Different circulating lymphocyte profiles in patients with different subtypes of juvenile idiopathic arthritis

C.H.P. Wouters[1], J.L. Ceuppens[2], E.A.M. Stevens

[1]Unit of Paediatric Rheumatology, Department of Paediatrics; [2]Division of Allergy and Immunology, Department of Internal Medicine, University Hospital Gasthuisberg, Leuven, Belgium.

Abstract

Objective

To determine the immunophenotypic profiles of circulating lymphocytes in patients with different disease types of Juvenile Idiopathic Arthritis (JIA).

Methods

Peripheral blood lymphocyte subsets from 19 patients with oligoarticular JIA (o-JIA), 10 patients with polyarticular JIA (p-JIA), 12 patients with systemic JIA (s-JIA) and from 41 age-matched healthy controls were characterized by two color immunofluorescence flow cytometry analysis.

Results

Patients with o-JIA and p-JIA had increased numbers of HLA-DR+ T cells and T cells co-expressing CD57 and CD16/56, indicating T cell activation and terminal differentiation of CD8+ T cells respectively. By contrast, in patients with s-JIA there was no increase in the activation or differentiation markers on T cells, but a profound decrease in circulating NK cells. All patients had hypergammaglobulinemia consistent with B cell hyperactivity, but increased numbers of CD5+ B cells were found only in o-JIA and p-JIA.

Conclusion

Distinct immunophenotypic lymphocyte profiles in patients with o-JIA and p-JIA compared to patients with s-JIA as demonstrated in this study, are consistent with a fundamental heterogeneity of the disease.

Key words

Juvenile idiopathic arthritis, lymphocytes, flow cytometry

Carine H.P. Wouters, MD, PhD; Jan L. Ceuppens, MD, PhD; Erik A.M. Stevens, MD, PhD.

Please address correspondence to: C. Wouters, MD, PhD, Department of Paediatrics, University Hospital Gasthuisberg, Herestraat 49, 3000 Leuven, Belgium. E-mail: Carine.Wouters@uz.kuleuven.ac.be

Received on May 31, 2001; accepted in revised form on December 21, 2001.

Introduction

Juvenile idiopathic arthritis (JIA) is a heterogeneous inflammatory disorder of unknown etiology that is currently divided into different subtypes based on the clinical manifestations at onset (1-3). The clinically heterogeneous nature of JIA is further defined by immunogenetic studies demonstrating different HLA associations in different disease types (4). More recently, an analysis of T cell receptor sequences on clonally expanded T cells from the joints of JIA patients led to the identification of disease-specific T cell repertoires that were relative to the disease subtype (5). Furthermore, analysis of cytokine expression in peripheral blood and synovial fluid from patients with JIA has shown differences between JIA subtypes (6-8).

The involvement of activated T cells in the pathogenesis of JIA is suggested by a predominance of CD4+ T cells infiltrating the synovial tissue and synovial fluid and showing an increased expression of both early (CD25) and late (HLA-DR, VLA-1) activation markers (9, 10). However, studies on the phenotype and function of peripheral blood T cells have shown some different results (11, 12). Investigations have been hampered by the heterogeneity of the disease itself and differences in the diagnostic criteria, disease activity and treatment of the patients studied and by the absence of age-matched healthy controls for flow-cytometric analysis of peripheral blood lymphocytes.

Hypergammaglobulinaemia in active disease and the presence of antinuclear antibodies (ANA) mainly in patients with oligoarticular and polyarticular onset JIA point to B cell hyperactivity, as was also evidenced by the observation of an increased number of IgG secreting lymphocytes in the peripheral blood of these patients (13). Most studies show no clear correlation of the clinical activity and duration of disease with a particular ANA immunofluorescence pattern or titer (11, 14) and at present it is unknown what – if any – role ANA play in the pathogenesis or perpetuation of chronic arthritis in JIA (4, 11, 14).

In the present study we have sought evidence for characteristic immunophenotypic profiles of peripheral blood lymphocytes in three major JIA subtypes compared with age-matched healthy children. In this context, we also measured humoral immune parameters including IgG and ANA, and analysed their relation to disease activity.

Materials and methods

Patients

Forty-one children (median age 5.6 years, range 1.9-16.8 years) fulfilling the ILAR criteria for the diagnosis of JIA (3) were recruited non-selectively from the Outpatient Clinic of Pediatric Rheumatology of the University Hospital. Twelve children suffered from systemic JIA (s-JIA), 10 had polyarticular JIA (p-JIA) and 29 had oligoarticular JIA (o-JIA). HLA B27 or rheumatoid-factor positive (by latex agglutination method) patients were excluded. Disease duration ranged from 0.26 to 10.3 (median 2.0) years in o-JIA, 0.24 to 10.3 (median 2.5) years in p-JIA and 0.24 to 10.8 (median 4.3) years in s-JIA. Two patients with o-JIA had a polyarticular disease course; s-JIA patients had either a systemic (6 patients), polyarticular (3 patients) or oligoarticular (1 patient) course; in 2 s-JIA patients with recent disease onset, the disease course could not be defined at the time of study.

