Polymorphic Glycoprotein-1 on Mouse Platelets: Possible Role of Pgp-1 and LFA-1 in Antibody-Dependent Platelet Cytotoxicity Involving Complement

By Peter J. McCaffery, An Seng Tan, and Michael V. Berridge

The presence of the Pgp-1 glycoprotein on mouse platelets is demonstrated by antibody-binding techniques, by immunoprecipitation, and by transblotting using the monoclonal antibody (MoAb) C71/26 against Pgp-1. C71/26 immunoprecipitates as a broad band of mol wt 87,000 to 100,000 as determined by radioiodination of the platelet cell surface and by the $^{3}$H-sodium borohydride labeling technique. Immunoblotting showed Pgp-1 expression on platelets to be quantitatively similar to its presence on macrophages and resolved platelet Pgp-1 into two bands of mol wt 87,000 and 97,000 whereas Pgp-1 on parasite-elicited peritoneal macrophages showed 82,000 and 87,000 mol wt species. Platelets and monocyte/macrophage cells from either peripheral blood or from the peritoneal cavity showed homogeneous binding of Pgp-1 antibody to greater than 97% of cells by flow cytometry. In contrast, lymphocytes from peripheral blood or from the spleen showed a heterogeneous binding pattern with 20% to 30% of cells being negative, and the majority weakly positive. In functional studies, MoAbs against CR1 and CR3 substantially inhibited platelet immune adherence, whereas C71/26 showed only marginal inhibition. In contrast, C71/26 and other MoAbs against Pgp-1 inhibited platelet-dependent cytotoxicity of antibody-coated sheep erythrocytes in the presence of C5-deficient mouse plasma whereas M1/70 against CR3 showed no effect. In this assay, MoAbs against the $\alpha$- and $\beta$-subunits of leukocyte functional molecule LFA-1 also inhibited platelet cytotoxicity. These results show that the platelet cell surface moieties Pgp-1 and LFA-1 are involved in or closely associated with antibody-dependent cellular cytotoxicity by platelets.

PLATELETS ARE the second most abundant cell type in circulating blood where their primary function is hemostasis. Another well-established function for platelets is immune adherence to microorganisms through complement receptors. This function appears to be restricted to certain species including rodents, but may be analogous to other hemostatic mechanisms involving platelet adhesion. For example, platelets bind to exposed collagen fibers following damage to blood vessel walls resulting in morphologic changes, release of $\alpha$-granules and dense bodies, and platelet aggregation. More recently, platelets have been demonstrated to be directly involved in antibody-dependent cell-mediated cytotoxicity involving complement. Thus, mouse platelets are cytotoxic toward antibody-coated sheep erythrocytes in the presence of C5-deficient mouse serum. Furthermore, platelets from parasite-infected rats and from human subjects were able to efficiently kill Schistosoma mansoni larvae, a process involving parasite-specific IgE. Together these findings suggest that platelets may be primary mediators of immunologic destruction of systematically infecting microorganisms in both rodents and man. Furthermore, an immunologic role for platelets in cell turnover and perhaps even in tumor cell metastasis is possible.

A search for platelet surface molecules which may be involved in cytotoxic mechanisms revealed the presence of the leukocyte functional molecule LFA-1 on mouse platelets. In addition, the presence of complement receptor type 3 (CR3) was also demonstrated by the binding of M1/70 monoclonal antibody (MoAb) and by inhibition of immune adherence. In this study we show that C71/26 MoAb against Pgp-1, a cell surface glycoprotein present on bone marrow cells and cells of the granulocyte-macrophage lineage, also binds to mouse platelets and present evidence that this molecule may play a role in antibody-dependent platelet cytotoxicity involving complement.

MATERIALS AND METHODS

Animals and cell preparation. DBA/2, CBA/T6T6, C57B1/6, and (CBAxC57B1/6)F<sub>1</sub>, mice were bred at Wellington Clinical School of Medicine. Platelets were prepared from whole cardiac blood of DBA or F<sub>1</sub>, mice, using heparin (25 U/mL) or trisodium citrate as an anticoagulant. Blood was centrifuged at 200 x g for ten minutes and platelet-rich plasma (PRP) aspirated with gentle stirring to increase the yield of platelets. Platelets were recovered by centrifuging PRP at 1,000 x g for five minutes and were washed in citrate-phosphate buffer pH 6.0 prior to use in fluorescent and solid phase assays and in immunoprecipitation. Platelet-poor plasma (PPP or plasma) was obtained either from whole blood centrifuged at 1,000 x g for ten minutes or as the supernatant from the purification of platelets from PRP. For cytotoxicity experiments platelets were resuspended in plasma from DBA mice.

