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This information is current as of October 19, 2019.

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J Immunol 2008; 181:4733-4741; ;
doi: 10.4049/jimmunol.181.7.4733
<http://www.jimmunol.org/content/181/7/4733>

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The American Association of Immunologists, Inc.,
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Print ISSN: 0022-1767 Online ISSN: 1550-6606.



Induction of IL-17⁺ T Cell Trafficking and Development by IFN- γ : Mechanism and Pathological Relevance in Psoriasis¹

Ilona Kryczek,^{2*} Allen T. Bruce,^{2†} Johann E. Gudjonsson,[†] Andrew Johnston,[†] Abhishek Aphale,[†] Linhua Vatan,^{*} Wojciech Szeliga,^{*} Yin Wang,^{*} Yan Liu,^{*} Theodore H. Welling,^{*} James T. Elder,^{3†‡§¶} and Weiping Zou^{3*}

Th1 and Th17 T cells are often colocalized in pathological environments, yet Th1-derived IFN- γ inhibits Th17 cell development *in vitro*. We explored the physiologic basis of this paradox in humans. In this study, we demonstrate increased the number of CD4⁺ and CD8⁺ IL-17⁺ T cells in skin lesions of psoriasis. Furthermore, we show that myeloid APCs potently support induction of IL-17⁺ T cells, and that this activity is greatly increased in psoriasis. We tested stimuli that might account for this activity. Th1 cells and IFN- γ are increased in psoriatic blood and lesional skin. We show that IFN- γ programs myeloid APCs to induce human IL-17⁺ T cells via IL-1 and IL-23. IFN- γ also stimulates APC production of CCL20, supporting migration of IL-17⁺ T cells, and synergizes with IL-17 in the production of human β -defensin 2, an antimicrobial and chemotactic protein highly overexpressed by psoriatic keratinocytes. This study reveals a novel mechanistic interaction between Th1 and IL-17⁺ T cells, challenges the view that Th1 cells suppress Th17 development through IFN- γ , and suggests that Th1 and IL-17⁺ T cells may collaboratively contribute to human autoimmune diseases. *The Journal of Immunology*, 2008, 181: 4733–4741.

Studies show that CD4⁺ IL-17⁺ T cells (Th17) and IL-17 play an active role in inflammation and autoimmune diseases in murine systems (1–9). Although Th17 and CD8⁺ IL-17⁺ T cells were found in both mouse and human tumors (10, 11), CD8⁺ IL-17⁺ T cells have not been described as increased in human autoimmune diseases. In mice, the cytokine mixture of TGF β and IL-6 promotes differentiation of Th17 (12–14) and CD8⁺ IL-17⁺ T cells (10, 11), but in humans, TGF β or this combination of cytokines is thought to suppress Th17 cell differentiation from naive T cells (15, 16). The cytokines IL-1 and IL-23 are important for amplifying and stabilizing Th17 cell differentiation from naive T cells in mice (2, 4–6, 11, 17–21) and in humans (16). However, it remains poorly understood which factors promote IL-23 production in humans and control the development and trafficking of human IL-17⁺ T cells (22). Furthermore, Th1 cells are reported to inhibit Th17 development through IFN- γ (12–14). Why, then, are Th1 and Th17 cells often colocalized in pathological environments (1–9, 23, 24), and what is the mechanism and pathological relevance of this colocalization?

To address these questions, we studied psoriasis, a common, chronic disease of the skin and joints mediated by T cell interactions with keratinocytes and other skin cells. Previous studies implicated Th1 cells and the Th1 promoting cytokine, IL-12, in psoriasis pathogenesis and maintenance (23–26). However, polymorphisms in IL-12p40 and the IL-23R, but not the IL-12R, have been associated with psoriasis (27, 28). Furthermore, an Ab that neutralizes IL-12p40, and thereby both IL-23 and IL-12, has potent effects in treating psoriasis (29). Recently, CD4⁺ IL-17⁺ T cells (Th17) have been isolated from psoriatic plaques (16, 24, 30). However, the relative number of Th17 cells in these lesions remains unquantified, and the proportion of CD8⁺ IL-17⁺ T cells is unknown. Furthermore, it has been reported that blocking IL-23 has no effects on Th17 cell development mediated by myeloid APCs in humans (15), whereas another study suggested that APC-derived IL-23 was associated with Th17 cell development in psoriasis (16). However, it has not been experimentally demonstrated whether psoriatic APC subsets induce IL-17⁺ T cells, and whether psoriatic APC-induced Th17 cells are mechanistically due to IL-23 or other cytokines.

In the current investigation, we explored the phenotype and function of IL-17-secreting T cells in psoriatic and healthy skin, and the factors supporting their trafficking to and induction in lesional skin. Specifically, we show that IFN- γ is demonstrated as a potent promoter of IL-17⁺ T cell trafficking, induction, and function. Our observations support a model wherein Th1 and IL-17⁺ T cells mechanistically interact and collaboratively contribute to human autoimmune disease pathogenesis.

Materials and Methods

Human subjects

All subjects provided written, informed consent. PBMC and skin biopsy samples were obtained from healthy donors and patients with psoriasis. Before biopsy, cases were required to be off all systemic therapies for at least 2 wk, and off topical antipsoriatic medications for at least 1 wk. Control donors were identified from respondents to advertisements in the area (Ann Arbor, MI), had no personal or family history of psoriasis, and were free of inflammatory skin disease at the time of biopsy. The study was

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Received for publication April 23, 2008. Accepted for publication July 23, 2008.

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¹ This work was supported by the Dermatology Foundation (to A.B. and J.E.G.), the National Psoriasis Foundation (to A.B.), the American Skin Association (to J.E.G.), Grant AR052889 from the National Institute of Arthritis, Musculoskeletal, and Skin Diseases (to J.T.E.), and Grant CA99985 from the National Cancer Institute (to W.Z.).

