Serum Levels of Soluble Intercellular Adhesion Molecule 1 Are Increased in Chronic B-Lymphocytic Leukemia and Correlate With Clinical Stage and Prognostic Markers

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The serum levels of soluble intercellular adhesion molecule 1 (ICAM-1) were significantly elevated ($P < .001$) in patients with chronic B-lymphocytic leukemia (B-CLL, $n = 113$) compared with healthy controls ($n = 31$). sICAM-1 levels in B-CLL were positively correlated to the tumor mass as reflected by the modified Rai and the Binet staging systems, lymphocyte counts, and isolated splenohepatomegaly. During disease progression or regression on cytoreductive therapy, the circulating sICAM-1 levels changed accordingly. sICAM-1 was also correlated to a kinetic parameter such as the lymphocyte doubling time. Furthermore, the serum sICAM-1 levels were inversely correlated to hemoglobin levels in patients with early clinical stage, and this may turn out to be of prognostic value. sICAM-1 was compared with other serum markers said to reflect disease activity in B-CLL, ie, soluble CD23, thymidine kinase, lactate dehydrogenase (LDH), and $\beta_2$-microglobulin. sICAM-1 was equally well or better correlated to clinical stage and lymphocyte doubling time. In univariate regression analysis, all serum markers but LDH correlated with survival, and in multivariate analysis, sICAM-1 was the only marker approaching significance for additional prognostic information when included after clinical stage and lymphocyte doubling time. Based on the present observations, it appears that prospective studies repeatedly monitoring serum sICAM-1 in B-CLL are justified.

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CHRONIC B-LYMPHOCYTIC leukemia (B-CLL)—the most common type of leukemia in Western countries—represents a monoclonal expansion of phenotypically small, slightly immature, and activated CD5$^+$ B cells expressing surface IgM frequently specific for autoantigen. The clinical course of B-CLL is quite variable, and consequently, attempts have been made to find methods for the prediction of prognosis and timing of therapy. Critical reviews of these methods have clearly established the value of clinical staging systems.$^6$ However, among B-CLL patients representing low-risk stages (Rai 0, Binet A), some 20% will progress more rapidly, and other methods predicting progression are needed.$^2$ Additional independent prognostic information may be obtained by determination of the blood lymphocyte doubling time,$^7$ hemoglobin (Hb) level,$^2$ marrow histopathology,$^5$ lymphocyte morphology,$^5$ cytogenetic patterns,$^4$ and probably also the blood lymphocyte count.$^3$ A number of serum markers including lactate dehydrogenase (LDH),$^5$ $\beta_2$-microglobulin ($\beta_2$m),$^5$ thymidine kinase (TK),$^8$ soluble CD23 (sCD23),$^9$ and recently, soluble CD27 (sCD27)$^{10}$ were reported to be positively correlated to clinical stage, but need to be evaluated further.

The intercellular adhesion molecule 1 (ICAM-1, CD54), a 90-kD member of the Ig supergene family, is the ligand for the integrin lymphocyte function-associated antigen 1 (LFA-1, CD11a/CD18).$^{11,12}$ ICAM-1/LFA-1 are widely distributed among hematopoietic and nonhematopoietic cells and regulate important cell-stromal and cell-cell interactions such as leukocyte adhesion and migration, mitogen- and allotype-induced T-cell proliferation, T/natural killer (NK) cell killing, and metastasis.$^{13,14}$ Recently, an 82-kD soluble form of ICAM-1 (sICAM-1) was detected in serum of normal subjects as well as patients with inflammatory conditions such as allograft rejection, rheumatoid arthritis, systemic lupus erythematosus, and Wegener’s granulomatosis.$^{15,16}$ Elevated levels of sICAM-1 were also reported in malignant disorders including melanoma, Hodgkin’s disease (HD), and childhood acute lymphoblastic leukemia (ALL).$^{17,18}$

We report here that serum sICAM-1 levels are elevated in B-CLL and correlate with the clinical stage, lymphocyte doubling time (LDT), isolated hepato/splenomegaly, and Hb level. The prognostic value of sICAM-1 was further compared with known serum markers of disease activity such as sCD23, TK, LDH, and $\beta_2$m.

