A Monoclonal Antibody That Detects Expression of Transferrin Receptor in Human Erythroid Precursor Cells

By Deborah Lebman, Massimo Trucco, Lisabianca Bottero, Beverly Lange, Silvana Pessano, and Giovanni Rovera

A monoclonal antibody, L5.1, obtained by immunizing a Balb/c mouse with HL60 human promyelocytic leukemia cells, was found to react with both HL60 cells and with the K562(S) cell line. This monoclonal antibody binds and immunoprecipitates a glycoprotein (Mr 87,000) present on the cell surface membrane of K562(S) as a disulfide bonded dimer. In competition experiments L5.1 competes with the cell surface membrane of K562(S) erythroleukemia cells. Binding of both L5.1 and transferrin to the surface of K562(S) is inhibited by treatment with 12-0-tetradecanoyl-phorbol-13-acetate. A monoclonal antibody, L5.1, was identified by radioimmunoassay (RIA) 2 wk after fusion by immunizing a Balb/c mouse with HL60 cells (5 x 10^5) were incubated for 30 min at room temperature with supernatant present in the colony containing wells. HL60 cells (5 x 10^5) were incubated for 30 min at room temperature with HL60 cell line. This monoclonal antibody detects expression of transferrin receptor in human erythroid precursor cells.

 MATERIAL AND METHODS

Immunization, Somatic Cell Hybridization and Selection of Specific Monoclonal Antibodies

A 2 mo old female Balb/c mouse was injected i.p. with 2 x 10^7 HL60 cells and injected i.v. 7 days later with an equal amount of cells. Three days after the second injection, the spleen was removed and somatic cell hybridization was done using a modification of the procedure of Kohler and Milstein. Approximately 10^7 spleen cells were fused with 2 x 10^7 P3-x63-Ag8 653 mouse myeloma cells (Ig non-producer) using 50% polyethylene glycol-1000. Hybrid cells were selected in HAT medium. Clones reactive with HL60 cells were identified by radioimmunoassay (RIA) 2 wk after fusion by testing the supernatant present in the colony containing wells. HL60 cells (5 x 10^5) were incubated for 30 min at room temperature with supernatant present in the colony containing wells. HL60 cell line. This monoclonal antibody detects expression of transferrin receptor in human erythroid precursor cells.

Isotype Characterization

The immunoglobulin isotype was determined by immunodiffusion in Ouchterlony plates using goat antimouse immunoglobulin class and subclass specific antibodies (Meloy Laboratories).
Indirect Immunofluorescence

Target cells (2 × 10⁶) were treated with 20 μl of the specific antibody at a chosen dilution, incubated at room temperature for 30 min and washed three times. The cells were then incubated in 20 μl of fluorescein isothiocyanate (FITC)—conjugated goat F(ab')₂, antinemouse F(ab')₂, antiseraum (Cappel). Control cultures were treated with the supernatant of the parental mouse myeloma and FITC—conjugated antibody.

The percentage of fluorescent cells was determined using either a Leitz fluorescence microscope or a fluorescence-activated cell sorter (Ortho Instruments 50HH).

Analysis and Sorting of Cells Reactive with Monoclonal Antibodies

The use of human marrow or peripheral blood for these investigations was approved by the Committee for the Protection of Human Subjects of the Children’s Hospital of Philadelphia. Informed consent was obtained from all donors.