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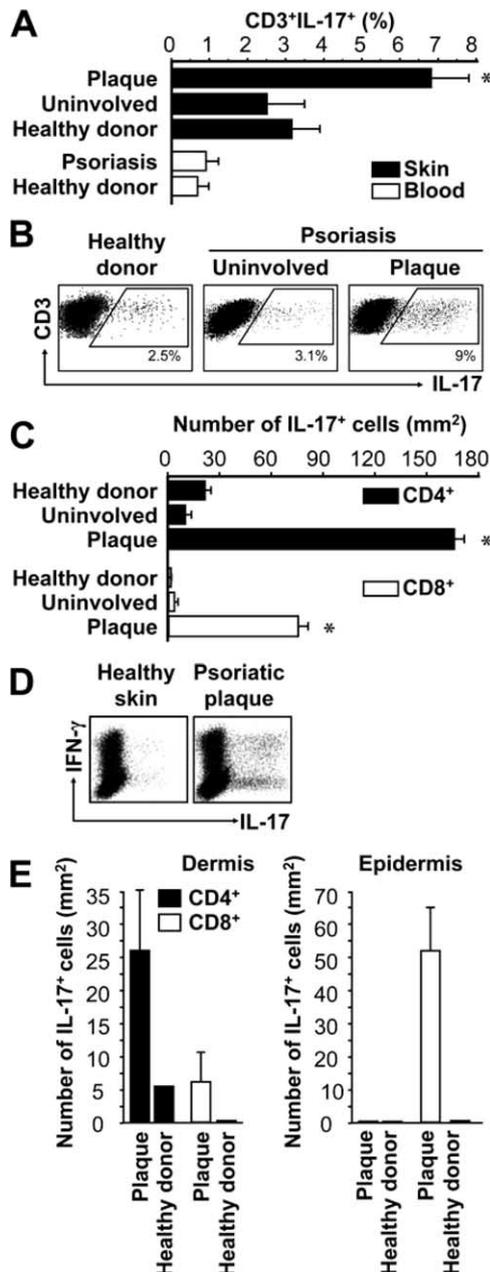


FIGURE 1. IL-17⁺ T cells in psoriasis lesions. Skin biopsy specimens and peripheral blood from healthy donors and patients with psoriasis were stained with specific Abs as described in *Materials and Methods*. IL-17⁺ T cells were analyzed with FACS. **A**, Results show mean percentage of IL-17⁺ T cells in T cells. Error bar indicates SEM, $n = 8$ –10 donors. **B**, Representative dot plots are shown. **C**, Total number of IL-17⁺ T cells in the skin. Total number of IL-17⁺ T cells was calculated by multiplying the percentage of IL-17⁺ T cells by the absolute number of T cells/mm² of skin, as determined previously (26). Results shown are mean number \pm SEM, for $n = 10$ donors. **D**, Coexpression of IL-17 and IFN- γ on IL-17⁺ T cells. Single cell suspensions were made from skin tissues in healthy donors and patients with psoriasis. The expression of IFN- γ and IL-17 was analyzed by intracellular cytokine staining gated on CD3⁺ T cells. **E**, Total number of IL-17⁺ T cell subsets in dermis and epidermis. Total number of CD4⁺ and CD8⁺ IL-17⁺ T cell subsets was calculated by multiplying the percentage of each IL-17⁺ T cell subset by the absolute number of T cell subsets/mm² of skin (26) in the separated dermis and epidermis. Results shown are mean number \pm SEM, for $n = 5$ donors. *, $p < 0.05$ (A, C, and E) compared with uninvolved and healthy donor skin.

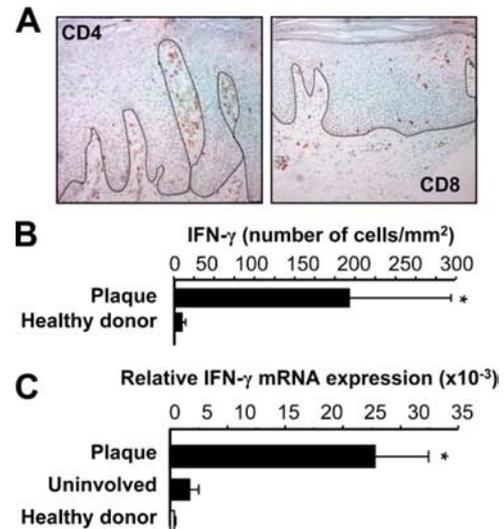


FIGURE 2. IFN- γ ⁺ T cells in psoriasis lesions. **A**, T cells in psoriasis lesions. Psoriasis plaques were stained with anti-CD4 and anti-CD8. One of five representative individuals is shown. **B** and **C**, IFN- γ ⁺ T cells in psoriasis lesions. CD45⁺CD3⁺ T cells were analyzed by FACS in cell suspensions obtained from healthy skin and psoriatic plaque. **B**, IFN- γ ⁺ T cells were analyzed by FACS and quantified in the skin. Results shown are the mean number of cells/mm² of skin \pm SEM for $n = 3$ donors. **C**, Expression of IFN- γ in skin from healthy donors and patients with psoriasis. IFN- γ was quantified by real-time PCR. Results shown are mean value of relative expression \pm SEM for $n = 5$ donors. *, $p < 0.03$ compared with uninvolved and healthy skin.

approved by the Institutional Review Board of the University of Michigan Medical School.

Immune cell isolation

Single cell suspensions were prepared from PBMC and skin tissue samples. Skin biopsy samples were incubated in 50 U/ml dispase (BD Biosciences) at 37°C for 90 min. Epidermis and dermis were teased apart with fine forceps. The dermal portions were then cut into 1-mm pieces and digested in collagenase for 2 h at room temperature. Single cell suspensions of epidermal portions were generated by incubation in Cell Dissociation buffer (Invitrogen) at 37°C for 2 h. Skin explant cultures of T cells from skin biopsies were prepared as described (31).

Immune cells including T cells and CD14⁺ or CD11c⁺ myeloid APCs were enriched using paramagnetic beads (StemCell Technologies) and sorted from stained single cell suspensions using a high-speed cell sorter (FACSARIA; BD Immunocytometry Systems) as we described (32, 33). Cell purity was >98% as confirmed by flow cytometry (LSR II; BD Immunocytometry Systems). CD14⁺ or CD11c⁺ myeloid APCs were used to stimulate T cells as indicated.

APC activation and cytokine production

Fresh peripheral blood CD11c⁺ APCs (0.5×10^6 /ml) were incubated for 72 h with or without recombinant human IFN- γ (200 ng/ml; R&D Systems). These cells were washed and used for T cell stimulation or activated for 12 h with LPS (1 μ g/ml; Sigma-Aldrich) or incubated 3 days (1×10^6 cells/ml) in the presence of LPS to detect cytokine levels in supernatants. All Abs were from R&D Systems, except IL-23p19 from eBioscience.

