

Increased prevalence of late stage T cell activation antigen (VLA-1) in active juvenile chronic arthritis

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SUMMARY The presence of activated T cells as judged from the reaction with monoclonal antibodies (MoAb) against (a) a late stage T cell activation antigen (VLA-1), (b) the interleukin 2 (IL2) receptor (CD25), and (c) four different HLA class II molecules (HLA-DR, DRw52, DQ, and DP) was studied in 15 patients with active juvenile chronic arthritis (JCA), 10 patients with JCA in remission, and 11 age matched, healthy controls. In addition, the distribution of 'helper/inducer' (CD4+), T 'suppressor/inducer' (CD4+,Leu8+), T 'suppressor/cytotoxic' (CD8+), and 'natural killer' (NK) cells (CD16+) was studied. Twenty patients and six controls were investigated for the capability to stimulate alloreactivated primed lymphocytes. The prevalence of VLA-1 positive, large cells was significantly increased to 5% (median value) in active JCA as compared with JCA in remission (2%, $p < 0.05$) and controls (1%, $p < 0.05$), whereas no significant difference between JCA in remission and controls was observed. Except for two patients with active JCA, less than 1% IL2 receptor bearing cells were found in patients with JCA and controls. No significant difference in the prevalence and expression of the various HLA class II antigens was observed between the groups. Similarly, no significant differences in stimulatory capability in secondary mixed lymphocyte culture (MLC) were seen. The distribution of T helper/inducer (CD4+), T suppressor/cytotoxic (CD8+), and NK cells was similar in active JCA, JCA in remission, and controls. The prevalence of T suppressor/inducer (CD4+,Leu8+) cells was higher in remission JCA (17%) than in active JCA (11%) and controls (10%). This increase, however, did not reach statistical significance. In conclusion, late stage but not early stage T cell activation antigens were increased in patients with active JCA as compared with patients with JCA in remission and controls, whereas some patients in remission had an increased prevalence of T suppressor/inducer cells.

Key words: HLA-DR, HLA-DQ, HLA-DP, T suppressor/inducer cells, secondary mixed lymphocyte reaction.

Only a small percentage of freshly isolated normal peripheral blood T lymphocytes are activated as judged by the expression of early T cell activation markers such as the IL2 receptor (CD25),^{1,2} transferrin receptor,³ HLA class II antigens (e.g., HLA-DR),^{4,5} and the recently described late stage T cell activation antigen (VLA-1).⁶⁻⁸

In active multiple sclerosis enhanced expression of a T cell activation antigen, Ta1, but not of other T cell activation antigens, such as the IL2 receptor, has been reported.⁹ In synovial fluid obtained from adults with rheumatoid arthritis raised levels of VLA-1 but not of CD25 positive T cells were observed, whereas VLA-1 positive cells were only detectable in peripheral blood in some patients.¹⁰ In juvenile chronic arthritis (JCA) high percentages of HLA-DR positive T cells in synovial fluid, but normal levels in peripheral blood, have been described.¹¹

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The present study was undertaken to investigate the possible presence in the blood of cells expressing early or late stage T cells activation antigens, or both, in patients with active JCA as compared with JCA in remission and controls. In addition, the distributions of HLA-DR, DRw52, DQ, and DP positive cells and of T helper/inducer, T suppressor/cytotoxic, T suppressor/inducer, and NK cells in JCA and controls were studied.

Patients and methods

PATIENTS AND CONTROLS

The study comprised 25 patients classified as having (a) pauciarticular (10 patients), (b) polyarticular (11), or (c) systemic (four patients) JCA according to the EULAR WHO criteria.¹² The patients were randomly selected from patients attending the department of paediatrics, Rigshospitalet. All were white Caucasoids of Danish extraction. Nineteen (76%) were female and six (24%) male with a mean age of onset of disease of 8.1 years (range 3–15 years). Disease activity (active synovitis) was defined as the presence of at least two of the following symptoms: (a) joint pain, (b) joint swelling (due to synovial swelling or effusion), and (c) restriction of joint movement. Remission was defined as absence of signs of active synovitis and a normal erythrocyte sedimentation rate, i.e., below 10 mm/min. Accord-

ing to these criteria, 15 patients (60%) were classified as having active disease at the time of investigation, while ten (40%) were in a state of remission. Controls comprised 11 healthy children matched ethnically and by age.

