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Biochemical Characterization and Cellular Distribution of a Polymorphic, Murine Cell-Surface Glycoprotein Expressed on Lymphoid Tissues

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Abstract. A murine leukocyte surface glycoprotein ($M_r = 95\,000$) has been defined by means of xenogeneic monoclonal antibodies. In normal hematopoietic tissues, the glycoprotein is found in highest amounts in the bone marrow. Flow cytometric analysis shows that essentially all bone-marrow cells express the glycoprotein and that it is a major component of a subpopulation of cells containing predominantly granulocytic precursors. In contrast, only about 5 percent of thymocytes express sufficient glycoprotein to be detected by flow cytometric analysis, although under stringent conditions up to 20 percent of thymocytes are susceptible to complement-mediated cytotoxicity using a monoclonal antibody against the glycoprotein. Functional assays showed that both prothymocytes and colony forming unit-spleen express the glycoprotein which is broadly distributed on murine hematopoietic tumor cell lines. However, although some Thy-1^+ (T) cell lymphomas express large amounts of the glycoprotein, others do not express detectable quantities of the molecule. The glycoprotein is not restricted to hematopoietic cells and can be detected on lung, kidney, brain, and liver as well as cultured fibroblasts. Monoclonal antibodies against the glycoprotein cross-react with an antigen present on human cells. As described in the accompanying paper, the glycoprotein exists in two antithetical allelic forms and we show that it is identical to a polymorphic surface molecule independently characterized by Colombatti and co-workers.

Introduction

In this report we describe the biochemical properties and cellular distribution of a murine leukocyte cell-surface glycoprotein identified by a series of xenogeneic rat monoclonal antibodies. The glycoprotein is broadly distributed on hematopoietic cells but shows striking quantitative differences in expression on different classes of leukocyte. Furthermore, as shown in the accompanying paper (Lesley and

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Trowbridge 1981), the glycoprotein is polymorphic and we have defined two allelic antigenic determinants by means of xenogeneic monoclonal antibodies. These alloantigens can be distinguished from previously described murine alloantigens of the Ly series but we show in this paper that one form of the glycoprotein is identical to an antigen first described on murine fibroblasts by Hughes and August (1980). The locus for this antigen was mapped by these investigators and was designated *Pgp-1* (Colombatti et al. 1981). We therefore refer to the glycoprotein as Pgp-1 glycoprotein. It appears likely that human hematopoietic cells express an antigen homologous to murine Pgp-1 glycoprotein.

Materials and Methods

Cell lines. Murine hematopoietic cell lines were grown in Dulbecco's modified Eagle's medium with 10% horse serum (Horibata and Harris 1970). The origin and properties of the murine cell lines are given in Hyman et al. (1980a, b), Hyman and Trowbridge (1981), and Hyman et al. (1981). Human hematopoietic cell lines were grown in RPMI 1640 medium supplemented with 10% fetal calf serum. The origin and properties of the human cell lines are given in Collins et al. (1978), Minowada et al. (1978), Klein et al. (1972), and Klein et al. (1974).

Monoclonal antibodies and serological procedures. Monoclonal antibodies against Pgp-1 glycoprotein were produced by fusion of murine S194/5.XXO.BU.1 myeloma cells with spleen cells from Lewis or Sprague-Dawley rats immunized against either whole murine hematopoietic cells or purified cell-membrane glycoprotein fractions using standard techniques (Köhler and Milstein 1975, Trowbridge 1978). Two antibodies against nonpolymorphic determinants of Pgp-1 glycoprotein were used in the present studies. I42/5 monoclonal antibody is an IgG2a from an immunization with BALB/c spleen-cell-membrane glycoproteins bound and eluted from a pea-lectin Sepharose. IM7 monoclonal antibody is an IgG2b and was from an immunization with the myeloid cell line M1 induced with 1×10^{-5} M dexamethazone (Ichikawa 1970, Sugiyama et al. 1979) and is cytotoxic for these cells in a direct cytotoxicity test employing rabbit complement. C72/42 monoclonal antibody is an IgG2b and was obtained from an immunization of a Lewis rat with membrane glycoproteins from the AKR/J T-cell lymphoma line BW5147 and is specific for a polymorphic H-2 determinant which is probably identical to the public specificity H-2.5. Monoclonal antibody I3/2 against murine T200 glycoprotein has been described previously (Trowbridge 1978). Trace antibody-binding assays (Morris and Williams 1975) and quantitative serological absorptions employing either trace antibody binding (Morris and Williams 1975) or direct cytotoxicity (Hyman and Stallings 1974) were essentially as described (Trowbridge 1978, Hyman et al. 1980a). Tissue homogenates were prepared from fresh tissues frozen in liquid nitrogen using an auto-pulverizer (Redi Industries Corp., Hempstead, New York). Red cells were removed by lysis with Tris-buffered ammonium chloride (Boyle 1968) from suspensions of mouse spleen, thymus, and bone-marrow cells and from human peripheral blood leukocytes obtained from the buffy coat of defibrinated venous blood.

