

Research article

T-cell activation without proliferation in juvenile idiopathic arthritis

Antony PB Black¹, Hansha Bhayani^{2†}, Clive AJ Ryder³, Janet MM Gardner-Medwin³ and Taunton R Southwood⁴

¹MRC Human Immunology Unit, Institute of Molecular Medicine, John Radcliffe Hospital, Headington, Oxford, UK

²Formerly of Department of Rheumatology, University of Birmingham

³Department of Paediatric Rheumatology, Birmingham Children's Hospital-NHS Trust, Birmingham, UK

⁴Department of Rheumatology, University of Birmingham, Edgbaston, Birmingham, UK

[†]Deceased.

Correspondence: Professor Taunton R Southwood, Department of Rheumatology, University of Birmingham, St Vincent's Drive, Edgbaston, Birmingham, B15 2TT, UK. Tel: +44 (0)121 414 6778; fax: +44 (0)121 414 6794; e-mail: t.r.southwood@bham.ac.uk

Received: 21 February 2001

Revisions requested: 4 April 2001

Revisions received: 7 August 2001

Accepted: 31 August 2001

Published: 21 November 2001

Arthritis Res 2002, **4**:177-183

© 2002 Black *et al.*, licensee BioMed Central Ltd
(Print ISSN 1465-9905; Online ISSN 1465-9913)

Abstract

A study was done to determine if the differentiation and activation phenotype of T cells in synovial fluid (SF) from patients with juvenile idiopathic arthritis (JIA) is associated with T-cell proliferation *in situ*. Mononuclear cells were isolated from 44 paired samples of peripheral blood and SF. Differentiation and activation markers were determined on CD4 and CD8 T cells by flow cytometry. Cell-cycle analysis was performed by propidium iodide staining, and surface-marker expression was also assessed after culture of the T cells under conditions similar to those found in the synovial compartment. The majority of the T cells in the SF were CD45RO⁺CD45RB^{dull}. There was greater expression of the activation markers CD69, HLA-DR, CD25 and CD71 on T cells from SF than on those from peripheral blood. Actively dividing cells accounted for less than 1% of the total T-cell population in SF. The presence or absence of IL-16 in T-cell cultures with SF or in a hypoxic environment did not affect the expression of markers of T-cell activation. T cells from the SF of patients with JIA were highly differentiated and expressed early and late markers of activation with little evidence of *in situ* proliferation. This observation refines and extends previous reports of the SF T-cell phenotype in JIA and may have important implications for our understanding of chronic inflammation.

Keywords: activation, chronic inflammation, differentiation, T cells

Introduction

The role of the T cell in initiating or perpetuating chronic inflammation is unknown. Juvenile idiopathic arthritis (JIA) encompasses a heterogeneous group of chronic inflammatory diseases, characterised by persistent arthritis beginning before the age of 16 years. It is classified into subgroups according to associated clinical features [1]. In several subgroups of JIA, there is indirect evidence that infectious

agents play a role in either initiation or perpetuation of the inflammatory process [2–4], but the role of one or more intra-articular antigens in contributing to the observed expansion of synovial T-cell numbers is unclear [5–7].

Previous research has shown that synovial T cells in JIA appear to have an activated memory phenotype [8–12], but only one study has addressed the role of *in situ* prolif-

Table 1

Clinical features of the patients with juvenile idiopathic arthritis (n = 31)

Subclass of arthritis	No. of patients	F:M	Mean age of onset (range) (years)	Mean duration of disease (range) (years)	B27+ (no.)	ANA+ (no.)
Oligoarthritis	8	5:3	4.5 (1–13.8)	4.1 (0.3–8)	1	4
Extended oligoarthritis	2	1:1	6.8 (4.5–9)	3.6 (1.2–6)	0	0
Systemic arthritis	2	0:2	6 (2.5–9.6)	6.7 (0.4–13)	0	0
Psoriatic arthritis	8	4:4	7.6 (1.1–10.8)	5.6 (0.5–14.2)	2	6
Enthesitis-related arthritis	4	1:3	11.3 (7.7–12.7)	1.7 (0.1–4)	3	1
Polyarthritis RF ⁻	5	2:3	6.8 (2–13)	1.9 (0.4–4.5)	1	3
Polyarthritis RF ⁺	1	1:0	13.9	1	0	0
Other	1	0:1	12.9	0.2	0	0

ANA = antinuclear antibodies; F = female; M = male; RF = rheumatoid factor.

eration in contributing to this phenotype [13]. We hypothesized that a proportion of T cells from the synovial fluid (SF) of patients with JIA were activated *in situ* by antigen-driven cell cycling. Such T cells would be expected to demonstrate a memory phenotype (CD45RO⁺) with markers of recent activation and impending or recently completed division, in particular CD69, CD71 and CD25. We also proposed that the differences in disease pathogenesis between JIA subgroups would be reflected in different patterns of T-cell phenotype and activation status.

