Response of Human Platelets to Activating Monoclonal Antibodies: Importance of FcγRII (CD32) Phenotype and Level of Expression

By Yoshiaki Tomiyama, Thomas J. Kunicki, Theodore F. Zipf, Sheila B. Ford, and Richard H. Aster

Certain monoclonal antibodies (MoAbs) specific for platelet membrane glycoproteins are known to be capable of activating platelets, and it is generally thought that platelets from normal subjects are equally susceptible to stimulation by such MoAbs. We found that platelets from 20 normal donors varied significantly in their sensitivity to three IgG1 murine MoAbs specific for membrane glycoproteins CD9, GPIV (CD36), and the GPIb/IIa complex (CD41), respectively. The response of platelets to these MoAbs was blocked by prior addition of MoAb IV.3 specific for the FcγRII receptor, indicating that activation was Fc receptor mediated. Platelets that responded poorly to these MoAbs failed to bind the MoAb 41H.16, specific for the “responder” form of FcγRII, but platelets that responded well reacted with this MoAb. The average number of FcγRII receptors on platelets from “responders” and “non-responders” was approximately the same. However, the number of FcγRII receptors expressed varied significantly on the basis of MoAb binding studies to be heterozygous for the two alleles of FcγRIIA. In contrast to their varying sensitivity to IgG1 MoAbs, members of the platelet panel responded equally well to 50H.19, an IgG2a MoAb specific for CD9, and these responses could not be blocked by MoAb IV.3 in the presence of plasma. This appears to be because of dual actions of 50H.19 on platelets: one FcR-dependent and the other complement-dependent. Our findings confirm previous reports that certain IgG1 MoAbs activate platelets through binding of their Fc domains to FcγRII receptors and demonstrate that this response is influenced both by FcγRII phenotype and (in the case of the anti-CD41 MoAb) by the number of FcγRII receptors expressed. The failure of nonresponding platelets to bind detectable amounts of MoAb 41H.16, which is thought to recognize all FcγRII receptors except for one allele of the FcγRIIA gene, is consistent with the possibility that FcγRIIA gene products, but not FcγRIIB or FcγRIIC gene products, are expressed on platelets.

C. Certain murine monoclonal antibodies (MoAbs) induce release and aggregation on binding to human platelets. Some MoAbs specific for glycoproteins IIb or IIa act directly on the GPIb/IIa complex to induce conformational changes leading to expression of the fibrinogen receptor. More often, MoAbs specific for these or other membrane glycoproteins trigger activation and release through conventional signal transduction pathways. For reasons not yet clear, MoAbs specific for CD9 or GPIV (CD36) are the most consistent platelet activators. In general, MoAb-induced transmembrane signalling requires an intact IgG Fc domain and can be blocked by MoAb IV.3 specific for the FcγRII receptor (CD32), implying that binding of monoclonal Fc to that receptor is important in the process of activation. It has been observed that platelets from normal subjects differ in their susceptibility to activation by murine MoAbs, but the basis for this has not been established.

Three families of receptors with specificity for the Fc region of human IgG have been described in humans. FcγRI receptors bind monomeric IgG Fc with high affinity. FcγRII and FcγRIII receptors bind monomeric IgG Fc weakly, but bind multimeric IgG Fc and IgG-containing immune complexes tightly. FcγRII molecules have the widest tissue distribution of the three FcγR families, being expressed on all FcγR-bearing blood cells except NK cells. Only FcγRII receptors have been demonstrated unequivocally on platelets.

FcγRII receptors in humans are the products of at least three different genes, designated A, B, and C, each coding for mature transmembrane proteins of about 40 Kd molecular mass. At least six different mRNA transcripts and four mature proteins are derived from these three genes. The FcγRIIA gene produces two transcripts as the result of alternative polyadenylation, the FcγRIIB gene produces three or four transcripts by alternative splicing of exons coding for cytoplasmic and signal sequences, and the FcγRIIC gene appears to produce a single transcript. The

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individuals can also be differentiated by their ability to form rosettes and mediate antibody-dependent cellular cytotoxicity (ADCC) against target cells sensitized with murine IgG, and by the isoelectric focusing patterns of their FcγRII receptors. IEF patterns of monocytes and platelets from the same individuals are concordant.