Forty-one age-matched healthy children hospitalised for minor elective surgical procedures served as normal controls. There was no double usage of patients nor controls. A history of infection in the previous 4 weeks was an exclusion criterion for patients and controls.

Clinical disease activity was assessed using: (1) the physician's global assessment of overall disease activity (a categorical rating from 1 = no active disease to 5 = very severe disease); and (2) the number of joints with active arthritis on examination (15, 16). Active uveitis was present in 3 patients with o-JIA. Clinical characteristics and laboratory parameters including the erythrocyte sedimentation rate (ESR), C-reactive protein (CRP), hemoglobin (Hb) level and thrombocyte (Thr) count, that

were measured as part of the routine evaluation of the patients, are shown in Table I. A typical inflammatory profile comprising anemia, thrombocytosis, elevated ESR and CRP was most pronounced in patients with s-JIA.

At the time of the study, 36 patients were receiving non-steroidal anti-inflammatory drugs (NSAIDs); 4 patients also received methotrexate (single weekly dose) and 6 of them low-dose prednisone (range 0.02 - 0.36 mg/kg daily). None of the patients had undergone an intra-articular corticosteroid infiltration within six months prior to this study.

Table I. Clinical and laboratory characteristics of 41 patients with JIA.

	Oligoarticular JIA (n=19)		Polyarticular JIA (n=10)		Systemic JIA (n=12)	
Age	3.8	(1.9 - 14)	7.5	(2.2 - 16.7) **	8.2	(3.9 - 16.8) ***
Active joints (n)	1	(0 - 8)	4.5	(0 - 55) **	5	(0 - 57) **
Global score	2	(1 - 4)	2.5	(1 - 4)	3	(1 - 5) *
Hb (g/dl)	11.7	(9.7 - 12.8)	11.5	(8.3 - 12.9)	10	(7.4 - 13.4) ***,°
Thr (x 10^9/l)	334	(228 - 461)	341	(285 - 497)	416	(207 - 750) *
ESR (mm)	11	(5 - 67)	17	(6 - 62)	41	(10 - 133) ***,°°

Results are expressed as the median and range.
Differences were calculated using the Mann Whitney U test.
*,**,*** = p < 0.1, p < 0.05, p < 0.01 compared with oligoarticular patients.
°,°°,°°° = p < 0.1, p < 0.05, p < 0 .01 compared with polyarticular patients.

Laboratory measurements

ESR was determined by the Westergren method. Antinuclear antibodies (ANA) were determined by indirect immunofluorescence on Hep-2 cells. Serum immunoglobulin G (IgG) was determined by nephelometry. Sera were analyzed immediately after sampling.

Monoclonal antibodies

Whole blood samples were stained with phycoerythrin (PE)- or fluorescein isothiocyanate (FITC)-conjugated monoclonal antibodies (Moab) from Becton Dickinson, California, USA. The monoclonal antibody panel consisted of the following combinations of reagents: anti-CD3-FITC and anti-CD8-PE, anti-CD4-FITC and anti-TCR gamma-delta-PE, anti-CD5-FITC and anti-CD19-PE, anti-CD3-FITC and anti-HLA DR-PE, anti-CD4-FITC and anti-HLA DR-PE, anti-CD57-FITC and anti-CD3-PE, anti-CD3-FITC and anti-CD16/56-PE.

Moab were used in dilutions as prescribed by the manufacturer. The combinations of CD45-FITC and CD14-PE, CD64-FITC and CD33-PE and IgG1-FITC and IgG1-PE were used as gating reagents and as isotype controls respectively.

Flow cytometry

FACSprep (Becton Dickinson) was used for the sample preparation. A Becton Dickinson FACScan analyser was used for fluorescence measurements and acquisition of data on hard disk. Analyses were performed on 2000 cells. The lymphocyte population was defined by gating using forward and side light scatter in the FACScan software program (FACScan Research Software Version 2.1 3/89, Becton Dickinson); the back-gating technique based on CD14/CD45 staining (Leucogate, Becton Dickinson) was used to verify the selected cell population. Appropriate gating was confirmed by exclusion of contaminating cells using the CD64/CD33 plot. Contamination of the lymphocyte gate was < 5%. The full blood count was used to calculate the absolute values. The measurements were analysed using Attractor software (version 3.0, Becton Dickinson). We previously demonstrated that the Attractor software program based on cluster analysis for the definition of cell populations, offers an advantage for the definition of small and/or activated lymphocyte subpopulations when compared to conventional software programs based on quadrant analysis (17). Results were transferred to a Hewlett Packard 900 computer for statistical analysis.

We measured the relative numbers and absolute counts of 6 lymphocyte subsets considered to represent discrete lineages (CD3+T cells, CD4+T cells, CD8+ T cells, / TCR T cells, CD19+ B cells, CD16/56+NK cells), 4 subsets defined by particular differentiation markers (CD57+CD3+ T cells, CD3+ CD16/56+ T cells, CD5+CD19+B cells, CD57+NK cells) and 3 subsets defined by activation markers (HLA-DR+ CD3+T cells, HLA-DR+CD4+T cells, HLA-DR+CD8+T cells).

