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Synovial fluid T cell clones from oligoarticular juvenile arthritis patients display a prevalent Th1/Th0-type pattern of cytokine secretion irrespective of immunophenotype

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SUMMARY

The aim of the present study was to investigate the patterns of cytokine production by T cell clones raised from *in vivo* activated synovial fluid (SF) mononuclear cells (MNC) of five patients with oligoarticular juvenile arthritis (JA). Freshly isolated SF T cells were cultured *in vitro* with low dose recombinant IL-2 and subsequently cloned by limiting dilution. Sixty-four clones were obtained from the five patients studied. Fifty-nine clones were TCR α/β^+, either $CD4^+$ ($n = 43$) or $CD8^+$ ($n = 15$). The remaining five clones were TCR γ/δ^+, $CD4^-$, $CD8^-$. Clone immunophenotypes differed in the individual patients. Forty-four T cell clones were stimulated with phytohaemagglutinin (PHA) and phorbol myristate acetate (PMA) and supernatants tested for the presence of IL-2, IL-4, IL-5 and interferon-gamma (IFN-γ) by ELISA or bioassays. Cytokine mRNA accumulation was tested by reverse transcriptase-polymerase chain reaction (RT-PCR). Most of 44 clones tested released large amounts of IFN-γ irrespective of the immunophenotype. Of these, 27 were classified as Th1-type and 17 as Th0-type based upon the IFN-γ/IL-4 ratio in culture supernatants. Finally, when 10 representative T cell clones were tested for pro- and anti-inflammatory cytokines, gene expression by RT-PCR, all of them were found to express the granulocyte-macrophage colony-stimulating factor (GM-CSF), tumour necrosis factor-alpha (TNF-α), IL-10 and transforming growth factor-beta 1 (TGF-β1) genes, and half of them IL-6 and IL-8 mRNA. In conclusion, T cell clones, that represent the progeny of *in vivo* activated SF T cells from oligoarticular JA patients, display heterogeneous immunophenotypes, but all share the ability to produce large amounts of IFN-γ, with a predominant Th1/Th0 pattern. The expression of pro- and anti-inflammatory cytokine genes in these clones suggests that *in vivo* activated SF T cells modulate joint inflammation in a complex fashion.

Keywords juvenile arthritis T cell clones Th1

INTRODUCTION

Oligoarticular juvenile arthritis (JA) is a peculiar entity in the group of rheumatic diseases of childhood. It is clinically characterized by the involvement of four joints or less, with a frequent positivity for serum antinuclear antibodies (ANA) and possible association with anterior uveitis [1].

The pathogenesis of JA is poorly understood. The finding that activated T cells are consistently detected in the synovial fluid or membrane of JA patients [2–6] may suggest that antigen-specific T lymphocytes [7–9] act as initiators of the inflammatory process. Cytokines locally released by activated T cells may play a role in tissue damage [10].

Production of IL-2, IL-4, IL-5 and interferon-gamma (IFN-γ) allows one to identify two discrete subsets of $CD4^+$ T cells, namely T helper (Th)1 and Th2, respectively [11–14]. Th1 cells produce IL-2 and IFN-γ, but little or no IL-4 or IL-5. Conversely, Th2 cells specialize in the production of IL-4 and IL-5, but not of IL-2 or IFN-γ. A third subpopulation of $CD4^+$ cells, named Th0, displays an unrestricted profile of cytokine production [15,16]. Th0 cells may represent precursors of Th1 or Th2 cells [17]. In disease states characterized by accumulation of activated T cells, the patterns of cytokines produced can influence the pathophysiology of the underlying disease as well as its clinical manifestations.

The latter issue has been addressed in a few studies which have

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shown a predominant Th1 pattern in synovial membrane [18–21] or fluid [19] T cells from rheumatoid arthritis (RA) patients. Virtually no information is available on the patterns of cytokine production by synovial T cells from children with JA, in particular with the oligoarticular subtype. Here we investigated the immunophenotype and the patterns of cytokine production of a panel of clones raised from IL-2-expanded synovial fluid (SF) T cells of five patients with oligoarticular JA.

