High Levels of the Shed Form of L-Selectin Are Present in Patients With Acute Leukemia and Inhibit Blast Cell Adhesion to Activated Endothelium

By Olivier Spertini, Patrizia Callegari, Anne-Sophie Cordey, Jacques Hauert, Jean Joggi, Vladimir von Frieden, and Marc Schapira

Circulating leukocytes use several adhesion molecules to bind to the vascular endothelium, leave the bloodstream, and migrate into tissues. L-selectin plays a critical role in the initiation of normal leukocyte attachment to activated endothelium, whereas other receptors (including integrins and immunoglobulin-like adhesion molecules) are involved in leukocyte subsequent firm adhesion and transmigration into tissues.

The primary structures of L-selectin and other members of the selectin family have been determined. The various selectins have in common the presence of an N-terminal lectin domain, an epidermal growth factor-like domain, and short consensus repeats similar to those found in complement regulatory proteins. L-selectin is expressed on most normal leukocytes and mediates the initial step of adhesion to vascular endothelium. A feature of this adhesion receptor is to be shed from the cell surface. We report here the presence of high levels of the shed form of L-selectin (sL-selectin) in plasma from patients with acute leukemia. We also show that sL-selectin purified from acute leukemia plasma exhibits functional activity. The mean (+1 SD) plasma level of sL-selectin among 100 healthy individuals was 2.1 ± 0.7 μg/mL. This value was increased (≥2 SD above the mean) in 63% of 58 patients with acute lymphoblastic leukemia (ALL) and 59% of 93 patients with acute myelogenous leukemia (AML) (P < .001). Repeated measurements in 24 patients showed normal-range values in 16 of 18 patients in complete remission and high levels in eight of eight patients with therapy-resistant acute leukemia or leukemia relapse. Furthermore, elevated sL-selectin levels were detected in cerebrospinal fluid of three patients with ALL suffering from a relapse limited to the central nervous system. Epitope mapping with monoclonal antibodies demonstrated that L-selectin shedding from leukemic blasts was accompanied by conformational changes of its epidermal growth factor–like domain. A functional role for sL-selectin purified from leukemic plasma was supported by its ability to completely inhibit L-selectin–dependent adhesion of blast cells to tumor necrosis factor-α (TNF-α)-activated endothelium in vitro. These results suggest that sL-selectin may have an important role in the regulation of leukemic cell adhesion to endothelium. In addition, monitoring of the sL-selectin level may be useful for evaluating leukemia activity, in particular for the detection of leukemia relapse.

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Communications

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vous system intrathecal prophylaxis, whereas intensification therapy included a first course of cytosine arabinoside and daunorubicin followed by a second course of cyclophosphamide and mitoxantrone. Patients with relapsed AML or ALL were treated using relevant protocols.

**Immunophenotyping.** Mononuclear cells were prepared from heparinized peripheral blood or bone marrow samples by centrifugation on Ficoll-Hypaque (Pharmacia, Uppsal, Sweden). Cell surface antigens were detected by standard immunofluorescence methods using a large panel of directly fluorescein isothiocyanate– or phycoerythrin-conjugated monoclonal antibodies. The expression of surface antigens was studied in double immunofluorescence using antibodies that react with CD1, -2, -3, -4, -5, -7, -10, -13, -14, -15, -19, -20, -22, -25, -33, -34, -41, -61, and -71, glycoporphin, surface immunoglobulins, and HLA-DR (Becton Dickinson, Mountain View, CA; Coulter, Hialeah, FL; and Dako, Glostrup, Denmark). The expression of L-selectin on blast cells was assessed with the anti-LAM1-3 monoclonal antibody.22 Double immunofluorescence analysis was performed with an Epics flow cytometer (Coulter Electronics, Hialeah, FL). A total of 5,000 cells were analyzed for each sample.

Acetone-methanol–fixed blast cells were examined by light microscopy for the presence of terminal deoxynucleotidyl transferase, intracytoplasmic immunoglobulins, and CD3. Rabbit polyclonal antibody against terminal deoxynucleotidyl transferase (Supertechs, Bethesda, MD), monoclonal antibody against human immunoglobulin (Dako), anti-CD3 Leu-4 monoclonal antibody (Becton Dickinson), and alkaline phosphatase–labeled antimouse or antirabbit antibodies (Dako) were obtained from the designated suppliers.

