γδT cells in Juvenile Idiopathic Arthritis: Higher Percentages of Synovial Vδ1+ and Vγ9+ T Cell Subsets Are Associated with Milder Disease

YACKOV BERKUN, ANNA BENDERSKY, MAYA GERSTEIN, ITAMAR GOLDSTEIN, SHAI PADEH, and ILAN BANK

ABSTRACT. ***Objective.*** To analyze γδT cell subsets in peripheral blood (PB) and synovial fluid (SF) of patients with juvenile idiopathic arthritis (JIA), and to correlate γδT cell subsets with clinical characteristics.
Methods. γδT cell subsets as percentages of CD3+ T cells in samples of PB (n = 25) and SF (n = 93) were analyzed by flow cytometry in 93 JIA patients. The percentage of Vγ9+ γδT cells after 10 days of *in vitro* expansion with either interleukin 2 (IL-2) or isopentenyl pyrophosphate (IPP) plus IL-2 was determined.
Results. Both Vδ1+ and Vγ9+ γδT cell subsets were detected in SF of all patients, but only the percentage of Vδ1+ cells was higher in SF compared to PB ($p < 0.01$). The distribution of γδT cell subsets was similar in different JIA subgroups, whereas antinuclear antibody (ANA)-positive patients had a higher percentage of SF Vδ1+ T cells than ANA-negative patients ($p < 0.01$). The percentage of SF Vδ1+ T cells was inversely associated with age at onset, recurrence of synovitis, and erythrocyte sedimentation rate; and that of SF Vγ9+ T cells was inversely correlated with age at onset and was higher in patients who recovered from disease (n = 15). IPP-induced expansion of SF Vγ9+ T cells correlated with disease remission, whereas the expansion of SF Vγ9+ T cells in media with IL-2 alone was significantly greater in patients with uveitis.
Conclusion. The percentage of Vδ1+ and Vγ9+ γδT cells among the SF T cells and their ability to respond to IPP or IL-2 correlated with specific outcomes of JIA, suggesting their role in the immunopathogenesis of this disease. (J Rheumatol First Release March 15 2011; doi:10.3899/jrheum.100938)

Key Indexing Terms:
GAMMA DELTA T CELLS JUVENILE IDIOPATHIC ARTHRITIS SYNOVIAL FLUID
PEDIATRIC RHEUMATOLOGY INTRAARTICULAR CORTICOSTEROID INJECTION

Juvenile idiopathic arthritis (JIA), a clinically heterogeneous group of different disorders characterized by chronic inflammatory arthritis in children, is the most common chronic rheumatic disease of childhood and an important cause of short- and longterm disability[1]. According to the current International League of Associations for Rheumatology (ILAR) classification, different subtypes of JIA can be distinguished on the basis of clinical manifestations (oligoarthritis, persistent and extended; rheumatoid factor (RF)-positive and RF-negative polyarthritis; systemic, psoriatic, enthesitis-related (ERA), and other undifferentiated arthritis)[2]. Each subtype is characterized by distinct clinical and laboratory manifestations and prognosis. Although the precise etiology is still unknown, JIA is an autoimmune disease, and T cells are thought to be key players in this process[3]. Indeed, quantitative and qualitative differences among functionally distinct T cell subsets may be linked to various subtypes of JIA[4,5].

In this report, we examine the role of γδT cells, a subset with both proinflammatory and immunoregulatory potential, in JIA. These lymphocytes differ from conventional γδT cells in several ways: they usually do not express CD4 or CD8, and their T cell receptor (TCR) is encoded by γ and δ TCR genes[6]. There are 2 major γδT cell subsets in humans. The first, variable region gene (V)δ1+, is mostly localized among epithelial cells lining mucosal surfaces. These cells

From the Department of Pediatrics A and Pediatric Rheumatology, Edmond and Lily Safra Children's Hospital; Department of Medicine, Rheumatology Unit, and Laboratory for Immunoregulation, Chaim Sheba Medical Center, Tel Hashomer; and Sackler School of Medicine, Tel Aviv University, Tel Aviv, Israel.

Y. Berkun, MD, Department of Pediatrics A and Pediatric Rheumatology, Edmond and Lily Safra Children's Hospital, Chaim Sheba Medical Center, and Sackler School of Medicine, Tel Aviv University; A. Bendersky, MSc, Laboratory for Immunoregulation, Chaim Sheba Medical Center, and Sackler School of Medicine, Tel Aviv University; M. Gerstein, MD, Department of Pediatrics A and Pediatric Rheumatology, Edmond and Lily Safra Children's Hospital, Chaim Sheba Medical Center; I. Goldstein, MD, Sheba Cancer Research Center, Rheumatology Unit, Chaim Sheba Medical Center, and Sackler School of Medicine, Tel Aviv University; S. Padeh, MD, Department of Pediatrics A and Pediatric Rheumatology, Edmond and Lily Safra Children's Hospital, Chaim Sheba Medical Center, Sackler School of Medicine, Tel Aviv University; I. Bank, MD, Department of Medicine, Rheumatology Unit, Laboratory for Immunoregulation, Chaim Sheba Medical Center, and Sackler School of Medicine, Tel Aviv University.

Dr. Berkun and Ms. Bendersky contributed equally to this report.