ISOLATION OF PERIPHERAL BLOOD MONONUCLEAR CELLS (PBMC)

PBMC suspensions were prepared by density gradient centrifugation, washed, and frozen as described elsewhere.¹³

MONOCLONAL ANTIBODIES (MoAbs)

Table 1 shows the specificity, immunoglobulin subclass, trade name, and references for the commercially available MoAbs used. The anti-VLA-1 MoAb (Coulter clone) recognises a surface antigen expressed on T cells after two to three weeks of activation.^{7,8} MoAbs detecting early T cell activation markers included one against the IL2 receptor (CD25) and the various MoAbs directed against HLA class II antigens (HLA-DR, DRw52, DQ, and DP; Table 1). Additionally, MoAbs detecting the CD3 (Leu4, pan-T), the CD4 (Leu3, T helper/inducer subset), and the CD8 (Leu2, T suppressor/cytotoxic subset) T cell surface molecules were used (Table 1). Double fluorescence experiments were performed with the anti-CD4 (Leu3, phycoerythrin conjugated) and the anti-Leu8 MoAb (fluorescein isothiocyanate conjugated) (Table 1).

Table 1 Characteristics of monoclonal antibodies

MoAb	Trade name	Molecular specificity	Immunoglobulin subclass	References
A-1A5	ACT-T-SET VLA-1 (T Cell Sciences)	Late stage T cell activation antigen (VLA-1)	IgG1	6-8
Anti-IL2 receptor	Anti-IL2 receptor (Becton-Dickinson)	The CD25 (IL2 receptor) complex	IgG1	1, 2
Anti-HLA-DR	Anti-HLA-DR (Becton-Dickinson)	Monomorphic HLA-DR	IgG2a	16
Anti-HLA-DQ	Leu10	HLA-DQw1, w3, blank and some w2 positive PBMC*	IgG1	14
Tü-39	Clonab DP-DR (Biotest)	Broad HLA class II, preferentially reacting with DP	IgG2a	14-16, 27
HLA-DRw52	HLA-DRw52 (Synbio-monosan)	A polymorphic determinant on HLA-DR which is associated with DR3, DR5, and DRw6	IgG2b	17, 18
T suppressor/cytotoxic	Anti-Leu2a (Becton-Dickinson)	The CD8 complex	IgG1	19, 20
T helper/inducer	Anti-Leu3a (Becton-Dickinson)	The CD4 complex	IgG1	19, 20
T suppressor/inducer	Anti-Leu8 (Becton-Dickinson)	Undetermined; reacts with a subpopulation of CD4+ lymphocytes	IgG2a	21, 22, 34
NK cells	Anti-Leu11 (Becton-Dickinson)	The CD16 complex	IgG1	22, 23

*PBMC=peripheral blood mononuclear cells.

CELL SURFACE ANTIGEN ANALYSIS

Cells were analysed by indirect immunofluorescence (fluorescence activated cell sorter (FACS) analyser, Becton-Dickinson) as described previously²⁴ using specific MoAbs (see above) and a second fluorescein conjugated goat antimouse immunoglobulin (Nei 504, New England Nuclear) or fluorescein

conjugated avidin (Vector) in the case of a biotin conjugated MoAb. The percentage of positive cells from FACS analysis was calculated as the percentage of specifically stained cells minus the percentage obtained with a control MoAb using a fixed cut off value. The cut off value was fixed to the right of background staining giving values of control staining