**sL-selectin enzyme-linked immunosorbent assay.** sL-selectin was assayed using a sandwich enzyme-linked immunosorbent assay (ELISA).23 The anti-LAM1-5 monoclonal antibody was used as the capture antibody. The presence of sL-selectin was revealed with biotinylated anti–LAM1-3 monoclonal antibody and avidin-horse-radish peroxidase, with 0-phenylene-diamine (0.125%, w/vol) in citrate buffer, pH 4.5, as the substrate. Plasma samples were run in duplicate or triplicate and diluted at 1/100 to 1/2,000 to obtain a measure in the linear range of our assay. Absorbances at 405 nm were measured using an MR 700 Microplate ELISA reader (Dynatech, Embrach, Switzerland). The concentration of sL-selectin was determined using a standard curve constructed for each ELISA plate with a reference plasma. The sL-selectin level in the reference plasma was measured using a recombinant L-selectin/igg heavy-chain y1 chimeric molecule as the standard.23 This chimeric protein was produced in COS-1 cells transfected with pL-selectin/y1 cDNA subcloned into the ApaM8 expression vector (the vector was provided by Lloyd Klickstein, Center for Blood Research, Boston, MA). After transfection, the cells were cultured in Dulbecco’s minimal essential medium containing 5% fetal calf serum. The fusion protein was purified from culture medium by salt fractionation and immunoadsorption to immobilized anti–LAM1-3 monoclonal antibody. After extensive washing with phosphate-buffered saline containing 0.5 mol/L NaCl and 0.05% Tween 20, the fusion protein was eluted with 4 mol/L imidazole, pH 7.5. Sodium dodecyl sulfate (SDS) polyacrylamide gel (10%) electrophoresis and silver staining of the eluate showed a large band with an Mr of 180,000 and two additional bands with MrS of 70,000 and 45,000. By Western blot analysis, the band with an Mr of 180,000 was identified as the L-selectin/y1 heavy chain of IgG chimeras. Analysis of the gel with a Clinitiscan 2 Scanner (Helena Laboratories, Beaumont, TX) indicated that the band with an Mr of 180,000 represented 50% of the sample total protein. The protein concentration was determined with the Micro BCA Protein Assay Reagent (Pierce, BA oud Beijerland, Holland) with purified mouse immunoglobulin used as the standard.

With this ELISA, sL-selectin levels were linearly correlated with the mean absorbance of triplicate dilutions of the standard plasma over a concentration range between 0.005 and 0.060 μg/mL (n = 63, r² > 98). Samples anticoagulated with heparin or EDTA yielded identical results. Since sL-selectin concentration is stable in frozen plasma,29 most determinations were performed in samples kept at −80°C.

**Shedding of L-selectin by blast cells and lymphocytes.** Mononuclear cells were isolated by Ficoll-Hypaque density-gradient centrifugation from three normal blood donors and three patients with L-selectin acute leukemia, two with ALL and one with AML. The six isolates were incubated in RPMI 1640 supplemented with 2% fetal calf serum for 0, 2, 5, and 18 hours. At these various times, the suspensions were centrifuged for 30 minutes at 13,000 × g. Mononuclear cells obtained from patients with leukemia contained more than 95% of L-selectin blast cells. The cell suspensions isolated from normal donors were depleted of monocytes by adherence on plastic. Cell viability was greater than 90% after 8 and 18 hours of incubation, as determined by trypan blue exclusion. The concentration of sL-selectin and L-selectin in cell supernatants and cell lysates was determined by ELISA.

**Detection of sL-selectin by Western blot analysis.** Plasma samples (1 mL) from eight patients with AML and six patients with ALL were fractionated with Na2SO4 (18% wt/vol) and centrifuged for 20 minutes at 10,000 × g at room temperature. Supernatants were dialyzed against distilled water, pH 8.0. The samples were then incubated with constant rotation for 12 hours at 4°C with 100 μL anti–LAM1-3–Sepharose 4B. After extensive washing, sL-selectin was eluted with 4 mol/L imidazole, pH 7.5. After dialysis against phosphate-buffered saline, sL-selectin samples were electrophoresed on 7.5% SDS-polyacrylamide gels according to the method of Laemmli,24 and transferred onto 0.2-mm nitrocellulose filters (Bio-Rad Laboratories, Glattbrugg, Switzerland). The presence of sL-selectin was detected with the anti–LAM1-14 monoclonal antibody,22 peroxidase-conjugated goat antimouse immunoglobulin antibody (Dako), and enhanced chemiluminescence detection reagents (Amersham, Zurich, Switzerland). MrS were determined using molecular weight marker standards (Bio-Rad Laboratories).

