Frequency and Phenotype of T Helper 17 Cells in Peripheral Blood and Synovial Fluid of Patients with Reactive Arthritis

HUI SHEN, JANE C. GOODALL, and J.S. HILL GASTON

ABSTRACT. ***Objective.*** To analyze the frequency and phenotype of peripheral blood mononuclear cells (PBMC) and synovial fluid mononuclear cell (SFMC) T helper (Th)17 cells in reactive arthritis (ReA).

Methods. T cell surface phenotype and cytokine production were measured following stimulation, using 8-color flow cytometry.

Results. The percentages of interleukin 17 (IL-17)–positive CD4+ T cells were increased in SFMC of patients with ReA compared with PBMC. All IL-17+ cells were CD4+CD45RO+; in SFMC most expressed CCR6, but only 50% expressed CCR4. IL-17+ cells sometimes coexpressed IL-22 and/or interferon-γ, but not IL-10.

Conclusion. These data support the hypothesis that Th17 cells are involved in ReA pathogenesis. (J Rheumatol First Release July 15 2010; doi:10.3899/jrheum.100146)

Key Indexing Terms:
T HELPER 17 INTERLEUKIN 17 INTERLEUKIN 22 REACTIVE ARTHRITIS

Interleukin 17 (IL-17)–producing cells (T helper; Th17 cells) have been implicated in a number of human inflammatory diseases, including arthritis[1,2], and we showed increased frequencies of Th17 cells in peripheral blood mononuclear cells (PBMC) of patients with ankylosing spondylitis (AS) or rheumatoid arthritis (RA) compared with healthy controls[3].

Reactive arthritis (ReA) is, like AS, a form of spondyloarthropathy, but is initiated by bacterial infection. In contrast to AS, large synovial effusions, containing large numbers of bacteria-specific T cells, are common in ReA, allowing us to examine IL-17+ cells in both PBMC and synovial fluid mononuclear cells (SFMC). Raised levels of IL-17 in synovial fluid have been reported[4], suggesting that IL-17 might play a role in the pathogenesis of ReA. Therefore we have measured the frequencies and phenotype of IL-17+ cells in PBMC and SFMC from patients with ReA, and as compared to PBMC from healthy controls.

MATERIALS AND METHODS

Patients. Paired PBMC and SFMC were obtained from 8 patients with ReA together with PBMC from 16 healthy controls. All patients fulfilled European Spondylarthropathy Study Group criteria for spondyloarthritis[5], and the diagnosis of ReA was based on clinical signs and symptoms, together with microbiologic evidence of preceding infection. Six of 8 patients had arthritis following gastrointestinal infection; 1 of these was culture-positive for *Salmonella virchow*, 4 had raised levels of antibodies (2 to *Campylobacter jejuni*, and 2 to *Yersinia enterocolitica*), and 1 had a clear history of preceding gastroenteritis without any organism being identified. One patient had proven *Chlamydia trachomatis* infection, and while in 1 case PBMC and SFMC had been cryopreserved some years before, no clinical details could be retrieved. All patients were examined in the acute phase of their arthritis, usually at their first presentation, and were not receiving disease-modifying drugs. All representative fluorescence-activated cell sorting (FACS) plots are from PBMC and SFMC of the patient who was *Salmonella* culture-positive and who was also positive for HLA-B27, and at presentation with a 2-day history of swollen knees had an erythrocyte sedimentation rate of 80 mm/h and C-reactive protein of 380 mg/l.

Flow cytometry. PBMC and SFMC were purified, stimulated with PMA and ionomycin, and stained with monoclonal antibodies as described[3]. Eight-color flow cytometry (FACSCanto system, Becton Dickinson, Franklin Lakes, NJ, USA) was used to analyze surface expression of CD4, CD45RO, CCR6, and CCR4, and intracellular production of IL-17, IL-22, interferon-γ (IFN-γ), and IL-10 using FACSDiVa software (Becton Dickinson).