Spleen, thymus, testis, bone marrow, and peripheral blood mononuclear cells isolated from the interface of Isopaque-Ficoll (p = 1.09) were prepared as washed single cell suspensions in phosphate-buffered saline (PBS). Macrophages were prepared from peritoneal cells of CBA or F<sub>1</sub>, mice chronically infected with 20 Mesocestoides corti larvae by repeated 1 x g sedimentation until the tissue through a sieve and washing extensively with PBS.

MoAbs and antisera. C71/26.3.6 against Pgp-1, I21/7.7 against LFA-1$\alpha$, C71/16.2 against LFA-1$\beta$, and I41/14.1 against T200 were obtained from Dr I. Trowbridge (Salk Institute, La Jolla, Calif); M1/70 against LFA-1$\alpha$ and M1/70 against Mac-1 (CR3) were obtained from Dr T. Springer (Dana-Farber Cancer Institute, Boston); Dako-C3bR against human CR1 was obtained from Dakopatts, Copenhagen, Denmark); 2.4G2 against FcRII from Dr J. Unkeless (Rockefeller University, New York).

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Antithymocyte serum (ATS) and antiamphicrase serum (AMS) were prepared in New Zealand white rabbits as described previously and absorbed with an equal volume of erythrocytes.

**Solid phase ELISA assay.** Platelet suspensions (0.1 mL, 10^6 per mL) were distributed in flat-bottomed microtiter wells (Nunclon, Roskilde, Denmark) and centrifuged at 1,000 x g for five minutes. Glutaraldehyde (50 μL, 0.25% in PBS) was added to each well without having removed the supernatant, and the plates centrifuged briefly at 150 x g for five minutes. Fixation was continued at 20 °C for 10 to 15 minutes. The fixative was then removed, and the cells were washed with PBS, then with PBS containing 0.05% Tween-20 (Sigma Chemical Co, St Louis) and stored frozen in this buffer. Duplicate wells were treated with the antibody to be tested (25 μL) followed by peroxidase-conjugated IgG sheep anti-rat IgG (Amer sham, Sydney) diluted to 1:800. Peroxidase substrate (0.1% a-chloro-l-naphthol (60 mg/100 mL) in Tris-buffered saline, pH 4.7) was added, and the reaction terminated after ten minutes by adding an equal volume of 4N H_2SO_4. Microtiter plates were read in an automated microplate reader (Dynatech, Sydney, Australia) at 490 nm using a reference wavelength of 410 nm.

**Cell surface labeling and immunoprecipitation.** Purified platelets (2 to 3 x 10^8) in PBS were labeled either with ^125_I-sodium iodide (Amersham, Sydney) by the glycolic oxide modification or with ^3H by mild oxidation of sialic acid residues followed by reduction with 3H-sodium borohydride. The radiolabeled cells were solubilized with 1% NP-40 in 50 mmol/L Tris-HCl buffer pH 7.2 containing 10 mmol/L phenylmethylsulfonyl fluoride and 0.3 U/mL aprotinin (Sigma). Following two preabsorptions with 0.2 vol of 10% v/v methylsulfonyl fluoride and 0.3 U/mL aprotinin (Sigma), Flow cytometry. Antibody binding to CBA mouse cells was determined using a fluorescence-activated cell sorter (FACS 420, Becton Dickinson, Sunnyvale, CA). Cell suspensions were treated with hybridoma supernatant, washed twice with PBS, then stained with affinity-purified IgM mouse anti-rat IgG coupled to fluorescein. Stained cells were analyzed directly or fixed with 10% paraformaldehyde in PBS and stored at 4 °C prior to analysis. Cell populations such as lymphocytes and monocyte/macrophage cells were electronically "gated" following positive identification in low angle v 90° scatter graphs and fluorescence intensity profiles measured on these cell populations using logarithmic amplification.

