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Genetics

Gene for a major cell surface glycoprotein of mouse macrophages and other phagocytic cells is on chromosome 2

(gene mapping/recombinant inbred/monoclonal antibody/polymorphism)

ALFONSO COLOMBATTI*†, EDWARD N. HUGHES*, BENJAMIN A. TAYLOR‡, AND J. THOMAS AUGUST*§

*Department of Pharmacology and Experimental Therapeutics, The Johns Hopkins University School of Medicine, Baltimore, Maryland 21205; and ‡Jackson Laboratory, Bar Harbor, Maine 04609

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ABSTRACT **A gene controlling the expression of a polymorphic 92,000-dalton glycoprotein of mouse macrophages and granulocytes has been identified. This glycoprotein was previously shown to be the major iodinated, trypsin-sensitive component of the murine phagocyte cell surface. The gene has been provisionally designated *Pgp-1* for phagocyte glycoprotein 1. Expression of the glycoprotein was measured by monoclonal antibody binding to a polymorphic antigenic determinant. Antibody binding to cells of positive strains of mice was proportional to cell number, whereas binding to cells of negative strains was insignificant. The concentration of the antigen in cells of heterozygous mice was approximately 50% of that in homozygous mice. Thirteen of 33 recombinant inbred strains of mice were positive, with binding values 100-fold over background, suggesting that a single gene controlled expression of the antigen. Segregation of the antigen correlated with markers on chromosome 2. The segregation of *Pgp-1*, with nonagouti coat color (*a*) and hemolytic complement (*Hc*) activity among progeny of (C3H/HeJ × DBA/2J)F_1 × DBA/2J mice confirmed the single gene control and the chromosomal assignment. Another gene on chromosome 2, *Ly-m11*, was also typed by using (BALB/cJ × C57BL/6J)F_1 × C57BL/6J mice. The data from both of these crosses indicated the following gene order: *Hc--Pgp-1--Ly-m11--a*.**

We have found a murine cell surface polymorphic glycoprotein of 92,000 daltons that was notable as the major iodinated trypsin-sensitive protein of surface-labeled macrophages (unpublished data). The glycoprotein was identified and characterized by use of xenogeneic monoclonal antibodies prepared with spleen cells of rats immunized with plasma membranes from NIH/3T3 cells (1). The antigen was specifically expressed on mature and immature cells of the myelomonocytic lineage and on a limited number of cell lines of mesenchymal origin, including fibroblasts and myoblasts (2). Cells of many common inbred strains of mice (A/J, AKR/J, C3H/HeJ, C57BL/6J, C58/J, NIH Swiss, NZB, RF/J, SJL/J, SWR/J, and 129/J) contained the determinant recognized by the monoclonal antibodies, whereas a few strains (BALB/cJ, DBA/1J, DBA/2J and CBA/J) were negative (2, 3).

We have now mapped the gene controlling the expression of the 92,000-dalton glycoprotein by measuring monoclonal antibody binding to cells of recombinant inbred (RI) strains and backcross segregant mice. The results indicated that a codominant gene located on chromosome 2 was responsible for the antigen expression and provided a gene order with respect to several other markers in chromosome 2. Because this polymorphic antigen was specifically expressed on macrophages, monocytes, and polymorphonuclear cells, the gene controlling expression has been provisionally designated phagocyte glycoprotein 1 (*Pgp-1*). The specificity "1.2" has been assigned by convention to the antigenic determinant found in the C57BL/6 strain. Strains bearing this specificity are provisionally assigned the allele designation *Pgp-1*^{a}; the allele found in negative strains such as BALB/c and DBA/2 is designated *Pgp-1*^{b}.

MATERIALS AND METHODS

Animals. Two sets of RI strains of mice derived from crosses of C57BL/6J with DBA/2J (B×D) and of BALB/cBy with C57BL/6By (C×B) were obtained from The Jackson Laboratory, as were C57BL/6J, BALB/cJ, C3H/HeJ, DBA/2J, (C57BL/6J × BALB/cJ)F_1 and (C3H/HeJ × DBA/2J)F_1 inbred and hybrid mice. Offspring of the backcross mating of (C3H/HeJ × DBA/2J)F_1 × DBA/2J and (C57BL/6J × BALB/cJ)F_1 × BALB/cJ were raised in the animal facilities of The Johns Hopkins University School of Medicine.

