Effect of Anti-P1^A1 Antibody on Human Platelets. II. Mechanism of the Complement-Dependent Release Reaction

By Alan D. Schreiber, Douglas B. Cines, Chester Zmijewski, and Robert W. Colman

We studied the mechanism by which complement activated by anti-P1^A1 antibody elicits the platelet release reaction. Anti-P1^A1 antibody mediates its action through the classic complement pathway, and its effect depends on the concentration of IgG antibody on the platelet surface. At relatively high concentrations of anti-P1^A1 antibody the release reaction was mediated by a mechanism in part independent of extracellular ADP and metabolic energy and inhibited by only high concentrations of PGE

At relatively high concentrations of anti-P1^A1 antibody the release reaction was mediated by a mechanism in part independent of extracellular ADP and metabolic energy and inhibited by only high concentrations of PGE. However, at lower concentrations of anti-P1^A1 antibody the release reaction was dependent on metabolic energy and ADP, and the concentration of PGE, required to inhibit platelet release was similar to that required to inhibit ADP-induced release. The cyclooxygenase inhibitor acetylsalicylic acid inhibited the release reaction at all nonlytic antibody levels studied. None of the agents studied inhibited the induction of platelet lysis by very high concentrations of anti-P1^A1 antibody, and no effect of antibody on platelet \(^{14}\)C-serotonin uptake was observed at antibody concentrations that did not mediate direct in vitro alteration. These studies suggest the possible use of pharmacologic agents in modifying some complement-mediated platelet alterations.

Platelet alteration by immune mechanisms is involved in the pathogenesis of several diseases. Antibody-induced platelet injury can lead to thrombocytopenia and to qualitative platelet disorders. Human antiplatelet antibodies can cause platelet lysis or aggregation, can initiate platelet serotonin release, and can make available platelet factor 3 and acid phosphatase at the membrane surface. Although activation of the complement system may occur in each of these disorders, the specific mechanism of complement interaction with human platelets is incompletely understood. Complement-platelet interaction can alter membrane fluidity and can cause platelet lysis. Additionally, deposition of the third component of complement on the platelet surface may play an independent role in the clearance of platelets in some patients with immune thrombocytopenic purpura. The platelet isoantibody anti-P1^A1 can also induce complement-dependent \(^{14}\)C-serotonin release in the absence of lysis and may decrease platelet survival or function by this mechanism.

In this report we explore the mechanism(s) by which human complement activated by anti-P1^A1 antibody induces the platelet release reaction. These mechanisms were observed to differ from those previously described with heterolo-
gous antibody. The results indicate the importance of antibody concentration in the observed complement-mediated effects and suggest the potential for pharmaco logic modification of such complement-platelet interaction.

MATERIALS AND METHODS

ADP, pyruvate kinase type III, trisodium phosphoenol pyruvate, 2-deoxy-D-glucose, and antimycin A were obtained from Sigma Chemicals, St. Louis, Mo. Prostaglandin E, (PGE,) was supplied by J. Pike, Upjohn, Detroit, Mich. Potato apyrase was a gift from Dr. S. Niewiarowski, Temple University, Philadelphia, Pa. Acetylsalicylic acid (ASA) was obtained from Fischer Scientific, Philadelphia, Pa. Indomethacin was kindly donated by Merck, Sharp & Dohme, West Point, Pa. Imipramine was purchased from Ciba-Geigy, Caldwell, N.J., and SH869 was obtained from Pharma-Research, Montreal, Canada. Highly purified human alpha thrombin (2500–2900 NIH units/mg) was a gift of Dr. John Fenton (New York State Division of Laboratories in Research, Albany, N.Y.).

Platelet Preparations

Platelet-rich plasma containing heparin (PRP), platelet-poor plasma (PPP), and gel-filtered platelets were prepared as previously described. Gel-filtered platelets were employed for studies involving ADP-utilizing enzymes, metabolic inhibitors, and thrombin. One-tenth milliliter of PPP containing heparin (final concentration 1.3 units/ml) was added, as a source of complement, to the gel-filtered platelets (0.3 ml) prior to their interaction with antibody. In all experiments the platelets released serotonin on addition of ADP.