Two well-studied MoAbs, IV.3 and 41H.16, appear to bind to the products of all three FcγRII genes. 41H.16 binds strongly to the HR allele of FcγRIIA, but weakly or not at all to the product of the LR allele. Strong binding of 41H.16 to the HR gene product was recently shown to be determined by an arginine, rather than a histidine, residue at position 131 in the second extracellular domain. Monocytes from about 25% of the general population carry the form of FcγRIIA that fails to bind 41H.16, ie, has a histidine residue at position 131.

In a number of clinical disorders, platelet destruction is thought to be mediated by IgG Fc-dependent mechanisms. A fuller understanding of platelet Fc receptors could provide clues to the pathogenesis of some of these conditions. Therefore, we characterized the FcγRII receptors of platelets using MoAbs IV.3 and 41H.16 and studied the relationship between FcγRII phenotype and level of expression and susceptibility of platelets to MoAb-induced activation.

**MATERIALS AND METHODS**

**Murine MoAbs.** Characteristics of the murine MoAbs used are summarized in Table 1. ALB-6 (α-CD9) was obtained from AMAC, Inc (Westbrook, ME); 8A6 (α GPIV, subclone D9) from Dr John Barnwell (New York University); 50H.19 (α-CD9) from Dr A.R.E. Shaw (University of Alberta, Canada); and IV.3 (α-FcγRII) from Dr Clark Anderson (Ohio State University). Literature citations are provided in Table 1. Monoclonal IgG was purified from ascites fluid by affinity chromatography on protein A Sepharose CL4B (Pharmacia, Piscataway, NJ).

**Preparation and radiolabeling of Fab fragments.** Fab fragments of MoAbs IV.3 and 41H.16 were produced by papain digestion of purified IV.3 IgG followed by chromatographic purification on Protein A Sepharose CL-4B. The final preparation yielded bands of molecular weight (MW) approximately 50 Kd (nonreduced) and 25 Kd (reduced) on sodium dodecyl sulfate (SDS) gel electrophoresis. The Fab fragments were labeled with 125I by the lactoperoxidase/glucose oxidase procedure using Enzymobeads (BioRad, Richmond, CA). Specific activity of the radiolabeled Fab was determined by comparing protein content with counts per minute (cpm) determined in a gamma scintillation counter. By trichloroacetic acid precipitation, it was found that more than 95% of the radioactivity was protein-bound.

**Binding of radiolabeled IV.3 Fab to platelets.** Binding of 125I-Fab from MoAb IV.3 to washed platelets was determined by the method of Court and LoBuglio with minor modifications.

<table>
<thead>
<tr>
<th>MoAb</th>
<th>Target GP</th>
<th>Subclass</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>OP-G2</td>
<td>CD41 (GPⅡb/Ⅲa)</td>
<td>IgG1</td>
<td>34</td>
</tr>
<tr>
<td>ALB-6</td>
<td>CD9</td>
<td>IgG1</td>
<td>4, 6</td>
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<tr>
<td>50H.19</td>
<td>CD9</td>
<td>IgG2a</td>
<td>3, 35</td>
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<tr>
<td>8A6</td>
<td>CD36 (GPIV)</td>
<td>IgG1</td>
<td>36</td>
</tr>
<tr>
<td>IV.3</td>
<td>CD32 (FcγRII)</td>
<td>IgGm</td>
<td>22, 27</td>
</tr>
<tr>
<td>41H.16</td>
<td>CD32 (FcγRII)</td>
<td>IgG2a</td>
<td>28, 37</td>
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Platelets were separated from freshly collected blood anticoagulated with EDTA and were washed three times in platelet suspension buffer (PSB) (0.01 mol/L phosphate-buffered isotonic saline, 1% bovine serum albumin, 4.0 mmol/L EDTA, pH 6.5). Final preparations contained fewer than one white blood cell per 10,000 platelets. Platelets, 1 × 10⁷, were suspended in 300 μL of buffer containing 2 μg of 125I-Fab fragments. Preliminary studies demonstrated that this quantity of Fab was in excess of the amount needed to achieve saturation binding. After incubation at room temperature for 1 hour, 50-μL aliquots were layered in triplicate on 200 μL of 20% Percoll in 400 μL polypropylene microcentrifuge tubes. The tubes were centrifuged at 10,000g for 4 minutes to allow pelleting of platelets and platelet-bound Fab. The tubes were then clamped, the tips cut off, and the pelleted radioactivity was measured in a gamma scintillation counter. Molecules of Fab bound were calculated from the formula:

\[
\text{cpm bound} \times 10^{-6} \times A \\
SA \times \text{No. of Platelets} \times \text{MW}
\]

where \(A = \text{Avogadro's number, } SA = \text{specific activity of Fab (cpm per microgram), and MW = 50,000.}\)

**Determination of IV.3 and 41H.16 binding by flow cytometry.** Platelet-rich plasma was prepared from freshly collected, citrated whole blood using excess acid-citrate-dextrose (ACD) anticoagulant to achieve a pH of about 6.4. The platelets were pelleted at 3,000g and washed twice in Ringer's citrate dextrose (RCD) buffer containing 2 mmol/L EDTA and 50 ng/mL prostaglandin E1 (Sigma Chemical Co, St Louis, MO), pH 6.5. Platelets, 2 × 10⁷, were resuspended in 100 μL of RCD buffer containing 1 μg/mL of MoAb IV.3 or 41H.16 and incubated for 60 minutes at room temperature. These quantities were more than 10-fold the amounts required to achieve saturation binding. After two washes in RCD buffer, the platelets were incubated with a 1:40 dilution of affinity-purified F(ab')2 fragments of fluorescein isothiocyanate (FITC)-conjugated goat antimouse IgG (Jackson Immunoresearch, West Grove, PA) for 30 minutes. The labeled platelets were washed twice and fixed with 1% paraformaldehyde. Bound FITC was analyzed by flow cytometry (FACStar-Plus; Becton Dickinson, Mountain View, CA). Antibody binding was expressed as mean fluorescence intensity in the linear mode.

**Platelet aggregation.** Platelet-rich plasma was prepared from freshly collected blood anticoagulated with one-tenth part of 3% sodium citrate. Donors had not ingested aspirin or other drugs known to inhibit platelet function for 1 week before study. The concentration of platelets was adjusted to about 300,000/μL by adding platelet-poor plasma from the same donor. The response to MoAbs and other platelet agonists was measured using a Bio/Data PAP-4 aggregometer (Bio/Data Corp, Hatboro, PA) at 37°C with a stirring rate of 1,000 rpm. Reactions were terminated after 20 minutes when platelets failed to aggregate. For reasons to be described (Results), we found it useful to measure the lag time required for platelets to respond to activating monoclonals. This was defined as the time required for light transmission to reach one half of its maximum value.

**Release of 14C-serotonin from labeled platelets.** Release of 14C-serotonin from platelets in plasma-free medium was determined by a modification of the method of Sheridan et al. Serumin-labeled...
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platelets were suspended in albumin-free Tyrode's buffer at a concentration of \(3 \times 10^5/\mu L\) and 75-\(\mu L\) aliquots were pipetted into the wells of a polystyrene microtiter tray (Dynatech, Chantilly, VA). Twenty-five microliters of test material or buffer was then added, and the trays were sealed and agitated gently on a horizontal rotator for 60 minutes at room temperature. Phosphate-buffered isotonic saline, 0.1 mL, pH 7.4 containing 0.5% EDTA was then added and the trays were spun at 2,500 rpm to pellet the platelets. Radioactive content of 100-\(\mu L\) aliquots of the supernatants was determined in a beta scintillation counter, and results were expressed as percent of total serotonin (determined from a detergent-lysed aliquot of labeled platelets). Release in control tubes containing labeled platelets and an irrelevant MoAb was less than 5%. Release of \(^{14}\)C-serotonin from platelets in platelet-rich plasma was determined according to Griffith et al.