Peripheral blood CD57+ and CD16/56+ T cells are mostly confined to the CD8+ T cell subset (18, 19). CD57 expression is associated with terminally differentiated cytotoxic-suppressor T cells and was found to be largely reciprocal with CD28 expression (18,20, 21, 22); CD16/56 expression is associated with the capability of T cells to mediate NK-like activities (23).

Statistical analysis

Data were analysed using the Wilcoxon and the Mann Whitney U test for paired and unpaired samples respectively. Correlations were measured using Spearman Rank test.

Results

Lymphocyte subset distribution in the peripheral blood of patients with JIA and age-matched healthy children

The total number of circulating lymphocytes was normal in 19 patients with o-JIA (median 4059/mm^3 (1372-7860) versus 3230/mm^3 (1302-6571) for 19 age-matched controls, ns), in 10 patients with p-JIA (2642/mm^3 (1151-4040) versus 2317 (1146-4465) for 10 age-matched controls, ns), and in 9 patients with systemic JIA (2360/mm^3 (1185-3136) versus 2443/mm^3 (1575-5501) for 9 age-matched controls, ns).

We measured the absolute and relative numbers of 13 lymphocyte subsets (summarized in Table IIa and b) in the peripheral blood of 19 o-JIA, 10 p-JIA and 9 s-JIA patients and age-matched healthy children. Because corticosteroid treatment is known to affect the peripheral blood lymphocyte subset profile (24), three children with s-JIA

Table IIa. Percentages of 13 lymphocyte subsets in the peripheral blood of patients with oligoarticular and polyarticular JIA compared to age-matched healthy controls.

Lymphocyte subset	% of lymphocytes				% of lymphocytes				% of lymphocytes			
	Healthy children (n=19)		Oligoarticular JIA (n=19)		Healthy children (n=10)		Polyarticular JIA (n=10)		Healthy children (n=29)		Oligo- + Polyarticular JIA (n=29)	
CD3+ T	71.7	(56.6-83)	67.9	(58.9-88.2)	70.1	(61.3-83)	66.7	(51.8-81.9)	71.7	(56.6-83)	67.9	(51.8-88.2)
CD4+ T	43.3	(28.1-61)	38.4	(27.6-51.8)	40.6	(29-59)	44.4	(37.3-50)	43.2	(28.1-61)	40.7	(27.6-51.8)
CD8+ T	20.8	(12.5-33.1)	24.4	(8.4-44)	22.9	(19.4-28)	22	(8.4-30.8)	22.5	(12.5-33.1)	22.9	(8.4-44)
g/d TCR T	6.0	(3-12)	4.9	(1.4-9.5)*	6.8	(4-11.5)	2.8	(1.2-7.3)**	6.0	(3-12)	3.9	(1.2-9.5)***
CD57+CD3+ T	1.5	(0.5-5)	3.8	(0-16.5)***	2.5	(0-6.1)	5.5	(1-16.6)**	2.0	(0-6.1)	4.6	(0-16.6)***
CD16/56+CD3+ T	1.0	(0.2-2)	1.27	(0.4-11.4)*	1.0	(0-2.8)	1.1	(0.4-7.2)*	1.0	(0-2.8)	1.2	(0.4-11.4)**
HLA-DR+ CD3+ T°	5.1	(3.4-14.6)	7.6	(3.9-40.6)*	5.5	(3.4-6)	8.3	(4.2-19.6)*	5.5	(3.4-14.6)	7.9	(3.9-40.6)**
HLA-DR+ CD4+ T°	4.4	(1.1-17)	7.7	(3.5-65.7)***	5.9	(1.1-10.7)	9.9	(3.7-20.4)**	4.4	(1.1-17)	8.9	(.5-65.7)***
HLA-DR+ CD8+T°	5.7	(0.4-16.9)	10.9	(2.7-69.9)**	5.1	(3.1-8.7)	16.9	2.5-47.4)***	5.3	(0.4-16.9)	12.3	(2.5-69.9)***
CD19+ B	20	(12.3-33)	25.3	(8.3-35.2)	20.6	(13-26.3)	22.1	(8.2-36.7)	20.5	(12.3-33)	22.7	(8.2-36.7)
CD5+ B	5.2	(1.5-15)	8.9	(0.2-18.6)*	4.3	(2-12.5)	10.5	(0.8-21.1)*	5.0	(1.5-15)	9.1	(0.2-21.1)**
CD16/56+NK	6.7	(1-20.9)	5.4	(1.4-13.8)	8.7	(2-12.3)	6.6	(2.6-10.4)	7.8	(1-20.9)	6.2	(1.5-12.8)
CD57+ NK	1.5	(0-5)	1.2	(0.5-6.5)	2.1	(0-4.5)	1.8	(0.9-7.1)	1.5	(0-5)	1.7	(0.5-7.1)

Results are expressed as median and range. P values were determined by Wilcoxon test. ° Percentages of activated subsets are expressed as a percentage of the parent cell population.
*, **, *** = $p < 0.1$, $p < 0.05$, $p < 0.01$.