Platelet 14C-Serotonin Release and Uptake

The release and uptake of 14C-serotonin from human platelets was assessed by a modification of the method of Jerushalmy and Zucker. PRP was incubated with 14C-serotonin for varying time periods to determine the kinetics of platelet serotonin uptake.

Platelet 51Cr Loss

The loss of 51Cr from human platelets was assessed by a modification of the method of Aster and Enright as previously described.

Low-Affinity Platelet Factor 4 and ATP Release

Low-affinity platelet factor 4 was determined by the method of Niewiarowski et al. and was expressed as percentage of the total PF4 as assayed by solubilization of the platelets in 0.8% Triton. ATP release was determined by the simultaneous monitoring of platelet aggregation and ATP release using luciferase-luciferin in a Lumi aggregometer (Chronolog, Broomall, Pa.). The results are expressed as micromoles of ATP released per 1011 platelets.

HLA Typing

HLA typing was performed according to the standard NIH microlymphocytotoxicity procedure. Sera detecting class I HLA antigens obtained from the NIH Serum Bank as well as our own collection were employed.

Antiplatelet Antibody

In these studies, naturally occurring human anti-P11 antibodies were employed. One antiserum also had antibody activity against HLA-B7; in all experiments with this antiserum, cells were specifically selected so as not to contain any HLA antigens of the HLA-B7-B27 cross-reactive group. In experiments involving ASA, rabbit antihuman platelet antisera were studied in parallel. These rabbit IgG antihuman platelet antibodies have been previously characterized.

Measurement of antibody-induced 14C-serotonin release was performed as previously described. All experiments were controlled by parallel incubations with nonimmune plasma that gave less than 7% platelet 14C-serotonin release. In selected experiments, PRP was simultaneously labeled with 51Cr and 14C-serotonin, and double-radiolabeled gel-filtered platelets were obtained.
IgG antibody per platelet was approximated using a modification of the IgG (gamma) Coombs test in which the antiglobulin reagent (anti-IgG) was radiolabeled with $^{125}$I. The results are expressed as anti-IgG combining sites per platelet.\textsuperscript{10,11}

**Effects of Nonsteroidal Antiinflammatory Agents on Action of Antiplatelet Antibody Effect**

Three-tenths milliliter of radiolabeled platelets was preincubated for 30 min at 37\degree C with ASA (final concentration 4 mM) or indomethacin (final concentration 100 $\mu$M) dissolved in ethanol (final concentration 0.2% ethanol). Two-tenths milliliter of antibody or ADP was added, and release was determined with the addition of equal volumes of Tyrode’s buffer. The percentage inhibition of serotonin or $^{51}$Cr release was calculated in this and in all experiments as follows: percentage inhibition equaled percentage $^{14}$C-serotonin release or $^{51}$Cr loss with buffer minus percentage $^{14}$C-serotonin release or $^{51}$Cr loss with ASA divided by percentage release or loss with buffer, multiplied by 100.

**Modification of Anti-P1\textsuperscript{A1} Antibody Effect by Removal of Extracellular ADP**

Three-tenths milliliter of $^{14}$C-serotonin-labeled gel-filtered platelets was preincubated at 37\degree C for 1 min with pyruvate kinase or apyrase, as previously described.\textsuperscript{12} In each experiment 0.2 ml of either anti-P1\textsuperscript{A1} antibody or ADP in modified Tyrode’s buffer was added with stirring at 37\degree C for 45 min and 15 min, respectively. The $^{14}$C-serotonin release was then determined as previously outlined.

**Modification of Anti-P1\textsuperscript{A1} Antibody Effect by Metabolic Inhibitors**

Three-tenths milliliter of $^{14}$C-serotonin platelets was preincubated for 30 min at 37\degree C with 2-deoxyglucose and antimycin A to deplete the metabolic pool of ADP.\textsuperscript{12} Two-tenths milliliter of anti-P1\textsuperscript{A1} antibody, ADP, or thrombin was then added as previously described, and the percentage $^{14}$C-serotonin release was determined.