**Immune adherence assay.** Immune adherence was determined using platelet-dependent agglutination of antibody-coated sheep erythrocytes (EA) in the presence of mouse complement. This assay has been described previously and erythrocyte agglutination shown to be dependent on platelets, complement, and antibody-coated indicator cells and to be inhibited by EDTA and heparin. Platelet-rich plasma was prepared from cardiac blood of F_3, mice collected into siliconized tubes containing trisodium citrate as an anticoagulant. After centrifugation at 200 x g for ten minutes, platelet-rich plasma was removed with gentle stirring to increase platelet yield, and the remaining suspension centrifuged at 1,200 x g for five minutes to produce PPP. PRP was diluted 1:4 in PBS prior to use (platelet concentration 3 x 10^10/mL). Platelets were mixed with PBS, or antibody (25 μL, final volume) for 30 minutes prior to addition of EA (25 μL, 1% v/v). EA were prepared by coating sheep erythrocytes (2% v/v) with subagglutinating concentrations of rabbit hemolysin (Wellcome Reagents, Beckenham, England) (1:1,600 dilution in PBS). Microtiter plates were agitated gently at 20-minute intervals and agglutination assessed at 60 to 90 minutes. Under these conditions, and using diluted platelet-rich plasma (1:4), no hemolysis of EA was observed over the period of assay.

**Platelet aggregation.** Cardiac blood was used to prepare PRP and PPP as described above. Aggregation studies were performed at 37 °C under continuous stirring in a Corning (Corning, NY) EEL-169 platelet aggregometer. PRP (400 μL) was mixed with antiserum or hybridoma supernatant (50 μL) and aggregation initiated by addition of 50 μL of 10 mmol/L ADP in saline. 100% transmission was determined with PPP.

**Platelet cytotoxicity.** Sheep erythrocytes from defibrinated blood were washed in Alsever's solution and stored at 4 °C. Erythrocytes were labeled with ^51_Cromium, washed three times with PBS, and coated with subagglutinating concentrations of rabbit hemolysin.

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**Table 1. Antibodies Used in This Study**

<table>
<thead>
<tr>
<th>Antibody</th>
<th>Specificity*</th>
<th>Mol Wt of Reduced Protein</th>
<th>Source Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>C71/26</td>
<td>Pgp-1</td>
<td>87,000–100,000</td>
<td>8</td>
</tr>
<tr>
<td>I4/2/5</td>
<td>Pgp-1</td>
<td>87,000–100,000</td>
<td>8</td>
</tr>
<tr>
<td>IM7</td>
<td>Pgp-1</td>
<td>87,000–100,000</td>
<td>8</td>
</tr>
<tr>
<td>I21/7</td>
<td>LFA-1α</td>
<td>170,000</td>
<td>9</td>
</tr>
<tr>
<td>M17/5</td>
<td>LFA-1α</td>
<td>170,000</td>
<td>10</td>
</tr>
<tr>
<td>M17/4</td>
<td>LFA-1α</td>
<td>170,000</td>
<td>10</td>
</tr>
<tr>
<td>C71/16</td>
<td>LFA-1β</td>
<td>95,000</td>
<td>9</td>
</tr>
<tr>
<td>M1/70</td>
<td>CR3(Mac-1)</td>
<td>160,000</td>
<td>11</td>
</tr>
<tr>
<td>Dako-C3br</td>
<td>CR1</td>
<td>210,000</td>
<td>12</td>
</tr>
<tr>
<td>I4/1/14</td>
<td>T200</td>
<td>200,000</td>
<td>Personal</td>
</tr>
</tbody>
</table>

*1 Pgp-1, polymorphic glycoprotein-1; LFA-1, leukocyte function antigen-1; α, alpha subunit; β, beta subunit; CR3, complement receptor type 3; CR1, complement receptor type 1; T200, leukocyte common molecule; lib/Illa, platelet glycoprotein expressing fibrinogen receptor; FcR, Fc receptor for IgG type II.**

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**Note:** The table lists antibodies used in the study, including their specificities, molecular weights, and sources of reference. The study involved the immunological analysis of platelet antigens and their interactions with various antibodies, providing a comprehensive understanding of platelet function and potential therapeutic applications.
(1/400 to 1/800) (Wellcome) prior to use. CBA or F1 platelets were purified from PRP and suspended in a 1:4 dilution of plasma from DBA mice (2 to 6 x 10^6 platelets/mL). Aliquots of this platelet suspension were incubated with control NS1 supernatant or hybridoma supernatant (25 μL) at 20 °C for 30 minutes prior to addition of ^51Cr-labeled antibody-coated sheep erythrocytes (0.25% v/v, 50 μL). Incubation was continued at 37 °C for three hours with gentle agitation at 15-minute intervals. Radioactivity released was determined in a γ-counter following centrifugation at 1,000 x g for five minutes to remove unlysed cells. Specific lysis was determined relative to controls lacking platelets or using heat inactivated plasma from DBA mice and complete release of radioactivity with plasma from CBA or F1 mice.

