MOLECULAR COMPLEXITY OF LEUKOCYTE SURFACE GLYCOPROTEINS RELATED TO THE MACROPHAGE DIFFERENTIATION ANTIGEN MAC-1*

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Since the early classic work on murine lymphocyte cell surface differentiation antigens (1), immunological analysis of the cell surface has been a powerful tool with which to distinguish and separate different classes of functional cell types (2). The development of the monoclonal antibody technique (3) has dramatically increased the ease with which cell surface antigenic determinants restricted to particular types of differentiated cells can be identified. If the objective in identifying differentiation antigens is solely to provide the means of discriminating one kind of cell from another, then the molecular basis of the antigenic difference between cells is of little practical consequence. If, however, one wishes to investigate the potential functional significance of a particular antigenic determinant restricted to surface structures of a particular cell type, then the structural basis of the antigenic determinant is of considerable importance. One striking example of the structural and antigenic complexity of cell surface glycoproteins is the family of high molecular weight leukocyte differentiation antigens found in several species, including man, referred to as T200 or leukocyte common antigen (4–9). Monoclonal antibodies have been described that react with all the structural variants of T200 glycoprotein (4, 6, 8), and distinguish hematopoietic cells from other differentiated cell types, while other monoclonal antibodies have been obtained that react only with the form of the glycoprotein found on bone marrow derived B cells and their precursors (10, 11), thus discriminating these cells from other classes of hematopoietic cells.

Recently a cell surface antigen known as Mac-1 has been identified by means of a monoclonal antibody designated M1/70 (12, 13). The antigenic determinant defined by the M1/70 monoclonal antibody is restricted in distribution to the surface of macrophages and it was inferred that the antibody recognized a molecule specific to phagocytes (13). Here we show that the glycoprotein bearing the Mac-1 antigenic determinant belongs to a family of antigenically and structurally related glycoproteins analogous to the T200 glycoprotein family. Variants of these glycoproteins are found on a much wider variety of hematopoietic cells than mononuclear phagocytes. This finding has implications for the biological function of Mac-1 and these related structures.

Materials and Methods

Cell Lines. The cell lines used in these studies were the murine T cell lymphoma, BW5147 (14), and the inducible murine myeloid cell line, M1 (15). Cells were maintained in Dulbecco's modified Eagle's medium supplemented with 10% horse serum. M1 cells were induced to differentiate along the macrophage lineage by exposure to 5×10^{-6} M dexamethasone (16) for 2–5 d.

Immunological Reagents and Procedures. The derivation and properties of monoclonal antibodies M1/70, defining Mac-1, and I3/2, defining an antigenic determinant of murine T200 glycoprotein, have been described previously (4, 12, 13). Monoclonal antibodies I21/7 and C71/16 are rat IgG monoclonal antibodies derived from hybridomas obtained by standard procedures (3, 4) from fusions of Lewis rat spleen cells with either MPC.11.TG.1.7.Oua^R myeloma cells, in the case of I21/7, or S194/5.XX0.BU.1 myeloma cells, in the case of C71/16. The immunogen was whole BW5147 cells for I21/7 monoclonal antibody and cell membrane glycoproteins derived from BW5147 cells for C71/16 monoclonal antibody. Hybridomas producing these monoclonal antibodies have been cloned and are stable.

Immunoprecipitation studies using ascitic fluid from tumor-bearing mice as the source of I21/7 and C71/16 monoclonal antibodies and culture supernates as the source of M1/70 antibody were carried out as described previously (17). A goat-anti-rat IgG serum was used as a second stage reagent, and antibody-antigen complexes were isolated from cell lysates by adsorption to fixed *Staphylococcus aureus*. Fluorescence-activated cell analysis was carried out on a Los Alamos type cell sorter (model BCS-176 biological cell sorter, Research Developments, Los Alamos, N. M.) using an argon laser at 488 λ essentially as described previously (18). The cell sorter is equipped with a three-decade logarithmic amplifier for fluorescence analysis, and relative fluorescence of stained cells was estimated from a standard curve constructed using fluorescent beads of known brightness.

