Monoclonal Antibodies Directed against the Human Le\(^b\) Blood Group Antigen*

Manfred Brockhaus\&frac12;, John L. Magnani\&frac12;, Magdalena Blaszczyk\&frac12;, Zenon Steplewski\&frac12;, Hilary Koprowski\&frac12;, Karl-Anders Karlsson\&frac12;, Göran Larsson\&frac12;, and Victor Ginsburg\&frac12;.

From the \&frac12;National Institute of Arthritis, Metabolism and Digestive Diseases, National Institutes of Health, Bethesda, Maryland 20205, the \&frac12;Wistar Institute of Anatomy and Biology, Philadelphia, Pennsylvania 19104, and the \&frac12;Department of Medical Biochemistry, University of Göteborg, S-400 33 Göteborg, Sweden.

Four monoclonal antibodies produced by hybridomas obtained from a mouse immunized with a human adenocarcinoma cell line SW1116 (Koprowski, H., Steplewski, Z., Mitchell, K., Herlyn, M., Herlyn, D., and Fuhrer, P. (1979) Somatic Cell Genet. 5, 957-972) are directed against the Le\(^b\) antigen of the human Lewis blood group system. Their specificities were established by binding studies using purified Le\(^b\)-active ceramide hexa saccharide and by hapten inhibition studies involving oligosaccharides obtained from human milk.

Nineteen monoclonal antibodies produced by hybridomas obtained from a mouse immunized with a human colon adenocarcinoma cell line have an apparent specificity for cell lines derived from human intestinal tumors (1). The antigen for one of these antibodies (1116NS-52a) has recently been characterized as a monosialoganglioside (2). Four other antibodies (1116NS-10, 1116NS-33a, 1116NS-38a, and 1116NS-43a) are identified by the data in the present paper as being directed against the Le\(^b\) antigen of the human Lewis blood group system (3). The Le\(^b\) antigen contains the terminal sugar sequence

\[
\text{Fuc}1\text{-2Gal1-3GlcNAc}\ldots
\]

and occurs in the glycolipids and glycoproteins of approximately 75% of the population who belong to the Le(a-b+) blood group (4). The remaining 25% of the population are unable to synthesize the Le\(^b\) sequence of sugars because they lack either the fucosyltransferase responsible for the formation of Fuc1-2Gal linkages (5) and belong to the Le(a-b-) blood group. The Le\(^b\) antigen is stable hybridoma clones, 19 of which secreted antibodies with apparent specificity for colon adenocarcinoma cells (1). Antibodies 1116NS-10, 1116NS-33a, and 1116NS-38a are of the immunoglobulin M type. Antibody 1116NS-43a is of the immunoglobulin G\(_1\) type. Serum-free supernatant fluids from hybridoma cell cultures (7) containing approximately 10 \(\mu\)g/ml of antibody described here. Oligosaccharides were isolated from human milk (8); Le\(^b\)-active ceramide hexa saccharide,

\[
\text{Fuc1-1-2Gal1-3GlcNAc1-3Gal1-4Glc1-1Cer},
\]

and an ALe\(^b\) ceramide heptasaccharide,

\[
\text{GalNAco-1-3Gal1-4Glc1-1-3Gal1-4Cer}.\]

were isolated from adult human small intestine (9). Their structures were confirmed with methods described elsewhere (10). The ALe\(^b\) glycolipid does not react with commercial human Le\(^b\) or A antisera but reacts with the Siedler antibody which is an AILeh antibody (3, 11). Other glycolipids were commercial products (Supelco, Bella- fonte, PA).

Lipid extracts of colon carcinoma line SW1116 and melanoma cell line WM 9 were prepared by the method of Folch et al. (12). The neutral glycolipids of human meconium were isolated as previously described (2, 10).

Anti-mouse Fab from rabbit \(^{125}\text{I}-\text{labeled F(ab')2}\) fragment (about 40 \(\mu\)Ci/\(\mu\)g) was obtained from The Radiochemical Centre, Amersham, England.

Solid Phase Radioimmunoassay—Binding of antibody to glycolipid is assayed by the procedure of Young et al. (13) with modifications as follows: glycolipid in 20 \(\mu\)l of methanol is added to wells in a polystyrene microtiter plate (Dynatech, Alexandria, VA) and the solutions dried by evaporation. After 90 min, the wells are filled with phosphate-buffered saline (0.15 M NaCl, 0.001 M sodium phosphate, pH 7.2) containing 1% bovine serum albumin (buffer A). After 2 h, the wells are emptied and to each is added 20 \(\mu\)l of buffer A and 5 \(\mu\)l of the monoclonal antibody solution. After covering the wells with parafilm, the microtiter plate is slowly rotated at a 45° angle to facilitate diffusion for 6 h at 22 °C. The wells are emptied, washed once with buffer A, and then to each well is added about 13,000 cpm of anti-mouse Fab from rabbit, \(^{125}\text{I}-\text{labeled F(ab')2,}\) in 20 \(\mu\)l of buffer A. After rotation of the microtiter plate for an additional 12 h, the wells are washed six times with cold phosphate-buffered saline, cut from the plate, and assayed individually for \(^{125}\text{I}\) in a Auto-Gamma spectrometer.