Patients and methods

Patients

Forty-four paired samples of SF and peripheral blood (PB) were obtained during therapeutic arthrocentesis (for intra-articular corticosteroid administration) from 31 patients who fulfilled criteria for the diagnosis of JIA (Table 1). Samples were collected from 12 patients on two separate occasions and from one patient on three occasions. Ethical approval for the study was obtained from the local research ethics committee and all patients and parents (as appropriate) gave their informed consent for sampling of SF and PB.

Isolation of mononuclear cells

Mononuclear cells (MCs) were isolated from SF and PB by the standard method of ficoll (Amersham Pharmacia Biotech, Sweden) density centrifugation [9].

Flow cytometry

Three-colour staining of MCs was carried out using a standard protocol [9]. The antibodies used were CD4-BIO, CD8-BIO, CD25-FITC, CD69-FITC and HLA-DR-FITC (Becton Dickinson, Oxford, UK); CD71-FITC, CD45RB-FITC, CD45RO-FITC and CD3-PE (Dako Ltd, Bucks, UK). Immunofluorescence was detected using a Beckman-Coulter flow cytometer (Bucks, UK). Dead cells were

excluded by gating on forward/side scatter and T cells were selected by gating CD3⁺ cells. Marker expression was recorded as either percentage of positive cells (% positive), if there was little overlap between the marked cell population and the control, or as median fluorescence intensity (MFI) if there was significant overlap between the two populations. Because of variation in the numbers of cells isolated from SF and PB, it was not possible to stain each sample using the full range of monoclonal antibodies (as noted in figures and tables).

Cell-cycle analysis

At least 5×10^6 MCs were analysed from PB and SF of five patients (two with psoriatic arthritis, two with polyarthritis and one with oligoarthritis). Cells were fixed in 70% ethanol for 24 hours. After washing, the cells were stained with propidium iodide for 30 min at 4°C. Staining was again detected on a Beckman-Coulter flow cytometer and data were analysed on WinMDI version 2.5 in accordance with 'Cell-cycle analysis: a pragmatic approach' (hoy@cf.ac.uk). Cells were gated to exclude dead cells, doublets and triplicates.