Antiserum. A hybridoma secreting the AMF-8 IgG2a monoclonal antibody directed against the polymorphic antigenic determinant of the phagocyte 92,000-dalton glycoprotein was derived from fusion of the murine myeloma cell line P3-NSl/1-Ag4-1 and spleen cells of rats immunized with NIH/3T3 plasma membrane (1). The source of the monoclonal antibody was the ascites fluids of BALB/c *nu/nu* mice bearing hybrid cell tumors. The antibody was purified by affinity chromatography with protein A-Sepharose (4) and was composed only of heavy and light chains of rat origin.

Typing Assay for *Pgp-1* and *Ly-m11*. The purified AMF-8 antibody was labeled with ^{125}I as described by Mason and Williams (5). The antibody binding assay for *Pgp-1* expression was carried out as follows. Freshly prepared cells (1×10^6) were incubated for 1 hr at 0°C with ^{125}I-labeled AMF-8 (0.15–0.4 μg/ml; 3.0–4.5 μCi/μg; 1 Ci = 3.7×10^{10} becquerels) in 100 μl of Dulbecco's phosphate-buffered saline containing 20 mg of bovine serum albumin per ml. The cells were collected by centrifugation and washed three times with Dulbecco's phosphate-buffered saline, and the bound radioactivity was measured. The antigen of RI strains was measured with spleen cell suspensions; inbred strains, F_1, and backcross mice were typed with thioglycollate-induced peritoneal polymorphonuclear cells.

An indirect immunofluorescence assay was used to determine the *Ly-m11* phenotype of (BALB/cJ × C57BL/6J)F_1 ×

Abbreviations: *Pgp-1*, phagocyte glycoprotein 1 gene; *a*, nonagouti coat color gene; *Hc*, hemolytic complement gene; RI, recombinant inbred; AMF, anti-mouse fibroblast; *B2m*, β-2-microglobulin gene; *Ea-6*, erythrocyte antigen 6 gene; *Cs-1*, catalase 1 gene; *H-3*, histocompatability 3 gene; *Ly-4*, lymphocyte antigen 4 gene; *Ly-m11*, lymphocyte antigen 11 (distinguished with a monoclonal antibody) gene; *Ir-2*, immune response 2 gene.

† Present address: Universitá Degli Studi, Istituto Di Istologia-Embriologia, Via Falloppio 50, 35100 Padova, Italy.
§ To whom reprint requests should be addressed.

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BALB/cJ mice. Freshly prepared peritoneal macrophage cells (1×10^6) were incubated for 1 hr at 0°C with 50 μl of tissue culture supernatant of the hybridoma cell line S 19.8 (6), washed with Dulbecco's phosphate-buffered saline, and incubated with 50 μl of a 1:40 dilution of fluorescein-conjugated goat anti-mouse IgG antiserum (Huntingdon Research Center, Brooklandville, MD). Fluorescent cells were detected by ultraviolet light microscopy.

Hemolytic Complement Assay. Hemolytic complement activity in serum was measured by the method of Rosenberg and Tachibana (7) with sheep erythrocytes labeled with ^{51}Cr. Packed erythrocytes (0.01 ml) were incubated with 250 μCi of ^{51}Cr for 1 hr at 37°C with gentle agitation. The cells were stored on ice for at least 1 hr, washed three times in Dulbecco's phosphate-buffered saline, and diluted in the same buffer to yield 4–5 $\times 10^4$ cpm in 50 μl. The ^{51}Cr-labeled cells (50 μl) were incubated at 0°C for 5–10 min with 50 μl of rabbit anti-sheep hemolysin (Cordis Laboratories, Miami, FL). A few drops of blood from the retro-orbital plexus of each mouse were added to the mixture, and incubation was continued for 60 min at 37°C with occasional mixing. The reaction was stopped by the addition of 1 ml of ice-cold Dulbecco's phosphate-buffered saline. The cell suspension was clarified by centrifugation, and the radioactivity in the supernatant was measured. Serum from mice expressing hemolytic complement activity released 15–40% of total radioactivity; serum from hemolytic complement-negative mice released 2–3%.

