Rapid Publications

Elevated Levels of Circulating Adhesion Molecules in IDDM Patients and in Subjects at Risk for IDDM

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Serum levels of recently discovered circulating forms of adhesion molecules, ICAM-1 and L-selectin, were found to be elevated in IDDM patients and in subjects at risk for developing IDDM compared with 100 normal, nondiabetic blood donors. Both adhesion molecules were determined by sandwich ELISA. Serum concentrations of either cICAM-1 or cL-selectin were >2SD of normal mean in 10 of 14 recent-onset IDDM patients ($P < 0.05$). Serum levels of cICAM-1 and cL-selectin did not correlate. In first-degree relatives, elevated adhesion molecule levels were observed in the 6 ICA$^+$ individuals and in the ICA$^-$ individuals all ($n = 14$) with a genetic risk of IDDM (sharing HLA-DR3 and/or -DR4 with the diabetic relative) but not in the HLA-DR3$^-$ and/or -DR4$^-$ relatives ($n = 13$). We conclude that elevated cICAM-1 and cL-selectin levels occur independently of ICA status and probably reflect ongoing immune processes in recent-onset IDDM patients and first-degree relatives at risk for IDDM.
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Adhesion of leukocytes to endothelium is an important step for migration into inflamed tissue. Several adhesion molecules have been identified and characterized over the past few years. ICAM-1 is a member of the immunoglobulin superfamily and can occur on various types of cells. Mediators of inflammation, such as LPS, IFN-γ, IL-1, and TNF-α, cause an induction of ICAM-1 expression on various cell types at sites of inflammation (1–3). Recently, a circulating form of ICAM-1 has been described (4). L-selectin (LECAM-1, MEL-14) is one of three membrane glycoproteins with an NH_2-terminal lectin domain. It is expressed on leukocytes, and it facilitates lymphocyte homing to peripheral lymph nodes as well as neutrophil and lymphocyte emigration at inflammatory sites (1–3).

The involvement of adhesion molecules in inflammatory processes has led us to characterize adhesion molecule expression in IDDM. In recent-onset IDDM patients, we observed the reduced expression of ICAM-1 and integrins on monocytes (5). This study describes an analysis of non–cell-bound adhesion molecules in recent-onset IDDM and in subjects at risk for IDDM.

RESEARCH DESIGN AND METHODS

Sera from first-degree relatives of IDDM patients were selected from the serum bank of the Düsseldorf-Essen Family Study according to their ICA status. Recent-onset IDDM patients are consecutive cases from the clinical department of the Diabetes Research Institute Düsseldorf. Blood was taken within 7 days after presentation. Normal values were defined by using sera of 100 healthy, nondiabetic blood donors from the Bone Marrow Transplant Unit Essen. All sera were stored at −20°C and tested in parallel. Basic characteristics of patients and probands are shown in Table 1.

Three groups—14 patients with recent-onset IDDM (mean age 25.9 yr; 8 men, 6 women), 27 first-degree relatives of IDDM patients without detectable ICAs (mean age 24.5 yr; 11 men, 16 women), and 6 first-degree relatives with ICA (mean age 8.3 yr; 1 boy, 5 girls)—were compared with healthy blood donors (mean age 25.7 yr; 58 men, 42 women). Four of the ICA$^+$ first-degree relatives had developed diabetes 2–20 mo after obtaining the serum sample tested here. ICAs were measured

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IDDM, insulin-dependent diabetes mellitus; ICAM-1, intercellular adhesion molecules; cICAM-1, circulating ICAM-1; cL-selectin, circulating L-selectin; HLA, human leukocyte antigen; ICA, islet cell antibody; IFN-γ, interferon-γ; IL-1, interleukin-1; TNF-α, tumor necrosis factor-α; JDF U, Juvenile Diabetes Foundation unit; DPBS, Dulbecco's phosphate-buffered saline; BSA, bovine serum albumin; ABTS, 2,2-azino-di(3-ethylbenz thiazoline) sulfonic acid.

TABLE 1
Characteristics of patients and probands

	n	Age (yr) mean (range)	Sex ratio m/f
Healthy blood donors	100	25.7 (18–32)	58/42
Recent-onset IDDM patients	14	25.9 (13–44)	8/6
First-degree ICA$^-$ relatives of IDDM patients	27	24.5 (1–57)	11/16
First-degree ICA$^+$ relatives of IDDM patients	6	8.3 (3–19)	1/5

by indirect immunofluoresence on sections of fresh frozen human pancreases of blood group O, as described previously (6). Results were expressed in JDF Us using the ICA workshop standard serum, and values >5 JDF U were considered positive. HLA-DR typing was performed by standard microlymphocytotoxicity technique (7) with B-lymphocytes enriched by nylon wool columns. cICAM-1 was measured as described previously (2).