RESULTS

Identification and characterization of Pgp-1 on platelets. The presence of Pgp-1 on mouse platelets is shown in Fig 1 where the titration curve of C71/26 MoAb is compared with that of several other MoAbs against platelet-cell-surface glycoproteins, some of which are known to be associated with cell-mediated cytotoxic reactions (Table 1). Similar results have also been obtained by radioimmunoassay and by indirect immunofluorescence (results not shown).

Immunoprecipitation of mouse platelets radiolabeled with ^125I-iodide (Fig 2) or ^3H-sodium borohydride (Fig 3) with C71/26 MoAb against Pgp-1 resulted in a broad band of radioactivity of mol wt 87,000 to 100,000. This band appeared to be different from the β-subunit of LFA-1 which immunoprecipitated a sharper band at mol wt 97,000. Immunoclearance experiments and peptide mapping showed these two glycoproteins to be quite distinct. Antiserum against mouse peritoneal macrophages immunoprecipitated LFA-1 from ^3H-labeled mouse platelets and a broad band of radioactivity which appeared to include Pgp-1. In contrast, antiserum against mouse thymocytes immunoprecipitated LFA-1, but not Pgp-1 from ^3H-labeled platelets. A nonspecific band at mol wt 150,000 was also observed in immunoprecipitates from ^3H-labeled platelets. Immunoblotting allowed direct comparison of Pgp-1 molecules on platelets, macrophages, and spleen cells (Fig 4). Platelets showed two Pgp-1 bands at mol wt 87,000 and 97,000 whereas peritoneal macrophages showed the 87,000 mol wt band and a prominent additional band at mol wt 82,000. A faint Pgp-1 band was also observed on spleen cells at mol wt 89,000. Pgp-1 was not observed on thymocytes or on a mast cell precursor line (P-cell), or on the nonhematopoietic tissues tested, which included liver, kidney, brain, and testis. In other immunoblotting experiments, neutrophils and bone marrow cells showed a strong Pgp-1 band whereas erythrocytes, erythroid precursor cells from the spleen of phenylhydrazine-treated mice, eosinophils from M. corti-infected mice, and skeletal muscle tissue were negative.

The relative abundance of Pgp-1 on peripheral blood monocyes is demonstrated in Fig 5. More than 97% of peripheral blood monocytes and peritoneal macrophages expressed Pgp-1, the mean relative fluorescence (MRF) being 7.0 and 7.5, respectively. In contrast, lymphocytes from peripheral blood and from spleen showed heterogeneous fluorescence intensity profiles, with 20% to 30% of cells fluorescence negative and 5% to 10% strongly fluores-
Fig 3. Glycosylation of platelet Pgp-1. Platelets surface-labeled with 3H-sodium borohydride were lysed with detergent and immunoprecipitated with 1. control rabbit serum; 2. M1/70; 3. C71/26; 4. I21/7: 5. antithymocyte serum; and 6. antimacrophage serum. Immunoprecipitates were analyzed by SDS-polyacrylamide gel electrophoresis under reducing conditions and the gel autoradiographed. Lane 7 shows 3H-labeled proteins in the whole platelet lysate.

lymphocytes suggests a role in some common function. Initial investigations of the effect of the MoAb C71/26 against Pgp-1 on platelet aggregation induced by adenosine diphosphate (ADP) showed no significant effect. Rodent platelets however are known to be involved in immune adherence, a function which is also shared with phagocytic cells and lymphocytes. Thus, MoAbs against Pgp-1, CR3, CR1, FcRII, T200, and LFA-1 were tested for their ability to inhibit platelet adherence to antibody-coated sheep erythrocytes in the presence of complement. Figure 6 shows that M1/70 against CR3 extensively inhibited platelet immune adherence whereas Dako-C3bR against human CR1 inhibited to a titer of 1:8. At the highest concentration of antibody

Fig 5. Comparison of binding of C71/26 MoAb against Pgp-1 to lymphocytes, monocytes, and platelets. Pgp-1 on gated cell populations in peripheral blood leukocytes and peritoneal cells and on spleen cells and platelets was determined by flow cytometry. Control cells were treated with NS1 plasmacytoma supernatant. A reference bar is included in each fluorescence intensity profile for comparative purposes.