Address correspondence to Dr. Y. Berkun, Department of Pediatrics, Safra Children's Hospital, Sheba Medical Center, Tel-Hashomer, 52621 Israel. E-mail: berkun@post.tau.ac.il

Accepted for publication January 13, 2011.

usually express V γ genes other than V γ9, and become activated by the stress-induced cell-surface major histocompatibility class I-related proteins MICA and MICB on neighboring epithelial cells[7]. The second subset expresses a TCR composed for the most part of the Vγ9 and Vδ2 genes and is predominantly found in the peripheral blood (PB) and the lymphatic system. Vγ9+Vδ2+ (here referred to as Vγ9+) cells are activated, in a TCR-dependent manner, by low molecular weight pyrophosphorylated intermediates [e.g., isopentenyl pyrophosphate (IPP)] in the classical mevalonate pathway of isoprenoid synthesis within eukaryotic and prokaryotic cells[8]. In addition, they respond with much higher affinity to (E)-4-hydroxy-3-methyl-but-2-enyl pyrophosphate (HMBPP), an intermediate in the nonmevalonate pathway of isoprenoid synthesis that is used by prokaryotes exclusively[9].

The broad functional repertoire of γδT cells includes *in vitro* non major histocompatibility complex (MHC) restricted cytotoxic effects against activated tumoral and nontumoral cells (e.g., activated CD4+ T cells, macrophages, and cultured synoviocytes), activation of dendritic cells, augmentation of autoantibody production, activation of polymorphonuclear cells, cytokine secretion, and even antigen presentation to TCR αß+ CD4+ T cells[10]. These properties suggest a central role of γδT cells in the mechanisms driving synovitis in JIA. We describe studies in which we prospectively evaluated the involvement of these unique T cells in different forms of JIA, and associated their percentage among the total T cell population with disease manifestations.

MATERIALS AND METHODS

Subjects included 93 consecutive patients with JIA attending the rheumatology clinic of Safra Children's Hospital, Sheba Medical Center, Tel Hashomer. All met the ILAR criteria for JIA[2] and had active disease, as PB and SF were taken during intraarticular corticosteroid injection (IACSI) of triamcinolone acetonide. SF was obtained from a knee in 84 and from other joints in 9 patients. In 25 patients both SF and peripheral venous blood samples were obtained concomitantly.

The ethical committee of our institute approved the study protocol, and signed informed consent was obtained from parents of all patients.

Sex, the age at examination and at JIA onset, presence of psoriasis, location of first joint involved, antinuclear antibody (ANA) and rheumatoid factor (RF) status, history of uveitis and of recently active uveitis, recent medications, and time elapsed since previous IACSI were recorded. Data collected prospectively included time to relapse in the injected joint and any other joint, months of followup after the IACSI, number of IACSI and number of joints injected, and the addition of new disease modifying antirheumatic drugs (DMARD). All patients attended our clinic at least every 4 months during the entire followup period. Clinical remission of medication was defined as absence of arthritis without medications for 12 months[11].

Isolation of mononuclear cells (MC). PBMC were isolated by Ficoll Hypaque (Sigma, St. Louis, MO, USA) density centrifugation as described[12,13]. MC were collected from the interface after centrifugation, washed 3 times with RPMI-1640 medium (Gibco, Invitrogen), and resuspended in RPMI-1640 supplemented with 10% heat-inactivated fetal bovine serum (FBS; Gibco BRL, UK) with 2 mM glutamine and penicillin-streptomycin solution (100 μg/ml) (Ta'assiot Biologiot, Beit Haemek, Israel).

Characterization of γδT cell subsets by flow cytometry. The immunostaining protocol and subsequent flow cytometric analysis of human T cells was carried out as described[12]. Mouse fluorochrome-conjugated monoclonal antibodies to human CD3-FITC were from BD Pharmingen, to human Vγ9, Vδ2 from Immunotech, and to human Vδ1 from Endogen (Pierce). We had found a 97.3% correlation of Vγ9 with Vδ2, the 2 genes predominantly coexpressed together in the γδT cell subset, in 45 samples of SF and PB from the patients in preliminary experiments; thus we have reported here only results for Vγ9 expression. The cells were analyzed on a FACSCalibur™ instrument using the Cellquest™ software (BD Biosciences). Viable lymphocytes were defined by their forward scatter/side scatter characteristics. Results are expressed as a percentage of the total CD3+ T cell count. Importantly, the number of the total T cells in samples of cultured cells, as measured in representative samples, increased by a factor of 2–3 fold routinely, showing that percentage of evaluated Vγ9 T cells was due to proliferation rather than death of non-Vγ9 T cells in the cultures (data not shown).

In vitro stimulation. SFMC or PBMC (1×10^6 cells/ml) were cultured in 96-well round-bottom tissue culture plates (Costar) in 200 μl RPMI-1640 medium supplemented with 10% FBS, 2 mM L-glutamine, 100 μg/ml penicillin-streptomycin solution, and 100 IU/ml recombinant human interleukin 2 (rhIL-2; Boehringer-Mannheim), and stimulated by addition of 0.5–4 μg/ml IPP (Sigma Aldrich). Medium was replaced on days 3 and 7 of culture. All cultures were maintained for 10 days, then cells were collected and analyzed by flow cytometry. Results reported here were at the dose of 2 μg/ml IPP, which we determined to yield the maximal proliferative response (data not shown).

Statistical analysis. Analysis of data was carried out using SPSS 11.0 statistical software (SPSS Inc., Chicago, IL, USA). For continuous variables, such as age at time of IACSI and laboratory data, normality of distribution was assessed using the Kolmogorov-Smirnov test (cutoff at $p = 0.01$). Descriptive statistics were calculated and described as appropriate for distribution. Categorical variables such as sex and presence of uveitis were described using frequency distributions and are presented as frequency (%). Associations between laboratory variables and continuous clinical data were described by calculating the Pearson or Spearman rho correlation coefficients as appropriate. Laboratory data were compared by categorical clinical data using the t-test for independent samples or the Mann-Whitney U-test for dichotomous clinical variables, and one-way analysis of variance (ANOVA) followed by the Bonferroni test or the Kruskal-Wallis test followed by Mann-Whitney U-test for clinical data with more than 2 categories. The chi-square test (exact as needed) was used to assess associations between categorical variables. All tests are 2-sided and considered significant at $p < 0.05$.