**Endothelial–leukemic blast cell attachment assay.** The attachment of leukemic blasts to endothelial cells was determined using previously published procedures.9,29 Human umbilical vein endothelial cells (passage 2 to 3) were grown to confluence in 0.5% gelatin-coated Petri dishes (Becton Dickinson). After an 8-hour stimulation with 100 U/mL tumor necrosis factor-α (TNF-α) Boehringer, Mannheim (Germany), endothelial cell monolayers were incubated for 30 minutes with medium (RPMI 1640/5% fetal calf serum) alone or medium containing purified sL-selectin (5 μg/mL) or the purified Fab' fragment of the W6/32 anti-HLA class I monoclonal antibody (5 μg/mL). sL-selectin was purified by salt-fractionation and affinity chromatography from plasma obtained from three patients with ALL and three patients with AML, as described above. Leukemic blast cells were isolated by Ficoll-Hypaque density gradient centrifugation from six patients with L-selectin acute leukemia, two with ALL and four with AML. The six isolates contained greater than 95% of L-selectin blast cells. Leukemic blasts (4 × 10⁵) were preincubated for 15 minutes on ice in 100 μL of medium alone or containing 5 μg/mL of the purified Fab' fragment of the W6/32 anti-HLA class I monoclonal antibody or 5 μg/mL of the purified anti–LAM1-3 monoclonal antibody. After 15 minutes of preincubation at 4°C, leukemia blasts were added to the endothelial monolayers kept under rotation at 72 rpm. After 30 minutes at 4°C or 15 minutes at 37°C, nonadherent cells were discarded and Petri dishes were placed vertically in 2% glutaraldehyde and fixed overnight. After washing, the number of adherent cells was determined by counting eight to 12 microscopic fields (0.08 mm² per field). Results were expressed as the mean ± 1 SD.

**Statistical analysis.** The relationship between parameters was evaluated by determination of the Spearman correlation coefficient.
Selectin Levels in Acute Leukemia

**Results**

*SL-selectin levels in plasma of patients with acute leukemia.* Plasma sL-selectin levels ranged from 0.65 to 3.6 μg/mL (mean ± 1 SD, 2.1 ± 0.7 μg/mL) among 100 healthy blood donors, from 0.7 to 68 μg/mL (median, 4.1 μg/mL) among 93 patients with AML, and from 0.11 to 98.3 μg/mL (median, 4.6 μg/mL) among 58 patients with ALL (Fig 1). Thus, both AML and ALL patients had increased sL-selectin levels (P < .001). Moreover, 59% of the patients with AML (55 of 93) and 66% of those with ALL (38 of 58) had sL-selectin levels exceeding 3.5 μg/mL (mean ± 2 SD among healthy blood donors). The following French-American-British subtype distribution was observed among the 93 patients with AML: 15, M1; 46, M2; 6, M3; 16, M4; 9, M5; and 1, M6. Increased sL-selectin concentrations were seen with the M1, M2, M4, and M5 subtypes, whereas levels within the normal range were observed in all the patients (6 of 6) with acute promyelocytic leukemia (M3). An L-selectin level within the normal range was also found in the only patient with erythroleukemia (M6). Among the patients with ALL, 14 had T-ALL and 44 had lymphoblasts expressing B-lineage markers. Similar sL-selectin levels were measured in the two groups of patients with ALL.

The level of expression of L-selectin by blast cells was compared with the plasma concentration of sL-selectin in 56 patients with acute leukemia (44 patients with AML and 12 patients with ALL). Statistical analysis did not disclose a significant correlation between these two parameters (r = .06). As previously reported, blast cells from most patients with AML expressed low levels of the receptor. Thus, the median level of L-selectin expression in 35 patients with AML was 20% (range, 2.5% to 78%). Significantly higher levels of L-selectin were observed in T-ALL (median value, 70%; n = 7; P < .01).