MATERIALS AND METHODS

Patients and Control Subjects

We studied 113 B-CLL patients (75 men and 38 women, mean age, 69; range, 44 to 88), all fulfilling the diagnostic criteria of the International Workshop on CLL (IWCLL).$^{19}$ Serum samples were collected at varying intervals after diagnosis (12 to 460 months) and stored at $-70^\circ$C until use. Seventy-one patients were untreated, five patients were splenectomized only (91, 76, 12, and 3 months before study), and 15 patients had received chemotherapy, but were off treatment (3 to 240 months). Two of the latter patients had been splenectomized (39 and 48 months earlier). Twenty-two patients were on chemotherapy (nine chlorambucil-prednisolone, five COP, two CHOP, two prednisolone, one cyclosporine-A, one teniposide, and one prednimustine). Three of these cases had been splenectomized (60, 12, and 2 months before study). At the time of serum sampling, all 113 patients were restaged according to the systems of Rai et al.$^{20,21}$ and Binet et al.$^{22}$ The LDT’s were calculated retrospectively in the 76 untreated patients all having observation times in excess of 1 year. All 22 patients undergoing chemotherapy had LDT’s $<1$ year before treatment, and were classified accordingly. In the 15 cases receiving intermittent chemotherapy, LDT’s were estimated a minimum of 3 months after treatment. At the time of serum sampling, analysis of routine hematologic variables including determination of serum LDH ($n = 95$) and serum TK ($n = 74$) were performed. Repeated sampling of sera was done in 10 patients with disease progression ($n = 8$) or regression ($n = 2$).

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Serum samples from 31 healthy elderly blood donors (mean, 51 years; range, 40 to 60 years) were analyzed for comparison.

**Assays**

*siCAM-1.* The siCAM-1 levels in serum were determined by enzyme immunoassay (EIA) kits purchased from British Biotechnology Products Ltd (Oxon, UK). The assay is based on two monoclonal antibodies (MoAbs) directed against different epitopes on the siCAM-1 molecule. The capture MoAb is bound to solid phase and the detection MoAb is conjugated to horseradish peroxidase (HRP). 3, 3, 5, 5-tetramethylbenzidine (TMB) is used as substrate. The colored product was quantified photometrically using a spectrophotometer (Titertek Multiskan MCC/340, Solna, Sweden) set at 450 nm with a correction wavelength of 620 nm. Data were evaluated by Deltasoft 3.3 MCC software for the Macintosh (both from Apple Computers, Cupertino, CA). The sensitivity of the assay was 0.35 ng/mL, the interassay coefficient of variance (CV) was less than 7.4% and the intra-assay CV varied between 3.3% and 4.8%. Serum samples from 10 patients were tested fresh and after freeze thawing. The siCAM-1 levels were unaltered.

**β₂-microglobulin.** Soluble β₂m levels in serum were determined by EIA kits (T Cell Diagnostics, Inc, Cambridge, UK) based on a competitive assay in which HRP-labeled β₂m competes with cold serum ligand for binding to solid phase antiserum. After incubation with TMB, the colored product was measured using the spectrophotometer set at 450 nm. The sensitivity of the assay was 0.25 μg/mL, the intra-assay CV was less than 6% and the interassay CV was less than 13%.

*sCD23.* sCD23 EIA kits (The Binding Site, Birmingham, UK) were based on three antibodies. The capture MoAb is bound to solid phase, and detection involves sheep-antihuman sCD23 followed by HRP-conjugated antsheep IgG. TMB is used as substrate and the colored product is measured by spectrophotometry. The intra-assay precision CV was 4.2% and the interassay CV was 18.9%.