RESULTS

Patient characteristics. The main clinical characteristics of the study cohort are shown in Table 1. Disease was oligoarticular in 57 patients (47 persistent; 10 extended), 12 had RF-negative polyarthritis, 11 the systemic subtype, 5 the psoriatic, 3 ERA, and 5 had the undifferentiated JIA subtype. In addition to the ILAR classification, we performed an independent analysis based on number of joints involved. This revealed that 36 patients had polyarticular, 51 pauciarticular, and 6 monoarticular disease. At the time of T cell studies, 22 patients (23.7%) were treated with methotrexate (MTX), 11 (11.8%) with low-dose systemic corticosteroids, and 11 (11.8%) with anti-tumor necrosis factor agents (combined with MTX in 8).

γδT cell subsets in SF and PB and clinical characteristics. γδT cell subset analysis in SF revealed that levels of Vδ1+ cells were significantly higher in females than males (5.60%

Table 1. Characteristics of the 93 patients with JIA enrolled in the study.

Clinical Variable	Mean ± SD
Age at the procedure, yrs	9.9 ± 5.1
Age at disease onset, yrs	5.8 ± 4.3
Females, n (%)	69 (74.2)
Uveitis, n (%)	18 (19.4)
Positive antinuclear antibody, n (%)	43 (45.2)
No. joints injected with corticosteroid	3.0 ± 2.7
Time to recurrence (mo) after IACSI	6.8 ± 7.2
Followup duration, mo	19.9 ± 11.4
Patients recovered, n (%)	15 (16.1)
No. IACSI	2.1 ± 2.3
No. joints injected with corticosteorid	4.6 ± 5.0

IACSI: intraarticular corticosteroid injection.

± 4.5% vs 3.36% ± 3.00%, respectively; $p < 0.032$). The percentage of each of the major subsets of γδT cells was independently inversely correlated with the patient's age at time of SF aspiration (Vδ1+, $r = –0.333$, $p = 0.002$; and Vγ9+, $r = –0.241$, $p = 0.025$) and also with the age at onset of JIA (Vδ1+, $r = –0.275$, $p = 0.01$; and Vγ9+, $r = –0.215$, $p = 0.048$). In the 25 patients in whom SF and PB were obtained simultaneously, Vδ1+ cells were significantly enriched among SF T cells relative to PB (5.06% ± 4.30% vs 1.78% ± 1.34%, respectively; $p < 0.01$)) compared with the Vγ9+ subset, which was represented equally in SF (5.39% ± 3.78%) and PB (4.66% ± 2.68%) samples (Figure 1).

Analyses in relation to JIA subtypes revealed, nevertheless, that both major subsets of γδT cells were represented in the SF of all JIA subtypes, with no significant differences (Table 2 and Figure 2). The proportions of the 2 major γδ subsets were also not associated with the number of joints involved (i.e., mono, oligo, and polyarthritis; data not shown). In addition, we found that the Vγ9+ T cells could be maintained *in vitro* for 10 days in the presence of IL-2 and expanded in response to IPP in SFMC cultures derived from patients of all clinical subsets (Figure 2 and Table 2). Interestingly, however, the percentage of Vγ9+ T cells stimulated with IL-2 for 10 days was increased in polyarticular JIA SFMC cultures compared to cultures from systemic JIA patients (6.99% ± 3.89% vs 2.44% ± 1.98%, respectively; $p < 0.05$). We also found that the percentage of Vγ9+ T cells in SFMC cultures after *in vitro* IL-2 stimulation was significantly higher in patients who had a history of uveitis (7.02% ± 3.14%), and particularly higher when the uveitis was active during the aspiration of sample (9.10% ± 2.24%) compared to patients without uveitis (4.62% ± 2.98% and 4.72% ± 2.91%; $p < 0.008$ and $p < 0.002$, respectively; Figure 3). In addition, the percentage of Vδ1+ T cells was significantly higher in the SF of ANA-positive patients compared to ANA-negative patients (6.44% ± 5.35% vs 3.98% ± 2.87%; $p = 0.033$). The percentage of Vγ9+ T cells was higher in ANA-positive patients as well, but this difference did not achieve statistical significance (Figure 4).

Relation of γδT cells to disease severity and prognosis. Associations between indices of disease severity, disease outcome, and SF γδT cells in JIA were assessed. The percentage of Vδ1+ T cells was significantly lower in patients with recurrence in any joint following the recent IACSI (4.40% ± 4.10% vs 7.00% ± 4.34%, respectively; $p = 0.015$), in patients with recurrence in the same joint (4.33% ± 4.22% vs 6.75% ± 4.04%; $p = 0.017$), and in patients starting DMARD therapy during the followup (3.51% ± 2.54% vs 5.50% ± 4.53%; $p = 0.043$). Inverse correlations between Vδ1+ cells and the number of IACSI during the followup ($r = –0.25$, $p = 0.022$), the number of joints injected ($r = –0.291$, $p = 0.007$), and the ESR ($r = –0.54$, $p = 0.025$) were also observed. Greater percentage of Vγ9+ T cells in SF was also associated with a better prognosis, since it correlated inversely with the number of joints injected with corticosteroids during followup ($r = –0.231$, $p = 0.032$). Most importantly, a higher frequency of SF Vγ9+ T cells, both at start of culture and after expansion on exposure to IPP *in vitro*, was found in patients who subsequently recovered compared to patients with active disease at the end of the followup (7.48% ± 4.14% vs 4.96% ± 3.61%, $p = 0.022$; and 43.1% ± 26.6% vs 26.2% ± 23.4%, $p = 0.035$, respectively). IPP-driven expansion of Vγ9+ T cells was also inversely associated with levels of C-reactive protein ($r = –0.609$, $p = 0.047$). We found no association between medication taken by the patient during the IACSI and the measures of γδT cells.