T cell culture system

Myeloid APCs were cocultured with peripheral blood T cells in ratios from 1:3 to 1:10 (0.5×10^6 T cells/ml) for 4 days in the presence of anti-CD3 (2.5–5 μ g/ml) and anti-CD28 (1.2–2.5 μ g/ml) mAbs (BD Biosciences). Different cytokines and neutralizing Abs or their combinations including IL-1 α (2.5 ng/ml), IL-1 β (2.5 ng/ml), IL-23 (10 ng/ml), anti-IL-4 (1 μ g/ml), anti-IFN- γ (2 μ g/ml), anti-IL-1 (1 μ g/ml anti-IL-1R plus 1 μ g/ml anti-IL-1 α) were used as indicated (all from R&D Systems). Cells were subjected to flow cytometric phenotyping, intracellular cytokine staining, and transcript detection by real-time PCR. Culture supernatants were collected for detection of IL-17 by ELISA (R&D Systems). For flow cytometry analysis, cells were first stained extracellularly with specific Abs, then

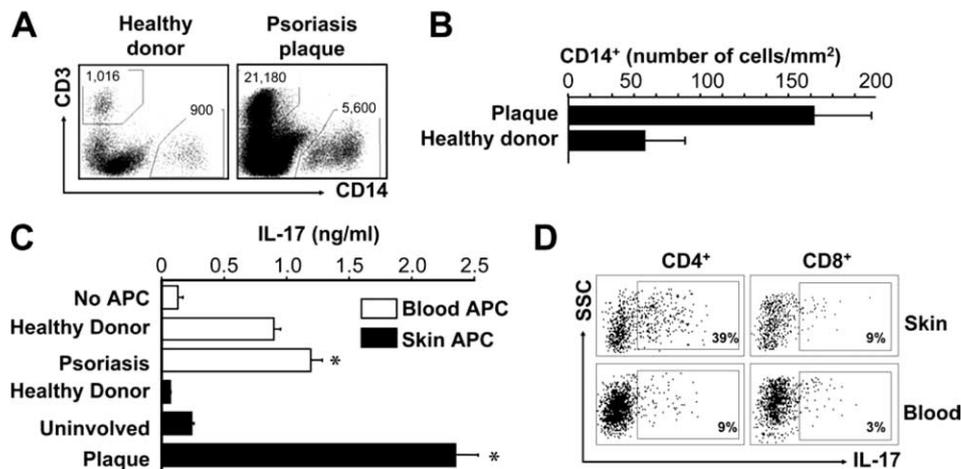


FIGURE 3. Psoriatic myeloid APCs stimulate memory IL-17⁺ T cell expansion. *A* and *B*, Myeloid APCs (CD45⁺CD14⁺) were analyzed by FACS in cell suspensions obtained from healthy skin and psoriatic plaque. *A*, One representative dot plot of CD45⁺ leukocytes from the skin of a healthy donor and a patient with psoriasis is shown. Total number of CD3⁺ or CD14⁺ cells is indicated. *B*, Myeloid APCs were analyzed by FACS and quantified in the skin. Results shown are mean number of cells/mm² of skin \pm SEM for $n = 3$ donors. *C* and *D*, Psoriatic myeloid APCs induce IL-17⁺ T cells. Normal peripheral blood T cells were stimulated for 5 days with myeloid APCs derived from skin and blood of healthy donors or patients with psoriasis in the presence of anti-CD3 and anti-CD28. *C*, IL-17 was detected in the culture supernatants. Results shown are mean \pm SEM, for $n = 5$ donors. *, $p < 0.05$ compared with healthy donor. *D*, One representative dot plot of IL-17⁺ T cells induced by myeloid APCs isolated from psoriatic skin or blood of one patient is shown.

fixed and permeabilized with Perm/Fix solution (eBioscience), and finally stained intracellularly with specific Abs against the indicated cytokines (BD Biosciences). Samples were acquired on a LSR II (BD Biosciences) and data were analyzed with DIVA software (BD Biosciences).

Small interfering RNA (siRNA)⁴ knockdown of human IL-23 gene expression

HEK293 cells were transfected with a Flag-tagged IL-23 expression plasmid and either a nonfunctional scrambled control siRNA or IL-23-specific siRNA using Lipofectamine 2000 (Invitrogen). After the siRNA treatment, the human IL-23 silencing efficiency was measured by Western blot analysis using anti-Flag tag (data not shown). Peripheral blood myeloid APCs were transfected with the siRNA or pmxGFP vector using Nucleofector technology (Macrophages Nucleofector kit; Amaxa) as we described (34). The transfection efficiency reached 60–80% as confirmed by pmxGFP vector transfection.

Analysis of human β -defensin (HBD)-2 induction in keratinocytes

Normal human keratinocytes were grown in serum-free medium optimized for high-density keratinocyte growth (medium 154; Cascade Biologics) and used for experiments in the second or third passage at subconfluence. All cells were plated at 5000 cells/cm², and switched to basal Medium 154 lacking growth factors at 24 h before assay. In some experiments, the calcium concentration was increased from 0.1 to 1.4 mM to enhance production of HBD-2 (35, 36). HBD-2 in supernatants was measured by ELISA using Abs and recombinant protein from PeproTech as described (37).

Migration assays

Migration assays were performed in a Transwell system with a polycarbonate membrane of 6.5 mm diameter with a 3- μ m pore size as we described (32). Purified T cell subsets were added to the upper chamber, and CCL20 (5 ng/ml; R&D Systems) was added to the lower chamber. After 4 h of incubation at 37°C, the phenotype and number of T cells in the upper and lower chambers was determined by FACS.

Quantification of gene expression

Quantitative real-time PCR was performed on control, lesional, and non-lesional skin samples from 10 psoriatic patients and 10 normal controls. Primers for the genes IL-1 α (catalog no. PPH00690A), IL-1 β (catalog no.

PPH00171A), DEFB4 (catalog no. PPH11010A), IL-12A (p35, catalog no. PPH00544B), and IL-12B (p40, catalog no. PPH00545A) were obtained from Superarray Biosciences. Results were normalized to the expression of the housekeeping gene, ribosomal protein, large PO (RPLPO, 36B4, catalog no. PPH21138E). Quantification of gene expression in the cultured myeloid APCs was performed as we described (10, 11). The gene transcripts were quantified in a MasterCycler RealPlex system (Eppendorf Scientific) and expressed as mRNA quantities normalized to GAPDH levels.

