EXTENDED REPORT

Comparison of soluble adhesion molecules in juvenile idiopathic arthritis between the active and remission stages

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Objective: To determine the serum levels of soluble adhesion molecules in patients with juvenile idiopathic arthritis (JIA), and to determine whether the levels of these molecules differ between active disease and remission in the same JIA subtype, and whether differences in these levels exist between controls and the three JIA subtypes.

Methods: The serum levels of soluble E-selectin (sE-selectin) and soluble intercellular adhesion molecule-1 (sICAM-1) were determined by enzyme linked immunosorbent assay (ELISA) in 40 patients with JIA (12 systemic, 13 polyarticular, and 15 oligoarticular) who had active disease or were in clinical remission and 16 healthy controls. Differences in the levels of adhesion molecules of the same JIA subtype during different disease activity were determined by the paired *t* test, and differences between the disease and control groups were calculated by one way analysis of variance. A value $p<0.01$ was considered significant.

Results: During the same disease stage (active or in remission), systemic JIA was associated with a significantly higher sE-selectin level than the oligoarticular JIA subtype, whereas this was not found for sICAM-1. Although the mean levels of sE-selectin and sICAM-1 in active systemic and polyarticular JIA were higher than those in remission, this did not reach statistical significance. The levels of sE-selectin and sICAM-1 of the three JIA subtypes, in both the active stage and clinical remission, were still significantly higher than in normal controls.

Conclusions: Systemic JIA is associated with a higher sE-selectin level than oligoarticular JIA both in active disease and in clinical remission. This may explain why the morbidity of systemic JIA is greater than that of oligoarticular JIA—namely, owing to increased endothelial cell activation. As significantly higher levels of sE-selectin and sICAM-1 were found in the active and remission stages of the three JIA subtypes compared with those in the control group, JIA may recur even when clinical remission has been achieved.

Juvenile idiopathic arthritis (JIA) is one of the most common rheumatic diseases of children and a major cause of chronic disability, especially the polyarticular and systemic subtypes. It is characterised by an idiopathic synovitis of the peripheral joints, associated with soft tissue swelling and effusion. The diagnosis of this disorder and evaluation of disease activity is based on clinical findings and laboratory tests which show the presence of autoantibodies, changes in serum immunoglobulins, and acute phase reactant proteins.[1] This inflammatory process is generated by a series of events, including the migration of leucocytes from the blood stream into the tissue, their activation to become effector cells and, finally, their local retention which facilitates the continuing immune reaction.[2] Adhesion molecules play a part in all these phases and have a major role in the disease process.

Many adhesion molecules appear to be shed during inflammation. The availability of enzyme linked immunosorbent assay (ELISA) kits has made it possible to measure the levels of circulating biologically active adhesion molecules. In adult rheumatoid arthritis (RA), raised levels of soluble E-selectin (sE-selectin) and soluble intercellular adhesion molecules-1 (sICAM-1) have been found.[3 4] However, sE-selectin is found only on activated endothelium[5] and is induced within hours of stimulation with interleukin 1, tumour necrosis factor, lipopolysaccharide,[6–8] C1q-fixing immune complexes,[9] or by CD40-CD40L interaction.[10] It is then rapidly (within 16–24 hours) down regulated.[8 11] ICAM-1 is widely distributed on leucocytes, endothelial cells, fibroblasts, and epithelial cells.[12] It is induced by proinflammatory cytokines such as interferon γ, tumour necrosis factor α, interleukin 1α, and interleukin 6.[13 14] In contrast with the selectins, which are rapidly down regulated after induction, once ICAM-1 is up regulated, it remains on the cell surface for more than 48 hours.[11]

The purpose of this study was to measure sE-selectin and sICAM-1 levels among different JIA subtypes and the control group. The study also investigated whether levels of soluble adhesion molecules (SAMs) correlate with disease activity or with the subtypes of JIA, or both, and whether these levels of SAMs return to normal during clinical remission.

MATERIALS AND METHODS

We studied children with JIA fulfilling the classification criteria, which was revised by the International League Against Rheumatism (ILAR) in 1997.[15] The children were divided into three subtypes (systemic, polyarticular, and oligoarticular). A series of blood samples were obtained every six weeks from patients with different disease status observed at outpatient clinics. All patients at the first evaluation were treated uniformly with naproxen only, and then the drugs were adjusted according to subsequent clinical responses and laboratory findings. At the moment of clinical remission, naproxen and/or one or more additional drug, such as corticosteroids,

Abbreviations: JIA, juvenile idiopathic arthritis; RA, rheumatoid arthritis; SAMs, soluble adhesion molecules; sICAM, soluble intercellular adhesion molecules; WBC, white blood cell count

Table 1 A summary of age, disease duration, and length of time between active and remission stages in the study subjects

