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A New Mouse Lymphoid Alloantigen (Lgp100) Recognized by a Monoclonal Rat Antibody

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Abstract. A new genetically polymorphic cell surface antigen recognized by a monoclonal rat anti-mouse antibody is expressed on mouse lymphoid cells. Fluorescence analysis on the fluorescence-activated cell sorter (FACS) locates the antigen on thymocytes, lymph node cells, and both T and B cells in the spleen. It also appears on approximately 40% of cells in the bone marrow.

Immune precipitations from surface iodinated spleen cells followed by 2-D gel electrophoresis demonstrate that the antigen is a glycoprotein of approximately 100,000 daltons. Since it is expressed in all lymphoid tissues and on both T and B cells, we designate it lymphoid glycoprotein 100 (Lgp100).

Strains with Lgp100 include A/J, AKR/J, AKR/Cu, BALB/c, 129/J, CBA/N, C3H/HeJ, CBA/2J, and SJL/J. Strains with no detectable antigen include C57BL/6J, C57BL/10J, C57BR/cdJ, C57L/J, and C58/J. Intercrosses and backcrosses establish a pair of alleles, a positive and a negative one, at a single locus. Heterozygotes display about 50% as much antigen as homozygotes by quantitative membrane immunofluorescence on the FACS. Tests for Lgp100 in 35 recombinant inbred strains from three crosses (CXB, BXB, and BXH) locate this locus on chromosome 1, closely linked to the *Mls* locus.

Introduction

One recent approach toward the analysis of the lymphocyte cell surface has been to produce highly specific probes in the form of monoclonal antibodies against lymphocyte cell surface antigens (Oi et al. 1978, Williams et al. 1977, Pearson et al. 1977, Springer et al. 1978 a and 1978 b, Trowbridge 1978, Hämmerling et al. 1978). We have chosen to examine the xenogeneic response to mouse lymphoid cells by immunizing rats with SJL/J spleen cells. Based upon the initial work by Köhler and Milstein (1975), monoclonal antibody secreting hybridomas were produced by fusion of rat spleen cells with the mouse myeloma cell line NS-1 (Köhler et al. 1976).

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From this initial hybridization we have obtained ten monoclonal antibodies that recognize seven distinct surface antigens. We used one of these monoclonal antibodies to discover and study an interesting, new, genetically polymorphic, lymphoid cell surface antigen. This is a report of the biochemistry, genetics, and cell distribution of this lymphocyte antigen.

Materials and Methods

Inbred strains. BALB/cNHz, C57BL/6J, C57BL/10J, and SJL/J mice were bred at Stanford University. A/J, AKR/J, C3H/HeJ, and DBA/2J mice were obtained from the Jackson Laboratory, Bar Harbor, Maine. AKR/Cu mice were obtained from Cumberland Farms, Clinton, Tennessee. The CXB recombinant inbred (RI) strains were derived by brother-sister inbreeding beginning with the F_2 generation from the cross of BALB/cBy and C57BL/6By (Bailey 1971). The BXH and BXD RI strains were similarly derived from the crosses of C57BL/6J × C3H/HeJ and C57BL/6J × DBA/2J respectively (Taylor et al. 1975, Taylor 1978). RI strains were obtained from the Jackson Laboratory. Breeding pairs of LOU/Ws1/M inbred rats were kindly provided by Dr. Eva Orlans. Chester Beatty Institute, London, England. Rats were bred at Stanford University.

Production of antibody secreting hybridomas. A LOU/Wsl/M rat was immunized IP with 10^7 SJL/J spleen cells and boosted in the same way 4 weeks later. Three days after the boost, 5×10^7 spleen cells from the rat were fused with 5×10^7 cells of the NS-1 BALB/c myeloma parental cell line (Köhler et al. 1976), with polyethylene glycol 1500, according to the procedure of Oi and Herzenberg (1979). After the fusion, the cells were plated into a 96-well culture dish (Costar ≠3596, Cooke Engineering, Alexandria, Virginia) and grown in HAT selective medium. Growing hybrids were detected in every well within 15 days.

Twenty days after the fusion, hybrid supernates were screened in cell binding assays (Tsu and Herzenberg 1979) using SJL/J spleen cells as targets and ^{125}I-conjugated mouse anti-rat IgG, labeled on rat IgG-Sepharose beads (Herzenberg and Herzenberg 1978 a), as the second-step reagent. Twenty-four of 96 supernates showed highly specific (five-fold over background) binding. Cells from these wells were cloned by sorting individual viable cells using a fluorescence-activated cell sorter into microculture wells containing BALB/c thymocytes as feeders (Parks et al. 1979). Clonal supernates were analyzed by quantitative indirect immunoflourescence using a FACS with SJL/J spleen cells as targets. Antibody secreting clones were obtained from 10 of the original 24 positive wells. Further analysis has reduced the number of distinct antigens recognized to seven. A clone derived from well C7 is used for the study described here.