**RESULTS**

**MoAb-induced platelet aggregation.** In preliminary studies, it was found that maximum aggregation of normal platelets could be induced by 10 \(\mu g/mL\) of monoclonal OP-G2 (\(\alpha\) GPIIb/IIIa), 20 \(\mu g/mL\) of ALB-6 (\(\alpha\)-CD9), 5 \(\mu g/mL\) of 8A6 (\(\alpha\)-GPIV), and 5 \(\mu g/mL\) of 50H.19 (\(\alpha\)-CD9). Higher incubations of MoAbs failed to further shorten the lag time of individual platelet preparations. The response of platelets to various amounts of MoAb 8A6 is shown in Fig 1A. Qualitatively similar dose-response curves were obtained with MoAbs OP-G2, ALB-6, and 50H.19. The response of platelets to each of the \(\operatorname{IgG}_{1}\) MoAb (8A6, ALB-6, OP-G2) was totally inhibited by prior addition of MoAb 41H.16 or Fab from 41H.16 (shown for MoAb OP-G2 in Fig 1B). The response to the \(\operatorname{IgG}_{2}\) MoAb 50H.19 (\(\alpha\)-CD9) was not inhibited by 41H.16 (see below).

Platelets from different normal subjects varied significantly in their response to the three activating MoAbs of the \(\operatorname{IgG}_{1}\) subclass (Fig 2). Platelets from some donors responded within 1 or 2 minutes; others never responded or aggregated only after a delay of 5 or 10 minutes. The extent of aggregation and the maximum slope of the aggregation curve were generally less in platelets requiring a longer time to respond. Further studies were conducted to determine the basis for these differences.

**Quantitation of FcγRII receptors on platelets.** Measurement of the binding of MoAb IV.3 Fab fragments to platelets of 20 unrelated normal subjects (10 male, 10 female) demonstrated a range of binding sites from 1,660 to 4,610 per platelet. The specificity of binding was confirmed by showing that platelet-associated radioactivity was reduced by more than 99% when platelets were preincubated with a 50-fold excess of nonlabeled IV.3 Fab. The number of IV.3 Fab binding sites was stable (±12% about the mean) in four individuals, two with relatively high and two with relatively low FcγRII expression, who were studied on four occasions over a 6-month period. Platelets from males and females did not differ significantly in the quantities of IV.3 Fab bound.

**Comparison of IV.3 and 41H.16 binding.** Binding of the \(\alpha\)-FcγRII MoAbs IV.3 and 41H.16 to platelets of the same group of normal subjects was measured by flow cytometry. Platelets from different donors varied considerably in the quantity of IV.3 bound as expected from the IV.3 Fab binding studies (see above). MoAb 41H.16 exhibited a different binding pattern in that no detectable amounts were bound to platelets from five members of the panel, while platelets from the remaining 15 donors bound variable amounts. When the ratio of 41H.16 fluorescence to IV.3 fluorescence was determined, the value was approximately zero for the five platelet preparations that failed to bind 41H.16. The binding ratios for the remaining 15 donors allowed them to be segregated into two additional groups with ratios ranging from 0.19 to 0.39 (10 donors) and from 0.58 to 0.63 (five donors) (Fig 3). The three groups will be referred to as groups 1, 2, and 3, respectively, in the following discussion. Varying ratios of 41H.16:IV.3 binding to platelets were also observed by Gosselin et al. in six selected normal subjects.

