Brief Definitive Report

T200 CELL SURFACE GLYCOPROTEIN OF THE MOUSE

Polymorphism Defined by the Ly-5 System of Alloantigens

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The Ly-5 alloantigen, originally believed to be restricted to thymocytes and thymus-derived (T) lymphocytes (1), has recently been shown to be present on most murine hematopoietic cells, including prothymocytes and pluripotent stem cells (2). Immunoprecipitation studies by Michaelson et al. (3) demonstrated that Ly-5 alloantisera react with high molecular weight surface proteins of thymocytes and spleen cells. Several components with apparent molecular weights that ranged from 175,000 to 220,000 could be resolved by sodium dodecyl sulfate (SDS)-polyacrylamide gel electrophoresis. The 220,000-mol wt species was found only in spleen cells and appeared to be derived from bone marrow-derived (B) cells.

The tissue distribution and biochemical properties of the Ly-5 alloantigen are very similar to the distribution and properties of the T200 glycoprotein, a major cell surface glycoprotein of murine hematopoietic cells that is a dominant antigen in xenoimmunization (4–6). We report here that Ly-5 alloantisera define a polymorphism of the T200 glycoprotein.

Materials and Methods

Cell Lines. The cell lines used in these studies were the murine T cell lymphoma cell line, BW5147, and two mutant cell lines, BW5147 ($T200^-$) and $BW5147PHA^R$ 1.8, that were derived from BW5147 cells. Further details of the mutant cell lines are given in Results. Cells were maintained in Dulbecco's modified Eagle's medium supplemented with 10% horse serum.

Immunological Reagents and Procedures. Ly-5.1 and Ly-5.2 alloantisera were prepared as described previously (2). Monoclonal T200 antibody was obtained from I3/2.3 hybridoma cells (6), and monoclonal Thy-1 antibody was obtained from the hybridoma T24/31.7 (7). For trace antibody binding assays, monoclonal antibodies were obtained from culture supernates of the appropriate hybridoma cells. For immunoprecipitation studies, a 50% ammonium sulphate fraction of ascitic fluid from tumor-bearing mice was the source of T200 antibody (8). Trace antibody binding assays were performed as previously described (6). Immunoprecipitation studies were carried out with 0.15 M NaCl-0.01 M phosphate buffer (pH 7.2) that contained 1% Nonidet P-40 (Shell Chemical Co., New York), 1% sodium deoxycholate, and 0.1% SDS to solubilize cells. Antibody-antigen complexes were isolated from cell lysates by adsorption to fixed *Staphylococcus aureus*. These procedures were as previously reported (8), and give low backgrounds of nonspecific binding. For precipitation of Ly-5 antigen, 5 μl of alloantiserum was used, and antibody-antigen complexes were bound directly to *S. aureus*.

Biochemical Procedures. Cells were labeled by the glucose oxidase modification of the lactoperoxidase technique (9). Immunoprecipitates were analyzed on 7.5% polyacrylamide gels with a discontinuous buffer system (10). Autoradiography was performed with fluorescent screens

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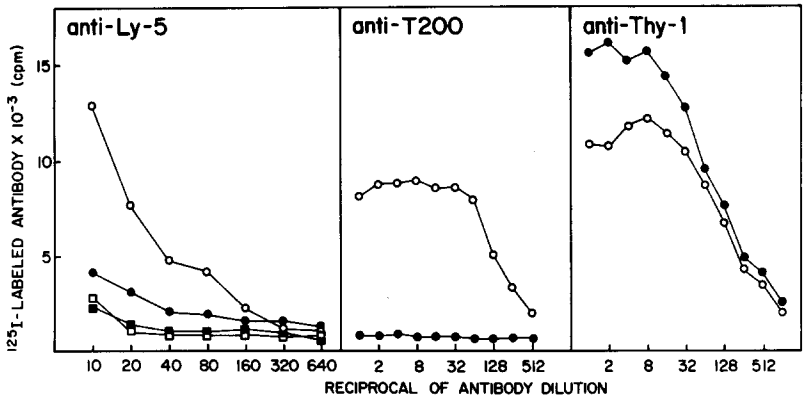