The presence of Pgp-1 on mouse platelets, monocytes, macrophages, and neutrophils and on-subpopulations of

Fig 4. Immunoblotting of cell proteins with C71/28 MoAb against Pgp-1. Cells or washed tissue (0.5 mL packed volume equivalent) were solubilized in NP-40 buffer, electrophoresed on SDS-polyacrylamide gels, and transblotted onto nitrocellulose. Pgp-1 was detected with C71/28 MoAb, peroxidase-conjugated second antibody, and 4-chloro-1-naphthol.
Table 2. Effects of MoAbs Against Pgp-1 and LFA-1 on Platelet Cytotoxicity

<table>
<thead>
<tr>
<th>Antibody</th>
<th>% Specific Lysis</th>
<th>% Inhibition Specific Lysis</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control (NS1)</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Pgp-1 (C71/26)</td>
<td>100</td>
<td>100</td>
</tr>
<tr>
<td>CR3 (M1/70)</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>CR1 (Dako-C3bR)</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>FcRII (2-4G2)</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>T200 (I41/14)</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>LFA-1&lt;1 (M17/4)</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>LFA-1β (C71/16)</td>
<td>-</td>
<td>-</td>
</tr>
</tbody>
</table>

Fig 6. Effect of MoAbs against Pgp-1 and other functionally-associated cell-surface molecules on platelet immune adherence. Platelet-rich plasma was diluted and incubated with antibody prior to the addition of opsonized erythrocytes. Inhibition of red cell agglutination is seen as a greatly reduced ability to form red cell buttons.
used, C71/26 against Pgp-1 inhibited aggregation. The possibility that this inhibition of immune adherence may have been caused by antibody-dependent agglutination of platelets was examined by phase contrast microscopy. The only antibody which caused significant platelet agglutination was C71/26, and this effect was only evident with undiluted hybridoma supernatant.

Another function held in common between most cells expressing Pgp-1 is antibody-dependent cell-mediated cytotoxicity involving complement. To test the involvement of Pgp-1, LFA-1, and CR3 in platelet cytotoxicity, a 51Cr-release assay similar to that described by Soper and Winn was used. The characteristics of this assay and the effect of MoAbs against Pgp-1, LFA-1, and CR3 on complement-dependent platelet cytotoxicity are shown in Table 2. In five experiments platelet-dependent lysis of 51Cr-labeled sheep erythrocytes varied between 14% and 94%. Specific lysis was determined relative to background release in the absence of platelets using C5-deficient plasma from DBA mice (Experiments 1 through 4) or in the presence of platelets using heat inactivated plasma from DBA mice (Experiment 5) and complete 51Cr-release with plasma from CBA or F1 mice. Under conditions where platelet agglutination was not observed, C71/26 against Pgp-1 inhibited lysis of antibody-coated erythrocytes by 44% to 100%. With either F1 or CBA platelets, inhibition was greater under conditions which favored less complete target cell lysis. Other MoAbs against Pgp-1, I42/5, and IM7 also inhibited platelet cytotoxicity (Table 2, Experiments 2 and 5). In addition, I21/7 against LFA-1a and C71/16 against LFA-1β reproducibly inhibited platelet cytotoxicity, although two other MoAbs against different epitopes of LFA-1, that is, M17/5 and M17/4, showed little or no effect. Similarly, M1/70 against CR3 did not significantly affect platelet cytotoxicity. The possibility that platelet-bound antibodies activated and depleted complement components indirectly causing inhibition of platelet cytotoxicity is ruled out by the observation that complement-fixing MoAbs such as M17/5 (Experiment 3) and 9F6 (Experiment 5), which bound strongly to platelets, did not inhibit platelet cytotoxicity.

**DISCUSSION**

The polymorphic cell surface glycoprotein Pgp-1 was first described by Hughes and August as a major antigenic component of mouse embryo 3T3 fibroblasts, but later was shown to be a constituent of myeloid and lymphoid cell membranes. We show here that Pgp-1 is also represented on purified mouse platelets and present evidence of a role for Pgp-1 in antibody-dependent cellular cytotoxicity mediated by platelets. In an enzyme-linked immunosorbent assay (ELISA), MoAbs against Pgp-1 and human glycoprotein 11b/11a showed high binding to mouse platelets relative to other MoAbs tested. Both the α- and β-subunits of LFA-1 and T200 were also present. These results confirm previous results which showed that EDU-3 cross-reacted with a small fraction of mouse platelets, and that LFA-1 and certain epitopes of the T200 family of cell surface glycoproteins were also expressed on mouse platelets. Both immunoprecipitation and immunoblotting procedures indicated heterogeneity of the Pgp-1 molecule on platelets, immunoblotting resolving discrete molecular forms on both platelets and macrophages. A minor platelet protein of mol wt 180,000 detected by C71/26 MoAb by immunoblotting has not been identified, but may represent a precursor form of Pgp-1, possibly of cytoplasmic origin as it was not detected by surface-labeling procedures. The Pgp-1 glycoprotein on platelets did not correspond with any of the major surface-labeled glycoproteins (Figs 2 and 3) although possible identity with platelet glycoprotein IVβ is suggested by similar mol wts and by lack of mol wt shift following complete reduction (results not shown).