Cell surface iodination, immune precipitation, and two-dimensional gel electrophoresis. Spleen or thymus cells were surface-labeled using lactoperoxidase and ^{125}I according to previously published procedures (Baur et al. 1971). Labelled cells were extracted with cell lysis buffer (0.5% NP40, 50 mM Tris, 150 mM NaCl, 0.02% NaN_3, 5 mM EDTA, pH 8.0) with gentle vortex mixing for 30 min on ice. Nuclei were then removed by centrifugation (10,000 × *g*, 20 min) and the extract was used immediately for immunoprecipitation.

Immunoprecipitations were done on ice using 200 µl of C7 supernate with NP40 lysates from 2×10^7 cells. After 30 min, 10 mg of *S. aureus*, Cowan I strain, was added (Kessler 1975). Because Staph A does not bind to antibody C7, it was pretreated with mouse anti-rat IgG serum. After an additional 30 min, the Staph A was washed three times in washing buffer (0.5% NP40, 0.45 M NaCl, 0.05 M Tris, 5 mM KI, 0.03% NaN_3, pH 8.3) and extracted into isoelectric focusing sample buffer (9.5 M urea, 2% NP40, 5% 2-mercaptoethanol, 1.6% pH 5–7 ampholines, 0.4% pH 3.5–10 ampholines).

Two-dimensional gels were run exactly as described (O'Farrell et al. 1977) using pH 3.5–10 ampholines (LKB-produkter AB, Bromma, Sweden) in the first dimension and 10% SDS polyacrylamide gels in the second dimension. The first dimension separation was a nonequilibrium pH gradient electrophoresis (NEPHGE) to resolve basic and acidic proteins (O'Farrell et al. 1977). Both first and second dimensions were run under reducing conditions.

Neuraminidase digestions. Neuraminidase (Worthington Biochemical Corp., Freehold, New Jersey) digestions were done with five units of neuraminidase with extract from 2×10^7 cells in 0.5 ml of cell lysis buffer and incubated for 30 min at 37° C.

Fluorescence analysis. Cells were stained with C7 antibody followed by an FITC-conjugated SJL/J anti-rat IgG serum as the second-step reagent. Quantitative flourescence analysis of stained cells was done by examining flourescence intensity on individual cells flowing past a laser light source and photo detectors using a modified FACS-II as previously described (Bonner et al. 1972, Herzenberg and Herzenberg 1978 b).

Microcytoxicity assays. These were performed as described (Murphy and Schreffler 1975) with the following modification: discrimination of live and dead cells was done by staining with a mixture of the nucleic acid binding dyes, acridine orange and ethidium bromide (Parks et al. 1979). A stock solution containing 1 μg/ml of each dye in phosphate buffered saline was mixed with an equal volume of cells. Acridine orange enters living cells giving the nuclei a green flourescence. Ethidium bromide is excluded by living cells but stains the nuclei of dead cells fluorescent orange. Both dyes are mutagenic (McCann et al. 1975) and should be handled carefully.

Results

Characteristics of the monoclonal C7 antibody

Production of C7 is described in the Methods section. The rat immunoglobulin heavy chain class of the monoclonal C7 antibody was typed by double diffusion using class-specific antisera (Miles Research Products, Elkhart, Indiana). C7 reacted only with the anti-IgG_{2a} serum. Two-dimensional gel analysis of the C7 antibody showed that the NS-1 κ light chain was present (Fig. 1) so this monoclonal antibody