**Influence of PGE\textsubscript{1} and SH869 on Action of Anti-P1\textsuperscript{A1} Antibody**

Three-tenths milliliter of $^{14}$C-serotonin platelets in PRP was preincubated for 1 min at 37\degree C with two concentrations of PGE\textsubscript{1} (0.28 or 5.6 mM) or for 10 min at 37\degree C with the phosphodiesterase inhibitor SH869 (4.0 or 8.0 $\mu$M). Two-tenths milliliter of anti-P1\textsuperscript{A1} antibody or ADP was added, and the percentage $^{14}$C-serotonin release was determined as described previously.

**RESULTS**

**Platelet Re-Uptake of $^{14}$C-Serotonin Released by Anti-P1\textsuperscript{A1} Antibody**

The time course of platelet serotonin release caused by low concentrations of anti-P1\textsuperscript{A1} antibody is such that release is not substantial until 20 min of antibody-complement-platelet interaction.\textsuperscript{11} Since this lag could represent reuptake of $^{14}$C-serotonin released during the early stages of the reaction, we studied whether platelets (PRP) incubated with anti-P1\textsuperscript{A1} antibody in the presence of imipramine have an altered pattern of $^{14}$C-serotonin release. Imipramine (5 $\mu$M), which inhibits platelet serotonin uptake\textsuperscript{20} (Table 1), did not substantially influence the kinetics of antibody-induced serotonin release (Fig. 1). In addition, imipramine (5 $\mu$M) did not affect platelet serotonin release caused by 5-$\mu$M ADP. At a higher dose, imipramine (100 $\mu$M) inhibited serotonin release by 5-$\mu$M ADP 90\%, but it did not affect anti-P1\textsuperscript{A1}-induced serotonin release with 6 X $10^5$ anti-IgG combining sites per platelet.

**Effect of Anti-P1\textsuperscript{A1} Antibody on Platelet Uptake of $^{14}$C-Serotonin**

Antiplatelet antibody has been reported to alter the platelet uptake of $^{14}$C-serotonin.\textsuperscript{18,19} Therefore, we examined whether or not anti-P1\textsuperscript{A1} antibody, at concentrations that do not mediate serotonin release, inhibits platelet $^{14}$C-serotonin uptake.
uptake. Platelets (PRP) were first sensitized with anti-P1A1 antibody (7.0 × 10^2 anti-IgG combining sites per platelet) at 37°C for 45 min, at which time ^14C-serotonin was added and the reactions were continued at 37°C (Table 1). At varying time intervals an aliquot was removed, 5% EDTA and imipramine were added, and the platelet ^14C-serotonin uptake was determined. As shown in two separate experiments, this nonaggregating concentration of antibody did not alter platelet uptake of ^14C-serotonin. However, imipramine (5 μM) did block ^14C-serotonin uptake by human platelets.

**Effect of ASA on Anti-P1A1-Induced ^14C-Serotonin Release**

Human antiplatelet antibody directed against the P1A1 antigen releases ^14C-serotonin. We studied the capacity of ASA to inhibit this effect of anti-P1A1 antibody (Table 2). In this, as in subsequent experiments, several different antibody concentrations were employed. With high antibody concentrations (12–24 × 10^3 anti-IgG combining sites per platelet) that mediate platelet lysis, ASA did not inhibit antibody-induced ^51Cr loss (not shown). However, ASA substantially inhibited antibody-induced serotonin release at concentrations of antibody (1.5–6 × 10^3 anti-IgG combining sites per platelet) not producing platelet lysis. At the lowest antibody concentration, inhibition was similar to that observed with ADP.

