

TNF was produced in >95% of CD11c⁺ DCs within untreated psoriasis plaques, as indicated by the yellow cells clustering near the dermal–epidermal junction and infiltrating the epidermis (Fig. 3 D). At weeks 2 and 12 of etanercept treatment, no visible overlap was apparent. iNOS protein in CD11c⁺ DCs is also down-modulated by etanercept treatment, as previously described by our group (30). Hence, iNOS, TNF, IL-20, and IL-23 are TipDC products down-modulated within the first 2 wk of etanercept treatment.

Myeloid DCs in the skin down-regulated maturation markers by week 2 of etanercept treatment

Single antigens specific for mature DC identification include CD83 and/or DC–lysosomal-associated membrane protein
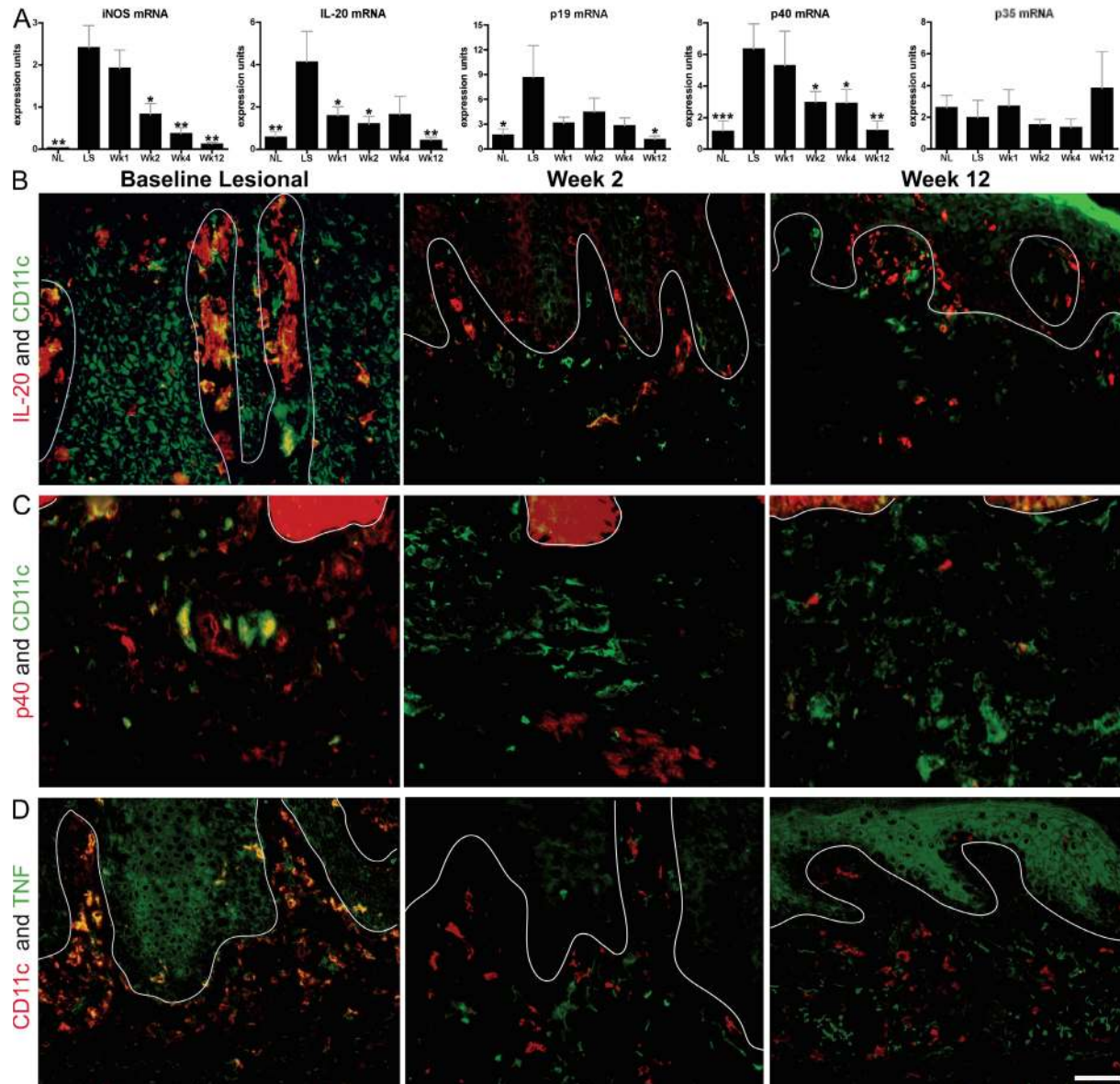
Figure 3.   Inflammatory DC products are rapidly down–modulated with etanercept treatment. (A) mRNA expression normalized to HARP for the inflammatory DC cell products iNOS, IL-20, IL-23 p19, IL-23/IL-12 p40, and IL-12 p35 in nonlesional skin (NL), lesional skin (LS), and in the lesional index plaque at weeks 1, 2, 4, and 12. Baseline lesional values were compared with other time points. *, $P < 0.05$; **, $P < 0.01$; ***, $P < 0.001$. (B–D) Double-label immunofluorescence of myeloid DCs (CD11c) and various mediators (IL-20, IL-12/23 p40, and TNF) demonstrating coexpression (yellow) in baseline lesional skin compared with weeks 2 and 12, showing a reduction in myeloid DCs and their products with etanercept treatment. (B) CD11c (green) and IL-20 (red); (C) CD11c (green) and IL-23/IL-12 p40 (red); and (D) CD11c (red) and TNF (green). The white lines identify the dermal epidermal junction. Auto-fluorescent keratinocytes appear in all panels. Bar, 100 μm.

(DC-LAMP). In responding patients, CD83^{+} DCs were scattered throughout the psoriatic epidermis and upper dermis, whereas DC-LAMP^{+} DCs aggregated together in clusters in the upper reticular dermis (Fig. 4 A). CD83 and DC-LAMP were significantly decreased by weeks 1 and 2 of etanercept treatment ($P < 0.01$ and 0.05, respectively; Fig. 4 B). The larger mean number of CD11c^{+} myeloid cells in lesional skin (247 cells/mm; SEM = 31.9; Fig. 1 C) compared with CD83^{+} (9 cells/mm; SEM = 3; Fig. 4 B) and DC-LAMP^{+} (49 cells/mm;

SEM = 6.8; Fig. 4 B) DCs suggests that mature DCs were a subset of lesional DC infiltrate.