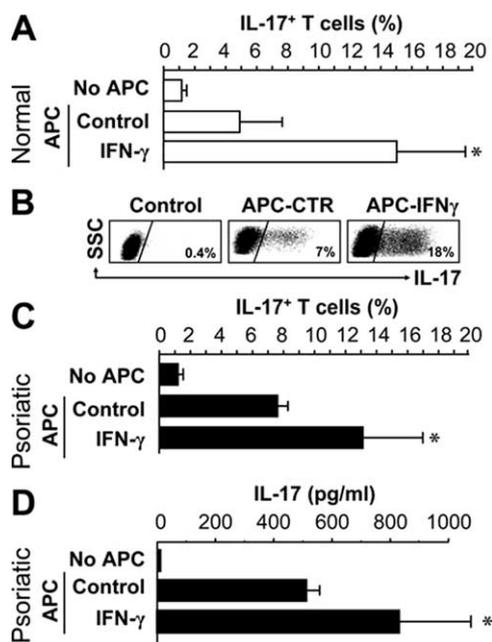


FIGURE 4. IFN- γ programs myeloid APCs to stimulate IL-17⁺ T cells. Normal (*A* and *B*) or psoriatic (*C* and *D*) blood-derived ex vivo CD11c⁺ cells were conditioned for 72 h with or without IFN- γ , and then cultured with normal T cells for 5 days in the presence of anti-CD3 and anti-CD28. *A*–*C*, IL-17⁺ T cells were detected by FACS. Results shown are mean percentage \pm SEM of IL-17⁺ T cells in T cells. *D*, IL-17 was measured in the supernatants by ELISA. Results shown are mean percentage \pm SEM of IL-17. *, $p < 0.05$ compared with control for $n = 11$ donors (*A*), one of $n = 11$ donors (*B*), and $n = 2$ donors (*C* and *D*).

⁴ Abbreviations used in this paper: siRNA, small interfering RNA; HBD, human β -defensin.

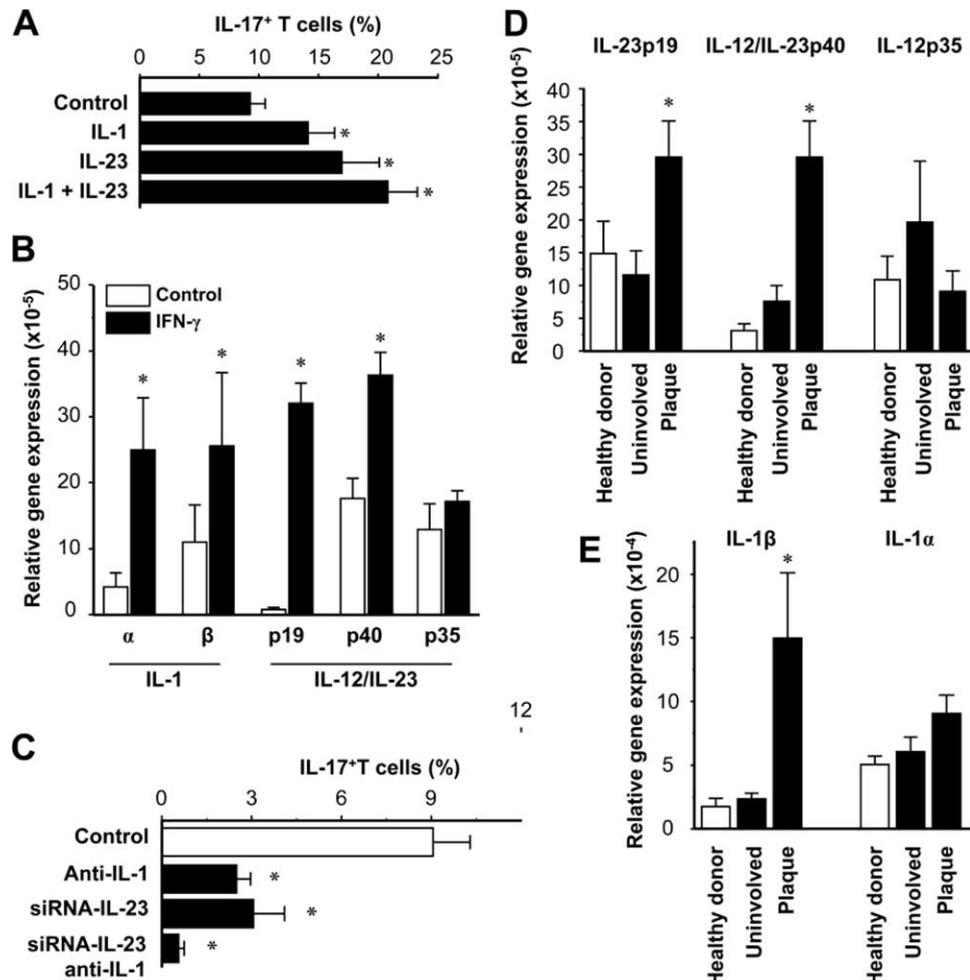


FIGURE 5. Myeloid APCs stimulate IL-17⁺ T cell expansion through IL-1 and IL-23. **A**, IL-1 and IL-23 enhance the induction of IL-17⁺ T cells by CD11c⁺ cells. Normal peripheral blood T cells were stimulated for 5 days with normal CD11c⁺ myeloid APCs with or without IL-1 and IL-23 in the presence of anti-CD3 and anti-CD28. IL-17⁺ T cells were determined by FACS. Results are expressed as mean \pm SEM for $n = 7$ donors. *, $p < 0.03$ compared with control. **B**, IFN- γ stimulates IL-1 and IL-23 expression by CD11c⁺ myeloid APCs. CD11c⁺ myeloid APCs were conditioned with or without IFN- γ . IL-1, IL-12, and IL-23 transcripts were quantified by quantitative RT-PCR. Results shown are mean of relative expression \pm SEM, for $n = 7$ donors. *, $p < 0.01$ compared with control. **C**, Blockade of IL-1 and IL-23 abrogates CD11c⁺-mediated IL-17⁺ T cell induction. Blood-derived CD11c⁺ cells were transfected with IL-23-specific siRNA or control siRNA, then conditioned with IFN- γ . T cells were stimulated for 5 days with these myeloid APCs with or without neutralizing IL-1R Abs. IL-17⁺ T cells were detected by FACS. Results shown are mean of IL-17⁺ T cells in T cells \pm SEM, $n = 5$ donors. *, $p < 0.05$ compared with control. **D** and **E**, Expression of IL-23, IL-12, and IL-1 were quantified by real-time PCR in skin from healthy donors and patients with psoriasis. Results shown are mean of relative expression \pm SEM, for $n = 5$ donors. *, $p < 0.03$ compared with uninvolved and healthy skin.

Immunohistochemistry

Skin biopsy samples were stained with anti-CD4 or anti-CD8 Ab as described (32). The staining was detected using the one-step avidin-biotin complex technique (Vectastain Elite ABC kit; Vector Laboratories).

Statistical calculations

The Wilcoxon rank-sum test was used to determine pairwise differences and the χ^2 test used to determine differences between groups. A value for $p < 0.05$ was considered as significant. Differences in phenotype of T cell subsets were tested with the paired Student's t test. Correlation was tested by Spearman's test. All statistical analysis was done on Statistica software (StatSoft).