DISCUSSION

The results presented here are the first to demonstrate significant associations between subsets of γδT cells in the SF and clinical characteristics of patients with JIA. The major findings are as follows: (1) both Vδ1+ and Vγ9+Vδ2+ subsets are represented in SF of all subsets of JIA patients; (2) Vδ1+ γδT cells are significantly enriched among SF T cells compared to PB; (3) ANA-positive patients have significantly higher levels of SF Vδ1+ cells than ANA-negative patients; (4) elevated levels of both Vδ1+ and Vγ9+ subsets appear to be associated with specific features of favorable disease outcomes; and (5) the relative expansion of the Vγ9+ subset *in vitro* in response to IL-2 stimulation is positively associated with the occurrence of clinical uveitis.

Previous studies addressed the issue of γδT cells in JIA[14,15,16,17]. Although healthy children display a gradual increase of Vγ9+ T cells with age (contrasting with our finding that in SF of JIA patients, the percentage of these cells decreased with age), no differences in the proportion of total γδT cells in the PB of patients and controls were found by some investigators[14,18]. However, Massa, *et al* reported that the proportion of γδT cells decreased upon treatment with MTX[17]. In contrast, Black, *et al* reported a higher proportion of γδT cells in SF than in PB in a cohort of 39 patients[19]. Moreover, the highest numbers were found in

