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Chronically Inflamed Human Tissues Are Infiltrated by Highly Differentiated Th17 Lymphocytes

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Chronic inflammatory diseases are characterized by local tissue injury caused by immunocompetent cells, in particular CD4+ T lymphocytes, that are involved in the pathogenesis of these disorders via the production of distinctive sets of cytokines. Here, we have characterized single CD4+ T cells that infiltrate inflamed tissue taken from patients with psoriasis, Crohn’s disease, rheumatoid arthritis, or allergic asthma. Results from a cytokine production and gene profile analysis identified a population of in vivo differentiated retinoid-related orphan receptor γ-expressing T cells, producing high levels of IL-17, that can represent up to 30% of infiltrating T lymphocytes. Activated Th17 cells produced IL-26, TNF-α, lymphotoxin-β, and IL-22. IL-17 and IL-22 concentrations secreted by tissue infiltrating Th17 cells could reach up to 100 nM and were inversely correlated with the production of Th1- and Th2-associated cytokines. In addition, tissue-infiltrating Th17 cells are also characterized by high cell surface expression of CCR6, a chemokine receptor that was not expressed by Th1 and Th2 cells, isolated from the same lesions, and by the production of CCL20/MIP3α, a CCR6 ligand, associated with tissue infiltration. Culture supernatants of activated Th17 cells, isolated from psoriatic lesions, induced the expression of gene products associated with inflammation and abnormal keratinocyte differentiation in an IL-17 and IL-22-dependent manner. These results show that tissue-infiltrating Th17 cells contribute to human chronic inflammatory disease via the production of several inflammatory cytokines and the creation of an environment contributing to their migration and sequestration at sites of inflammation. The Journal of Immunology, 2008, 180: 7423–7430.

The CD4+ T lymphocytes play a pivotal role in orchestrating the physiopathological immune reactions that underlie the pathogenesis of chronic inflammatory diseases via the production of distinctive sets of cytokines, as well as the expression of cell surface molecules, that are involved in their functional activity (1). Because of the proinflammatory nature of its signature cytokine, IFN-γ, the activity of Th1 lymphocytes has traditionally been linked to the induction and progression of tissue damage that is a common dominator in these diseases, and the pathogeneses of psoriasis vulgaris, rheumatoid arthritis, or Crohn’s disease are generally considered to be associated with Th1 type immune responses. Recently, however, a novel subpopulation of mouse memory CD4+ T lymphocytes has been identified that produces high levels of IL-17 (2) and results from experimental models of tissue injury have underscored a major role for these so-called Th17 cells in the induction of inflammation and tissue destruction in various disorders, such as experimental autoimmune encephalomyelitis, collagen-induced arthritis, dermal inflammation, and inflammatory bowel disease (3–10). These reports corroborate results from earlier literature showing an association between the presence of IL-17-producing cells and inflammatory disease (reviews in Refs. 11 and 12).

IL-17 belongs to a recently discovered family of cytokines that contribute to the crosstalk between adaptive and innate immunity (5). IL-17 and its relative IL-17F have strong proinflammatory properties on a broad range of cellular targets, including epithelial and endothelial cells, fibroblasts, keratinocytes, osteoblasts, and monocytes/macrophages (12). In the mouse, IL-17 has been shown to induce the production of the proinflammatory cytokines IL-6 and TNF-α, the chemokines CCL2 and CCL3, as well as matrix metalloproteases that are effector molecules involved in leukocyte diapedesis and tissue destruction (13). IL-17 also mediates the proliferation, maturation, and chemotaxis of neutrophils (14).

Recently, the identification of peripheral blood-derived (15, 16) human Th17 cells, as well as Th17 cells differentiated in vitro from naive peripheral blood T cells (17), has been reported. However, although human T lymphocyte populations with different cytokine production profiles, including Th17 cells, can be obtained following in vitro differentiation from naive peripheral blood-derived precursor cells, this approach does incompletely mimic the in vivo-inflammatory environment that drives the generation and differentiation of those T cells that mediate tissue inflammation, and little information is available on human IL-17-producing effector T cells that infiltrate inflammatory lesions. In the present study, we have phenotypically and functionally characterized in vivo differentiated Th17 lymphocytes isolated from inflamed tissues of patients with chronic inflammatory disease.