Biochemical Procedures. Cells were labeled by the glucose oxidase modification of the lactoperoxidase technique (17, 19). Immunoprecipitates were analyzed on 7.5% polyacrylamide gels using a discontinuous buffer system (20). Autoradiography was performed using intensifying screens (21). Peptide "mapping" was carried out exactly as described previously except that carboxymethylation was carried out before sodium dodecyl sulphate (SDS)-polyacrylamide gel electrophoresis (17). Briefly, iodinated polypeptides were purified by immunoprecipitation and SDS-polyacrylamide gel electrophoresis. The labeled species were separately extracted from the gels and digested with trypsin. Tryptic peptides were then analyzed on 20 × 20-cm thin-layer cellulose plates by electrophoresis in the first dimension followed by chromatography in the second dimension.

Results

Mac-1 is a differentiation antigen found on macrophages defined by the monoclonal antibody M1/70 (12, 13). This monoclonal antibody precipitates two labeled polypeptides (apparent M_r of 190,000 and 105,000, respectively) from lysates of surface-iodinated macrophages (13). In the course of characterizing the surface glycoproteins of murine hematopoietic cells, we obtained two other monoclonal antibodies, designated I21/7 and C71/16, that appeared to precipitate similar polypeptides to those detected with M1/70 monoclonal antibody. In contrast to M1/70 monoclonal antibody, however, both these antibodies reacted with virtually all thymocytes and a substantial fraction of spleen and bone marrow cells, as judged by fluorescence-activated cell analysis. Moreover, these two antibodies reacted with many different murine hematopoietic tumor cell lines, including T lymphomas, Abelson-virus-induced B cell lines, and Friend virus-induced erythroleukemias. Antibody-blocking experiments showed that although all three antibodies precipitate similar polypeptides, each antibody reacted with distinct antigenic determinants. This was further documented in a striking fashion by the reactivity of the antibodies with BW5147, a

murine T lymphoma cell line (14), and M1, an inducible macrophage cell line (15, 16). As shown in Fig. 1, Mac-1 was expressed on the macrophage cell line but not BW5147 cells, as might be predicted from its known distribution on normal leukocytes (12, 13). In contrast, I21/7 antibody reacted with BW5147 cells but not with M1 cells. C71/16 monoclonal antibody, on the other hand, reacted with both cell lines.

Despite the disparate reactivity of the monoclonal antibodies with BW5147 cells and M1 cells, a comparison of the labeled polypeptides precipitated from lysates of
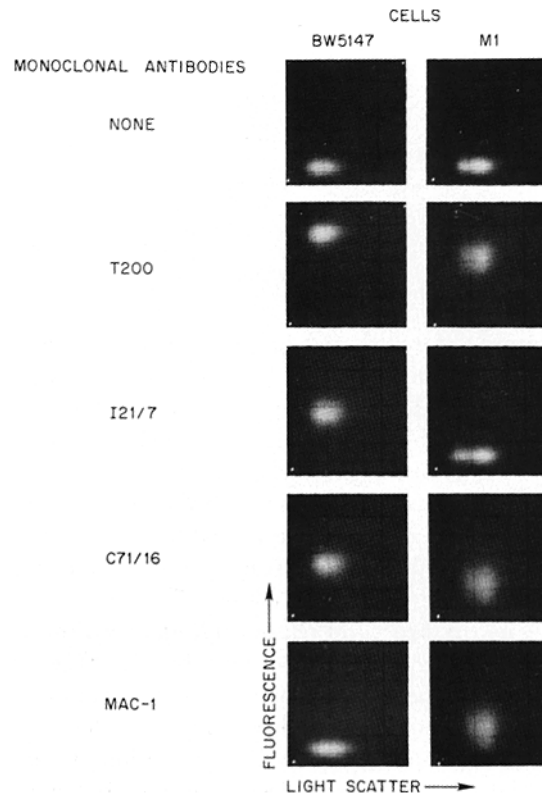

FIG. 1. Quantitative binding of monoclonal antibodies to BW5147 and M1 cells. Fluorescence-activated cell analysis was carried out as described in Materials and Methods using saturating amounts of antibodies. M1 cells were grown in the presence of 5×10^{-6} M dexamethasone for 3 d before analysis to induce differentiation along the macrophage pathway. The results are presented as two-dimensional displays using a three-decade logarithmic amplifier for fluorescence. The mean relative fluorescence was determined by relating the peak fluorescent channel for each cell type stained with various reagents to the absolute fluorescence of standard beads analyzed under the same conditions. The staining of BW5147 cells with anti-T200 monoclonal antibody was set at 1.0. * Indicates relative binding of at least four times background values.

