Oncoplacental Protein SP₁—A Constitutive and Inducible Late Differentiation Marker of the Human Myelomonocytic Lineage

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The oncoplacental protein SP₁, is found in large quantities in human placenta, amniotic fluid, and pregnancy serum. Low levels have been reported in association with malignancy but also in healthy nonpregnant individuals. By indirect immunofluorescence, fluorescence-activated cell sorting, and immunoprecipitation we here demonstrate the presence of SP₁ both on the surface and in the cytoplasm of human granulocytes but not in earlier myeloid progenitor cells in bone marrow. Lymphocytes did not contain the protein, and only trace amounts could be found in the cytoplasm of blood monocytes. A major glycoprotein with an apparent mol wt of 90,000 was obtained by immunoprecipitation of surface-labeled granulocytes. Cultivated blood monocytes, adhering to surfaces or forming multinuclear giant cells, displayed a strong membrane and cytoplasmic expression of SP₁. Treatment of the myeloid leukemia cell line ML-2 with tetraphorbol acetate (TPA) strongly induced SP₁ in the membrane and cytoplasm as revealed by immunofluorescence and polyacrylamide gel electrophoresis (PAGE) of immunoprecipitates from lysates of surface radiolabeled cells. The induction of synthesis of SP₁ in TPA-activated cells was confirmed by immunoprecipitation from lysates of cells metabolically labeled with ³⁵S-methionine. Human lymphoblastoid and erythroleukemic cell lines did not express SP₁, either before or after induced differentiation. Thus SP₁ provides a late differentiation marker for the myelomonocytic lineage and is strongly induced during macrophage differentiation or by TPA treatment of ML-2 cells.

Cell culture and induction of cell differentiation. Monocytes were cultured in RPMI 1640 medium supplemented with 10% fetal bovine serum (FBS) and allowed to attach on glass coverslips that were used for immunofluorescent staining. Primary monolayer cultures of monocytes were detached with a rubber policeman and subcultured at high density for five days to form multinuclear giant cells on coverslips. HL-60 and ML-2 cells were cultivated at optimal densities for three to five days in the presence of 10⁻⁴ mol/L tetraphorbol acetate (TPA) or 10⁻⁴ mmol retinoic acid as described. K562 and HEL cells were treated with 1.2 µg/mL sodium butyrate or bemin at 50 µg/mL as described. Immunochemistry. Cytocentrifuged smears were fixed for five minutes in cold methanol (−20°C) to permeabilize the plasma membrane. The indirect immunofluorescence staining was done with 1:30 diluted rabbit anti-SP₁ (Dakopatts, Copenhagen, Denmark) followed by 1:20 diluted fluorescein isothiocyanate (FITC)-conjugated goat antirabbit antisemur. Double immunofluorescence staining was performed with the monoclonal antibody (MoAb) OKM-1 (Ortho, Raritan, NJ) and rabbit anti-SP₁ antisemur by using FITC-conjugated goat antimouse and rhodamine-conjugated goat antirabbit antisemur, respectively.

Fluorescence-activated cell sorting. Normal bone marrow cells in suspension were stained in indirect immunofluorescence by rabbit anti-SP₁ antisemur. The cell suspension was fractionated on a FACS IV (Becton-Dickinson) cell sorter, and the positively stained and

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MATERIALS AND METHODS

Hematopoietic cells. Normal blood leukocytes were isolated fromuffy coats of blood units kindly donated by the Finnish Red Cross Blood Transfusion Service. Normal bone marrow was recovered from pieces of ribs that had to be resected during open thorax surgery.

Fractionation of cells. Blood granulocytes were isolated by dextran sedimentation ofuffy coats. The leukocyte-rich suspension was subjected to Ficoll-Isopaque density gradient centrifugation at 400 g for 30 minutes. The lymphocytes and monocytes were recovered from the interface, while the granulocytes were recovered from the pellet as described. Monocytes were purified by Percoll density gradient centrifugation of blood mononuclear cells recovered by Ficoll-Isopaque density gradient centrifugation.

Cell lines. The following human hematopoietic cell lines were studied: the erythroleukemic lines K562 and HEL, the myeloid cell lines ML-2 and HL-60, and lymphoblastoid cell lines established locally by Epstein-Barr virus (EBV) infection of normal blood B cells.

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unstained populations were identified from May-Grünwald–Giemsa-stained cytocentrifuged smears.