Approximately 0.2 ml of human bone marrow cells was obtained a) from normal donors during harvest for transplantation; b) from patients with solid tumors during diagnostic search for metastases; c) from patients with acute lymphoblastic leukemia (ALL) in remission during routine surveillance. Morphological examination of these samples confirmed that the marrow was normal. Marrows from patients with leukemias in which leukemic cell replacement was more than 70% were also used. The diagnosis of leukemia was based on clinical, morphological and histochemical criteria. Thymus was obtained at autopsy within 4 hr of death. Cells were partially purified by Ficoll-Hypaque gradient centrifugation (d = 1.078). The residual in the interface were suspended in phosphate buffered saline and incubated with the monoclonal antibodies and stained with indirect immunofluorescence as described above. The control cell population consisted of a cell suspension treated with parental myeloma supernatant and FITC—conjugated antibody. Approximately 2 × 10⁶ cells per sample were used for flow cytofluorimetric analysis and cell sorting. The threshold fluorescence intensity was established at which 99% of the total control population was negative. Sorting was performed at a rate of 3000 cells per second, and the efficiency of sorting was 85% with an anticoincidence setting of three. Both positive and negative cell populations were collected separately in tubes containing 1 ml of a solution containing 50% PBS and 50% FCS. Cytocentrifuged slides were prepared using a Shandon cytocentrifuge, and the cells were stained with May-Grundwald-Giemsa. At least 300 cells per slide were identified morphologically and at least three separate sorting experiments on different bone marrows were performed with the monoclonal antibody in question.

Cell Lines

Human promyelocytic leukemia cell lines, human monocytic leukemia, human histiocytoma cell, human erythroleukemia, human lymphoid cell lines, the P3-x63-Ag8 653 mouse myeloma line, and the nonhemopoietic cell lines described in the text were grown in RPMI medium containing 10% fetal bovine serum and incubated in a humidified 5% CO₂ atmosphere at 37°C.

Radiolabeling and Immunoprecipitation of Cell Surface Proteins

Exponentially growing K562 cells were incubated with [35S]-methionine (200 μCi/ml) in methionine free medium overnight or with [3H]-glucosamine for 2 days with 2 μCi/ml. The labeled cells were washed three times in PBS, solubilized in a buffer containing 0.5% NP40 and used for immunoprecipitation. Lactoperoxidase surface labeling was done using the method of Marchalonis as modified by Pink and Ziegler.

Lysates (300 μl) of 2 × 10⁶ labeled cells were centrifuged at high speed in a microcentrifuge and the supernatant was incubated with 5 μl of ascitic fluid or in some cases with Sepharose protein A purified monoclonal antibody for 30 min. Sepharose protein A (BioRad, 200 μl) was added instead of Staphylococcus aureus. The immunoprecipitate was dissolved in SDS sample buffer under reducing and nonreducing conditions and separated electrophoretically using 8% acrylamide gel (SDS-PAGE) as described by Laemmli. Molecular weights were calibrated using 14C-labeled methylated molecular weight marker mixtures (Amersham). The methylated protein mixture contained: myosin (Mr 200,000), phosphorylase B (Mr 92,500), bovine serum albumin (Mr 69,000), ovalbumin (Mr 46,000) and carbonic anhydrase (Mr 30,000). After SDS-PAGE, the gel was stained, subjected to fluorography according to the method of Laskey and Mills and exposed at −70°C to Kodak RP X-Omat film.

Competition and Binding Assays

Three types of competition experiments were done. In the first case, cells (5 × 10⁶ in 25 μl) were incubated at 37°C with 25 μl of increasing concentrations of either cold transferrin, Sepharose protein A affinity purified L5.1 antibody, or albumin. After 30 min, 251 labeled transferrin (2.5 × 10⁶ cpm, specific activity 1 × 10⁷ cpm/μg) or 125I-labeled L5.1 (1.4 × 10⁶ cpm, specific activity 0.8 × 10⁷ cpm/μg) was added. After incubation at 37°C for another 30 min, the cells were washed four times and the radioactivity determined in a gamma counter.

In the second group of experiments, cells were coincubated with either 125I-labeled transferrin or 125I-labeled L5.1 and with cold transferrin, cold L5.1, and cold albumin as indicated in Results.