Table IIb. Absolute counts of 13 lymphocyte subsets in the peripheral blood of patients with oligoarticular and polyarticular JIA compared to age-matched healthy controls.

Lymphocyte subset	Lymphocytes (absolute counts)				Lymphocytes (absolute counts)				Lymphocytes (absolute counts)			
	Healthy children (n=19)		Oligoarticular JIA (n=19)		Healthy children (n=10)		Polyarticular JIA (n=10)		Healthy children (n=29)		Oligo- + Polyarticular JIA (n=29)	
CD3+ T	2332	(742-7299)	3084	(963-5338)	1637	(817-3080)	1921	(944-4027)	2018	(742-7299)	2480	(944-5338)
CD4+ T	1514	(579-4399)	1804	(502-2981)	1025	(437-1830)	1083	(570-3175)	1156	(437-4399)	1397	(502-3175)
CD8+ T	767	(162-2599)	990	(374-2220)	555	(281-937)	619	(192-1000)	646	(162-2599)	823	(192-2220)
g/d TCR T	212	(65-425)	171	(47-480)*	153	(87-262)	66	(24-208)**	204	(65-425)	150	(25-480)*
CD57+CD3+ T	54	(6-299)	160	(0-666)***	59	(0-267)	145	(36-653)*	55	(0-299)	150	(0-666)***
CD16/56+CD3+ T	31	(6-91)	53	(15-263)**	25	(0-65)	29	(11-180)	31	(0-91)	46	(11-263)***
HLA-DR+ CD3+ T°	127	(70-269)	229	(47-1095)	119	(63-138)	171	(119-545)	120	(63-269)	191	(47-1095)**
HLA-DR+ CD4+ T°	62	(6-243)	157	(33-1186)***	45	(21-107)	107	(38-315)**	59	(6-243)	120	(33-1186)***
HLA-DR+ CD8+T°	49	(6-199)	116	(12-1076)***	29	(14-44)	82	(12-474)**	40	(6-199)	86	(12-1076)***
CD19+ B	612	(240-2249)	941	(172-2106)	468	(202-937)	617	(104-2577)	547	(202-2249)	840	(104-2577)*
CD5+ B	186	(45-942)	439	(10-1246)*	109	(49-395)	311	(10-932)**	147	(45-942)	440	(10-1246)***
CD16/56+NK	240	(97-516)	190	(44-920)	222	(49-516)	170	(47-508)	227	(49-516)	177	(44-920)
CD57+ NK	47	(0-135)	45	(21-215)	49	(0-105)	45	(21-153)	47	(0-135)	45	(21-215)

Results are expressed as the median and range. P values were determined by the Wilcoxon test. ° Percentages of activated subsets are expressed as a percentage of the parent cell population.
*, **, *** = $p < 0.1$, $p < 0.05$, $p < 0.01$

Table IIc. Percentages and absolute counts of 13 lymphocyte subsets in the peripheral blood of patients with systemic JIA compared to age-matched healthy controls.

Lymphocyte subset	% of lymphocytes				Lymphocytes (absolute counts)			
	Healthy children (n=9)		Systemic JIA (n=9)		Healthy children (n=9)		Systemic JIA (n=9)	
CD3+ T cells	68.0	(52.2-79.6)	75.5	(68.8-83.3)*	1860	(1169-3133)	1719	(397-2480)
CD4+ T cells	33.8	(30.5-54.1)	47.7	(32.0-57.7)*	1020	(627-1821)	1005	(244-1604)
CD8+ T cells	24.5	(14.8-33.2)	26.8	(19.3-31.9)	727	(419-1083)	578	(123-986)
TCR g/ T cells	6.9	(4.4-12.5)	4.6	(2.5-10.9)	243	(83-446)	102	(37-251)**
CD57+CD3+ T cells	2.5	(1.2-15.1)	3.7	(0.4-18.3)	71	(35-396)	88	(12-499)
CD16/56+CD3+ T cells	1.1	(0.5-2.1)	1.25	(0.4-3.8)	26	(15-66)	31	(6-87)
HLA-DR+ CD3+ T cells°	7.4	(4.2-15.3)	10.2	(3.6-15.1)	131	(91-265)	147	(52-326)
HLA-DR+ CD4+ T cells°	7.2	(2.9-12.5)	8.5	(2.3-13.3)	61	(36-143)	49	(20-133)
HLA-DR+ CD8+ T cells°	9.6	(1.3-23.4)	11.5	(3.8-28.5)	58	(11-165)	63	(30-192)
CD19+ B cells	15.8	(9.5-24)	14.7	(5.3-20.5)*	379	(289-1320)	339	(33-642)*
CD5+ B cells	4.1	(2-11.5)	3.4	(1.7-8.2)	106	(46-632)	77	(8-199)
CD16/56+NK cells	12.6	(6-30)	5.0	(1.7-11.9)**	330	(156-1243)	97	(31-283)**
CD57+ NK cells	3.5	(0.5-10.5)	0.85	(0-2.8)**	132	(18-456)	14	(0-81)***

Results are expressed as the median and range. P values were determined by the Wilcoxon test. °Percentages of activated subsets are expressed as a percentage of the parent cell population. *, **, *** = $p < 0.1$, $p < 0.05$, $p < 0.01$

receiving supraphysiological dosages of prednisone (i.e. more than 0.04 mg/kg prednisone equivalent daily) at the time of study were excluded from the flow cytometry analysis.