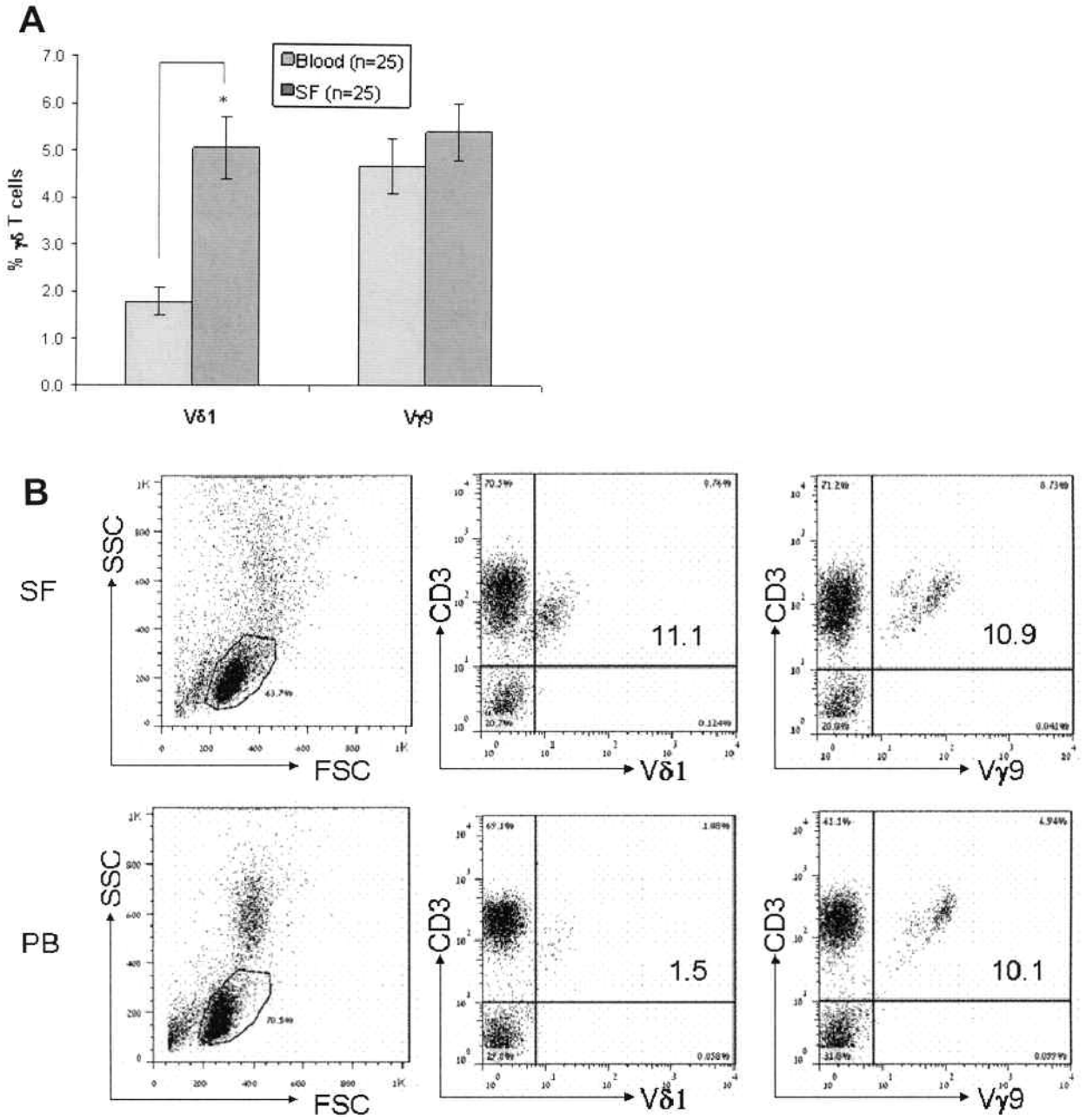

Figure 1. A. Vδ1+ and Vγ9+ T cells in peripheral blood and synovial fluid (SF). Mean ± 1 SD of γδ subset as a percentage of total CD3+ T cells, in freshly isolated peripheral blood and SF in paired samples from 25 JIA patients. *$p < 0.05$ comparing peripheral blood and SF. B. FACS analysis of γδ subsets in peripheral blood (PB) and synovial fluid (SF) of a single patient; dot plots on the left represent sidescatter (SSC)/forward scatter (FSC) of PB and SF mononuclear cells; the areas gated for analysis of γδ subsets (lymphocytes) are enclosed. For each gated area, dot plot FACS analysis of cells stained with monoclonal antibody to the indicated markers is shown. Numbers represent percentage of the γδ subset/CD3+ T cells.

patients with strong responses to GroEL, Yersinia, and human heat shock protein and, similar to our findings, correlated with lower systemic acute-phase reactions. Doherty, *et al* studied Jγ rearrangements in SF samples, and concluded that the γδT cells in synovia might be oligoclonal and identical in different joints of the same patient, implying antigenic or superantigenic selection[20]. These authors also reported a lower expression of the joining region JγP (Jγ1.2), which is required for IPP reactivity, in the SF than in the PB of the patients. Kjeldsen-Kragh, *et al* found that both Vδ1+ and Vγ9+ cells in the SF displayed increased activation markers; the former were CD45RA+CD69+, whereas the latter expressed the CD45RO isoform, suggesting different levels of activation of the respective subsets[15]. Although these studies indicated that both subsets of γδT cells are localized in SF in an activated state and differ from PB γδT

Table 2. Vδ1+ and Vγ9+ T cells in synovial fluid in different JIA subtypes, as percentage of total T cells. Data are mean ± SD of percentage Vδ1+ and Vγ9+ subsets among CD3+ T cells in freshly isolated synovial fluids of 93 patients with JIA subtypes or (for Vγ9+ T cells only) in 10-day synovial fluid mononuclear cell cultures triggered with 100 IU/ml recombinant human IL-2 alone (IPP0) or with 2 μg/ml isopentenyl pyrophosphate (IPP2).

Subtype	N (% of total)	Vδ1+	Vγ9	IPP0 (n)	IPP2 (n)
All cohorts	93 (100)	5.06 ± 4.30	5.39 ± 3.78	5.13 ± 3.15 (70)	29.1 ± 24.4 (70)
Persistent	47 (50.5)	5.68 ± 4.69	6.45 ± 4.28	5.59 ± 3.10 (32)	33.4 ± 26.7 (32)
Extended	10 (10.8)	5.86 ± 3.29	3.57 ± 1.97	4.93 ± 2.84 (8)	30.6 ± 21.5 (8)
Polyarthritis	12 (12.9)	4.70 ± 3.50	5.57 ± 2.48	6.99 ± 3.89* (9)	20.0 ± 20.6 (9)
Psoriatic	5 (5.4)	5.35 ± 6.75	6.20 ± 3.76	6.14 ± 2.52 (5)	45.6 ± 22.0 (5)
Systemic	11 (11.8)	3.34 ± 3.93	3.02 ± 3.13	2.44 ± 1.98* (10)	18.7 ± 22.1 (10)
Enthesitis-related arthritis	3 (3.2)	1.50 ± 0.89	4.57 ± 2.74	4.85 ± 0.64 (2)	41 ± 13.7 (2)
Other	5 (5.4)	3.47 ± 3.22	2.90 ± 2.79	3.35 ± 2.62 (4)	12.6 ± 15.7 (4)

* $p < 0.05$ (ANOVA) between subgroups of patients for the respective measurement. N: number of patients studied.