**Thymidine kinase.** TK is a routine analysis at our institution. In a radioenzymatic technique (Prolifigen assay; Sangtec Medical, Bromma, Sweden), TK converts [³²P] deoxyuridine into [³²P] deoxyuridine monophosphate, and the reaction product, but not the unconverted substrate, is absorbed to a granulate. The radioactivity of the granulate is then measured after several washings. The mean serum TK level in healthy adults was 2.4 ± 1.3 U/L. TK values above 5 U/L (mean + 2 SD) were considered elevated.

**Lactate dehydrogenase.** LDH is a routine analysis at our institution. LDH (EC 1.1.1.27) was analyzed on a Hitachi 717 spectrophotometer (Boehringer Mannheim Diagnostica, Titzing, Germany) using an LDH reagent from the same manufacturer (Catalogue No. 191 353 ). In short, LDH catalyzes pyruvate to lactate + nicotinamide adenine dinucleotide (NAD) in the presence of reduced NAD (NADH) + H⁺. Kinetic measurement of NAD at 340 nm proportionally reflects the concentration of LDH. The serum levels in healthy subjects ranged from 3.8 to 6.7 μkat/L.

**Statistical Methods**

Data are presented as mean ± SD. P values less than .05 were considered significant. Analysis of variance was used to compare serum siCAM-1 levels with the clinical classification systems of Rai and Binet. The paired Student’s t-test was used to compare serum siCAM-1 levels to lymphocyte counts, LDT, and Hb levels. Spearman’s correlation coefficient was used to determine the association between siCAM-1 and other serum markers, and the association between lymphocyte count and serum markers. The Cox proportional hazard regression model was used in univariate and multivariate analyses.

**RESULTS**

*Serum siCAM-1 Is Elevated in B-CLL.*

All 113 B-CLL patients and 31 control subjects had detectable serum siCAM-1 (Fig 1). Circulating siCAM-1 levels were significantly elevated (P = .0009) in the patient group (533 ± 293 ng/mL, mean ± SD) compared with the control group (329 ± 132 ng/mL), although values were widely scattered in B-CLL. The siCAM-1 levels in patients representing the lowest Rai stage 0 (395 ± 169 ng/mL) were not different from controls (P = .06). However, when comparing Binet stage A patients (412 ± 205 ng/mL) with controls, a significant difference was found (P = .03) (see below).

**siCAM-1 in Relation to Clinical Staging Systems**

When applying the modified Rai classification system (Fig 2), B-CLL patients representing high-risk stages had significantly higher (P < .0001) serum levels of siCAM-1 (711 ± 336 ng/mL) compared with intermediate-risk (443 ± 217)
Fig 2. Serum sICAM-1 in relation to the corrected Rai classification. B-CLL patients in high-risk stages have significantly higher (P < .0001) levels of sICAM-1 than patients in low- and intermediate-risk stages. Horizontal bars indicate mean values.

and low-risk (380 ± 185) patients. There was a trend toward higher sICAM-1 levels in the intermediate-risk versus low-risk group, but the difference was not statistically significant. Using the Binet staging system, we likewise found that the sICAM-1 levels in stage C (739 ± 335 ng/mL) were significantly elevated (P < .0001) compared with stages B and A (481 ± 245 and 448 ± 233, respectively). Again, no difference was found between stages A and B (data not shown).

Among B-CLL patients in Binet stage A, a survival advantage has been reported for patients with Hb values greater than 120 g/L compared with cases having Hb values 120 g/L or less.11 Therefore, we compared circulating sICAM-1 levels with Hb levels in 48 stage-A patients. Patients with Hb values ≥120 g/L had significantly higher (P = .001) serum sICAM-1 levels compared with patients having higher Hb values (583 ± 241 vs 366 ± 163 ng/mL) (Fig 3).

sICAM-1 Versus Lymphocyte Doubling Time, Lymphocyte Count, and Organomegaly

Patients having an LDT less than 1 year had significantly higher levels of serum sICAM-1 compared with cases having longer LDT’s (640 ± 331 vs 434 ± 213 ng/mL, P = .01) (Fig 4A).

