

Cytokine Flexibility of Early and Differentiated Memory T Helper Cells in Juvenile Idiopathic Arthritis

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ABSTRACT. *Objective.* It has been suggested that CD45RO+CD27+ T cells represent recently activated memory cells, whereas CD45RO+CD27- T cells are activated memory T cells in the process of differentiating into effector cells. We investigated (1) CCR7 and CCR5 expression and (2) modulation of cytokine expression in "early" (CD27+) and "differentiated" (CD27-) memory CD4+ T cells from peripheral blood and synovial fluid (SF) of patients with juvenile idiopathic arthritis (JIA). *Methods.* SF CD4+CD45RO+CD27+ and CD27- memory T cells from 6 patients with JIA were tested by flow cytometry for intracellular interferon- γ (IFN- γ) and interleukin 4 (IL-4) after *in vitro* priming with CD3 and CD28 mAb in the presence of IL-4, and subsequent culture with IL-2. *Results.* SF CD4+CD45RO+CD27+ cells contained higher proportions of CCR7+ (median 46% vs 23%) and lower proportions of CCR5+ (73% vs 90%) cells than paired CD27- T cells. Both CD27+ and CD27- memory T helper cells from SF displayed a higher IFN- γ /IL-4 ratio than their peripheral blood counterparts. No significant difference was observed in the percentage of IFN- γ -expressing cells between CD27+ (32%, range 4-47%) and CD27- (29.4%, range 5-52%) memory T helper cells from SF. *Conclusion.* Irrespective of their differentiation stage, both CD27+ and CD27- SF memory T helper cells were found to switch from a proinflammatory to an antiinflammatory pattern of cytokine production. (J Rheumatol 2004;31:2048-54)

Key Indexing Terms:

MEMORY T LYMPHOCYTES

IMMUNE DEVIATION

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JUVENILE IDIOPATHIC ARTHRITIS

In idiopathic arthritides, as well as in other chronic inflammatory disorders, quiescent memory T cells are thought to migrate to inflamed tissues and to undergo reactivation by antigenic peptides¹. Helper (CD4+) memory T cells are indeed abundant in the synovial fluid (SF) and in the synovial membrane of patients with rheumatoid arthritis (RA) and juvenile idiopathic arthritis (JIA) and are thought to play a pivotal role in initiating and sustaining chronic inflammation¹⁻³.

The RO isoform of CD45 is a reliable surface marker of memory T cells, but its expression is independent of their functional differentiation stage. Therefore additional markers have been searched for with the aim of characterizing memory T cells generated in the course of immune responses⁴.

CD27 is a member of the tumor necrosis factor receptor (TNFR) family constitutively expressed on both naive and memory T cells; its surface expression is upregulated

following T cell interaction with antigen-presenting cells⁵. CD27 binds to its specific ligand, CD70, expressed on the activated T cells themselves⁶, supporting the clonal expansion of antigen-specific T lymphocytes^{7,8}. *In vitro* studies have shown that prolonged stimulation of both naive and memory T cells results in the irreversible loss of CD27 from the surface membrane, suggesting that the CD27-negative phenotype marks T cells at advanced differentiation stages⁹.

CD27 has been used in combination with CD45RO to trace the functional differentiation of memory T cells^{10,11}. It has been shown that CD45RO+CD27+ T cells represent recently activated memory cells, whereas CD45RO+CD27- T cells are activated memory T cells in the process of differentiating into effector cells⁸.

An enrichment of CD4+CD45RO+CD27- T cells has been reported in SF from patients with RA¹⁰ and JIA¹¹. Tak and coworkers have reported that in RA synovium early memory CD45RO+CD27+ T cells localize mainly in perivascular lymphocytic infiltrates, whereas the more differentiated CD45RO+CD27- T cells are found preferentially in diffuse lymphocytic synovial infiltrates¹².

Recently, expression of certain chemokine receptors, such as CCR7 and CCR5, has been found to represent an additional important marker of the functional differentiation of memory T cells^{13,14}. According to these studies, differentiation of CCR7+ "central" memory T cells to "effector" memory T cells is accomplished by CCR7 downregulation

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and CCR5 upregulation. Eventually, such changes could allow these cells to migrate to peripheral tissues^{13,14}. However, CCR7+ memory T cells have also been found to infiltrate peripheral tissues, especially those showing a clear lymphoneogenesis, as in the inflamed synovia¹⁵.