**Response of platelets to the \(\operatorname{IgG}_{1}\) MoAbs OP-G2, 8A6, and ALB-6.** Platelets in the three groups defined by the 41H.16:IV.3 binding ratios (Fig 3) differed in respect to the time required for MoAbs OP-G2 (\(\alpha\)-GPIIb/IIIa), 8A6 (\(\alpha\)-GPIV), and ALB-6 (\(\alpha\)-CD9) to induce an aggregation response (lag time) as shown in Fig 4. Platelets from donors
Fig 3. Ratio of 41H.16:IV.3 binding in platelets from 20 normal subjects. MoAb binding at saturation was determined by flow cytometry using FITC-labeled Fab', specific for mouse IgG as a secondary antibody. Values shown are the average of two or three independent measurements with each MoAb on platelets of each donor. Donors were divided into groups 1, 2, and 3 on the basis of 41H.16:IV.3 binding ratios.

Fig 4. Aggregation responses of platelets from 20 normal subjects to platelet-activating MoAbs OP-G2 (10 μg/mL), ALB-6 (20 μg/mL), and 8A6 (5 μg/mL). Platelets were assigned to groups 1, 2, and 3 on the basis of MoAb 41H.16:IV.3 binding ratios (see Fig 3 and text). Ordinate depicts inverse lag time of aggregation response in minutes⁻¹ × 10². A value of 0 indicates an "infinite" lag time, ie, no response within 20 minutes. Values shown are the average of two or three independent measurements on platelets from each donor. Horizontal bars indicate average values for each group. Groups 1 and 3 differed significantly from each other for each of the three MoAbs (P < .01).

Fig 5. Aggregation responses of platelets from group 2 donors to MoAb OP-G2 (α-GPIIb/IIIa) at 10 μg/mL. Ordinate depicts inverse lag time of aggregation response in minutes⁻¹ × 10². Response was roughly proportional to the number of binding sites for IV.3 Fab, ie, the number of FcγRII receptors (P < .01) (y = .02x - 34.8, r² = .756). Similar, but less striking, relationships were observed between FcγRII expression and response to MoAb 8A6 (α-GPIV) and ALB-6 (α-CD9) (not shown).
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Serotonin release was also induced by MoAb 50H.19 (IgG2a, α-CD9). This response was completely blocked by MoAb IV.3, in contrast to aggregation induced by this MoAb (Fig 7A). Because the serotonin release studies were performed in the absence and the aggregation studies in the presence of plasma, it seemed possible that plasma affected the ability of IV.3 to inhibit activation by this MoAb. This was confirmed by demonstrating that IV.3 failed to inhibit serotonin release triggered by 50H.19 in platelet-rich plasma (Fig 7B). In contrast, IV.3 completely inhibited serotonin release induced by the IgG1 MoAbs ALB-6, 8A6, and OP-G2 in platelet-rich plasma (not shown). Plasma and serum heated to 56°C for 30 minutes and unheated plasma containing 10 mmol/L EDTA failed to support serotonin

Induction of serotonin release by MoAbs. 14C-serotonin release from platelets was induced by about one tenth the amount of MoAb required to induce an aggregation response. As shown in Figs 6, A and B, at low concentrations, the IgG1 MoAbs caused release of 14C-serotonin from group 3 platelets more readily than from group 1 platelets. At higher concentrations of MoAb, group 1 platelets also released serotonin. As with aggregation, serotonin release was blocked by prior addition of MoAb IV.3 (α FcyRII) at 10 μg/mL (not shown).

Fig 6. Release of 14C-serotonin from platelets of a donor from group 3 (■) and a donor from group 1 (▲) in response to different amounts of MoAbs OP-G2 (A) and ALB-6 (B) (average of three determinations). Error bars indicate ±1 SD. Significance of differences is *** < .001, ** < .01, * < .05. Results shown are typical of three paired studies in which responses of group 1 and group 3 platelets were compared. Similar results were obtained with MoAb 8A6 (not shown).