Because sL-selectin could influence the final homing of blast cells by inhibiting their attachment to activated endothelium, the clinical characteristics (ie, presence of splenomegaly, hepatomegaly, enlargement of lymph nodes, skin or mucosal infiltration) of 41 patients with AML were related to the plasma concentration of sL-selectin at diagnosis. The 15 patients with splenomegaly had significantly higher plasma levels of the shed receptor than the 26 patients who did not have an enlarged spleen (P < .01). It was not possible to assess the impact of sL-selectin on the development of hepatomegaly and/or lymphadenopathies because only 7 patients were found to have enlarged liver or lymph nodes.

A weak correlation was noted between plasma sL-selectin levels in patients with acute leukemia and their venous blood blast cell counts (r = .47, n = 69, P < .01). Furthermore, observations made with 3 patients with aleukemic acute leukemia indicated that plasma sL-selectin reflected the total mass of blast cells in the body. Two patients with ALL who had no detectable blast cells in their peripheral blood had increased plasma levels of sL-selectin (5.2 and 17.4 μg/mL, respectively) and more than 50% lymphoblasts in the bone marrow. In addition, a patient with therapy-resistant aleukemic M1 AML was evaluated for 42 days. During the entire follow-up period, he had continuing marrow infiltration by leukemic blasts, almost no blast cells in the peripheral blood, and elevated sL-selectin levels (data not shown).

*SL-selectin levels and the clinical course of acute leukemia.* Thirteen patients with ALL and three with AML, all of whom had increased sL-selectin levels at diagnosis (from 4.5 to 73.5 μg/mL), were reevaluated after having entered a complete remission. In all the remission samples (16 of 16), the concentration of sL-selectin was within the normal range (<3.5 μg/mL; Fig 2). Additional samples were obtained from 5 of these 16 patients after their leukemia had relapsed;

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**Fig 1.** SL-selectin levels in plasma of 100 normal blood donors (○), 93 patients with AML (■), and 58 patients with ALL (□). Median values are indicated by horizontal bars.

**Fig 2.** SL-selectin levels in plasma of 16 patients with acute leukemia at diagnosis and at remission. The area under the broken horizontal line indicates the 95% confidence interval of SL-selectin concentration in normal plasma.
increased sL-selectin levels (from 4.8 to 16.2 μg/mL) were observed in all of them (5 of 5). sL-selectin levels and circulating blast cell counts of a patient receiving chemotherapy for relapsed pre-B-ALL are shown in Fig 3. Leukemia relapse was established on a bone marrow aspirate obtained 6 months after the patient had entered a complete remission. At relapse, no circulating leukemic blasts were detectable, but sL-selectin was increased to 9.4 μg/mL (Fig 3, day 1). A second remission, as determined by marrow examination at day 42, was observed after reinduction chemotherapy. Between day 2 and day 10, sL-selectin decreased from 17.9 to less than 3.5 μg/mL, and concentrations within the normal range were observed until day 80. The patient relapsed a second time at day 90, and complete remission could not be observed despite aggressive chemotherapy. sL-selectin remained elevated (>3.5 μg/mL) throughout the second relapse, although blast cells were not detectable in the peripheral blood from day 105 to day 128 (Fig 3).

**High concentrations of sL-selectin in the cerebrospinal fluid from patients with meningeal leukemia.** sL-selectin levels in the cerebrospinal fluid of 41 patients with various neurologic conditions (see Materials and Methods) ranged from 0.003 to 0.044 μg/mL (mean ± 1 SD, 0.020 ± 0.010 μg/mL). Similar sL-selectin concentrations were found in the cerebrospinal fluid of 23 patients with ALL without central nervous system involvement (mean ± 1 SD, 0.018 ± 0.010 μg/mL). Thirteen to 34 months after the initial diagnosis, 3 patients with ALL suffered from a relapse limited to the central nervous system, as assessed by the presence of leukemic lymphoblasts on cerebrospinal fluid examination. Cerebrospinal fluid samples obtained at that time contained increased levels of sL-selectin (0.066 to 0.202 μg/mL). In 1 patient, a cerebrospinal fluid sL-selectin increase (0.2 μg/mL) was observed 8 weeks before the appearance of clinical and cytologic signs of meningeal involvement. It should be emphasized that these 3 patients with relapses confined to the central nervous system had plasma sL-selectin levels within the normal range.