### Table 1. Effect of Anti-P1A1 Antibody on Platelet Uptake of ^14C-Serotonin

<table>
<thead>
<tr>
<th>Time (min)</th>
<th>Experiment A</th>
<th></th>
<th>Experiment B</th>
<th></th>
<th>Imipramine†</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Antibody</td>
<td>Buffer</td>
<td>Antibody</td>
<td>Buffer</td>
<td></td>
</tr>
<tr>
<td>0</td>
<td>10</td>
<td>16</td>
<td>9</td>
<td>6</td>
<td>2</td>
</tr>
<tr>
<td>3</td>
<td>46</td>
<td>50</td>
<td>35</td>
<td>32</td>
<td>—</td>
</tr>
<tr>
<td>6</td>
<td>49</td>
<td>55</td>
<td>59</td>
<td>59</td>
<td>—</td>
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<tr>
<td>10</td>
<td>55</td>
<td>57</td>
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<td>57</td>
<td>3</td>
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<td>58</td>
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<td>61</td>
<td>61</td>
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<td>30</td>
<td>58</td>
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<tr>
<td>40</td>
<td>60</td>
<td>63</td>
<td>63</td>
<td>62</td>
<td>7</td>
</tr>
</tbody>
</table>

*Each value represents percentage incorporation of ^14C-serotonin into platelets at the time shown. Platelets preincubated with anti-P1A1 antibody were compared with platelets preincubated with modified Tyrode’s buffer.

†Mean of 2–5 experiments.

![Fig. 1. Effect of imipramine on anti-P1A1-antibody-induced ^14C-serotonin release.](https://example.com/f1)
Table 2. Effect of ASA on Anti-P1 A1-Antibody-Mediated Serotonin Release*

<table>
<thead>
<tr>
<th>Human Anti-P1 A1 Antibody (anti-lgG sites/platelet)</th>
<th>Rabbit Antiplatelet Antibody Dilution</th>
<th>Percentage Inhibition of 14C-Serotonin Release</th>
</tr>
</thead>
<tbody>
<tr>
<td>1.5 × 10³</td>
<td>—</td>
<td>86 ± 7</td>
</tr>
<tr>
<td>3 × 10³</td>
<td>—</td>
<td>78 ± 14</td>
</tr>
<tr>
<td>6 × 10³</td>
<td>—</td>
<td>62 ± 21</td>
</tr>
<tr>
<td>—</td>
<td>1:32</td>
<td>10 ± 4</td>
</tr>
<tr>
<td>—</td>
<td>1:64</td>
<td>11 ± 7</td>
</tr>
<tr>
<td>—</td>
<td>1:128</td>
<td>24 ± 5</td>
</tr>
</tbody>
</table>

ASA (4-mM) was incubated with PRP for 30 min prior to addition of anti-P1 A1 antibody. Each value represents the mean of three experiments ± SEM. Controls with ADP (30-μM) revealed 92% inhibition of 14C-serotonin release by ASA. In the absence of ASA, anti-P1 A1 antibody caused 91% 14C-serotonin release with 6 × 10² anti-IgG combining sites per platelet and 48% release with 1.5 × 10³ anti-IgG combining sites per platelet. The rabbit antibody caused 93% 14C-serotonin release at 1:32 dilution, 60% release at 1:128 dilution, and 9% release at 1:256 dilution.

Effect of Removal of Extracellular ADP

To further investigate the relationship between the platelet release induced by human anti-P1 A1 antibody and ADP, studies were performed to examine the requirement for ADP. Two reaction systems that deplete ADP were studied (Table 3). Both apyrase, which converts ADP to AMP, and pyruvate kinase, which in the presence of its substrate phosphoenolpyruvate converts ADP to ATP, completely inhibited the release reaction caused by 20-μM ADP. This indicates a sufficient capacity and velocity to transform any endogenous ADP released during antibody-induced alteration. At 1.5 × 10³ anti-IgG combining sites per platelet, substantial inhibition of the release reaction was observed with both ADP-removing systems. With 6 × 10³ anti-IgG combining sites per platelet, platelet 14C-serotonin release was only partially inhibited. There was no inhibition of platelet lysis at the higher concentrations of antibody (12–24 × 10³ anti-IgG combining sites per platelet) that mediate 51Cr loss (not shown).