Statistical analysis. All data are presented as mean ± SD. Differences in frequency were analyzed by paired and unpaired Student's t-test as appropriate, using SPSS 17 and GraphPad Prism 5 software.

RESULTS

Increased frequency of IL-17+ and CD4+ T cells in ReA SFMC. Following stimulation, clearly increased percentages of CD4+ IL-17+ T cells were apparent within ReA SFMC (4.81% ± 1.31% of all CD4+ T cells) as compared to paired PBMC (0.85% ± 0.11%) or PBMC of healthy controls (0.50% ± 0.08%; Figure 1A, 1B), but numbers in ReA and control PBMC were not significantly different. The percentages of IL-22+CD4+ T cells were also increased in ReA

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Supported by the National Institute for Health Research, Cambridge Biomedical Research Centre, and Arthritis Research UK.

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Accepted for publication May 17, 2010.

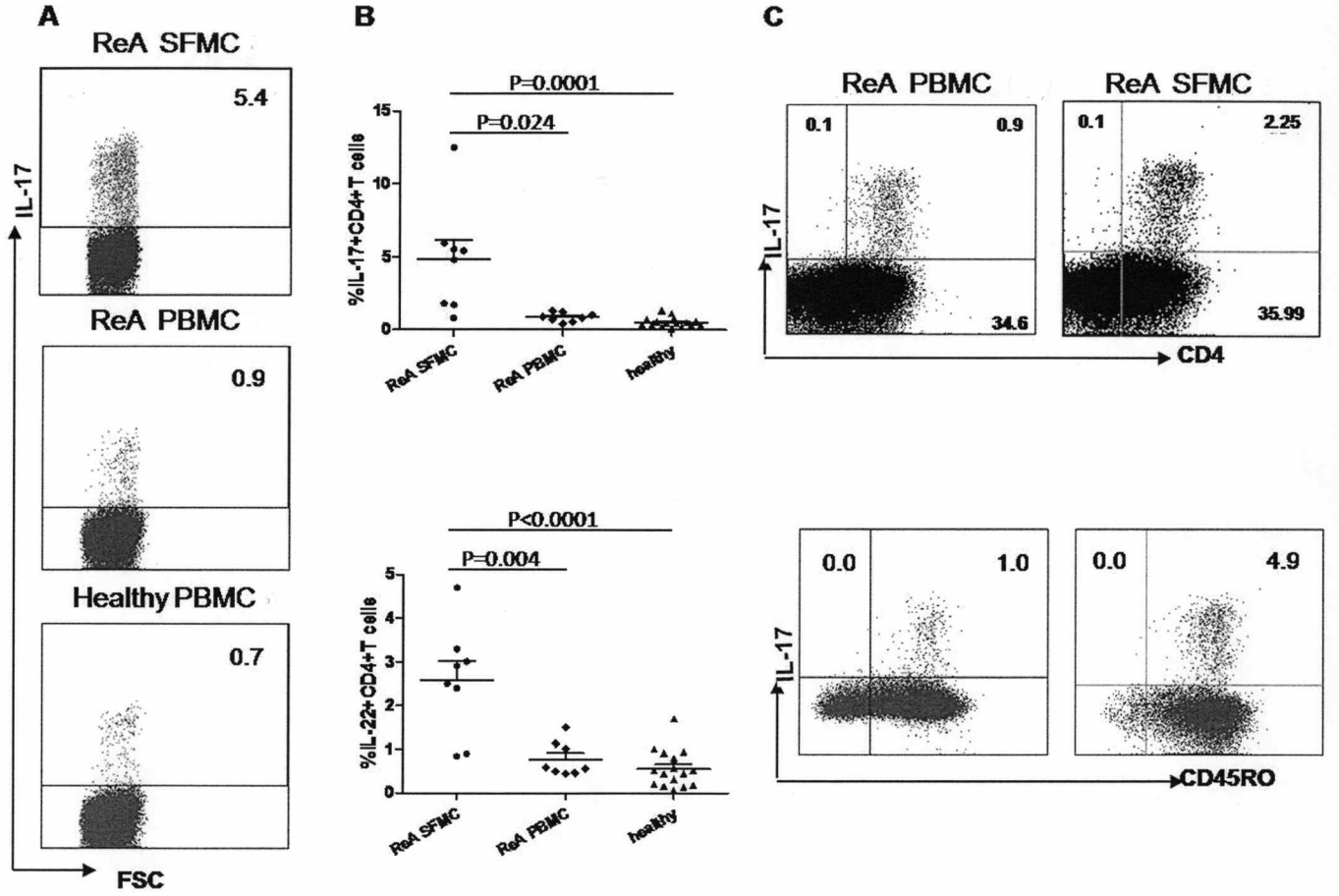

Figure 1. A. Intracellular staining for IL-17 in representative SFMC and PBMC. B. Frequency of IL-17+ (upper panel) and IL-22+ (lower) CD4+ T cells in SFMC and PBMC from patients with ReA, and PBMC from healthy donors. C. Representative examples of costaining for intracellular IL-17 and CD4 (upper panel) or CD45RO (lower).

SFMC (2.57% ± 0.44%) compared to paired PBMC (0.78% ± 0.14%) or control PBMC (0.55% ± 0.11%; Figure 1B). In contrast, there were no differences in the percentages of either IFN-γ+ or IL-10+ cells in SFMC or PBMC in ReA, and in control PBMC (data not shown).