Comparison of the measurements in o-JIA and p-JIA patients versus healthy children revealed an identical pattern of lymphocyte subset changes for both patient groups (Table IIa, b; furthermore, the absolute numbers and percentages of lymphocyte subsets were not different between 10 o-JIA and 10 age-matched p-JIA patients (not shown). Therefore, for further analyses the results of lymphocyte subset measurements from o-JIA and p-JIA patients were combined.

As shown in Table IIa-c, the absolute numbers and percentages of CD3+ T cells, CD4+ T cells and CD8+ T cells were not different between patients of the different JIA subtypes and healthy children.

Circulating T cells expressing T cell receptors (TCRs) composed of and chains, are almost uniformly CD4-CD8-; these T cells were found to provide help for autoantibody production and to be implicated in a number of autoimmune diseases in mice (25). The numbers of / TCR T cells were found to be decreased in all JIA patients.

T cell subsets coexpressing CD57 and/or CD16/56+ are considered to represent terminally differentiated suppressor-cytotoxic effector T cell populations (18, 20-22). Our analysis revealed that o-JIA and p-JIA patients had significantly higher absolute numbers and percentages of CD57+CD3+ T cells and CD16/56+CD3+ T cells. By contrast, the absolute and relative numbers of these subsets were not different between s-JIA patients and healthy children (Table IIa-c).

T cells expressing early (CD25) and late (VLA-1, HLA-DR) activation markers are present within the inflamed joints in most arthritic diseases in man, in experimental animal arthritis models and also in JIA patients (9, 10, 26). We found significantly increased numbers and percentages of circulating HLA-DR+CD4+ T cells and HLA-DR+CD8+ T cells in o-JIA and p-JIA patients indicating systemic activation of the T cell compartment in these subtypes (Table IIa-b). In contrast, the absolute and relative numbers of activated T cell subsets were not significantly different between s-JIA patients and age-matched healthy children (Table IIc).

As shown in Table IIa-c, analysis of the absolute numbers of CD19+ B cells showed a trend to an increase in o-JIA and p-JIA patients, but not in the s-JIA group. We also measured CD5+ B cells, which are a known source of natural autoantibodies (27) and found this subset to be selectively increased in o-JIA and p-JIA patients.

Patients with s-JIA had significantly decreased numbers and percentages of CD16/56+ NK cells and CD57+ NK cells (Table IIc). In contrast, levels of these subsets were similar in patients with o-JIA/p-JIA and healthy children. The numbers of NK cell subsets in s-JIA were inversely correlated to disease activity using the global score (for CD16/56+ NK cells: Rs = -0.61, p = 0.07; for CD57+ NK cells: Rs = -0.57, p= 0.1) and serum levels of CRP (for CD16/56+ NK cells: Rs = -0.75, p= 0.004; for CD57+ NK cells: Rs = -0.54, p = 0.08). No other correlations were found between clinical or laboratory parameters of disease activity and lymphocyte subsets in any JIA subtype nor in the total group of patients.

The number of patients with s-JIA was small. We therefore performed a comparative analysis of all lymphocyte subsets in 10 patients with p-JIA and 9 patients with s-JIA in the same age range [median 7.5 (range: 2.2-16.7) years for p-JIA versus 8.2 (3.9-16.8) years for s-JIA, ns]. The analysis revealed the same differences,be it trend-wise, as described above, i.e. an increase in the levels of T cell subsets expressing HLA-DR, CD57 and CD16/56 and of CD19+ B cells and CD19+ CD5+ B cells in patients with p-JIA