	Total	Systemic	Polyarticular	Oligoarticular
Number of cases	40	12	13	15
Mean age (years)	9.4 (3–16)	8.2 (3–16)	10.3 (6–14)	9.7 (6–15)
Duration of disease at first evaluation (mo)	2.1 (6 wks–7.5 mo)	1.8 (2 mo–6.8 mo)	2.3o (2 mo–7.5 mo)	2.1o (6 wks–5.7 mo)
Average duration to remission (months)	11.3 (3–33)	9 (3–22)	13.5 (3–28)	11.3 (3–33)

disease modifying antirheumatic drugs, methotrexate, cyclosporin, was used in different patients. Patients with concomitant infection or other systemic diseases were excluded. The study period was June 1996 to December 2000, and a total of 40 patients (12 systemic, 13 polyarticular, and 15 oligoarticular) with complete data were finally enrolled. All the patients had active disease, defined for polyarticular or oligoarticular disease as at least one joint that was either swollen, tender, and limited by examination. Systemic disease was defined as arthritis, as described above, or fever ⩾38.5°C at least four days a week, without definable infection or other source besides JIA, or a systemic manifestation such as serositis. Patients were considered to be in clinical remission when they had morning stiffness not exceeding 15 minutes, no fatigue, no joint pain, no joint tenderness, no joint or tendon sheath swelling, and an erythrocyte sedimentation rate of less than 20 mm/1st h (based on the American College of Rheumatology (ACR) criteria for remission of adult RA).[16]

Assays for adhesion molecules were performed on frozen serum samples. Levels of sE-selectin and sICAM-1 were measured by sandwich ELISA using commercial kits (R&D systems, Minneapolis, MN, USA). Values were determined by interpolation of values from a standard curve determined by spectrophotometric readings from a dilution series of positive controls with a known concentration of each molecule. Control serum samples were obtained from 16 healthy children aged 3–16 years among a group attending the hospital for vaccination.

The paired *t* test was used to assess probability levels between active disease and remission for the same JIA subtype. Differences in levels of SAMs among JIA subtypes and the control group were assessed by one way analysis of variance. A value of $p<0.01$ was considered significant.

RESULTS

Table 1 shows the average age, duration of disease at the initial evaluation, and length of time between the active stage and remission. Age and duration of disease at onset were not significantly different among the subtypes. In the polyarticular JIA group, only two patients had a positive rheumatoid factor.

It was found that no matter whether the disease was active or in remission, levels of sE-selectin and sICAM-1 in the three subtypes of JIA were significantly higher than the levels in the control group (figs 1 and 2).

When compared during active disease or remission, systemic JIA (fig 1) was always associated with a significantly higher level of sE-selectin than the oligoarticular JIA subtypes, whereas there were no differences in the levels of sICAM-1 in the different JIA subtypes (fig 2).

DISCUSSION

JIA is a systemic inflammatory disease with dysregulation of normal immune responses that lead to chronic tissue inflammation and damage. Synovial neoangiogenesis, one of the pathologic hallmarks of JIA, was recently found to be caused mainly by vascular endothelial cell growth factor and was associated with increased infiltration of inflammatory cells.[17] The activation, migration, and penetration of leucocytes into local inflammatory tissues are dependent on attachment to the adhesion molecules on endothelial cells. For this reason, adhesion molecules are believed to play a part in the initiation

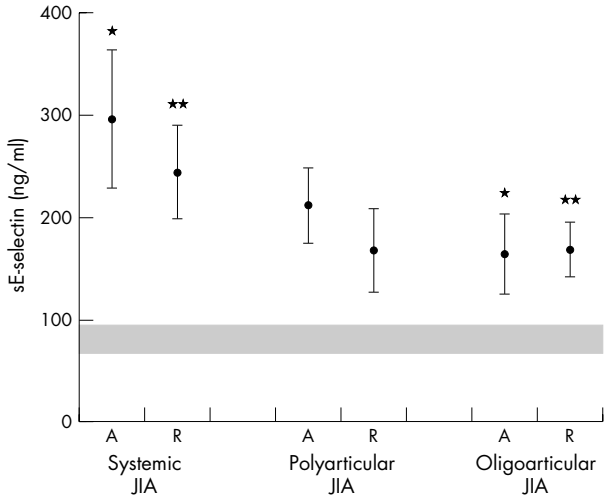

Figure 1 The serum levels of sE-selectin in different JIA subtypes and in active disease and remission. Mean values of sE-selectin in the different subtypes are represented by black circles, with ±2SEM represented by the upper and lower confidence bars. The range of normal control (mean ±2SEM) is represented by the shaded band. A, active disease; R, remission. *In active disease, systemic *v* oligoarticular, $p<0.01$; **in remission, systemic *v* oligoarticular, $p<0.01$.