Biochemical procedures. Metabolic labeling with 2-^{3}H-mannose and 1-^{3}H-galactose and lactoperoxidase-catalyzed, cell-surface iodination were performed as described previously (Trowbridge et al. 1978, Omary and Trowbridge 1980). Chromatography on pea-lectin Sepharose, immunoprecipitation using fixed *S. aureus* to collect the antibody-antigen complexes, and SDS-polyacrylamide gel electrophoresis were as described in Trowbridge et al. (1977) and Omary and Trowbridge (1980). Tryptic digestion of intact BW5147 cells (2×10^7 cells per ml) was at 23 °C using L-tosylamido-2-phenylethyl chloromethyl ketone-treated trypsin (Worthington, 50 μg/ml) for 20 min and was terminated by the addition of ovomucoid trypsin inhibitor in tenfold molar excess (Omary and Trowbridge 1981). Immunoprecipitates were prepared from the cell pellet and incubation supernatant after centrifugation of the trypsin-treated cells.

Flow cytometric analysis. Flow cytometric analysis was carried out on a Los Alamos multiparameter cell sorter equipped with a three-decade logarithmic amplifier (Hiebert 1975, Steinkamp et al. 1973, Coulter

1976) using a Spectra Physics 164-05 argon laser tuned to 488 n. m. Dead cells were gated out using propidium iodide at 25 μg/ml in the sample buffer (Horan and Kappler 1977). Staining procedures were as described in Dennert et al. (1980) except that cells were suspended in Hepes-buffered Eagle's medium containing 2% fetal calf serum and washed with 0.15 M NaCl-0.015 M NaN_3-0.01 M Na phosphate buffer (pH 7.2) containing 0.1% bovine serum albumin. The results of the analysis of 20 000 viable cells are presented as 64 × 64 channel isocontour displays of fluorescence (> 520 nm) on a logarithmic scale versus either forward narrow angle or right angle 488 nm light scatter. Relative mean fluorescence was measured by means of a standard curve constructed from the analysis of 10 μm fluorespheres (Coulter Electronics) run with the same laser output, high voltage, and filters as the cell samples.

Hematopoietic stem cell assays. Assays for prothymocytes (Kadish and Basch 1976) and CFU-S (Till and McCulloch 1961) were carried out as described previously (Dennert et al. 1980), except that a two-step complement-mediated cytotoxic procedure was used. Cells (1×10^7 for the CFU-S assay and 1×10^8 for the prothymocyte assay) were centrifuged in 15 ml tubes and resuspended in the appropriate monoclonal antibody-containing tissue culture supernatant. The tubes were gassed with CO_2 and incubated at 0 °C for 30 min. At the end of the incubation Hepes-buffered Eagles medium containing 2% newborn calf serum was added and the tubes were centrifuged. The pellets were resuspended in a 1:200 dilution of rabbit anti-rat immunoglobulin (Miles) in Hepes-buffered Eagles medium containing 2% newborn calf serum and rabbit complement in Hepes-buffered Eagles medium containing 2% newborn calf serum was added. After incubation at 37 °C for 45 min, the tubes were centrifuged, washed once in Hepes-buffered Eagles medium containing 2% newborn calf serum, and resuspended at 2×10^6 cells/ml (CFU-S assay) or 2.5×10^7 cells/ml (prothymocyte assay).

Results

1. Biochemical properties of Pgp-1 glycoprotein. Monoclonal antibody against a nonpolymorphic antigenic determinant of Pgp-1 precipitates a single radioactive species from lysates of BW5147 lymphoma cells labeled by lactoperoxidase-catalyzed iodination with an apparent molecular weight of 95 000 on 7.5% or 10% polyacrylamide gels. As shown in Figure 1, treatment of intact cells with trypsin releases a labeled soluble fragment of the molecule ($M_r = 65\,000$) into the supernatant. A similar 95 000 M_r species was precipitated from a variety of lymphoid tumor cell lines and bone marrow, spleen, and thymus with Pgp-1-specific monoclonal antibody. Consistent with the relative paucity of the antigen on thymocytes (see below), only a relatively weak radioactive band was obtained from lysates of iodinated thymocytes. SDS-polyacrylamide gel analysis of Pgp-1 under nonreducing conditions showed that there were no interchain disulfide bonds. Pgp-1 was shown to be a glycoprotein by metabolic labeling experiments and lectin affinity chromatography. The antigen obtained from BW5147 cells could be labeled with [^{35}S]-menthionine, 2-[^{3}H]-mannose, and [^{3}H]-galactose. Passage of a Nonidet P-40 lysate of iodinated BW5147 cells through pea-lectin Sepharose resulted in about 50 percent of the labeled Pgp-1 glycoprotein being bound. A comparison of the Pgp-1 glycoprotein fraction which failed to bind to the pea-lectin Sepharose with that retained on the column by SDS-polyacrylamide gel electrophoresis showed that the pea-lectin-reactive fraction migrated more rapidly than the unreactive fraction as noted previously for mouse thymocyte Thy-1 glycoprotein (Trowbridge et al. 1977). Recently the structural requirements for high affinity binding of oligosaccharides to pea lectin have been investigated (Kornfeld et al. 1981) and shown to involve a fucose residue attached to the asparagine-linked N-acetyl-glucosamine residue of complex oligosaccharides as well as α-mannosyl residues (Trowbridge 1974).