**sL-selectin is shed from leukemic blast cells.** Additional experiments were undertaken to study the shedding of L-selectin from leukemic blasts and to characterize the structural properties of sL-selectin in acute leukemia. The kinetics of L-selectin shedding were examined using L-selectin' blasts obtained from three patients with AML or ALL. After 5 to 18 hours of incubation, 50% to 70% of the L-selectin initially expressed at the surface of blast cells was released in the medium in the form of sL-selectin (Fig 4). For comparison, normal peripheral blood lymphocytes cultured under the same conditions released 30% to 40% of cell surface L-selectin. The molecular characteristics of sL-selectin in plasma samples collected from leukemic patients were evaluated by SDS-polyacrylamide gel electrophoresis and Western blotting using the anti-LAM1-14 monoclonal antibody directed against the sL-selectin short consensus repeat domain. Samples were obtained from 14 patients with acute leukemia, 6 with ALL and 8 with AML. In contrast to the pattern seen with normal plasma (which contains two sL-selectin isoforms mainly derived from neutrophils and lymphocytes), the various leukemic plasma samples contained only a single form of sL-selectin (Fig 5). The ALL samples contained an sL-selectin form with an Mr ranging from 60,000 to 90,000, whereas sL-selectin from AML plasma had an Mr ranging from 70,000 to 95,000 (Fig 5). These results are consistent with earlier data on the sL-selectin isoforms released from normal peripheral blood leukocytes, which indicated that sL-selectin from lymphocytes had an Mr of 62,000, whereas fragments shed from neutrophils had Mr ranging from 80,000 to 105,000. Additional information on the structure of sL-selectin was provided by epitope mapping. A series of binding studies used the anti-LAM1-18 and anti-LAM1-5 monoclonal an-
attachment of L-selectin+ blast cells to TNF-α-activated endothelium. The attachment of leukemic blasts was determined under rotation, since the L-selectin-mediated component of this reaction is better detected under nonstatic conditions. Only a few leukemic blast cells were attached to unactivated endothelium at 4°C (9 ± 7 blasts per field, n = 3) and at 37°C (21 ± 12 blasts per field, n = 3). On the other hand, endothelial cells activation with TNF-α for 8 hours induced a significant increase in leukemic blast cell attachment at 4°C (21- to 59-fold, n = 3) and at 37°C (eightfold to 39-fold, n = 3). In three experiments performed at 4°C, where the shedding of L-selectin is minimal and the family of β2-integrins are not active, the preincubation of blast cells with the anti-LAM1-3 monoclonal antibody inhibited the cytokine-induced increase in blast cell adhesion by 42% ± 7% (P < 0.005, n = 3). At 37°C, the anti-LAM1-3 monoclonal antibody inhibited it by 51% ± 5% (P < 0.005, n = 3). Under the same conditions, the preincubation of endothelium with 5 μg/mL purified sL-selectin inhibited 52% ± 5% of blast cell adhesion at 4°C (P < 0.005, n = 3) and 43% ± 10% at 37°C (P < 0.005, n = 3), indicating that the L-selectin-dependent adhesion of leukemic blasts was entirely prevented by the binding of the shed form of L-selectin to endothelium (Fig 6). Furthermore, these results indicate that L-selectin acts in conjunction with other adhesion receptors to mediate blast cell attachment to cytokine-activated endothelium. sL-selectin was purified from plasma obtained from three patients with AML and three patients with ALL. The different sL-selectin forms had the same capacity to inhibit blast cell adhesion to endothelium. Furthermore, the lymphoblastic and myeloid shed forms had the same inhibitory effect on the lymphoblast and myeloblast attachment to activated endothelium without respect to blast cell origin (data not shown). Additional experiments examined the effect of substituting L-selectin+ leukemic blasts for the L-selectin+ cells. The adhesion of L-selectin+ blast cells to the activated endothelium was not influenced by the presence of 5 μg/mL sL-selectin (data not shown). This observation further demonstrated that sL-selectin is a specific inhibitor for the attachment of L-selectin+ blasts to activated endothelial monolayers.