Several studies have pointed out the predominant T_H1-like pattern of cytokine production by synovium-infiltrating memory T helper cells in RA and JIA¹⁶⁻¹⁹. Functional deviation of these cells may represent a promising strategy for the treatment of many autoimmune conditions²⁰, and some novel approaches to idiopathic chronic arthritis therapy are based upon tilting the Th1/Th2 balance^{21,22}. However, whether committed Th1 cells infiltrating nonlymphoid tissues may be switched to a Th0/Th2 cytokine profile is still debated.

Our aim was (1) to characterize “early” (CD45RO+CD27+) and “effector” or “differentiated” (CD45RO+CD27-) SF memory T helper cells from patients with JIA in terms of cytokine production and expression of chemokine receptors related to different stages of T cell differentiation; and (2) to test *in vitro* the possible flexibility of their cytokine production in relation to their differentiation stage.

MATERIALS AND METHODS

Patients. We studied 15 patients (12 female, 3 male) meeting the Durban criteria for the diagnosis of JIA²³. Their mean age at disease onset was 6.1 years (range 1.2–11.4) and disease duration ranged from 4 months to 3 years (mean 1.6 yrs). At the time of the study, 9 patients displayed a persistent oligoarticular disease course (involvement of < 5 joints), whereas 6 patients displayed a more aggressive (polyarticular) disease, since they experienced involvement of > 4 joints within the first 6 months from onset²³. None of these latter patients was positive for rheumatoid factor or HLA-B27. In polyarticular patients mononuclear cells (MNC) from SF and peripheral blood (PB) were obtained before they started taking methotrexate. Twelve patients were receiving nonsteroidal antiinflammatory drugs (NSAID), 2 were untreated, and one was treated with NSAID and salazopyrin. SF was obtained at the time of steroid joint injection. Any previous steroid infiltration at the same joint was a criterion for exclusion from the study. Venipuncture was performed with consent from parents or patients at the same time as joint injection. Informed consent for the study was obtained from all parents and children.

Monoclonal antibodies and reagents. The following mAb were used: CD3 [OKT3; American Type Culture Collection (ATCC), Rockville, MD, USA], CD28 (CK248, a gift of Dr. S. Poggi, Laboratory of Immunopathology, IST Genova, Italy), CD8 (OKT8; ATCC), CD27 and CD45RA (Caltag, Burlingame, CA, USA), and CD19 (Dako, Glostrup, Denmark). CD45RA-FITC, CD45RO-TC, and CD4-TC mAb were from Caltag; CD27-PE, CD27-FITC, and CCR5-PE were from Pharmingen (San Diego, CA, USA); anti-CCR7-PE was from R&D Systems (Minneapolis, MN, USA); anti-IL-4-FITC and anti-IFN- γ -FITC were from Caltag. Recombinant human IL-4 was kindly provided by Dr. G. Trinchieri, Schering-Plough Laboratory of Immunological Research, Dardilly, France. Recombinant human IL-2 (rhIL-2) was from Chiron Corp., Emeryville, CA, USA.

Memory T cell separation and culture. PB and SF MNC were isolated from heparinized blood and SF samples by Ficoll (Biochrom KG, Berlin, Germany) density gradient centrifugation. Cells were washed, resuspended in complete medium (RPMI 1640 with L-glutamine, penicillin/streptomycin, nonessential amino acids, and 10% fetal bovine serum (FBS; Sigma Chemical Co., St. Louis, MO, USA) and depleted of adherent cells by adherence to plastic for 1 h at 37°C in 5% CO₂. In some experiments, to isolate the CD45RO+CD4+, CD27+, and CD27- cell subsets, T cells were

first incubated with saturating amounts of CD45RA, CD8, and CD19 for 30 min on ice, washed, and allowed to adhere to goat anti-mouse Ig-coated Petri dishes for negative selection panning. Then to further isolate CD27+ and CD27- subsets, cells were treated with saturating amounts of CD27 mAb, washed, and allowed to bind to goat anti-mouse Ig-coated (Southern Biotechnology, Birmingham, AL, USA) Petri dishes. The nonadherent CD27- cells were aspirated, the plates were gently washed, and the CD27+ adherent cells were recovered by scraping.