Monoclonal antibody	Relative binding to	
	BW5147	M1
None	0.02	0.02
T200	1.0*	0.8*
I21/7	0.3*	0.02
C71/16	0.2*	0.08*
Mac-1	0.02	0.09*

a   b   c   d

MONOCLONAL   C71/16   I21/7   C71/16   M1/70
ANTIBODIES:

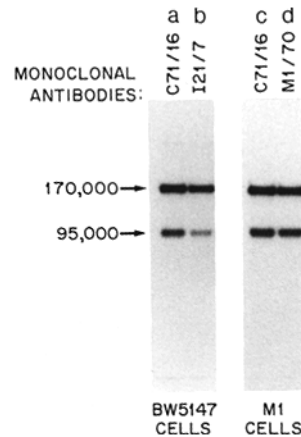170,000→

95,000→

BW5147   M1
CELLS   CELLS

Fig. 2.   SDS-polyacrylamide gel analysis of the labeled polypeptides precipitated by M1/70, C71/16, and I21/7 monoclonal antibodies from lysates of ^{125}I-labeled BW5147 cells and dexamethasone-induced M1 cells.

the iodinated cells confirmed that similar species were precipitated from BW5147 cells by I21/7 and C71/16 antibodies (Fig. 2a, b) and by C71/16 and M1/70 antibodies from M1 cells (Fig. 2c, d). In each case, two polypeptides, both of which were glycosylated as judged by metabolic labeling with [2-^{3}H]mannose, were detected. The apparent molecular weights of the smaller species from each cell line were identical (M_r = 95,000), whereas the larger polypeptide from BW5147 cells and precipitated by both I21/7 and C71/16 antibodies migrated with a slightly higher apparent molecular weight (M_r = 170,000) than the species obtained from M1 cells by precipitation with M1/70 and C71/16 antibodies.

To further investigate the structural relationship between the various glycosylated polypeptides precipitated from the two cell lines by the different monoclonal antibodies, each radioiodinated species was isolated, tryptic peptides prepared, and then each digest analyzed on thin-layer cellulose plates by electrophoresis in the first dimension and chromatography in the second dimension. As shown in Fig. 3, the labeled tryptic peptides of the lower molecular weight polypeptide from M1 and BW5147 cells precipitated by each antibody were indistinguishable (Fig. 3e-h). In contrast, the labeled tryptic peptides of the higher molecular weight polypeptides from M1 and BW5147 cells appeared to be quite different by the same criterion. C71/16 monoclonal antibody, which reacts with both cell lines, therefore precipitates a different high molecular weight polypeptide from each cell type (Fig. 3b, c). These conclusions were confirmed by analysis of the appropriate mixtures of tryptic peptides by the same methods (data not shown).

Discussion

The results reported in this communication show that Mac-1 is an antigenic determinant expressed on a glycoprotein complex that is a member of a structurally and antigenically related family of molecules found on hematopoietic cell types other than mononuclear phagocytes. This is most clearly illustrated by the results obtained with monoclonal antibody C71/16, which reacts with an antigenic determinant