In the third group of experiments, 25 μl of 125I-labeled L5.1 (50,000 cpm) and 25 μl of 1:10 dilution of ascites of monoclonal antibodies (titer 1:10², concentration range 1–1.5 mg/ml) or 20 μl of supernatant of the parental myeloma concentrated 20 times by precipitation with 300 Ml of ascitic fluid or in some cases with Sepharose protein A purified monoclonal antibody for 30 min. Sepharose protein A (BioRad, 200 μl) was added instead of Staphylococcus aureus. The immunoprecipitate was dissolved in SDS sample buffer under reducing and nonreducing conditions and separated electrophoretically using 8% acrylamide gel (SDS-PAGE) as described by Laemmli. Molecular weights were calibrated using 14C-labeled methylated molecular weight marker mixtures (Amersham). The methylated protein mixture contained: myosin (Mr 200,000), phosphorylase B (Mr 92,500), bovine serum albumin (Mr 69,000), ovalbumin (Mr 46,000) and carbonic anhydrase (Mr 30,000). After SDS-PAGE, the gel was stained, subjected to fluorography according to the method of Laskey and Mills and exposed at −70°C to Kodak RP X-Omat film.

Table 1. Reactivity of Established Cell Lines of Hemopoietic Origin to L5.1 by Indirect Immunofluorescence and RIA

<table>
<thead>
<tr>
<th>Cell Line</th>
<th>Origin</th>
<th>Percentage of Positive Cells*</th>
<th>RIA†</th>
</tr>
</thead>
<tbody>
<tr>
<td>HL60</td>
<td>promyelocytic leukemia</td>
<td>5–85</td>
<td>4.4</td>
</tr>
<tr>
<td>ML3</td>
<td>promyelocytic leukemia</td>
<td>0–10</td>
<td>1.8</td>
</tr>
<tr>
<td>KG1</td>
<td>myeloblastic leukemia</td>
<td>0–10</td>
<td>1.9</td>
</tr>
<tr>
<td>GM1500</td>
<td>B cell lymphoma</td>
<td>0</td>
<td>NT²</td>
</tr>
<tr>
<td>Daudi</td>
<td>B cell lymphoma</td>
<td>0</td>
<td>1.5</td>
</tr>
<tr>
<td>Raji</td>
<td>B cell lymphoma</td>
<td>0</td>
<td>1.3</td>
</tr>
<tr>
<td>WT 52</td>
<td>EBV-transformed B lymphocyte</td>
<td>0</td>
<td>4.2</td>
</tr>
<tr>
<td>SB</td>
<td>EBV-transformed B lymphocyte</td>
<td>10</td>
<td>2.7</td>
</tr>
<tr>
<td>Jurkat</td>
<td>T cell leukemia</td>
<td>15</td>
<td>1.6</td>
</tr>
<tr>
<td>Molt 4</td>
<td>T cell leukemia</td>
<td>15</td>
<td>5.0</td>
</tr>
<tr>
<td>NALM1</td>
<td>non-T, non-B cell leukemia</td>
<td>0</td>
<td>NT²</td>
</tr>
<tr>
<td>K562(S)</td>
<td>erythroleukemia</td>
<td>10–50</td>
<td>5.8</td>
</tr>
<tr>
<td>THP1</td>
<td>monocytic leukemia</td>
<td>0</td>
<td>2.0</td>
</tr>
<tr>
<td>U937</td>
<td>histiocytic leukemia</td>
<td>0</td>
<td>1.6</td>
</tr>
</tbody>
</table>

*The percentage of positive cells was determined either by visual fluorescence or by cell sorter analysis in which case a baseline of 99% negative cells was determined using the cell line treated only with FITC second antibody as a negative control.
†Values are the number of times above background (approximately 200 cpm).
‡Cell lines were tested at various stages of growth.
§Not tested.

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precipitation with 50% (NH₄)₂SO₄ were incubated with K562(S) cells (5 x 10⁶ cells in 25 μl) for 45 min at 4°C.

Binding assays using ¹²⁵I-labeled L5.1 and ¹²⁵I-labeled transferrin with K562(S) cells were done as follows: cells were treated for different lengths of time with 12-O-tetradecanoyl-phorbol-13-acetate (TPA) (1.7 x 10⁻⁷ M). Cells were incubated at 4°C for 45 min with labeled L5.1 (130,000 cpm) or at 37°C for 30 min with labeled transferrin (190,000 cpm). Preliminary experiments indicated that the binding of the antibody and of transferrin reached saturation under these conditions.