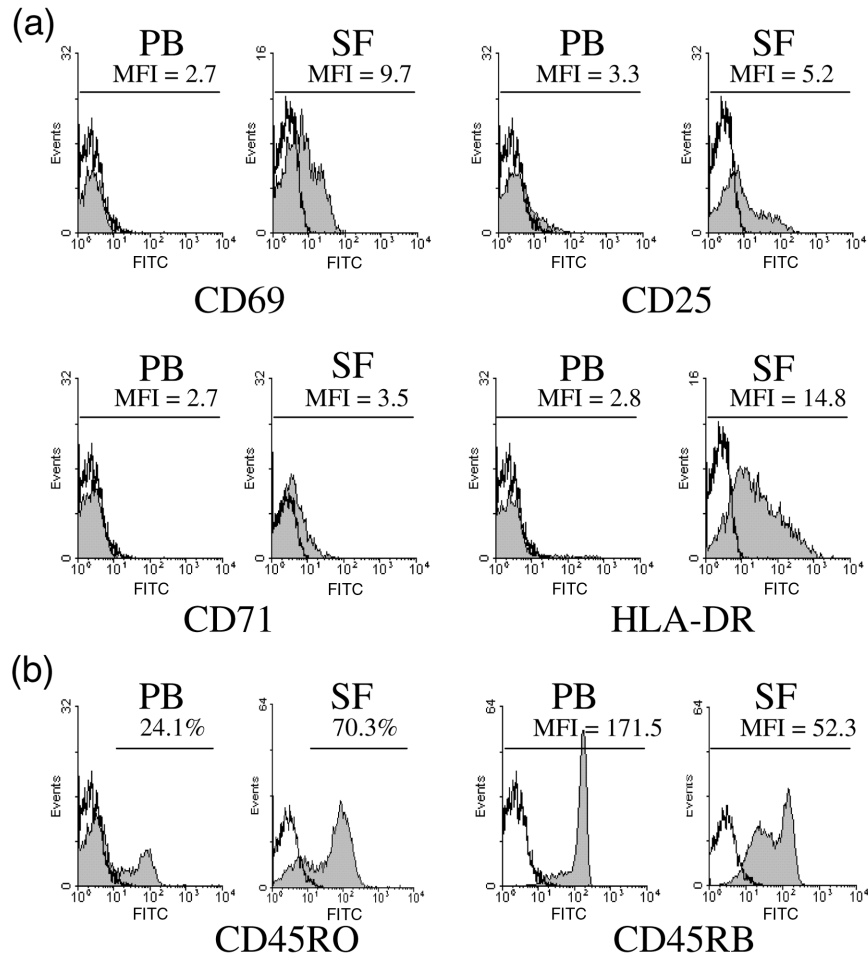
Culture conditions

We attempted to upregulate expression of activation markers on T cells from PB by exposing them to a microenvironment similar to that found in the synovial compartment. T cells were cultured overnight in a 1:2 dilution of autologous, preservative-free and fetal-calf-serum-free SF with or without hypoxic conditions (95% nitrogen 5% CO₂) for 24 hours, and with or without 200 ng/ml IL-16 (Serotec, Oxford, UK) for 48 hours.

Statistical analysis

Statistical analysis was performed using GraphPad prism software. Mann-Whitney tests and Wilcoxon *t* tests were performed where appropriate.

Figure 1



Expression of activation and differentiation markers on T cells from peripheral blood (PB) and synovial fluid (SF) of a patient with juvenile idiopathic arthritis. **(a)** Expression of activation markers. **(b)** Expression of differentiation markers. Each histogram is a representative example of marker expression on PB or the paired SF sample. Marker expression was recorded as either percentage positive cells (CD45RO only), or as median fluorescent intensity (all other markers). The clear peak on each histogram represents the staining from an isotype-matched, negative control. FITC = fluorescein isothiocyanate; MFI = median fluorescence intensity.

Results

T-cell activation state

A greater proportion of T cells from SF expressed activation markers than of T cells from PB (Figs 1a and 2a). The greatest differences were seen for CD69 (MFI for T cells from SF and PB 13.2 and 2.7, respectively; $P = 0.0002$) and HLA-DR (MFI for SF and PB cells 19.8 and 2.9, respectively; $P < 0.0001$). CD25 and CD71 were also significantly increased in the SF T-cell population (respective SF:PB MFI 4.1:3.3 and 3.7:2.7), but the differences between SF and PB were not as dramatic as for CD69 or HLA-DR. CD8 T cells expressed significantly higher levels of CD69 and HLA-DR than did CD4 T cells, whereas expression of CD25 and CD71 was higher on CD4 than CD8 T cells (Table 2).