PATIENTS AND METHODS

Patients

Five oligoarticular JA patients were studied. The diagnosis was established according to the new Classification Criteria for Idiopathic Arthritides of Childhood [1]. According to the above mentioned criteria, the oligoarthritis subgroup includes patients with idiopathic arthritis affecting one to four joints during the first 6 months of disease. Spondyloarthopathy or positive family history of spondyloarthopathy, rheumatoid factor and/or HLA-B27 positivity are specific exclusion criteria.

Four patients were female, one was male. Their ages ranged from 6 to 13 years. Disease duration ranged from 7 months to 2·9 years (Table 1). At study, no patient presented iridocyclitis.

Cell separation and culture

SF and peripheral blood mononuclear cells (PBMC) were separated by centrifugation on a Ficoll–Hypaque gradient and suspended in RPMI 1640 medium (Seromed Biochrom KG, Berlin, Germany) supplemented with L-glutamine, penicillin-streptomycin (Seromed) and 10% fetal calf serum (FCS; Seromed Biochrom KG) (complete medium). SF PBMC (1×10^6/ml) were subsequently cultured in 24-well plates with 50 U/ml rIL-2 (Genzyme, Milan, Italy) in an atmosphere containing 5% CO_2 in air [22]. After 7 days in culture, lymphoid blasts were purified (>95%) on a Percoll (Pharmacia, Uppsala, Sweden) gradient, as previously reported [22] and cloned by limiting dilution (see below).

The cloning procedure was performed as follows. Percoll-enriched blast cells were seeded in 96-well U-bottomed plates at a concentration of 0·5–1 cell/well in 0·2 ml of complete medium supplemented with 50 U/ml rIL-2 in the presence of 10^5 gamma-irradiated (60 Gy) allogeneic PBMC and 0·5 μg/ml phytohaemagglutinin (PHA-P; Wellcome Burroughs, London, UK) [22]. Cells were fed weekly with fresh, complete medium containing 50 U/ml rIL-2. After 15–20 days each well was checked microscopically and proliferating microcultures were expanded in rIL-2-containing medium [22]. By this procedure, the cloning efficiency was approximately 15%. Cytokine production by SF-derived clones was investigated following non-specific stimulation. Cloned T cells were washed twice, resuspended at the concentration of 1×10^6/ml in complete medium and cultured for 24–48 h with 1 μg/ml PHA-P plus 5 ng/ml phorbol myristate acetate (PMA; Sigma Chemical Co., St Louis, MO). Supernatants were collected and stored at −80°C until tested.

Immunophenotypic analyses

Surface marker analysis was performed on freshly isolated SF and PBMC and cultured lymphocytes (after 7 days) by direct immunofluorescence using a panel of FITC- or PE-conjugated murine MoAbs. Peripheral blood was obtained from five healthy donors (age range 5–15 years), following the informed consent of the parents. The following MoAbs were used: CD3, CD4 and CD8 from Dako (Glostrup, Denmark); HLA-DR and CD69 from Becton Dickinson (Mountain View, CA); CD20 and CD25 from Coulter (Hialeah, FL); CD16 from Serotec (Oxford, UK). Cells were incubated with saturating amounts of MoAbs for 30 min on ice, washed twice and analysed by a FACScan flow cytometer (Becton Dickinson) setting the gate on the lymphocyte population [23]. Both single and double staining experiments were carried out. Isotype-matched, fluorochrome-conjugated MoAbs of unrelated specificities were used as controls to assess the non-specific binding of test MoAbs to each cell preparation and to set the threshold between positively and negatively staining cell populations [23].

Cytokine detection in culture supernatants

IL-2 and IL-5 were detected by biological assays. IL-2 was measured according to the capacity of the culture supernatants to promote the proliferation of the IL-2-dependent CTLL-2 murine cell line [24]. IL-5 was detected by the same principle using the murine LyH7.B13 target cell line, kindly donated by Dr Ronald Palacios [25]. For both cytokines, the amounts contained in test supernatants were quantified by comparison with the dose–response curves obtained by culturing the two cell lines with different concentrations of human rIL-2 or rIL-5 (Genzyme), respectively. IFN-γ and IL-4 were assayed using ELISA kits from Genzyme. The minimum threshold of detection of these assays is 100 pg/ml for IFN-γ and 45 pg/ml for IL-4.