RESULTS

Genetic Control of *Pgp-1* Expression. The expression of the 92,000-dalton plasma membrane glycoprotein was measured by the binding of purified ^{125}I-labeled AMF-8 antibody to thioglycollate-induced, peritoneal polymorphonuclear cells of C57BL/6J, BALB/cJ, and (C57BL/6J × BALB/cJ)F_1 mice (Fig. 1). Antibody binding to C57BL/6J cells was proportional to cell number. Antibody binding to BALB/cJ cells was insignificant. The binding curves with cells from (C57BL/6J × BALB/cJ)F_1 mice were proportional to cell number and were approximately 50% lower than those with C57BL/6J cells. Similar binding curves were obtained with cells of C3H/HeJ (positive), DBA/2J (negative), and (C3H/HeJ × DBA/2J)F_1 mice (data not reported). These results indicated that *Pgp-1* is expressed in a codominant fashion.

Linkage Analysis of the Locus Controlling *Pgp-1* Expression. RI strains of mice, produced by inbreeding from the F_2 generation progeny of two dissimilar strains, have been used to assign linkage relationships of many genetic loci (8, 9). Cosegregation of parental alleles at any given pair of loci provides evidence for their genetic linkage.

In this study, the segregation of *Pgp-1* alleles with alleles of other genetic loci was examined in RI strains derived from crosses of C57BL/6J and DBA/2J (B×D) and of BALB/cBy and C57BL/6By (C×B) mice (Table 1). Spleen cells from 13 of 33 strains yielded antibody-binding values that were 100-fold over the background obtained with the progenitor BALB/cBy and DBA/2J mice. The remaining 20 strains yielded only background binding values. This finding suggested that a single gene was responsible for the regulation of *Pgp-1* expression.

The pattern of segregation of *Pgp-1* among the different RI strains indicated that the gene was located on chromosome 2. Among the genetic markers on chromosome 2 for which the C × B strains have been typed are *Ea-6*, *Cs-1*, *H-3*, *Ly-4*, *Ly-m11*, *B2m*, and *a*. The B×D strains have been typed for *Ly-m11*. A significant association was observed between *Pgp-1* and *Ly-m11* (8 discordant strains among 33; $P < 0.01$) when the segregation data from the B×D and C×B strains were combined. From this the mean (± SEM) recombination frequency is estimated to be 9.5 ± 4.6% (12). *Pgp-1* showed a close association with *Cs-1* (0/7 discordant), *Ea-6* (1/7 discordant), and *a* (1/7 discordant) in the C×B RI strains. However, due to the small number of C×B strains and ambiguities concerning the order of the marker genes, *Pgp-1* could not be mapped precisely on the basis of the RI strains alone.

A three-point backcross linkage analysis with *a*, *Pgp-1*, and hemolytic complement activity (*Hc*) was carried out to confirm the chromosome 2 assignment of *Pgp-1* and to establish the correct gene order. (C3H/HeJ × DBA/2J)F_1 × DBA/2J backcross mice were typed for the three markers. There was no effect of sex on the segregation of these markers, and the data for males and females were combined (Table 2). The AMF-8 monoclonal