Table 3. Effect of ADP Removal on Anti-P1 A1-Antibody-Induced Serotonin Release*

<table>
<thead>
<tr>
<th>Inhibitors</th>
<th>Anti-lgG Sites/Platelet</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>1.5 × 10³</td>
</tr>
<tr>
<td>Apyrase (450 units/ml)</td>
<td>76</td>
</tr>
<tr>
<td>Phosphoenolpyruvate (0.45 mM) + pyruvate kinase (90 units/ml)</td>
<td>88</td>
</tr>
</tbody>
</table>

*Each value represents the percentage inhibition 14C-serotonin release based on the mean of two experiments. ADP (20 μM) stimulated 37% platelet 14C-serotonin release in the absence of inhibitors. Antiplatelet antibody caused 84% platelet serotonin release (6 × 10² anti-IgG combining sites per platelet) and 64% 14C-serotonin release (1.5 × 10³ anti-IgG combining sites per platelet).
Table 4. Effect of Metabolic Inhibitors on Anti-PI Antibody-Induced 14C-Serotonin Release*

<table>
<thead>
<tr>
<th>Anti-IgG Sites/Platelet</th>
<th>Thrombin (0.2 NIH U/ml)</th>
</tr>
</thead>
<tbody>
<tr>
<td>2-Deoxyglucose (20 mM) + antimycin A (10 μg/ml)</td>
<td>79 ± 16</td>
</tr>
</tbody>
</table>

*Each value represents the percentage inhibition of serotonin release recorded of the mean ± SEM of three replicate experiments in gel-filtered platelets. 14C-serotonin release was 82% with thrombin at 0.2 NIH U/ml. Antiplatelet antibody caused a mean of 76% release with 6 × 10^3 anti-IgG combining sites per platelet and 67% release with 1.5 × 10^3 anti-IgG combining sites per platelet.

Requirements for Metabolic Energy

The requirements for metabolic energy in the antibody–platelet interactions were next studied (Table 4). An inhibitor of aerobic metabolism (2-deoxyglucose) and an inhibitor of anaerobic metabolism (antimycin A) were both employed. In confirmation of the work of others, the combination of 2-deoxyglucose (20 mM) and antimycin A (10 μg/ml) inhibited ADP-induced 14C-serotonin release 94% and thrombin-induced serotonin release 85% (Table 4). Control experiments using platelets preincubated with metabolic inhibitors for 30 min at 37°C did not reveal any 14C-serotonin release after 45 min of incubation at 37°C in the absence of antibody, ADP, or thrombin. The results with human anti-PI antibody were entirely dependent on antibody concentration. Release of 14C-serotonin was not affected by metabolic inhibitors with 6 × 10^3 anti-IgG combining sites per platelet. However, at lower antibody concentrations, considerable inhibition of serotonin release was observed in the presence of inhibitors of both aerobic and anaerobic metabolism. With higher concentrations of antibody (12–24 × 10^3 anti-IgG combining sites per platelet), inhibition of 51Cr loss was only 15% with 2-deoxyglucose and antimycin A (not shown).

Influence of PGE₁ and SH869 on Effect of Anti-PI Antibody

The mechanism of the human antibody effect was further pursued by investigating the consequence(s) of the addition of PGE₁, which increases the activity of adenylate cyclase in platelets, and SH869, which inhibits phosphodiesterase (unpublished observation), an enzyme necessary for cyclic AMP catabolism. SH869 has been observed to potentiate the effect of PGE₁ on platelets. At the lowest antibody concentration, inhibition was virtually complete at a PGE₁ concentration of 0.28 μM (Fig. 2). Higher concentrations of PGE₁ (5.6 μM) were required for substantial inhibition of platelet serotonin release with 6 × 10^3 anti-IgG combining sites per platelet. PGE₁ (0.28 μM) totally inhibited ADP-induced serotonin release (not shown). Higher antibody concentrations (12–24 × 10^3 anti-IgG combining sites per platelet) that caused platelet lysis (loss of 51Cr) were not inhibited by either concentration of PGE₁. SH869 also inhibited antibody-mediated platelet 14C-serotonin release at nonlytic concentrations of antibody (Table 5). As with PGE₁, at both concentrations of SH869 studied the extent of inhibition of serotonin release depended on the concentration of anti-PI antibody employed. Only partial inhibition of antibody-induced serotonin release was observed with an antibody concentration of 6 × 10^3 anti-IgG combining sites per platelet.
platelet. Complete inhibition of the release reaction caused by ADP was observed with SH869 (4 μM).