Results

IL-17⁺ T cells are increased in psoriatic plaques

Th17 cells are thought to play a role in psoriatic pathogenesis (16, 30). However, the phenotype and distribution of IL-17⁺ T cells in patients with psoriasis remain poorly defined. We investigated the number and distribution of CD4⁺ and CD8⁺ IL-17⁺ T cells in peripheral blood (Fig. 1A) and skin (Fig. 1, A–C)

of healthy donors and patients with psoriasis. The highest percentage (Fig. 1, A and B) and absolute number (Fig. 1C) of CD4⁺ and CD8⁺ IL-17⁺ T cells were observed in psoriatic skin lesions (plaques). Notably, we observed an increased number of CD8⁺ IL-17⁺ T cells in psoriatic skin for the first time (Fig. 1C). We further analyzed the expression of IFN- γ and IL-17 per single cell level. Interestingly, 30–50% IL-17⁺ T cells coexpressed IFN- γ in the skin from psoriatic plaque (Fig. 1D). After enzymatic separation of epidermis and dermis, we found an increased number of both CD4⁺ and CD8⁺ IL-17⁺ T cells in lesional psoriatic dermis, relative to uninvolved skin from persons with psoriasis or healthy donors. However, in plaque epidermis most of the IL-17⁺ T cells were CD8⁺ (Fig. 1E), whereas CD8⁺ IL-17⁺ T cells were virtually absent from healthy donor epidermis (Fig. 1E). The data demonstrate the prevalence, phenotype, and distribution of IL-17⁺ T cells in patients with psoriasis, and indicate that psoriatic skin is an environment containing abundant IL-17⁺ T cells including Th17 cells and CD8⁺ IL-17⁺ T cells.

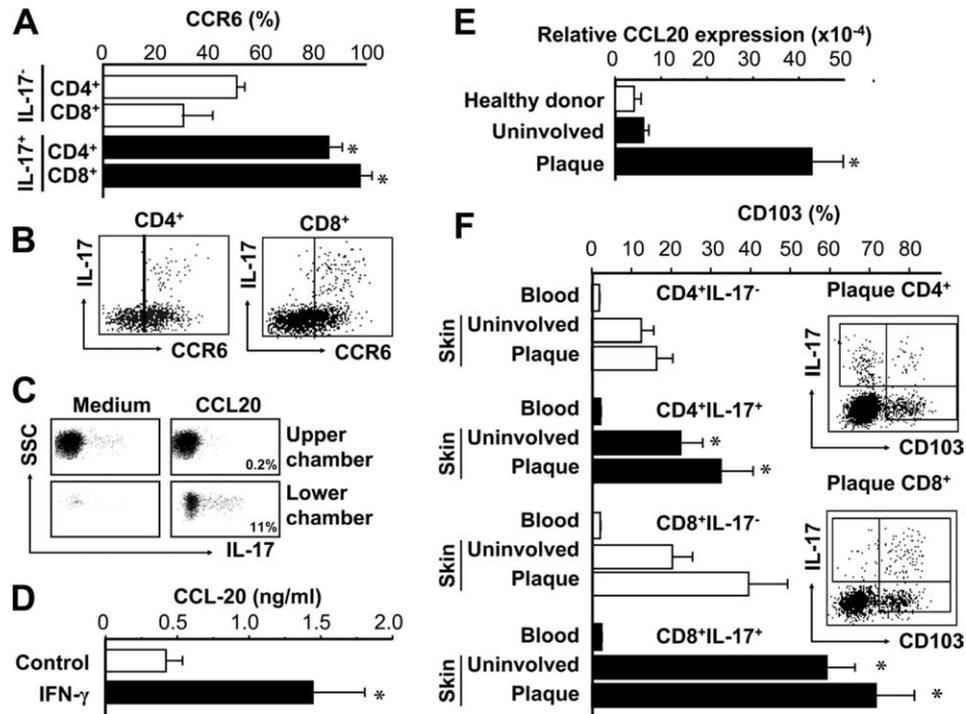


FIGURE 6. CCR6⁺ IL-17⁺ T cells and CCL20 in the psoriatic environment. *A* and *B*, Psoriatic IL-17⁺ T cells highly express CCR6. Expression of CCR6 was determined by FACS on IL-17⁺ and IL-17⁻ T cells in psoriatic skin. *A*, Results shown are mean of CCR6⁺ T cells in IL-17⁺ T cells or IL-17⁻ T cells \pm SEM for $n = 6$ donors. *, $p < 0.05$ compared with IL-17⁻ T cells. *B*, One representative dot plot of six is shown. Gated on CD4⁺ and CD8⁺ T cells. *C*, IL-17⁺ T cells migrate in response to CCL20. Migration assay was performed as described in *Materials and Methods*. The migrated T cells were subjected to intracellular staining for IL-17. The percentage of IL-17⁺ T cells in the upper and lower chambers is shown. *D*, IFN- γ induces CCL20 production. Blood CD11c⁺ cells were stimulated for 3 days with or without IFN- γ . CCL20 was detected by ELISA in the supernatants. Results shown are mean value \pm SEM for $n = 7$ donors. *, $p < 0.01$ compared with control. *E*, High expression of CCL20 in psoriatic skin. CCL20 transcript was quantified by real-time PCR. Results shown are mean value of relative expression \pm SEM for $n = 5$ donors. *, $p < 0.03$ compared with uninvolved and healthy skin. *F*, High expression of CD103 on psoriatic IL-17⁺ T cells. CD103 expression was determined by FACS on IL-17⁺ and IL-17⁻ T cell subsets. Results shown are mean \pm SEM of CD103⁺ T cells in each T cell subset, for $n = 5$ donors. *, $p < 0.05$ compared with IL-17⁻ T cells. One representative dot plot of psoriatic CD103⁺ IL-17⁺ T cells (*right inset*) is shown gated each on CD4⁺ and CD8⁺ T cells.

IFN- γ ⁺ T cells in patients with psoriasis

As a control for relative IL-17⁺ T cell quantification, we examined the distribution of CD4⁺, CD8⁺, and IFN- γ ⁺ T cells in psoriatic skin in patients with psoriasis. Consistent with previous reports (23, 26, 38), we observed a large number of CD4⁺ and CD8⁺ T cells in psoriatic dermis and an increased number of primarily CD8⁺ T cells in psoriatic epidermis (Fig. 2A). The epidermal-dermal distribution of CD4⁺ and CD8⁺ IFN- γ ⁺ T cells is similar for T cells expressing IFN- γ and IL-17 in psoriatic skin (Fig. 1). It has been hypothesized that Th1 cells promote psoriatic pathogenesis (23, 26, 38). Consistent with these observations, high levels of IFN- γ ⁺ T cells (Fig. 2B) and IFN- γ transcripts (Fig. 2C) were also detected in psoriatic plaques. In our experiments, we therefore analyzed the relationship between IFN- γ and IL-17⁺ T cells in psoriatic pathogenesis.