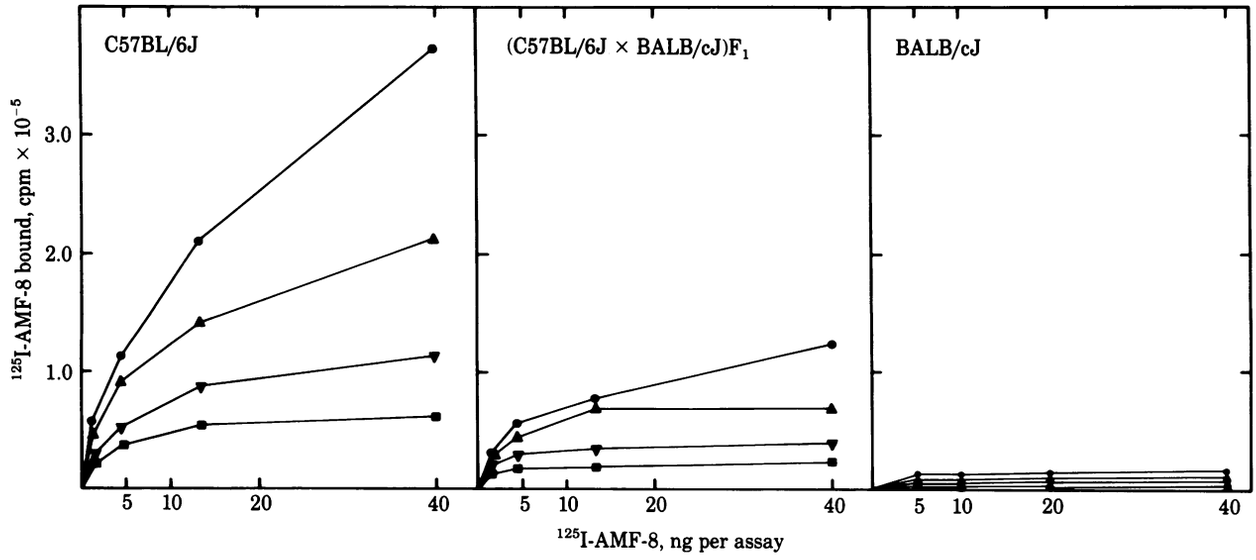

FIG. 1. Monoclonal antibody AMF-8 binding to thioglycollate-induced peritoneal polymorphonuclear cells. Cells from 8-week-old mice obtained 16 hr after intraperitoneal injection of Brewer's broth were washed in phosphate-buffered saline and adjusted to the following numbers in 50 μl: 8.0×10^5 (●), 4.0×10^5 (▲), 2.0×10^5 (▼), 1.0×10^5 (■).

Table 1. Inheritance of *Pgp-1* and other chromosome 2 markers in RI strains

	B×D RI strains																									
Locus	1	2	5	6	8	9	11	12	13	14	15	16	18	19	20	21	22	23	24	25	27	28	29	30	31	32
Pgp-1	B	D	D	B	D	B	D	B	D	D	B	D	D	D	D	D	B	D	D	B	D	D	D	D	B	D
		x	x					x	x	x	x															
Ly-m11	B	B	B	B	D	B	D	D	B	B	D	D	D	D	D	D	B	D	D	B	D	D	D	D	B	D

	C×B RI strains						
	D	E	G	H	I	J	K
Ea-6	C	B	C	B	B	C	B
						x	
Pgp-1, Cs-1	C	B	C	B	B	B	B
			x	x			
Ly-4, H-3	C	B	B	C	B	B	B
Ly-m11, B2m							
		x	x	x			
a	C	C	C	B	B	B	B

The letters B, D, and C indicate alleles inherited from C57BL/6J or C57BL/6By, DBA/2J, and BALB/cBy strains, respectively. x designates a region of recombination. Strain distribution patterns have been published for *Ly-m11* (cell surface antigen), *Ea-6* (erythrocyte cell surface antigen), *Cs-1* (erythrocyte catalase), *Ly-4* (lymphocyte cell surface antigen), *H-3* (minor histocompatibility locus), *B2m* (β_2-microglobulin), and *a* (nonagouti) (9–11). The B×D-6 strain was originally typed "D" for *Ly-m11*, presumably because of a mixup in the identity of the mice. The strain has subsequently been typed "B" (N. Tada, personal communication).

antibody reacted with approximately 50% of the progeny (59 of 112 mice), confirming the monogeneic nature of *Pgp-1*. The mean (± SEM) recombination frequency was estimated to be 19.6 ± 3.8% between *Pgp-1* and *a*, 28.6 ± 4.3% between *Pgp-1* and *Hc*, and 37.0 ± 4.6% between *Hc* and *a*. These data were consistent with the gene order *Hc--Pgp-1--a*. Six double crossovers were scored.