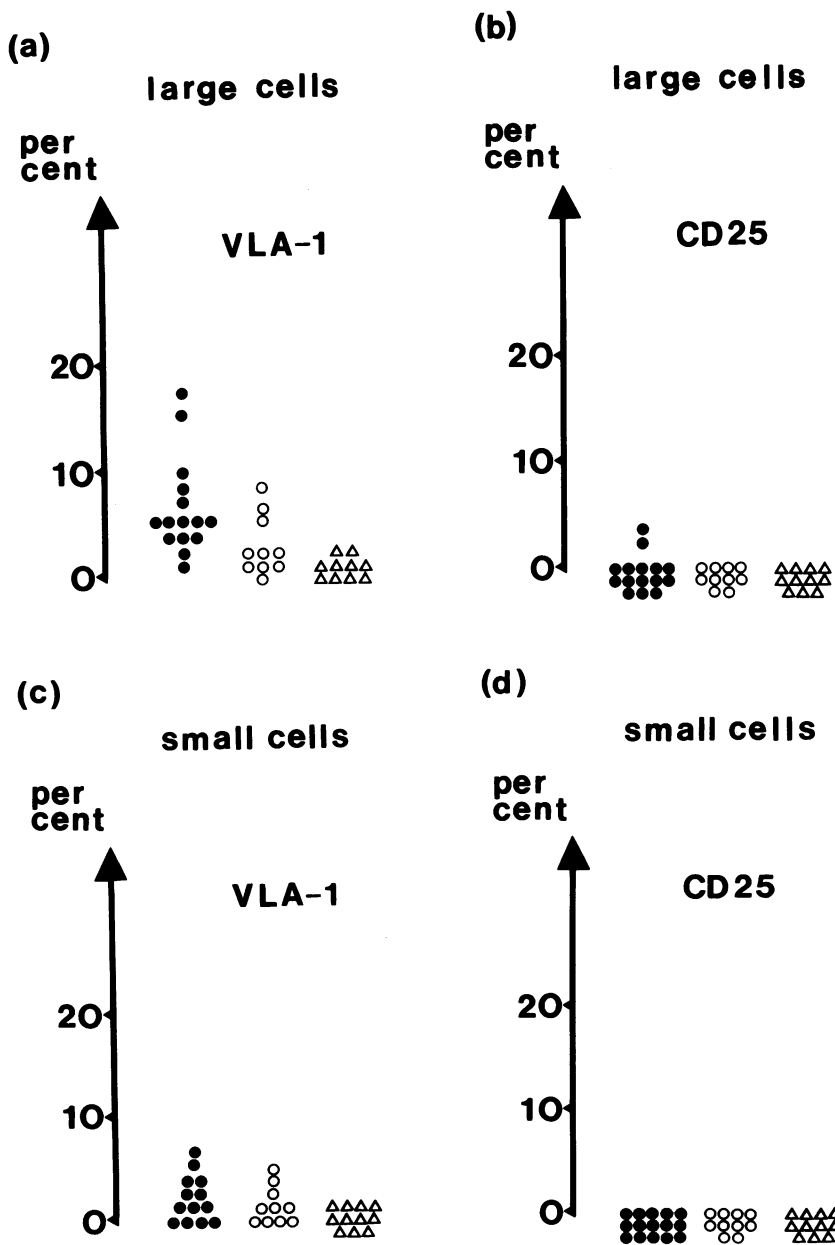


Fig. 1 Fractions (%) of large and small cells positive for VLA-1 and CD25 (IL2 receptor) antigens in patients with active JCA (●), with in remission (○), and in healthy controls (△). The prevalence of VLA-1 positive large cells was significantly higher in patients with active disease than in patients in remission ($p < 0.05$) or in controls ($p < 0.05$). No other differences between the groups reached statistical significance.

of less than 4%. The channel numbers corresponding to median fluorescence intensity were determined as the median fluorescence intensity (MFI) of the positive cells on an arbitrary scale from 1 to 256. The percentages and MFI values were analysed on large and small cells, separately. In all individuals it was possible to make the distinction between large and small cells from the volume signals from the FACS analysis, which clearly showed a dichotomy of the cell populations.

STIMULATION OF ALLOACTIVATED PRIMED LYMPHOCYTES IN SECONDARY MIXED LYMPHOCYTE CULTURE (MLC)

PBMC from patients with JCA and controls were used as stimulator source in secondary MLC experiments using HLA-DP and DR reactive primed lymphocytes as described in detail elsewhere.¹³

STATISTICS

The Wilcoxon rank sum test for paired differences and the Mann-Whitney U test were used. A value of $p < 0.05$ was considered significant. In the case of no a priori expectations the p values were corrected for (multiplied by) the number of comparisons and further multiplied by two in order to obtain a double sided test. For example, when comparing the distribution (prevalence and fluorescence intensity) of the four HLA class II antigens in active JCA, JCA in remission, and controls the p values were multiplied by $2^4 \times 3 \times 2 \times 2 = 192$.

Results

VLA-1 AND CD25 EXPRESSION IN JCA AND CONTROLS

Fig. 1 shows the percentage of large (a and b) and small cells (c and d) expressing the VLA-1 and the CD25 antigen in JCA patients with active synovitis and JCA in remission as well as in age matched controls. In 10 of 15 patients with active disease more than 3% of the large cells were VLA-1 positive (Fig. 1a), whereas only three of 10 patients in remission had more than 3% VLA-1 positive cells (Fig. 1a). In contrast, none of the healthy children expressed more than 3% VLA-1 positive cells. In two patients with active JCA 15% or more VLA-1 positive large cells were found. The prevalence of VLA-1 positive large cells was significantly increased in active JCA as compared with JCA in remission ($p < 0.05$) and with controls ($p < 0.05$), whereas no significant difference between JCA in remission and controls was observed. Similar results, although less pronounced, were obtained when VLA-1 on small cells were analysed (Fig. 1c).