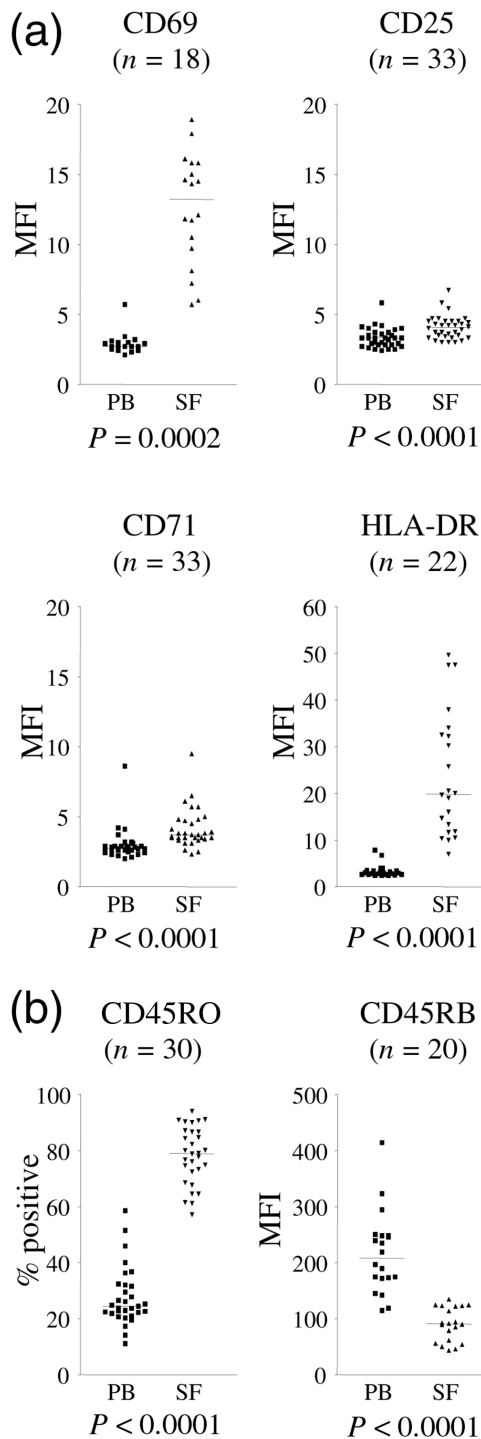
Cell-cycle status

The majority of the SF MCs were in phase G_0/G_1 , with fewer than 1% in S phase (Fig. 3). There were no detectable differences in the percentage of dividing SF cells in comparison with cells from the PB compartment or between different disease subgroups (data not shown).

Lack of influence on T-cell phenotype of two microenvironmental factors, hypoxia and IL-16

In the absence of T-cell proliferation, we hypothesized that factors within the synovial microenvironment, such as hypoxia, which may induce the expression of the CD71 gene [14,15], or IL-16, which is thought to upregulate CD25 expression [16], would influence the state of the T cells. The expression of activation markers on T cells

Figure 2



Phenotype of peripheral blood (PB) and synovial fluid (SF) T cells from patients with juvenile idiopathic arthritis. **(a)** Expression of activation markers. **(b)** Expression of differentiation markers. Bars represent median expression on paired PB and SF samples. Because of variation in cell numbers isolated from each sample, not all samples could be stained for every antibody available. Each datum point represents an individual sample (n = number of samples studied). MFI = median fluorescence intensity.

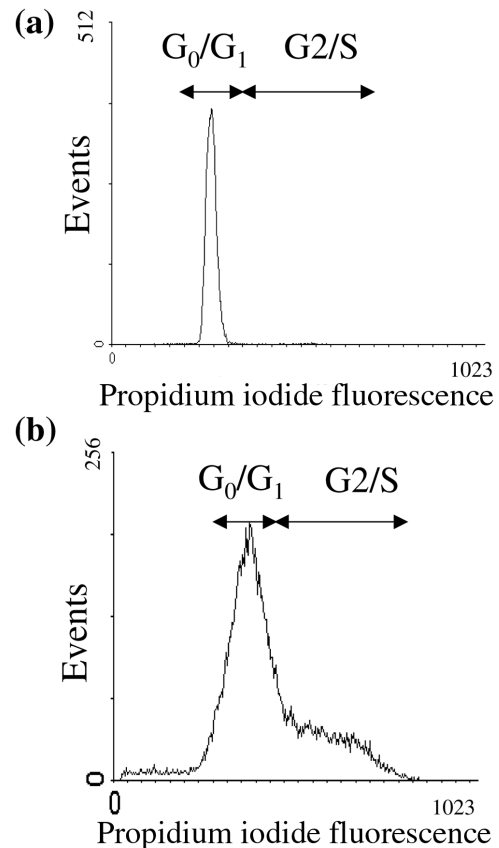
Table 2

Expression of activation and differentiation markers on T cells (CD3⁺) from peripheral blood (PB) and synovial fluid (SF) of patients with juvenile idiopathic arthritis

Marker	CD4		CD8	
	PB	SF	PB	SF
CD69	2.8	9.1***	3.5	14.6***
CD25	3.7	6.0***	2.9	3.3*
CD71	2.8	5.0***	3.1	3.9**
HLA-DR	2.7	19.3***	3.5	26.7***
CD45RO	27.1	97.1***	23.6	73.9***
CD45RB	179.4	49.1***	273.8	142.0***

Values are median fluorescence intensity of the markers (except for CD45RO, which is expressed as % positive) on CD4⁺ or CD8⁺ T cells. * $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$, versus PB.

Figure 3



Cell-cycle analysis of mononuclear cells from synovial fluid. **(a)** Representative example from a patient with oligoarthritis. **(b)** Positive control: peripheral blood mononuclear cells stimulated with phytohaemagglutinin for 3 days.