Maturation of migrant dermal DCs, as measured by levels of surface co-stimulatory molecules, was also decreased by week 2 of etanercept treatment (Fig. 4 C). Using FACS analysis, we gated on cells that met the classic definition of DCs (Lin^{−}CD11c^{+}HLA-DR^{+}) and determined the expression levels of CD86, HLA-DR, CD40, and CD11c on cells emigrating from the dermis at baseline (week 0) and week 2 ($n = 5$ patients).
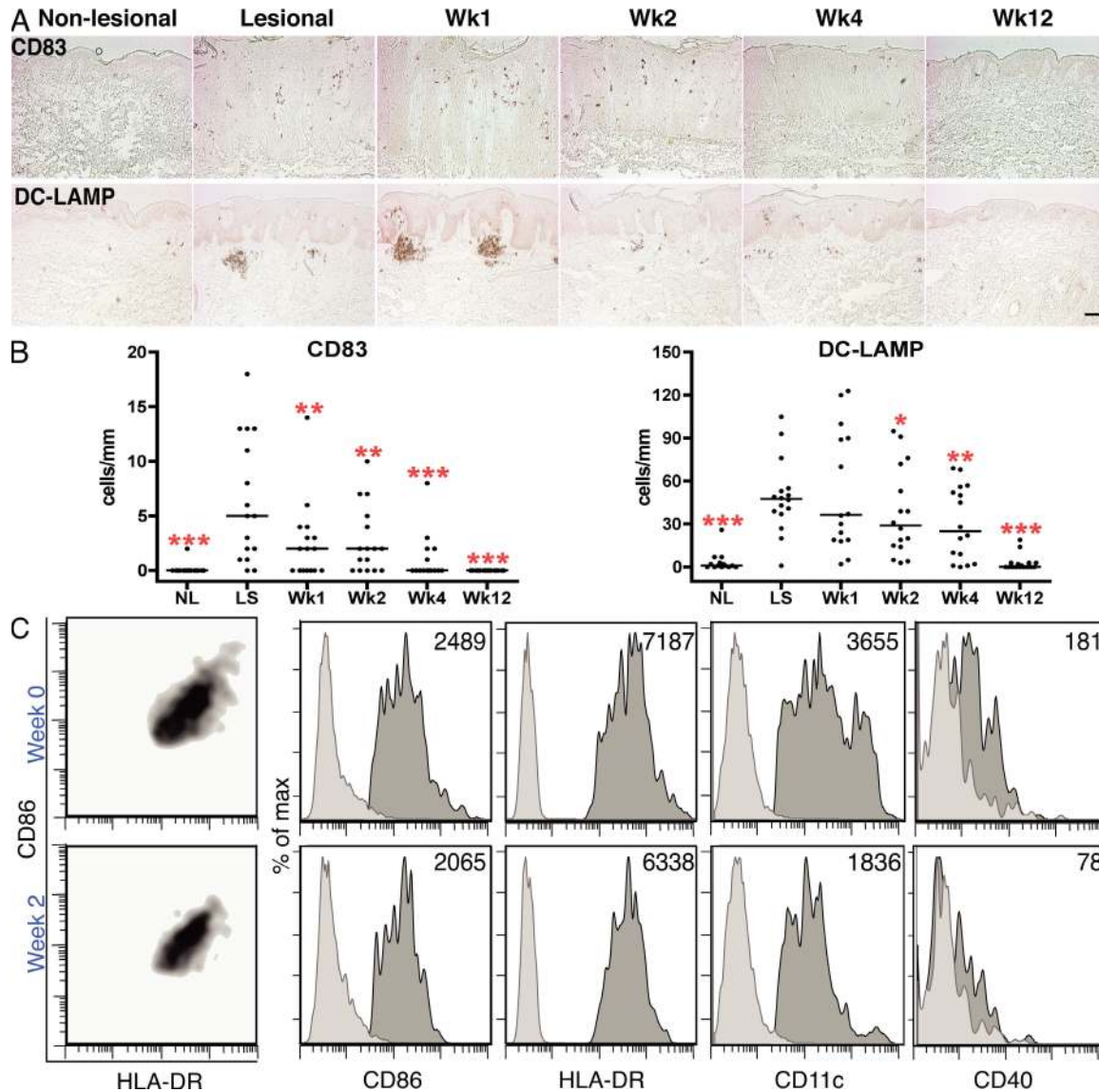
Figure 4. DCs down–regulate maturation and co-stimulatory molecules with etanercept treatment. (A) Immunohistochemistry for the mature DC markers CD83 and DC-LAMP in nonlesional skin (NL), lesional skin (LS), and in the lesional index plaque at weeks 1, 2, 4, and 12. Bar, 100 μm. (B) Quantification of CD83+ and DC-LAMP+ cells per millimeter (n = 16) during etanercept treatment. Horizontal bars represent the mean. Baseline lesional values were compared with other time points. *, P < 0.05; **, P < 0.01; ***, P < 0.001. CD83 and DC-LAMP baseline lesional cell counts have been previously reported (reference 50). (C) FACS analysis at baseline (week 0) and matched week 2 etanercept-treated lesional dermal single-cell suspensions. Acquired cells were gated on myeloid DCs (Lin−HLA-DR+CD11c+; dark gray). MFI is indicated in the top right corner of each histogram; isotypes are shown in light gray.

At baseline, there was a subset of CD86hiHLA-DRhi cells that was not present after 2 wk of etanercept treatment. Mean fluorescence intensity (MFI) of the DC activation markers CD86, HLA-DR, and CD40 decreased in all week–2 samples (a representative patient is shown in Fig. 4 C). The myeloid lineage marker CD11c was decreased in three samples and increased in two samples. Cell size (foward scatter–height) and complexity (side scatter–area) decreased in all samples (unpublished data). Thus, myeloid dermal DC activation (CD86, HLA-DR, and CD40) and cell size/complexity are reduced by week 2 of etanercept treatment compared with baseline.

