Soluble Adhesion Molecules in Pediatric Rheumatic Diseases

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ABSTRACT. *Objective.* To determine serum levels of adhesion molecules ICAM-1, ICAM-3, VCAM-1, L-selectin, and E-selectin in children with a variety of pediatric rheumatic diseases and investigate their relationship to clinical disease activity.

Methods. Retrospective review of records of 18 children with rheumatic diseases who had banked sera available for study. Eight children had systemic lupus erythematosus (SLE), 2 mixed connective tissue disease, 4 dermatomyositis (DM), and 4 various forms of vasculitis. Levels of the soluble adhesion molecules were determined by sandwich ELISA. Levels were compared among patients with the various diagnoses and between patients with active vs inactive disease. Levels were also correlated with erythrocyte sedimentation rate in all patients; C3, C4, and total hemolytic complements and anti-dsDNA antibodies in SLE; and creatine phosphokinase, aldolase, and von Willebrand factor (vWF) antigen levels in DM. Levels also correlated with disease activity scores, which varied by diagnosis.

Results. A trend toward higher levels of sE-selectin was found in vasculitis vs other diagnoses (p = 0.08). sICAM-1 was higher in patients with active vs inactive disease (p = 0.05) across all diagnoses. L-selectin levels correlated with C4 complement levels in SLE patients (r = 0.76, p = 0.03), and there was a trend toward an inverse correlation between levels of sE-selectin and vWF (r = –0.93, p = 0.08). There was no direct correlation of the adhesion molecule levels with any of the disease activity scores.

Conclusion. The small number of patients and retrospective design of this study mean that any results must be interpreted with caution. We conclude: (1) Elevated E-selectin levels in vasculitis likely reflect the high degree of endothelial activation and possibly overt vascular damage in those conditions. (2) The correlation of sL-selectin with C4 in SLE may indicate that downregulation of shedding of cell surface L-selectin is involved in continued adherence of leukocytes to endothelium, possibly causing further damage and immune complex deposition in this condition. (3) The trend toward inverse correlation between sE-selectin and vWF:Ag in DM is curious, but may show that the role of endothelium in the pathophysiology of this disease is different from those such as vasculitis. (4) Levels of sICAM-1 may be a useful marker of active vs quiescent disease in general in the pediatric rheumatic diseases, although lack of correlation with disease activity indices may indicate that it is too insensitive to smaller differences in disease activity to be recommended for routine clinical use. (J Rheumatol 2002;29:832–6)

Key Indexing Terms:

ADHESION MOLECULES	ENDOTHELIUM	CHILDREN
VASCULITIS	SYSTEMIC LUPUS	DERMATOMYOSITIS

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We reported a pilot study of levels of soluble forms of several adhesion molecules (intercellular adhesion molecule, ICAM-1, ICAM-3, E-selectin, L-selectin, vascular cellular adhesion molecule, VCAM-1) in children with juvenile rheumatoid arthritis[1]. That study suggests that ICAM-1 and E-selectin are relatively elevated in patients with systemic disease when compared to other subtypes, and that correlation of E-selectin levels with other disease related variables might suggest the utility of that molecule as a clinical marker of disease activity. While studies of soluble adhesion molecules in rheumatic diseases of adulthood have been quite extensive, there have been few other studies of soluble adhesion molecule levels in pediatric rheumatic diseases. In studies of children with Kawasaki disease, levels of sICAM-1 and sE-selectin were elevated, especially

in patients with coronary aneurysms[2,3]. Another study showed that levels of sE-selectin were not elevated in children with localized and systemic forms of scleroderma[4]. An additional study of sICAM-1 levels in pediatric rheumatic diseases showed that levels were elevated in both juvenile rheumatoid arthritis (JRA) and systemic lupus erythematosus (SLE), and that levels correlated with disease activity over time in a subset of the patients with SLE[5]. Another study contradicted these findings in SLE. sICAM-1 levels were not different among SLE patients, patients with Henoch-Schönlein purpura, and controls[6]. However, sVCAM-1 levels were higher in SLE patients, and correlated with erythrocyte sedimentation rate (ESR) and lymphocyte counts, and inversely with C4 complement. A recent study confirmed our initial findings that sE-selectin was elevated during active periods of systemic JRA, but indicated that sICAM-1 levels were not different than controls in any JRA subtype[7].