RESULTS

Reactivity of Leukemic Cell Lines with L5.1

L5.1 was found by immunodiffusion to produce IgG2a antibody. Table 1 shows the reactivity of monoclonal antibody L5.1 with a variety of malignant human hemopoietic cell lines. The extent of L5.1 reactivity with HL60 and K562(S) cells was dependent upon the growth conditions of the cells. The percentage of positive HL60 cells and K562(S) cells was higher when the cells were in log phase than with cells at saturation density. With the exception of HL60 and K562(S) cells, most of the cell lines tested were negative. One of five B cell lines and two of three T cell lines were weakly positive.

Biochemical Characteristics of the Erythroid Cell Surface Antigen Recognized by L5.1

Fig. 1 shows immunoprecipitation analysis of an NP40 lysate of K562(S) cells metabolically labeled with ³⁵S-methionine or surface labeled by using the ¹²⁵I-lactoperoxidase method. In both cases, L5.1 immunoprecipitated a polypeptide with an apparent molecular weight of ≈87,000 when the SDS gel was run under reducing conditions. Under nonreducing conditions, L5.1 precipitates a molecule of about 180,000 daltons. This molecule could be labeled by incubating the cells with ¹³C-glucosamine. Thus, L5.1 appears to immunoprecipitate a glycoprotein (gp87), which exists as a disulfide bonded dimer of two polypeptides with identical molecular weights (Mr 87,000).

Fig. 1. SDS-PAGE and autoradiography of the antigen present in K562(S) cells reacting with the monoclonal antibody L5.1. a) Lane 1: Immunoprecipitate of ¹²⁵I-labeled cells run under nonreducing conditions. Lane 2: Immunoprecipitate of ¹²⁵I-labeled cells run under reducing conditions. Lane 3: Immunoprecipitate of ³⁵S-labeled cells. b) Lane 1: Marker ¹³C protein standards: Myosin (Mr 200,000); Phosphorylase B (Mr 92,500); Bovine serum albumin (Mr 69,000); Ovalbumin (Mr 46,000); Carbonic anhydrase (Mr 30,000). Lane 2: Immunoprecipitate of ¹³C-glucosamine-labeled cells.
Competition Studies

Because the molecular structure and distribution of gp87 on erythroid cells suggest that gp87 is the transferrin receptor,29,33 the ability of L5.1 to compete with transferrin for binding to K562(S) cells was tested. Cells were preincubated for 30 min at 37°C with various concentrations of cold L5.1, transferrin or albumin and then incubated for an additional 30 min at 37°C with either 125I-labeled transferrin or 125I-labeled L5.1. The results are shown in Fig. 2A and B. The irrelevant protein (albumin) does not compete with either 125I-transferrin or 125I-labeled L5.1. Both L5.1 and transferrin compete with themselves. L5.1 can compete partially with transferrin (Fig. 2A) but transferrin cannot compete with L5.1 (Fig. 2B). Similar results, i.e., a one way competition, are obtained when there is no preincubation (Fig. 2C and D). That L5.1 cannot totally compete with transferrin can be explained by a difference in affinity for the receptor and/or a difference in the nature of antigen-antibody binding versus protein-receptor binding. The one way competition can also be explained by steric hinderance, i.e., L5.1 can block the site for transferrin but transferrin cannot block the site for L5.1.

Since the monoclonal antibody OKT9 has been reported to bind to the transferrin receptor,33 it was also investigated for its ability to compete with labeled L5.1 for the same binding site on K562(S) cells. The results (Fig. 3) indicate that OKT9 strongly competes with labeled L5.1 for binding to K562(S) cells.

Binding of L5.1 and Transferrin to K562(S) Cells

Binding of L5.1 and transferrin to K562(S) after different times of treatment with TPA was studied. As shown in Fig. 4, when K562(S) cells were treated with TPA, the level of surface antigen recognized by L5.1 decreases as a function of time with a characteristic multiphase pattern. An identical rate and pattern of decrease was observed for transferrin binding.