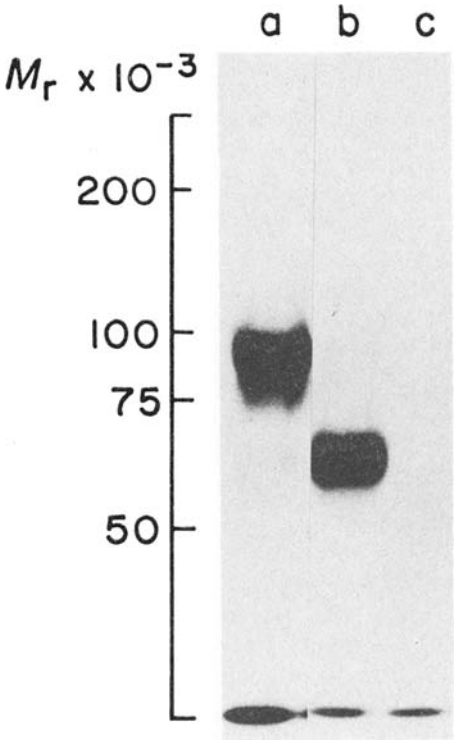

Fig. 1. SDS-polyacrylamide gel analysis of Pgp-1 glycoprotein. BW5147 cells were iodinated by lactoperoxidase catalyzed iodination. Pgp-1 glycoprotein was then immunoprecipitated from a lysate of untreated cells or the supernatant from an equal portion of cells treated with trypsin as described in *Materials and Methods.* Immunoprecipitates were analyzed on 7.5% polyacrylamide gels and the exposure of the autoradiograph was for 2 days with an intensifying screen. (lane a) Pgp-1 glycoprotein from BW5147 cells (M_r = 95 000), (lane b) soluble tryptic fragment of Pgp-1 glycoprotein (M_r = 65 000), (lane c) control immunoprecipitation from intact cells in which Pgp-1 antibodies were omitted from the first incubation step.

2. Quantitative distribution of Pgp-1 glycoprotein on murine hematopoietic tissues and tumor cell lines. The distribution of Pgp-1 glycoprotein on normal murine hematopoietic cells was examined by quantitative serological absorption and flow cytometric analysis. Large quantitative differences in the expression of the glycoprotein on the various lymphoid tissues were found. Quantitative absorption studies showed that bone marrow contains the highest quantities of Pgp-1, 25–80 percent on a per cell basis of the amount found on BW5147 cells used as the positive control (Table 1). In contrast, thymus contains only 1–4 percent of the amount of Pgp-1 found on the lymphoma cells. Spleen cells contained 7–22 percent of the amount of Pgp-1 expressed by BW5147 cells. Erythrocytes were Pgp-1 negative. Flow cytometric analysis of the distribution of Pgp-1 on bone marrow, thymus, and spleen gave results consistent with the data obtained in the quantitative absorption studies and provided additional information about the expression of the antigen on specific cell types (Fig. 2). Although essentially all bone-marrow cells expressed Pgp-1, three subpopulations of cells could be distinguished on the basis of narrow-angle light scatter and fluorescence analysis. The most brightly stained subpopulation represented about 30 percent of bone-marrow cells. Both on the basis of light scatter properties and morphological examination of Giemsa-stained cytocentrifuge preparations of cells fractionated by flow cytometry, the predominant cell type within the brightly stained cell population was the immature granulocyte. By comparson with the staining obtained with T200-specific monoclonal antibody, Pgp-1 antigen is clearly a major cell-surface glycoprotein on bone-marrow

Table 1. Quantitative expression of Pgp-1 glycoprotein on hematopoietic cells

Cells	Relative amount of Pgp-1 (%)			
	Experiment 1	Experiment 2	Experiment 3	Experiment 4*
BW5147	100	100	100	100
C58(NT)D	<2	<5	<3	N. D.
Bone marrow	25	N. D.	26	80
Thymus	1.4	<5	3	4
Spleen	10	13	7	22
Erythrocytes	N. D.	N. D.	N. D.	<0.2

* Experiments 1–3 were quantitative absorptions using I42/5 Pgp-1-specific monoclonal antibody at limiting dilution in trace antibody binding assays (Morris and Williams 1976). Experiment 4 was a quantitative cytotoxic absorption (Hyman and Stallings 1974) using IM7 Pgp-1 monoclonal antibody. C58(NT)D is a rat lymphoma cell line (see Hyman and Trowbridge 1981) used as a negative control. N. D. = not determined.

granulocytic precursors. The remaining bone-marrow cells consisted of about 40 percent of weakly fluorescent cells with the lowest light scatter profile and a subpopulation of cells of intermediate brightness and light scatter profile representing about 20 percent of the total bone-marrow cells (Fig. 2a). Thymocytes expressed very little Pgp-1 antigen and only 5 percent of the cells gave weak staining above background (Fig. 2d), while the majority of spleen cells were clearly positive but less intensely fluorescent than bone-marrow granulocytes (Fig. 2). The presence of the Pgp-1 glycoprotein on a subpopulation of thymocytes was confirmed by direct cytotoxicity testing. Under stringent conditions using a two-step procedure in which rabbit complement and rabbit anti-rat IgG antibodies were added in the second incubation period (Hyman and Trowbridge 1981), approximately 20 percent of thymocytes could be killed.