Phenotype of IL-17+ T cells. In all individuals, in both PBMC and SFMC, the majority of IL-17+ T cells were CD4+ and expressed CD45RO (Figure 1C). In addition, a high proportion of IL-17+ T cells from both PBMC and SFMC expressed CCR6 (Figure 2A), but IL-17+ T cells in SFMC expressed lower levels of CCR4 than their counterparts in PBMC (Figure 2B).

Coexpression of inflammatory cytokines by IL-17+CD4+ T cells in ReA. Analysis of IL-22 and IFN-γ production by IL-17+CD4+ T cells in SFMC showed that a significant proportion coexpressed IL-22 or IFN-γ although cells that secreted only IL-22 were commonly observed and the proportions of IFN-γ+ cells were always much higher than those expressing the other cytokines (Figure 3A). No cells producing both IL-17 and IL-10 were observed (Figure 3B). Interestingly, 2 patients with ReA had high proportions of IL-10+CD4+ T cells in SFMC, and most of these coexpressed IFN-γ (Figure 3C).

DISCUSSION

Data on IL-17 levels and IL-17+ T cells are limited in ReA or other forms of spondyloarthritis (SpA), and are sometimes contradictory. IL-17 expression has been described in synovial tissue in psoriatic arthritis (PsA)[6], and increased numbers of IL-17+ cells in peripheral blood of patients with PsA and AS[7]. Melis, *et al*[8] reported no significant differences in serum and synovial fluid levels of patients with IL-17 SpA who have peripheral arthritis (excluding PsA) compared to controls, while others showed elevated IL-17 levels in AS serum[9] and elevated SF levels of IL-17 and IFN-γ in ReA and patients with undifferentiated SpA[4]. To date, no study has characterized IL-17+ cells in SF from patients with ReA.

By analyzing IL-17+ T cells in paired ReA SFMC and PBMC, we have shown that IL-17+ T cells are highly enriched within SF as compared to peripheral blood. The IL-