T cell clones were subdivided into three groups based upon the IFN-γ/IL-4 ratio, calculated by dividing the concentrations of the two cytokines detected in clone supernatants [13,26]. An IFN-γ/IL-4 ratio ranging from 0·1 to 10 was taken as evidence for Th0-type of clones; when the same ratio was >10 or <0·1, the clones

Table 1. Clinical characteristics of oligoarticular juvenile arthritis (JA) patients and treatment at the time of synovial fluid aspiration

Patient	Sex	Age at onset, years	Duration of disease	Duration of arthritic flare-up, days	ANA	IgM RF	HLA-B27	Treatment
A	F	6	8 months	32	+	–	–	NSAID
B	F	10	1 year	15	+	–	–	–
C	M	11	7 months	13	+	–	–	–
D	F	13	2.9 years	37	+	–	–	NSAID
E	F	8	1.8 years	24	+	–	–	NSAID

ANA, Antinuclear antibodies; RF, rheumatoid factor; NSAID, non-steroidal anti-inflammatory drug.

were defined as Th1-type or Th2-type, respectively. High levels in culture supernatants of either IL-4 or IFN-γ *per se* were not considered as sufficient criteria for the definition of the Th2- or Th1-type pattern, respectively.

Detection of cytokine mRNA by reverse transciptase-polymerase chain reaction

The expression of IL-2, IL-4, IL-5, IL-6, IL-8, IL-10, IFN-γ, transforming growth factor-beta 1 (TGF-β1) and granulocyte-macrophage colony-stimulating factor (GM-CSF) genes was investigated using the reverse transcriptase-polymerase chain reaction (RT-PCR) procedure. Total RNA was extracted from 10^6 cloned T cells that had been cultured for 6 h with 1 μg/ml PHA-P and 5 ng/ml PMA. RNA was extracted by a modified Chomczynsky–Sacchi method [27] and reverse-transcribed into cDNA for PCR amplification using the 1st strand cDNA Synthesis Kit (Clontech Labs Inc., Palo Alto, CA) and the oligo dT primer supplied with the kit according to the instructions of the manufacturer. The resulting cDNA mixture was diluted to a final volume of 100 μl and 3 μl were amplified with forward and reverse primers specific for the glyceraldehyde-3-phosphate dehydrogenase (G3PDH) housekeeping gene to check the efficiency of reverse transcription. Forward (F) and reverse (R) primers specific for IL-2, IL-4, IL-5, IL-6, IL-8, IL-10, IFN-γ, TGF-β1 and GM-CSF genes were: IL-2 F ACTCACCAGGATGCTCACAT, IL-2 R AGGTAATCCATCTGTTCAGA; IL-4 F CCGAGTTGACCGTAACAGAC, IL-4 R TTCCTGTCGAGCCGTTTCAG; IL-5 F GAAATTCCCACAAGTGCATTG, IL-5 R CTATTATCCACTCGGTGTTCA; IL-6 F ATGTAGCCGCCCCACACAGA, IL-6 R CATCCATCTTTTTCAGCCAT [28]; IL-8 F CAGCCTTCCTGATTTCTGCA, IL-8 R CCCTCTGCACCCAGTTTTCC; IL-10 F ATGCCCCAAGCTGAGAACCAAGACCCA, IL-10 R TCTCAAGGGGCTGGGTCAGCTATCCCA; IFN-γ F ATGCAGGTCATTCAGATGTAG, IFN-γ R GTCAGTTACCGAATAATTAGTC; TGF-β1 F GCCCTGGACACCAACTATTGC, TGF-β1 R GCAGGAGCGCACGATCATGT; GM-CSF F ATCTCTGCACCCGCCCGCTCG, GM-CSF R CCCTGCTTGTACAGCTCCAGG. Three microlitres of the cDNA mixture for each sample were amplified using 25 pmoles of each cytokine-specific primer and 2 U of *Taq* polymerase (Boehringer, Mannheim, Germany) by 35 cycles of denaturation of 94°C for 1 min, annealing and extension at 55°C (IL-2, IL-6), 62°C (IFN-γ) and 70°C (IL-10, GM-CSF) for 2 min; denaturation of 94°C for 1 min, annealing at 60°C (IL-4, IL-5, IL-8) for 1 min or 65°C (TGF-β1) and extension at 72°C for 1 min in a total volume of 50 ml. PCR products were analysed by electrophoresis on 2% agarose gel and visualized by ethidium bromide staining.

