CONCISE REPORT

My-1, the Human Myeloid-Specific Antigen Detected by Mouse Monoclonal Antibodies, Is a Sugar Sequence Found in Lacto-N-Fucopentaose III

By Laura C. Huang, Curt I. Civin, John L. Magnani, Joel H. Shaper, and Victor Ginsburg

The My-1 cell surface antigen is expressed on human granulocytes and granulocytic precursor cells. The sugar sequence

\[ \text{Gal} \beta_1-4\text{GlcNAc} \beta_1-3\text{Gal} \ldots, \quad \text{Fucal} \]

which occurs in the glycolipid, lacto-N-fucopentaose III ceramide, in several higher glycolipids, and in glycoproteins, contains the epitope recognized by anti-My-1 and 17 other monoclonal antibodies with the same cellular specificity.

THE ANTI-My-1 MONOCLONAL antibody, produced by a hybridoma obtained from a mouse immunized with HL60 human promyelocytic leukemia cells,\(^1\) reacts with granulocytes and granulocytic precursor cells but not with normal peripheral blood lymphocytes, monocytes, platelets, or red cells.\(^2,4\) The antigen (designated My-1) detected by this monoclonal antibody has been characterized by the data in this article as a sugar sequence found in the human milk oligosaccharide lacto-N-fucopentaose III.\(^5\)

\[ \text{Gal} \beta_1-4\text{GlcNAc} \beta_1-3\text{Gal} \beta_1-4\text{Glc} \]

A glycolipid containing lacto-N-fucopentaose III was first isolated from human adenocarcinoma.\(^6\) The same sugar sequence minus the glucosyl residue occurs in higher glycolipids\(^7\) and also in glycoproteins.\(^8\) Many other monoclonal antibodies directed against this sugar sequence have been produced by hybridomas obtained from mice and rats immunized with various tumor cells.\(^7,9,10\)

MATERIALS AND METHODS

Materials

Lacto-N-fucopentaose I (Fucol-2Galβ1-3GlcNAcβ1-3Galβ1-4Glc), lacto-N-neotetraose (Galβ1-4GlcNAcβ1-3Galβ1-4Glc), and lacto-N-fucopentaose III were isolated from human milk.\(^11\) Globose (GbOseCer) (GalNAcβ1-3-Galβ1-4Glcβ1-1 ceramide) was obtained from Supelco, Inc., Bellefonte, PA. Lacto-N-fucopentaose III ceramide was a gift from Dr. David A. Zopf (National Cancer Institute, Bethesda, MD). The higher glycolipids used as chromatographic standards for Fig. 1 were kindly supplied by Dr. S.-I. Hakomori (Fred Hutchinson Cancer Research Center, Seattle, WA).

HL60 cells were cultured and prepared as described, as were normal peripheral blood granulocytes and mononuclear cells.\(^2,12\) Monoclonal antibodies were the products of twice-cloned murine hybridoma cells, prepared as described.\(^2,12\) Spent hybridoma culture supernatant was the source of monoclonal antibody.

Isolation of Neutral Glycolipid

Glycolipids were extracted from HL60 cells, granulocytes, and peripheral blood mononuclear cells and phase-partitioned as described by Folch et al.\(^13\) The upper-phase glycolipids were applied to a column of DEAE Sepharose CL-6B, and the neutral glycolipids that emerged were used for solid-phase radioimmunoassay and for autoradiography after thin-layer chromatography.

Solid-Phase Radioimmunoassay

The binding of antibody to glycolipid was measured by solid-phase radioimmunoassay as previously described.\(^14\) Glycolipid in 20 µl of methanol was added to wells of a round-bottom polystyrene microtiter plate (Dynatech, Alexandria, VA) and the solutions dried by evaporation. The wells were then filled with phosphate-buffered saline (0.15 M NaCl, 0.01 M sodium phosphate, pH 7.4) containing 1% bovine serum albumin (buffer A). After 30 min, the wells were emptied, and to each was added 20 µl of monoclonal antibody solution diluted 1:4 with buffer A. The wells were covered with paraffin, incubated for 3 hr at 22°C, washed once with buffer A, and then to each was added about 20,000 cpm of anti-mouse Fab from rabbit, \(^{125}\)I-labeled F(ab')2, in 20 µl of buffer A. After 3 hr, the wells were washed 6 times with cold phosphate-buffered saline, cut from the plate, and assayed for \(^{125}\)I in an Auto-Gamma spectrometer.
Autoradiography of Glycolipid Antigens

Glycolipid antigens were detected on thin-layer chromatograms by autoradiography as previously described. Glycolipids were chromatographed on aluminum-backed high performance thin-layer chromatography plates (Silica Gel 60, Merck, Darmstadt, West Germany; American Supplier, Applied Analytical Services, Wilmington, NC) in chloroform:methanol:0.2% CaCl₂ (60:40:9 by volume). The dried chromatogram was soaked for 30 sec in a saturated solution of polyisobutylmethacrylate (Polysciences, Inc., Warrington, PA) in hexane. After drying in air, the chromatogram was sprayed with buffer A and immediately soaked in buffer A until all of the silica gel was wet (about 10 min). The plate was then removed and overlaid with monoclonal antibody solution diluted 1:4 with buffer A (about 55 µl/sq cm) and incubated for 3 hr at 4°C. The chromatogram was washed by dipping in 4 successive changes of cold phosphate-buffered saline at 1-min intervals and overlaid with buffer A containing 10⁶ cpm/ml of ¹²⁵I-labeled F(ab')₂ of rabbit anti-mouse immunoglobulin G antibodies (about 40 µCi/mg; Amer sham Corp., Arlington Heights, IL). After 3 hr at 4°C, the chromatogram was washed as before in cold phosphate-buffered saline, dried, and exposed to X-R 5 x-ray film (Eastman Kodak, Rochester, NY) against an intensifying screen (Cronex, Lightning Plus AH, DuPont, Wilmington, DE) for 2-3 hr at -90°C.