Because neither *Hc* nor *a* is near *Pgp-1*, another marker was sought for a more precise mapping. Ly-m11 is an alloantigen whose controlling locus is situated between *Hc* and *a* in the *H-3*, *Ly-4*, and *B2m* cluster (6). Because analysis of the RI strain data indicated that *Ly-m11* is approximately 9 centimorgans from *Pgp-1*, offspring of the (BALB/cJ × C57BL/6J)F_1 × BALB/cJ cross were typed for *Pgp-1* and *Ly-m11* (Table 3). There were 6 recombinant mice among 51 progeny, for an estimated map distance of 11.8 ± 4.5 centimorgans, in good agreement with the RI data. The two estimates may be combined for a weighted average of 10.7 ± 3.2 centimorgans.

DISCUSSION

The chromosomal locus of a gene (*Pgp-1*) controlling the expression of a major cell surface differentiation antigen of murine macrophages and other phagocytic cells has been identified by use of xenogeneic monoclonal antibodies. *Pgp-1* satisfied the criteria of a polymorphic gene: it was present in some strains and absent in others, it was expressed in half-quantity in heterozygotes, and it segregated as a mendelian trait. Analysis of RI strains of mice showed that the gene for antigen expression was located on chromosome 2. This chromosomal assignment was confirmed by analysis of progeny of the backcross (C3H/HeJ × DBA/2)F_1 × DBA/2J, segregating for one gene proximal (*Hc*) and one gene distal (*a*) to *Pgp-1*. The cell surface alloantigen segregated with the *a* allele for recombination frequency of 19.6% and with the Hc^1 allele for a recombination frequency of 28.6%. Because the *Hc* and *a* loci exhibited a recombination frequency of 37%, the gene order was inferred to be *Hc--Pgp-1--a*. Localization of *Pgp-1* between the *Hc* and *a* loci was obtained by analysis of the linkage of *Pgp-1* with *Ly-m11* (6) with a monoclonal antibody provided by N. Tada and S. Kimura (Sloan–Kettering Institute for Cancer Research). The recombination frequency of *Pgp-1* and *Ly-m11*, estimated from analysis of RI and backcross data, was 10.7 ± 3.2%. These data from the RI strains and the backcross mice indicated that the tentative gene order and estimated map distances were *Hc*--28.6 ± 4.3--*Pgp-1*--10.7 ± 3.2--*Ly-m11*--8.9 ± 5.0--*a*.

Closely associated with *Ly-m11* on chromosome 2 is a gene complex of *H-3* (encoding the transplantation antigen) (13, 14), *Ly-4* (the differentiation alloantigen) (15), *Ir-2* (the immune response function) (16, 17), and *B2m* (the structural gene for β_2-microglobulin) (11, 18, 19). These genes appear to be tightly

Table 2. Progeny of (C3H/HeJ × DBA/2J)F_1 × DBA/2J backcross mice typed for alleles at the *Pgp-1*, *Hc*, and *a* loci

Genotype	Mice, no.	Genotype	Mice, no.
Nonrecombinant:		Recombinant *Pgp-1* to *a*:	
Hc^1--$Pgp\text{-}1^a$--+	34	Hc^1--$Pgp\text{-}1^a$--*a*	6
Hc^o--$Pgp\text{-}1^b$--*a*	30	Hc^o--$Pgp\text{-}1^b$--+	10
Double recombinant:		Recombinant *Pgp-1* to *Hc*:	
Hc^o--$Pgp\text{-}1^b$--*a*	4	Hc^o--$Pgp\text{-}1^a$--+	15
Hc^1--$Pgp\text{-}1^a$--+	2	Hc^1--$Pgp\text{-}1^b$--*a*	11

Cross:

$$\frac{Hc^1\text{--}Pgp\text{-}1^a\text{--}+}{Hc^o\text{--}Pgp\text{-}1^b\text{--}a} \times \frac{Hc^o\text{--}Pgp\text{-}1^b\text{--}a}{Hc^o\text{--}Pgp\text{-}1^b\text{--}a}$$

Recombination values (mean ± SEM): *Pgp-1* to *Hc*, 32/112 = 28.6 ± 4.3%; *Pgp-1* to *a*, 22/112 = 19.6 ± 3.8%; *Hc* to *a*, 48/112 = 42.8 ± 4.7%.