Pgp-1 glycoprotein was also found on a wide variety of murine hematopoietic tumor cell lines including myelomas, erythroleukemias, myeloid cell lines, Ia^+ Friend-virus-induced cell lines and an Abelson-virus-induced immature B-cell line (Fig. 3). However, while two T-cell lymphomas, EL4 and BW5147, showed strong binding of Pgp-1-specific monoclonal antibody in trace antibody-binding assays, five of nine T-cell lymphomas tested, S49, S1A, AKR1, T1M1, and SAKRTLS12, did not bind detectable amounts of the antibody. Quantitative absorption studies showed that the Pgp-1-negative lymphomas tested (S49, T1M1, and S1A) expressed less than 3 percent of the amount of the glycoprotein found on BW5147 cells.

3. Expression of Pgp-1 glycoprotein on hematopoietic stem cells. The predominant expression of Pgp-1 glycoprotein on bone-marrow cells raised the issue of whether the molecule was expressed on hematopoietic stem cell populations. In particular, the fact that T-cell lymphomas differ markedly in their expression of Pgp-1 glycoprotein and the majority of thymocytes are Pgp-1-negative raised the question of whether Pgp-1 glycoprotein is expressed on thymic precursors in the bone marrow.

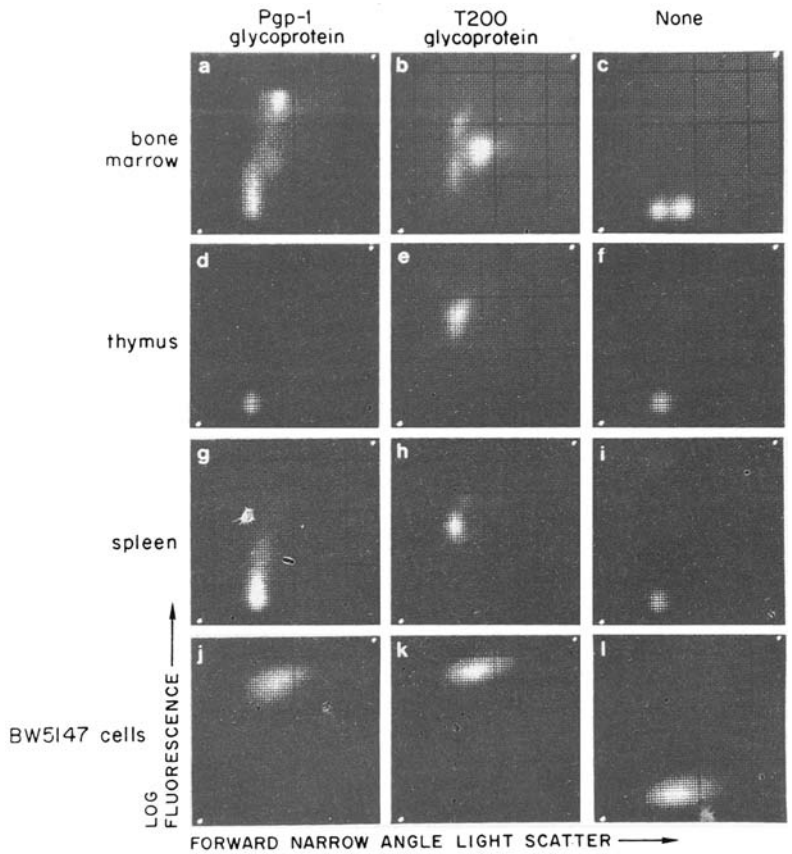

Fig. 2a–l. Quantitative expression of Pgp-1 glycoprotein on murine hematopoietic cells measured by flow cytometric analysis. Shown are the histograms of various murine hematopoietic cells incubated with either Pgp-1 monoclonal antibody (I42/5), anti-T200 monoclonal antibody (I3/2) or tissue culture medium and then stained with 50 μg/ml affinity-purified fluorescein isothiocyanate-conjugated goat anti-rat IgG. For each panel, 20 000 viable cells were analyzed and fluorescence was measured using a 3-decade log amplifier. Mean fluorescence is expressed relative to background fluorescence which for all cells was 1. For bone marrow the relative fluorescence and percentage of cells in each subpopulation is given in order of increasing fluorescence (see Table 2). At the settings used to calculate the percentage of positive cells stained by each antibody, 1.8 percent thymocytes, 4.7 percent spleen, 4.1 percent bone marrow, and 2.9 percent BW5147 cells were scored as positive in control samples containing tissue culture medium as the primary staining reagent.