Table 2 HLA class II antigens in JCA and controls

MoAbs reactive with HLA	Large cells				Small cells							
	Active JCA (n=15)		Remission JCA (n=10)		Controls (n=11)		Active JCA (n=15)		Remission JCA (n=10)		Controls (n=11)	
	Positive (%)	MFI (channel No)	Positive (%)	MFI (channel No)	Positive (%)	MFI (channel No)	Positive (%)	MFI (channel No)	Positive (%)	MFI (channel No)	Positive (%)	MFI (channel No)
DR	58	132	57	133	58	139	10	131	11	125	13	132
DRw52	53‡	114	50§	123	49	122	13‡	123	12§	116	14	115
DQ	25	108	26	112	25	114	12	113	13	109	11	112
Dp*	74	143	73	141	64	155	12	131	14	132	11	140

*MoAb used (Clonab DP/DR (Tu-39)) preferentially reacts with HLA-DP molecules and to a lesser extent with HLA-DR molecules.¹⁴⁻¹⁶

†MFI=median fluorescence intensity (see 'Patients and methods').

‡Median of 10 DRw52 positive individuals.

§Median of eight DRw52 positive individuals.

||Median of seven DRw52 positive individuals.

||Median of 14 Leu10 positive individuals.

Table 3 Prevalence of CD4 positive cells in JCA and healthy children*

	Percentage of cells positively stained with:				Ratio		
	CD4+ (%)	Leu8+ (%)	CD4+.Leu8+ T suppressor/inducer (%)	CD8+ (%)	CD16+ (%)	CD4+/CD8+	CD4+.Leu8+/CD4+
Active JCA (n=15)	33	41	11	25	11	1.50	0.44
Remission JCA (n=10)	30	42	17	23	7	1.38	0.53
Controls (n=11)	28	37	10	18	12	1.87	0.42

*Results are shown as median values.

In contrast with VLA-1, no patients or controls had more than 4% CD25 positive large or small cells (Figs 1b and d). Indeed, with the exception of two patients with active JCA, CD25 positive cells were detectable in neither JCA nor controls (Figs 1b and d). There was no significant difference in the distribution of large and small cells between the groups, and no correlation between the percentage of VLA-1 positive cells and any clinical subgroup of JCA was observed (data not shown).

HLA CLASS II ANTIGENS IN HEALTHY CHILDREN

Table 2 shows the median values of the percentage of positive cells and the median fluorescence intensity (MFI) on large and small cells obtained from 11 healthy children when investigated with MoAbs directed against HLA-DR, DRw52, DQ, and DP.

The percentages of HLA-DP (64%) and DR (58%) positive large cells were significantly higher ($p<0.01$) than those of HLA-DRw52 (49%) and DQ (25%) positive cells (Table 2). In contrast, when the results for small cells were compared no differences between the percentages of HLA-DP (11%), DR (13%), DRw52 (14%), and DQ (11%) were observed.

HLA CLASS II ANTIGENS IN JCA

As shown in Table 2, in patients with JCA the percentages of HLA-DP positive, large cells (74% in active JCA and 73% in remission JCA) were not significantly higher than those of DR positive large cells (58% and 57% respectively); uncorrected p value <0.01 , but not significant when corrected. The fractions of HLA-DP positive large cells (74% in active JCA and 73% in remission JCA) and DR positive large cells (58% and 57%) were significantly higher than those of HLA-DRw52 (53% and 50%, $p<0.05$) and DQ (25% and 26%, $p<0.01$) respectively.

HLA CLASS II ANTIGENS IN ACTIVE JCA, JCA IN REMISSION, AND CONTROLS

No significant differences between active and remis-

sion JCA were observed with MoAbs against the various HLA class II antigens when analysed for MFI and fractions of positive large and small cells. The fraction of HLA-DP positive large cells was insignificantly increased and the MFI values obtained from DP positive cells insignificantly decreased, however, in JCA as compared with controls. No differences between JCA and controls in expression or percentage of positive cells were observed when analysed for reaction with HLA-DR, DRw52, and DQ reactive MoAbs.