Etanercept blocked in vitro–derived DC maturation and IL-23 production and immunostimulatory capacity, and shifted differentiation toward a macrophage–like phenotype

Monocyte-derived DCs (MoDCs) cultured with etanercept decreased CD86 expression threefold and HLA-DR expression fivefold (Fig. 5 A). CD11c expression decreased slightly, as did cell complexity (side scatter–area). RT-PCR on three paired biological replicates showed a significant decrease in IL-23 subunits p19 and p40 (P = 0.02 and 0.05, respectively), but there was no significant decrease in IL-12 subunit p35 (P = 0.25; Fig. S2, available at http://www.jem.org/cgi/content/full/
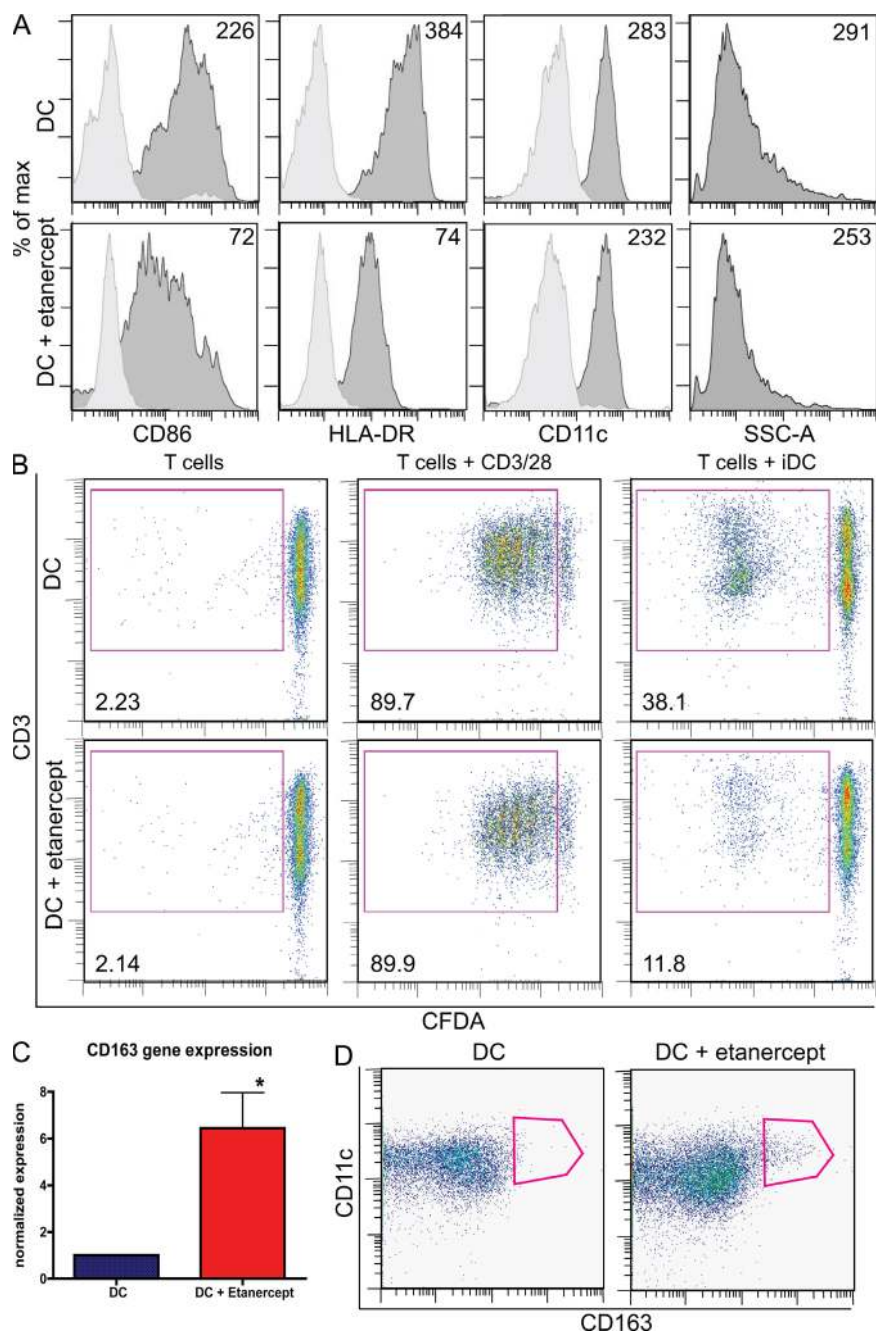
Figure 5. In vitro MoDCs generated in the presence of etanercept are less mature and less immunostimulatory, and express macrophage antigen CD163. (A) FACS analysis of MoDCs generated without or with etanercept. Acquired cells were gated on myeloid DCs (Lin−HLA-DR+CD11c+; dark gray). MFI is indicated in top right corner of each histogram; isotypes are shown in light gray. (B) MLR comparing MoDCs matured with and without etanercept (T cells + iDC). T cells alone and T cells + CD3/28 beads serve as negative and positive controls, respectively. The percentage of proliferation is indicated in the bottom left corner of each FACS plot. (C) Comparison of CD163 mRNA expression (gene array) in MoDCs generated without (blue) or with (red) etanercept. Error bars represent the mean ± SEM. *, P < 0.05. (D) Increased surface expression of CD163 on MoDCs generated with etanercept was confirmed by flow cytometry. CFDA, carboxyfluorescein diacetate; iDC, inflammatory DC.

jem.20071094/DC1). Likewise, IL-6 was down-regulated (P = 0.04), whereas TGF-β1 was up-regulated (P = 0.05). MoDCs cultured with etanercept were also an average of two to threefold less stimulatory than control DCs in a mixed leukocyte reaction (MLR; n = 2). Etanercept did not affect

the stimulation of T cells alone or T cells stimulated with CD3/CD28 beads (Fig. 5 B).

Gene array on control MoDCs compared with those cultured with etanercept revealed that CD163, a macrophage scavenger receptor, was strongly up-regulated (6.5-fold increase;

P < 0.05; Fig. 5 C). We confirmed these results using FACS analysis and identified up-regulation of CD163 protein on CD11c^{+} cells matured with etanercept compared with control DCs (Fig. 5 D). Etanercept had no significant effect on the expression of Th1 (IFN-γ) or Th17 (IL-17 and IL-22) cytokine mRNAs in activated T cells with or without etanercept (n = 3; unpublished data).

The small number of nonresponders in this trial (n = 4) limits statistical comparison with responders (n = 16). However, for interest, we have included data from nonresponders in Fig. S3 (available at http://www.jem.org/cgi/content/full/jem.20071094/DC1). Of note, the IL-17 response genes CCL20 and DEFB4 are not down-modulated as rapidly or consistently in nonresponders (Fig. S3 C) as they are in responders (Fig. 2 D). Reactive epidermal hyperplasia is also not suppressed to the same extent as in responders.

DISCUSSION

This study contains new information that informs two separate but related topics: the therapeutic mechanisms of the TNF inhibitor etanercept, and the network of inflammatory cytokines and leukocytes that drive psoriasis pathogenesis. Presently, there are three TNF inhibitors in widespread use for the treatment of psoriasis, psoriatic arthritis, rheumatoid arthritis, IBD, and ankylosing spondylitis: infliximab and adalimumab, which are monoclonal TNF antibodies, and etanercept, which is a dimeric TNFRII Fc fusion protein (31, 32). Although often considered as a therapeutic class, these agents are structurally different, have different affinities for TNF, and are not uniformly effective for all inflammatory diseases (33). Although more than one million patients have been treated with these drugs, there are surprisingly little data on therapeutic mechanisms in human inflammatory diseases. In this paper, we show that psoriasis disease improvement correlated with early reduction in DC and Th17 cell products and downstream effector molecules, and final disease resolution correlated with late down-modulation of Th1 cells.