Fig 7. Serotonin release induced by MoAb 50H.19 in group 1 and group 3 platelets in plasma-free (A) and plasma-containing media (B). Release was inhibited by MoAb IV.3 in buffer (A), but not in plasma (B).
release in response to 50H.19 in the presence of IV.3. These findings suggest that complement activation by 50H.19 accounts for its ability to induce the release reaction in platelets whose FcγRII receptors are blocked by IV.3.

**DISCUSSION**

Our studies show that platelets from different normal subjects vary greatly in their responsiveness to IgG1 MoAbs specific for membrane glycoproteins CD9, GP14, and GPIIb/IIIa. Inhibition of the action of these MoAbs by MoAb IV.3 specific for the FcγRII receptor is confirmatory of previous reports 4-8,11 and consistent with the view that the FC domains of activating MoAbs react with platelet FcγRII receptors to induce release and aggregation. Recent reports indicate that this occurs both on an intra-platelet and inter-platelet basis.9,10

Our quantitative studies of FcγRII expression on platelets using radiolabeled Fab from MoAb IV.3 showed stable differences in the number of FcγRII expressed on platelets of normal individuals, in confirmation of the findings of Rosenfeld et al.12 who used intact IV.3. Platelets from the 20 subjects studied bound 1,660 to 4,610 IV.3 Fab (average 3,060), about twice the value (1,350) obtained by Karas et al.13 using a dimeric IgG probe.4,14 With intact IV.3, we obtained values for FcγRII expression about half as great as those found with Fab (data not shown). King et al.14 also found that IV.3 Fab binding to platelets was twice that of intact IV.3. The total number of binding sites per platelet identified using Fab labeled IV.3 Fab (4,700) was about twice the number we measured. However, their binding studies were performed at low ionic strength, which they found doubled the apparent number of Fc receptor sites. Lower values for Fc receptors obtained with intact MoAbs than with Fab may reflect a bivalent binding of the intact molecule to FcγRII.

On the basis of the 41H.16:IV.3 binding ratios, it was possible to divide our panel into three groups (Fig 3). Using nomenclature applied to monocytes,12-24 these appear to correspond to homozygotes for the LR form of FcγRII (group 1), homozygotes for the HR form (group 3), and LR/HR heterozygotes (group 2). Platelets judged to be HR homozygous on this basis all responded well to activating MoAbs of the IgG1 subclass, and those judged to be LR homozygous all responded poorly (Fig 4). However, platelets from apparent heterozygotes (group 2) varied widely in their sensitivity to MoAbs. Responsiveness of group 2 platelets to OP-G2 correlated fairly well with the number of FcγRII molecules expressed (Fig 5). No such relationship was observed with 8A6 or ALB-6. These findings indicate that both FcγRII phenotype and the number of FcγRII molecules carried on the platelet surface influence the responsiveness of platelets from heterozygous (group 2) individuals. The variation in response of group 2 platelets to 8A6 and ALB-6 implies that other, unrecognized factors are also important. Rosenfeld et al.12 found that the response of washed platelets to heat-aggregated IgG (HALgG) correlated with the number of FcγRII receptors expressed. We confirmed this finding with our platelet panel (data not shown). However, platelets from donors in groups 1 and 3 responded similarly to HALgG, indicating that this particular response is not affected by FcγRII phenotype. Similar observations were made by Looney et al.28

Target molecules recognized by the three IgG1 MoAbs used in our study are expressed in different numbers on the platelet surface (CD36 about 20,000, CD41 about 40,000 to 50,000, and CD9 about 65,000 molecules per platelet). However, MoAb 8A6 specific for CD36 produced a maximum platelet response at a concentration lower than that required for OP-G2 or ALB-6. Therefore, within the range from 20,000 to 65,000, the number of target molecules per platelet does not appear to be an important determinant of response. CD9, CD36, and CD41 are not known to vary significantly on platelets of normal subjects except in rare individuals with genetically determined deficiencies of CD3647 or CD41 (type I Glanzmann’s thrombasthenia). The concordant behavior of group 1 platelets in response to all three of the IgG1 MoAbs essentially rules out the possibility that their poor responses were caused by a deficiency of target protein.