Psoriatic myeloid APCs induce IL-17⁺ T cells

APCs contribute to T cell polarization. It is thought that myeloid-derived gene products contribute to pathogenic inflammation in psoriasis (39). We therefore investigated a potential role for myeloid APCs in inducing IL-17⁺ T cells. Myeloid APCs were found in psoriatic skin (Fig. 3, A and B). Although numerically CD14⁺ cells are the major APCs in the skin, CD11c⁺ cells may be functionally the most powerful APCs in the skin. Therefore, we initially sorted both CD14⁺ and CD11c⁺ myeloid APCs from peripheral blood and skin plaques of people with psoriasis and healthy donors. We observed that CD14⁺ and CD11c⁺ myeloid

APCs induced similar levels of Th17 cells (Fig. 3C and data not shown). Interestingly, APCs from psoriatic peripheral blood and skin lesions were significantly more efficient than those from healthy donors in inducing IL-17 production (Fig. 3C) and IL-17⁺ T cells (Fig. 3D). Myeloid APCs isolated from psoriatic plaques were superior to those isolated from blood in IL-17⁺ T cell induction (Fig. 3, C and D), although these APCs induced comparable levels of IFN- γ production. Myeloid APCs induced both CD4⁺ and CD8⁺ IL-17⁺ T cells (Fig. 3D). We performed similar experiments with responder T cells from normal donors and psoriatic patients. We observed that APCs from psoriatic skin lesions induced comparable Th17 cell development in responder T cells from normal donors and psoriatic patients (Fig. 3, C and D, and data not shown). Altogether, these data indicate that psoriatic myeloid APCs potentially induce IL-17⁺ T cells, and may thereby stimulate and maintain the IL-17⁺ T cell pool in patients with psoriasis.

IFN- γ programs myeloid APCs to stimulate IL-17⁺ T cells

We next studied why psoriatic myeloid APCs are potent inducers of IL-17⁺ T cells. The contribution of IFN- γ was investigated, as IFN- γ is increased in serum of persons with psoriasis (40). IFN- γ ⁺ T cells are enriched in psoriatic skin (Fig. 2), and previous studies implicated IFN- γ and Th1 cells in psoriasis pathogenesis (23, 25, 26). To test the hypothesis that IFN- γ may program myeloid APCs to stimulate IL-17⁺ T cells, CD11⁺ cells from the blood of donors who did not have psoriasis were conditioned with IFN- γ and tested

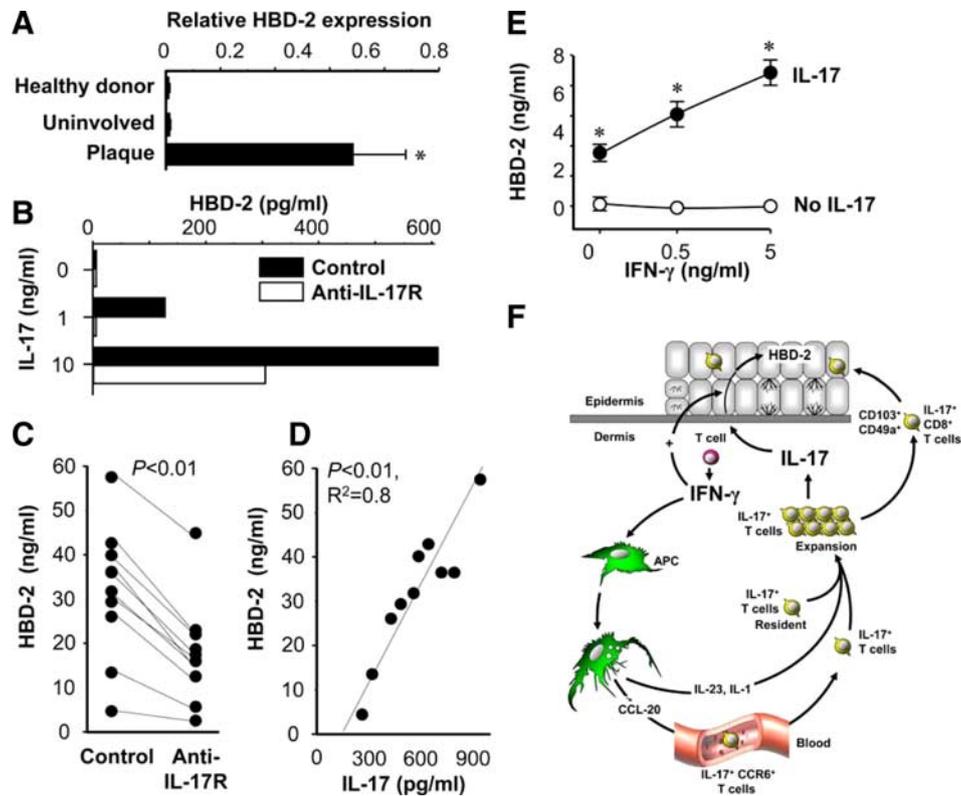


FIGURE 7. Function of IL-17⁺ T cells in psoriasis. **A**, High levels of HBD-2 transcript in psoriatic plaque. Expression of HBD-2 was quantified by real-time PCR in the skin from healthy donors and patients with psoriasis. Results shown are mean value of relative expression \pm SEM for $n = 5$ donors. *, $p < 0.03$ compared with uninvolved and healthy skin. **B**, IL-17 induces HBD-2 production by normal human keratinocytes. Keratinocytes were cultured for 48 h with variable concentrations of IL-17 with or without anti-IL-17R. HBD-2 in the supernatants was detected by ELISA. One representative experiment of three is shown. **C** and **D**, IL-17⁺ T cells stimulate normal human keratinocyte HBD-2 production through IL-17. T cells were stimulated with psoriatic myeloid APCs for 5 days to generate IL-17⁺ T cells. **C**, Normal human keratinocytes were cultured for 48 h in the presence of 20% concentration of these T cell supernatants in the absence (control) or presence of anti-IL-17R. HBD-2 in normal human keratinocyte supernatants was detected by ELISA in $n = 10$ donors. *, $p < 0.01$ compared with control. **D**, Strong correlation was observed between the amount of T cell derived IL-17 in the supernatants and keratinocyte HBD-2 production in $n = 10$ donors ($R^2 = 0.8$, $p < 0.01$). **E**, Synergistic effect of IFN- γ on IL-17-mediated HBD-2 production. Normal human keratinocyte cells were cultured with IL-17 (10 ng/ml) in the presence of variable concentrations of IFN- γ . HBD-2 was detected in the normal human keratinocyte cell supernatants by ELISA. Results shown are mean value of \pm SEM for $n = 3$ donors. *, $p < 0.05$. **F**, Schematic model of interaction between Th1, IL-17⁺, and myeloid APCs. IFN- γ derived from Th1 cells induces CCL20 production by local APCs, which in turn promotes IL-17⁺ T cell trafficking to the psoriatic environment. IFN- γ also programs APCs and expands memory IL-17⁺ T cells through APC-derived IL-1 and IL-23. Additionally, IFN- γ derived from Th1 acts synergistically with IL-17 on keratinocytes. Thus, Th1 and IL-17⁺ T cells collaboratively contribute to psoriasis pathogenesis.