To measure cL-selectin, DREG-55 and DREG-200 MAb directed against L-selectin were prepared as described previously (8). A capture ELISA for the detection of cL-selectin has been described in detail (T.K.K., R.R., E.A.M., unpublished observations). Briefly, anti–L-selectin mAb, DREG 55(2.5 μg/ml) was added to 96-well flat-bottom E.I.A. microtiter plates (Limbro) at 50 μl/well at room temperature for 1 h. Wells were washed three times with DPBS and then blocked with 200 μl of 2% BSA-DPBS for 1 h at 37°C. Wells were flicked empty, and a titration of standard sera (twofold dilutions 1:4–1:32) and serum samples (diluted in 1% BSA-DPBS) were added (50 μl/well) in duplicate for 1 h at 37°C. Wells then were washed three times with DPBS. The biotinylated anti–L-selectin mAb (DREG 200) was added at 0.63 μg/ml (50 μl/well) for 30 min at 37°C. Wells were washed three times with DPBS. Horseradish peroxidase-conjugated streptavidin (Zymed, San Francisco, CA) (1:4000) was added (50 μl/well) for 30 min at 37°C. Wells were washed three times with DPBS and once with ABTS substrate buffer (Zymed). Then, ABTS substrate was added (50 μl/well), and the plates were read on a Molecular Devices reader (410 nm) until maximum OD readings were obtained. Mean OD readings were calculated, and the cL-selectin levels were expressed as the ratio to a standard control sera and as cL-selectin units.

Statistical analyses. The χ^2 test with α and contingency correction or Student's *t* test were performed as appropriate to test for statistical significance of differences between groups. We used Statview 512+ and StatWorks software packages on an Apple Macintosh.

RESULTS

Results are as follows: First, mean serum levels of cICAM-1 were 153 ± 54.4 ng/ml in 100 blood donors, the upper limit of the normal range was 262 ng/ml (mean ± 2 SD) (Fig. 1*A*). cICAM-1 levels above the normal range were observed in 4 of 14 recent-onset IDDM patients, in 3 of 6 ICA$^+$ first-degree relatives, and 13 of 27 ICA$^-$ first-degree relatives (χ^2 test, $P < 0.05$ for all groups vs. controls).

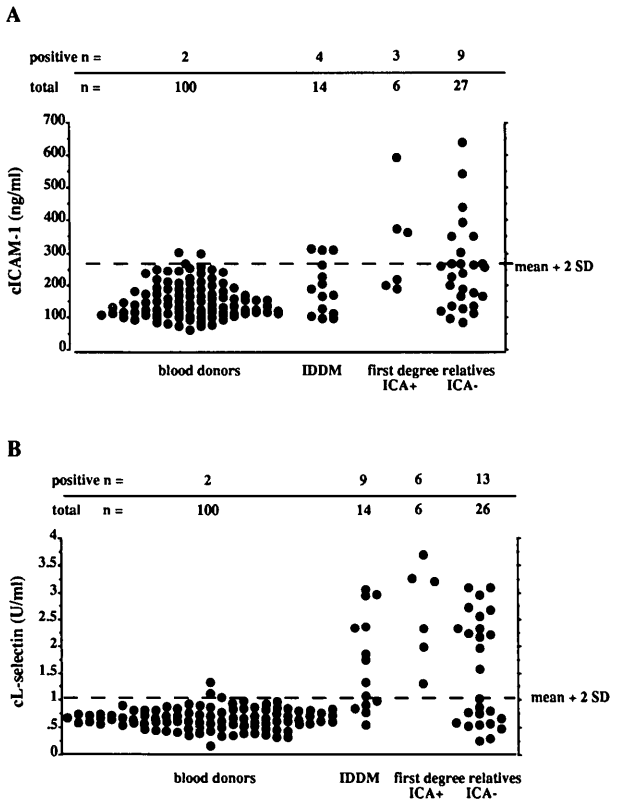

FIG. 1. Individual results of cICAM-1 (*A*) and cL-selectin (*B*) measurements in healthy blood donors, IDDM patients, and diabetes-related risk groups. (– – – –), upper limit of normal (mean ± 2 SD of control subjects).