Cell surface labeling, metabolic labeling, and immunoprecipitation. Purified granulocytes and uninduced and induced ML-2 cells (50 x 10⁶ cells) were radioactively surface-labeled by the periodate/NaB₃H₄ technique, as described previously. After labeling, the cells were solubilized at 0°C in 1 mL of 0.15 mol/L NaCl-0.01 mol/L sodium phosphate, pH 7.4, containing 1% Triton X-100. The samples were centrifuged at 10,000 g for 15 minutes and the supernatants recovered. Twenty-five microliters were used for slab gel electrophoresis and 100-μL aliquots for immunoprecipitation. For metabolic labeling of ML-2 cells the cultured cells were incubated in 5 ml methionine-free Eagle’s minimum essential medium (MEM) containing 1 mCi ³⁵S-methionine for one hour. After a two-hour chase in normal medium the cells were collected, washed twice, and lysates prepared for immunoprecipitation. Part of the ³⁵S-methionine-labeled samples were subjected to lentil lectin-Sepharose chromatography and the α-methylmannoside-eluted fractions used for immunoprecipitation.

Immunoprecipitations with protein A-containing staphylococci were performed using 10 μL rabbit anti-SP₁ antiserum in the absence or presence of 10 μg SP₁ protein. The procedure has been described in detail previously.

Polyacrylamide gel electrophoresis of H-labeled cells and the immunoprecipitates were electrophoresed on 8% polyacrylamide slab gels, and the radioactive proteins were visualized by fluorography as described.

RESULTS

When cytocentrifuge smears from normal blood leukocytes were stained by immunofluorescence with rabbit anti-SP₁ antiserum, a strong intracytoplasmic staining was seen in granulocytes. No staining was obtained with lymphocytes. The eosinophilic granulocytes also remained unstained. In bone marrow a strong positive reactivity was obtained only with cells of the granulocytic lineage. The earliest myeloid cells staining with anti-SP₁ antiserum were found to be promyelocytes. Metamyelocytes and mature neutrophils showed a strong reactivity. No staining was seen in the erythroid or megakaryocytic lineages (Fig 1 A).

Purified blood monocytes were negative in immunofluorescent surface staining, while a very faint, cytoplasmic reactivity, probably mainly in the Golgi area, could be seen in smears of monocytes (Fig 1 B).

Double-immunofluorescence surface staining with OKM-1 antibody and rabbit anti-SP₁ antiserum revealed a codistribution of these antigens as regards the granulocytic lineage, except that eosinophilic granulocytes were OKM-1 positive but SP₁ negative (figure not shown). Neither reagent detected early myeloblasts or cells of the erythroid lineage. Lymphoid cells were negative.

Sorting of anti-SP₁-stained bone marrow cells by a FACS IV revealed two populations (Fig 2 A). The positively staining cells consisted of mature granulocytes concentrating in the right shoulder of the histogram and their late precursors (Fig 2 C), while other bone marrow cells sorted in the unstained population (Fig 2 B).

When monocytes were allowed to adhere to a glass surface and were stained with anti-SP₁ antiserum, a strong intracytoplasmic reactivity was seen after only one day in culture (Fig 3 A). The staining intensity increased during the subsequent days of culture. Adherent monocytes forming multinuclear giant cells also displayed an intense and often asymmetrically distributed cytoplasmic and surface reactivity with the anti-SP₁ antiserum (Fig 3 C).

In the hematopoietic cell lines studied, SP₁ positivity was confined to the myeloid cell lines (ML-2 and HL-60). A very faint, hardly detectable, intracytoplasmic fluorescence like that in monocytes was seen in uninduced ML-2 (Fig 3 E) and HL-60 (not shown) cells. After treatment with TPA a very
**DISCUSSION**

The SP, glycoprotein belongs to the family of glycoproteins that show elevated serum levels during pregnancy. Placental tissue has been shown to synthesize this protein in vitro and is therefore considered as the main source of the SP, during pregnancy. In this study we have shown that the SP, glycoprotein is present in blood leukocytes and constitutes a late differentiation marker for the myelomonocytic lineage. Although most of the antigen, as judged by immunoreactivity, is located intracytoplasmically, a surface expression can also be observed that allows positive selection of the later stages of the granulocyte lineage by fluorescent-activated cell sorting.