**DISCUSSION**

In this study we show that sL-selectin, the cleaved and shed form of the cell adhesion receptor L-selectin, is present at high concentrations in plasma samples of a majority of patients with AML or ALL. We also provide evidence that adhesion of L-selectin+ leukemic blasts to the cytokine-activated endothelium can be inhibited by sL-selectin. These observations support the conclusion that cleavage of cell surface L-selectin and inhibition of leukemic cell adhesion to the endothelium by the shed receptor provide opportunities for regulating the traffic of blast cells out of the bloodstream of patients with acute leukemia.

The majority (62%) of the 151 patients with acute leukemia who were studied had sL-selectin levels more than 2 SD above the mean value observed among healthy blood donors (Fig 1). sL-selectin concentrations are likely to reflect the total mass of leukemic cells, as indicated by observations showing that high plasma sL-selectin levels can be seen in
than 95% of blast cells were isolated from the peripheral blood of a patient with an M1 (A) and an M4 (B) AML. Assays were performed with...mAb, monoclonal antibody.

Impressively, a perfect correlation was found in patients with sL-selectin acute leukemia between sL-selectin levels and the clinical course of acute leukemia, with normal-range levels being observed in 16 of 16 patients in complete remission and high levels in eight of eight patients with therapy-resistant acute leukemia or leukemia relapse (Fig 2). Thus, monitoring sL-selectin levels was helpful for the early diagnosis of leukemia relapse in patients with sL-selectin acute leukemia. Furthermore, an absence of normalization of the sL-selectin level after induction chemotherapy was associated with a failure to achieve a complete remission (Fig 3, days 105 to 128). It should be emphasized that apart from L-selectin acute leukemia, elevated concentrations of sL-selectin can also be seen in patients with other malignant hematologic disorders (O. Spertini, M. Schapira, unpublished observations, June 1993). Slightly increased levels are also observed in acute inflammation, sepsis, or the acquired immunodeficiency syndrome.

Lymphoblasts and monoblasts frequently infiltrate the central nervous system. In symptomatic individuals, leukemic infiltration of the meninges can often be demonstrated by the presence of blast cells in the cerebrospinal fluid. However, in asymptomatic patients, cerebrospinal fluid examination may not disclose any leukemic cells or may yield nondiagnostic results. We have found that 41 patients with various vascular, inflammatory, or mechanical neurologic disorders had a mean cerebrospinal fluid sL-selectin level of 0.020 ± 10 µg/mL. Similar values were seen in 23 ALL patients who did not have meningeal leukemia (0.005 to 0.045 µg/mL). In contrast, higher values (0.066, 0.150, and 0.202 µg/mL) were found in cerebrospinal fluid samples of three patients with a relapsed ALL limited to the central nervous system. The only other condition in which an increased sL-selectin level was observed in the cerebrospinal fluid is bacterial meningitis (O. Spertini, A. Stucki, M. Schapira, unpublished observation). Thus, elevated concentrations of sL-selectin in the cerebrospinal fluid of ALL patients may facilitate the diagnosis of central nervous system involvement.

Whereas normal plasma contains two sL-selectin isoforms, only one form of the shed receptor is detectable in leukemic plasma. sL-selectin from patients with ALL has an M, of 60,000 to 90,000, whereas sL-selectin from AML plasma has an M, between 70,000 and 95,000 (Fig 5). This difference in M, is likely to result from posttranslational modifications of the L-selectin molecule. A single and identical L-selectin mRNA species, which encodes a protein of 62,000, is shed from lymphocytes and the other (M, 80,000 to 105,000) from neutrophils. In contrast, only one isoform is detectable in leukemic plasma, most certainly because normal hematopoietic cells are replaced by monoclonal leukemic cells (Fig 5). Furthermore, a decrease of L-selectin expression should be demonstrated at the surface of peripheral blood lymphocytes if the increase of sL-selectin plasma concentration resulted from a lymphocytic reaction to blast overload. This was not the case, since lymphocytes and neutrophils isolated from peripheral blood of two patients with aleukemic leukemia expressed the same levels of L-selectin as normal leukocytes (70% to 80% and 85% to 95%, respectively).