RESULTS

Establishment and characterization of T cell clones from IL-2-expanded SF PBMC

PBMC were isolated from the SF of the five children with oligoarticular JA, stained with MoAbs and analysed by flow cytometry. Lymphocytes comprised predominantly $CD3^+$, TCR α/β^+ cells, with a CD4/CD8 ratio ranging from 0·79 to 1·6. TCR γ/δ^+ cells and natural killer (NK) ($CD16^+$) cells were also detected (ranges 3–11% and 6–18%, respectively) (not shown and [4]).

Double staining experiments showed that approximately one third of the T lymphocytes from all SF samples expressed the CD69 and/or HLA-DR activation markers, whereas CD25 was detected on a minor T cell fraction only (Fig. 1). These markers were found on both $CD4^+$ and $CD8^+$ lymphocytes (not shown and [29]).

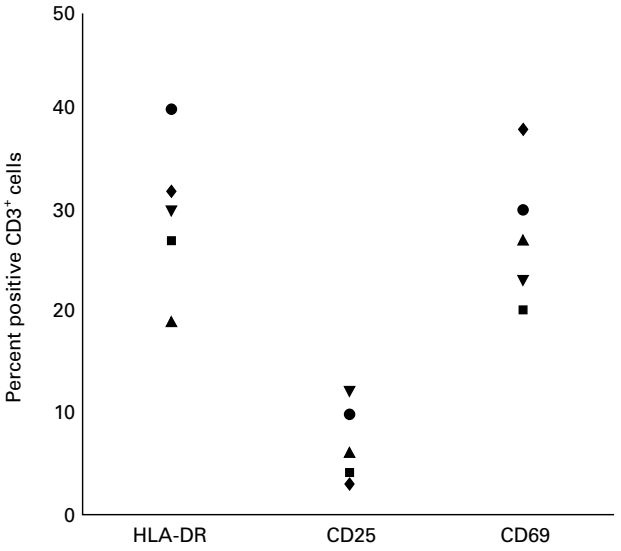

Fig. 1. Expression of activation markers (HLA-DR, CD25 and CD69) on freshly isolated synovial fluid (SF) T cells from five patients with oligoarticular juvenile arthritis. ♦, Patient A; ▲, patient B; ●, patient C; ■, patient D; ▼, patient E.

Immunophenotypic studies carried out with paired peripheral blood samples from the five patients showed no increase in the proportion of $CD69^+$, $CD25^+$ or HLA-DR^+ T cells compared with normal, age-matched controls.

In subsequent experiments, SF PBMC were cultured for 7 days with low-dose rIL-2, enriched for blasts by a Percoll density gradient and cloned by limiting dilution. This procedure was selected with the aim of expanding and cloning *in vitro* those lymphocytes that were already activated *in vivo*. Sixty-four clones were obtained from the five SF samples; 44 T cell clones were TCR α/β^+, $CD4^+$, 15 were TCR α/β^+, $CD8^+$ and the remaining five were TCR γ/δ^+, $CD4^-$, $CD8^-$.

Cytokine production by T cell clones derived from in vivo *activated SF T cells*

In order to investigate cytokine secretion by SF PBMC-derived T cell clones, cells from 44/64 clones (29 $CD4^+$, 10 $CD8^+$ and five TCR γ/δ^+, $CD4^-$, $CD8^-$, Table 2) were stimulated with PHA and PMA for 24–48 h and supernatants tested for the presence of IFN-γ, IL-2, IL-4 and IL-5 using immuno- or bioassays. These cytokines were selected for study since their production helps to delineate the Th1, Th2 or Th0 profiles of cytokine secretion [11–17].

As shown in Table 2, most of the clones produced high amounts of IFN-γ irrespective of the immunophenotype. In contrast, only six clones, all of which were $CD4^+$, released IL-5 (Table 2). IL-2 was detected in variable amounts in the supernatants of 19 clones; 13 of them were $CD4^+$, four $CD8^+$ and two TCR γ/δ^+ (Table 2). IL-4 was released by 23 T cell clones; 19 of them were $CD4^+$, three $CD8^+$ and one TCR γ/δ^+ (Table 2).

Taken together, these results suggest that the major feature of SF-derived T cell clones was IFN-γ production, whereas IL-2 and IL-4 were released predominantly by subsets of $CD4^+$ clones and IL-5 was detected only occasionally.

