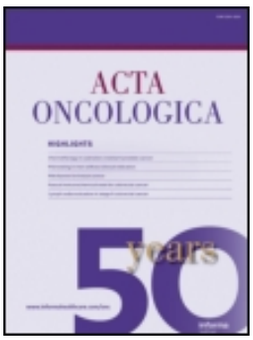

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Syndecan-1 (CD138) Expression in Acute Myeloblastic Leukemia Cells

An Immuno Electron Microscopic Study

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Syndecan-1 (CD138), an important transmembrane heparan sulfate proteoglycan is expressed in distinct stages of cell differentiation. Although its expression in acute lymphoblastic leukemia (ALL) cells is well known; its function or presence in acute myeloblastic leukemia (AML) cells is still largely unknown. The expression of syndecan-1 was studied in bone marrow biopsies of three patients with AML using electron microscopic immunocytochemistry. Positive expression of syndecan-1 was found in AML cells. These results suggest that syndecan-1 expression is not only a characteristic phenotypic marker for ALL, but is also expressed in AML cells.

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Heparan sulfate proteoglycans (HSPGs) are complex molecules that display various functions in the cellular environment. They can interact with many different ligands and thereby participate in cell adhesion, proliferation and differentiation (1). Among HSPGs expressed on the surface of most types of cells, syndecans form a family that includes four members, three of them (syndecan-1, -2 and -4) being cloned in human cells (2, 3). Syndecans are expressed in cell-, tissue- and development-specific patterns. In mesenchymal tissues, syndecan-1 (CD138) is only expressed transiently at particular stages of morphogenesis and cell differentiation (4). These transmembrane proteoglycans appear to display two main functions. One of these functions is to bind extracellular ligands such as matrix adhesive proteins, cell–cell adhesion molecules, enzymes, and growth factors (5, 6). The second function of syndecans may be to promote signaling events that are associated with the ligand-dependent activation of high-affinity receptors (7, 8).

The expression of syndecan-1 in acute lymphoblastic leukemia (ALL) cells is well known (9, 10); and it might also be expressed in acute myeloblastic leukemia (AML) cells. The present study was designed to determine whether syndecan-1 is also expressed in AML cells. In our study we evaluated periodate-lysine-paraformaldehyde fixation and four types of syndecan-1 (B-B2, B-B4, MI15, 1D4) immunoreactivity on electron microscopic sections of AML cells.

MATERIAL AND METHODS

Bone marrow aspiration and biopsy samples were obtained from three untreated patients with AML aged between 40 and 45 years. Light microscopic examination revealed blastic infiltration ($>30\%$ of the nucleated cells). Blastic cells contained 'auer rods', and showed a positive peroxidase and negative non-specific esterase stain. The results of immunophenotyping and cytogenetic analyses were CD13 (+), CD33 (+), CD14 0 and t(8;21) (+), respectively. Depending on the morphological and cytochemical criteria of the French–American–British classification, we diagnosed AML-M2 subgroups in three patients. For electron microscopy, samples were immediately fixed for 4 h in freshly prepared periodate-lysine-paraformaldehyde (PLP) fixative in 0.037 mol/L phosphate