To elucidate the role of adhesion molecules in the pathophysiology of pediatric rheumatic diseases, and assess their potential utility as markers of activity in these diseases, we performed a cross sectional pilot study of several soluble adhesion molecules in a variety of pediatric rheumatic diseases. We chose the following soluble adhesion molecules to study because their role in leukocyte adhesion to endothelium has been determined to be critical in the inflammatory process, and because they have been studied the most in adult rheumatic diseases, thus allowing comparison: ICAM-1, E-selectin, L-selectin, ICAM-3, and VCAM-1[8].

MATERIALS AND METHODS

Patients. A total of 18 children were studied retrospectively based on availability of banked sera. Eight children had SLE, 2 had mixed connective tissue disease (MCTD), 4 had dermatomyositis (DM), and 4 had various forms of systemic vasculitis [one Wegener's granulomatosis (WG), 2 Behçet's disease (BD), one unspecified]. Age, sex, time since diagnosis, and medications were recorded. No patient had a concurrent infection, thrombosis, or other acute inflammatory condition, except one patient with DM who had inflamed skin around an area of calcinosis on her great toe, which was thought to possibly be partly due to cellulitis.

Evaluation of disease activity. *SLE*. Levels of C3, C4, and CH50 (total hemolytic) complement and antibodies to native DNA (anti-dsDNA) were recorded. Score on the Systemic Lupus Erythematosus Disease Activity Index (SLEDAI), a tool assessing current manifestations of active disease, was recorded. For the purpose of analysis, anyone with a SLEDAI score > 0 was considered to have active disease, with the exception that a patient was not considered active if they had depressed complement levels alone.

Dermatomyositis. Levels of creatine phosphokinase (CPK) and aldolase were recorded, as was von Willebrand factor (vWF) antigen activity. A composite strength score based on manual muscle testing was recorded. Strength in each of the 4 following muscle groups was recorded and rated on a scale of 0–5, as described: sternocleidomastoid, anterior deltoid, rectus abdominus, quadriceps femoris[9]. A rash score was also recorded, which rated 5 types of rash (Gottron's papules, periorbital heliotrope, periungual telangiectasias, calcinosis, and "other," including erythematous rash in "shawl" distribution, etc.) on a scale of 0–3. On this scale, 0 = no rash, 1 = minimal or atrophic rash, 2 = moderate rash, and 3 = severe rash.

MCTD. SLE or DM manifestations were recorded as above. Arthritis activity was measured by recording number of inflamed joints. Manifestations consistent with scleroderma (e.g., sclerodactyly, esophageal dysmotility) were recorded, but not specifically rated.

Vasculitis. Measurement of disease activity was disease-specific. For patients with BD this included rating of each of the primary manifestations (oral ulceration, genital ulceration, rash, uveitis, and "other": systemic involvement on a scale of 0–3 (absent, mild, moderate, severe). The patient with WG had each of 3 primary types of systemic involvement (upper respiratory, pulmonary, and renal) rated on a 0–3 severity scale as above. ESR and antineutrophil cytoplasmic antibody (ANCA) titers were also recorded. The patient with unspecified vasculitis had each of his manifestations (rash/nodules and constitutional symptoms) rated on a 0–3 severity scale as above, and a total inflamed joint score was also recorded. Based on activity of disease-specific manifestations, each of these patients was also then assigned a global disease activity score, rated on a scale of 0–4.

Laboratory testing. We measured levels of soluble forms of ICAM-1, ICAM-3, VCAM-1, E-selectin, and L-selectin by sandwich ELISA (R&D Systems, Minneapolis, MN, and Biosource International, Camarillo, CA, USA). ESR and complete blood count variables were measured in all patients by standard methods. Von Willebrand factor antigen activity was determined by ELISA.