To study this point, we used a modification (Dennert et al. 1980) of the cytotoxic ablation assay for prothymocytes described by Kadish and Basch (1976). Bone-marrow cells from AKR/J mice (Thy 1.1^+) were treated with Pgp-1 monoclonal antibody and complement and the ability of the remaining viable cells to repopulate the thymus of irradiated AKR/Cum mice (Thy 1.2^+) was measured. As shown in Figure 4, prior treatment of bone marrow with Pgp-1 monoclonal antibody and complement led to a marked delay in the appearance of cells of donor origin in the thymus of irradiated recipients that was only slightly less profound than that

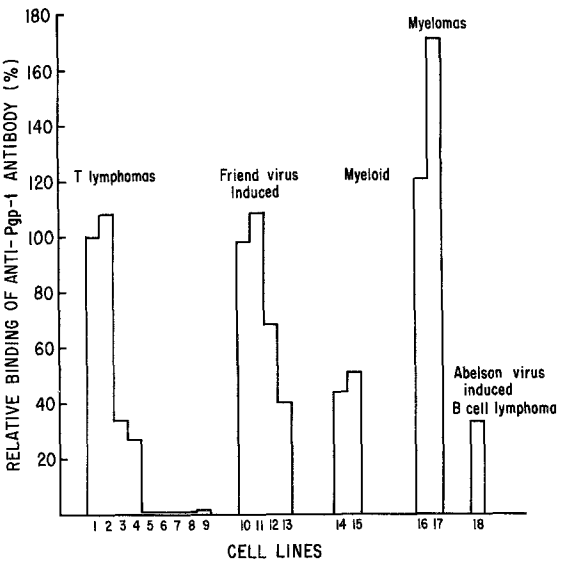

Fig. 3. Distribution of Pgp-1 glycoprotein on murine hematopoietic tumor cell lines. The data shown is from trace antibody binding assays in which BW5147 and C58(NT)D, a rat lymphoma cell line (see Hyman and Trowbridge 1981), were included as positive and negative controls, respectively. After subtraction of nonspecific radioactivity (10–20 percent of specific binding for BW5147 cells), measured in controls in which cells were incubated with tissue culture medium followed by ^{125}I-labeled goat anti-rat IgG, the results were expressed relative to binding by BW5147 cells. Cell lines: 1, BW5147; 2, EL4; 3, ASL1.1; 4, R1.1; 5, S49; 6, T1M1; 7, SAKRTLS12.1; 8, AKR1; 9, 9, S1A; 10, 745.6; 11, D1B.10; 12, T27A; 13, D2N; 14, WEH1 3; 15, C1498; 16, S194/5.XXO.BU.1; 17, C1.18; 18, RAW 253.

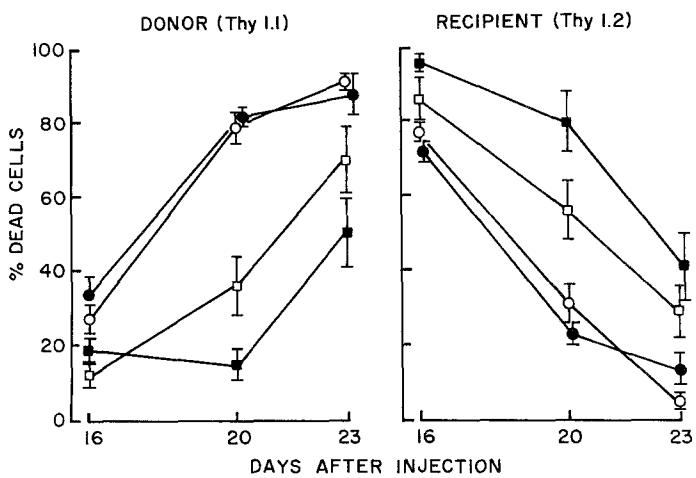

Fig. 4. Effect of cytotoxic ablation of bone-marrow cells by Pgp-1-specific monoclonal antibody on prothymocytes. The figure shows the fraction of thymocytes of donor (Thy 1.1) and recipient (Thy-1.2) origin measured by direct cytotoxicity in irradiated AKR/Cum mice (3 months old) at various times after reconstitution with AKR/J bone-marrow cells either untreated (●), treated with rabbit complement and anti-rat IgG alone (○), Pgp-1 (IM7) monoclonal antibody followed by rabbit complement and anti-rat IgG (□) or H-2 monoclonal antibody followed by rabbit complement and anti-rat IgG (■). Each *point* is the mean ± standard error of five individual animals. Further experimental details are given in *Materials and Methods.*

obtained with bone marrow treated with H-2-specific monoclonal antibody used as a positive control. If a mean generation time of 6–8 h for repopulating thymocytes is assumed (Metcalf 1967), then, from the delay of more than 3 days in the appearance of donor cells in the thymuses of mice receiving anti-Pgp-1 or H-2-treated bone