Neither the modified Rai nor the Binet classification accounts for hepatomegaly and/or splenomegaly as single factor. Because this might be relevant when considering adhesion molecules and their soluble counterparts, we studied the relationship between splenomegaly/hepatomegaly and sICAM-1 (n = 39, of which only 4 had hepatomegaly, excluding splenectomized cases). Patients with organomegaly had significantly (P = .0006) higher levels of sICAM-1 (707 ± 317 vs 430 ± 240 ng/mL), as shown in Fig 4B.

A correlation existed between the lymphocyte counts and serum levels of sICAM-1 (r = .32, P = .0009) but data were widely distributed (not shown). Excluding patients on therapy or off treatment with cytostatics, the same pattern was seen (not shown).

Serial Determinations of sICAM-1

In 10 B-CLL patients, sera were obtained prospectively at 2 to 8 occasions and analyzed for sICAM-1. According to the original Rai classification, 8 of 10 patients exhibited a change in clinical stage (7 progressions and 1 regression on therapy). As shown in Fig 5, a switch in Rai stage was consistently paralleled by a corresponding change in serum sICAM-1 level in all individuals studied. The same results were registered using the Binet (9/10 changes in stage) and
sICAM-1 IS ELEVATED IN B-CLL

Fig 4. (A) Serum sICAM-1 levels are correlated with the lymphocyte doubling time (LDT; P = .01). (B) Patients with splenomegaly and/or hepatomegaly have higher serum levels of sICAM-1 than patients without organomegaly (P = .0006). Horizontal bars indicate mean values.

IWCLL 1992 (10/10 changes in stage) classifications (data not shown). One patient was treated with peroral 2-chlorodeoxyadenosine (CdA) × 8 according to a recent protocol. A good partial remission was recorded, the patient changing in stage from C(IV) to A(0) at the end of therapy. The serum sICAM-1 levels rapidly dropped to normal levels and stayed low during therapy (from 724 to 325 ng/mL). One B-CLL patient was monitored before and after splenectomy. Serum sICAM-1 rapidly dropped from 818 to 470 ng/mL after the operation (data not shown).

sICAM-1 Compared With Other Serum Markers

Among serum markers claimed to be associated with clinical stage and disease activity in B-CLL, we chose to consider sCD23, TK, β₂m, and LDH and compared these with sICAM-1 (Tables 1 through 3). The correlation between each individual serum marker and the corrected Rai classification is shown in Table 1. sICAM-1 was equal or superior to sCD23, TK, and LDH. No significant correlation between β₂m and corrected Rai stage was registered. Table 2 shows the correlation between each individual serum marker and the LDT. With the exception of LDH, the other serum markers (sICAM-1, sCD23, TK, and β₂m) were correlated to LDT, with marginal significance for β₂m. Table 3 shows the cross-correlations between serum levels of the five markers and the lymphocyte count. sICAM-1 was most strongly correlated to TK and sCD23, followed by β₂m and the lymphocyte count. No correlation was seen with LDH.

sICAM-1 and Prognosis

Table 4 shows the prognostic value (survival) of sICAM-1 and the four other serum markers in univariate and multivariate Cox regression analysis. A significant (P < .002) survival advantage was registered in patients with lower serum sICAM-1 levels. However, no cutoff value was found. With the exception of LDH, the other markers sCD23, TK, and β₂m also showed significant correlation to survival. Mul-
corrected Rai staging plus LDT versus each serum marker. The results reflecting disease activity have been proposed. These changes were identical, and therefore, only the analyses testing the only marker approaching significance (P < .0001) in the present patient material.