Statistical analysis. Differences in soluble adhesion molecule levels among diagnoses were analyzed by analysis of variance (ANOVA) and between patients with active vs inactive disease by t tests. Correlations of soluble adhesion molecules with clinical variables were analyzed by linear regression analysis.

RESULTS

Patient demographics, clinical and laboratory variables, and patient medications at the time of study are presented in Table 1. This includes SLEDAI scores for patients with SLE, and strength and rash scores for patients with DM. As disease activity scoring was more complex and individualized in MCTD and vasculitis, only a global activity score is given in Table 1. Further details of disease activity in these patients are listed below.

MCTD. One patient had mild arthritis activity only (one swollen knee) and mild sclerodactyly. She had previously had active dermatomyositis–like manifestations, but at this time had normal rash and strength scores, with normal muscle enzymes. Complements were normal. SLEDAI yielded a score of 0, consistent with her previous lack of SLE manifestations other than lymphopenia and ANA positivity. ESR was also normal. She was given a global score of 1/4. The second patient had moderately active polyarthritis in about 25 joints. She had mild sclerodactyly and a mild restrictive defect on pulmonary function tests. She had a SLEDAI score of 6 for her SLE-like manifestations (arthritis and mouth ulcers), but had normal complement levels. She had never had myositis. Her ESR was 33. She was given a global score of 2/4.

Vasculitis. The patient with WG had clinically quiescent pulmonary disease, although some residual pulmonary nodules and interstitial abnormalities were noted by chest radiograph and computed tomography. Similarly, previous renal biopsy had documented necrotizing glomerulonephritis, but her urine sediment was currently inactive

Table 1. Demographics, clinical and laboratory variables, and medications according to diagnosis group. See text for explanation of scoring of disease activity.

	SLE	MCTD	Juvenile DM	Vasculitis
N	8	2	4	4
Age, yrs	14.6 (5–21)	11.5 (11–12)	11 (6–15)	14 (11–17)
Sex, F:M	7:1	2:0	3:1	3:1
Disease duration, yrs	5 (1–17)	1.3 (1–2.3)	3.5 (0–6)	1.7 (0.5–3.5)
WBC, × 10^3 cells/mm^3	6.4 (4–11)	4.5 (4.2–4.7)	7.2 (6–8.2)	7.6 (4.6–12.2)
ESR, mm/h	15 (4–38)	24 (15–33)	8 (4–10)	14 (4–33)
C3, IU	0.97 (0.6–1.24)			
C4, IU	0.16 (0.1–0.21)			
CH50, IU	182 (95–239)			
SLEDAI score	4.6 (0–16)			
CPK, IU			121 (63–184)	
Aldolase, IU			9.7 (5.3–14.5)	
Strength score			14–19 (16.8)	
Rash score			4.3 (3–6)	
Global score		1.5 (1–2)		2.3 (2–4)
VWF:AG, % activity			147 (119–176)	
Medications				
Prednisone	6	1	2	2
Hydroxychloroquine	3	2		
Cyclophosphamide	2			1
Chlorambucil				1
Naproxen		1		
None	2		2	

Data in parentheses are ranges.

and her serum creatinine was mildly elevated but stable at 1.3 mg/dl. She had never had upper respiratory disease. Her ANCA test was positive in a titer of 1:16 in a cytoplasmic pattern. ESR was mildly elevated at 33. She was given a global activity score of 2/4.

Of the 2 patients with BD, one had active uveitis and genital ulcers. The other had skin pustules and 2 arthritic joints, with a positive ANCA in a perinuclear pattern in a titer of 1:256. Global activity scores were 3/4 and 2/4, respectively.

The last patient, with an undifferentiated systemic vasculitis, had active polyarthritis and nodular and leukocytoclastic skin vasculitis, with low grade fevers at the time of study. Global activity score was 2/4.