FIG. 1. Binding of Ly-5 alloantisera and rat monoclonal antibodies to BW5147 cells and BW5147(T200$^-$) cells. Trace antibody binding assays were carried out as described in Materials and Methods. ^{125}I-labeled affinity-purified goat anti-mouse IgG antibody was used to detect binding of mouse alloantisera, whereas goat anti-rat IgG antibody was used to detect binding of rat monoclonal antibodies. The left-hand panel shows the binding of Ly-5.1 antibody to BW5147 cells (○), and BW5147(T200$^-$) cells (●). Also shown is the binding of Ly-5.2 antibody to BW5147 cells (□) and BW5147(T200$^-$) cells (■). The middle panel shows the binding of T200 antibody to parental (○) and mutant cells (●). The right-hand panel shows the binding of anti-Thy-1 to parental (○) and mutant (●) cells.

(11). Peptide mapping was carried out exactly as described previously (8). Briefly, iodinated molecules were purified by immunoprecipitation and SDS-polyacrylamide gel electrophoresis. The labeled species were extracted from the gels, carboxymethylated, 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

To investigate the relationship between the Ly-5 alloantigen and the T200 glycoprotein, we took advantage of the availability of a T200-negative mutant cell line derived from BW5147 cells by immunoselection with monoclonal T200 antibody. As described elsewhere (R. Hyman, I. S. Trowbridge, and V. Stallings. Manuscript in preparation.), this mutant, BW5147(T200$^-$), appears to have a defect in the structural gene for T200 glycoprotein, and other surface glycoproteins are expressed in normal amounts on the mutant cells. If Ly-5.1 (BW5147 is derived from an AKR mouse, an Ly-5.1$^+$ strain) were expressed on the parental cells but not on the T200-negative mutant cells, then this would be strong evidence that the Ly-5 alloantisera detect a polymorphism of T200 glycoprotein.

The presence of the Ly-5 alloantigen on parental BW5147 cells and the T200-negative mutant cells was tested by trace antibody binding and by immunoprecipitation studies. As shown in Fig. 1, significant binding of Ly-5.1 antibodies to the parental cell line was detected in the trace antibody binding assay. In contrast, the binding of Ly-5.1 antibodies to BW5147(T200$^-$) cells was only slightly higher than that of Ly-5.2 antibodies used as a control for nonspecific binding. Monoclonal T200 antibody bound only to the parental cells, whereas the binding of a monoclonal Thy-1 antibody to the mutant cells was somewhat higher than to the wild-type cells. In contrast to the Ly-5.1 alloantiserum, plateau levels of maximal binding were obtained with both monoclonal antibodies.