Table 2. Cytokine production by synovial fluid (SF)-derived T cell clones after phorbol myristate acetate (PMA)-phytohaemagglutinin (PHA) stimulation

Clones	Phenotype	IFN-γ (pg/ml)	IL-2 (U/ml)	IL-4 (pg/ml)	IL-5 (pg/ml)	Pattern
Patient A						
1	CD8	3670	2	80	0	Th1
2	CD8	3560	0	0	0	Th1
3	CD8	700	0	0	0	Th1
4	CD4	1110	0	100	0	Th1
5	CD4	700	0	0	0	Th1
6	CD4	5200	0	1150	0	Th0
Patient B						
7	CD4	1600	12	70	100	Th1
8	CD4	3000	0	0	ND	Th1
9	CD4	4600	ND	2100	ND	Th0
10	CD4	3400	0	160	120	Th1
11	CD8	5200	2	2600	ND	Th0
12	CD4	3800	0	1600	0	Th0
13	CD4	4300	0	1450	80	Th0
14	CD4	3400	2	2300	0	Th0
15	CD4	3800	0	100	0	Th1
16	CD4	850	50	1480	600	Th0
17	γδ	500	2	0	ND	Th1
18	CD4	3700	6	700	0	Th0
Patient C						
19	CD8	0	0	0	0	–
20	CD4	4100	0	1850	0	Th0
21	CD4	200	0	50	0	Th0
22	CD8	3100	2	70	0	Th1
23	CD4	5100	0	2150	0	Th0
24	CD4	2600	50	1650	200	Th0
25	CD8	3260	4	0	0	Th1
26	CD4	4200	4	0	0	Th1
27	CD4	3500	100	0	0	Th1
Patient D						
28	CD4	3100	0	0	0	Th1
29	CD8	0	0	0	0	–
30	γδ	2500	0	0	0	Th1
31	γδ	4350	0	40	0	Th1
32	CD8	3800	0	0	0	Th1
33	CD4	5470	0	0	0	Th1
34	CD4	6800	2	2100	0	Th0
35	CD4	1500	10	0	0	Th1
36	γδ	3650	0	0	0	Th1
Patient E						
37	CD4	1100	200	0	0	Th1
38	CD4	650	0	0	0	Th1
39	CD4	620	12	0	0	Th1
40	CD8	2600	0	0	0	Th1
41	CD4	3600	0	1500	0	Th0
42	CD4	3600	10	270	0	Th1
43	γδ	3800	2	0	0	Th1
44	CD4	4500	200	1500	250	Th0

Nineteen of 44 clones were classified as Th1, since they produced IFN-γ, but not at all IL-4 (Table 2); of these, 10 were $CD4^+$, five were $CD8^+$ and four were TCR γ/δ^+, $CD4^-$, $CD8^-$.

Twenty-three of 44 clones released IFN-γ, IL-2, IL-4 and IL-5 in variable amounts; eight of such clones, five $CD4^+$, two $CD8^+$ and one TCR γ/δ^+ (clone nos 1, 4, 7, 10, 15, 22, 31 and 42 of Table 2) were operationally classified as Th1-type based upon an IFN-γ/IL-4 ratio >10. In Fig. 2, a recapitulative subdivision of Th1- and Th0-type clones according their immunophenotype is shown.

Altogether, 27 out of 44 clones displayed a Th1-type profile of cytokine production, whereas the remaining clones had a Th0-type pattern, as indicated by the IFN-γ/IL-4 ratio <10 (Table 2 and Fig. 2).

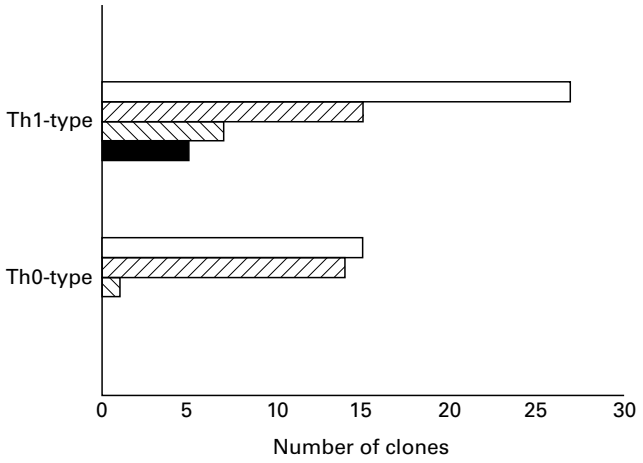

Fig. 2. Subdivision of the Th1- and Th0-type clones according to their immunophenotype. The $CD4^-$ $CD8^-$ clones were all TCR γ/δ^+, as shown by staining with the specific MoAb (see Results). □, Total; ▨, $CD4^+$; ▧, $CD8^+$; ■, $CD4^-$ $CD8^-$.