ELISA results are presented in Table 2. "Normal" refers to range of values in a group of normal, healthy adult subjects tested by the kit manufacturers (personal communication with R&D Systems and kit package inserts). In summary, relative to normal, ICAM-1 was elevated in all diagnoses except SLE, L-selectin was elevated in all diagnosis groups, E-selectin was elevated in MCTD and vasculitis, ICAM-3 was normal in all groups, and VCAM-1 was elevated in DM and MCTD. When results among groups were analyzed by ANOVA, there was a trend toward E-selectin levels being elevated in vasculitis vs other diagnoses ($p < 0.08$). When results were compared across all diagnoses between patients with active vs inactive disease, only ICAM-1 differed significantly ($p < 0.05$; Table 3). There was no difference in adhesion molecule levels according to medications used [prednisone vs no prednisone, immunosuppressive agents (cyclophosphamide, chlorambucil) vs no immunosuppressive agents].

When soluble adhesion molecule levels were compared to other laboratory variables, the following significant

Table 2. Soluble adhesion molecule levels by diagnosis. All values are ng/dl. Mean values (ranges in parentheses) are given for pathologic diagnoses, mean ± SD for normal values.

Diagnosis	ICAM-1	L-selectin	E-selectin	ICAM-3	VCAM-1
SLE	245.3 (61–465.4)	1108.4 (544.7–1532.4)	41.1 (36.2–54.1)	41.6 (12.4–68.2)	640.2 (327.4–1158.8)
MCTD	684.8 (329.3–710.9)	1816.2 (1332.1–2300.3)	51.4 (47.4–55.3)	53.4 (50.7–56.1)	1103.9 (915.2–1292.5)
DM	365.6 (260.9–465.8)	1412.8 (611.1–2034.7)	47.6 (30.5–64.3)	51.8 (30.5–64.3)	888.7 (580.3–1095.4)
Vasculitis	355.3 (122.8–559.4)	1485.3 (879.1–2372)	57.2 (29.1–62.1)	41.9 (24.8–62.1)	768.1 (434.9–1028.3)
Normal	230 ± 47	842 ± 169	31.6 ± 12.8	50 ± 13.8	553 ± 160

Table 3. Levels of soluble adhesion molecules (ng/ml) in patients with active vs inactive disease across all diagnoses. Only levels of sICAM-1 were significantly different in active disease.

	Active	Inactive	p
ICAM-1	398.4 (74.1–710.9)	230.1 (61–462.4)	0.05*
E-selectin	50.3 (35.9–73.8)	42.5 (36.2–54.1)	0.11
L-selectin	1491.7 (683.9–2300.3)	1097.8 (544.7–1721)	0.13
ICAM–3	48.8 (24–64.3)	39.6 (12.4–68.2)	0.35
VCAM-1	862.9 (327.4–1292.5)	636.4 (334.7–1158.8)	0.11

* Statistically significant.

correlations were found. L-selectin levels correlated with C4 complement levels in SLE (r = 0.76, p < 0.03), and E-selectin levels showed a trend toward an inverse correlation with vWF:Ag levels in DM (r = –0.93, p < 0.08).

DISCUSSION

Infiltration of leukocytes from the peripheral circulation into tissues is key to the inflammatory response. This process is primarily facilitated by adhesion molecules, a group of receptors and counter-receptors on a variety of cells including leukocytes and endothelium. Soluble forms of these molecules are generally formed by proteolytic cleavage of the extracellular domains of these molecules. Although their precise role is unclear, they provide a potentially useful reflection of this aspect of the inflammatory process that is easily accessible for study. As we had done in JRA, we performed this pilot study of soluble adhesion molecule levels in a variety of other pediatric rheumatic diseases to assess the role of these molecules in the pathophysiology of these conditions. Further, since others have sometimes found levels of these molecules to correlate with clinical events or disease activity, we also investigated whether soluble adhesion molecules correlated with disease activity in the studied conditions.