HIGHER
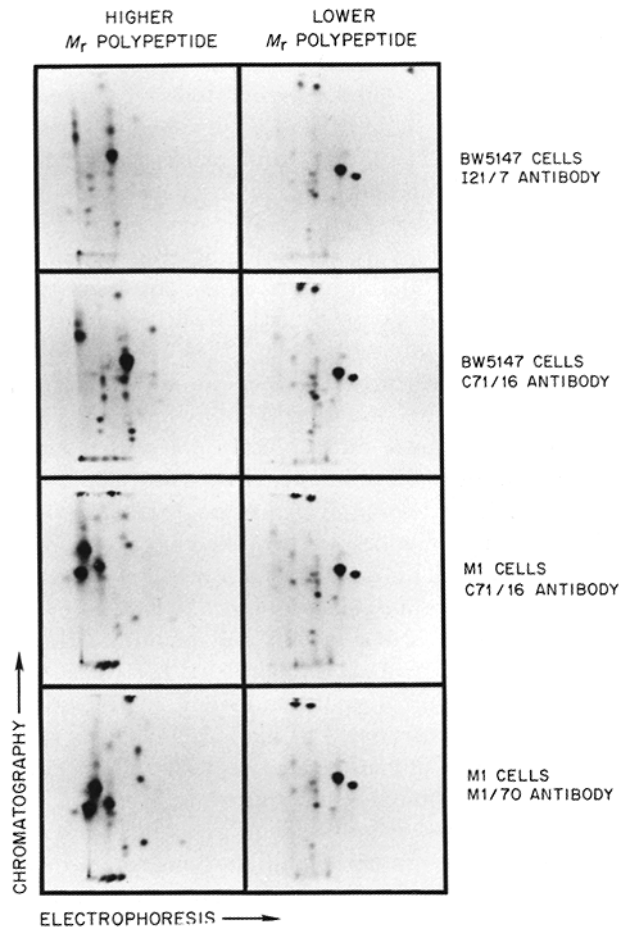M_r POLYPEPTIDE

LOWER
M_r POLYPEPTIDE

FIG. 3.   Comparison of the labeled tryptic peptides from the polypeptides precipitated by M1/70, C71/16, and I21/7 monoclonal antibodies from lysates of ^{125}I-labeled M1 and BW5147 cells. Polypeptides were immunoprecipitated and separated by SDS-polyacrylamide gel electrophoresis (see Fig. 2). The 170,000-mol wt polypeptides (higher M_r polypeptide) and the 95,000-mol wt polypeptides (lower M_r polypeptide) were then extracted from the gel and separately processed for peptide mapping as described in Materials and Methods. Approximately 10,000 cpm of the high molecular weight protein digests and 6,000 cpm of the low molecular weight protein digests were loaded onto the cellulose plates and exposure of the autoradiographs was for 3 d.

common to the forms of the glycoprotein found on the macrophage cell line M1 and the T lymphoma cell line BW5147.

Whereas a plausible interpretation of the serological and structural data presented is that C71/16 monoclonal antibody reacts with an antigenic determinant on the lower molecular weight polypeptide common to the glycoprotein complexes of both M1 and BW5147 cells, and that I21/7 and M1/70 monoclonal antibodies react with the higher molecular weight polypeptides that are dissimilar, further work is required. For example, there is no definitive evidence that the high and low molecular weight polypeptides are physically associated, and it cannot be excluded that each monoclonal antibody reacts with both despite the data from peptide mapping. A separate question is whether the antigenic differences detected reflect either post-translational

modifications of a single gene product such as glycosylation or an expression of distinct but related genes in the different hematopoietic cell types.

The difference in structure between the glycoproteins recognized by I21/7 and M1/70 monoclonal antibodies may be of functional significance since I21/7 monoclonal antibody binds to cytotoxic T cell lines and blocks their cytolytic activity (M. Sarmiento and I. S. Trowbridge, manuscript in preparation). An antibody with similar properties has recently been described by Springer and his colleagues (22). Since it is known that M1/70 monoclonal antibody recognizes an antigenic determinant on human myeloid cells (23), this also raises the possibility that a similar family of glycoproteins defined in the mouse by M1/70, I21/7, and C71/16 monoclonal antibodies exists in man.

It is evident that the results reported in this communication raise many interesting questions concerning the structure and function of these related cell surface glycoproteins shared by macrophages and T cells. It has been reported that during induction of M1 cells there is an increase in a prominent 180,000 surface glycoprotein found on normal macrophages (24), and some evidence has been presented suggesting this molecule may play a role in cell adhesion (16). The glycoprotein complex defined by Mac-1 and C71/16 monoclonal antibodies also increases in expression during induction of M1 cells with dexamethasone (M. B. Omary and I. S. Trowbridge, unpublished results) and thus the high molecular weight polypeptide of this complex may be identical to this previously described glycoprotein. It is possible, therefore, that variants of these glycoproteins on other hematopoietic cell types are also involved in cell-cell or cell-substratum interactions. The major point that is clearly documented, however, is that the structural and antigenic complexity of this family of cell surface glycoproteins accounts for the limited cellular distribution of the Mac-1 differentiation antigen. This situation closely resembles that previously observed for the T200 glycoprotein family (4–11) and emphasizes both the power of the monoclonal antibody technique in the analysis of cell surfaces, and the potential complications that may be encountered in such studies. From the viewpoint of the possible involvement of cell surface structures in cellular functions, it is of considerable importance to establish whether monoclonal antibodies that react with specific cell types define surface antigenic determinants or molecules unique to particular differentiated cells.

Summary

Monoclonal antibodies have been obtained that react with the murine leukocyte surface glycoproteins bearing the macrophage differentiation antigen Mac-1. Structural and antigenic analysis shows that related glycoproteins are found on other murine hematopoietic cell types. The data not only illustrate the complexity of cell surface structures that can be detected by means of monoclonal antibodies, but also raise questions as to the functional significance of this family of molecules within the hematopoietic system.

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