When considering previous research on the TNF inhibitor mechanism, it is useful to divide response into early (hours to days) versus late (weeks to months) effects. In the case of infliximab and adalimumab, there are studies suggesting that broad apoptosis of inflammatory leukocytes is induced within hours of drug delivery (34, 35). With these agents, the reduction of cytokine-driven inflammation is likely a combination of inhibition of TNF-dependent cytokine production, as well as reducing cytokine-producing cells via apoptosis. Early apoptosis, however, is not a feature of etanercept treatment. Experiments on psoriasis lesions show some leukocyte apoptosis after 1 mo of treatment (36), suggesting that apoptosis is a secondary mechanism after growth factor/TNF withdrawal.

In this paper, we propose that an early mechanism of etanercept is to inhibit inflammatory DC cytokine production and maturation, leading to a reduction in the activity of Th17 cells. Recently, a new type of inflammatory myeloid CD11c^{+} DC was described in psoriasis, the TipDC (27). This cell type was first identified in a mouse model of innate immune response

to *Listeria monocytogenes* infection (37). In a previous clinical trial using etanercept, iNOS mRNA and protein, along with various other DC and T cell inflammatory cytokines and chemokines, were decreased by 1 mo of treatment (the earliest time point in that study) (30). Our current study uses even earlier time points to recreate the hierarchy of TNF-dependent mediators and separate primary (early) versus secondary (late) responses. We now show that multiple inflammatory products of TipDCs, including iNOS, TNF, IL-20, and IL23 p40 subunit, are reduced within 1–2 wk after beginning etanercept, whereas the number of CD11c^{+} DCs in the tissue is minimally affected during this time, suggesting an initial blockade of cytokine production by these cells rather than cell reduction. This suggests that TNF is an autocrine or paracrine inducer of TipDC inflammatory products that is blocked by etanercept. This direct effect on DCs is supported by our in vitro studies with MoDCs showing that etanercept blocked up-regulation of co-stimulatory and MHC class II molecules, IL-23 production, and immunostimulatory capacity.

The early modulation of TipDCs by etanercept may rapidly affect Th17 cells, beginning the process of molecular resolution before reduction in cellular infiltrates and long before clinical resolution. Our proposed psoriatic inflammatory pathway involves the production of IL-23 from these inflammatory TipDCs causing proliferation of Th17 cells, with subsequent induction of IL-17, IL-22, and other products (Fig. 6). IL-17 appears to serve as an inducer of keratinocytes to produce antimicrobial peptides like DEFB4, S100 acute-phase proteins, and chemokines such as IL-8 (38). Models of psoriasis suggest that IL-22 strongly induces keratinocyte hyperplasia and mediates IL-23–induced dermal inflammation and acanthosis (7). All of these products were down-modulated within 1–2 wk of etanercept treatment. The involvement of Th17 cells in psoriasis may now help explain the following: hyperplasia of psoriatic keratinocytes (IL-22); why psoriatics are relatively protected from bacterial infection (defensins); and why neutrophils that are normally reserved for acute inflammatory processes appear in a chronic inflammatory disease (IL-8). Moreover, histological resolution of the disease, as defined by decreased epidermal thickness and normalization of keratinocyte proliferation (Ki67) and differentiation (K16), correlates with rapidly decreased TipDC and Th17 cell products. Thus, these results suggest that Th17 cells are important for disease pathogenesis and may be modified by etanercept at an early time point.

Finally, although there is an emerging role for Th17 cells driving inflammation in psoriasis, Th1 cells may still be important for final disease resolution. Although TipDC and Th17 cell products are down-modulated within 2 wk of etanercept treatment, IFN-γ is not decreased until week 12, and STAT-1 (an IFN-γ–dependent transcription factor) is not significantly decreased until several months of treatment (30). Therefore, although histological disease resolution begins within weeks, complete remission does not occur until after several months of treatment, when both Th17 and Th1 cell products have been down-modulated. IFN-γ is a major inducer of MHC class II and acts synergistically with IL-17
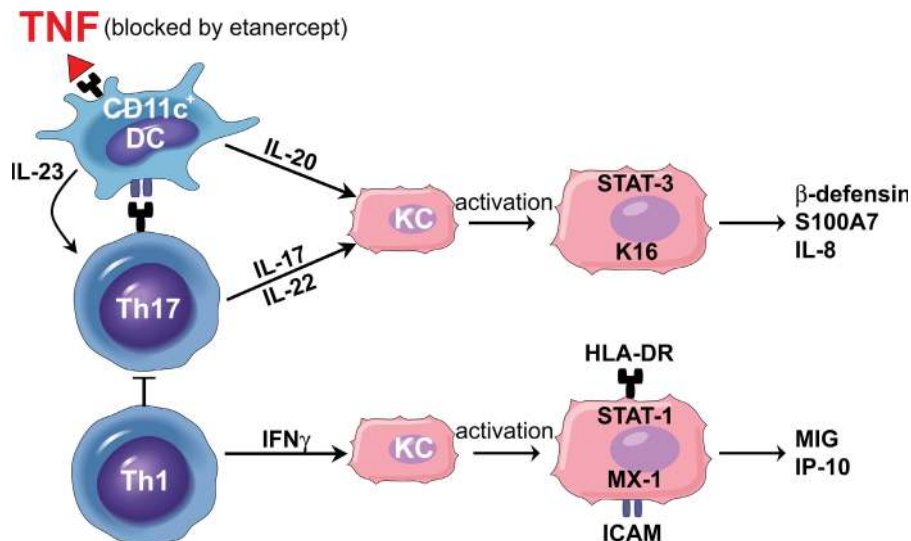
Figure 6. Proposed role of Th17 and Th1 cells in psoriasis pathogenesis. TNF stimulates CD11c^{+} inflammatory DCs to produce IL-23 and IL-20. DC activation and production of IL-23 supports Th17 cell survival and proliferation and induces the production of IL-17 and IL-22. DC and Th17 cell products activate keratinocytes, promoting the release of innate inflammatory molecules such as DEFB4, S100A7, and IL-8. Concurrently, Th1 cells producing IFN-γ activate keratinocytes to up-regulate MHC class II molecules (HLA-DR) and integrins (ICAM), and release cytokines including membrane Ig (MIG) and IFN-inducible protein 10 (IP-10). Th1 and Th17 cells may suppress each other's development, but IFN-γ can also act synergistically with IL-17 to increase ICAM expression and IL-8 release from keratinocytes. In psoriasis, etanercept may proximally inhibit this IL-23–IL-17 pathway to normalize keratinocyte proliferation and leukocyte infiltration.