Our results lead us to speculate the following. First, the correlation of soluble L-selectin levels with C4 complement levels in SLE may indicate that downregulation of shedding of cell surface L-selectin is involved in continued adherence of leukocytes to endothelium, causing further damage and complement activation/immune complex deposition in SLE. This is similar to observations by Kanekura, *et al* in Kawasaki disease[10], and Blann, *et al* in systemic sclerosis and various vasculitic diseases, that showed low levels of sL-selectin during active disease periods[11]. Indeed, in Kawasaki disease, the lowest L-selectin levels were found in patients with coronary artery aneurysms, the gravest complication of this condition. This concept is supported by functional studies, such as that in which stimulation of nociceptive neurons in an animal model led to increased shedding of L-selectin from circulating neutrophils, and there was decreased accumulation of neutrophils in tissues, indicating a downregulated inflammatory response[12]. Similarly,

immune complexes from adult patients with rheumatoid vasculitis have the ability to downregulate expression of L-selectin on neutrophils[13]. It is thus possible that sL-selectin could serve as a useful marker of active versus quiescent disease in systemic lupus, although there was not a direct correlation of these levels with the SLEDAI in our patients.

The trend toward inverse correlation of soluble E-selectin and vWF in DM is curious. In vasculitic conditions, such as Kawasaki disease, both molecules are often highly elevated, and correlate positively with each other[3]. In juvenile DM specifically, vWF:Ag is often elevated, but not consistently so[9]. One study of E-selectin in adult inflammatory myositis found elevated levels in active disease that correlated with levels of CPK[14]. Also, these molecules do not always correlate with each other in inflammatory conditions. For example, in Henoch-Schönlein purpura and hemolytic-uremic syndrome, both conditions that involve considerable endothelial activation, vWF:Ag was elevated, but sE-selectin was not[15,16]. Similar findings have been reported in a number of studies of nonrheumatic conditions in which adhesion molecules and endothelial activation play an important role, including stroke, diabetes mellitus, peripheral vascular disease, menopause, and transplant rejection[17-22]. Authors of these studies propose that both molecules may be elevated in diseases that involve more overt vascular damage, while disparate results in tests of endothelial activation may reflect different degrees or mechanisms of endothelial activation in these conditions. Also, it is possible that, as in our patients, E-selectin is more elevated in active myositis, while vWF:Ag is more of a marker of active skin disease. Since in our study patients with active DM had the least active myositis, a spurious inverse correlation between the 2 molecules may have been produced. Further study of subjects with juvenile DM will be needed to clarify these observations.

The elevated levels of sE-selectin in vasculitis are intuitive, given the high degree of endothelial activation and overt vascular inflammation and damage occurring in these diseases. They are consistent with results in adults with vasculitic conditions such as WG[6].

Levels of soluble ICAM-1 may be a useful marker of active vs quiescent disease in general in the pediatric rheumatic diseases. When data from patients with all diagnoses were analyzed dichotomously, patients with active disease had higher levels of sICAM-1 vs patients with inactive disease. However, since there was no direct correlation with disease activity measures such as the SLEDAI in patients with SLE, these levels might not be sensitive enough to routinely detect or predict smaller but clinically important changes in disease activity. This is consistent with data from a variety of conditions, most notably Wegener's granulomatosis[6].

There are some weaknesses of this pilot study. The number of subjects was small. The retrospective, cross

sectional design makes applicability of results to general, prospective clinical use difficult. Similar pilot studies in adults have sometimes shown promising results that have not necessarily been substantiated by further, in-depth studies[6]. Yet the study of sICAM-1 in pediatric SLE[5] tends to confirm our findings about ICAM-1 as a marker of disease activity. We also did not use pediatric controls. However, previous studies have shown that normative adult values are applicable to children who are at least 6 years old, and only 1/18 of our patients was < 6 years old[3,23].

The design of this pilot study precludes any definite conclusions about the role of soluble adhesion molecules in pediatric rheumatic diseases. However, it provides some insights into possible pathophysiologic mechanisms in these diseases. This study also identifies possible candidates for markers of disease activity in these conditions. In particular, soluble ICAM-1 levels may help differentiate active from quiescent disease.

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