Materials and Methods

T lymphocyte cultures

In vivo differentiated human T cell populations were directly isolated from biopsies taken from inflammatory sites of patients suffering from chronic...
inflammatory or autoimmune disease. Cutaneous biopsies were obtained from active lesions of patients with psoriasis vulgaris (kindly provided by Dr. Nadia Raison-Peyron, Service de Dermatologie, Hôpital St. Eloi, Montpellier, France), bronchial biopsies from patients during an acute episode of severe asthma (from Dr. Pascal Chanez, Centre Hospitalier de l’Université Arnaud de Villeneuve, Montpellier, France), intestinal biopsies from patients with active Crohn’s disease (from Prof. Jean-Michel Fabre, Service de Chirurgie Digestive, Hôpital St. Eloi), and synovial tissue from patients with rheumatoid arthritis undergoing surgery for joint replacement (from Dr. François Canovas, Centre Hospitalier de l’Université Lapeyronie, Montpellier, France). All biopsies were obtained after informed consent forms were signed, according to a protocol established by the Ethics Committee of the University Hospitals of Montpellier. Punch biopsies were washed and transferred to a well of a 24-well tissue culture plate (Nunc) in the presence of anti-CD3 and anti-CD28-coated magnetic beads (Ref. 18; Invitrogen) in Yssel’s medium (19), supplemented with 1% human AB serum and 2 ng/ml rIL-2 (R&D Systems). After 3 days of culture, 2 ng/ml rIL-2 were added to the cultures, and growing T cells were collected after 7–10 days, stained with an anti-CD4 mAb, cloned using a flow cytometer (FACSVantage; BD Biosciences), and subsequently cultured in the presence of rIL-2 (2 ng/ml). From 10 to 14 days later, T cells were collected and used in subsequent experiments.

Keratinocyte cultures and in vitro-reconstituted epidermis
Primary human keratinocytes were cultured in a 12-well plate in keratinocyte growth medium (Promocell) and were used at a concentration of $1.5 \times 10^5$ cells/well in experimental procedures. Reconstituted human epidermis was generated as described previously (21).

ELISA
IL-4, IL-5, and IFN-γ production was analyzed by cytokine-specific ELISA, as described (20) or using kits for IL-10, IL-13 (Diaclone), IL-17, IL-22, and MIP-3α (R&D Systems).

FIGURE 1. Correlations of cytokine production profiles of tissue-infiltrating Th cells. CD4+ tissue-infiltrating T cell clones were stimulated with immobilized anti-CD3 and anti-CD28 Abs for 24 h, and the production of cytokines was quantified in the culture supernatants by ELISA.

A, Analysis of IL-4, IFN-γ, and IL-17 production by Th1, Th2, and Th17 clones. B, Number of Th1, Th2, and Th17 clones according to the magnitude of IL-4, IFN-γ, and IL-17 production. C, Comparison of IL-17 production with that of the other cytokines (units on x- and y-axis are in picograms per milliliter). Statistical correlations were determined according to Spearman’s rank correlation test, and differences between pairs of cytokines were determined by Student’s t test.

![Correlation of cytokine production profiles](http://www.jimmunol.org/)
Immunofluorescence and flow cytometry analysis

Reconstituted epidermis samples were collected and embedded in OCT compound (Miles), frozen in liquid nitrogen-precooled isopentane; and immunofluorescence was analyzed as described (21), using the anti-psoriasin mAb clone 47C1068 (Santa Cruz Biotechnology) in association with a secondary AlexaFluor 488-conjugated goat anti-mouse IgG (Invitrogen). Flow cytometry analysis of T cells was conducted as described (20), using PE-conjugated anti-CD4, -CCR5, and -CCR6 mAbs or an isotype control mAb (R&D Systems). Cell fluorescence intensity was analyzed on a FACSCalibur flow cytometer (BD Biosciences) using CellQuest software.