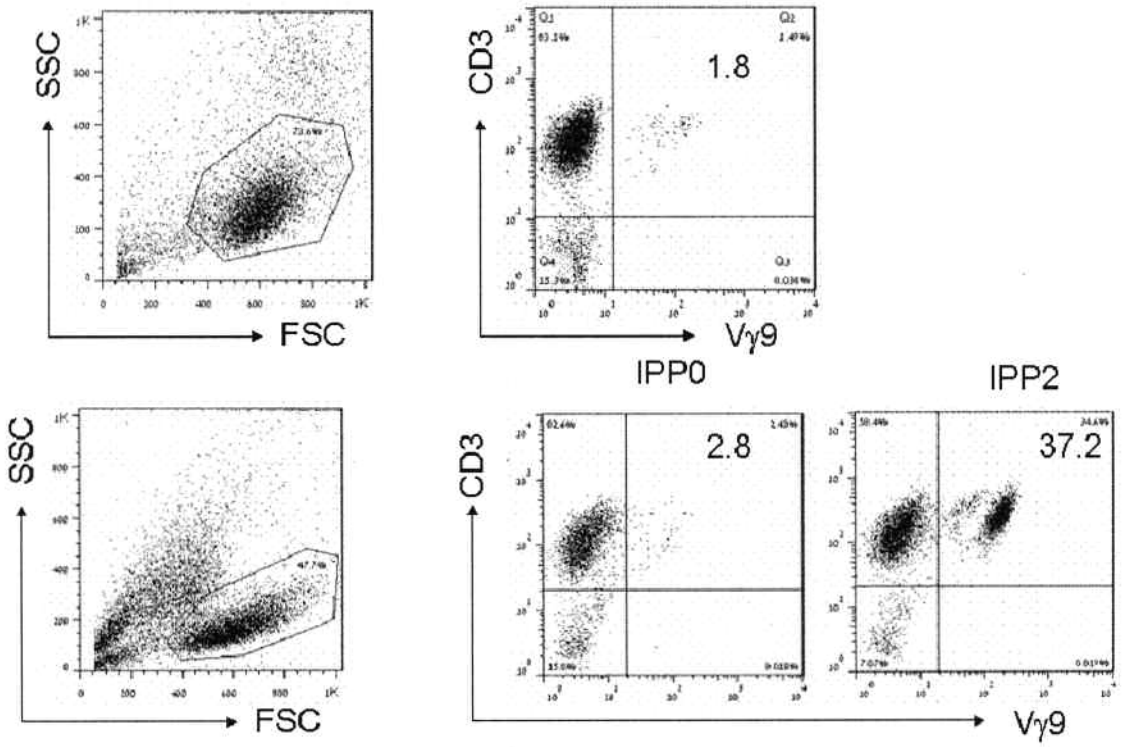

Figure 2. IPP response of Vγ9+ T cells in SF. Left: representative forward scatter (FSC)/side scatter (SSC) of fresh SFMC (upper panel) and SFMC cultured 10 days (lower panel) and gates selected for analysis of γδ T cells. Dot plots of cells within the gates stained with monoclonal antibody to CD3 and Vγ9 are shown on the right. IPP0 and IPP2 indicate cells cultured with IL-2 alone and IL-2 plus IPP 2 μg/ml. Large digits indicate percentages of positive Vγ9+ T cells as a proportion of all CD3+ T cells.

cells, none systematically analyzed these lymphocytes in different subtypes of JIA.

Our first major finding of comparable percentages of the Vγ9+ and Vδ1+ T cell subsets in the SF *in situ* suggests that both subsets play a role in the immunopathogenesis of synovitis in all subtypes of JIA (Figures 1 and 2). Nevertheless, the analysis of variance revealed a significantly lower percentage of IL-2-responsive Vγ9+ T cells in systemic JIA, compared to RF-negative polyarticular JIA (Table 2). This suggests that in systemic JIA Vγ9+ T cells are defective in their ability to proliferate in response to IL-2, which may be related to the unique mechanisms underlying systemic JIA, currently perceived as autoinflammatory rather than autoimmune. Indeed, our findings complement recent observations of a decrease of γδT cells in the PB of patients with systemic but not other forms of JIA during disease flares, and suggest

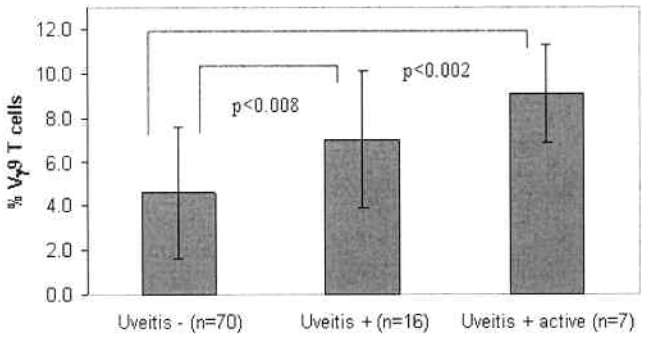

Figure 3. Synovial fluid Vγ9+ T cells stimulated with IL-2 in JIA patients with and without uveitis. Mean percentages (± 1 SD) of Vγ9+ T cells in the CD3+ T cells in synovial fluid mononuclear cell cultures stimulated for 10 days with 100 IU/ml IL-2 are shown. The p values compare means.

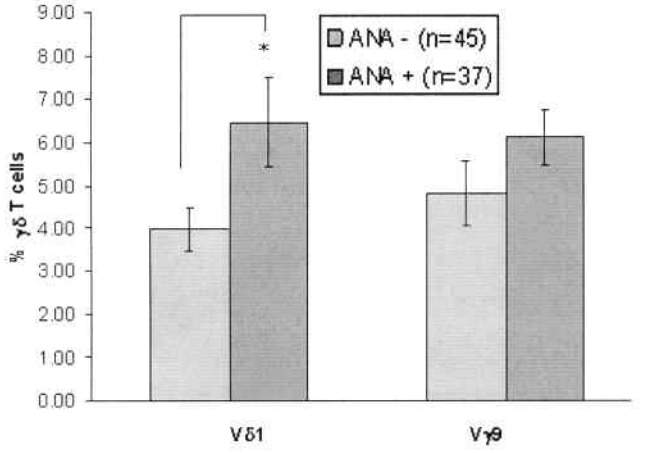

Figure 4. Vδ1+ and Vγ9+ T cells in freshly isolated synovial fluid in ANA-negative and ANA-positive patients with JIA. γδ subset is shown as mean percentages (± 1 SD) of total T cells. *p < 0.05 for comparison of indicated values.

that this JIA subtype is associated with a unique Vγ9+ γδT cell response[21].