**Fig 4.** Polyacrylamide slab gel electrophoretic patterns of granulocyte surface glycoproteins reacting with anti-SP, antiserum. (A) Pattern of ¹⁴C-labeled standard proteins: PH₅, phosphorylase b; BSA, bovine serum albumin; OA, ovalbumin; CA, carbonic anhydrase (B) Triton X-100 lysate is presenting the total surface-labeled glycoproteins of granulocytes. (C) Pattern of immunoprecipitate obtained with anti-SP, antiserum from surface-labeled granulocytes. (D) Pattern of immunoprecipitate obtained with anti-SP, antiserum from surface-labeled granulocytes in the presence of 10 µg unlabeled SP; GP90, surface glycoprotein with an apparent mol wt of 90,000; GP54, surface glycoprotein with an apparent mol wt of 54,000.

When immunoprecipitation was performed with anti-SP, antiserum from ³⁵S-methionine, were immunoprecipitated with anti-SP, two bands of 80,000 and 90,000 apparent mol wt were seen (Fig 6 F). The lower band was enriched on lentil lectin-Sepharose columns (Fig 6 L), which shows its glycoprotein nature. The two bands are apparently not components of a heterodimeric molecule linked by disulfide bridges, since a similar polyacrylamide gel pattern was obtained under nonreducing conditions (not shown).
The surface expression of SP, on granulocytes could further be substantiated by immunoprecipitation from lysates of surface-labeled, purified granulocytes. Analysis of the antigen precipitated by the anti-SP, antiserum on PAGE revealed a single major band of 90,000 apparent mol w. This corresponds to the mol wt reported for SP, of placental origin. Antigenic identity of the membrane protein in granulocytes and the SP, occurring in serum during pregnancy could be confirmed, since the addition of highly purified placental SP, could completely inhibit the immunoprecipitation with anti-SP, antiserum from surface-labeled granulocytes.

The availability of hematopoietic cell lines that phenotypically represent clonal expansions of certain lineages arrested at different stages of differentiation offers a useful tool for studies on biosynthetic events that are difficult to perform on limited amounts of available bone marrow cells. In this study we used a panel of hematopoietic cell lines that can be induced to further differentiation in vitro. Of these the promyelocytic cell line HL-60 and the myeloleukemia cell
line ML-2 expressed appreciable amounts of SP, after induced differentiation. The pluripotent leukemia cell line K-562 which, like the HEL line, has capacity of erythroid differentiation, and the lymphoid lines remained negative before and after induced differentiation.

As TPA treatment in vitro of ML-2 induced the most dramatic increase in the expression of SP, this model was selected for further investigations. As seen for differentiating granulocytes in normal bone marrow and for monocytes in culture, induction of ML-2 cell differentiation led to a strongly increased expression of SP, intracytoplasmically, but reactivity was also found with the cell surface. Metabolic labeling of the TPA-induced ML-2 cells by U-S-methionine followed by immunoprecipitation and PAGE revealed two closely spaced bands of 90,000 and 80,000 apparent mol wt, respectively. The two bands did not represent components of a heterodimeric molecule linked by disulfide bridges, since two distinct similar bands were obtained by gel electrophoresis under nonreducing conditions. Immunoprecipitation from surface labeled ML-2 cells, on the other hand, revealed only a single band of 90,000 mol wt corresponding to that of higher mol wt obtained from metabolically labeled cells. It is possible that SP, occurs in two forms in hematopoietic cells where the intracytoplasmic protein has a slightly lower apparent mol wt. Alternatively, the 80,000-mol wt band observed after metabolic labeling might represent an incompletely glycosylated precursor of the membrane-expressed 90,000-mol wt antigen. Pulse-chase experiments with TPA-induced ML-2 cells are now in progress to distinguish between these two possibilities.

TPA is commonly used in induction of differentiation both of hematopoietic and nonhematopoietic cells. The mechanism of action of this compound is still incompletely understood. The ability of TPA to activate protein kinase C might be involved in the transduction of the differentiation signals. We have earlier shown that treatment of ML-2 cells with TPA induces distinct alterations in the surface glycoprotein pattern. While untreated ML-2 cells contain hardly detectable amounts of SP, treatment with TPA led to a strong increase in the synthesis of this glycoprotein. This phenomenon offers a new model for studies on the TPA-induced regulation of gene expression.

REFERENCES

Oncoplastical protein SP1--a constitutive and inducible late differentiation marker of the human myelomonocytic lineage

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