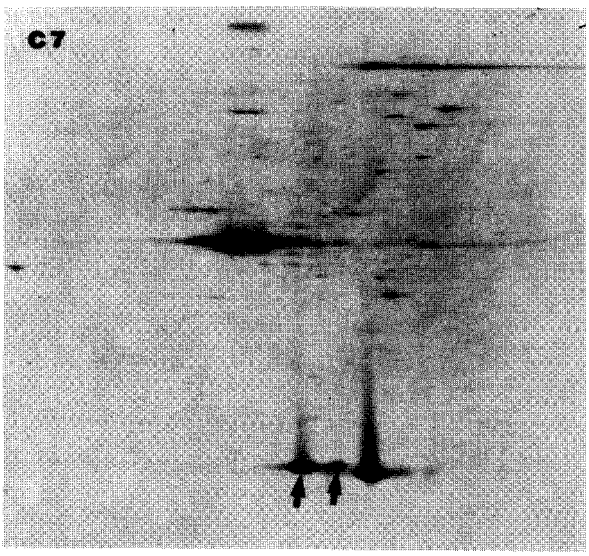

Fig. 1. Two dimensional gel electrophoresis of light and heavy chains secreted by clone H30-C7. The antibody was labeled by growth of 10^6 cells for 16 h in the presence of 50 μCi ^{35}S-methionine. The autoradiogram represents the total secreted products contained in the supernate. The first dimension separation was by nonequilibrium pH-gradient electrophoresis (acidic proteins are on the right and basic proteins are on the left). The second dimension was by SDS-PAGE (from top to bottom). The *arrows* point to the NS-1 κ light chain, which was identified by two-dimensional separation of MOPC-21 myeloma protein on a gel run in parallel, with ovalbumin run in each gel as a standard.

is designated an HLK (rat heavy chain, rat light chain, NS-1 κ light chain). With either guinea pig or rabbit complement, C7 is not cytotoxic.

Flow fluorescence analysis of Lgp100 in lymphoid tissues

Lgp100 is expressed on thymocytes, lymph node cells, and peripheral blood lymphocytes (PBL) of many mouse strains (Fig. 2) and on approximately 80% of spleen cells and 40% of bone marrow cells. The expression of Lgp100 in the bone marrow was mainly on cells in the smaller cell light-scatter fraction containing lymphocytes (Loken and Herzenberg 1975) with a small number of the bone marrow cells in the larger cell light-scatter fraction also specifically stained (Fig. 3). Since the fluorescence staining was done with saturation levels of monoclonal C7 antibody and second-step reagent, the relative fluorescence intensities reflect the amount of antigen. Whereas the intensity of staining in bone marrow and thymus was quite dull, the intensity of staining of spleen, lymph node, and PBL was much brighter. Therefore, the amount of Lgp100 per cell is higher in the peripheral lymphoid tissues than in the bone marrow or thymus.

The analysis of Lgp100 expression on spleen cells was continued with efforts to determine which lymphoid cells contain Lgp100 on their surface. For this purpose, spleen cells were stained with both rhodamine-conjugated rabbit anti-mouse μ chain and C7 followed by fluorescein-conjugated mouse anti-rat IgG. The cells were analyzed for rhodamine and fluorescein simultaneously (Loken et al. 1977). The results (Fig. 4) indicate that Lgp100 is expressed on all of the μ-bearing cells plus nearly all of the non-μ-bearing cells in the spleen. C7 antibody also reacts with nylon-purified splenic T cells (Julius et al. 1973). Together these results demonstrate that Lgp100 is expressed on both splenic T and B cells.

Trypsin sensitivity of Lgp100

Treatment of SJL/J spleen and thymus cells with 0.05% trypsin ($\neq$ 610–5300, Grand Island Biological Co., Grand Island, New York) for 10 min at 37° C completely abolished fluorescent staining using C7 antibody. The trypsin treatment had no

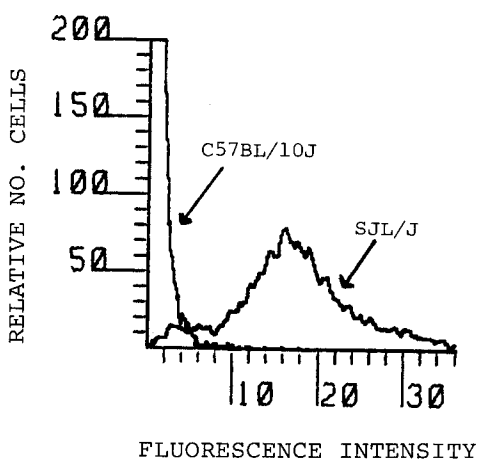

Fig. 2. Immunofluorescent staining of peripheral blood lymphocytes (PBL) from a Lgp100 positive strain (SJL/J) and a Lgp100 negative strain (C57B1/10J) with monoclonal C7 antibody. PBL were purified on Ficoll-Paque (Pharmacia) prior to reaction with C7 antibody and then FITC-conjugated mouse anti-rat IgG. Stained cells were analyzed on the FACS-II.