Table 3**Synovial fluid T-cell expression of differentiation and activation markers in disease subgroups of patients with juvenile idiopathic arthritis**

SF T cells	Oligo	Poly	JPsA	B27 ⁻	B27 ⁺	ANA ⁻	ANA ⁺	APR hi	APR lo	Drugs ⁺	Drugs ⁻
CD69	10.1	15.8	13.4	14.3	11.8	13.1	15.8	14.4	8.9	14.6	14.3
CD25	3.9	3.9	4.4	4.2	3.6	4.2	3.9	4.0	4.2	4.2	4.0
CD71	3.5	3.4	4.5	3.7	3.5	3.7	3.6	3.8	3.2*	3.7	3.7
HLA-DR	13.1	33.5	30.2	20.5	11.8	18.9	47.4	16.0	19.6	19.2	30.2
CD45RO	74.6	78.8	81.1	77.8	80.1	76.4	78.9	78.9	77.8	77.3	79.0
CD45RB	78.4	88.8	92.2	89.4	106.7	88.3	89.8	85.1	89.8	92.2	69.9

All values represented as median fluorescence intensity (MFI) except CD45RO, which is represented as median % positive. Patients were grouped according to disease subgroup (oligoarthritis, polyarthritis or psoriatic arthritis), and expression or absence of HLA-B27, ANA, a high or low acute-phase response (high: erythrocyte sedimentation rate >20 or C-reactive protein >5), and whether they had received intra-articular triamcinolone hexacetonide earlier in their disease process (drugs⁺ versus drugs⁻). There was a statistically significant difference between those groups of patients with a high or low APR (* $P < 0.05$). ANA = antinuclear antibodies; APR = acute-phase response; hi = high; lo = low; JPsA = juvenile psoriatic arthritis; Oligo = oligoarthritis; Poly = polyarthritis.

from PB was unaffected by culture in the presence of autologous, cell-free SF under normal or hypoxic conditions. Culture of PB T cells for 48 hours in the presence of IL-16 did not influence activation marker expression (data not shown).

T-cell differentiation status

The SF T-cell population consisted mainly of highly differentiated memory cells: 78.9% expressed CD45RO, compared with 24.4% in the PB ($P < 0.0001$). This was reflected in the reduced expression of CD45RB on SF T cells (SF MFI 90.6, PB MFI 207.5; $P < 0.0001$) (Figs 1b and 2b).

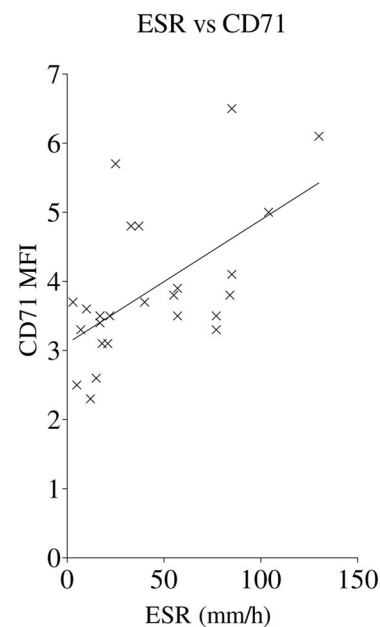
CD8⁺ T-cell numbers were higher (SF 45.3%, PB 29.4%, $P < 0.0001$) and CD4⁺ T cells lower (SF 39.9%, PB 58.3%, $P < 0.0001$) in SF than in PB. Interestingly, expression of CD45RO was significantly higher on SF CD4 T cells than on SF CD8 T cells (97.1% and 73.9%, respectively). Similarly, expression of CD45RB was lower on SF CD4 than on CD8 T cells (MFI 49.1 and 142, respectively) (Table 2).

Clinical parameters

No consistent variation of T-cell activation or differentiation status was observed for most of the clinical parameters (Table 3). There was, however, an association between CD71 expression on SF T cells and the vigour of the acute-phase response as measured by the erythrocyte sedimentation rate ($P = 0.0005$; Fig. 4).

Discussion

In the chronic inflammatory process of JIA, it is unclear whether the observed accumulation of synovial T cells reflects local activation by one or more intra-articular antigens or reflects non-antigen-specific migration of memory

Figure 4

Relation between erythrocyte sedimentation rate and expression of CD71 on T cells from synovial fluid from patients with juvenile idiopathic arthritis. Points represent individual synovial fluid samples. Data show line of linear regression ($P = 0.0005$). ESR = erythrocyte sedimentation rate; MFI = median fluorescence intensity.