STIMULATORY CAPABILITY IN SECONDARY MLC OF PBMC FROM PATIENTS WITH JCA AND CONTROLS

All patients and controls stimulated alloreactive primed lymphocytes in secondary MLC. No significant difference in stimulatory capability was found between active JCA (median cpm=25.0), JCA in remission (median cpm=26.6), and controls (median cpm=24.2) (data not shown).

T HELPER/INDUCER (CD4+), T SUPPRESSOR/CYTOTOXIC (CD8+) AND NK (CD16+) CELLS IN JCA AND CONTROLS

Table 3 shows the prevalence of various T subpopulations in active JCA, JCA in remission, and controls. No significant differences in fractions of CD4+ (33%, 30%, and 28% respectively), CD8+ (25%, 23%, and 18%), CD16+ (11%, 7%, and 12%) and Leu8+ (41%, 42%, and 37%) cells were observed between the three groups (active JCA, JCA in remission, and controls respectively). Similarly the ratio of cells CD4+/CD8+ did not differ significantly between the groups. The prevalence of CD4+.Leu8+ cells (T suppressor/inducer) and the ratio CD4+.Leu8+/CD4+ were higher in JCA in remission (17% and 0.53) than in active JCA (11% and 0.44) and controls (10% and 0.42). None of these differences, however, was significant.

Discussion

In this study we demonstrated an increased pre-

valence of cells reactive with a MoAb detecting a late stage T cell activation antigen (VLA-1)⁶⁻⁸ in patients with active JCA as compared with JCA in remission and controls. In contrast, early activation markers such as IL2 receptor and HLA class II antigens were not increased. It may be speculated that the reaction of the VLA-1 (IgG1) MoAb with large cells in active JCA could be due to non-specific, Fc mediated binding to monocytes, activated T cells, or other cells bearing Fc receptors. Although this possibility cannot be ruled out, it seems unlikely, as no non-specific binding was observed with other MoAbs of the same immunoglobulin isotype, e.g., the anti-IL2 receptor MoAb. To our knowledge the present data yield the first evidence of an increased prevalence of cells expressing a late stage T cell activation antigen (VLA-1) in patients with active JCA. In eight adults with rheumatoid arthritis raised levels of VLA-1 but not CD25 positive T cells were observed in synovial fluid, whereas levels in peripheral blood of VLA-1 positive cells above control values were only seen in some patients.¹⁰

High percentages of HLA-DR positive T cells in synovial fluid have been demonstrated in JCA,¹¹ whereas no increased prevalence of HLA-DR positive T cells was observed in peripheral blood.¹¹ Similarly, no differences in MFI or the prevalence of HLA-DR, DRw52, or DQ positive cells between the groups could be shown in the present study. In contrast, the prevalence of HLA-DP positive cells was significantly increased in JCA as compared with controls. No significant difference in the stimulatory capability was observed between JCA and controls when tested in secondary MLC with HLA-DP reactive primed lymphocytes.

Conflicting data on the distribution of T helper/inducer (CD4+) and T suppressor/cytotoxic (CD8+) subpopulations in JCA have been reported.²⁵⁻²⁸ No significant difference in the prevalence of CD4+ and CD8+ lymphocytes could be shown in the present study. The prevalence of the T suppressor/inducer subpopulation, however, defined as CD4+,Leu8+ cells, was insignificantly increased in JCA in remission as compared with patients with active disease. In healthy individuals the T suppressor/inducer subpopulation was originally identified as the subpopulation of CD4+ T cells that reacted with autoantibodies found in sera of patients with JCA.^{29, 30} Recently, the T suppressor/inducer subset has also been defined as CD4+ T cells reactive with various monoclonal antibodies (Leu8,³¹ TQ1,³² and RH³³). In contrast with our findings in JCA, very low levels of T suppressor/inducer cells (<2%) were observed in infants with autoimmune neutropenia or haemolytic

anaemia as compared with age matched, healthy controls (6% v 25%), whereas the number of CD8+ cells in the patients was normal.³⁴

In conclusion, the analysis of peripheral blood mononuclear cells may be of importance in the evaluation of the disease activity and the underlying immunopathological mechanism in JCA as VLA-1 positive cells appear in vitro after long term stimulation.⁸ It may be speculated that VLA-1 positive cells in JCA represent lymphocytes chronically stimulated by ectoantigen or autoantigen. Functional in vitro studies such as lymphokine production and cytotoxicity may elucidate an immunopathogenic importance of VLA-1 positive cells in JCA.

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