Table 3. Progeny of (BALB/cJ × C57BL/6J)F_1 × BALB/cJ backcross mice typed for alleles at the *Pgp-1* and *Ly-m11* loci

Phenotype	Mice, no.	Phenotype	Mice, no.
Nonrecombinant:		Recombinant:	
Ly-m11.2$^-$--Pgp-1.2$^-$	29	Ly-m11.2$^-$--Pgp-1.2$^+$	2
Ly-m11.2$^+$--Pgp-1.2$^+$	16	Ly-m11.2$^+$--Pgp-1.2$^-$	4

(BALB/cJ × C57BL/6J)F_1 mice inherited both Ly-m11.2 and Pgp-1.2 specificities from the C57BL/6J parent. The backcross mice were typed for the presence of these specificities. Recombination value (mean ± SEM): 6/51 = 11.8 ± 4.5%.

linked because there is no reported recombination between *H-3*, *Ly-4*, *Ly-m11*, and *B2m*; *Ir-2* is located slightly distal to *H-3* (16, 17). The erythrocyte alloantigen gene *Ea-6* (20) is about 8 map units proximal to *H-3*. Because *Pgp-1* is about 10.7 map units proximal to *Ly-m11*, it appears to be closely linked to *Ea-6*, a deduction consistent with the finding of a single recombinant between *Pgp-1* and *Ea-6* among the C×B RI strains.

The possible significance of this cluster of genes on chromosome 2 is unknown; however, it is of interest that they each encode a cell surface antigen. Several other clusters of genes specifying leukocyte cell surface molecules have been identified by use of alloantisera or monoclonal antibodies: (*i*) *H-2--Qa--Tla* on chromosome 17 (21); (*ii*) *Mls*--Lgp 100 (*Ly-m9*)--*LyM* on chromosome 1 (22); (*iii*) *Lyb-2--Lyb-4--Lyb-6* on chromosome 4 (23–25); (*iv*) *Lyt-1--Ly-m10* on chromosome 19 (26); and (*v*) *Lyt-2--Lyt-3* on chromosome 6 (27).

Cell surface glycoproteins of macrophages mediate a number of immune response functions including phagocytosis, antigen processing, and cell–cell interactions (reviewed in refs. 28–30). A few of these functions can be assigned to specific proteins. An immunoglobulin Fc receptor has been identified as a glycoprotein of M_r 47,000–70,000 (31, 32). The Ia antigens, glycoproteins of M_r 33,000, 31,000, and 30,000 (33), appear to be involved in determinant selection and presentation of antigen for recognition by T lymphocytes (34). The functions of other characterized proteins are unknown: the Mac-1 antigen, composed of polypeptides of M_r 190,000 and 105,000; Mac-2, M_r 32,000; Mac-3, M_r 110,000; and the leukocyte common or T-200 antigen, M_r 150,000–200,000 (35–39).

Likewise, the role of the polymorphic differentiation antigen described in this study remains to be elucidated. This glycoprotein, of M_r 80,000 in NIH/3T3 cells and M_r 92,000 in leukocytes, is noteworthy in several respects (refs. 1–3; unpublished data): (*i*) it was a polymorphic differentiation antigen of macrophages and other cells of the mononuclear/phagocytic lineage; (*ii*) it was a major plasma membrane component, with about 10^6 antibody binding sites per cell and was distinguished as the predominant iodinated cell surface polypeptide; (*iii*) the molecule on the cell surface was remarkably sensitive to proteolytic cleavage and treatment of intact cells with trypsin yielded a water-soluble, antigenically active fragment of about 65,000 daltons; and (*iv*) it was uniformly distributed throughout the cell surface, except for the coated pits (40). Further structural and biological characterization of this glycoprotein has now been augmented by the preparative purification of the molecule. We have obtained milligram quantities of pure glycoprotein and have prepared monospecific polyclonal antiserum, in addition to hybridoma monoclonal antibodies. These tools, including a genetic system, the purified glycoprotein, monoclonal antisera, and monospecific polyclonal antisera, are now available for studies of the biological function of this molecule.

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