marrow compared with untreated bone marrow, it can be conservatively estimated that more than 95 percent of prothymocytes express both antigens. Because of differences in sensitivity of various cell types to complement-mediated lysis, however, it is not clear whether prothymocytes fall into the subpopulation of bone-marrow cells with high, intermediate or low amounts of Pgp-1 glycoprotein. Approximately 45 percent of bone-marrow cells were killed by Pgp-1-specific antibody (compared with 70 percent by anti-H-2) under the cytotoxic conditions employed, and flow cytometric analysis showed that the bone-marrow population expressing moderate amounts of Pgp-1 (see Fig. 2a) was enriched among the surviving cells relative to both the cells expressing high or low amounts of the antigen. A further consideration precluding more detailed conclusions is that it is also possible that binding of Pgp-1 antibody to prothymocytes alone is sufficient to prevent thymic repopulation (see below for CFU-S assay).

Similar cytotoxic ablation experiments were carried out to determine if evidence could be obtained that pluripotent stem cells estimated in the CFU-S assay (Till and McCulloch 1961) expressed Pgp-1 on their cell surface. As shown in Table 3, treatment of bone-marrow cells with Pgp-1-specific monoclonal antibody followed by rabbit complement and rat IgG-specific antibody has a profound effect on the number of spleen colonies obtained in irradiated recipients (group C versus groups A and B). However, it is clear from a comparison of groups C and D that incubation with Pgp-1 monoclonal antibody in the absence of complement was equally effective. This was also the case for the H-2 antibody used as a positive control. Thus, it appears that CFU-S express both Pgp-1 and H-2 and the binding of Pgp-1 and H-2 antibodies to the surface of the progenitor cells is sufficient to interfere with their capacity to form spleen colonies in irradiated recipients.

4. Distribution of Pgp-1 glycoprotein on nonhematopoietic tissues. The distribution of Pgp-1 antigen on murine nonhematopoietic tissues was assessed by quantitative serological absorption and trace antibody binding using tissue homogenates from mice irradiated 48 h previously with 1000 rad to reduce passenger lymphoid cells.

Table 2. Fluorescent staining of cells from various organs with Pgp-1-and T200-specific antibodies (see Fig. 2)

Cells	Antibody	Percent positive cells	Mean relative fluorescence
Bone marrow	Pgp-1	41, 21, 32	1.5, 5.5, 50
Spleen	Pgp-1	53	1.5
Thymus	Pgp-1	5.7	<1
BW5147	Pgp-1	98	70
Bone marrow	T200	26, 53, 20	5, 12.5, 25
Spleen	T200	98	25
Thymus	T200	99	12.5
BW5147	T200	99	85

Absorption of Pgp-1-specific antibodies was observed with lung, brain, kidney, and liver homogenates but about tenfold more of these tissue homogenates were required on a volume basis than bone-marrow cells for 50 percent absorption. Less than 50 percent absorption was obtained with muscle and heart homogenates at 50-fold greater volumes than bone-marrow cells. The presence of Pgp-1 on non-hematopoietic cells was most clearly demonstrated by trace antibody binding to cultured murine cells. Strong binding of Pgp-1 antibodies to 3T3 fibroblasts (Todaro and Green 1963) and primary cultures of baby mouse kidney epithelial cells (Winocour 1963) and mammary epithelial cells (Emerman et al. 1977) was observed.

5. Human cells express an antigen that cross-reacts with murine Pgp-1 glycoprotein. It appears that human cells express a cell-surface antigen related to murine Pgp-1. As shown in Figure 5, when the binding of one Pgp-1-specific monoclonal antibody, I42/5, directed against a nonpolymorphic determinant was tested on a panel of human hematopoietic cell lines, a broad pattern of reactivity was obtained similar to that obtained with murine hematopoietic tumor cell lines. Thus, among the positive

Table 3. Effect of treatment of bone-marrow cells with Pgp-1-specific monoclonal antibody on CFU-S

Group	Treatment		C′	No. of spleen colonies
	1° Antibody	Anti-rat IgG		
A*	–	–	–	42.6±2.1 (5)
B	–	+	+	37.8±4.6 (5)
C	Anti-Pgp-1	+	+	7.6±3.4 (5)
D	Anti-Pgp-1	+	–	7.3±1.3 (4)
E	Anti-H-2	+	+	0.4±0.2 (5)
F	Anti-H-2	+	–	8.8±1.8 (4)
G	No cells (irradiation control)			0.0 (2)

* Bone-marrow cells were treated as shown with IM7 Pgp-1-specific monoclonal antibody or C72/42 H-2 monoclonal antibody and CFU-S assayed as described in *Materials and Methods*. The results are given as the mean ± standard error of spleen-cell colonies with the number of recipients given in parentheses.