T cells into the joint. To address this issue, we comprehensively studied the numbers of differentiated, activated and cycling cells within the SF compartment in JIA.

In the JIA population studied, expression of all the activation markers on T cells was higher in cells from SF than

than in cells from PB. Although this observation has been reported previously using CD25 and HLA-DR expression [9–13], the use of the very early activation marker (CD69) and the transferrin receptor (CD71) in our study has added an extra dimension to the understanding of the process of JIA. Antigen-induced T-cell activation *in vitro* is known to result in the expression of activation markers in an orderly sequence: CD69, CD25, CD71 and HLA-DR [17,18]. In our study, the upregulation of CD69 and HLA-DR appeared greater than that of CD25 or CD71, suggesting that these changes were not the result of antigen-specific activation. A similar T-cell activation pattern has been reported in adults with rheumatoid arthritis [19,20]. Expression of CD69 may be the result of cell–cell contact after transendothelial migration into the joint [20,21], and expression of HLA-DR does not necessarily imply recent activation, as it may persist on memory T cells [22].

To further elucidate whether the expression of activation markers was induced by T-cell proliferation, we measured propidium iodide staining of SF T cells. The majority of the SF MCs were in phase G₀/G₁, and fewer than 1% were in S phase. MC proliferation was no greater in SF than in PB, confirming the observations Bergroth *et al.* made by a manual count of ³H-thymidine-labelled MCs in SF [13]. This finding suggests that SF T cells in JIA express activation markers as a result of a proliferation-independent process. It is possible, however, that actively proliferating cells were lost during the cell-separation process. It is also possible that small numbers of SF T cells were actively dividing and driving a local inflammatory process, as antigen-specific T cells may be present at frequencies of fewer than 1/1000 in inflammatory arthropathies [23]. Differences in the frequency of dividing cells between patient subgroups may emerge if greater numbers of patients are investigated.

Alterations in the microenvironment have been associated with changes in expression of activation markers in the absence of proliferation [16]. The SF compartment is relatively hypoxic and this may alter T-cell behaviour *in vivo* [24]. Hypoxia has been shown to induce the expression of the CD71 gene [14,15], and IL-16 has been shown to increase T-cell expression of CD25 [16]. In the JIA population studied, however, no detectable change in the expression of any activation markers on T cells was observed after culture in hypoxic conditions, or in the presence of IL-16. Additionally, overnight culture of SF or PB T cells with autologous SF did not induce upregulation of the activation markers. It is likely that many other factors within the joint, including the presence of synoviocytes, influence the activation state of synovial T cells.

Research observations of relevance to the aetiology and pathogenesis of JIA may be masked by the heterogeneity

of the diseases under the umbrella term of JIA [25]. There was little evidence that different subgroups of JIA in this study were characterised by different patterns of marker expression, although the relatively small number of patients and samples may have obscured subtle changes. Further study of biochemical indices of cell activation in larger numbers of JIA patients may help to elucidate differences in disease pathogenesis in JIA patient populations with distinct clinical phenotypes.

This study has revealed a number of other interesting findings of potential relevance for T-cell recruitment to the synovial space. The majority of SF T cells were CD45RO⁺CD45RB^{dull}, a finding also made in adults with rheumatoid arthritis [26,27]. Such highly differentiated T cells are likely to be recruited to the arthritic joint because of an intrinsic increase in ability to bind to, and migrate across, inflamed endothelium [12,28]. When the T-cell subsets were examined, 74% of SF CD8 T cells expressed CD45RO, versus 97% of SF CD4 T cells. The observation that 26% of SF CD8 T cells did not express CD45RO raises the intriguing possibility that there is a large intra-articular effector population of CD8 T cells [29] or that a proportion of the memory CD8 T cells have reverted to the expression of CD45RA [30]. The influence of the general inflammatory milieu on T-cell recruitment or retention in the synovial space has not been extensively reported. In this study, an association between CD71 and erythrocyte sedimentation rate was observed. This finding could indicate that factors governing the acute-phase response, such as proinflammatory cytokines, influence the activation state, and possibly the migrating capacity, of the T cells in SF.