To avoid possible bias due to activation of CD27+ cells during the isolation procedures, an alternative strategy was also followed. Briefly, T cells were isolated by rosetting with neuraminidase-treated sheep red blood cells and further purified by negative selection panning. Cells were incubated with saturating amounts of CD45RA mAb for 30 min on ice, washed, and allowed to adhere to plastic Petri dishes coated with goat anti-mouse Ig. The recovered cells were 95% enriched for CD45RO+ cells. Cells were resuspended in complete medium and processed for intracellular cytokine analysis.

Cytokine expression repolarizing conditions. In some experiments, an aliquot of purified SF memory T cells was primed for 1 week before being stained for intracellular cytokine expression, as described²⁴. Briefly, T cells were cultured in U-bottom 96-well plates (2 × 10⁵ cells/well; Falcon), and precoated with CD3 mAb in the presence of 10 U/ml of rIL-2, 25 ng/ml rIL-4, and CD28 mAb. After 4 days, cells were washed, then cultured 3 additional days in medium containing rIL-2 (10 U/ml). Cells were then processed for intracellular cytokine staining.

Flow cytometry. For intracellular cytokine staining, freshly purified or “primed” CD45RO+ memory T cells (1 × 10⁶) were incubated for 5 h in PMA (20 ng/ml; Sigma), the A-23187 Ca-ionophore (250 ng/ml; Sigma), and brefeldin-A (5 μ g/ml; Sigma). Cells were washed in phosphate buffered saline (PBS) with 1% fetal calf serum (FCS; staining buffer) and subsequently double-stained with CD4-TC and CD27-PE mAb for 30 min at 4°C in the dark. Cells were washed in staining buffer and fixed in 4% paraformaldehyde for 20 min at 4°C in the dark. Then the cells were washed twice with permeabilization buffer (PBS, 1% FCS, 0.1% saponin; Sigma) and stained with FITC-conjugated mAb to human IL-4 and IFN- γ for 30 min at 4°C in the dark. Cells were washed in staining buffer and analyzed by flow cytometry (FACScan, BD Biosciences, Mountain View, CA, USA) gating on the CD4+CD27+ or CD4+CD27- T cell subsets.

In some experiments, intracellular cytokine analysis was performed as described above with purified CD45RO+CD4+CD27+ or CD27- cell subsets, either immediately following isolation or after *in vitro* priming.

CCR7 and CCR5 expression was investigated by tricolor staining of freshly isolated CD45RO+ memory T cells with CD4-TC, CD27-FITC, and CCR7-PE or anti-CCR5-PE mAb, gating on the CD4+CD27+ or CD4+CD27- T cell subsets.

PE-, FITC-, and TC-conjugated mouse Ig control mAb (Caltag) were used as negative controls in all these experiments. CellQuest software (BD Biosciences) was used for analyses. The results of flow cytometry experiments were expressed as percentage positive cells.

Statistical analysis. Differences in the expression of chemokine receptors and of intracellular cytokine and their variations upon stimulation were calculated with the nonparametric Wilcoxon rank test.

RESULTS

Expression of CCR7 and CCR5 chemokine receptors in SF CD27+ and CD27- memory T cells. SF and PB MNC from patients with JIA were isolated and stained with CD4, CD45RO, and CD27 mAb to gate on lymphocytes in 10 patients. As expected, an enrichment in CD4+CD45RO+ memory T cells was found in synovial fluid (median 43%, range 25–53%) compared to paired peripheral blood samples (median 9%, range 6–15%; $p = 0.01$)³. Consistent with a previous report, the median percentage of CD27+ cells within the CD4+, CD45RO+ T cell subset was 83.5%

(range 66–96%) in PB and 58.5% (range 43–79%) in the SF ($p = 0.005$)¹¹.

To better characterize memory T cell differentiation stages, the expression of CCR7 and CCR5 on PB and SF CD4+CD45RO+CD27+ and CD4+CD45RO+CD27– T cells was investigated by tricolor staining of freshly isolated CD45RO+ cells with CD4, CD27, and anti-CCR7 or anti-CCR5 mAb.

Although there was wide variability, in SF the percentage of CCR7+ cells was significantly higher in CD27+ (median 46.2%, range 18–80%) than in CD27– T cells (median 23%, range 8–72%) ($p = 0.007$, Wilcoxon rank test) from all patients. This finding was even more evident when corresponding PB CD4+CD45RO+ cells were analyzed in 5 patients (Figure 1A).