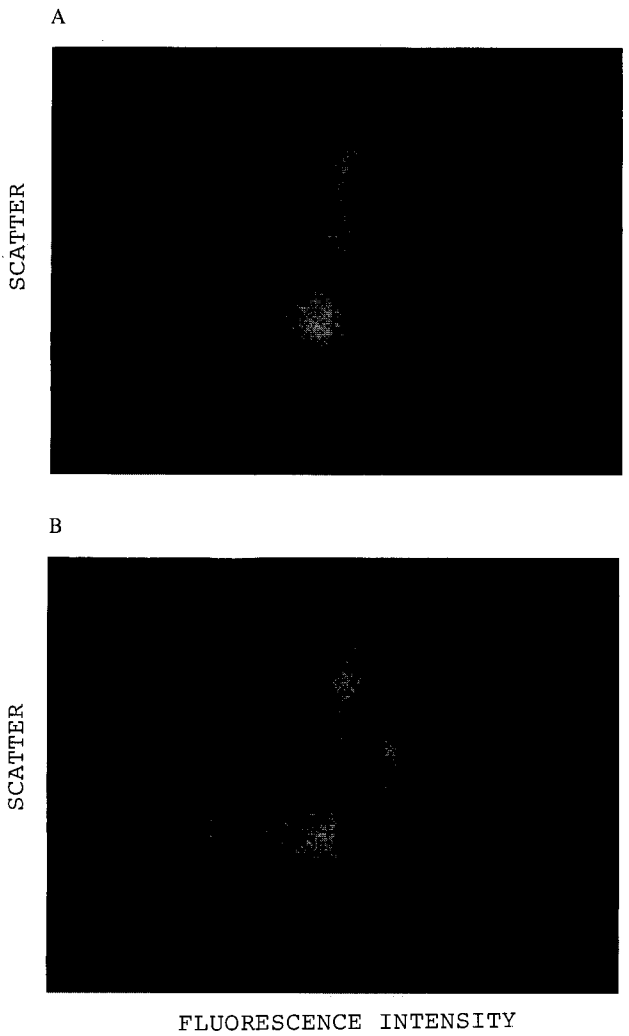

Fig. 3 A and B. Expression of Lgp100 on bone marrow cells. The *dot plot* shows scatter versus fluorescences of SJL/J bone marrow stained with (**A**) FITC conjugated mouse anti-rat IgG alone, and (**B**) C7 antibody followed by FITC-conjugated mouse anti-rat IgG.

effect on cell binding of other monoclonal antibodies to Thy 1.2 or T200 (Trowbridge and Mazauskas 1976) which were derived from the same hybridization experiment that generated C7. Lgp100 therefore is very sensitive to trypsin digestion.

Immunoprecipitation and two-dimensional gel analysis of Lgp 100

C7 antibody precipitates a surface protein of approximately 100,000 daltons (Fig. 5c). This protein was not precipitated with medium alone or with unrelated monoclonal antibodies (data not shown). From the size and charge heterogeneity of

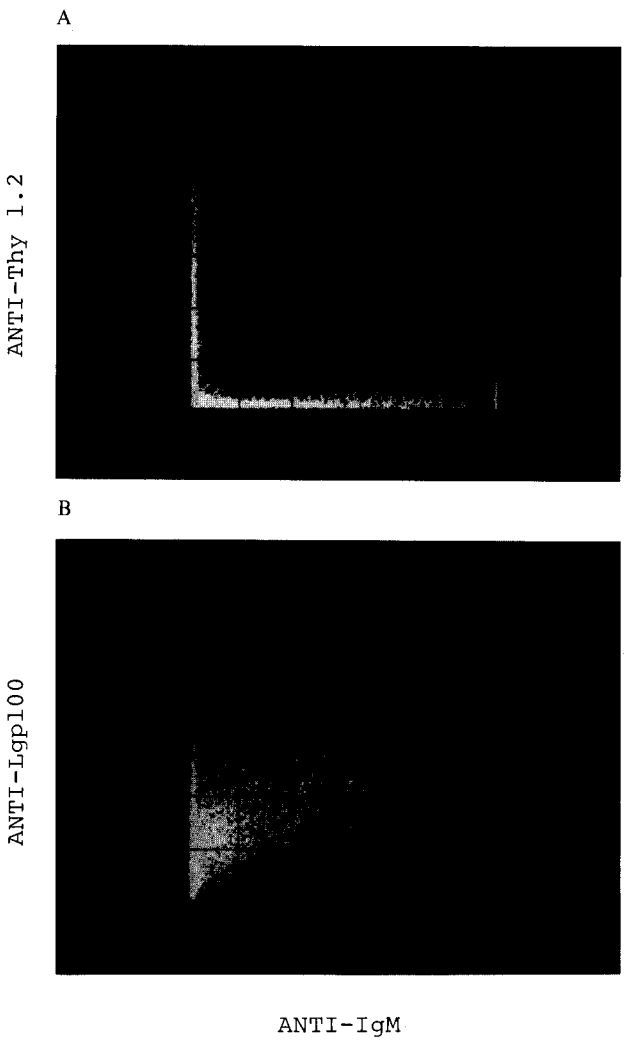

Fig. 4 A and B. Correlation of anti-Lgp100 and anti-IgM stains on BALB/c spleen cells. Cells were first labeled with rhodamine-conjugated rabbit anti-μ chain followed by **(A)** monoclonal anti-Thy 1.2 and fluorescein-conjugated mouse anti-rat IgG, and **(B)** monoclonal anti-Lgp100 and fluorescein-conjugated mouse anti-rat IgG.

the precipitated protein it appears to be a glycoprotein (Ledbetter 1979). Further evidence that it is a glycoprotein is presented below.