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buffer (pH 7.4) containing 10 mmol/L sodium metaperiodate, 75 mmol/L lysine and 2% paraformaldehyde. PLP-fixed tissues were then rinsed in 0.05 mol/L sodium phosphate buffer, pH 7.4 containing 5% sucrose, and were immunostained according to the method described elsewhere (11). Briefly, mouse monoclonal primary antibodies against four syndecan-1 (CD138) molecules (B-B2, B-B4, MI15, 1D4) obtained from Dr Mason (Department of Clinical Laboratory Sciences, John Radcliff Hospital, Oxford, UK) were diluted in 1:100 in phosphate buffer solution (PBS) containing 1% bovine serum albumin (BSA) and 5% sucrose. Four anti-syndecan-1 mAbs were tested in every sample, one by one. Fixed tissue pieces were incubated overnight at 4°C with solutions having monoclonal primary antibodies. Tissue pieces were then washed 3–5 times with PBS/sucrose and incubated for 6 h with peroxidase-conjugated goat anti-mouse IgG secondary antibody (Sigma, St. Louis, MO, USA) that was diluted in 1:150 in PBS/BSA/sucrose. After repeated washing in PBS/sucrose, the tissue pieces were immersed for 15 min in PBS containing 1% glutaraldehyde, washed extensively with PBS/sucrose, and then immersed for 20 min in 0.005M Tris buffer (pH 7.6) containing 0.0006% H_2O_2, 0.2 mg/ml diaminobenzidine (DAB) and 5% sucrose. After rinsing in PBS/sucrose, the tissue pieces were fixed in 1% OsO_4. They were then washed, dehydrated and embedded in araldite. To test the antibody specificity, we conducted a preadsorption control test before the incubation. By omitting either primary or secondary antibodies from the incubation media, control incubations were also performed. Ultrathin sections (50–70 nm thick) were obtained from araldite-embedded tissue blocks, using an ultramicrotome (LKB, Uppsala, Sweden). Ultrathin sections were stained with lead citrate and uranyl acetate and examined under a LEO 906 E electron microscope (Oberkochen, Germany).

RESULTS

During the electron microscopic examination, immunolabeling with four anti-sydecan-1 monoclonal antibodies (mAbs) (B-B2, B-B4, MI15, 1D4) was observed in AML cells. Immunoreactivity with these mAbs was only membrane-associated in AML cells. Surface staining was observed as diffuse and homogeneous electron-dense reaction deposits along the cell membranes (Figs. 1–4). The syndecan-1 positive AML cells were in close contact with each other, and had short and long microvilli, and bulbous extensions on their surfaces (Fig. 2). Some AML cells were also in close contact with venous sinusoids (Fig. 3) and capillaries (Fig. 4). A preadsorption control test performed before the incubation did not reveal any immunoreactivity specific to the antigen. Control incubations did not result in specific staining for AML cells at the electron microscopic level (Fig. 5).

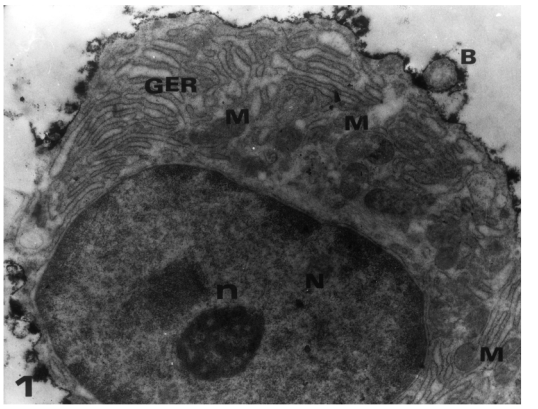

Fig. 1. Immuno electron micrograph of a syndecan-1 (B-B2)-positive acute myeloblastic leukemia (AML) cell rich in granular endoplasmic reticulum (GER) showing strong surface membrane-associated labeling. N: nucleus, n: nucleolus, M: mitochondrion, B: bulbous protrusions. (× 20000).

DISCUSSION

A variety of immunohistochemical markers can potentially improve the specificity and sensitivity of leukemia cell detection on routine histological sections obtained from bone marrow biopsies, but most of them are not entirely satisfactory (9, 10). Syndecan-1 is a glycoprotein with carbohydrate residues (2) and an optimal marker for detection of ALL and multiple myeloma cells on bone marrow aspirates by flow cytometry (12, 13). In this study, we investigated whether syndecan-1 antibody can also be used successfully for the identification of AML cells on PLP-fixed biopsies.

The immunohistochemical localization of tissue antigens in tissues preserved for immuno electron microscopy is difficult. Various fixation procedures have been used in an attempt to preserve antigenicity as well as to maintain ultrastructural integrity (14–17). McLean & Nakane suc-