to up-regulate keratinocyte intracellular adhesion molecule (ICAM)–1 and IL-8 production (39), suggesting that Th1 cells may be important for leukocyte activation. Activated T cells are required in the epidermis for psoriasis to develop (40), and most epidermal T cells are type 1 CD8^{+} cells (41); it follows that they must be ablated for disease resolution. Thus, although Th17 cells may be the major drivers of keratinocyte hyperplasia and inflammatory cytokine production, Th1 cells may be important for leukocyte activation and for sustaining a network of >100 genes linked to IFN-γ signaling (42). In addition, there may be important functional interactions between Th17 and Th1 cells, as cross-regulation has been recently demonstrated in model systems (15, 43). More work needs to be done to delineate the specific roles of Th17 and Th1 cells in psoriasis and in other examples of autoimmune inflammation.

MATERIALS AND METHODS

Patient studies and classification. 20 adult patients with moderate to severe psoriasis were treated with 50 mg etanercept (ENBREL; Amgen) subcutaneously biweekly for 12 wk under a Rockefeller University Institutional Review Board–approved protocol. Patients did not receive topical or systemic psoriasis therapy for a minimum of 1 mo before dosing. No patient was experiencing flare at the initiation of etanercept treatment. At baseline, 6-mm (diameter) punch biopsies were taken from an uninvolved area (nonlesional) and from an index psoriasis lesion. Punch biopsies were obtained again from the index lesion at weeks 1, 2, 4, and 12 of etanercept treatment. All biopsies were cut in half: one piece was frozen in liquid nitrogen for RNA extraction, and the other was frozen in cutting media for immunostaining. 3-cm² shave biopsies used for FACS analysis on dermal émigrés were obtained from lesional areas at baseline and week 2. Patients were classified as histological responders based on frozen section epidermal thickness, K16 immunostaining, and Ki67 cell counts decreasing to

nonlesional magnitude by week 12 (30). All data shown are from the 16 responder patients.

Immunohistochemistry. Tissue sections were stained with hematoxylin (Thermo Fisher Scientific) and eosin (Shandon) or with purified mouse anti–human monoclonal antibodies to K16 (clone K8.12, 1:1,000; Sigma-Aldrich), Ki67 (Mib-1, 1:100; Immunotech), CD11c (B-ly6, 1:100; BD Biosciences), CD3 (Sk7, 1:100; BD Biosciences), CD163 (5C6-FAT, 1:100; Acris Antibodies), CD83 (HB15e, 1:100; BD Biosciences), or DC-LAMP (104.G4, 1:50; Beckman Coulter). Biotin-labeled horse anti–mouse antibodies (Vector Laboratories) were amplified with avidin–biotin complex (Vector Laboratories) and developed with chromogen 3-amino-9-ethylcarbazole (Sigma-Aldrich). Positive cells per millimeter were counted manually using computer-assisted image analysis software (Image, version 6.1; National Institutes of Health [NIH]). Appropriate isotype controls were used.

Immunofluorescence. Frozen skin sections were fixed with acetone and blocked in 10% normal goat serum (Vector Laboratories) for 30 min. Primary antibodies CD11c (B-ly6, 1:100) or CD11c-FITC (3.9, 1:100; Invitrogen) were incubated overnight at 4°C, followed by secondary antibodies IL-20 (158609, 1:10; R&D Systems), IL-23/IL-12 p40 (31052.11, 1:50; R&D Systems), or TNF-α–FITC (6401.111, 1:25; BD Biosciences) again overnight at 4°C. FITC-labeled antibodies were amplified with anti–FITC Alexa Fluor 488, whereas other antibodies were amplified with goat anti–mouse IgG1 conjugated to Alexa Fluor 568. All primary and secondary antibodies were IgG1 isotype. Images were acquired using the appropriate filters from a microscope (Axio-plan 2I; Carl Zeiss, Inc.) with a numerical aperture lens (Plan-Apochromat 20×/0.7; Carl Zeiss, Inc.) and a cooled charge-coupled device camera (ORCA-ER; Hamamatsu) controlled by MetaVue software (MDS Analytical Technologies). Dermal collagen fibers gave green autofluorescence. FITC-conjugated antibodies gave background epidermal fluorescence.

Tissue mRNA gene expression. RNA was extracted from skin biopsies frozen in liquid nitrogen using the RNeasy Mini Kit (QIAGEN). RT-PCR was performed using EZ PCR core reagents, primers, and probes (Applied Biosystems), as previously described (44). The primers and probes for TaqMan

RT-PCR assays for K16, iNOS, IL-23 p19, IL-12/IL-23 p40, IFN-γ, IL-8, and human acidic ribosomal protein (HARP) were also previously described (44). Sequences of other primers and probes used in this study were as follows: CCL20 (MIP-3α) forward, GCTTTGATGTCAGTGCT-GCTACTC; CCL20 reverse, GTATCCAAGACAGCAGTCAAAGTTG; CCL20 probe, FAM-TGCGGCGAATCAGAAGCAGCAA-TAMRA; IL-6 forward, CCAGGAGCCCAGCTATGAAC; IL-6 reverse, CCCAGGG-AGAAGGCAACTG; IL-6 probe, FAM-CCTTCTCCACAAGCGCC-TTCGGT-TAMRA; IL-4 forward, CGACTGCACAGCAGTTCCA; IL-4 reverse, AGGTTCCTGTCGAGCCGTTT; IL-4 probe, FAM-AGGCA-CAAGCAGCTGATCCGATTCC-TAMRA; IL-20 (Hs00218888_m1); IL-12 p35 (Hs00168405_m1); LTA-1 (Hs00236874_m1); MX-1 (Hs00182073_m1); IL-17A (Hs00174383_m1); IL-22 (Hs00220924_m1); defensin (Hs00175474_m1); IL-1β (Hs00174097_m1); and TGF-β1 (Hs00171257_m1; all assays from were obtained from Applied Biosystems). The data were analyzed and samples were quantified by software provided with the sequence detection system (PRISM 7700, version 1.7; Applied Biosystems). Data were normalized to HARP housekeeping mRNA.