C7 immunoprecipitates from ^{125}I lacteperoxidase-labeled thymus and spleen (Fig. 5 b, c) show a striking difference in two-dimensional gel patterns. In addition to the 100,000 dalton species, the thymus had a species with a higher molecular weight bearing the Lgp100 antigenic determinant.

The two-dimensional gel patterns were identical when obtained under both reducing and nonreducing conditions. Thus Lgp100 contains only one polypeptide chain.

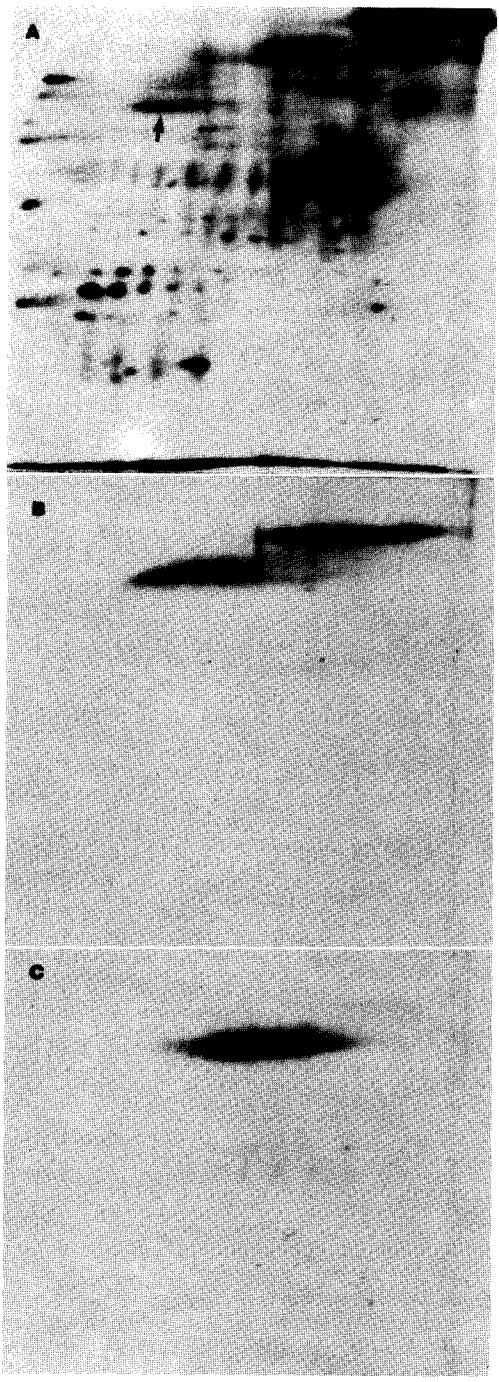

Fig. 5 A–C. Two-dimensional gels of Lgp100 from thymus and spleen. Lgp100 was precipitated from NP-40 extracts of ^{125}I lactoperoxidase-labeled BALB/c cells with C7 antibody; **(B)** Lgp100 precipitated from labeled thymocytes; **(C)** Lgp100 precipitated from labeled spleen cells. The gel in **(A)** shows the total ^{125}I lactoperoxidase-labeled thymocyte proteins, with the *arrows* pointing to the thymus forms of Lgp100. The autoradiograms are positioned as described in the legend to Figure 1.

Two-dimensional separation of total cellular proteins from surface-labeled thymocytes (Fig. 5 a) demonstrated that Lgp100 can be seen without immunoprecipitation as a minor species among the total labeled proteins.

We were next interested in investigating the relationship between the 100,000 dalton form of Lgp100 and the larger form precipitated from thymocytes but not spleen cells (Fig. 5). Since Lgp100 is highly susceptible to trypsin cleavage, it could be argued that the higher molecular weight form of Lgp100 is also present on spleen cells but is degraded by splenic proteases after NP-40 extraction. Unlabeled spleen cells were mixed with labeled thymocytes prior to NP-40 extraction and C7 immunoprecipitation. This resulted in no change in the two-dimensional gel pattern