Conclusion

The T cells within the SF of patients with JIA have a phenotype that would support selective recruitment from the blood. Highly differentiated T cells, which have increased capacity to migrate across inflamed endothelium, may be selectively recruited to the joint, a process which, of itself, may alter the expression of activation markers on the cells in the absence of cell proliferation. This is the first time that such a T-cell phenotype has been demonstrated in patients with JIA.

Acknowledgements

We thank Professor M Salmon and Dr D Pilling for helpful advice during this research. We also thank Professor P Bacon, Dr C Buckley and Dr L Wedderburn for their critical reading of the manuscript. This work was funded partly by the Arthritis Research Council and supported by the Henry Smith's Charity.

References

1. Petty RE, Southwood TR, Baum J, Bhetty E, Glass DN, Manners P, Maldonado-Cocco J, Suarez-Almazor M, Orozco-Alcala J, Prieur A-M: **Revision of the proposed classification criteria for juvenile idiopathic arthritis: Durban, 1997.** *J Rheumatol* 1998, **25**: 1991-1994.
2. Pugh MT, Southwood TR, Gaston JSH: **The role of infection in juvenile chronic arthritis.** *Br J Rheumatol* 1993, **32**:838-844.

3. Mielants H, Veys EM, Cuvelier C, De Vos M, Goemaere S, Maertens M, Joos R: **Gut inflammation in children with late onset pauciarticular juvenile chronic arthritis and evolution to adult spondyloarthritis - a prospective study.** *J Rheumatol* 1993, **20**:1567-1572.
4. Life P, Hasell A, Williams K, Young S, Bacon P, Southwood T, Gaston JSH: **Responses to gram negative enteric bacterial antigens. Synovial T cells from patients with juvenile chronic arthritis: recognition of Heat Shock Protein HSP 60.** *J Rheumatol* 1993, **20**:1388-1396.
5. Zhang H, Phang D, Laxer RM, Sliverman ED, Pan S, Doherty PJ: **Evolution of the T cells receptor β repertoire from synovial fluid T cells of patients with juvenile onset rheumatoid arthritis.** *J Rheumatol* 1997, **24**:1396-1402.
6. Thompson SD, Murray KJ, Grom AA, Passo MH, Choi E, Glass DN: **Comparative sequence analysis of the human T cell receptor β chain in juvenile rheumatoid arthritis and juvenile spondyloarthropathies.** *Arthritis Rheum* 1998, **41**:482-497.
7. Scotet E, Peyrat M-A, Saulquin X, Retiere C, Couedel C, Davodeau F, Dulphy N, Toubert A, Bignon J-D, Lim A, Vie H, Hallet M-M, Liblaur R, Weber M, Berthelot J-M, Houssaint E, Bonneville M: **Frequent enrichment for CD8 T cells reactive against common herpes viruses in chronic inflammatory lesions; towards a reassessment of the physiopathological significance of T cell clonal expansions found in autoimmune inflammatory processes.** *Eur J Immunol* 1999, **29**:973-985.
8. Murray KJ, Luyrink L, Grom AA, Passo MH, Emery H, Witte D, Glass DN: **Immunohistological characteristics of T cell infiltrates in different forms of childhood onset chronic arthritis.** *J Rheumatol* 1996, **23**:2116-2124.
9. Silverman ED, Isacovics B, Petsche D, Laxer RM: **Synovial fluid cells in juvenile arthritis: evidence of selective T cell migration to inflamed tissue.** *Clin Exp Immunol* 1993, **91**:90-95.
10. Forre O, Dobloug JH, Natvig JB: **Augmented numbers of HLA-DR-positive T lymphocytes in the synovial fluid and synovial tissue of patients with rheumatoid arthritis and juvenile rheumatoid arthritis.** *Scand J Immunol* 1982, **15**:227-231.
11. Thoen J, Forre O, Waalen K, Kass E: **Phenotypes of T lymphocytes from peripheral blood and synovial fluid of patients with rheumatoid arthritis and juvenile rheumatoid arthritis.** *Scand J Rheumatol* 1987, **16**:247-256.
12. Wedderburn LR, Robinson N, Patel A, Varsani H, Woo P: **Selective recruitment of polarized T cells expressing CCR5 and CXCR3 to the inflamed joints of children with juvenile idiopathic arthritis.** *Arthritis Rheum* 2000, **43**:765-774.