As noted, the FcγRII gene family encodes a number of mature proteins expressed differently in various cell types. 41H.16 is thought to bind to all FcγRII gene products except the allelic form of FcγRIIA that contains a histidine, rather than an arginine residue, at position 131.15 Therefore, the failure of group 1 platelets to bind detectable amounts of 41H.16 is consistent with the possibility that only products of the FcγRII gene and not those of the FcγRII B and C genes are expressed on platelets.

Activation of platelets by the IgG2a MoAb 50H.19 specific for CD9 requires special comment because this MoAb, in contrast to OP-G2, ALB-6, and 8A6, failed to differentiate between HR and LR platelets and because, in a plasma medium, activation by 50H.19 could not be blocked by IV.3, even at concentrations as high as 50 μg/mL. However, IV.3 readily blocked the action of 50H.19 on platelets in a plasma-free medium (Fig 7). The IgG2a subclass of murine Ig is a potent activator of the classical complement pathway, in contrast to the IgG1 subclass,46 and it is known that release can be triggered by assembly of the terminal complement components in the platelet membrane.47 These facts, together with our finding that heated plasma and serum and plasma containing 10 mmol/L EDTA were unable to support 50H.19-induced release in the presence of IV.3, strongly suggest that 50H.19 activates platelets by two mechanisms: one complement-dependent and the other dependent on binding of its Fe region to FcγRII. Griffith et al.15 have recently shown that immobilized F(ab')2 from 50H.19, but not soluble F(ab')2, can activate platelets by a direct action on CD-9.35

Together with previous reports, our findings indicate that MoAbs activate platelets by several different mechanisms: (1) Fe-mediated activation of FcγRII; (2) complement activation; and (3) in the case of certain MoAbs,1,8,12 direct activation via the target protein to which they bind. Platelets from normal subjects vary greatly in their susceptibility to Fe-mediated activation by IgG1 MoAbs, and failure to respond to these MoAbs is associated with homozygosity for one of the two defined alleles of the FcγRIIA gene (Figs
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3 and 4). In the absence of plasma, "non-responder" platelets can be induced to release serotonin by increasing the concentration of stimulating MoAbs (Fig 6). However, high concentrations of MoAbs did not cause group 1 platelets to aggregate in platelet-rich plasma. These observations suggest that the two alleles of FcγRII differ in their affinity for murine IgG, Fc and that high levels of monomeric IgG in human plasma modulate the response to these MoAbs by competing with their Fc domains for binding to FcγRII. Thus, in the presence of plasma, only the allele of FcγRII with higher affinity for IgG1 Fc is activated.

Although FcR of classes I, II, and III appear to be redundant in their functions, it has been argued that FcγRII has special functional significance. Whether the FcγRII phenotype of platelets is relevant to platelet physiology or pathophysicsiology in humans therefore deserves consideration. A recent report showed that the LR form of FcγRIIA preferentially binds multimers of human IgGs, the IgG subtype mainly involved in the human immune response to saccharide antigens. Alloantibodies specific for class I HLA antigens are capable of inducing platelet activation and aggregation, and may bind to platelets at a site close to the FcγRII receptor. Therefore, further studies of platelet FcR may be relevant to platelet destruction mediated by alloantibodies reactive with Class I HLA alloantigens. A number of reports of platelet activation and thrombosis induced by platelet-reactive autoantibodies have appeared, and it seems possible that platelet activation occurs via FcR in some of these patients. In one study, a mild hemostatic defect was observed in three patients whose platelets responded poorly to an MoAb specific for CD9. Finally, heparin-induced thrombocytopenia/thrombosis appears to be mediated by immune complexes reactive with platelet FcR and platelets from different normal subjects differ widely in their sensitivity to heparin-dependent antibodies. Further studies to determine whether the FcγRII receptor phenotype of platelets predisposes to any of these conditions also appear warranted.

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