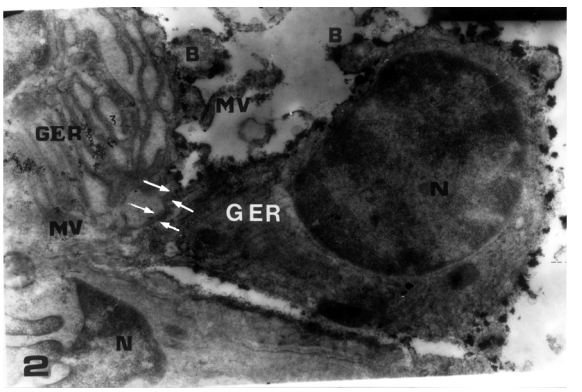

Fig. 2. Immuno electron micrograph of three syndecan-1 (B-B4)-positive acute myeloblastic leukemia (AML) cells having strong membrane-associated labeling, granular endoplasmic reticulum (GER) and bulbous extensions (B). The cells were in close contact with each other (arrows). Mv: microvillus, N: nucleus. (× 16000).

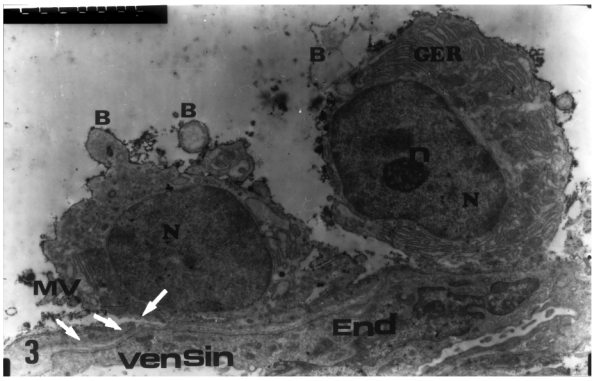

Fig. 3. Immuno electron micrograph of two syndecan-1 (1D4) positive acute myeloblastic leukemia (AML) cells in close proximity to the sinusoidal endothelium (End) (arrows). N: nucleus, n: nucleolus, Mv: microvillus, Vensin: venous sinusoid. (× 10000).

cessfully used a periodate-lysine-paraformaldehyde (PLP) mixture in order not only to improve fixation of carbohydrate moieties but also to avoid the denaturing effect of glutaraldehyde on protein structure and antigenicity (18). Since stabilization of cell surface antigens and preservation of ultrastructural integrity are important for immuno electron microscopic studies, we preferred PLP fixative and indirect pre-embedding electron microscopic immunocytochemistry to examine syndecan-1 immunoreactivity in the present study, and this method seems to preserve both the antigenicity and structural integrity of leukemic myeloblastic cells, since it allowed us to detect membrane-associated expressions of four syndecan-1 molecules. We therefore believe that PLP fixative may be one of the most promising fixative solutions available for electron microscopic immunocytochemical studies of leukemic cells.

Sobol et al. described a large series of prospectively studied patients with adult ALL whose lymphoblasts expressed myeloid antigen (19). Their findings indicated that

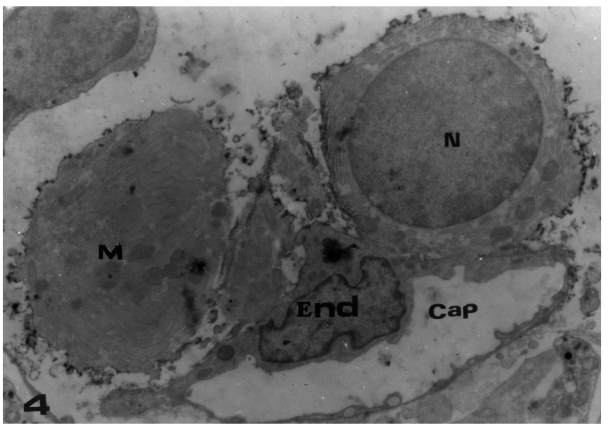

Fig. 4. Immuno electron micrograph of three acute myeloblastic leukemia (AML) cells immunolabeled with anti-syndecan-1 (MI 15) mAb showing close contact with the capillary (Cap) endothelium (End), N: nucleus, M: mitochondria. (× 10791).