Conversely, CD4+CD45RO+CD27+ T cells were found to contain a lower proportion of CCR5+ cells (median 73.5%, range 65–86%) than paired CD27– cells (median 90%, range 84–94%; $p = 0.007$) (Figure 1B). Again, the same behavior was observed in paired PB samples with a significantly lower percentage of positive cells in both cell subtypes (Figure 1B).

Thus, the putative subset of effector CD4+CD45RO+CD27– T cells isolated from SF showed a prevalent CCR7–CCR5+ phenotype.

Intracellular cytokine expression in SF and PB CD4+CD45RO+CD27+ and CD27– memory T cells. Next, CD45RO+ cells were isolated from the SF of 10 JIA patients and stained with CD4, CD27, and anti-IFN- γ or anti-IL-4 mAb. Both CD4+CD45RO+CD27+ and CD4+CD45RO+CD27– T cells displayed a Th1 pattern of cytokine production, with no difference between the 2 subgroups (Table 1).

Thus, an IFN- γ /IL-4 ratio greater than 1 was observed in both CD4+CD45RO+CD27+ (median 5.6, range 0.5–36) and CD27– T cells (median 5.7, range 1.2–14). Of note, consistent with previous reports, in 2 patients with a persistent oligoarticular form of disease (Patients 7 and 8, Table 1) an IFN- γ /IL-4 ratio lower than 1 was observed in the CD4+CD45RO+CD27+ cell subpopulation^{18,19}.

In 4 out of 10 patients the same experiments were performed after purification of CD4+, CD45RO+, CD27+, and CD27– SF T cells and subsequent intracellular staining with anti-IFN- γ or anti-IL-4 mAb. The results obtained were similar to those reported in Table 1 (data not shown).

In 4 patients, memory T helper cell subsets were tested for intracellular accumulation of IL-4 and IFN- γ in paired SF and PB samples (Table 1). Both CD4+CD45RO+CD27+ and CD4+CD45RO+CD27– cells from SF displayed higher levels of cytokine expression and higher IFN- γ /IL-4 ratio than their PB counterparts (Table 1), indicating the presence of a higher percentage of “effector” cytokine-producing cells in the context of the inflamed tissue.

Immune deviation of SF memory T helper cells in JIA. Attempts at modulating T_H1 cytokine production *in vitro* have had limited success in studies on adult RA^{25,26}. We investigated this issue in SF CD4+CD45RO+CD27+ and CD27– memory T cells from 6 JIA patients by flow cytometric analysis of intracellular IFN- γ or IL-4, either immediately after cell purification or following repolarizing stimulation.

Figure 2 illustrates one representative experiment in which, after priming (panels C and D), both CD45RO+CD27+ and CD45RO+CD27– helper T cells were found to display increased expression of IL-4, as