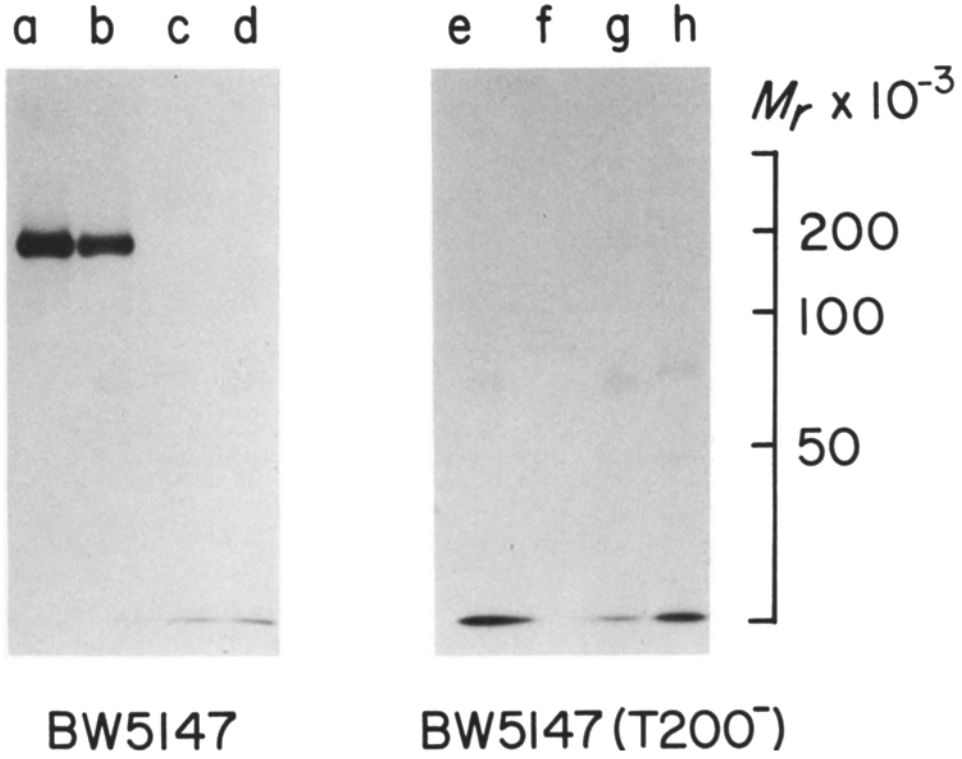

FIG. 2. Ly-5.1 alloantibody precipitates an iodinated molecule from BW5147 cells but not from BW5147(T200$^-$) cells. The autoradiograph (8-h exposure) of a 7.5% SDS-polyacrylamide gel of immunoprecipitates prepared from lysates of iodinated BW5147 cells and BW5147(T200$^-$) cells is shown. Greater than 10-fold more radioactivity was precipitated from BW5147 cells by monoclonal T200 antibody (52,000 cpm) than by Ly-5.1 alloantiserum (4,680 cpm). Only one-tenth the amount of the anti-T200 precipitate from BW5147 cells was loaded relative to the other samples. Gel tracks a-d contain immunoprecipitates from BW5147 cells, and gel tracks e-h contain immunoprecipitates from BW5147(T200$^-$) cells. Antibodies: a and f, anti-T200; b and e, anti-Ly-5.1; c and g, normal C57BL mouse serum; and d and h, normal Lewis rat serum. M_r, apparent molecular weight.

The results of immunoprecipitation studies that employed Ly-5.1 and monoclonal T200 antibody are shown in Fig. 2. BW5147 cells and BW5147(T200$^-$) mutant cells were labeled by lactoperoxidase-catalzyed idonation and cell lysates prepared as described in Materials and Methods. Anti-Ly-5.1 serum specifically precipitated a labeled molecule from lysates of BW5147 cells with an apparent molecular weight of 200,000 that comigrated on SDS-polyacrylamide with T200 glycoprotein. Neither antibody precipitated a labeled species from lysates of BW5147(T200$^-$) mutant cells.

To obtain additional evidence that Ly-5 antibodies react with T200 glycoprotein, tryptic peptides were prepared from the iodinated species precipitated by Ly-5 alloantisera and T200 antibodies and analyzed by two-dimensional peptide mapping. The source of labeled antigens for these experiments was the cell line BW5147.PHA^R 1.8, a phytohemagglutinin-resistant mutant derived from BW5147 cells. As described previously (12), this mutant cell line has a defect in the synthesis of *N*-asparagine-linked complex oligosaccharides. Tryptic peptides of iodinated T200 glycoprotein from BW5147.PHA^R 1.8 cells are more satisfactorily resolved than those of T200

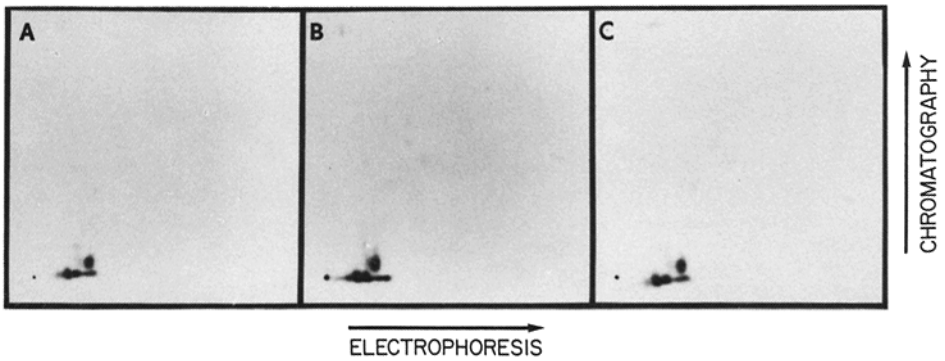

FIG. 3. Comparison of the peptide maps of Ly-5 antigen and T200 glycoprotein. T200 glycoprotein and Ly-5.1 antigen were isolated from BW5147.PHA^R 1.8 cells labeled by lactoperoxidase-catalyzed iodination by immunoprecipitation and SDS-gel electrophoresis. Tryptic peptides were prepared and analyzed on thin-layer cellulose plates by electrophoresis in the first dimension and chromatography in the second dimension. Additional details are given in Materials and Methods.