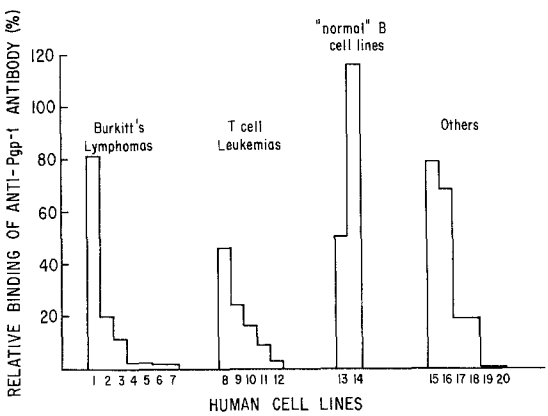

Fig. 5. Cross-reactivity of Pgp-1-specific monoclonal antibody with human hematopoietic cell lines. Trace antibody binding assays were carried out as described in the legend to Figure 3. Cell lines: 1, BJAB; 2, DAUDI; 3, NAMALVA; 4, SULUBO; 5, JIJOYE; 6, P3HR1; 7, RAJI; 8, RPM1 8402; 9, JM; 10, HPB-ALL; 11, CCRF-CEM; 12, HSB2; 13, B85; 14, CCRF-SB; 15, K562; 16, NALM 16; 17, NALM 1; 18, HL-60; 19, REH; 20, NALM-6.

human cell lines were T and B cell lines, the erythroid cell line K562, HL-60, and some Burkitt's lymphoma cell lines. Several human hematopoietic cell lines were negative, including the majority of Burkitt's lymphoma cell lines tested.

The reactivity of monoclonal Pgp-1-specific antibodies with human leukocytes was tested by flow cytometric analysis. As shown in Figure 6, monoclonal antibody IM7 directed against a nonpolymorphic determinant of Pgp-1 glycoprotein reacted with both human lymphocytes and granulocytes. More than 90 percent of lymphocytes and granulocytes which were discriminated on the basis of their light scatter properties (Hoffman et al. 1980) were positive with the IM7 monoclonal antibody. Similar results were obtained with I42/5 monoclonal antibody. However, neither of the two monoclonal antibodies defining the Pgp-1.1 and Pgp-1.2 allodeterminants in the mouse reacted with peripheral leukocytes from this individual (Fig. 6).

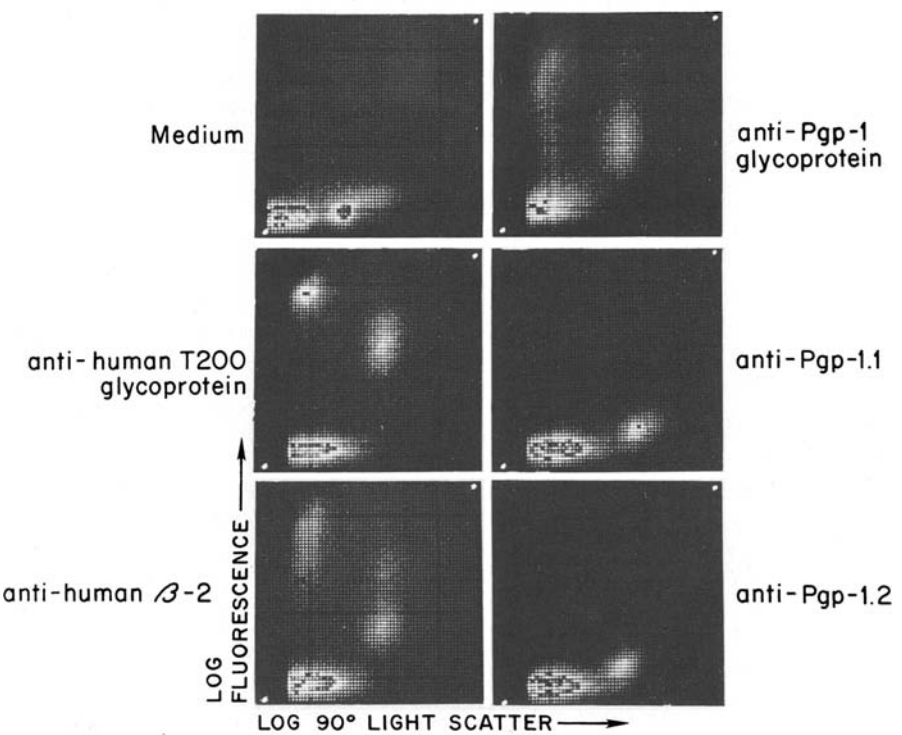

Fig. 6. Flow cytometric analysis of anti-Pgp-1 binding to human peripheral blood leukocytes. Buffy coat leukocytes were stained with the monoclonal antibodies shown as described in *Materials and Methods*. Anti-human T200 glycoprotein is monoclonal antibody T29/33 (Omary et al. 1980) and anti-human β-2 is monoclonal antibody A3/10 (Omary and Trowbridge 1981). Anti-Pgp.1.1 and anti-Pgp.1.2 monoclonal antibodies recognize the allelic determinants of Pgp-1 glycoprotein and are described in Lesley and Trowbridge (1981). Shown are histograms of log fluorescence (> 520 nm) versus log right angle 488 nm light scatter. The low right-angle light scatter fluorescent cells are lymphocytes, the high right-angle light scatter fluorescent cells are granulocytes. The low light-scatter negative cells are erythrocytes. These assignments were confirmed by analyzing the forward narrow angle and right angle 488 nm light scatter properties of each cell population (see Hoffman et al. 1980).