Fig 6. L-selectin-mediated attachment of leukemic blast cells to cytokine-activated endothelium. Mononuclear cells containing more than 95% of blast cells were isolated from the peripheral blood of a patient with an M1 (A) and an M4 (B) AML. Assays were performed at 4°C for 30 minutes (A) and at 37°C for 10 minutes (B). Confluent endothelial monolayers were cultured for 8 hours at 37°C with medium or TNF-α. sL-selectin was purified by salt-fractionation and affinity chromatography from plasma obtained from two patients with AML, as described in Materials and Methods. The figure shows the number of myeloblasts attached per field. Results are expressed as the mean ± 1 SD and are representative of six similar experiments. mAb, monoclonal antibody.
approximately 37,000 daltons, is found in cells that differentiate along the myeloid and lymphocytic pathways. Because AML and ALL plasmas contain distinct sL-selectin isoforms, the M₃ value of the shed receptor could be considered, in addition to conventional criteria, as a marker of myeloid or lymphoid differentiation.

sL-selectin isolated from plasma of patients with acute leukemia expressed the same epitopes as sL-selectin from normal plasma, but conformational differences were observed between the shed form of L-selectin and cell surface L-selectin. The epidermal growth factor–like domains of cell surface L-selectin and sL-selectin have an identical primary structure. However, the epitope defined on the epidermal growth factor–like domain of L-selectin by the anti–LAM-1 monoclonal antibody is not detectable on sL-selectin. As previously reported for normal leukocytes, the loss of this epitope upon L-selectin shedding from the cell surface demonstrates that this reaction is associated with a change in the conformation of the epidermal growth factor–like domain. Since the LAM-1 epitope seems to be involved in the regulation of L-selectin affinity for its ligand, and the loss of this epitope may reduce the affinity of L-selectin for its counterreceptor. If such a scenario is operative, one might expect that the affinity between sL-selectin and its endothelial cell ligand would be weaker than the affinity between the ligand and cell surface L-selectin. This would provide a mechanism for detaching sL-selectin from its endothelial ligand and replacing it by higher-affinity L-selectin molecules present at the surface of normal activated leukocytes. Since the number of L-selectin receptors expressed by normal leukocytes is lower than the number of sL-selectin molecules present in normal plasma, this mechanism may be in regulating the attachment of normal leukocytes to the vascular endothelium. Different conditions are observed in acute leukemia since leukemic blast cells express much less L-selectin than normal leukocytes, and we show here that leukemic plasma contains much higher concentrations of sL-selectin than normal plasma. Under these conditions, the inhibitory effect of sL-selectin on the attachment of blast cells to their endothelial ligands is likely to exceed the capacity of blast cells to attach via the L-selectin mechanism. Direct support for this hypothesis was obtained by experiments examining the effect of sL-selectin on blast cell attachment to cytokine-activated endothelium under nonstatic conditions. Preincubation of endothelial cell monolayers with 5 μg/mL sL-selectin purified from plasma samples of patients with ALL or AML almost completely inhibited L-selectin–mediated attachment of leukemic blast cells to the activated endothelium (Fig 6).

We have shown here that in acute leukemia, interactions between leukemic blasts and endothelial cells via the L-selectin mechanism can be inhibited in vitro by high levels of sL-selectin. Since in vivo experiments have shown that leukocyte rolling, mediated through L-selectin, is a prerequisite for subsequent steps of migration into tissue, high plasma sL-selectin levels in patients with acute leukemia may have an important role in regulating the initiation of blast cell adhesion to endothelium. The capacity of sL-selectin to inhibit leukemic cell attachment to endothelium in vitro suggests that the elevated concentrations of this circulating receptor in acute leukemia may contribute in vivo to maintain these cells in the bloodstream. However, further in vivo studies are needed to establish definitively the role of sL-selectin in the regulation of blast cell migration into tissues.

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REFERENCES

27. Ley K, Tedder T, Kansas G: L-selectin can mediate leukocyte rolling in untreated mesenteric venules in vivo independent of E- or P-selectin. Blood 82:1632, 1993
41. Jung TM, Dailey MO: Rapid modulation of homing receptors (gp90MAd) induced by activators of protein kinase C. Receptor shedding due to accelerated proteolytic cleavage at the cell surface. J Immunol 144:3130, 1990
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O Spertini, P Callegari, AS Cordey, J Hauert, J Joggi, V von Fliedner and M Schapira