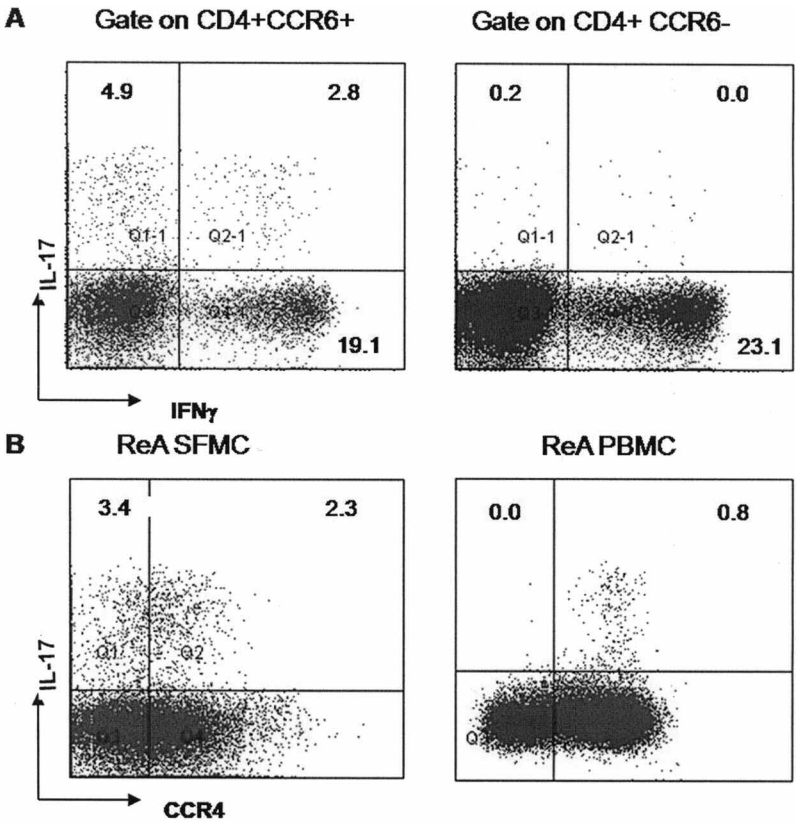

Figure 2. A. Intracellular IL-17 and IFN-γ in CD4+ cells gated according to expression of CCR6. B. Intracellular IL-17 and CCR4 in CD4+ T cells from ReA SFMC (left) and PBMC (right).