Two of 44 clones (clones 19 and 29 of Table 2) did not secrete any of the four cytokines tested.

In subsequent experiments, 10 representative T cell clones, six $CD4^+$ and four $CD8^+$, were tested by RT-PCR for the expression of the IFN-γ, IL-2, IL-4 and IL-5 genes (Table 3) in order to investigate the relationships between the accumulation of cytokine mRNA and the presence of the corresponding proteins in T cell clone supernatants. These clones included the two which did not secrete appreciable amounts of any cytokine (see above, clones 19 and 29 of Table 2).

As shown in Table 3, 10/10 clones expressed IFN-γ mRNA, 8/10 displayed IL-2 transcripts, 7/10 expressed the IL-4 gene and only 2/10 were positive for IL-5 mRNA. When these results were compared with those of the assays with clone supernatants, a good correspondence between mRNA and protein production was found for each cytokine (Table 3). The only exceptions were: two clones that expressed IFN-γ mRNA but did not release the corresponding protein; three clones that expressed IL-2 mRNA but did not secrete the protein; one clone that was IL-4 $mRNA^+$ but did not produce the protein (Table 3). It is of note that 8/10 clones tested were negative for the production of IL-5 mRNA and protein (Table 3).

Finally, the two clones which did not produce detectable cytokines in culture supernatants (see above) were found to express the IFN-γ, but not the IL-4 or IL-5 genes; the IL-2 gene was expressed in one clone only (Table 3, clone 29).

Expression of pro- and anti-inflammatory cytokine genes

Rheumatoid synovitis is characterized by the local release of numerous cytokines from different cell types, such as, for example, macrophages and synoviocytes. Therefore it was of interest to investigate whether cloned T cells could express the genes encoding a panel of proinflammatory and anti-inflammatory cytokines. To this end, 10 representative T cell clones (seven $CD4^+$, two $CD8^+$, one TCR γ/δ^+, $CD4^-$, $CD8^-$), eight of which had already been tested for IFN-γ, IL-2, IL-4 and IL-5 gene expression (see above), were stimulated with PHA-PMA and subjected to RT-PCR to investigate the expression of the following genes: IL-6, IL-8, IL-10, TNF-α, GM-CSF and TGF-β1.

As shown in Fig. 3, all of the clones tested expressed TNF-α, TGF-β1 and IL-10 mRNA, 6/10 clones (nos 16, 23, 24, 30, 32 and 34) expressed GM-CSF mRNA, 5/10 clones (21, 24, 30, 32, 34) expressed IL-6 mRNA, 5/10 clones (21, 22, 23, 24, 32) displayed IL-8 transcripts (Fig. 3). Although assays to ascertain the presence of the corresponding proteins in culture supernatants were not carried out, these results suggest that *in vivo* activated T cells from SF of pauciarticular JA patients may influence joint inflammation in a complex manner.

DISCUSSION

There is general consensus that T lymphocytes act as initiators of joint inflammation in RA [30,31] by migrating to the affected joints, recognizing peptides deriving from processed antigens and releasing a host of cytokines [32,33]. Such cytokines, in turn, may influence the function of other cell types, such as macrophages, dendritic cells, synoviocytes and neutrophils. Candidate antigens capable of activating synovial T lymphocytes from JA patients are heat shock proteins from Gram-negative bacteria and viral peptides [6–8,34].