Although the γδT cell subsets were equally distributed among JIA subtypes, ANA-positive patients had a significantly higher percentage of SF Vδ1+ T cells than ANA-negative patients (Figure 4). Vδ1+ T cell activation is, at least in part, driven by interactions with MICA/MICB molecules induced on stressed cells, and synoviocyte MICA has been shown to be functional and induce cytokine secretion from CD4+CD28– SF T cells in patients with rheumatoid arthritis[7]. This suggests that these molecules could similarly affect SF Vδ1+ T cells in JIA. Further, certain MICA alleles are more highly expressed in JIA patients than in controls, and an association between MICA-5 and systemic lupus erythematosus, the prototypic ANA-positive disease, has also been shown[22,23,24]. Thus, it is possible that the higher level of Vδ1+ T cells in the synovium of ANA-positive patients may reflect different levels and/or subtypes of MICA molecules in the synovium of ANA-positive versus ANA-negative JIA patients, a topic which will be tested in future studies. In Lyme-induced arthritis, SF Vδ1+ T cells induce the apoptosis of the Lyme-reactive SF CD4+ T cells, while enhancing maturation of dendritic cells, suggesting that Vδ1+ T cells may perform both antiinflammatory and proinflammatory roles in JIA as well[25]. In our study, however, elevated levels of Vδ1+ T cells were associated with less recurrences of arthritis. Together with the better longterm prognosis of ANA-positive JIA patients, who have higher levels of Vδ1+ SF T cells, this suggests that an increase in levels of these cells may play a regulatory rather than a proinflammatory role in JIA synovium. Of note, we also found higher levels of Vδ1+ cells in female patients; this finding is probably related to the higher prevalence of ANA-positive females versus males. Anterior uveitis is a serious extraarticular manifestation of JIA, occurring in 10%–20% of the patients, mainly in oligoarticular and RF-negative polyarticular subtypes and ANA-positive patients[26,27]. Patients with uveitis had a higher percentage of IL-2-responsive SF Vγ9+ T cells (Figure 3). Patients with polyarticular JIA, in whom uveitis is more severe, also exhibited an elevated percentage of SF IL-2-responsive Vγ9+ T cells, whereas in systemic JIA, in which uveitis is rare, a low response was observed (Table 2). These findings, suggesting a role of IL-2-responsive Vγ9+ T cells in uveitis in JIA, are consistent with other reports showing that γδT cells activated IL-17-producing uveitogenic CD4+ T cells in an animal model, and that Vγ9+ T cells are found in intraocular fluid of Behçet disease patients with uveitis[28].

Finally, recovery from disease was found to be positively correlated with the percentage of SF Vγ9+ T cells and the degree of expansion of the Vγ9+ T cells in response to stimulation with IPP. Although these 2 variables may be related, it should be noted that only a subset of Vγ9+ T cells, i.e., those expressing the Vγ9J1.2 gene rearrangement, can respond to IPP and expand[29]. Thus, our data suggest that patients whose SF Vγ9+ T cell populations are enriched for Vγ9Jγ1.2 cells have a greater chance of recovery[20]. A potential beneficial role for IPP-responsive Vγ9+ T cells in the synovium could be attributable to immunoregulatory effects and/or to the ability of IPP-activated Vγ9+ T cells to mediate cytotoxicity against proliferating synovial fibroblasts[12]. Further, a potentially protective role for Vγ9+ T cells in JIA is consistent with our previous report of an association between a depleted Vγ9+ subset in PB and development of a more severe arthritis in adult patients with Felty syndrome[30]. Studies to elucidate the mechanisms underlying the association of Vγ9+ T cells with better outcomes of arthritis are clearly warranted. Measurements of Vγ9+ T cells and their response to IPP *in vitro* may serve as novel prognostic markers in JIA, suggesting that larger-scale studies are indicated to validate these hypotheses.

Our results show for the first time a significant correlation between relative numbers of γδT cell subsets in the SF

and their ability to expand in response to IPP or IL-2 and distinct clinical forms of JIA and the prognosis. Studies of these cells might yield important new insights into pathogenetic mechanisms of these diseases as well as new prognostic markers and therapeutic tools.