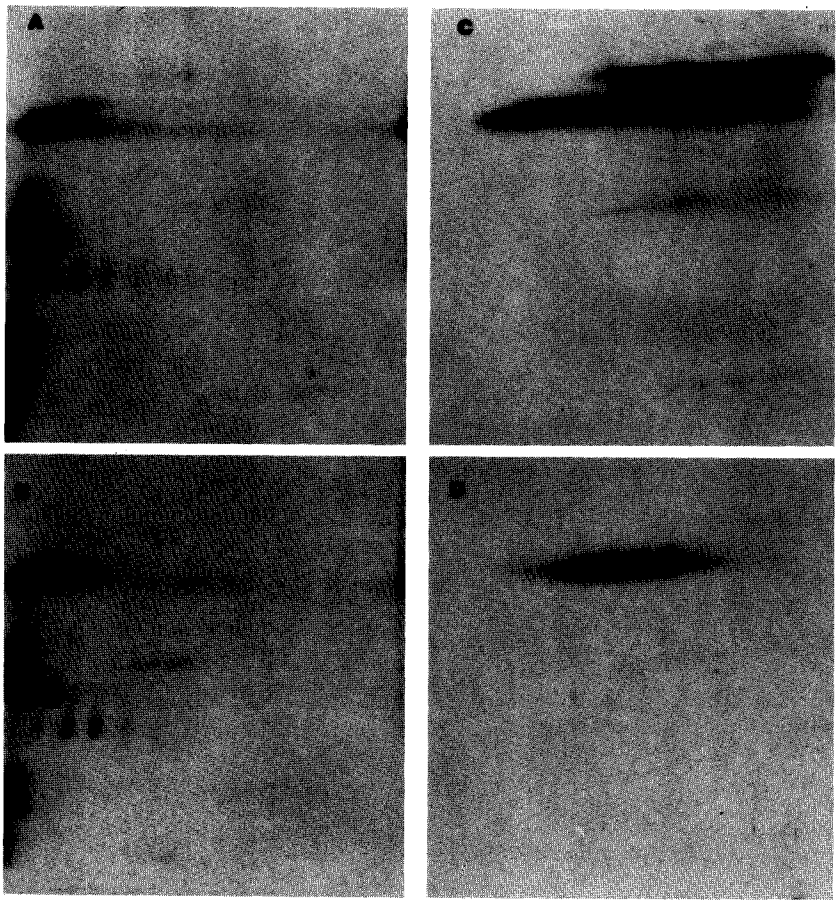

Fig. 6 A–D. Neuraminidase digestions of Lgp100 from thymus and spleen. NP-40 extracts from ^{125}I lactoperoxidase-labeled BALB/c thymus and spleen cells were treated with or without neuraminidase before immunoprecipitation with C7 antibody. **(A)** Thymocyte extract treated with neuraminidase before C7 immunoprecipitation. **(B)** Spleen extract treated with neuraminidase before C7 immunoprecipitation. **(C)** Thymocyte extract treated without neuraminidase before C7 immunoprecipitation. **(D)** Spleen extract treated without neuraminidase before C7 immunoprecipitation. Autoradiograms of the two-dimensional gels are positioned as described in the legend to Figure 1

of C7 immunoprecipitates from labeled thymocytes (not shown), strongly suggesting that the 100,000 dalton species is not a breakdown product of the larger form.

Neuraminidase treatments of thymocyte and spleen extracts provided evidence that Lgp100 is a glycoprotein with terminal sialic acid residues and that the higher molecular weight species in the thymus is a more highly glycosylated form of the 100,000 dalton species. Removal of the sialic acid residues with neuraminidase altered the charge characteristics of Lgp100, causing it to migrate close to the basic end of the gel (Fig. 6). The first conclusion from this result is that Lgp100 is a glycoprotein with terminal sialic acid residues.

The second effect of neuraminidase treatment was a partial digestion of the C7 immunoprecipitates, most likely caused by contaminating proteases in the neuraminidase. The pattern given by these breakdown products provides a kind of peptide map. Since these patterns from thymus and spleen are virtually identical, the higher molecular weight species in the thymus most likely is a more glycosylated form of Lgp100. It is worth emphasizing that the neuraminidase digestion was done prior to C7 immunoprecipitation, so that each of the breakdown products must be expressing the antigenic determinant recognized by the C7 monoclonal antibody.

Genetic polymorphism of Lgp100 expression

Although cells from many mouse strains have Lgp100, a group of strains score negative for this surface antigen. Table 1 shows the strain distribution of Lgp100 on PBL. In all mice tested, reaction of C7 on PBL correlated with the presence or absence of C7 reactivity in spleen and thymus.

The genetic control of Lgp100 expression was investigated by typing F_1 and backcross mice using flourescence analysis of PBL with the FACS (Table 2). All F_1 mice were positively stained, whereas of 21 backcross mice, 10 were positive and 11 were negative. This indicates a single dominant gene control of Lgp100 expression.

