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A high proportion of the Vδ1+ synovial fluid γδ 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)

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SUMMARY

We have previously shown that γδ T cells in the synovial compartment of patients with juvenile rheumatoid arthritis (JRA) express activation antigens (CD69 and HLA-DR) and that they are predominantly of the Vδ1 subset. In this study we have analysed the expression of activation antigens (CD69 and HLA-DR) and different isoforms of the leucocyte common antigen (CD45RO and CD45RA) on the Vδ1 and the Vδ2 subsets of γδ T cells in paired samples of synovial fluid and peripheral blood of nine patients with JRA, and in the peripheral blood of five children with idiopathic scoliosis. In the synovial fluid of children with JRA, there were significantly more Vδ1+CD69+ and Vδ2+CD69+ cells compared with the peripheral blood of the same patients. In contrast, however, in the synovial fluid the Vδ1 and the Vδ2 subsets differed with respect to the expression of the two isoforms of the leucocyte common antigen. The majority of the Vδ1+ cells expressed the high molecular weight isoform (CD45RA+) while most of the Vδ2+ cells carried the low molecular weight variant (CD45RO+) of this molecule.

Keywords juvenile rheumatoid arthritis γδ T cells activation antigens CD69 leucocyte common antigen CD45RA CD45RO

INTRODUCTION

Juvenile rheumatoid arthritis (JRA) is a chronic inflammatory disease which mainly involves the peripheral joints. Although the cause of JRA and rheumatoid arthritis (RA) is unknown, the pathogenesis is assumed to be of an autoimmune nature. One of the major findings supporting this view is the extensive infiltration of the inflamed synovial membrane by T cells which exhibit the characteristics of activated cells [1]. Most of these cells express the αβ T cell receptor whereas a small proportion (5–15%) express the γδ T cell receptor. It is established that the αβ T cells recognize antigen in context with MHC class I or class II molecules, but for the γδ T cells little is known concerning expression of activation markers, activation requirements, antigenic repertoire and restriction elements used. γδ T cells have been studied in a number of pathological conditions such as RA and JRA [2–7], leprosy [8], polymyositis [9], coeliac disease [10–11], cancer [12–14], primary immunodeficiency syndrome [15,16], Behçet's disease [17], and infections with Epstein–Barr virus [18] and HIV-1 [19]. Promiscuous cytotoxicity exerted by γδ T cells has been documented *in vitro* [20,21] and may play a role in polymyositis [8], however, for the other diseases the pathological role for these cells remains a mystery.

In the peripheral blood (PB) of normal individuals the dominant V genes used by γδ T cells are Vδ2 followed by Vδ1 [22]. We have previously shown that the majority of synovial γδ T cells in JRA patients express the Vδ1Jδ1/2 gene products, whereas only a minor proportion uses the Vδ2 gene segment [2]. Similar results have also been reported in RA [3–7]. In JRA patients we also detected HLA-DR and the very early activation antigen CD69 [23] on synovial γδ T cells. The selective increase in Vδ1Jδ1/2+ γδ T cells in the inflamed joint and the expression of activation antigens on these cells suggest a pathogenic role for this cell type. In this study we have used dual colour immunofluorescence to assess the expression of the activation antigens CD69 and HLA-DR, and two different isoforms of the leucocyte common antigen (CD45RA and CD45RO) on αβ T cells and the two major subsets of γδ T cells (Vδ1 and Vδ2) in the synovial fluid (SF) and PB of patients with JRA.

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PATIENTS AND METHODS

Patients and controls

Paired samples of SF and PB were analysed from nine patients with JRA. According to the criteria defined by Brewer *et al.* [24],

eight patients were classified as having the pauciarticular type of the disease and one patient had the polyarticular form. For each patient the age, sex, disease duration, medication, and the presence of rheumatoid factor (Waaler test) and anti-nuclear antibodies is shown in Table 1.

Blood was also drawn from five children who were due to undergo elective surgery due to idiopathic scoliosis. These children were otherwise healthy and did not take any medicine. The age and sex of these children are shown in Table 1.

Preparation of mononuclear cells

Synovial fluid was pretreated with 20 U/ml Penetrase (Leo, Copenhagen, Denmark) for 20 min at 37°C. The mononuclear cells (MNC) were isolated from the pretreated SF and from PB by gradient centrifugation on Lymphoprep (Nycomed, Oslo, Norway). Finally the cells were washed three times with Hank's balanced salt solution (GIBCO, Glasgow, UK) and stained immediately.