REFERENCES

1. Mason TG, Reed AM. Update in juvenile rheumatoid arthritis. Arthritis Rheum 2005;53:796-9.
2. Petty RE, Southwood TR, Baum J, Bhettay E, Glass DN, Manners P, et al. Revision of the proposed classification criteria for juvenile idiopathic arthritis: Durban, 1997. J Rheumatol 1998;25:1991-4.
3. Berkun Y, Padeh S. Environmental factors and the geoepidemiology of juvenile idiopathic arthritis. Autoimmun Rev 2010;9:319-24.
4. Ruprecht CR, Gattorno M, Ferlito F, Gregorio A, Martini A, Lanzavecchia A, et al. Coexpression of CD25 and CD27 identifies FoxP3+ regulatory T cells in inflamed synovia. J Exp Med 2005;201:1793-803.
5. de Kleer IM, Wedderburn LR, Taams LS, Patel A, Varsani H, Klein M, et al. CD4+CD25bright regulatory T cells actively regulate inflammation in the joints of patients with the remitting form of juvenile idiopathic arthritis. J Immunol 2004;172:6435-43.
6. Bank I, DePinho RA, Brenner MB, Cassimeris J, Alt FW, Chess L. A functional T3 molecule associated with a novel heterodimer on the surface of immature human thymocytes. Nature 1986;322:179-81.
7. Groh V, Bruhl A, El-Gabalawy H, Nelson JL, Spies T. Stimulation of T cell autoreactivity by anomalous expression of NKG2d and its MIC ligands in rheumatoid arthritis. Proc Natl Acad Sci USA 2003;100:9452-7.
8. Gober HJ, Kistowska M, Angman L, Jeno P, Mori L, De Libero G. Human T cell receptor gamma-delta cells recognize endogenous mevalonate metabolites in tumor cells. J Exp Med 2003;197:163-8.
9. Kabelitz D, Glatzel A, Wesch D. Antigen recognition by human gamma-delta T lymphocytes. Int Arch Allergy Immunol 2000;122:1-7.
10. Moser B, Brandes M. Gamma-delta T cells: An alternative type of professional APC. Trends Immunol 2006;27:112-8.
11. Wallace CA, Ruperto N, Giannini E. Preliminary criteria for clinical remission for select categories of juvenile idiopathic arthritis. J Rheumatol 2004;31:2290-4.
12. Bendersky A, Markovits N, Bank I. V-gamma-9+ gamma-delta T cells in systemic sclerosis patients are numerically and functionally preserved and induce fibroblast apoptosis. Immunobiology 2010;2010:380-94.
13. Goldstein I, Ben-Horin S, Koltakov A, Chermoshnuk H, Polevoy V, Berkun Y, et al. Alpha-1-beta-1 integrin+ and regulatory FoxP3+ T cells constitute two functionally distinct human CD4+ T cell subsets oppositely modulated by TNF-alpha blockade. J Immunol 2007;178:201-10.
14. Kjeldsen-Kragh J, Quayle A, Kalvenes C, Førre O, Sørskaar D, Vinje O, et al. T gamma delta cells in juvenile rheumatoid arthritis and rheumatoid arthritis. In the juvenile rheumatoid arthritis synovium the T gamma delta cells express activation antigens and are predominantly v delta 1+, and a significant proportion of these patients have elevated percentages of T gamma delta cells. Scand J Immunol 1990;32:651-9.
15. Kjeldsen-Kragh J, Quayle AJ, Vinje O, Natvig JB, Forre O. A high proportion of the v delta 1+ synovial fluid gamma delta T cells in juvenile rheumatoid arthritis patients express the very early activation marker CD69, but carry the high molecular weight isoform of the leucocyte common antigen (CD45RA). Clin Exp Immunol 1993;91:202-6.
16. Lepore L, Pennesi M, Saletta S, Perticarari S, Presani G, Prodan M. Study of Il-2, Il-6, TNF alpha, IFN gamma and beta in the serum and synovial fluid of patients with juvenile chronic arthritis. Clin Exp Rheumatol 1994;12:561-5.
17. Massa M, de Benedetti F, Robbioni P, Ramenghi B, Albani S, Martini A. Association of methotrexate treatment with a decrease of double negative (CD4-CD8-) and gamma/delta T cell levels in patients with juvenile rheumatoid arthritis. J Rheumatol 1993;20:1944-8.
18. Caccamo N, Dieli F, Wesch D, Jomaa H, Eberl M. Sex-specific phenotypical and functional differences in peripheral human v gamma 9/v delta 2 T cells. J Leukoc Biol 2006;79:663-6.
19. Black AP, Bhayani H, Ryder CA, Pugh MT, Gardner-Medwin JM, Southwood TR. An association between the acute phase response and patterns of antigen induced T cell proliferation in juvenile idiopathic arthritis. Arthritis Res Ther 2003;5:R277-84.
20. Doherty PJ, Yang SX, Laxer RM, Silverman ED, Inman R, Pan S. Evidence for clonal expansion of T cell receptor v gamma II+ T cells in the synovial fluid of patients with arthritis. J Immunol 1992;149:295-9.
21. Macaubas C, Nguyen K, Deshpande C, Phillips C, Peck A, Lee T, et al. Distribution of circulating cells in systemic juvenile idiopathic arthritis across disease activity states. Clin Immunol 2010; 134:206-16.
22. Sanchez E, Torres B, Vilches JR, López-Nevot MA, Ortego-Centeno N, Jiménez-Alonso J, et al. No primary association of MICA polymorphism with systemic lupus erythematosus. Rheumatology 2006;45:1096-100.
23. Gambelunghe G, Gerli R, Bocci EB, Del Sindaco P, Ghaderi M, Sanjeevi CB, et al. Contribution of MHC class I chain-related a (MICA) gene polymorphism to genetic susceptibility for systemic lupus erythematosus. Rheumatology 2005;44:287-92.
24. Nikitina Zake L, Cimdina I, Rumba I, Dabadghao P, Sanjeevi CB. Major histocompatibility complex class I chain related (MIC) a gene, TNF-a microsatellite alleles and TNF-b alleles in juvenile idiopathic arthritis patients from Latvia. Hum Immunol 2002;63:418-23.
25. Collins C, Wolfe J, Roessner K, Shi C, Sigal LH, Budd RC. Lyme arthritis synovial gamma-delta T cells instruct dendritic cells via fas ligand. J Immunol 2005;175:5656-65.
26. Kanski JJ. Uveitis in juvenile chronic arthritis. Clin Exp Rheumatol 1990;8:499-503.
27. Sim KT, Venning HE, Barrett S, Gregson RM, Amoaku WM. Extended oligoarthritis and other risk factors for developing JIA-associated uveitis under ILAR classification and its implication for current screening guideline. Ocul Immunol Inflamm 2006;14:353-7.
28. Verjans GM, van Hagen PM, van der Kooi A, Osterhaus AD, Baarsma GS. V gamma 9 v delta 2 T cells recovered from eyes of patients with Behcet's disease recognize non-peptide prenyl pyrophosphate antigens. J Neuroimmunol 2002;130:46-54.
29. Evans PS, Enders PJ, Yin C, Ruckwardt TJ, Malkovsky M, Pauza CD. In vitro stimulation with a non-peptidic alkylphosphate expands cells expressing v gamma 2-j gamma 1.2/v delta 2 T-cell receptors. Immunology 2001;104:19-27.
30. Bank I, Cohen L, Mouallem M, Farfel Z, Grossman E, Ben-Nun A. Gamma delta T cell subsets in patients with arthritis and chronic neutropenia. Ann Rheum Dis 2002;61:438-43.