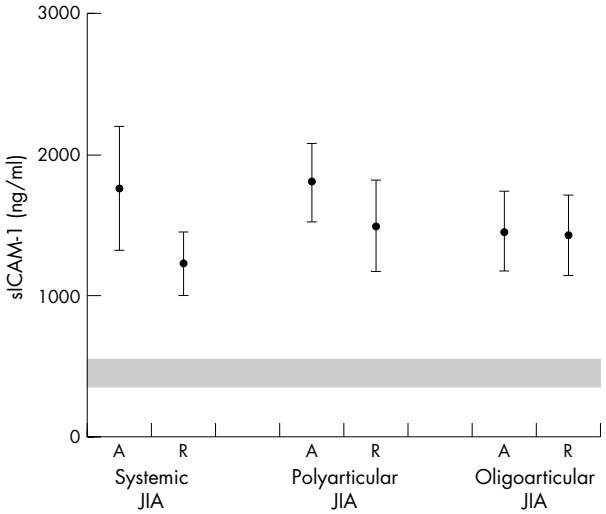

Figure 2 Serum levels of sICAM-1 in different JIA subtypes and in active disease and remission. Mean values of sICAM-1 in the different subtypes are represented by black circles, with ±2SEM represented by the upper and lower confidence bars. The range of normal control (mean ±2SEM) is represented by the shaded band. A, active disease; R, remission.

and propagation of autoimmune diseases, although there are limited data available about the correlation of SAM levels with indicators of disease activity.[18]

Our study showed that the sE-selectin levels in active disease or remission of systemic JIA are significantly higher than the levels in the same status of oligoarticular JIA. These findings may explain why systemic JIA is associated with greater morbidity and is more refractory to medical treatment than the oligoarticular JIA subtype. Additionally, a large proportion of patients with oligoarticular JIA (particularly those with monarthritis) do remit fully and require no further treatment, but it is rare to see the same fully drug-free remission in systemic JIA, probably because systemic JIA causes more inflammation through the reaction of adhesion molecules.

Our study also showed that the levels of sE-selectin and sICAM-1 in the three JIA subtypes, no matter whether in the active stage or in clinical remission, were significantly higher than those in the control group. A possible explanation may be that although we clinically define patients as in remission, the inflammatory processes may still continue insidiously and this is expressed by an increase of SAMs, resulting in recurrence. The definition of remission for JIA should thus be considered from this point of view. To our knowledge, there are no uniform criteria for defining clinical remission from JIA. In our study we used the preliminary criteria for clinical remission of adult RA from the ACR.[16] The main purpose of these criteria is to provide a preliminary evaluation for "relative" remission, rather than to define an "absolute" or "complete" remission of adult RA. When applied to patients with JIA, the criteria may also provide a useful guideline for clinical remission, but they do not appear to correlate with the actual activity of JIA assessed by the presence of adhesion molecules. It seems reasonable to include normal levels of SAMs as a criterion in the definition of complete remission; however, further studies are necessary to confirm this.

Many studies have debated which kind of adhesion molecules are prominently related to different JIA subtypes. Bloom *et al* analysed a total of 16 patients with active disease and reported that sE-selectin was significantly higher in patients with systemic juvenile rheumatoid arthritis than the other subtypes.[19] They did not have a control group for comparison. Laucella *et al* reported on six patients in remission and 31 with active juvenile chronic arthritis, compared with 25 healthy controls. They found that sICAM-1 was higher in systemic and polyarticular juvenile chronic arthritis than in the pauciarticular group.[20] However, they did not follow up and compare the sICAM-1 levels between active disease and remission. Kolopp-Sarda *et al* carried out a six year cohort study, which showed a sustained increase of sVCAM-1 in adult RA in comparison with control subjects.[21] The criteria they used for definition of clinical remission—namely, Ritchie's index[22] and Larsen score,[23] are not suitable for children, because the subjective grades of tenderness are not easily expressed by young children. In addition, the joint damage shown by radiography is also rare in patients with JIA. To our knowledge, until now there has been no study that analysed patients longitudinally according to the different disease status of all three JIA subtypes, and compared the SAMs among the three subtypes and the control group simultaneously.

tk;3Our findings showed additionally that when the correlations were measured between SAMs and disease parameters, such as erythrocyte sediment rate, C reactive protein, total white blood cell count (WBC), packed cell volume, and platelet count, sE-selectin correlated with WBC (r=0.318, p=0.046) and packed cell volume (r=0.349, p=0.028) in the active stage and with WBC (r=0.425, p=0.01) and platelet count (r=0.458, p=0.003) in the remission stage. There were no correlations between sICAM-1 and disease parameters. Some authors have suggested that the SAMs may provide additional markers for disease activity.[19 24 25] In our study, the SAMs did not correlate with some of the sensitive markers of inflammation, such as C reactive protein or erythrocyte sedimentation rate. A further large cohort study is needed to provide more information about the value of SAMs for monitoring inflammatory arthritis. Thus, at present, the more costly determination of SAMs for monitoring disease activity is not recommended.

Perhaps, the most interesting aspect of this study is the persistent increase of SAMs, despite "remission" having been achieved in the patients with JIA of various subtypes. It seems reasonable to include the presence of normal levels of SAMs in the definition of complete remission. Increased production of adhesion molecules in JIA means increased cytokine induction and endothelial cell activation, which cause tissue inflammation and damage. Recently, great therapeutic potential has been seen in modulating inflammation by interfering with leucocyte-endothelial cell adhesion and using specific antibodies or non-specific inhibitors.[26] Further study is necessary.

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