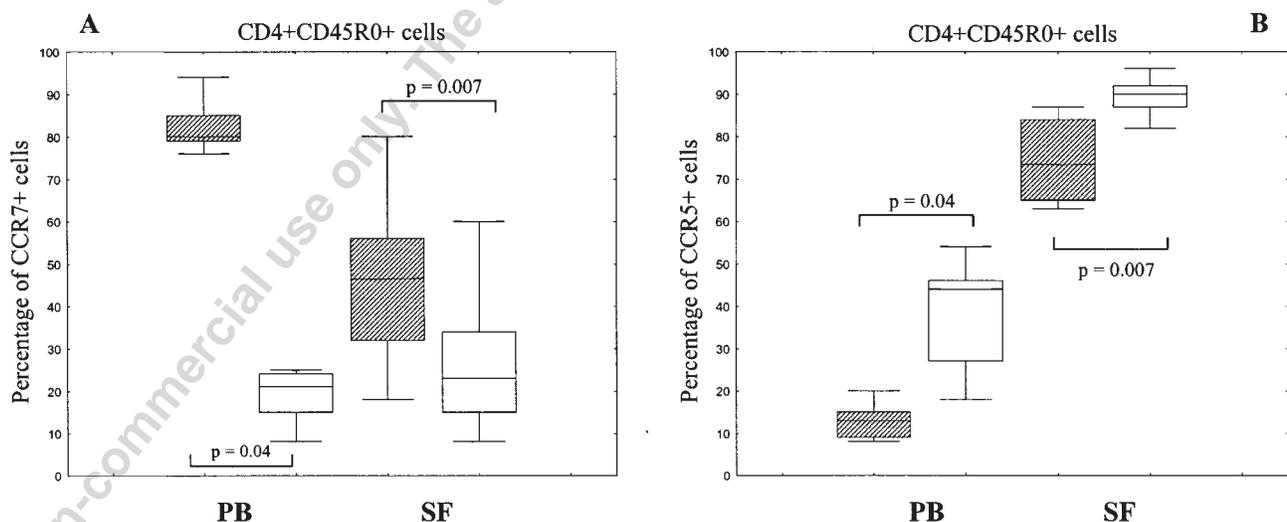


Figure 1. CCR7 (A) and CCR5 (B) expression in paired CD27+ (shaded boxes) and CD27– (white boxes) samples of peripheral blood (PB, 5 patients) and synovial fluid (SF, 10 patients) memory T helper cells. Freshly isolated CD45RO+ T cells were triple-stained with CD4-TC, CD27-FITC, and anti-CCR7-PE or anti-CCR5-PE mAb and analyzed by flow cytometry gating on the CD4+CD27+ or CD4+CD27– T cell subsets. Boxes indicate values between the 25th and 75th percentiles, whisker lines represent highest and lowest values for each subgroup. Lines represent median values. Differences in CCR7 and CCR5 expression between CD27+ and CD27– cells were evaluated by Wilcoxon rank test.

Table 1. IFN- γ and IL-4 expression in SF and PB CD4+CD45RO+CD27+ and CD27- T cells.

Patient	Age, yrs	Sex	Disease Course	Disease Duration, yrs	SF CD4+CD45RO+*				PB CD4+CD45RO+*			
					CD27+ [†]		CD27- [†]		CD27+ [†]		CD27- [†]	
					IFN- γ	IL-4						
1	2	F	Oligoarticular	0.8	32	4	13	2	3	1	2.5	4
2	10	F	Polyarticular	0.7	13	<1	7	0	5	0	9	7
3	10	F	Polyarticular	0.6	56	2	37	2	10	0	14	4.4
4	7	F	Polyarticular	1.2	54	3	34	4	2.5	1	5	3
5	12	F	Polyarticular	0.6	36	<1	40	7	—	—	—	—
6	9	M	Oligoarticular	1.6	46	2	51	21	—	—	—	—
7	4	F	Oligoarticular	1.5	4	8	5	4	—	—	—	—
8	5	F	Oligoarticular	3.2	24	37	34	24	—	—	—	—
9	12	M	Polyarticular	1	34	16	52	4	—	—	—	—
10	7	F	Oligoarticular	3.4	28	5	29	25	—	—	—	—

* Purified SF or PB CD45RO+ T cells were stimulated 5 h with PMA and Ca-ionophore in the presence of brefeldin-A. Cells were double-stained with CD4 and CD27 mAb. Intracellular cytokines were detected with anti-IL-4 and anti-IFN- γ mAb. Cytokine expression was analyzed by flow cytometry gating on the CD4+CD27+ or CD4+CD27- T cell subsets. [†] Percentage of positive cells.