Single-cell suspension from shave biopsy and FACS analysis. Lesional shave biopsies from baseline and week 2 etanercept-treated patients were obtained and incubated in 1 mg/ml dispase (Invitrogen) overnight at 4°C. The epidermis was peeled off and discarded, and the dermis was transferred to fresh RPMI 1640 supplemented with 10% pooled human serum (Mediatech Inc.), 0.1% gentamicin reagent solution (Invitrogen), and 1% 1-M Hepes (Sigma-Aldrich). The dermis was incubated for 48 h at 37°C, and the supernatant was collected and filtered with 40-μm cell strainers (BD Biosciences). Cells were centrifuged and frozen in RPMI 1640 (Invitrogen) and 10% DMSO (American Type Culture Collection) for future FACS analysis.

Five baseline shave biopsy samples and five matched week 2 samples were stained with the following anti–human, mouse monoclonal antibodies: CD40-FITC (5C3, IgG1, 1:20; BD Biosciences), CD11c–PE-Cy5 (3.9, IgG1, 1:20; BioLegend), Lin-PE (CD3/CD19/CD20/CD56, IgG1 and IgG2b, 1:20), CD86–Alexa Fluor 647 (IT2.2, IgG2b, 1:33; BioLegend), and HLA-DR–Alexa Fluor 700 (L243, IgG2a, 1:50; BioLegend). In brief, cells were stained for 30 min at 4°C, washed with FACSwash (PBS with 0.1% sodium azide and 2% FBS), and resuspended in 1.3% formaldehyde (Thermo Fisher Scientific) in FACSwash. Samples were acquired using a flow cytometer (LSR II; BD Biosciences) and analyzed with FlowJo software (Tree Star, Inc.). Appropriate isotype controls were used.

In vitro etanercept blocking assays, MLR, and gene array. The process for making MoDCs has been previously described (45). All analysis was performed on day 5 immature DCs. 10 μg/ml etanercept was added to experimental wells on days 0, 2, and 4. We chose this concentration of etanercept as it approximates the plasma concentration of the drug when given 50 mg biweekly. Three biological replicates of each condition were prepared. For the MLR, responding T cells were obtained from a normal volunteer by density centrifugation over Ficoll–Paque Plus (GE Healthcare), followed by T cell purification using RosetteSep (StemCell Technologies Inc.). T cells were labeled with 10 μm CFSE and co-cultured with DCs matured with or without 10 μg/ml etanercept. T cell proliferation was analyzed on day 6 with a DC/T cell ratio of 1:50. The cultures were harvested and stained with 250 ng/ml propidium iodide, to label dead cells, and CD3-APC (Becton Dickenson). The CFSE-low cells were quantified as a percentage of live cells in the culture (46). T cells for cytokine FACS staining were purified from peripheral blood using T cell–negative select beads (Invitrogen), activated using PMA and ionomycin as previously described (47), and cultured for 24 h with or without 10 μg/ml etanercept. Three biological replicates of each condition were prepared.

RNA was extracted using the RNeasy Mini Kit, and DNA was removed with on-column DNase digestion using the RNase-free DNase Set (QIAGEN) and used for either RT-PCR or gene array. For each GeneChip (Affymetrix), 4 μg of total RNA was reverse transcribed, amplified, and labeled as previously described using an RNA transcript labeling kit (BioArray HighYield; Enzo Life Sciences) (48). 15 μg of the biotinylated complementary RNA was then hybrid-

ized to the Human Genome U133A 2.0 Array (14,500 probe sets; Affymetrix). The chips were washed, stained with streptavidin-PE, and scanned with a scanner (GeneArray; Hewlett-Packard Company). Raw fluorescence intensity values were analyzed using GeneChip operating software (version 1.2; Affymetrix) and GeneSpring software (Agilent Technologies). Data for triplicates were averaged. Microarry data have been deposited in the National Center for Biotechnology Information Gene Expression Omnibus under accession no. GSE9239.

Statistical analysis. All clinical variables were analyzed using repeated measures analysis of variance models using the MIXED procedure (available from SAS). The within-subjects correlation that best modeled the data was an AR(1) structure that considered each time measurement as dependent on the previous one. Differences between lesional (baseline) and weeks 1, 2, 4, and 12 were estimated, and the one-tail p-values are designated as follows: *, $P < 0.05$; **, $P < 0.01$; and ***, $P < 0.001$. To assess the correlation between IL-17/IL-22 or IFN-γ/LTA-1 with epidermal thickness/K16/Ki67, the muStat package (available at www.r-project.org) was used. U scores were computed for histological response and gene expression, taking into account the clustered structure of the data (time points for each patient), as previously described (49). Variables were normalized within patients to make all patients comparable. Correlation between the histological response score and the expression score was calculated, and its significance is presented in the figures. In vitro gene array data that passed the Benjamini and Hochberg correction and had p-values <0.05 were considered relevant.

Online supplemental material. Fig. S1 shows additional RT-PCR data for responders ($n = 16$). Fig. S2 shows RT-PCR from in vitro–derived DCs matured without (control) and with etanercept (+etanercept). Fig. S3 includes RT-PCR data and histology from nonresponding patients ($n = 4$). Online supplemental material is available at http://www.jem.org/cgi/content/full/jem.20071094/DC1.

REFERENCES

1. Lowes, M.A., A.M. Bowcock, and J.G. Krueger. 2007. Pathogenesis and therapy of psoriasis. *Nature.* 445:866–873.
2. Blauvelt, A. 2007. New concepts in the pathogenesis and treatment of psoriasis: key roles for IL-23, IL-17A and TGF-β1. *Expert Rev. Dermatol.* 2:69–78.
3. Prinz, J.C., B. Grob, S. Vollmer, P. Trommler, I. Strobel, M. Meurer, and G. Plewig. 1994. T cell clones from psoriasis skin lesions can promote keratinocyte proliferation in vitro via secreted products. *Eur. J. Immunol.* 24:593–598.
4. Bettelli, E., M. Oukka, and V.K. Kuchroo. 2007. T(H)-17 cells in the circle of immunity and autoimmunity. *Nat. Immunol.* 8:345–350.
5. Liang, S.C., X.Y. Tan, D.P. Luxenberg, R. Karim, K. Dunussi-Joannopoulos, M. Collins, and L.A. Fouser. 2006. Interleukin (IL)-22 and IL-17 are coexpressed by Th17 cells and cooperatively enhance expression of antimicrobial peptides. *J. Exp. Med.* 203:2271–2279.
6. Wolk, K., E. Witte, E. Wallace, W.D. Docke, S. Kunz, K. Asadullah, H.D. Volk, W. Sterry, and R. Sabat. 2006. IL-22 regulates the expression

of genes responsible for antimicrobial defense, cellular differentiation, and mobility in keratinocytes: a potential role in psoriasis. *Eur. J. Immunol.* 36:1309–1323.