Reactivity of L5.1 with Normal Hemopoietic Cells

Peripheral blood leukocytes, platelets or erythrocytes did not react with L5.1 as determined by RIA and/or by immunofluorescence. However, PHA-stimulated lymphocytes were reactive (Table 2). Reticulo-
cytes (obtained from a sickle cell anemia patient, having 40% reticulocytes in peripheral blood) reacted strongly (more than 20% of total erythrocytes positive) and agglutinated in the presence of L5.1 (not shown).

As monoclonal antibody L5.1 was found to react with immature myeloid (HL60) and erythroid (K562) cells, it was of interest to determine the distribution of this surface antigen in bone marrow cells during normal hemopoietic differentiation. When the marrow population was incubated with antibody L5.1 and the cells sorted using a Cytofluorograf 50HH, a characteristic distribution of the positive and negative cells was observed. Fig. 5 shows the analysis (forward angle scatter vs. right angle scatter vs. number of cells) of the

**Table 2. Reactivity of Peripheral Blood Cells and Thymocytes with L5.1**

<table>
<thead>
<tr>
<th>Cell Type</th>
<th>Percentage of Positive Cells *</th>
</tr>
</thead>
<tbody>
<tr>
<td>Granulocytes</td>
<td>0</td>
</tr>
<tr>
<td>Lymphocytes</td>
<td>0</td>
</tr>
<tr>
<td>Monocytes</td>
<td>0</td>
</tr>
<tr>
<td>Platelets</td>
<td>NT†</td>
</tr>
<tr>
<td>Erythrocytes</td>
<td>0</td>
</tr>
<tr>
<td>PHA-stimulated lymphocytes</td>
<td>40</td>
</tr>
<tr>
<td>Thymocytes†</td>
<td>2.5</td>
</tr>
</tbody>
</table>

*Percentage of positive cells was determined using multiparameter analysis as described by Hoffman et al. (35) for peripheral blood buffy coat. Background positivity was subtracted from the values indicated.
† Not tested for fluorescence, but negative in RIA.
‡ Total cellular suspension of thymocytes.

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**Fig. 4.** Binding of 125I-labeled L5.1 (○-○) and 125I-labeled transferrin (○-○) to K562(S) cells at different times after treatment with 12-O-tetradecanoyl-phorbol-13-acetate (TPA). Abscissa: Hours after addition of TPA (1.7 x 10^-4 M); Ordinates: cpm 125I bound to cells.

**Fig. 5.** Multiparameter analysis of human marrow cells reactive with L5.1 monoclonal antibody. A) Bone marrow cell population; B) Population of cells from A reacting with L5.1 antibody. 1) erythrocytes; 2) lymphocyte + erythroid cells; 3) myeloid cells. x-axis, right-angle light scatter; y-axis, forward-angle scatter; z-axis, number of cells.
population in the bone marrow that reacts with L5.1. The data indicate that a homogeneous population of relatively small nucleated cells contains the antigen defined by L5.1.

The positive bone marrow population was 22.9% of the total population. Morphological analysis of the sorted cells (Fig. 6) indicated that all the positive cells consisted of pronormoblasts, basophilic normoblasts, and polychromatic and orthochromatic normoblasts. A very small population of normal promyelocytes, representing less than 20% of the total marrow promyelocytes also consistently reacted with L5.1. The negative bone marrow population contained myeloid cells, monocytes and lymphocytes and essentially no erythroid cells.

When L5.1 was tested against normal thymocytes, a very small fraction (~2.5%) was found to be positive by cell sorting analysis (Table 2).

Reactivity of Leukemic Cells with Monoclonal Antibody L5.1

Leukemic cells obtained from bone marrow of patients with different types of acute leukemia were tested for reactivity with monoclonal antibody L5.1. All were consistently negative except for one case of non-B, non-T-ALL (Table 3). These results indicate that the surface antigen is not usually expressed in acute human leukemias, and thus it is not a suitable marker for distinguishing subtypes of leukemias.