The distribution of Pgp-1 on mouse splenic lymphocytes (Fig 5) shows close similarity to previously published results in which the majority of cells exhibited low relative fluorescence. However, we have detected a minor population (5% to 10%) of more brightly fluorescent lymphocytes in both splenic and peripheral blood. These results were supported by the immunoblotting studies which showed low levels of Pgp-1 on spleen cells relative to macrophages and platelets (Fig 4). Flow cytometry also indicated low relative fluorescence per platelet, a result which reflects the small surface area of the platelet compared to lymphocytes and macrophages.

Despite the isolation and biochemical and genetic characterization of Pgp-1, its function has remained elusive. As the Pgp-1 on platelets provided a system in which to test several functional associations. Initial experiments indicated no effect of antibodies against Pgp-1 on platelet aggregation and a marginally significant inhibition of immune adherence of platelets to antibody-coated sheep erythrocytes in the presence of complement (Fig 6), an effect that correlated with antibody-induced platelet agglutination. That immune adherence is mediated through complement receptors is suggested by the fact that MoAbs against both CR3 (Mac-1) and human CR1 inhibited platelet-erythrocyte agglutination under conditions where no platelet agglutination was observed. Using C5-deficient plasma from DBA mice, platelets were shown to lyse antibody-coated sheep erythrocytes. Specific lysis varied between 14% to 94% and was determined relative to controls lacking platelets and to complete release via the complement cascade using plasma from F1 or CBA mice. The reason specific lysis varied in different experiments is not fully understood, but possibly relates to the state of platelet activation in individual experiments, to the quality of the target erythrocytes, to the mouse strain used to prepare platelets, and to the nature and frequency of shaking which has been shown to influence platelet cytotoxicity. The experiments described in Table 2 used purified CBA or (CBAxCS7B1/6)F1 platelets in the presence of C5-deficient plasma from DBA mice to effect cytotoxicity of antibody-coated erythrocytes. Platelets were washed prior to use so that plasma carryover was at least two orders of magnitude lower than that required to effect cytotoxicity by the complement cascade. Furthermore the presence of plasma from DBA mice is necessary to cause platelet-dependent cytotoxicity; omission of plasma or heat-
treatment of the plasma negates the killing effect. In some experiments we have observed cytotoxicity with PRP from DBA mice, but in general cytotoxicity is lower and the effect more variable than with CBA or F1 platelets. Inhibition of platelet cytotoxicity with C71/26 MoAb against Pgp-1 was highly significant within each experiment, more extensive at low specific lysis with a particular mouse strain, and titratable (Table 2). Furthermore, the possibility that the inhibition of platelet cytotoxicity seen with C71/26 MoAb was caused by platelet agglutination by virtue of its IgM isotype was ruled out both visually and by using other Pgp-1 MoAbs of IgG2a and IgG2b isotypes (Table 2).28 No significant effect of M1/70 MoAb against CR3 was observed. In contrast, MoAbs against LFA-1 produced variable inhibition of platelet cytotoxicity, I21/7 against the α-subunit and C71/16 against the β-subunit inhibiting specific lysis by 25% to 103%, M1/74 inhibiting by 21%, and M17/5 showing no inhibition. Thus, certain epitopes on both Pgp-1 and LFA-1 on platelets appear to be involved in or closely associated with the cytolytic process. The variability of inhibition of cytotoxicity observed with MoAbs against LFA-1 has been observed previously with CTL-mediated killing in both human34 and mouse10,35 studies. Inhibition of cytolysis appeared to be dependent on the target cell used35 and no correlation of the effect of LFA-1 monoclonal antibodies on platelet cytotoxicity and CTL-mediated killing were evident. It will be of interest to determine the effect of MoAbs against Pgp-1 on other antibody-dependent cytotoxic events—for example, those involving macrophages and neutrophils, which have both been shown to express Pgp-1. Furthermore, it would also be of interest to investigate functionally human cells, which may also express Pgp-1.28 In this context, human platelets have recently been shown to exert cytotoxic effects on tumor cells.36

ACKNOWLEDGMENT

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