In this study, a fraction of SF T cells from patients with oligoarticular JA were found to express activation markers [2–5]. Therefore, such cells were preferentially expanded in the presence of low-dose rIL-2 and cloned. This procedure was not applied to

Table 3. Correlation between IFN-γ, IL-2, IL-4 and IL-5 gene expression and release of the corresponding protein by synovial fluid (SF)-derived T cell clones

	IFN-γ		IL-2		IL-4		IL-5	
Clone	Protein	mRNA	Protein	mRNA	Protein	mRNA	Protein	mRNA
16	+	+	+	+	+	+	+	+
19	−	+	−	−	−	−	−	−
21	+	+	−	−	+	+	−	−
22	+	+	+	+	+	+	−	−
23	+	+	−	+	+	+	−	−
24	+	+	+	+	+	+	+	+
27	+	+	+	+	−	−	−	−
29	−	+	−	+	−	−	−	−
32	+	+	−	+	−	+	−	−
34	+	+	+	+	+	+	−	−

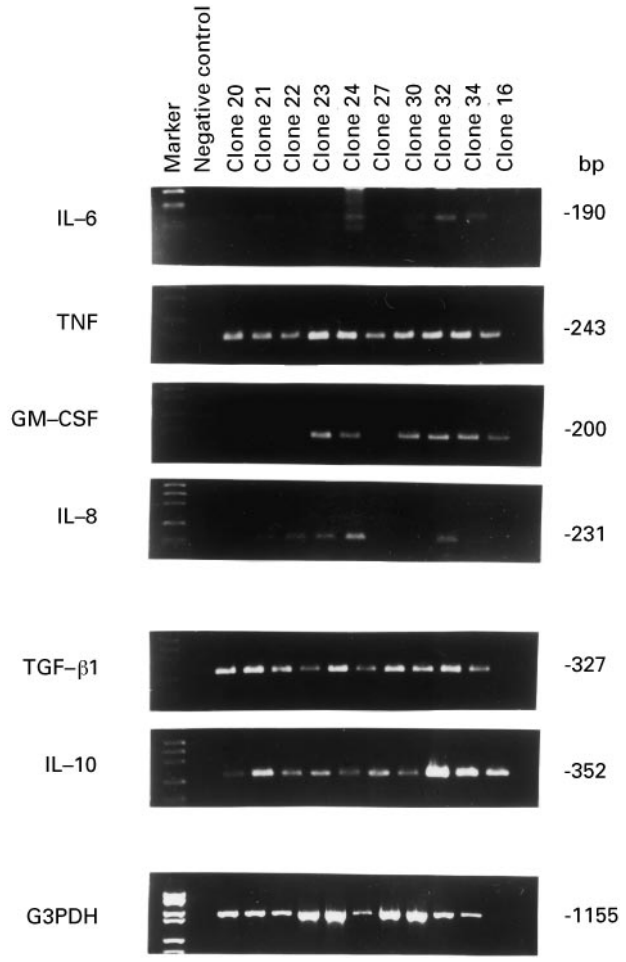

Fig. 3. Cytokine gene expression in a panel of synovial fluid of (SF)-derived T cell clones. The immunophenotypes of the clones investigated are shown in Table 2. RNA was extracted, reverse transcribed and amplified with pairs of cytokine-specific primers. The expected size of the amplification products is shown on the right.

paired PBMC samples, due to the low number of cells that expressed activation markers. However, the possibility that circulating T lymphocytes contained some IL-2 selectable T cells related to SF T cells cannot be excluded.

The strategy of raising T cell clones after the bulk expansion of *in vitro* activated lymphocytes has advantages as well as limitations. The main advantage is the opportunity of characterizing T cell clones which are predominantly the progeny of cells already activated *in vivo*. A major drawback is that, also under the best culture conditions, the cloning efficiency is limited, and therefore the clones obtained are not fully representative of all the cells present *in vivo*. Furthermore, the possibility that some 'sister' clones originating from *in vivo* clonally expanded T cell populations were raised in this study cannot be ruled out.

Most of the clones raised here were TCR α/β^+, $CD4^+$, but a proportion of them were either TCR α/β^+, $CD8^+$ or TCR γ/δ^+ $CD4^-$ $CD8^-$. As for the $CD4^+$ T cell clones, it can be assumed that they were indeed derived from *in vivo* activated T lymphocytes, since resting $CD4^+$ cells never express the CD122 component of the IL-2 receptor in the absence of CD25 [35]. Therefore, the latter cells cannot mount a proliferative response upon exposure to rIL-2 alone.