Phenotypic analysis

We used FITC-conjugated MoAbs specific for CD3 (UCHT1), αβ TcR (WT31), and Vδ1Jδ1/2 (δTCS1) [25,26]. PE-conjugated MoAbs against the following antigens were used: CD45RO (UCHL1), CD45RA (2H4), CD69 (Leu-23) and HLA-DR. Dr L. Moretta kindly supplied the MoAb BB3 [27] as ascites fluid, which has been shown to be specific for Vδ2 [28]. WT31, Leu-23 and HLA-DR were purchased from Becton Dickinson (Mountain View, CA); δTCS1 from T Cell Diagnostics (Cambridge, MA); 2H4 from Coulter Immunology (Hialeah, FL); and UCHT1 and UCHL1 from Dakopatts (Copenhagen, Denmark). The MoAb TIB96 specific for the Ig-5b allotype of IgD (American Type Culture Collection, Rockville, MD) served as an irrelevant control for BB3, and FITC- and PE-conjugated MoAbs with an irrelevant specificity were used as isotype-matched control antibodies for the other MoAbs. To develop BB3 we used a FITC anti-mouse IgG $F(ab')_2$ fragment (Sigma, St Louis, MO). The stainings were carried out as previously described [2].

Table 1. Characteristics of patients and controls

	Sex	Age (years)	Disease duration (years)	Diagnosis	ANA/RF	Medicine
Patients						
1	M	14·1	4·0	Pauci	+/+	N+C
2	F	13·8	7·0	Pauci	−/−	N+M
3	F	13·5	11·0	Pauci	−/−	N+M
4	F	7·3	4·7	Pauci	−/−	N+M
5	F	4·3	4·2	Pauci	−/−	N
6	M	13·1	3·8	Pauci	−/−	N+C
7	M	12·8	5·3	Poly	−/−	N+C+M
8	F	13·5	3·0	Pauci	−/−	N
9	F	14·7	1·0	Pauci	−/−	N
Controls						
1	F	14·1	—	IS		—
2	M	13·5	—	IS		—
3	F	14·0	—	IS		—
4	F	12·4	—	IS		—
5	F	11·8	—	IS		—

Pauci, Pauciarticular form of juvenile rheumatoid arthritis; poly, polyarticular form of juvenile rheumatoid arthritis; IS, idiopathic scoliosis; N, non-steroidal anti-inflammatory drugs; C, hydroxy chlorocine; M, methotrexate.

Statistical analysis

Wilcoxon's signed rank sum test was used for comparing paired data and the Mann–Whitney *U*-test was used for unpaired data.

RESULTS

As previously reported [2], we found a shift in the predominant usage of the Vδ2 gene product by the PB γδ T cells to the Vδ1Jδ1/2 gene product by the γδ T cells in the SF. Thus there was a significant difference in the ratio of δTCS1⁺/BB3⁺ between PB and SF in the JRA patients ($P < 0.007$).

All the patients except for patient 7 had a high number of Vδ1⁺ cells (δTCS1⁺) in SF which expressed the CD45RA isoform (2H4⁺) (Table 2). The CD45RO isoform (UCHL1⁺) could only be detected on the Vδ1⁺ cells in four patients and only as a small percentage in two of these patients. The opposite was the case for the Vδ2 subset (BB3⁺) in which the CD45RO⁺ cells dominated in SF of the JRA patients. The expression of CD45RA and CD45RO differed significantly between those two subsets of γδ T cells ($P < 0.005$ and $P < 0.02$ respectively).

Only three patients had detectable levels of Vδ1⁺ cells in PB, and most of these cells were CD45RA⁺ and CD45RO⁻. Concerning the expression of the two isoforms of the leucocyte common antigen on Vδ2⁺ cells in PB, the picture varied from patient to patient. In accordance with a previous study [29] we found that the proportion of αβ T cells which were CD45RO⁺ and CD45RA⁻ were much higher in SF compared with PB.

In the SF there were significantly more Vδ1⁺CD69⁺ and Vδ2⁺CD69⁺ cells in SF as compared with PB ($P < 0.008$ and $P < 0.02$ respectively). HLA-DR could only be detected on the two γδ T cell subsets in SF in approximately half of the patients and in those cases the percentages were generally lower than for CD69. Neither CD69 nor HLA-DR could be detected on γδ T cells of normal controls. These antigens, however, were expressed in high percentages on SF αβ T cells as previously shown [2].

DISCUSSION

In this study we have confirmed our previous findings; first, that there is a shift in the V-gene usage of γδ T cells from Vδ2 in PB to Vδ1 in SF, and second, that a high proportion of the γδ T cells express activation antigens such as CD69 and HLA-DR. Dual-colour immunofluorescence was used to study the distribution of activation antigens and two of the isoforms of the leucocyte common antigen on the two major subsets of γδ T cells.