Quantitative PCR analysis

Total RNA was extracted using an RNeasy microkit (Qiagen), according to the manufacturer’s protocol. One microgram of RNA was reversed transcribed into cDNA using random hexamer primers and Superscript II reverse transcriptase (Invitrogen). Primers were designed using Primer3 software (http://frodo.wi.mit.edu/cgi-bin/primer3/primer38www.cgi). PCR amplifications reactions were conducted in duplicate with iQ-SYBR Green Supermix (Bio-Rad) in a 15-μl reaction volume containing 200 nM primers and 5 ng of cDNA, and using a Chromo4 System (Bio-Rad). Thermal cycling was initiated with a 3-min incubation at 95°C followed by 40 cycles of 95°C for 10 s, 55°C for 15 s, and 72°C for 15 s. The ΔCt method was retained for quantification and GAPDH, HPRT1, and HSPCR housekeeping genes used for multiple normalization as described previously (22).

Western blotting analysis

One million Th17 lymphocytes were incubated for 15 min with rIL-23, rIL-6, rIFN-γ, or rIL-4 (25 ng/ml) and were subsequently lysed in Laemmli loading buffer. Cell lysates were sonicated, submitted to SDS-PAGE gel electrophoresis, and transferred onto an Immobilon P transfer membrane (Millipore). The membranes were incubated overnight with a polyclonal Ab raised specific for phospho-705-Stat3, phospho-701-Stat1, or tubulin, respectively (Cell Signaling Technology) and stained with a HRP-conjugated goat anti-rabbit Ab (Biosource).

Software and statistical analysis

Three-dimensional scatter plots were drawn using Spotfire DecisionSite version 9.0 software. Correlations were determined by Spearman’s rank correlation test, and statistical significance was determined by Student’s t test using GraphPad Prism version 5.0 software (GraphPad Software). Results were statistically significant for p < 0.05.

Results

Identification of Th17 cells infiltrating inflamed tissues

CD4+ T cells were directly isolated from inflamed lesions of patients with chronic inflammatory disease, including psoriasis vulgaris, rheumatoid arthritis, or Crohn’s disease, as well as from bronchial biopsies taken from patients with severe asthma. After cloning by electronic sorting, the cytokine production profiles of >500 in vivo differentiated T cell clones was determined after their activation via CD3 and CD28. In addition to classic Th1 and Th2 cells, characterized by the reciprocal production of IFN-γ or IL-4, IL-5, and IL-13, respectively, IL-17-producing T cells were isolated from the inflammatory sites (Fig. 1, A and B). The use of short-term culture conditions, in the absence of exogenous polarizing cytokines, allowed a direct ex vivo comparison of production of IL-17 with that of IFN-γ and IL-4. The production of IL-17 was found to inversely correlate with the production of IFN-γ, as well as with the production of the Th2 response-associated cytokines IL-4, IL-5, and IL-13, with p values ranging from 10^-5 to 10^-10 depending on the studied cytokine (Fig. 1C). A large number of T cell clones producing Th1 or Th2 cytokines, in addition to IL-17, were isolated from inflamed tissue as well. No significant correlation, neither positive nor negative, could be established between the production of IL-17 and that of IL-10 (Fig. 1C). In contrast, however, the production of IL-17 by human CD4+ T cells was strongly and quantitatively correlated with that of IL-22 (p < 2 × 10^-5; Fig. 1C).

Infiltrating Th17 cells specifically express retinoid-related orphan receptor γ (RORγ)2 transcription factor

Expression of Th lineage-specific transcription factors is a distinctive feature of subpopulations of cytokine-producing T lymphocytes. In the mouse, the nuclear receptor retinoid-related orphan receptor γ (RORγ) has been shown to be necessary and sufficient to control IL-17 transcription and to drive the differentiation of IL-17-producing cells from naive T cells (23). All T cell clones, selected for their high levels of IL-17 and IL-22 production, specifically and constitutively expressed the gene product of RORγ, the human ortholog of mouse RORγ (Fig. 2; p < 0.002). None of these Th17 clones expressed the Th1-specific transcription factor T-bet which, as expected, was prominently expressed in the Th1 clones. In contrast, however, the transcription factor GATA-3 factor was found to be expressed preferentially in Th2 cells and to a lesser level in both Th1 and Th17 populations.