Reactivity of L5.1 with Nonhemopoietic Cell Lines

A number of nonhemopoietic cell lines were tested (Table 4) for reactivity with L5.1 to determine whether the antibody reacted with cell lines derived from a variety of tissues. A melanoma and a colon carcinoma cell line were found to be strongly positive, whereas other tumor cell lines and WI38 cells, human embryonal fibroblasts, were negative both by RIA and by immunofluorescence.

DISCUSSION

We report here the characterization of the L5.1 monoclonal antibody found to react primarily with the K562(S) erythroleukemic cell line and with the immature nucleated red cell population of the bone marrow. The surface antigen recognized by L5.1 is a glycoprotein with an apparent molecular weight of 180,000 formed by a disulfide bonded dimer of two 87,000 dalton polypeptides.

The structural characteristics and molecular weight are similar to those reported for the transferrin receptor isolated from human placenta, from tissue culture cell lines, from a T cell line, and from PHA

<table>
<thead>
<tr>
<th>Type of Leukemia</th>
<th>Cases Positive* / Number of Cases</th>
</tr>
</thead>
<tbody>
<tr>
<td>Acute undifferentiated</td>
<td>0/13</td>
</tr>
<tr>
<td>Acute myeloblastic</td>
<td>0/1</td>
</tr>
<tr>
<td>CLL</td>
<td>0/1</td>
</tr>
<tr>
<td>Acute erythroleukemia</td>
<td>0/3</td>
</tr>
<tr>
<td>CML</td>
<td>0/10</td>
</tr>
<tr>
<td>B-ALL</td>
<td>0/2</td>
</tr>
<tr>
<td>T-ALL</td>
<td>0/3</td>
</tr>
<tr>
<td>Non-B, Non-T-ALL</td>
<td>1/12</td>
</tr>
</tbody>
</table>

*Positive cases were those in which more than 20% of the cells fluoresced as determined in the cell sorter. The leukemic bone marrow contained at least 80% leukemic infiltrate. Positive controls included unrelated monoclonal antibodies reactive with various types of leukemias (Perussia et al., submitted for publication).

<table>
<thead>
<tr>
<th>Cell Line</th>
<th>Type</th>
<th>Reactivity*</th>
</tr>
</thead>
<tbody>
<tr>
<td>WI38</td>
<td>embryonal fibroblasts</td>
<td>**</td>
</tr>
<tr>
<td>LNSV</td>
<td>SV40-transformed fibroblasts</td>
<td></td>
</tr>
<tr>
<td>WM9</td>
<td>melanoma, metastatic</td>
<td>+</td>
</tr>
<tr>
<td>SW1116</td>
<td>colon adenocarcinoma</td>
<td>+</td>
</tr>
<tr>
<td>KATO</td>
<td>gastric adenocarcinoma</td>
<td></td>
</tr>
<tr>
<td>Astro 1783</td>
<td>astrocytoma</td>
<td></td>
</tr>
<tr>
<td>Hep G2</td>
<td>hepatoma</td>
<td></td>
</tr>
<tr>
<td>Hep 38</td>
<td>hepatoma</td>
<td></td>
</tr>
<tr>
<td>HT1080</td>
<td>osteosarcoma</td>
<td></td>
</tr>
<tr>
<td>N2102</td>
<td>teratocarcinoma</td>
<td></td>
</tr>
<tr>
<td>SW900</td>
<td>lung carcinoma</td>
<td></td>
</tr>
</tbody>
</table>

*Binding of L5.1 to cells was determined by indirect immunofluorescence or by radioimmunoassay (RIA) as described in Material and Methods.

- ** = >70% of cells positive (by fluorescence) or >2 times above background (by RIA)
- * = <99% of cells negative (by fluorescence) or <1.5 times above background (by RIA)
stimulated T cells. The competition experiments with transferrin and with OKT9, a monoclonal antibody reported to bind the transferrin receptor, confirm that L5.1 recognizes the transferrin receptor in cells of the erythroid lineage. The presence of the surface antigen defined by L5.1 in immature erythroid cells and in reticulocytes, and its absence in erythrocytes, further supports the notion that L5.1 is recognizing the transferrin receptor, as this receptor is present in reticulocytes but absent in mature erythrocytes.