Second, similar results were obtained with cL-selectin. Mean serum levels of cL-selectin in blood donors were 0.66 ± 0.21 U/ml with an upper limit of the normal range at 1.07 U/ml (Fig. 1*B*). Elevated cL-selectin levels were observed in 9 of 14 recent-onset IDDM patients, in 6 of 6 ICA$^+$ first-degree relatives, and in 14 of 27 ICA$^-$ first-degree relatives (χ^2 test, $P < 0.05$ for all groups vs. controls).

Third, a combined analysis of cICAM-1 and cL-selectin shows that elevated levels of either of the adhesion molecules occur in 4% of normal individuals, 71% of IDDM patients, 100% of ICA$^+$ first-degree relatives, and 52% of ICA$^-$ first-degree relatives.

Serum levels of cICAM-1 and cL-selectin do not correlate (Fig. 2, $R^2 = 0.36$). However, the lack of correlation is only partial, that is, elevated cL-selectin levels may occur beside normal cICAM-1 levels. All subjects with extremely high (>3 SD) levels of cICAM-1 also had extremely high (≥3 SD) cL-selectin levels ($P < 0.05$).

The group of 27 ICA$^-$ first-degree relatives was divided into two subgroups by HLA typing: 14 subjects shared either HLA-DR3 and/or -DR4 with the diabetic relative and thus carried some genetic predisposition for IDDM, 13 subjects were negative for HLA-DR3 and/or -DR4. Elevated levels of circulating adhesion molecules occurred only in the HLA-DR3 and/or -DR4 subgroup

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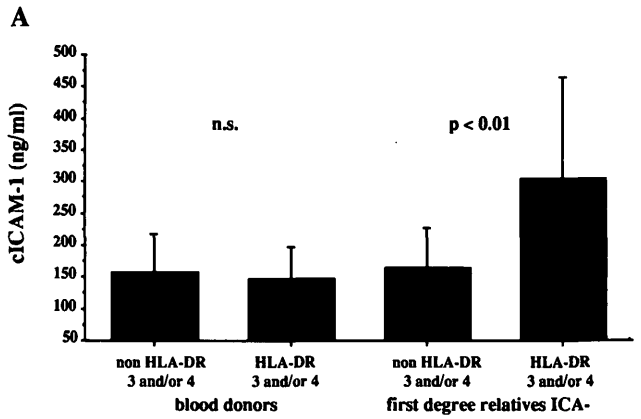

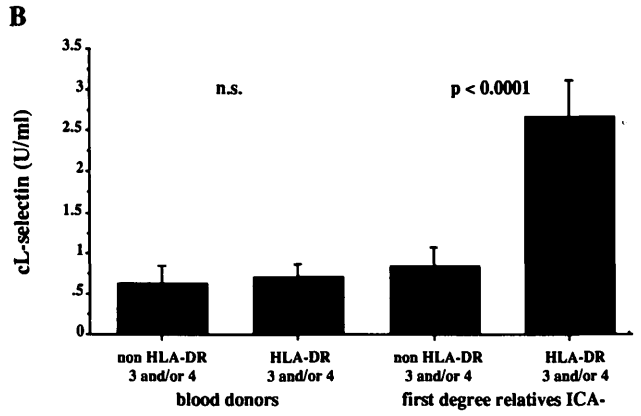

FIG. 2. Comparison of cICAM-1 (*A*) and cL-selectin (*B*) levels in healthy blood donors and first-degree ICA$^-$ relatives of IDDM patients, with respect to their HLA-DR types. cICAM-1 and cL-selectin were significantly elevated in HLA-DR3$^+$ and/or DR4$^+$ first-degree relatives of IDDM patients (by Student's *t* test).

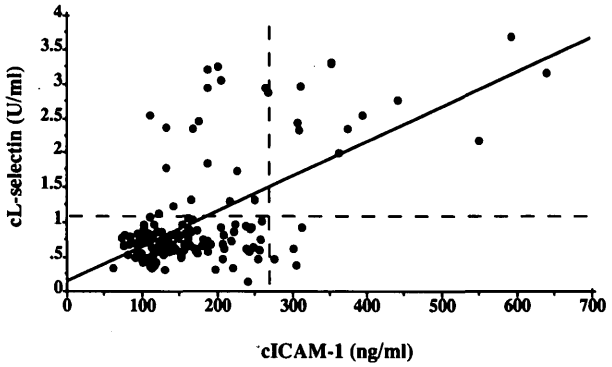

FIG. 3. cICAM-1 and cL-selectin values from all groups are not correlated (R^2 = 0.36, regression line: $y = 0.005x + 0.143$). (– – –), upper limit of normal (mean ± 2 SD of control subjects).