In contrast, circulating TCR γ/δ^+ cells [36,37] and a minor subset of TCR α/β^+, $CD8^+$ cells which contains lymphokine-activated killer (LAK) cell precursors [38] express constitutively CD122 in the absence of CD25. This feature allows both cell types to proliferate following *in vitro* treatment with high-dose rIL-2. Thus, although low concentrations of rIL-2 were used in this study, the possibility that some of our TCR γ/δ^+ and TCR α/β^+, $CD8^+$ T cell clones originated from IL-2-responsive LAK precursors cannot be ruled out.

The most striking feature of all the clones obtained from the SF of the different patients was the ability to release huge amounts of IFN-γ irrespective of the immunophenotype. When the IFN-γ/IL-4 ratio from culture supernatants was employed to assign T cell clones to the Th1-type, Th2-type or Th0-type categories, it became apparent that more than half of the clones displayed a Th1-type profile, since they released little or no IL-4 [11–13]. The latter conclusion was strengthened by the observation that most of the clones did not produce IL-5 at all [14]. RT-PCR analysis of selected clones demonstrated that the failure to detect IL-5 in culture supernatants was attributable to the lack of transcription of the corresponding gene.

The Th1-type profile was detected in the majority of TCR α/β^+, $CD8^+$ and TCR γ/δ^+, $CD4^-$, $CD8^-$ clones, but only in approximately a half of TCR α/β^+, $CD4^+$ clones. The remaining clones were all classified as Th0-type. The finding that TCR α/β $CD8^+$ or TCR γ/δ $CD4^-$, $CD8^-$ T cell clones displayed patterns of cytokine secretion previously ascribed to $CD4^+$ cells only is not surprising in the light of previous results [14,16,39,40].

Studies carried out in humans [41–44] and in experimental animals [45,56] suggest that lymphocytes infiltrating inflamed tissues comprise a mixture of antigen-specific and antigen-non-specific activated T cells. It is conceivable that a similar scenario applies also to our study. Whatever the mechanism(s) responsible for *in vivo* T cell activation in oligoarticular JA, it may be hypothesized that special stimuli, such as for example IL-12 [47] or the recently cloned IFN-γ-inducing factor (IGIF) [48], trigger IFN-γ overproduction by activated T and NK cells within the inflamed joints [49].

The availability of large amounts of IFN-γ might influence the joint microenvironment in different ways. For example, IFN-γ can activate tissue macrophages to release a wide spectrum of cytokines and inflammatory molecules [50]. Furthermore, IFN-γ up-regulates the surface expression of HLA-class I and II antigens, thus enhancing the antigen-presenting cell (APC) capabilities of various cell types [51]. Finally, IFN-γ may favour the differentiation of Th1-type cells and, in the meanwhile, inhibit the generation of Th-2-type cells [52–54].

An alternative hypothesis to explain the enrichment for IFN-γ-producing T cell clones in our study is that they were selected by the system for expansion and cloning of *in vivo* activated T cells. This hypothesis is, however, unlikely, since recent studies have disclosed a Th1-type pattern both in the synovial membrane [20] and in T cell clones derived from the SF [19] or membrane [18–21] of RA patients.

In principle, a final possibility is that IFN-γ overproduction by SF T cells resulted from a generalized abnormality of the patient immune system. This hypothesis, which is made unlikely by the failure to detect circulating activated T cells in our patients, is not supported by the results of a study carried out recently in adult

RA patients, where IFN-γ overproduction was observed selectively in synovial T lymphocytes compared with mitogen-activated peripheral blood T cell clones.

In order to gain more insight into the potential pathogenic role played *in vivo* by activated T cells, the expression of pro- and anti-inflammatory cytokines in a selected panel of T lymphocyte was investigated. These experiments demonstrated that most of the clones tested expressed the transcripts of inflammatory cytokines such as GM-CSF, TNF-α, IL-6 or IL-8. This finding supports the hypothesis that *in vivo* activated SF T cells, from which the clones were derived, may be involved in the pathogenesis of tissue damage. Conversely, the same clones were found to express the genes of IL-10 and TGF-β1, two cytokines which display potent immunosuppressive and anti-inflammatory properties.

In conclusion, these results provide the first demonstration that SF T cell clones from patients with oligoarticular JA display predominantly Th1/Th0 patterns of cytokine secretion. Further studies are warranted to better define the actual role played by *in vivo* activated T cells in the pathogenesis of synovial tissue damage.

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