### DISCUSSION

The most commonly used methods for predicting prognosis in B-CLL are clinical staging systems that estimate the static tumor mass, whereas additional information is obtained by studying the kinetics of leukemic cell expansion, ie, lymphocyte doubling time, the lymphocyte and marrow morphology, and cytogenetic aberrations. The prediction of prognosis is particularly difficult in patients representing early clinical stages and serial monitoring of different serum markers and activated lymphocyte phenotypes reflecting disease activity have been proposed.

The present investigation is, to our knowledge, the first to show that serum levels of 82-kD sICAM-1 are elevated in B-CLL and correlate with survival in univariate analysis. However, elevated serum levels of sICAM-1 have recently been described in patients with other hematopoietic tumors such as HD and also in malignant melanoma. In HD patients, serum sICAM-1 levels were related to tumor mass, B symptoms, circulating interleukin-2 (IL-2) receptor (CD25) levels, and disease-free survival. In melanoma, increased sICAM-1 serum levels and tumor cell ICAM-1 expression were typical for metastatic disease. In the case of HD, it was shown by histochemistry that the malignant cells overexpress ICAM-1 and that HD cell lines release sICAM-1 when exposed to various cytokines.

In the present study, the serum sICAM-1 levels in B-CLL patients were positively correlated to the tumor mass as reflected by the modified Rai and Binet stagings, lymphocyte counts and isolated splenomegaly/hepatomegaly. In patients repeatedly monitored during disease progression or regression on cytoreduceptive therapy, the circulating sICAM-1 levels changed accordingly. sICAM-1 was also correlated to a kinetic parameter represented by the lymphocyte doubling time. This indicates that sICAM-1 may not simply reflect the static tumor mass in B-CLL. Further, the serum sICAM-1 levels were inversely correlated to Hb levels in patients with early clinical stage, and this may turn out to have prognostic significance. We also compared sICAM-1 with other serum markers said to reflect disease activity in B-CLL, ie, sCD23, TK, LDH, and β2m. sICAM-1 was equally well or better correlated with clinical stage and LDT. In univariate regression analysis, all serum markers but LDH correlated with survival, and in multivariate analysis, sICAM-1 was the only marker approaching significance (P = .08) for prognostic information when included after clinical stage and lymphocyte doubling time. Based on all the present observations, Table 3 shows that serum levels of sICAM-1 are elevated in B-CLL and correlate with survival in univariate analysis.
tions, we think that a prospective study repeatedly monitoring serum sICAM-1 in B-CLL is justified.

The cellular origin of sICAM-1 in B-CLL is presently unknown. Soluble ICAM-1 is thought to be released from the cell surface through proteolytic cleavage. Some 40% of B-CLL patients were reported to have surface ICAM-1+ leukemic cells in the blood. However, in our experience the surface expression of ICAM-1 on circulating B-CLL cells is low and poorly correlated with serum sICAM-1 levels (unpublished observations). Further, circulating B-CLL cells are arrested in the G0 phase of the cell cycle, and the proliferative tumor cell compartment is likely represented by the bone marrow, lymph nodes, and spleen. Several inflammatory mediators including IL-1, tumor necrosis factor α (TNFα), interferon γ, and IL-4 enhance cell surface expression of ICAM-1, and B-CLL cells have been reported to release at least TNFα, IL-1, and possibly IL-2. Furthermore, T-cells and NK-cells in these patients are phenotypically activated.

Thus, circulating sICAM-1 in B-CLL may originate from either tumor cells, regulatory cell types, or both, and this is presently under investigation.

ACKNOWLEDGMENT

We are grateful to Dr J. Bring and A. Taube at the Department of Statistics, Uppsala University, for their expert help with the statistical analysis.

REFERENCES

Serum levels of soluble intercellular adhesion molecule 1 are increased in chronic B-lymphocytic leukemia and correlate with clinical stage and prognostic markers [see comments]

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