**Low-Affinity Platelet Factor 4 and ATP Release**

Complement also induced release of 92% of the total platelet low-affinity PF4 as measured immunochemically with 1.5–3.0 × 10^3 anti-IgG combining sites per platelet. In addition, ATP release was 1.8–2.1 μmoles per 10^11 platelets with similar concentrations of anti-P1^A^ antibody.

**DISCUSSION**

We studied the mechanism of action of human complement activated by anti-P1^A^ antibody on human platelets. Anti-P1^A^ antibody causes platelet lysis ([Cr] Cr loss), and at lower concentrations it mediates a platelet release reaction ([4C-serotonin release]). These reactions are mediated through the classic complement pathway. We studied the mechanism of this complement-dependent release reaction when initiated by different concentrations of antibody.

The data indicate that complement initiates a release reaction in human platelets by at least two different mechanisms, depending on the concentration of anti-P1^A^ antibody employed. With lower concentrations of anti-P1^A^ antibody, the complement-mediated release reaction involves a mechanism that requires the release of

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**Table 5. Effect of SH869 on Anti-P1^A^ Antibody-Mediated [4C-Serotonin Release**

<table>
<thead>
<tr>
<th>Antibody Concentration (anti-IgG sites/platelet)</th>
<th>SH869 4 μM</th>
<th>SH869 8 μM</th>
</tr>
</thead>
<tbody>
<tr>
<td>3 × 10^3</td>
<td>83 ± 3</td>
<td>86 ± 8</td>
</tr>
<tr>
<td>4 × 10^3</td>
<td>83 ± 6</td>
<td>92 ± 1</td>
</tr>
<tr>
<td>6 × 10^3</td>
<td>35 ± 7</td>
<td>37 ± 6</td>
</tr>
</tbody>
</table>

*Each value represents the mean percentage inhibition of serotonin release as determined from three experiments ± SEM. ADP (10 μM) stimulated 55% [4C-serotonin release in the absence of inhibitors. In the presence of SH869, ADP-induced [4C-serotonin release was completely inhibited. [4C-serotonin release was 72% and 29% at antibody concentrations of 6 × 10^2 and 1.5 × 10^2 anti-IgG combining sites per platelet, respectively, in the absence of SH869.*
ADP (Table 3), that requires metabolic energy (Table 4), and that is more readily inhibitable by PGE$_1$ (Fig. 2). This pathway is quite different from that mediated by heterologous antibody,$^{12}$ but it is similar to, if not identical with, that mediated by exogenous ADP and epinephrine. In addition, similar to ADP- and epinephrine-induced release, low-affinity PF4 and ATP are also released by these concentrations of anti-P1$^\text{A1}$ antibody. A further similarity to ADP- or epinephrine-mediated platelet serotonin release is the substantial inhibition achieved with ASA (Table 2). The results were similar for all three anti-P1$^\text{A1}$ antibodies studied, and they suggest that this complement effect on platelets is, in part, mediated through the prostaglandin-forming pathway.