Table 1. Expression of Lgp100*

Positive strains	Negative strains
A/J	C57BL/6J
AKR/J	C57BL/10J†
AKR/Cu	C57L/J
129/J	C57BR/cdJ
BALB/cNHz†	C58/J
CBA/N	
C3H/HeJ	
DBA/2J	
SJL/J†	
CXB‡-E,H,I	CXB-D,G,J,K
BXD‡-2,8,9,11,24,25,27,28,30	BXD-1,6,12,13,14,15,18,19,23,29
BXH‡-6,9,11,12,14	BXH-4,8,10,19

* Assayed by fluorescence (FACS) analysis of peripheral blood lymphocytes using monoclonal C7 antibody.

† These strains were also tested by immunoprecipitation using monoclonal C7 antibody.

‡ CXB, BXD, and BXH are recombinant inbred strains derived from crosses of (Balb/cBy × C57BL/6By), (C57BL/6J × DBA/2J) and (C57BL/6J × C3H/HeJ).

Table 2. Segregation of Lgp100 expression*

	Fluorescence/cell†	Lgp100 genotype
BALB/c	21.9	+/+
C57BL/10	3.8	−/−
(BALB/c × C57BL/10)F_1	11.2	+/−
Backcross: F_1 × C57BL/10		
Average of positives (10 mice)	11.7	+/−
Range	10.7 – 13.0	
Average of negatives (11 mice)	3.3	−/−
Range	2.4 – 4.3	
2nd step only	3.1	
Range	2.7 – 3.4	

* Assayed by quantitative fluorescence analysis of peripheral blood lymphocytes using the FACS.
† Mean fluorescence of 10,000 cells stained with C7 antibody followed by FITC conjugated mouse anti-rat IgG.

The mean fluorescence of the F_1 and positive backcross mice was close to half of the mean fluorescence of the parental BALB/c strain (Table 2).

Among the strains tested (Table 1) were thirty-five CXB, BXD, and BXH recombinant inbred (RI) strains. Seventeen of these were positive for Lgp100, whereas 18 were negative, adding to the evidence for single gene control of Lgp100 expression.

These RI typing data establish the Lgp100 gene on mouse chromosome 1, closely linked to the Mls locus (Festenstein et al. 1977). Only 3 recombinants (BXD 23, BXD 29, BXH 11) between Mls and Lgp100 were evident in the 28 RI strains typed for both of these markers. The exact location of Lgp100 with respect to Mls on chromosome 1 is under further investigation.

Discussion

In this report we describe a new lymphoid cell surface antigen detected by a monoclonal xenogeneic antibody. Immunoprecipitation from surface-labeled spleen cells and two-dimensional gel analysis have demonstrated that the molecular species bearing this antigen is a glycoprotein of approximately 100,000 daltons. Since the antigen is expressed on both T and B cells in all lymphoid tissues, it has been designated lymphoid glycoprotein 100 (Lgp100).

Lgp100 immunofluorescence is considerably brighter on lymphocytes in the peripheral lymphoid tissues (spleen, lymph node, and blood) than in the thymus or bone marrow. It may be that the brightness of staining is related to the degree of glycosylation, the thymus being most glycosylated. This higher degree of glycosylation in the thymus may mask some antigenic determinants giving the duller fluorescence staining. This suggests that protease-free glycosidase treatment will increase thymic staining with antibodies to Lgp100.

From partially completed studies, the expression of Lgp100 on lymphoid tumors appears to parallel its expression on normal lymphoid cells. The results to

date show that Lgp100 is expressed on a wide variety of lymphoid tumors (Warner, personal communication), including both B and T cell leukemias and plasmocytomas. It has not been found on myeloid tumors or on a fibrosarcoma.

Interestingly, the five mouse strains that do not express Lgp100 are closely related in origin; they can be traced back to common ancestry hundreds of generations ago (Morse 1978). The C57BL/10J, C57BL/6J, C57BR/cdJ, and C57BL/J strains were derived from matings made by Little in 1921 using mouse female-57 and mouse male-52 obtained from Lathrop. The C57/J strain was derived from matings made by MacDowell in 1921 using mouse female-58, also obtained from Lathrop, and the same male-52. Thus all five of the Lgp100-negative strains have a common ancestor in Lathrop's male-52. Therefore, the mutation determining loss of Lgp100 probably occurred only once among the ancestry of all the common inbred strains.

In studies using 35 CXB, BXD, and BXH RI strains we have established that the gene controlling Lgp100 expression is located on mouse chromosome 1, closely linked to the *Mls* locus (Festentein et al. 1977). Since recombinants between Mls and Lgp100 were found among the RI strains, Lgp100 does not appear to be controlled by the *Mls* locus. The recombination frequency between Lgp100 can be estimated from the fraction of RI strains in which the two markers have recombined. Three recombinants found among 28 informative RI strains gives a crossover distance of about 0.03 (Taylor 1978).