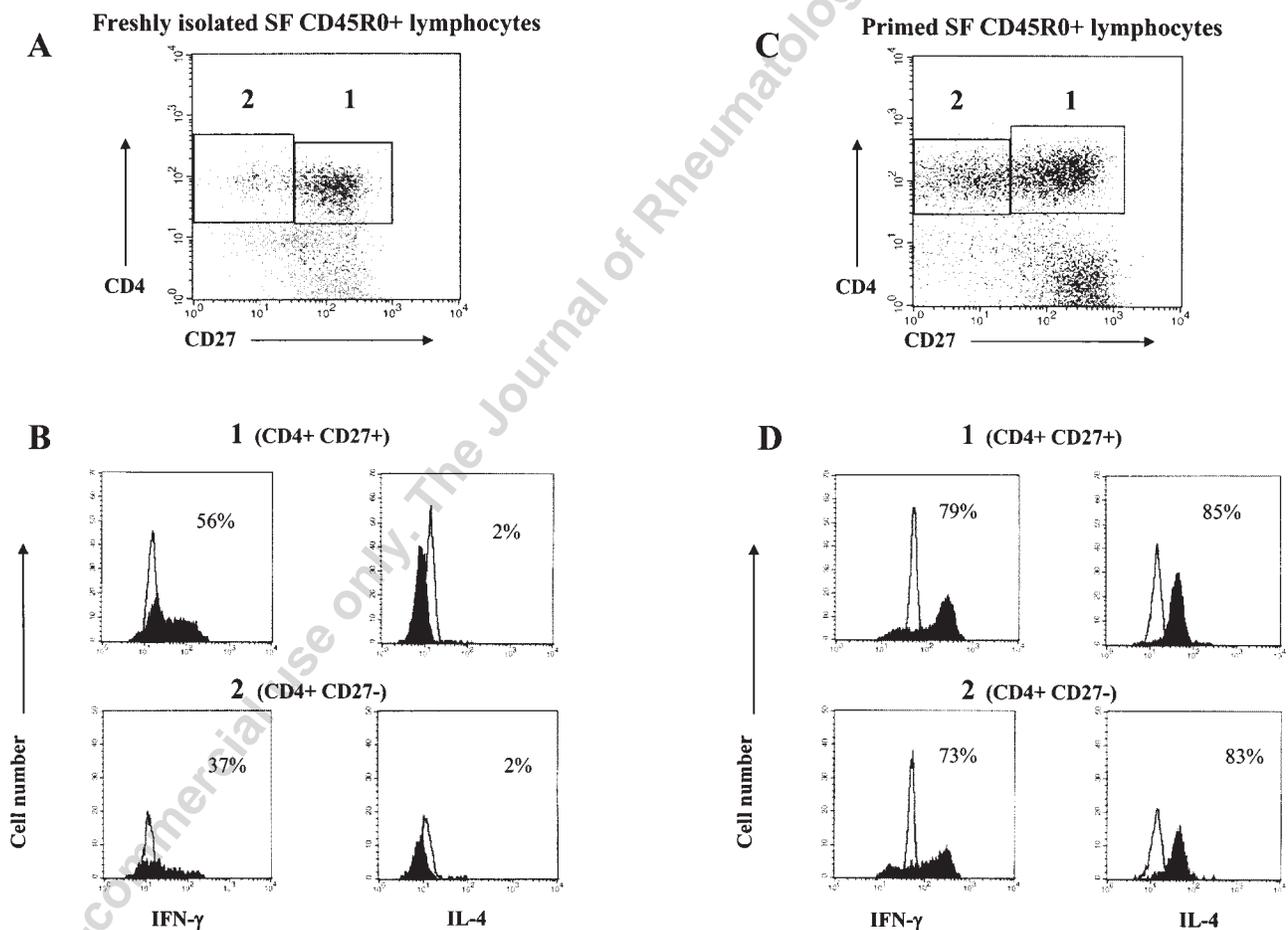


Figure 2. Flow cytometric analysis of intracellular IFN- γ and IL-4 in freshly isolated and primed SF CD45RO+CD4+CD27+ and CD27- cells from one representative patient (Patient 3, Table 1). SF CD45RO+ T cells, freshly isolated (A) or primed *in vitro* with CD3 and CD28 mAb in the presence of IL-4 (C), were stimulated 5 h with PMA and Ca-ionophore in the presence of brefeldin-A. Cells were double-stained with CD4 and CD27 mAb and intracellular cytokines were detected with anti-IL-4 and anti-IFN- γ mAb by flow cytometry. CD4+CD27+ (panels B and D, 1) and CD4+CD27- (panels B and D, 2) subpopulations were gated. Cytokine expression analysis on the gated cell populations (B and D). Positive cell fractions are shown in black, isotype-matched controls in white; percentage of positive cells is indicated.

compared to their freshly isolated counterparts (panels A and B), with an evident increase of the double-positive (IFN- γ and IL-4) subpopulation (data not shown). This pattern was consistently observed in all the JIA cases studied (Figure 3), irrespective of disease severity at the time of the study and the clinical course.

A significant decrease of the IFN- γ /IL-4 ratio after *in vitro* priming was detected in both the CD4+CD45RO+CD27+ and the CD4+CD45RO+CD27- SF cell subsets in comparison to the corresponding prestimulated cell suspensions ($p = 0.04$ and $p = 0.02$, respectively, Wilcoxon rank test); a clear inversion of the IFN- γ /IL-4 ratio was observed in some patients (Figure 3).

Taken together, these results indicate that SF memory T helper cells from patients with JIA retained the ability to differentiate into IL-4-producing cells after *in vitro* Th2 priming. The immune deviation was successful both in early memory CD27+ and in more differentiated CD27- memory T helper cells.