7. Zheng, Y., D.M. Danilenko, P. Valdez, I. Kasman, J. Eastham-Anderson, J. Wu, and W. Ouyang. 2007. Interleukin-22, a T(H)17 cytokine, mediates IL-23–induced dermal inflammation and acanthosis. *Nature.* 445:648–651.

8. Sa, S.M., P.A. Valdez, J. Wu, K. Jung, F. Zhong, L. Hall, I. Kasman, J. Winer, Z. Modrusan, D.M. Danilenko, and W. Ouyang. 2007. The effects of IL-20 subfamily cytokines on reconstituted human epidermis suggest potential roles in cutaneous innate defense and pathogenic adaptive immunity in psoriasis. *J. Immunol.* 178:2229–2240.

9. Mangan, P.R., L.E. Harrington, D.B. O'Quinn, W.S. Helms, D.C. Bullard, C.O. Elson, R.D. Hatton, S.M. Wahl, T.R. Schoeb, and C.T. Weaver. 2006. Transforming growth factor-beta induces development of the T(H)17 lineage. *Nature.* 441:231–234.

10. Sutton, C., C. Brereton, B. Keogh, K.H. Mills, and E.C. Lavelle. 2006. A crucial role for interleukin (IL)-1 in the induction of IL-17–producing T cells that mediate autoimmune encephalomyelitis. *J. Exp. Med.* 203:1685–1691.

11. Annunziato, F., L. Cosmi, V. Santarlasci, L. Maggi, F. Liotta, B. Mazzinghi, E. Parente, L. Fili, S. Ferri, F. Frosali, et al. 2007. Phenotypic and functional features of human Th17 cells. *J. Exp. Med.* 204:1849–1861.

12. Vanden Eijnden, S., S. Goriely, D. De Wit, F. Willems, and M. Goldman. 2005. IL-23 up-regulates IL-10 and induces IL-17 synthesis by polyclonally activated naive T cells in human. *Eur. J. Immunol.* 35:469–475.

13. Komiyama, Y., S. Nakae, T. Matsuki, A. Nambu, H. Ishigame, S. Kakuta, K. Sudo, and Y. Iwakura. 2006. IL-17 plays an important role in the development of experimental autoimmune encephalomyelitis. *J. Immunol.* 177:566–573.

14. Nakae, S., Y. Komiyama, A. Nambu, K. Sudo, M. Iwase, I. Homma, K. Sekikawa, M. Asano, and Y. Iwakura. 2002. Antigen-specific T cell sensitization is impaired in IL-17-deficient mice, causing suppression of allergic cellular and humoral responses. *Immunity.* 17:375–387.

15. Park, H., Z. Li, X.O. Yang, S.H. Chang, R. Nurieva, Y.H. Wang, Y. Wang, L. Hood, Z. Zhu, Q. Tian, and C. Dong. 2005. A distinct lineage of CD4 T cells regulates tissue inflammation by producing interleukin 17. *Nat. Immunol.* 6:1133–1141.

16. Nakae, S., A. Nambu, K. Sudo, and Y. Iwakura. 2003. Suppression of immune induction of collagen-induced arthritis in IL-17-deficient mice. *J. Immunol.* 171:6173–6177.

17. Uyttenhove, C., and J. Van Snick. 2006. Development of an anti-IL-17A auto-vaccine that prevents experimental auto-immune encephalomyelitis. *Eur. J. Immunol.* 36:2868–2874.

18. Yen, D., J. Cheung, H. Scheerens, F. Poulet, T. McClanahan, B. McKenzie, M.A. Kleinschek, A. Owyang, J. Mattson, W. Blumenschein, et al. 2006. IL-23 is essential for T cell–mediated colitis and promotes inflammation via IL-17 and IL-6. *J. Clin. Invest.* 116:1310–1316.

19. Fujino, S., A. Andoh, S. Bamba, A. Ogawa, K. Hata, Y. Araki, T. Bamba, and Y. Fujiyama. 2003. Increased expression of interleukin 17 in inflammatory bowel disease. *Gut.* 52:65–70.

20. Nielsen, O.H., I. Kirman, N. Rudiger, J. Hendel, and B. Vainer. 2003. Upregulation of interleukin-12 and -17 in active inflammatory bowel disease. *Scand. J. Gastroenterol.* 38:180–185.

21. te Velde, A.A., F. de Kort, E. Sterrenburg, I. Pronk, F.J. ten Kate, D.W. Hommes, and S.J. van Deventer. 2007. Comparative analysis of colonic gene expression of three experimental colitis models mimicking inflammatory bowel disease. *Inflamm. Bowel Dis.* 13:325–330.

22. Kotake, S., N. Udagawa, N. Takahashi, K. Matsuzaki, K. Itoh, S. Ishiyama, S. Saito, K. Inoue, N. Kamatani, M.T. Gillespie, et al. 1999. IL-17 in synovial fluids from patients with rheumatoid arthritis is a potent stimulator of osteoclastogenesis. *J. Clin. Invest.* 103:1345–1352.

23. Ikeuchi, H., T. Kuroiwa, N. Hiramatsu, Y. Kaneko, K. Hiromura, K. Ueki, and Y. Nojima. 2005. Expression of interleukin-22 in rheumatoid arthritis: potential role as a proinflammatory cytokine. *Arthritis Rheum.* 52:1037–1046.

24. Li, J., D. Li, and Z. Tan. 2004. The expression of interleukin-17, interferon-gamma, and macrophage inflammatory protein-3 alpha mRNA in patients with psoriasis vulgaris. *J. Huazhong Univ. Sci. Technolog. Med. Sci.* 24:294–296.

25. Arican, O., M. Aral, S. Sasmaz, and P. Ciragil. 2005. Serum levels of TNF-alpha, IFN-gamma, IL-6, IL-8, IL-12, IL-17, and IL-18 in patients with active psoriasis and correlation with disease severity. *Mediators Inflamm.* 2005:273–279.

26. Piskin, G., R.M. Sylva-Steenland, J.D. Bos, and M.B. Teunissen. 2006. In vitro and in situ expression of IL-23 by keratinocytes in healthy skin and psoriasis lesions: enhanced expression in psoriatic skin. *J. Immunol.* 176:1908–1915.

27. Lee, E., W.L. Trepicchio, J.L. Oestreicher, D. Pittman, F. Wang, F. Chamian, M. Dhodapkar, and J.G. Krueger. Increased expression of interleukin 23 p19 and p40 in lesional skin of patients with psoriasis vulgaris. *J. Exp. Med.* 199:125–130.

28. Tyring, S., A. Gottlieb, K. Papp, K. Gordon, C. Leonardi, A. Wang, D. Lalla, M. Woolley, A. Jahreis, R. Zitnik, et al. 2006. Etanercept and clinical outcomes, fatigue, and depression in psoriasis: double-blind placebo-controlled randomised phase III trial. *Lancet.* 367:29–35.