Perhaps it is more than coincidence that the gene controlling expression of a lymphoid surface antigen (Lgp100) is closely linked to a locus, *Mls*, controlling a lymphocyte function. Four alleles of *Mls* are known, based upon mixed lymphocyte stimulation (Festenstein 1976), but none of these alleles correlates in strain distribution with Lgp100. For example, both BALB/c and C57BL/6 mice are Mls^a, whereas of these two only BALB/c mice are positive for Lgp100. There have been several attempts to find antibodies which react with Mls determinants (Tonkonogy and Winn 1976, Festenstein et al. 1972, Sachs et al. 1973). In one of these attempts (Tonkonogy and Winn 1976), an antiserum was obtained with which cytotoxic reactivity segregated with Mls^a reactivity in backcross mice. This antiserum, a C3H/HeJ (Mls^b, $Lgp100^+$) anti-CBA/J (Mls^a, $Lgp100^+$) was also cytotoxic for other, non-Mls^a strains. This suggested two possibilities: either *Mls* alleles crossreact, or Mls is closely linked to genes controlling lymphocyte antigens, such as Lgp100. Mls may be part of a chromosome region containing genes that specify lymphocyte surface antigens and are involved in lymphocyte function, reminiscent of the major histocompatibility complex on chromosome 17.

None of our evidence bears on the question of whether Lgp100 is a polymorphic antigen. The monoclonal antibody, C7, since it is xenogeneic, may react with a framework (or species) determinant. The two known alloantisera recognizing Lgp100 (see below) have been assayed only by immunoprecipitation. Whether absorption by cells of one Lgp100-positive strain and testing on a second would reveal polymorphic determinants has not been tested.

In fact, we have seen three examples of rat anti-mouse monoclonal antibodies that do not distinguish between allelic products of antigenically polymorphic cell surface molecules. These include a monoclonal anti-Thy 1 antibody that reacts with both mouse Thy 1.1 and Thy 1.2 but not with rat Thy 1, and monoclonal anti-Lyt 1

and anti-Lyt 2 antibodies. The anti-Lyt 1 antibody reacts equally well with both Lyt 1.1 and Lyt 1.2, and the anti-Lyt 2 antibody reacts equally well with both Lyt 2.1 and Lyt 2.2. The rat anti-mouse antibodies thus react with framework determinants on these antigenically polymorphic molecules.

While this work was in progress we became aware that Lgp100 has been detected by immunoprecipitation because of an additional antibody present in a noncongenic anti-Lyt 2.1 serum (Durda et al. 1979). The evidence that this alloantibody reacts with the same molecule that C7 detects can be summarized as: (a) the apparent molecular weight on SDS gels is similar; (b) the strain distribution is identical, including the (C57BL/6 × BALB/c) recombinant inbred strains; and (c) the segregation of reactivity in backcross mice is consistent with single dominant gene control. We have found yet another alloantiserum, prepared by immunization of C57BL/6 mice with the AKR leukemia K36, which immunoprecipitates Lgp100.

The strains which type negative with the C7 antibody may express an allelic form of Lgp100. To examine this possibility we have prepared an alloantiserum of C3H.SW (Lgp100$^+$, H-2^b) anti-C57BL/6 thymocytes (Lgp100$^-$, H-2^b). This antiserum immunoprecipitates proteins from surface-iodinated C57BL/6 thymocytes that are similar to Lgp100 in mobility on two-dimensional gels. Although further experiments are required, it is likely that these proteins represent an allelic form of Lgp100. It is worth emphasizing that any immunizations made between Lgp100-positive and Lgp100-negative strains are likely to contain antibodies to Lgp100.

The rat-x-mouse hybridoma cell lines we have produced grow quite differently from the mouse-x-mouse hybridomas. The former grow best attached to the culture flask at high cell densities with frequent feeding, whereas the latter grow mainly in suspension.

The hybridoma cell line producing the monoclonal C7 antibody has been delivered to the Cell Distribution Center, Salk Institute, La Jolla, California, for distribution to others interested in Lgp100, along with detailed instructions on how to grow the cell line. Since our laboratory is not equipped to produce large amounts of the antibody, those interested should obtain the cell line H30-C7, which can be expected to produce approximately 10 μg/ml of monoclonal antibody in tissue culture. The H30-C7 cells have not grown in mice, whereas several other rat-x-mouse hybridomas we have produced (including a cell line secreting monoclonal anti-Thy 1.2) have been successfully grown in BALB/c mice that were irradiated with 350 rad.

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