DISCUSSION

We carried out a phenotypic and functional characterization of memory T helper cells in paired SF and PB samples from children with JIA. Immunophenotypic characterization of memory T helper cells was accomplished by mAb-based flow cytometric analyses, while functional studies

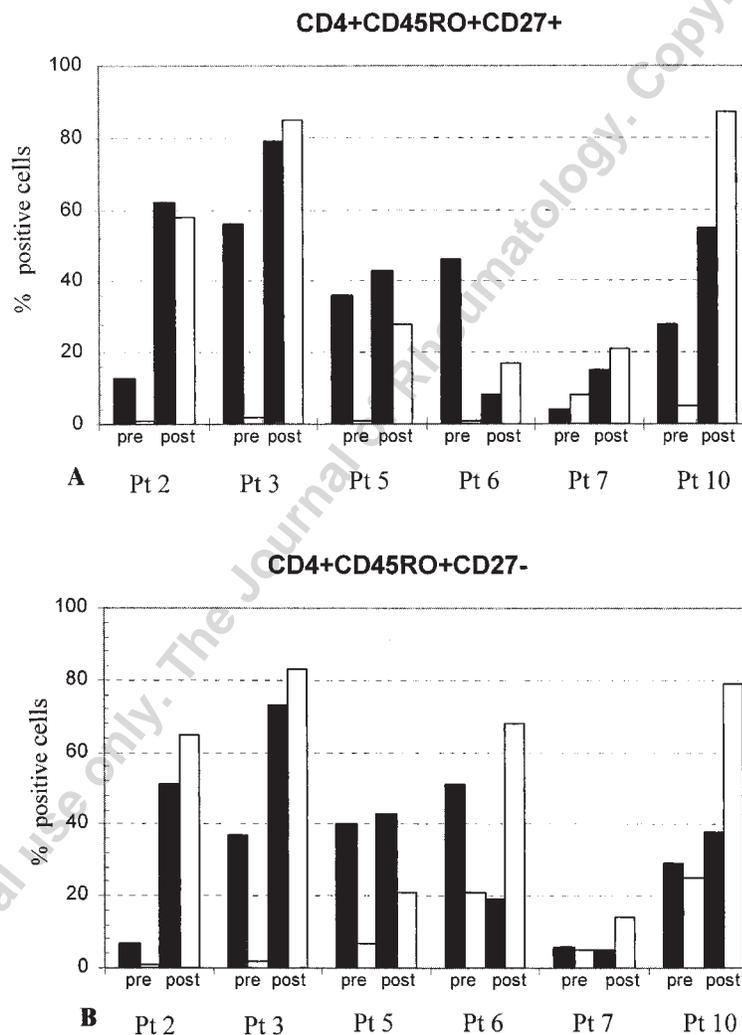


Figure 3. IFN- γ and IL-4 expression in freshly isolated and primed SF CD45RO+CD4+CD27+ and CD27- cells from the 6 patients tested. SF CD45RO+ T cells freshly isolated (pre, gray bars) or primed *in vitro* with CD3 and CD28 mAb in the presence of IL-4 (post, white bars) were stimulated and incubated 5 h in the presence of PMA, the A-23187 Ca-ionophore, and brefeldin-A. Cells were double-stained with α CD4 and CD27 mAb, and intracellular cytokines were detected with anti-IFN- γ and anti-IL-4 mAb by flow cytometry gating on the CD4+CD27+ (A) or CD4+CD27- (B) T cell subsets.

addressed intracellular cytokine expression shortly after cell isolation and following *in vitro* stimulation.

In a previous study, we reported that memory T cells from the SF of JIA patients contained a higher proportion of CD45RO+CD27- cells than their PB counterparts¹¹. Loss of CD27 from the cell surface accompanies differentiation of recently activated CD45RA+ naive and antigen-reactivated CD45RO+ memory T cells into effector cells²⁷⁻²⁹. It has been postulated that circulating CD4+CD45RO+CD27+ represents a population of "resting" memory cells that are recruited to inflamed tissues, where they undergo reactivation ("early" memory) and subsequent differentiation into "effector" CD4+CD45RO+CD27- memory T cells³⁰.