13. Bergroth V, Kontinen YT, Pelkonen P, Haapala M, Haapasaari J, Nordstrom D, Kunnamo I, Friman C: **Synovial fluid lymphocytes in different subtypes of juvenile rheumatoid arthritis.** *Arthritis Rheum* 1988, **31**:780-783.
14. Tacchini L, Bianchi L, Bernelli-Zazzera A, Cairo G: **Transferrin receptor induction by hypoxia.** *J Biol Chem* 1999, **274**:24142-24146.
15. Nam Lok C, Ponka P: **Identification of a hypoxia response element in the transferrin receptor gene.** *J Biol Chem* 1999, **274**:2417-24152.
16. Cruikshank WW, Center DM, Nisar N, Wu M, Natke B, Theodore AC, Kornfield H: **Molecular and functional analysis of a lymphocyte chemoattractant factor: association of biological function with CD4 expression.** *Proc Natl Acad Sci U S A* 1994, **91**:5109-5113.
17. Biselli R, Matricardi PM, D'Amelio R, Rattorossi A: **Multiparametric flow cytometric analysis of the kinetics of surface molecule expression after polyclonal activation of human peripheral blood T lymphocytes.** *Scand J Immunol* 1992, **35**:439-447.
18. Caruso A, Licenziati S, Corulli M, Canaris AD, De Francesco MA, Fiorentini S, Peroni L, Fallacara F, Dima F, Balsari A, Turano A: **Flow cytometric analysis of activation markers on stimulated T cells and their correlation with cell proliferation.** *Cytometry* 1997, **27**:71-76.
19. Afeltra A, Galeazzi M, Ferri GM, Amoroso A, De Pita O, Porzio F, Bonomo L: **Expression of CD69 antigen on synovial fluid T cells in patients with rheumatoid arthritis and other chronic synovitis.** *Ann Rheum Dis* 1993, **52**:457-460.
20. Fernandez-Gutierrez B, Hernandez-Garcia C, Banares AA, Jover JA: **Characterisation and regulation of CD69 expression on rheumatoid arthritis synovial fluid T cells.** *J Rheumatol* 1995, **22**:413-420.
21. Iannone F, Corrigan VM, Kingsley GH, Panayi GS: **Evidence for the continuous recruitment and activation of T cells into the joints of patients with rheumatoid arthritis.** *Eur J Immunol* 1994, **24**:2706-2713.
22. Johannisson A, Festin R: **Phenotype transition of CD4+ T cells from CD45RA to CD45RO is accompanied by cell activation and proliferation.** *Cytometry* 1995, **19**:343-352.
23. Sieper J, Braun J, Wu P, Kingsley G: **T cells are responsible for the enhanced synovial cellular immune response to triggering antigen in reactive arthritis.** *Clin Exp Immunol* 1993, **91**:96-102.
24. Bodamyali T, Stevens CR, Billingham MEJ, Ohta S, Blake DR: **Influence of hypoxia on inflammatory synovitis.** *Ann Rheum Dis* 1998, **57**:703-710.
25. Petty RE, Southwood TR: **Classification of childhood arthritis: divide and conquer.** *J Rheumatol* 1998, **25**:1869-1870.
26. Matthews N, Emery P, Pilling D, Akbar A, Salmon M: **Subpopulations of primed T helper cells in rheumatoid arthritis.** *Arthritis Rheum* 1993, **36**:603-607.
27. Salmon M, Pilling D, Borthwick NJ, Viner N, Janossy G, Bacon PA, Akbar AN: **The progressive differentiation of primed T cells is associated with an increasing susceptibility to apoptosis.** *Eur J Immunol* 1994, **24**:892-899.
28. Borthwick NJ, Akbar AN, Maccormac LP, Lowdell M, Craigen JL, Hassan I, Grundy JE: **Selective migration of highly differentiated primed T cells, defined by low expression of CD45RB, across human umbilical vein endothelial cells: effects of viral infection on transmigration.** *Immunology* 1997, **90**:272-280.
29. Sallusto F, Lenig D, Forster R, Lipp M, Lanzavecchia A: **Two subsets of memory T lymphocytes with distinct homing potentials and effector functions.** *Nature* 1999, **401**:708-712.
30. Wills MR, Carmichael AJ, Weekes MP, Mynard K, Okecha G, Hicks R, Patrick Sissons JG: **Human virus-specific CD8+ CTL clones revert from CD45RO^{high} to CD45RA^{high} *In Vivo*: CD45RA^{high} CD8+ T cells comprise both naive and memory cells.** *J Immunol* 1999, **162**:7080-7087.