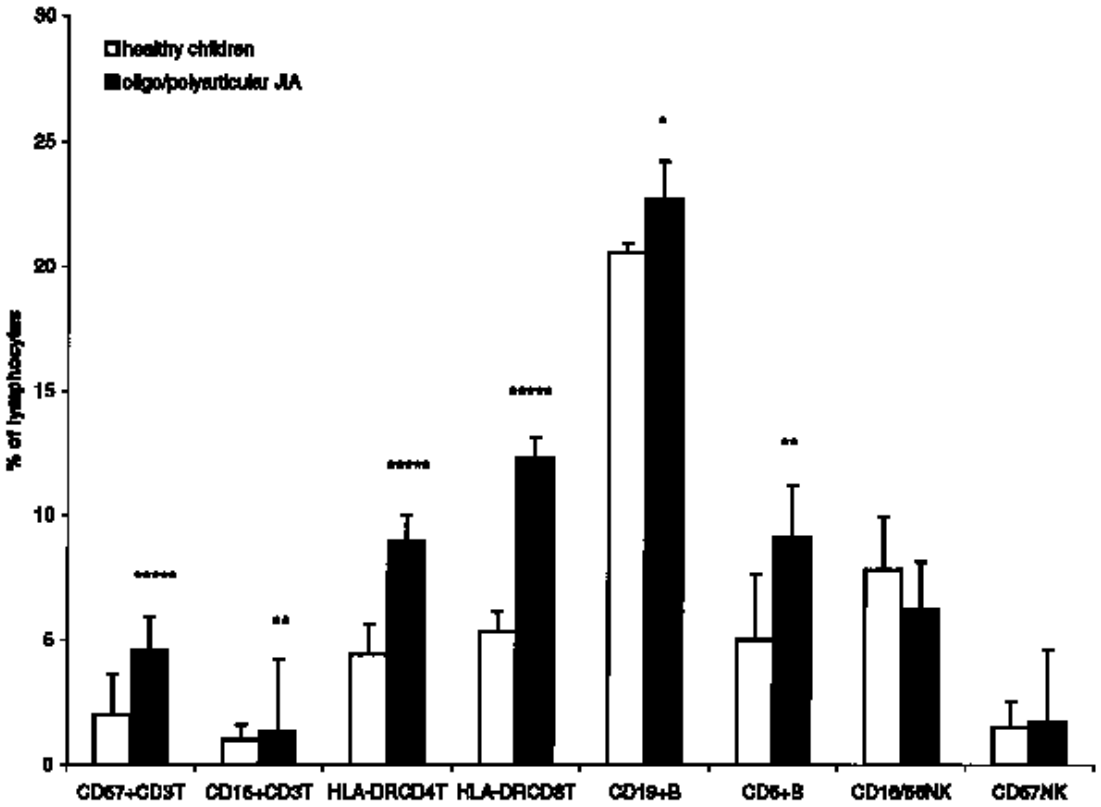

Fig. 1. Peripheral blood lymphocyte subsets (expressed as percentages of the total lymphocyte count) in 29 patients with oligoarticular or polyarticular JIA and 29 age-matched healthy controls.

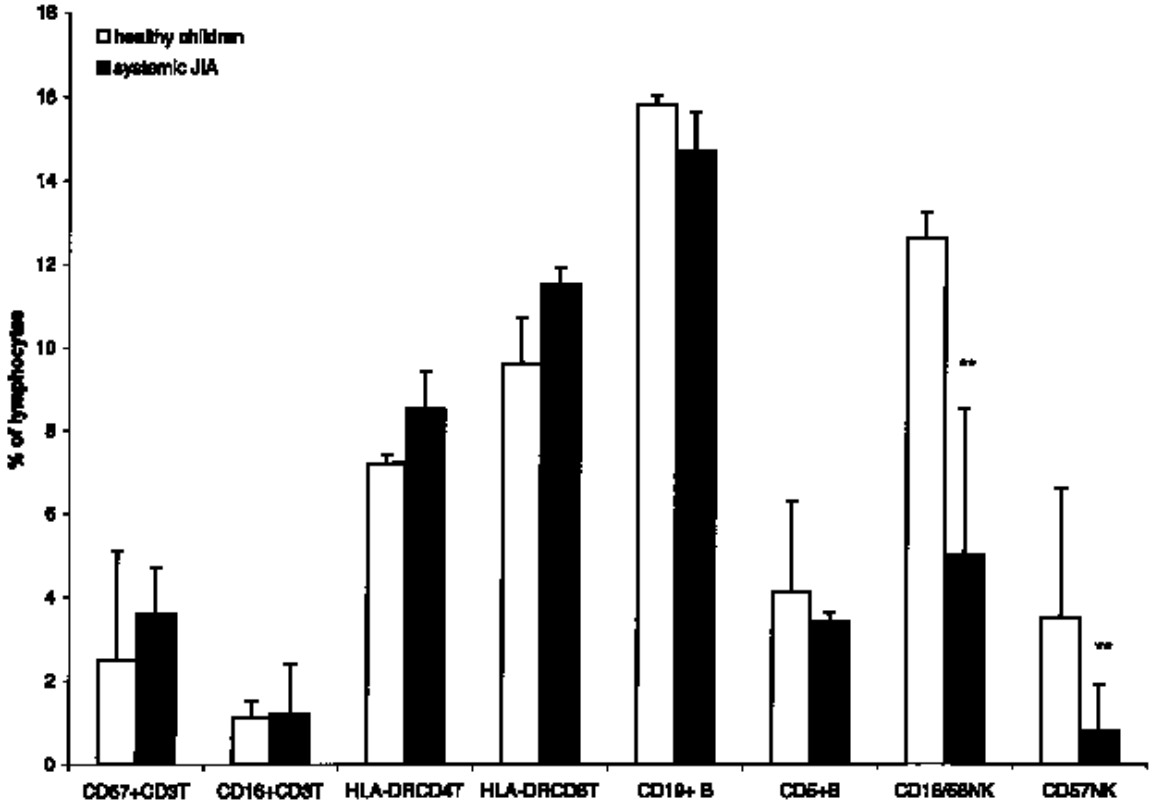

Fig. 2. Peripheral blood lymphocyte subsets (expressed as percentages of the total lymphocyte count) in 9 patients with systemic JIA and 9 age-matched healthy controls.

when compared to patients with s-JIA. On the other hand, lower levels of NK cell subsets were found in patients with s-JIA when compared to the patients with p-JIA (not shown).