glycoprotein from wild-type cells by the peptide mapping procedures employed, presumably because of differences in their oligosaccharide moieties (8). As shown in Fig. 3, the peptides obtained from Ly-5 antigen and T200 glycoprotein appeared to be identical (Fig. 3 a and b). This was confirmed by analysis of a mixture of both tryptic digests (Fig. 3 c).

Discussion

The results reported here establish that alloantisera that defined Ly-5 detect a polymorphism of T200 glycoprotein, a major cell surface glycoprotein of murine hematopoietic cells originally detected by surface labeling techniques and immunoprecipitation with xenoantisera (4, 5). We have previously estimated by saturating binding assays with a rat monoclonal T200 antibody that there are, on average, ~50,000 molecules of T200 glycoprotein on thymocytes and spleen cells and ~25,000 molecule/cell on bone marrow cells (6). Fluorescence-activated cell analysis shows most cells in these tissues express similar amounts of antigen (7). Thus, Ly-5 is similar to other alloantigens of murine lymphocytes, including Thy-1, Lyt-2, and Lyt-1 in that it represents a polymorphism of a glycoprotein present in relative abundance on the cell surface (13–15).

Although the present studies demonstrate that the Ly-5 alloantigen is a polymorphism of T200 glycoprotein, and thus confirm estimates of the molecular weight of the antigen given previously by Michaelson et al. (3), Ewald and McKenzie (16) have suggested that Ly-5 was associated with a 130,000-mol wt glycoprotein. The reason for the lower estimate of the apparent molecular weight of Ly-5 antigen found in this study is not clear, although one possibility is that partial proteolysis of T200 glycoprotein occurred during isolation.

A high molecular weight glycoprotein, referred to as leukocyte-common antigen, has been isolated in the rat (17). This molecule appears to be analogous to murine T200 glycoprotein, and, recently, a polymorphism of this rat glycoprotein comparable with that of the Ly-5 alloantigen in the mouse has been demonstrated (18). Alloantisera that define the rat alloantigens ART (19) and Ly-1 (20) were found to react

with leukocyte-common antigen even though in direct cytotoxic tests these antisera selectively killed thymocytes and T cells. It is striking that not only these alloantisera in the rat, but also Ly-5 alloantisera and monoclonal T200 antibody (1, 2, 7) in the mouse, selectively kill thymocytes and T cells despite the fact that in both species the glycoprotein with which the antibodies react are found in similar amounts on most hematopoietic cells. The basis of this selective cytotoxicity is not known, although the trivial explanation that there is a synergistic interaction of each of these antibodies with sublytic amounts of anti-rodent T cell antibodies in the complement source can probably be discounted (7).

Clarification of the relationship between Ly-5 antigen and T200 glycoprotein allows a choice to be made in future studies between the use of alloantisera against Ly-5 and xenogeneic T200 monoclonal antibodies, such as the one used in this investigation, which does not discriminate between the Ly-5.1 and Ly-5.2 phenotypes (I. S. Trowbridge. Unpublished results.). Whereas Ly-5 alloantisera should prove useful for cell lineage studies within the murine hematopoietic system, xenogeneic monoclonal antibodies, which are generally of higher affinity than the corresponding alloantibodies, are likely to be more valuable for structural studies of T200 glycoprotein (8).

Summary

The cell line BW5147, and a mutant T200-negative cell line derived from BW5147, were studied by immunoprecipitation and peptide mapping, with xenogeneic monoclonal anti-T200 serum and with Ly-5 alloantiserum. It appears that the Ly-5 system defines a structural polymorphism of the cell surface glycoprotein T200, and that the monoclonal anti-T200 serum defines a feature of T200 that is common to mice of both $Ly\text{-}5^a$ and $Ly\text{-}5^b$ genotypes and may be invariable in the species.

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