(Fig. 3). This was most impressive with cL-selectin, where all 14 HLA-DR3$^+$ and/or -DR4$^+$ individuals were above the normal range, whereas the other subjects were in the normal range. An interaction between HLA-DR3 and/or -DR4 and adhesion molecule levels was not observed in the normal blood donors (Fig. 3).

DISCUSSION

In this study, we have shown that cICAM-1 and cL-selectin levels are increased in patients with recent-onset IDDM and in subjects at risk for IDDM.

We measured a cohort of 100 healthy blood donors for serum cICAM-1 and cL-selectin. This enabled us to define normal ranges for healthy adults. The upper limit of the normal range for cICAM-1 was 261 ng/ml, which is similar to that described previously (4). Published data for comparison of the normal range of cL-selectin levels as described herein are unavailable. Both serum L-selectin and cICAM-1 appeared stable in spite of repeated freezing and thawing of sera (at least three times).

Elevated cICAM-1 has been described during ongoing inflammation or tissue damage (4). The highest cICAM-1 we have observed was in a patient with recent-onset IDDM and acute pneumonia (boy, 12 yr old, cICAM-1 of 1270 ng/ml, cL-selectin of 3.16 U/ml—excluded from study because of infection). In the absence of any known infection, we observed elevated (>2 SD of normal mean) levels of cICAM-1 in 4 of 14 and cL-selectin in 9 of 14 newly diagnosed IDDM patients. To our knowledge, this is the first report of increased cICAM-1 and cL-selectin in an autoimmune disorder.

Probands with increased risk of diabetes can be defined by the appearance of ICA and by genetic relation to a subject with IDDM. We have grouped our probands according to this criteria. In the extremely high-risk group (ICA$^+$ first-degree relatives), numerous individuals had elevated cL-selectin and cICAM-1 levels (6 of 6 and 3 of 6, respectively). Similarly, ICA$^-$ first-degree relatives exhibited raised cL-selectin and cICAM-1 levels (14 of 27 and 9 of 27, respectively). When we split this group further into those who share the risk genes HLA-DR3 and/or -DR4 with the diabetic relative and those who do not, all subjects with increased genetic risk had elevated cL-selectin levels, and 9 of 14 also had elevated cICAM-1 levels. Blood donors with HLA-DR3 and/or -DR4 did not have abnormal levels of adhesion molecules. This indicates that no interaction exists between HLA-DR type and cICAM-1/cL-selectin. Rather, the elevated adhesion molecule levels correlate with the genetic risk of IDDM. The lack of dramatic difference between ICA$^-$ and ICA$^+$ first-degree relatives and IDDM patients suggests that the changes observed may be genetic rather than associated with active autoimmunity. cICAM-1 and L-selectin thus may qualify as new markers of diabetes risk in IDDM, independent of ICA status. Currently, little is known about a possible functional role of soluble adhesion molecules.

Soluble ICAM-1, after shedding from melanoma cells, abrogates non–MHC-restricted cytotoxicity by NK and lymphokine-activated killer cells (9). This may be one mechanism by which neoplastic cells escape immunosurveillance. Similarly, soluble L-selectin chimera inhibit leukocyte accumulation at extravascular sites in vivo (10). Thus, both cICAM-1 and cL-selectin may interfere with lymphocyte homing and leukocyte extravasation.

A major source of cICAM-1 are mononuclear cells. In a previous study, we showed that expression of adhesion

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molecules on monocytes is decreased in recent-onset IDDM (5). In light of this study, both the elevation in serum and reduced expression on cell surfaces can result from increased shedding of ICAM-1. It already has been shown that cL-selectin is cleared from activated neutrophils (11).

Therefore, we assume that the increased levels of cICAM-1 and cL-selectin in IDDM and in subjects at risk for IDDM reflect ongoing immune processes during which soluble adhesion molecules are released. Further studies are needed to determine the time course of cICAM-1/cL-selectin production during prediabetes and to analyze for similar abnormalities in other patient cohorts.

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