Discussion

We report in this paper the biochemical properties and cellular distribution of a polymorphic murine cell-surface glycoprotein that is expressed on lymphoid cells. As shown in the accompanying paper (Lesley and Trowbridge 1981), the glycoprotein, although distinct from previously described murine antigens of the Ly series, is identical to another glycoprotein first identified on murine fibroblasts and designated phagocyte glycoprotein 1 or Pgp-1 (Colombatti et al. 1981). Although our results indicate that the glycoprotein is not restricted to phagocytic cells, to avoid confusion we have adopted the same nomenclature.

Within the murine hematopoietic system, the Pgp-1 glycoprotein is found on many different cell types, judged by the reactivity of monoclonal Pgp-1-specific antibodies with both hematopoietic tumor cell lines and normal tissues. One striking feature of the cellular distribution of the glycoprotein is that it is a major cell-surface component of granulocytes in the bone marrow. As judged by fluorescence-activated cell analysis, Pgp-1 glycoprotein is present on granulocytic bone-marrow cells in greater amounts than T200 glycoprotein. It can be estimated that there are on the order of 2×10^5 molecules of Pgp-1 glycoprotein per granulocyte by a comparison of the relative fluorescence of bone marrow stained with Pgp-1 monoclonal antibody with that of thymocytes stained with anti-T200 glycoprotein (Fig. 3 using a value of 50 000 molecules of T200 glycoprotein per thymocyte (Trowbridge 1978).

A second point of interest is the expression of Pgp-1 glycoprotein within the T-cell lineage. Only 20 percent of thymocytes express sufficient amounts of Pgp-1 glycoprotein to be detectable by any method and only 5 percent of thymocytes show weak staining above background by flow cytometric analysis (Fig. 3). Although many Thy-1^+ T-cell lymphomas also do not express detectable amounts of the glycoprotein, BW5147, an AKR Thy-1^+ lymphoma, and EL4, a C57BL Thy-1^+ lymphoma, in particular express Pgp-1 in an amount similar to that of bone-marrow granulocytes. It is not clear at present whether the heterogeneity in expression of Pgp-1 on Thy-1^+ lymphomas reflects the fact that tumors may arise from cells of distinct T-cell lineages or at different stages of T-cell differentiation. There seems to be no clearcut correlation between the expression of Pgp-1 on T-cell lymphomas and other cell-surface antigens such as Lyt-1, Lyt-2 or TL. The very small amount of antigen detectable on the subpopulation of Pgp-1^+ thymocytes makes it difficult to investigate whether they differ from Pgp-1^- thymocytes in maturity or in any other respect. It is of some interest, however, that, as judged by the marked delay in thymic repopulation by bone-marrow cells treated with Pgp-1-specific monoclonal antibody and complement, virtually all prothymocytes are Ly-Pgp-1 positive. At present, H-2, Ly-5 (as judged by an in vitro induction assay, Scheid and Triglia 1979) and Pgp-1 are the only well characterized cell-surface glycoproteins known to be expressed on prothymocytes.

Not only can Pgp-1 glycoprotein be distinguished from previously described murine Ly antigens, its properties also differ from most other cell-surface glycoproteins of similar molecular weight found on leukocytes in other species. Williams and his colleagues have described a rat cell-surface glycoprotein, W3/13, that migrates on SDS-polyacrylamide gels with an apparent molecular weight of

95 000 (Brown et al. 1981). This glycoprotein is similar to glycophorin in having many small O-glycosidically linked oligosaccharides. It is unlikely that Pgp-1 is the mouse homologue of rat W3/13 glycoprotein since the rat glycoprotein is abundant on thymocytes and brain and, in contrast to Pgp-1, lacks N-asparagine-linked oligosaccharides and consequently fails to bind to lentil lectin. Pgp-1 glycoprotein can also be distinguished from the transferrin receptor, which is a 95 000 molecular weight glycoprotein under reducing conditions in SDS-polyacrylamide gels (Trowbridge and Omary 1981) but which, in its native state, exists as a dimer linked by a disulfide bond. Nor, on the basis of marked differences in cellular distribution, does murine Pgp-1 appear to be homologous with the CALLA antigen found on some human leukemic cells and probably a subpopulation of normal bone-marrow cells (Greaves and Janossy 1978, Ritz et al. 1980). Since a number of rat antibodies against murine Pgp-1 glycoprotein bind to human cells, it is likely that a homologous glycoprotein does exist in man. However, so far, we have been unable to immunoprecipitate a cell-surface molecule from lysates of human cells with the Pgp-1-specific antibodies that bind to the cells. The most probable explanation for this is that the monoclonal antibodies have lower affinity for the antigenic determinant on human cells than for murine Pgp-1 glycoprotein. Until biochemical data on the nature of the molecule recognized by the Pgp-1 antibodies on human cells is available, it cannot be stated with certainty that it is in fact the homologue of murine Pgp-1 glycoprotein.

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