Thin Layer Chromatography and Autoradiography—Thin layer chromatography is carried out on HPTLC plates precoated with Silica Gel 60 (E. Merck, West Germany) developed with chloroform-methanol-water (60:35:5 by volume) or butanol. Glycolipid antigens are detected by autoradiography using a previously published procedure (2) with minor modifications as follows: after chromatography, the chromat-
Monoclonal Antibodies against the Human Le$^b$ Antigen

![Graph 1](image1.png)

**FIG. 1.** Binding of antibody 1116NS-10 to total lipid extracts of cultured cells and to isolated glycolipids. Solid phase radioimmunoassays were carried out as described under "Experimental Procedures." A, binding of antibody to extracts of colorectal carcinoma cells SW1116 (O ○ O) and to extracts of melanoma cells WM9 (● ● ●); B, binding of antibody to Le$^b$-active ceramide hexasaccharide (□ □ □) and GalNAcβ1-3Galα1-4Galβ1-4Glcβ1-1Ceramide (globoide) (● ● ●).

![Graph 2](image2.png)

**FIG. 2.** Binding of antibody 1116NS-10 to thin layer chromatograms of the neutral glycolipids of meconium and of purified Le$^b$-active ceramide hexasaccharide as detected by autoradiography as described under "Experimental Procedures." Lane 1, 1.0 μg of neutral glycolipids from the meconium of an individual belonging to the Le(a+b−) blood group; lane 2, 1.0 μg of neutral glycolipids from pooled meconium; and lane 3, 0.5 μg of Le$^b$-active ceramide hexasaccharide. The positions of some standard gangliosides and neutral glycolipids after chromatography are shown on the right. The abbreviations used are: CMH, Galβ1-1Ceramide; CDH, Galβ1-4Glcβ1-1Ceramide; CTH, Galα1-4Galβ1-4Glcβ1-1Ceramide; paragloboside, Galβ1-4-GlcNAcβ1-3Galα1-4Glcβ1-1Ceramide; GM1, Galβ1-3Galα1-4[NeuAcα2-3]Galβ1-4Glcβ1-1Ceramide; and Gm1, NeuNAcα2-3Galβ1-3Galα1-4[NeuAcα2-3]Galβ1-4Glcβ1-1Ceramide.

**RESULTS AND DISCUSSION**

Total lipid extracts of adenocarcinoma cell line SW1116 but not melanoma cells WM 9 contain antigen for monoclonal antibody 1116NS-10 as evidenced by solid phase radioimmunoassay (Fig. 1A).

The glycolipids in the extract of adenocarcinoma cells were fractionated by DEAE-Sephrose chromatography and thin layer chromatography as previously described (2) followed by autoradiography. The major antigen of antibody 1116NS-10 in the extract was revealed by this technique to be a neutral glycolipid with the mobility of Gm1 under the conditions of thin layer chromatography described under "Experimental Procedures." An antigen with the same mobility is present in the neutral glycolipid fraction obtained from pooled meconium including samples from individuals belonging to the Le(a+b−) blood group (Fig. 2, lane 2) but not in the neutral glycolipid fraction obtained from an individual belonging to the Le(a+b−) blood group (Fig. 2, lane 1). As this finding suggested that antibody 1116NS-10 recognizes the Le$^b$ antigen, purified Le$^b$-active ceramide hexasaccharide was tested for binding by solid phase radioimmunoassay. As shown in Fig. 1B, the glycolipid effectively binds antibody 1116NS-10 with maximal binding attained by 5 ng of glycolipid. No binding was observed with other glycolipids such as globoide (Fig. 1B). Similar results were obtained with antibodies 1116NS-33a, 1116NS-38a, and 1116NS-43a. None of the four bound to the ceramide heptasaccharide that contains the sugar se-
quences of both the A antigen and the Le\textsuperscript{b} antigen.

 Autoradiography of the Le\textsuperscript{b}-active ceramide hexasaccharide after thin layer chromatography using antibody 1116NS-10 is shown in Fig. 2, lane 3. Its mobility is the same as the major antigen in meconium and in colorectal carcinoma cells. The slower running, minor antigen in meconium (Fig. 2, lane 2) is probably Le\textsuperscript{b}-active ceramide octasaccharide reported previously (10, 14). Autoradiography using antibody 1116NS-43 revealed the same two antigens in meconium as those detected by antibody 1116NS-10. Antibodies 1116NS-33a and 1116NS-38a indicated that they agglutinate Le(a-b+) erythrocytes but not Le(a+b-) or Le(a-b-) erythrocytes. More extensive studies, however, are required to evaluate the usefulness of these antibodies for routine blood grouping.

Monoclonal anti-Le\textsuperscript{b} antibodies should be useful for histochemical studies and may offer an advantage over affinity-purified polyclonal antibodies with Le\textsuperscript{b} specificity which have recently been used for this purpose (17).

Acknowledgments—We are grateful to Mary H. McGinniss for help in blood typing. We would also like to thank Urner Chase for technical assistance and Julie Reddick for preparing the manuscript.

REFERENCES