Trowbridge and Omary have shown that the B3/25 monoclonal antibody binds to the transferrin receptor. B3/25 reacted with all T cell, B cell, and non-T, non-B cell lines tested as well as with HL60 and K562 cells. Sutherland et al. have shown that the OKT9 anti-T cell monoclonal antibody recognizes the transferrin receptor in T cell lines. Haynes has shown that another antitransferrin receptor (5E9) binds to all T-, B-, promyelocytic, and histiocytic cell lines, and with the choriocarcinoma, epidermoid carcinoma and hepatoma cell lines tested.

Thus, differences in the patterns of reactivity of L5.1, OKT9, B3/25 and 5E9 with cell lines can be seen. Antibody L5.1 seems more restricted in its reactivity than the other three monoclonal antibodies. Antibody L5.1 does not react with W138 cells, which have been shown to express the transferrin receptor, nor does it react with most of the nonhemopoietic cell lines tested. It is therefore possible that transferrin receptors are heterogeneous, and/or the various monoclonal antibodies against transferrin receptors bind with different affinity.

The expression of the transferrin receptor can be modulated by treatment of cells with agents affecting cellular differentiation. Omary et al. have reported that treatment of HL60 with DMSO causes disappearance of the transferrin receptor detected by B3/25. We have shown here that the binding of both L5.1 and of transferrin to K562(S) cells decreases as a function of time in an identical way, which further supports the interpretation that the surface antigen defined by L5.1 and the transferrin receptor are the same molecule.

The fact that L5.1 reacts with only a few nonerythroid cell lines and with some nonhemopoietic malignant cell lines in no way detracts from its usefulness in the identification and rapid purification of erythroid precursor cells from the bone marrow using flow cytfluorimetry. The extent of myeloid contamination in this case is minimal and contrasts with the reactivity reported for other antitransferrin monoclonal antibodies. The reactivity with a fraction of promyelocytes is of interest because L5.1 was originated from the HL60 human promyelocytic leukemia cell line.

In summary, the studies presented here indicate that L5.1 is a monoclonal antibody directed against the transferrin receptor. Unlike the other antitransferrin receptor antibodies, L5.1 binds almost exclusively to erythroid cell precursors. However, L5.1 also reacts with a number of nonerythroid established cell lines. Using L5.1, we have also shown that the transferrin receptor is expressed on the surface of all the erythroid precursor cells in the bone marrow, from the pronormoblast to the orthochromatric normoblast. Approximately 50% of the reticulocytes and a small fraction of promyelocytes have detectable transferrin receptors.

The relationship between transferrin receptors and heme metabolism in erythroid cell differentiation, and between transferrin receptors and the requirement for transferrin for cell growth in culture make these antitransferrin receptor monoclonal antibodies valuable reagents to study these processes in detail.

ACKNOWLEDGMENT

We thank Dr. B. Perussia and Dr. G. Trinchieri for the gift of P3-x63-Ag8 653 mouse myeloma cells, and for helpful suggestions during the preparation and screening of monoclonal antibodies; and J. Faust for help in using the cell sorter. We thank Drs. B. Knowles, Z. Steplewski, D. Santoli, V. Cristofalo, J. Minowada, H. P. Koeller and R. C. Gallo for the gift of the various cell lines used throughout this work. This work was supported by grants CA-10815, CA-24273, CA-25875 and AI-17636 from the National Institutes of Health; CH-202 from the American Cancer Society; and by a grant from the W. W. Smith Foundation. B. Lange is a recipient of JFCF 564 from the American Cancer Society. We thank Marina Hoffman for the editing and Ann McNab for the typing of this manuscript.

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A monoclonal antibody that detects expression of transferrin receptor in human erythroid precursor cells

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