29. Papp, K.A., S. Tyring, M. Lahfa, J. Prinz, C.E. Griffiths, A.M. Nakanishi, R. Zitnik, P.C. van de Kerkhof, and L. Melvin. 2005. A global phase III randomized controlled trial of etanercept in psoriasis: safety, efficacy, and effect of dose reduction. *Br. J. Dermatol.* 152:1304–1312.

30. Gottlieb, A.B., F. Chamian, S. Masud, I. Cardinale, M.V. Abello, M.A. Lowes, F. Chen, M. Magliocco, and J.G. Krueger. 2005. TNF inhibition rapidly down-regulates multiple proinflammatory pathways in psoriasis plaques. *J. Immunol.* 175:2721–2729.

31. Scott, D.L., and G.H. Kingsley. 2006. Tumor necrosis factor inhibitors for rheumatoid arthritis. *N. Engl. J. Med.* 355:704–712.

32. Atzeni, F., M. Turiel, F. Capsoni, A. Doria, P. Meroni, and P. Sarzi-Puttini. 2005. Autoimmunity and anti–TNF-alpha agents. *Ann. NY Acad. Sci.* 1051:559–569.

33. Furst, D.E., R. Wallis, M. Broder, and D.O. Beenhouwer. 2006. Tumor necrosis factor antagonists: different kinetics and/or mechanisms of action may explain differences in the risk for developing granulomatous infection. *Semin. Arthritis Rheum.* 36:159–167.

34. Lugering, A., M. Schmidt, N. Lugering, H.G. Pauels, W. Domschke, and T. Kucharzik. 2001. Infliximab induces apoptosis in monocytes from patients with chronic active Crohn's disease by using a caspase-dependent pathway. *Gastroenterology.* 121:1145–1157.

35. Shen, C., G.V. Assche, S. Colpaert, P. Maerten, K. Geboes, P. Rutgeerts, and J.L. Ceuppens. 2005. Adalimumab induces apoptosis of human monocytes: a comparative study with infliximab and etanercept. *Aliment. Pharmacol. Ther.* 21:251–258.

36. Malaviya, R., Y. Sun, J.K. Tan, A. Wang, M. Magliocco, M. Yao, J.G. Krueger, and A.B. Gottlieb. 2006. Etanercept induces apoptosis of dermal dendritic cells in psoriatic plaques of responding patients. *J. Am. Acad. Dermatol.* 55:590–597.

37. Serbina, N.V., T.P. Salazar-Mather, C.A. Biron, W.A. Kuziel, and E.G.P. Am. 2003. TNF/iNOS-producing dendritic cells mediate innate immune defense against bacterial infection. *Immunity.* 19:59–70.

38. Kao, C.Y., Y. Chen, P. Thai, S. Wachi, F. Huang, C. Kim, R.W. Harper, and R. Wu. 2004. IL-17 markedly up-regulates beta-defensin-2 expression in human airway epithelium via JAK and NF-kappaB signaling pathways. *J. Immunol.* 173:3482–3491.

39. Teunissen, M.B., C.W. Koomen, R. de Waal Malefyt, E.A. Wierenga, and J.D. Bos. 1998. Interleukin-17 and interferon-gamma synergize in the enhancement of proinflammatory cytokine production by human keratinocytes. *J. Invest. Dermatol.* 111:645–649.

40. Conrad, C., O. Boyman, G. Tonel, A. Tun-Kyi, U. Laggner, A. de Fougerolles, V. Kotelianski, H. Gardner, and F.O. Nestle. 2007. alpha(1)beta(1) integrin is crucial for accumulation of epidermal T cells and the development of psoriasis. *Nat. Med.* 13:836–842.

41. Gudjonsson, J.E., A. Johnston, H. Sigmundsdottir, and H. Valdimarsson. 2004. Immunopathogenic mechanisms in psoriasis. *Clin. Exp. Immunol.* 135:1–8.

42. Lew, W., E. Lee, and J.G. Krueger. 2004. Psoriasis genomics: analysis of proinflammatory (type 1) gene expression in large plaque (Western) and small plaque (Asian) psoriasis vulgaris. *Br. J. Dermatol.* 150:668–676.

43. Nakae, S., Y. Iwakura, H. Suto, and S.J. Galli. 2007. Phenotypic differences between Th1 and Th17 cells and negative regulation of Th1 cell differentiation by IL-17. *J. Leukoc. Biol.* 81:1258–1268.

44. Chamian, F., M.A. Lowes, S.L. Lin, E. Lee, T. Kikuchi, P. Gilleaudeau, M. Sullivan-Whalen, I. Cardinale, A. Khatcherian, I. Novitskaya, et al. 2005. Alefacept reduces infiltrating T cells, activated dendritic cells, and inflammatory genes in psoriasis vulgaris. *Proc. Natl. Acad. Sci. USA*. 102:2075–2080.

45. Dhodapkar, K.M., J. Krasovsky, B. Williamson, and M.V. Dhodapkar. 2002. Antitumor monoclonal antibodies enhance cross-presentation of-cellular antigens and the generation of myeloma-specific killer T cells by dendritic cells. *J. Exp. Med*. 195:125–133.

46. Zaba, L.C., J. Fuentes-Duculan, R.M. Steinman, J.G. Krueger, and M.A. Lowes. 2007. Normal human dermis contains distinct populations of CD11cBDCA-1 dendritic cells and CD163FXIIIA macrophages. *J. Clin. Invest*. 117:2517–2525.

47. Vugmeyster, Y., T. Kikuchi, M.A. Lowes, K. Howell, F. Chamian, M.H. Kagen, P. Gilleaudeau, E. Lee, W. Dummer, S. Pippig, et al. 2004. Efalizumab (anti-CD11a)-induced increase in leukocyte numbers in psoriasis patients is preferentially mediated by blocked entry of memory CD8+ T cells into the skin. *Clin. Immunol*. 113:38–46.

48. Zhou, X., J.G. Krueger, M.C. Kao, E. Lee, F. Du, A. Menter, W.H. Wong, and A.M. Bowcock. 2003. Novel mechanisms of T-cell and dendritic cell activation revealed by profiling of psoriasis on the 63,100-element oligonucleotide array. *Physiol. Genomics*. 13:69–78.

49. Wittkowski, K.M., and X. Liu. 2002. A statistically valid alternative to the TDT. *Hum. Hered*. 54:157–164.

50. Guttman-Yassky, E. 2007. Atopic dermatitis. *Curr. Probl. Dermatol*. 35:154–172.