Figures 1 and 2 show the numbers and percentages of T cell subsets expressing activation or differentiation markers, B cell and NK cell subsets in patients with o-JIA/p-JIA and s-JIA compared to healthy children.

Humoral immune parameters in patients with JIA

We found increased serum levels of total IgG in s-JIA [median: 1848 (range: 1060-2858) mg/dl), p-JIA (1640 (931-3156 mg/dl) and o-JIA patients (1348 (633-2751) mg/dl] with respectively 8 (66%), 7 (70%) and 9 (48%) patients exceeding the laboratory reference range for this age group (492-1493 mg/dl). Comparison of IgG levels between 10 o-JIA, 10 p-JIA and 10 s-JIA patients matched for age revealed no significant differences (not shown), indicating that B cell hyperactivity is a characteristic feature of JIA, regardless of the subtype.

ANA were present in 9 (48%) oligoarticular and 9 (90%) polyarticular patients, and serum titers were positively correlated to levels of IgG (Rs = 0.36, p = 0.06).

Interestingly, the proportion of activated HLA-DR+ CD4+ T cells was positively correlated to serum levels of IgG in patients with o-JIA and p-JIA (Rs = 0.54, p = 0.002), but not in s-JIA. No other correlations were found between levels of IgG nor ANA and the numbers of B cell nor the T cell subsets (not shown).

Serum levels of IgG were correlated to clinical disease activity expressed using the global score (Rs = 0.41, p = 0.02) and the active joint score (Rs = 0.60, p = 0.0007). We found that in o-JIA and p-JIA patients, levels of ANA were also correlated to the global score (Rs = 0.36, p = 0.06) and the active joint score (Rs = 0.40, p = 0.03).

Discussion

In the present study, we have defined characteristic immunophenotypic profiles of circulating lymphocytes in patients with o-JIA and p-JIA and s-JIA, and compared them to age-matched healthy controls. The clinical expression and immunogenetic associations of o-JIA and RF negative p-JIA are clearly different (4, 28). Some features, such as female preponderance, peak age at onset in early childhood, the presence of antinuclear antibodies and the occurrence of chronic anterior uveitis, are characteristic – even if to a different extent – of both disease subtypes (4). Moreover, 10%-40% of o-JIA patients evolve to an extended oligoarticular or polyarticular pattern, which resembles polyarticular onset JIA in joint pattern, radiological destruction of joints, the incidence of chronic anterior uveitis and outcome (2, 29).

We found an identical peripheral blood lymphocyte subset profile in o-JIA and p-JIA which is consistent with a similar dysregulation of cellular immune function. In contrast, children with s-JIA displayed a clearly different lymphocyte subset profile, suggesting a different dysregulation of cellular immune function in this subtype.

We found normal numbers of circulating CD3+ T cells, CD4+ T cells and CD8+ T cells in all JIA subtypes which is in agreement with most earlier reports (4, 9,10, 13), with the exception of an increased percentage of CD8+ T cells observed by Alarcon (30). However, in this study of 23 JIA patients, 10 healthy controls were not age-matched; furthermore, the absolute numbers of T cells were not analysed.

Patients with o-JIA and with p-JIA had a significant expansion of CD57+ T cells and CD16/56+ T cells representing chronically activated differentiated effector T cell populations. Our findings are in line with previous reports of long-lived CD57+ T cell expansions (31-33) in other immune-inflammatory disorders including sarcoidosis, rheumatoid arthritis, human immunodeficiency virus or human cytomegalovirus infection, allotransplantation, inflammatory bowel disease and various hematological malignancies (18,19, 21, 34-36). Analysis of the cytokine secretion pattern of these T cell subsets both in healthy controls and in hemato-oncological patients revealed a predominant Th1-type cytokine profile (20, 34); interestingly, it has been shown recently that synovial fluid T cell clones from o-JIA patients also display a predominant Th0 or Th1 cytokine secretion pattern and one author found higher levels of the Th1-inducing cytokine IL-12 in the sera of JIA patients (37-40).

Further evidence for the activation of circulating T cells in o-JIA and p-JIA was provided by the significant expansions of CD4+ T cells and CD8+ T cells expressing the late activation marker HLA-DR. Many studies have shown that T cells within inflamed joints display an activated phenotype (9, 10, 26), but the expression of HLA-DR on peripheral blood T cells was found to be normal by most investigators (9, 41). Only one study by Tsokos mentioned an increased proportion of HLA-DR+ T cells (13). The lack of use of age-matched healthy children as controls has been a major shortcoming of all earlier studies on the subject and may have contributed to the contradictory results.