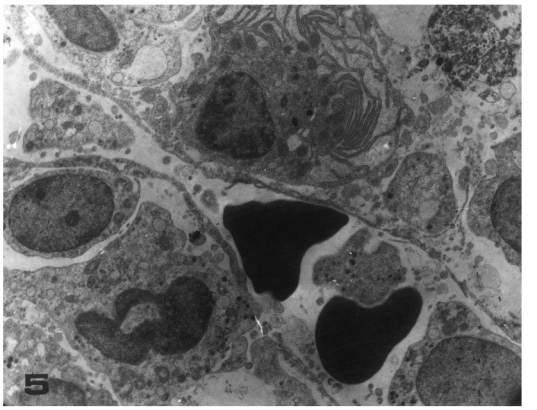

Fig. 5. Control of acute myeloblastic leukemia (AML) cells by omitting the primary antibody. (× 7 194).

myeloid antigen expression occurs more frequently in adult ALL than previously realized, and such expression defines major high-risk subgroups not identified by conventional cytologic and cytochemical evaluations. At the same time, in a subgroup of children with AML, expression of lymphoid-associated antigen has been reported (20). In our study we demonstrated that AML cells expressed four syndecan-1 molecules at the electron microscopic level. The observed immunoreactivity in the present study may be attributable to the success of the fixation and conservation of the antigenic structures in leukemic cells, and we believe that our method may be a reliable strategy in preserving the myeloid features present in AML cells and that it facilitates research on this cell type. In addition, because of its clear-cut cell membrane localization, we suggest that CD138 can be used in double-marker immunostaining reactions to evaluate nuclear prognostic markers such as Ki67 and p53 in AML.

Syndecan-1 is a cell surface proteoglycan that binds cells to the matrix and undergoes changes in expression during development, during differentiation of some cell types, and following malignant transformation in some tumors (21). In hemopoietic cells, syndecan-1 was shown to be expressed only in B cells at certain differentiation stages (pre-B and plasma cells), and in lymphoproliferative conditions this selective expression was reported to be retained in myelomas/plasmacytomas (12) and syndecan-1 was therefore proposed to be a useful phenotypic marker to identify cells with plasmacytic differentiation from a diagnostic point of view (13). On the other hand, cell lines of the myeloid lineage, like the myeloblastic/promyelocytic cell lines B1 and B2, were reported as not expressing HS proteoglycans (22). Our results do not support these views, in that AML cells also express syndecan immunoreactivity. We believe that the importance of syndecan-1 expression in AML still requires further study.

Adhesion of normal and leukemic B-cell precursors to bone marrow stroma is largely mediated by the integrins

VLA-4 and VLA-5, although other adhesion molecules such as CD44 may also be implicated (23). Cell migration is a complex activity that is dependent on interaction of cells with an adhesive surface and mediated by directed membrane and cytoskeleton assembly. The motility and migration of precursor ALL cells are significant both for the regulation of their growth, and for the dissemination of the disease (24). In this study, leukemic cells were in close contact with each other. They expressed surface membrane-associated immunoreactivity with anti-syndecan-1 mAbs. Large numbers of these cells were also in close proximity with venous sinusoids and capillaries. In the light of these findings, we suggest that signaling and adhesion events occur largely between myeloid cell precursors through the interaction of heparan sulfate glycosaminoglycan chains present in the cell membrane.

The ultrastructural changes in leukemic cells on initial contact with, and during migration into the layers of bone marrow stroma in vitro have been examined in a variety of acute leukemic cell types. It has been demonstrated that precursors of B-ALL cells undergo rapid and complex morphological changes after contact with fibroblasts in the adjacent layers of bone marrow (25). The formation of extended microvilli was demonstrated by electron microscopy within minutes of contact by leukemic cells with marrow stroma, and further changes in membrane, cytoplasmic and nuclear shape occur during migration of lymphoblasts into the adherent layer (25). In our findings, syndecan-1-positive AML cells displayed short and long microvilli and blebs. These findings suggest that morphologic alteration is a common phenomenon in all forms of leukemia, which is associated with an immunologic response that is capable of producing lymphocyte transformation.

We conclude that a combination of PLP fixation and pre-embedding electron microscopic immunocytochemistry is a useful method for the detection of cell surface syndecan molecules in leukemic bone marrow cells. We also conclude that syndecan-1 (CD138), a heparan sulfate proteoglycan, is not only a characteristic phenotypic marker for ALL, it is also expressed in AML cells.

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