At relatively high antibody concentrations complement-mediated platelet alteration proceeds by a different mechanism, one that is partly independent of ADP (Table 3) and metabolic energy (Table 4), that requires a high concentration of PGE$_1$ (Fig. 2) and phosphodiesterase inhibitor (Table 5) for significant inhibition, and that proceeds in the presence of 100-$\mu$M imipramine. These results are very similar to those we observed with heterologous antibody$^{12}$ in a reaction that is independent of complement.$^{13}$ A similar pathway to platelet secretion has also been described recently with thrombin and a cation ionophore (A23187).$^{26,27}$ The different pattern of inhibition depending on antibody concentration may represent either quantitative or qualitative differences in the mechanism of the release reaction. The lack of significant inhibition by ASA of the release reaction caused by a full range of heterologous antibody concentrations (Table 2) suggests that this mechanism of complement-mediated alteration is not identical with that mediated by heterologous antibody. The inhibition of ASA of the release reaction caused by a single concentration of a human autoantibody$^6$ and an HLA antibody thought to be complement-independent$^{28}$ further suggests that a prostaglandin-forming pathway to platelet release may be generally applicable to human antibodies.

At low antibody concentrations the kinetics of the release reaction are slow, without substantial release before 20 min.$^{11}$ This time lag could represent platelet reuptake of $^{14}$C-serotonin released during the early stages of the release reaction. Our results suggest that this is not the case, as the addition of imipramine, an agent that inhibits platelet serotonin uptake,$^{29}$ did not alter the kinetics of platelet serotonin release (Fig. 1). Therefore, it appears more likely that the time required to initiate serotonin release is caused largely by the slow activation of complement in the presence of limiting amounts of antibody. A similar time course to platelet release has been observed with activation of the alternative complement pathway by immune complexes.$^{12}$ We also observed that a concentration of antibody insufficient to cause platelet aggregation and serotonin release, but yet sufficient to activate the classic complement pathway and place C3 on the platelet surface,$^{11}$ did not inhibit $^{14}$C-serotonin uptake by platelets (Table 1). Higher concentrations of antibody could not be evaluated because they stimulate serotonin release. Human antibody has been observed in some studies to alter the uptake of $^{14}$C-serotonin.$^{18,19}$ However, it appears that antibody concentrations that in themselves cause platelet aggregation and release were employed in these studies.

Several investigators have explored the mechanism by which human antibodies mediate alteration of allogeneic platelets in systems believed to be complement-independent. A requirement for ADP,$^{28}$ as well as endogenous prostaglandin
formation has been observed. Exposure of human platelets to isoantibody has also been noted to stimulate glycolysis and anaerobic metabolism. However, the relationships of these events to functional changes have not been previously studied. Shulman et al. observed that platelet aggregation caused by a single concentration of isoantibody was inhibited by PGE. Our work extends these studies by examining the effect of antibody concentration in a complement-dependent system.

It is clear from our studies as well as from the work of others that antibody can alter human platelets in several ways. The amount of antibody or complement on the platelet surface is clearly one important factor in determining the nature of the alteration. Antibody specificity and avidity and the location of antibody or complement components on the cell membrane may also be important. A diverse number of nonimmunologic molecules also alter human platelets. The site of antibody or complement deposition on the platelet may influence platelet interaction with such nonimmune agonists. Studies using monospecific antibodies of a defined class in the presence and absence of complement should help to delineate these interactions.

Complement activation can cause the release of mediators from other cells, such as leukocytes and mast cells. These complement-dependent release mechanisms have also been observed to involve prostaglandins and are modified by agents that increase the level of intracellular cyclic AMP. Similarly, ADP appears to be required for complement-mediated platelet release initiated by immune complexes or zymosan. Our analysis of a platelet release reaction dependent on the classic complement pathway and initiated by anti-platelet antibody extends this knowledge with human platelets. It is quite possible that other complement-mediated release reactions proceed by similar mechanism(s). Thus, our studies may be relevant to an understanding of the mechanism of complement-mediated damage in other cell systems. Pharmacologic interference with certain of these complement--cellular alterations may be possible. Such intervention might serve to decrease in situ immune damage and/or interfere with the clearance of such altered cells.

ACKNOWLEDGMENT

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REFERENCES

7. Hirschman RJ, Shulman NR: The use of platelet serotonin release as a sensitive method for detecting antiplatelet antibodies and a plasma
Effect of anti-P1A1 antibody on human platelets. II. Mechanism of the complement-dependent release reaction

AD Schreiber, DB Cines, C Zmijewski and RW Colman