Cytokine and cytokine receptor profile of tissue-infiltrating Th17 cells

To further characterize human tissue-infiltrating Th17 cells, the expression profile of 230 genes corresponding to all known cytokines and chemokines, as well as their respective receptors, was analyzed by quantitative PCR and compared with that of Th1 and Th2 cells activated via CD3 and CD28 (n = 8 for each subpopulation). Sixty-six percent of the analyzed genes displayed a similar distribution profile among the three Th lymphocyte lineages (Fig. 3A). The Th17 lymphocytes predominantly expressed 12 genes that were either absent or strongly decreased in Th1 and Th2 lymphocytes. In addition to IL-17 and IL-22 mRNA, a strong expression IL-17F, IL-26, TNF-α, and lymphotoxin (LT)-β transcripts was detected (Fig. 3B). Another gene that was specifically

2 Abbreviations used in this paper: RORC, retinoid-related orphan receptor γ; LT, lymphotoxin; RORγ, retinoid-related orphan receptor γ.
expressed by the Th17 but by neither the Th1 nor the Th2 cell clones was the CCR6 ligand CCL20. The exclusive production of this chemokine by human Th17 cells was confirmed at protein level in the culture supernatants of the latter cells after their activation by via CDC3 and CD28, yet was undetectable in the culture supernatants of the other T cell subpopulations (Fig. 3C).
The cytokine receptor profile showed a preferential expression by Th17 cells of transcripts for the IL-1, IL-6, and IL-23 receptor chains (Fig. 3D). A specific recruitment of the Stat3 signaling pathway was observed in Th17, but neither Th1 nor Th2, cells in response to stimulation with IL-23, thereby demonstrating the functionality of the corresponding receptor (Fig. 3E).

Chemokine receptor expression

Human tissue-infiltrating Th17 cells expressed a distinctive pattern of chemokine receptors. A 5-fold enhanced expression of CCR5 transcripts, as compared with the expression of this chemokine receptor by tissue-infiltrating Th1 and Th2 cells (Fig. 3D), was detected and confirmed by flow cytometry analysis (Fig. 3F). Importantly, however, a strongly enhanced expression of CCR6 transcripts was observed in human Th17 cells (Fig. 3D), which was completely absent from Th1 and Th2 cells. The specific cell surface expression of CCR6 by Th17 cells was confirmed by flow cytometry analysis (Fig. 3F).

CCR6 expression defines IL-17-producing T cells

To determine whether expression of CCR6 on T cells could be used to identify IL-17-producing T cell populations, we sorted and cloned primary CD4+ T cells, isolated from an active lesion of psoriatic skin, based on the presence or absence of this chemokine receptor. All CD4+ CCR6+ T cell clones were found to produce high levels of IL-17 and IL-22, whereas these two cytokines were not produced by the CD4+ CCR6- T clones. Production levels of IL-4, IL-5, and IL-13 by the latter clones were superior to those produced by the CD4+ CCR6+ T cell clones in a statistically significant manner, whereas the production of IL-10 was not statistically different between both populations (Table I).

The frequency of tissue-infiltrating Th17 cells isolated from the various biopsies, as measured by the production of IL-17 and IL-22 by individual T cell clones, was variable and between 10 and 30% of such cells could be detected in addition to IL-4-, IFN-γ-, and IL-10-producing cells (Table II). To validate these results and to exclude any effect of the cloning procedure, primary tissue-infiltrating T cells were propagated for 7 days under neutral culture conditions with anti-CD3/CD28 mAbs and the presence of cytokines in the culture supernatants was quantified by ELISA.

| Table I. Cytokine production profiles of CCR6+ and CCR6- T cell clones
<table>
<thead>
<tr>
<th>Clones Tested</th>
<th>Cytokines Produced (ng/ml)</th>
</tr>
</thead>
<tbody>
<tr>
<td>CCR6+</td>
<td>IL-17</td>
</tr>
<tr>
<td>No. 19</td>
<td>13.5 ± 17.1b</td>
</tr>
<tr>
<td>CCR6-</td>
<td>0.04 ± 0.05</td>
</tr>
<tr>
<td>p value</td>
<td>≤0.0001</td>
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</table>

* T cells obtained from the skin biopsy of a psoriatic patient were double-stained with an anti-CD4 FITC and an anti-CCR6 PE mAb. CD4+ CCR6+ and CD4+ CCR6- T cells were cloned using a cell sorter and expanded in culture conditions using IL-2 only as a growth factor. T cell clones were then activated using immobilized anti-CD3 and anti-CD28 mAbs and the presence of cytokines in the culture supernatants was quantified by ELISA.