for their capacity to induce IL-17⁺ T cells. IFN- γ profoundly increased the capacity of these CD11c⁺ cells to elicit IL-17⁺ T cells (Fig. 4, A and B). We also conditioned peripheral blood myeloid APCs from psoriatic patients with exogenous IFN- γ , and observed that IFN- γ was able to further enhance the ability of normal and psoriatic myeloid APCs to induce IL-17-secreting T cells (Fig. 4, C and D). The data suggest that IFN- γ released by psoriatic T cells (Fig. 2) may condition myeloid APCs to induce IL-17⁺ T cells.

IL-1 and IL-23 mediate IFN- γ -stimulated induction of IL-17⁺ T cells by myeloid APCs

We further investigated the mechanism by which IFN- γ programs myeloid APCs to induce IL-17⁺ T cells. We showed that exogenous IL-1 and IL-23 were each capable of enhancing the induction of IL-17⁺ T cells by blood CD11c⁺ cells (Fig. 5A). We next analyzed the expression of IL-1 and IL-23 in CD11c⁺ cells treated with IFN- γ . IFN- γ moderately increased the expression of IL-12p35. However, IFN- γ dramatically increased the expression of IL-1, IL-12p40, and IL-23p19 (Fig. 5B). Similar effects of IFN- γ were observed on CD11c⁺ and CD14⁺ APCs. The data indicate that the effects of IFN- γ are more pronounced on IL-23 than IL-12p70.

We next examined the role of CD11c⁺-derived IL-1 and IL-23 in inducing memory IL-17⁺ T cells. Reliable and specific neutralizing Abs against IL-23p19 are not commercially available, and anti-IL-12p40 neutralizing Ab blocks both IL-12 and IL-23. Therefore, to conclusively block IL-1 and IL-23, we used neutralizing Abs against IL-1 α and IL-1R and designed three siRNA sequences, which specifically block human IL-23 (data not shown). Blockade of IL-1 and IL-23 separately each reduced CD11c⁺-mediated induction of IL-17⁺ T cells, and simultaneous blockade of both cytokines abrogated the IL-17 response (Fig. 5C). We extended these observations to patients with psoriasis. Consistent with the high levels of IL-17⁺ T cells (Fig. 1) and of IL-17⁺-inducing myeloid APCs (Fig. 3), we detected high levels of IL-1 β and IL-23 transcripts in psoriatic plaques (Fig. 5, D and E). Taken together, our data suggest that IFN- γ programs myeloid APCs to induce IL-17⁺ T cells through IL-1 and IL-23 in patients with psoriasis.

CCR6⁺ IL-17⁺ T cells and CCL20 in the psoriatic environment

We next examined how IL-17⁺ T cells traffic to the psoriatic environment. We found that CD4⁺ and CD8⁺ IL-17⁺ T cells derived from psoriatic plaque highly expressed CCR6 (Fig. 6, A and B).

We therefore asked whether IL-17⁺ T cells could migrate toward CCL20, the ligand for CCR6. We observed that T cells efficiently migrated in response to CCL20, and that the migrating cells were enriched for IL-17⁺ T cells (from 0.2% IL-17⁻ cells in the upper chamber to 11% IL-17⁺ T cells in the lower chamber) (Fig. 6C). We further tested the role of IFN- γ in CCL20 production. We observed that IFN- γ stimulated CCL20 production from CD11c⁺ APCs (Fig. 6D). High levels of CCL20 mRNA were detected in lesional psoriatic skin (39, 41) (Fig. 6E). The data suggest that IFN- γ derived from psoriatic T cells induces CCL20 and promotes homing of IL-17⁺ T cells to the psoriatic environment.

In addition to CCR6, we observed that psoriatic IL-17⁺ T cells highly expressed CD103 as compared with IL-17⁻ T cells (Fig. 6F). CD103 has been implicated in localization of T cells to psoriatic epidermis (42, 43), and may play a specific role in IL-17⁺ T cell trafficking.

Psoriatic IL-17⁺ T cells are functional

We next asked whether myeloid APC-induced IL-17⁺ T cells are functional. HBD-2 is an important component of skin innate immunity (35, 44). Increased HBD-2 copy number has recently been shown to be a risk factor for development of psoriasis (45). We confirmed that high levels of HBD-2 transcripts are present in psoriatic plaques (Fig. 7A). Furthermore, IL-17 induced a dose-dependent increase in secreted HBD-2 protein in keratinocytes, which could be blocked by a neutralizing Ab specific for the human IL-17R (anti-IL-17R) (Fig. 7B). We next generated IL-17⁺ T cells with psoriatic myeloid APCs, collected the conditioned medium from these cultures, assayed them for IL-17 protein, and treated normal human keratinocytes with a 1/5 dilution of conditioned medium. The T cell supernatants strongly induced human HBD-2 secretion by keratinocytes (Fig. 7C). Moreover, anti-IL-17R blocked the induced HBD-2 expression (Fig. 7C). Furthermore, the concentrations of IL-17 in the T cell supernatants were positively correlated with the levels of HBD-2 secreted by keratinocytes (Fig. 7D). We next examined whether IFN- γ has an effect on HBD-2 production. IFN- γ alone had no detectable effect on HBD-2 secretion by keratinocytes. However, IFN- γ synergistically increased HBD-2 production induced by IL-17 (Fig. 7E). The data indicate that IL-17⁺ T cells generated by psoriatic myeloid APCs are functional, and that IFN- γ and IL-17 synergistically promote the innate immune function of keratinocytes. Based on all our findings, we propose a novel mode of interaction among Th1, IL-17⁺ T cells, and APCs in human autoimmune disease (Fig. 7F): IFN- γ derived from Th1 and other cells promotes tissue homing of IL-17⁺ T cell by stimulating local CCL20 production and stimulates IL-17⁺ T cells by programming IL-17-inducing myeloid APCs.