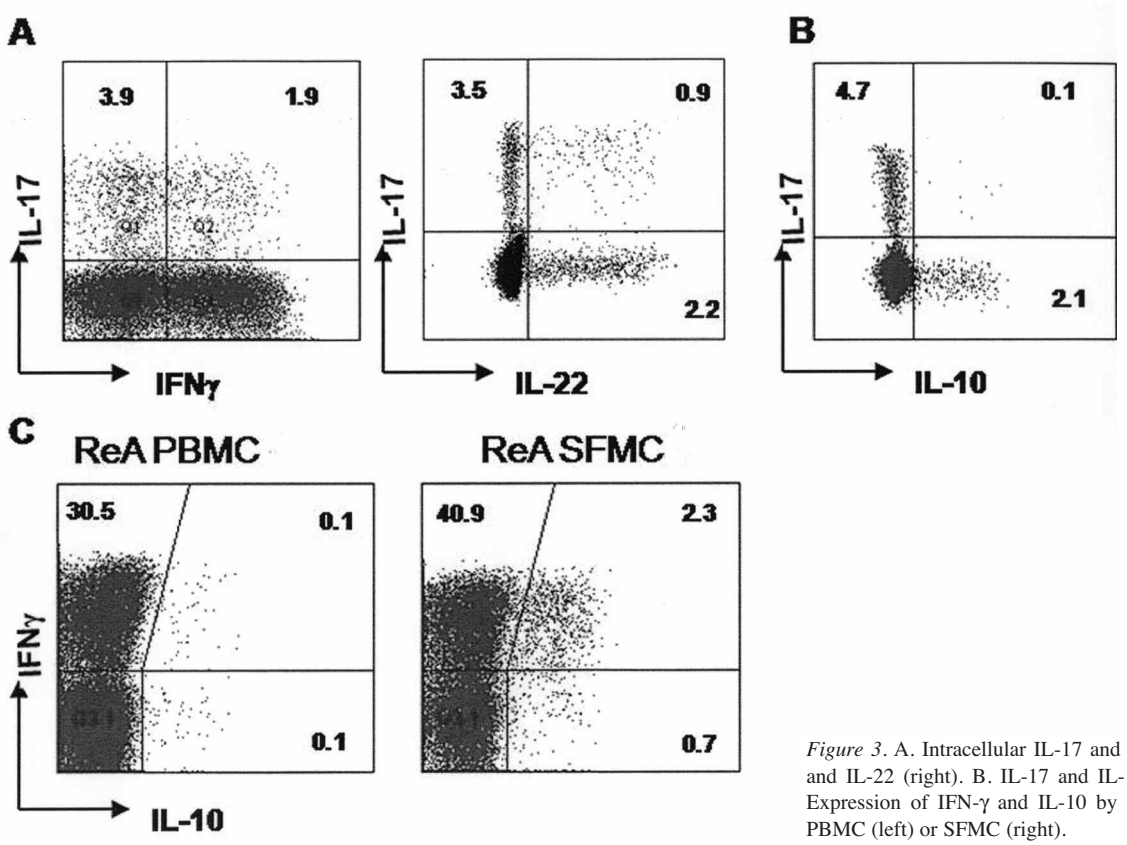

Figure 3. A. Intracellular IL-17 and IFN-γ (left) and IL-17 and IL-22 (right). B. IL-17 and IL-10, in ReA SFMC. C. Expression of IFN-γ and IL-10 by CD4+ T cells in ReA PBMC (left) or SFMC (right).

17+ T cells were uniformly CD4+CD45RO+, consistent with their being a CD4+ memory population. In addition, the vast majority of both SFMC and PBMC IL-17+ T cells expressed CCR6. While in PBMC the expression of CCR4 on IL-17+ T cells mirrored that of CCR6, CCR4 expression by SF IL-17+ T cells was variable and low in a significant proportion, raising the possibility of loss or downregulation in the joint. These data are consistent with those on SFMC in juvenile idiopathic arthritis[10].

We also examined IL-22+ cells, since IL-22 has been implicated in the pathogenesis of several autoimmune inflammatory diseases[11]. Again, we showed enrichment of IL-22+CD4+ T cells in ReA SFMC, and that some, but not all, of the IL-17+ cells coexpressed IL-22, consistent with our previous report of IL-17+ cells in AS and RA PBMC[3]. It is interesting that the difference observed in PBMC of patients with AS and RA compared to healthy controls was not evident in the patients with ReA (albeit in a small cohort). Nevertheless, cells with similar phenotype were readily detected in SFMC in acute ReA; it may require longer periods of chronic inflammation, as in RA and AS, for these cells to become evident in PBMC.

One group has shown that ReA SF CD4+ cells produce lower amounts of IFN-γ than equivalent cells from RA SF[12], but another study of synovial tissue showed high levels of expression of IFN-γ in ReA[13]. In our study, we did not find any difference in numbers of IFN-γ+CD4+ T cells between SFMC and PBMC. It has been suggested that RA SF T cells can coexpress IFN-γ and IL-17, and in that study, about 30% of IL-17+ cells coexpressed IFN-γ[14]. We showed that, unlike IL-17+ cells, ReA SF IFN-γ+ T cells could coexpress IL-10, consistent with a report that IL-10 can be produced by Th1 cells[15]. IL-10+ T cells among those responding to specific bacterial antigens have also been reported in SFMC of patients with ReA[16]. While IL-10 is generally regarded as an antiinflammatory cytokine, in the context of ReA it could have a negative effect on disease outcome by interfering with bacterial clearance, allowing persistence of bacterial antigens and continued production of IL-17.

This study is the first to report the frequency and characteristics of Th17 cells in ReA and shows a significantly higher prevalence of these cells in synovial fluid, in keeping with the hypothesis that IL-17–producing cells contribute to the pathogenesis of ReA.

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