To date, the effector role of CD27- memory T cells has been supported by the following observations: (1) the CD4+CD45RO+CD27- T cell subset may serve as a potent inducer of B cell differentiation and Ig production²⁹; (2) it expresses organ-specific homing receptors, such as cutaneous leukocyte antigen (CLA) and the $\alpha_E\beta_7$ integrin^{30,31}; and (3) it is enriched for cells producing either IL-4 or IFN- γ ³².

In our study, expression of CD27 on CD4+CD45RO+ PB and SF T cells from JIA patients was correlated to 2 chemokine receptors (CCR7 and CCR5) expressed in different stages of memory T cell differentiation^{13,33,34}.

CCR7 and CCR5 were detected on CD27+ and CD27- cells in PB and SF with different patterns of distribution in the 2 sets of samples. Indeed, a higher proportion of CCR7+ "central" memory T cells were detected in PB compared to SF, especially in the CD27+ subpopulation. Conversely, both early CD27+ and more differentiated CD27- memory T cells from SF displayed a clear enrichment of the "effector" CCR5+ phenotype compared to PB^{13,14}.

Notably, SF CD45RO+CD4+CD27- cells contained definitely higher proportions of CCR7- and CCR5+ cells in comparison with paired CD27+ memory T cells. These findings may provide additional evidence for a greater degree of differentiation of CD27- memory T cells at the site of inflammation.

Detection of SF CCR7+ memory T helper cells in RA patients¹⁵ and in our JIA patients suggests that CCR7 expression is not restricted to memory T cells homing to the secondary lymphoid tissues, nor does it prevent the migration of polarized CCR7+ T cells into inflamed tissues, especially when diffuse lymphoid neogenesis is present^{35,36}.

In the second part of the study, the potential ability of CD27+ and CD27- memory T cells from JIA patients to switch from a proinflammatory to an antiinflammatory pattern of cytokine production was investigated.

Reversion of the cytokine expression profile in effector T cells infiltrating peripheral tissues is still a matter of debate. Th1 and Th2 differentiation programs are considered mutually exclusive, and a number of intracellular mechanisms have been described that limit T cell repolarization once Th1

or Th2 commitment has been achieved. However, some effector T cells produce both IFN- γ and IL-4 (Th0 phenotype), indicating that both Th1 and Th2 transcriptional programs can be activated simultaneously. Moreover, repolarization of Th1 or Th2-committed cells has been described in some experimental settings^{37,38}.

Aarvak and coworkers have shown that polyclonal T cell lines with a memory phenotype and a Th1 profile isolated from the synovial tissue of RA patients could be induced *in vitro* to express Th2 related chemokine receptors without a change in the cytokine profile²⁵. Circulating and synovial effector memory T cells from patients with early stage RA displayed a reduced ability to differentiate into IL-4-producing cells after priming with CD3 and CD28 mAb and IL-4, compared to healthy controls^{26,39}.

In our study, freshly isolated CD4+CD45RO+CD27+ and CD4+CD45RO+CD27- cells synthesized IFN- γ in relative excess over IL-4, with a slightly higher percentage of IL-4-positive cells in those patients showing a persistent oligoarticular course^{18,19,34,40}. SF memory T cells from JIA patients were found *in vitro* to retain the ability to switch to IL-4 production after priming with Th2-polarizing stimuli, irrespective of disease severity and T cell differentiation stage.

Notably, in a recent study, Messi and coworkers have shown that effector Th1 memory T cells derived from CCR5-positive cell lines are able to revert to IL-4-producing cells upon appropriate restimulation. Moreover, the same cells retained the ability to express the Th2-restricted transcription factors (*GATA-3*) and to undergo histone hyperacetylation at the *Ii4* promoter locus⁴¹. Our data as well support the hypothesis that synovium-infiltrating Th1-oriented memory T cells may retain the flexibility of cytokine gene expression programming. This topic is now being investigated.

Our study demonstrates that CD27+ and CD27- memory CD4+ T cells express a similar pattern of cytokine production. However, CD27+ memory T cells display a profile of chemokine receptor expression (CCR7+, CCR5-) consistent with an early stage of differentiation, whereas that of CD27- memory T cells (CCR5+, CCR7-) pointed to a more differentiated stage. Irrespective of these features, both CD27+ and CD27- SF memory T helper cells retain the potential to switch from a proinflammatory to an antiinflammatory pattern of cytokine production. This finding may have important therapeutic implications, since reprogramming of the Th1/Th2 balance represents a promising strategy for treatment of many autoimmune diseases.

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