** As determined by the Mann-Whitney nonparametric test. NS: Non-significant.

Table II. Presence of IL-17-producing cells in inflamed tissues

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<table>
<thead>
<tr>
<th>Pathologies</th>
<th>CCR6+ T Cells (%)</th>
<th>Cytokines Produced (% of Producing Clones – Mean Production in ng/ml)*</th>
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</thead>
<tbody>
<tr>
<td></td>
<td>CD4+ Cells (%)</td>
<td>IL-17</td>
</tr>
<tr>
<td>Psoriasis</td>
<td>29</td>
<td>27% – 12.3</td>
</tr>
<tr>
<td>Crohn’s disease</td>
<td>21</td>
<td>29% – 28.1</td>
</tr>
<tr>
<td>Rheumatoid arthritis</td>
<td>10</td>
<td>13% – 1.9</td>
</tr>
<tr>
<td>Asthma</td>
<td>18</td>
<td>21% – 19.9</td>
</tr>
</tbody>
</table>

* Tissue-infiltrating T lymphocytes were isolated from biopsies taken from various inflammatory pathologies (two patients in each group except for bronchial asthma: n = 3) and expanded in culture conditions using IL-2 only as a growth factor. After a 2-wk culture period, the frequency of CD4+ CCR6+ T cells was estimated by double immunofluorescence and FACS.

** T cell clones were activated using immobilized anti-CD3 and anti-CD28 mAbs and the presence of Th17-, Th2-, and Th1-associated cytokines, as well as IL-10, in the culture supernatants was quantified by ELISA. For each cytokine analyzed, the values represent the percentage of cells producing this cytokine. Numbers in parentheses, mean amount (nanograms per milliliter) produced by the cells.
shown by the effect of neutralizing Abs specific for these cytokines (Fig. 4C), as well as the addition of the recombinant cytokines themselves (Fig. 4D). Moreover, IL-17, but not IFN-γ, in these culture supernatants also induced the expression of transcripts for IL-1F9, an IL-1 family member expressed by keratinocytes, and of human β-defensin 2 (HBD-2), in primary keratinocytes was measured by quantitative PCR. SD was <10% of two independent experiments.

Discussion

In this present study, we have characterized CD4+ tissue-infiltrating human Th17 cells isolated from inflamed sites of patients with chronic inflammatory disease by comparing their gene expression profile with that of Th1 and Th2 cells isolated from the same lesions and generated under identical experimental conditions. After a single stimulation via CD3 and CD28, these cells were expanded under neutral culture conditions in the presence of IL-2 and single cloned cells were classified, according to their cytokine production profile, into different Th subpopulations. In agreement with the notion that the inflammatory environment contains T cells producing not only inflammatory but also anti-inflammatory cytokines, production of IFN-γ, IL-4, and IL-17 was observed among the more than 500 CD4+ T cells clones that were analyzed.

It has been hypothesized, based on the evidence of independent regulation of each cytokine gene, that cytokine-producing effector cells display a spectrum of cytokine profiles of which Th1 and Th2 cells are only two possible extremes, thereby representing the universal end points of continued Ag exposure (25). In agreement with this notion, many tissue-infiltrating T cells were found to be heterogeneous in the number and the cytokine combinations that they produce, independent of the type of inflammatory sites the cells were isolated from. However, T cell clones producing combinations of cytokines were generally those with low production levels, whereas the degree of polarization increased with T cell clones producing higher cytokine levels of IL-4, IFN-γ, or IL-17. In this respect, in contrast to in vitro differentiated peripheral blood-derived Th17 cells that were reported to produce both IFN-γ and IL-17 (16, 17), many of the IL-17-producing T cell clones

![FIGURE 4.](image)

**FIGURE 4.** Th17 cells induce the expression of gene products associated with inflammation in primary keratinocytes. A, Expression of cytokine and chemokine transcripts in activated psoriatic lesion-infiltrating T lymphocytes, as determined by quantitative PCR. The effect of culture supernatants of these T lymphocytes (10% v/v), activated for 24 h via CD3 and CD28, in the absence (B) or presence (C) of neutralizing anti-IL-17, TNF-α, or IFN-γ mAbs or the direct effect of recombinant cytokines (10 ng/ml; D) on the expression of the CXCR2 and CXCR3 ligands, IL-1F9 and β-defensin 2 (HBD-2), in primary keratinocytes was measured by quantitative PCR. SD was <10% of two independent experiments.