RESULTS AND DISCUSSION

As protein reacting with anti-My-1 antibody was not detected by immunoprecipitation¹⁵ of ¹²⁴I- or ³⁵S-labeled proteins from HL60 cells or granulocytes, glycolipids were tested as possible antigens. In solid-phase radioimmunoassay, the antibody binds to the neutral glycolipids of HL60 cells and lacto-N-fucopentaose III ceramide but not to the neutral glycolipids of peripheral blood mononuclear cells or to globoside (Table 1).

The specificity of anti-My-1 antibody for a sequence of sugars in lacto-N-fucopentaose III was confirmed by hapten inhibition (Fig. 1). Binding of the antibody to the neutral glycolipids of HL60 cells in solid-phase radioimmunoassay was specifically inhibited by lacto-N-fucopentaose III but not by the closely related oligosaccharides, lacto-N-fucopentaose I and lacto-N-neotetraose (see Materials and Methods for structures). About 0.3 mM lacto-N-fucopentaose III inhibits binding by 50%.

The binding of anti-My-1 antibody to authentic lacto-N-fucopentaose III ceramide and to glycolipids in extracts of HL60 cells and granulocytes was detected by radioautography after chromatography (Fig. 2). In contrast to colon adenocarcinoma and small cell lung cancer,¹⁰ HL60 cells and granulocytes contain little if any lacto-N-fucopentaose III ceramide. From a comparison with the chromatographic mobilities of the standard glycolipids shown in Fig. 2, the approximate size of the glycolipid antigens from HL60 cells and granulocytes that are detected by the anti-

<table>
<thead>
<tr>
<th>Target</th>
<th>¹²⁵I Bound (cpm)</th>
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<tr>
<td>Neutral glycolipids from 0.5 mg wet weight of HL60 cells</td>
<td>2,200</td>
</tr>
<tr>
<td>Neutral glycolipids from 0.5 mg wet weight of peripheral blood mononuclear cells</td>
<td>140</td>
</tr>
<tr>
<td>Lacto-N-fucopentaose III ceramide (80 ng)</td>
<td>3,700</td>
</tr>
<tr>
<td>Globoside (100 ng)</td>
<td>70</td>
</tr>
<tr>
<td>None</td>
<td>60</td>
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Solid-phase radioimmunoassay was performed on microtiter plates as described in Materials and Methods.
My-1 antibody can be estimated to range from a heptasaccharide to larger than a dodecasaccharide. Granulocytes contain more antigen than do HL60 cells on the basis of wet weight. In addition, granulocytes have two glycolipid antigens that migrate between standard ceramide decasaccharide and ceramide dodecasaccharide that are not detected in the extract from HL60 cells (Fig. 2). These differences may be related to granulocyte maturation or to the fact that HL60 cells are leukemic.

Seventeen additional monoclonal antibodies with specificity for granulocytes and granulocytic precursor cells were produced by hybridomas obtained from four independent fusions using mice immunized with HL60 cells or normal human granulocytes. These antibodies were selected for binding to whole viable granulocytes but not to lymphocytes as described. When examined by the methods described above, all of these 17 antibodies were also found to react with a sugar sequence in lacto-N-fucopentaose III. Apparently this sugar sequence is extremely immunogenic in mice. All of the antibodies reported in this article and all of the antibodies with the same specificity reported in previous papers are of the immunoglobulin M type.

Although My-1 is strongly expressed only on granulocytic cells in the human hematopoietic system, it is also strongly expressed on some nonhematopoietic human and murine cells as well (My-1 and the murine stage-specific embryonic antigen, SSEA-1, are the same antigens).

Chang et al. have recently reported the transient appearance of the My-1 antigen on My-1-negative mouse fibroblasts following transfer of DNA from human myeloblastic or lymphoblastic leukemia cells. As My-1 is a carbohydrate antigen, it is not a direct gene product. Possibly DNA transfer transiently alters the pattern of glycosyltransferases normally present in the recipient cells, and it is these altered glycosyltransferases that catalyze the synthesis of My-1.

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Fig. 2. Autoradiography of the glycolipid antigens of anti-My-1 antibody from human promyelocytic leukemia cell HL60 and normal granulocytes after thin-layer chromatography. Extraction of glycolipids, chromatography, and autoradiography were carried out as described in Materials and Methods. Lane 1, glycolipid extract from 10 mg wet weight of HL60 cells; lane 2, glycolipid extract from 10 mg wet weight of granulocytes; and lane 3, 25 ng of lacto-N-fucopentaose III ceramide. The positions of some standard glycolipids are shown on the right. The abbreviations used are: LNF III Cer, lacto-N-fucopentaose III ceramide; Ose10Cer, a branched glycolipid containing 10 glycosyl residues; Ose12Cer, a branched glycolipid containing 12 glycosyl residues; and Ose14Cer, a branched glycolipid containing 14 glycosyl residues.
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