Systemic JIA patients had a different immunophenotypic lymphocyte profile from the o-JIA/p-JIA patients, supporting the concept that they represent a different disease entity (4, 7, 42-44). There was no increase in terminally differentiated T cells subsets (i.e., CD57+ T cells, CD16/56+ T cells) nor of the T cell subsets expressing HLA-DR when compared to age-matched healthy children; however, increased serum levels of soluble IL-2 receptors reported by others (6, 7) point to activation of the circulating T cell compartment also in this subtype. The number of s-JIA patients was small and the absence of significant differences should be interpreted accordingly. However, we found the same differences, be it trendwise, when these subsets were compared between 10 p-JIA and 9 s-JIA patients of the same age range. Some recent findings suggest that increased monocyte activity may play an important role in the pathogenesis of s-JIA. Indeed, s-JIA is characterized by high levels of circulating Il-6, which may be responsible for most of the clinical and laboratory features of the disease (44). Evidence for increased acti-

vation of monocytes, which are the main source of Il-6, in s-JIA has been put forward by some investigators (45-49).

We found an expansion of circulating CD19+ B cells, and particularly CD5+ B cells, in patients with o-JIA and p-JIA, but not in the systemic subtype. An increase of CD5+ B cells was found by Martini *et al*. in all JIA subtypes irrespective of disease activity (50), whereas Jarvis *et al*. found increased percentages of this subset to be significant only in p-JIA (51). Our finding of increased levels of CD5+ B cells specifically in patients with o-JIA and p-JIA is in accordance with the potential of these cells to produce antibodies with a broad range of specificities, as well as autoantibodies, in mice and in humans (27).

The most striking feature in patients with s-JIA was the profound reduction in the numbers of circulating CD16/56+ NK cells and CD57+ NK cells, which is a new and unexplained observation. Because we found a strong and inverse relation of NK cell numbers to the clinical and laboratory parameters of disease activity, we presume this to be a secondary phenomenon. Several potential mechanisms, such as suppression of NK cell proliferation by increased serum IL-6 levels (52), by binding of haptoglobin to the surface CR3 (CD11b) receptor (53), or increased apoptosis by ligation of surface FC RIII with aggregated IgG (54, 55) are at present only hypothetical.

We realize that an analysis of the immunophenotypic profile of peripheral blood lymphocytes may not reflect completely the nature of the immune-inflammatory process in the joint space; a concomitant analysis of circulating and synovial fluid lymphocytes in different JIA subtypes certainly would contribute to the relevance of the present data.

Increased serum levels of IgG consistent with increased B cell activity were found in most of the JIA patients and were correlated with clinical disease activity, as has been reported by others (4, 11). Patients with o-JIA and p-JIA demonstrated B cell hyperactivity concomitantly with increased numbers of circulating HLA-DR+ T cells, and showed a positive correlation between the proportion of HLA-DR+ CD4+ T cells and IgG. These findings point to the importance of the interaction between hyperactive T cells and B cells resulting in hypergammaglobulinaemia in these JIA subtypes. In contrast, B cell hyperactivity in patients with s-JIA was not associated with increased numbers of HLA-DR+ T cells, and may conceivably result from excessive stimulation by cytokines such as IL-6, produced by B cells or activated macrophages, on B cell differentiation and IgG secretion (56, 57). The low numbers of NK cells in s-JIA may be of relevance as well; NK cells have indeed been reported to inhibit B cell proliferation and IgG production through interferon- production (58).

ANA were demonstrated in 62% of oligoarticular and polyarticular patients in the present study, and were correlated to levels of IgG. We found a positive correlation of ANA titers with clinical disease activity, whereas most investigators found no clear correlation of ANA positivity or titer with the activity of eye or joint disease (11, 59-61). Our data are in agreement however with the observation by Leak *et al*., who found ANA titers to be correlated with ESR, active joints and active uveitis (62). The relatively low frequency of ANA positivity (47%) in patients with o-JIA in our study might then be explained by a relatively large number of patients with inactive or mildly active disease (13 patients with a global score of 1 or 2) at the time of testing.

In conclusion, we have demonstrated characteristic immunophenotypic lymphocyte profiles in children with o-JIA and p-JIA, that were different from the lymphocyte profiles in children with s-JIA. These results further support the heterogeneity of the disease. We found o-JIA and p-JIA to be characterized primarily by a sustained activation and differentiation of circulating T cells and by an expansion of CD19+ B cells and CD19+CD5+ B cells. Patients with s-JIA had hypergammaglobulinaemia consistent with B cell hyperactivity but without overt signs of chronic T cell activation. Decreased numbers of NK cell subsets were only found in s-JIA.

Acknowledgements

We thank Mrs Lisette Meurs (Laboratory of Clinical Immunology) for excellent technical assistance. We thank Mrs Magda Minnart (Division of Clinical Immunology) for secretarial assistance.

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