![FIGURE 5.](image)

**FIGURE 5.** IL-17 and IL-22 produced by Th17 cells induce, in a synergetic manner, the expression of S100A7 in reconstituted human epidermis. Reconstituted human epidermis was incubated in medium only or with 10 ng/ml rIL17 and rIL-22, alone, in combination, or with 10 ng/ml rIFN-γ, with 10% (v/v) of the culture supernatant of two anti-CD3-CD28 mAb-activated tissue-derived Th17 clones, and one of these culture supernatants was depleted for the presence of IL17 and IL-22. After 4 days, the expression of S100A7 was determined by immunofluorescence analysis.
isolated from chronically inflamed lesions did produce only very weak levels of IFN-γ, IL-4, or other Th2-associated cytokines, whereas IL-17 and the Th17-associated cytokine IL-22 were produced at much higher levels by the latter cells and could reach up to nanomolar range concentrations. An inverse correlation between IL-17 and IFN-γ production was also observed when comparing the production of both cytokines by all CD4+ T cell clones isolated from different inflamed sites. Low frequencies of Th17 clones isolated from biopsies of patients with rheumatoid arthritis were accompanied by a high frequency of IFN-γ-producing cells, whereas the opposite situation was observed for T cells isolated from biopsies from the other inflammatory disease types (Table II). The lower frequency of Th17 cells isolated from synovial biopsies could be due to the clinical status of the disease, given that they were taken from patients undergoing joint replacement who generally present lower inflammatory symptoms at that stage of the disease. These results indicate that the cytokine profile of Th17 cells, derived from either peripheral blood cells or infiltrating patient tissues (15–17), express similar signatures but that a part of tissue-infiltrating Th17 is strongly polarized with very high secretion levels of Th17-type cytokines, most likely reflecting the chronic and repetitive activation of this cell lineage at the inflammatory site. The polarized phenotype of these Th17 cells was furthermore underscored by their specific expression of the human ortholog of RORγt, RORC, irrespective of the type of disease they were generated from, unlike peripheral blood-derived Th17 cell clones that were reported to express both RORC and the Th1 cell-specific transcription factor t-bet (16).

The production of IL-17 by tissue-infiltrating Th17 cells was strongly associated with that of IL-22, a class II cytokine belonging to the IFN-IL-10 family of cytokines (review in Ref. 26). These results confirm previously published studies showing that IL-22 is produced preferentially by activated mouse (27) as well as human (16) Th17 lymphocytes. However, and as reported in the mouse (27), it is shown here that the production of human IL-22 is also not restricted to IL-17-producing cells, because not only Th1, but also Th2 cells were found to produce this cytokine, albeit at much lower levels (data not shown). The general conclusion therefore is that whereas the production of IL-17 is strongly correlated with that of IL-22, the production of the latter cytokine is not always correlated with that of IL-17. IL-22 has initially been identified as an inflammatory cytokine, able to induce the production of acute-phase reactant by hepatocytes (28). IL-22 also has strong inflammation-inducing effects on keratinocytes of IL-22 (29), and it was reported very recently that IL-22 mediates dermal inflammation and acanthosis of mouse epidermis (9). Indeed, culture supernatants derived from an activated Th17 clone, isolated from a psoriatic lesion, strongly induced the expression of psoriasin or S100A7, a proinflammatory protein belonging to the S100 family of calcium-binding proteins, in reconstituted human epidermis in an IL-22-dependent manner. Interestingly, whereas IL-17 used alone had little effect, it strongly enhances, in a synergistic manner, IL-22 mediated induction of S100A7 protein expression in reconstituted epidermis.