Discussion

Th17 cells are thought to play a role in psoriatic pathogenesis (16, 30). In this report we have investigated the phenotype, distribution, trafficking, development, and function of IL-17⁺ T cells in healthy humans and patients with psoriasis. We show that both CD4⁺ and CD8⁺ T cells express IL-17 in normal donors as well as in people with psoriasis. Although the phenotype of human Th17 cells has not yet been fully defined, CD4⁺ IL-17⁺ T cells (Th17) are postulated to play a role in psoriasis pathogenesis (16, 30). Entry of CD8⁺ T cells into the epidermis is necessary for the epidermal hyperproliferative response in psoriasis (43, 46). We observed high levels of CD8⁺ IL-17⁺ T cells in the epidermis of psoriatic lesions. CD8⁺ IL-17⁺ T cells are thus ideally positioned to respond to potential keratinocyte autoantigens on HLA class I molecules, which have been genetically implicated in psoriasis (47).

Our observations support the hypothesis that CD8⁺ IL-17⁺ T cells are critical mediators of the persistently altered epidermal growth and differentiation and the local inflammation that is characteristic of psoriasis. We have also observed CD8⁺ IL-17⁺ T cells in cancers (10, 11) and colitis (W. Zou et al., unpublished observation). Our data provide the first evidence that CD8⁺ IL-17⁺ T cells are at least as important as Th17 cells (16, 30) in autoimmune diseases, including but not limited to psoriasis.

We further characterize the phenotype of human psoriatic IL-17⁺ T cells: CD103⁺CCR6⁺ IL-17⁺ T cells are effector T cells often found in environments with chronic inflammation (1, 3). It is therefore not surprising that human IL-17⁺ T cells highly express CD103, which may facilitate trafficking of IL-17⁺ cells into inflammatory tissues (42, 43). We confirm that psoriatic skin is an environment enriched with CCL20, and show that CCL20 is triggered by IFN- γ in myeloid APCs. Furthermore, we show that psoriatic Th1 cells are one of the major sources of IFN- γ , and that IL-17⁺ T cells efficiently migrate toward a CCL20-enriched psoriatic environment via CCR6. Our data lead us to propose a first mode of potential interaction between Th1 and IL-17⁺ T cells in psoriasis: IFN- γ derived from Th1 cells (and other cells) promotes trafficking of IL-17⁺ T cells to the psoriatic environment through the induction and maintenance of local CCL20 production.

There are <1% CD4⁺ and <0.5% CD8⁺ IL-17⁺ T cells in peripheral blood of healthy humans (10, 11). In contrast, high levels of Th17 and CD8⁺ IL-17⁺ T cells are observed in psoriatic plaques. How does this dramatic induction occur? In addition to migration from peripheral blood, IL-17⁺ T cells may be induced within the psoriatic environment. It has been reported that myeloid cell-derived genes contribute to pathogenic inflammation in psoriasis (39). We demonstrate that myeloid APCs including macrophages and myeloid dendritic cells potently induce human IL-17⁺ T cells. Our observation may explain why IL-17⁺ T cells are often found in inflammatory tissues and organs. In support of this concept, we show that myeloid APCs isolated from psoriatic plaques potently stimulate IL-17⁺ T cells. Additionally, our observation that circulating myeloid APCs from people with psoriasis promote IL-17⁺ T cell development may partially explain why persons with psoriasis experience inflammation at distinct anatomical sites, including skin plaques, arthritis, and atherosclerosis. Increased circulating IFN- γ (48) may activate circulating APCs, allowing them to enter tissue and promote expansion of IL-17⁺ T cells. Interestingly, recent studies in mouse models of chronic psoriasiform skin inflammation support pathogenic involvement of activated macrophages, although IL-17⁺ T cells have not been specifically investigated in these models (49, 50).

How and why do activated myeloid APCs contribute to psoriatic skin inflammation? Th1 cells can suppress Th17 cell differentiation through IFN- γ (1–3, 5, 51). However, this presents the paradox of why Th17 and Th1 cells often coexist in autoimmune diseases (17, 18, 20) including psoriasis (24). We demonstrate that psoriatic T cells produce IFN- γ , IFN- γ triggers myeloid APCs to produce IL-1 and IL-23, and in turn induce IL-17⁺ T cells. Our data therefore demonstrate a second mode of potential interaction between Th1 and IL-17⁺ T cells in psoriasis: IFN- γ derived from Th1 cells (and other cells) activates psoriatic myeloid APCs, and the latter promote IL-17⁺ T cell development through IL-1 and IL-23 in the psoriatic environment. Therefore, IFN- γ may possibly play dual roles in regulating the IL-17⁺ T cell pool: IFN- γ targets APCs to initiate and promote Th17 polarization (this study) whereas it targets naive T cells (1–3, 5, 51) to suppress Th17 polarization.

In addition to promoting the trafficking and development of IL-17⁺ T cells, IFN- γ also regulates the function of IL-17⁺ T cells. HBDs are greatly enriched in the psoriatic environment (35) and

play a role in increasing genetic susceptibility to psoriasis (45). Interestingly, we demonstrate that HBD-2 is induced by IL-17 and its expression is synergistically enhanced by IFN- γ , similar to synergistic effects of IFN- γ and IL-17 on induction of IL-6, IL-8, GRO- α , and GM-CSF (52, 53). Our data thereby illustrate a third mode of potential interaction between Th1 and IL-17⁺ T cells in psoriasis: IFN- γ derived from Th1 (and other cells), and IL-17 derived from IL-17⁺ T cells, may synergistically promote keratinocyte function. The three modes of interaction described in this study suggests a novel, collaborative contribution of IL-17⁺ T cells and IFN- γ ⁺ cells including Th1 cells to inflammatory reactions and autoimmune diseases, including psoriasis.

In summary, we show that IFN- γ may promote trafficking, induction, and function of IL-17⁺ T cells in people with psoriasis. This study suggests a novel mechanistic interaction between Th1 and Th17 cells, challenges the view that Th1 cells suppress Th17 cell development, and suggests a collaborative contribution of Th1 and Th17 to human autoimmune diseases. Improved mechanistic insight into the collaborative interactions between these central regulatory players promises novel approaches to management of autoimmune diseases.

Acknowledgments

We thank the volunteers who provided blood and skin samples for this study, and acknowledge the skilled technical assistance of Lynda Hodges, Kathleen McCarthy, Suzan Rehbine, and Thy Thy Do.

Disclosures

The authors have no financial conflict of interest.

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