No difference was observed with regard to expression of activation antigens on the two γδ T cell subsets in SF. In contrast, in SF the majority of the Vδ1⁺ cells expressed the high molecular weight isoform of the leucocyte common antigen (CD45RA) and the Vδ2⁺ cells the low molecular weight isoform of this molecule (CD45RO). Our results are in contrast to the results reported by Bucht *et al.* [6] who found that the SF Vδ1⁺

Table 2. Expression of activation antigens and different isoforms of the leucocyte common antigen on the $\gamma\delta$ T cell subpopulations and on the $\alpha\beta$ T cells in children with juvenile rheumatoid arthritis (JRA) and in controls

	Per cent CD3$^+$ cells expressing		Per cent Vδ1Jδ1/2$^+$ cells expressing				Per cent Vδ2$^+$ cells expressing				Per cent TcR$\alpha\beta^+$ cells expressing			
	Vδ1Jδ1/2	Vδ2	CD45RO	CD45RA	CD69	HLA-DR	CD45RO	CD45RA	CD69	HLA-DR	CD45RO	CD45RA	CD69	HLA-DR
Patients														
1 PB	—	1·3	—	—	—	—	—	—	—	—	17	67	6	6
SF	3·6	2·0	—	75	64	—	86	—	54	26	69	31	57	62
2 PB	—	2·2	—	—	—	—	36	77	—	—	17	59	6	2
SF	13·3	2·4	—	67	52	28	64	—	63	—	76	26	62	58
3 PB	—	1·5	—	—	—	—	47	54	—	—	15	82	13	2
SF	2·3	2·6	—	78	36	—	82	23	25	—	83	15	34	74
4 PB	2·2	1·4	—	75	55	25	61	49	—	—	27	61	8	10
SF	9·5	1·8	9	85	63	6	82	37	73	37	88	19	40	64
5 PB	1·3	0·9	—	83	—	—	—	100	—	—	17	61	35	5
SF	9·9	1·6	26	81	60	18	49	64	77	45	87	22	74	84
6 PB	1·9	8·2	—	85	—	—	62	12	ND	ND	28	35	3	12
SF	5·0	5·0	—	55	45	—	49	ND	ND	67	74	7	58	38
7 PB	—	9·3	—	—	—	—	74	—	8	21	97	—	9	30
SF	1·4	3·5	71	—	32	44	86	—	82	—	94	—	69	84
8 PB	—	3·7	—	—	—	—	84	23	—	—	37	59	13	8
SF	2·4	2·1	40	56	73	—	100	—	49	—	90	10	66	65
9 PB	—	8·8	—	—	—	—	ND	ND	ND	ND	56	43	43	4
SF	3·1	3·7	—	69	63	57	ND	ND	ND	ND	89	5	50	92
Controls														
1 PB	3·8	2·0	65	30	—	—	52	50	—	—	26	74	4	4
2 PB	2·2	0·7	53	63	—	—	ND	ND	—	—	9	65	1	3
3 PB	2·8	1·4	56	45	—	—	—	50	—	—	21	69	—	3
4 PB	1·5	4·8	—	44	—	—	ND	45	—	—	ND	45	13	6
5 PB	3·1	3·7	23	94	—	—	35	65	—	—	14	72	6	5

PB, Peripheral blood; SF, synovial fluid; ND, not done; —, below detectable level (0·5% positive cells in total gated population).

cells in patients with RA expressed the low molecular weight isoform of the CD45 antigen. However, it is known that the proportion of CD45RO⁺ cells increase with age [30], and since the age of the patients studied by Bucht *et al.* were higher than in the present study this age difference could probably explain the discrepancy.

The leucocyte common antigen is present on all leucocytes and exists in different isoforms due to alternate splicing of mRNA. T cells expressing the high molecular weight isoform are assumed to represent virgin, unprimed T cells, while T cells expressing the low molecular weight variant are considered to be memory T cells [31–33].

Although the SF Vδ1⁺ cells expressed activation antigens we found that most of this subset expressed CD45RA; presumably representing virgin, unprimed γδ T cells. An obvious question is how virgin T cells can express activation antigens. There could be two explanations for these findings. First, the distinction between virgin and memory T cells is based on experiments with T cell populations with a predominance of αβ T cells, and although it has been suggested also to be valid for γδ T cells [34], the experimental evidence is still scarce for this T cell population. As long as the ligand for CD45 is unknown and the mechanism for the alternate mRNA splicing in relation to priming of the T cell has not been disclosed, it may be more appropriate just to refer to the CD45 isoforms as tyrosine phosphatases [35], and not as markers for memory or virgin T cells. Second, it is possible that γδ T cells do not need to be triggered via the γδ antigen receptor in order to express activation markers. We have recently discovered profound differences between γδ and αβ T cells with regard to their responses to rIL-2. After short term *in vitro* culture in rIL-2 a large proportion of the γδ but not the αβ T cells expressed activation antigens such as CD69 and HLA-DR (manuscript in preparation). These findings could indicate that the expression of activation antigens on γδ T cells in the rheumatoid inflammation and in other inflammatory conditions [6,18,36] is merely an epiphenomenon secondary to interleukins produced by activated αβ T cells.

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