Th17 cells also express increased levels of transcripts for IL-26, a proinflammatory cytokine belonging, like IL-22, to the IFN-IL-10 family. Little is known on the biology of IL-26 other than that it targets colon epithelial cells and that it is likely to be involved in mucosal immunity (30). Finally, the finding that Th17 cells also express strongly enhanced transcripts for the proinflammatory genes, TNF-α and LT-β, generally thought to be associated with Th1 lymphocyte responses, further points to the inflammatory capacity of these cells and their potential for tissue destruction.

Culture supernatants, derived from activated psoriatic skin-infiltrating T cells induced the expression of transcripts for the CXCR2-binding chemokines, CXCL1, CXCL2, CXCL3, CXCL5, and CXCL8, as well as the CXCR3 ligands CXCL10 and CXCL11 in primary keratinocytes. All CXCR2-binding chemokines are strong chemotactic factors for neutrophils, whereas CXCR3 is specifically expressed on Th1 cells that via the production of IFN-γ favor their own recruitment into the inflammatory sites. The induction of each of these two chemokine receptor-binding ligands on keratinocytes is under the control of IL-17 and TNF-α or IFN-γ, respectively (31, this study), thereby functioning as a positive feedback loop for the recruitment of inflammatory cells. Furthermore, these results show that tissue-infiltrating Th1 and Th17 cells differ in their chemokine expression-inducing activity on keratinocytes, the latter subpopulation essentially reinforcing the recruitment of neutrophils and macrophages to the site of inflammation via its effect on CXCR2.

Among the various chemokine receptors that were analyzed, transcripts for CCR5 were strongly enhanced in Th17 cells, as compared with Th1 and Th2 cells, which was confirmed by a strong expression at the cell surface of these cells. This contrasts with data from previous studies in the literature, in which the expression of CCR5 has been reported to be specific for Th1 lymphocytes (32). However, these results were obtained with rather ill-defined in vitro differentiated Th1 cell clones and, similar to the reported in vitro differentiated IFN-γ-producing Th17 cells, probably do not reflect the polarized in vivo differentiated cell populations described here. Moreover, although T cells infiltrating the synovial tissue from patients with rheumatoid arthritis were found to express CCR5 (32), in the light of emerging knowledge on the involvement of T cell subpopulations in inflammatory disease, these synovium-infiltrating cells might represent Th17, rather than Th1 lymphocytes. In contrast, a strongly enhanced and specific expression of CCR6 by human Th17 cells was observed which is in keeping with results from recently published reports (15–17, 33) and which shows that this chemokine receptor can be considered as a cell surface marker for the identification of Th17 cells. CCR6 is a receptor that mediates leukocyte homing to skin and mucosal tissue (34). Moreover, its ligand, CCL20, triggers integrin-dependent arrest of memory, but not naive, CD4+ T cells onto endothelial cells, thereby controlling lymphocyte-endothelial cell recognition and recruitment of CCR6-expressing T cells (35). CCL20, which is constitutively expressed in normal skin and mucosa-associated tissues, is up-regulated by IL-17 (36). Activated Th17 cells were found to express strongly enhanced transcription levels of CCL20, and the exclusive production of this chemokine by human Th17 cells was confirmed at protein level in their culture supernatants. The results are in line with the observation that CCL20 and its receptor CCR6 are markedly up-regulated in inflammatory lesions such as those observed in psoriasis (36). The finding that activated human tissue-infiltrating Th17 cells not only express CCR6 but also produce its ligand CCL20 suggests that these cells create a particular inflammatory environment favoring their own migration and sequestration and therefore the perpetuation of chronic inflammatory disease.

Taken together, the results from the present study demonstrate that tissue-infiltrating Th17 lymphocytes are highly differentiated cells with a particular and restricted cytokine secretion profile. Because the IL-22R, IL-17R, TNFRs, and possibly IL-26R are expressed in a wide range of tissue-resident cells, including keratinocytes, fibroblasts, epithelial cells, astrocytes, and synovocytes (37, 38), they are likely to be the targets of the deleterious effects of Th17 lymphocytes. Furthermore, the capacity of IL-17 to induce
HIGHLY DIFFERENTIATED TH17 CELLS IN INFLAMED HUMAN TISSUE


