LYSIS OF ANTIBODY-COATED CELLS BY PLATELETS*

BY WILLIAM D. SOPER,† SCOTT P. BARTLETT, AND HENRY J. WINN

From the Transplantation Unit, General Surgical Services, and the Department of Surgery, Harvard Medical School at the Massachusetts General Hospital, Boston, Massachusetts 02114

In the course of our studies on the role of complement in antiserum-mediated damage to allografts and xenografts of skin, we have observed that, whereas serum from C5 deficient mice fails, as expected, to lyse sensitized sheep erythrocytes (SRBC),1 whole blood from these animals causes measurable lysis even when diluted 1:100 in saline. This lytic activity occurs only if the cells have reacted with antibody, and only if platelets and early acting components of complement (C) are present. It appears to involve mechanisms not previously described in the literature and it may be useful in studying the functions of platelets as well as in delineating the cytotoxic potential of antibody. We describe here the conditions under which the phenomenon occurs and we present evidence for the essential involvement of antibody, complement, and platelets.

Materials and Methods

Animals. Mice were purchased from The Jackson Laboratory, Bar Harbor, ME. A/J, AKR/J, B10.D2 (old), DBA/2J, and AKD2F1 mice are C5 deficient, and lack, therefore, hemolytic C; C3H/HeJ, B10.D2 (new), and CAF1 have readily detectable levels of C5 and hemolytic C. Rabbits, guinea pigs, and chickens, which served as donors of erythrocytes in one experiment, were obtained from local dealers.

Antisera. Mouse anti-SRBC sera were produced in A/J (C5−) and CAF1 mice. The mice received 20 intraperitoneal injections of a 2% suspension of washed cells at weekly intervals. They were bled each week just before injection, beginning 1 wk after the fifth injection. Serum obtained from each group of mice was made into a separate pool. Similar procedures were used for the preparation, in A/J (C5−) mice, of antisera to erythrocytes of guinea pigs (GRBC), rabbits (RRBC), and chickens (CRBC). A/J (C5−) mice were used in the preparation of antisera to avoid the inadvertent addition of C5 to experimental systems during the process of sensitization of the SRBC. CAF1 (BALB/c × A/J) anti-SRBC serum was initially prepared for titrating hemolytic C in C5+ mice and was used in the early phases of the study that led to the observation that C5− whole blood can lyse sensitized cells. Because BALB/c mice have hemolytic C, the CAF1 serum probably contains small amounts of C5. However, this serum clearly did not reconstitute the hemolytic activity of C5− mice, as shown in the results of the two experiments in which it was used (Fig. 1). In all of the other experiments, erythrocytes were sensitized with A/J (C5−) antiserum. Rabbit anti-mouse thymus serum (RAMTS) was prepared by injecting rabbits first with thymocytes in Freund’s adjuvant and 3 wk later with cells alone intravenously. 7 d later the animals were bled to form a single large pool of serum. Monoclonal anti-SRBC antibodies were prepared by injecting BALB/c mice with hybridoma cells and

---

* Supported by grants CA17800 and CA20044 from the National Cancer Institute.
† Present address: Department of Surgery, Northwestern University Medical School, Chicago, IL 60611.
1 Abbreviations used in this paper: ACD, acid citrate dextrose; C, complement; Cof, cobra venom factor; CRBC, chicken erythrocytes; GRBC, guinea pig erythrocytes; ppp, platelet-poor plasma; prp, platelet-rich plasma; RAMTS, rabbit anti-mouse thymocyte serum; RRBC, rabbit erythrocytes; SRBC, sheep erythrocytes; VBS.G, veronal-buffered saline with 0.1% gelatin.

1210 J. Exp. Med. ©The Rockefeller University Press • 0022-1007/82/10/1210/12 $1.00
Volume 156 October 1982 1210-1221
harvesting the resulting ascites. Lines N-S.4.1 (IgM) and N-S.7 (IgG3) were obtained from the Salk Institute for Biological Studies; Cell Distribution Center, San Diego, CA.

**SRBC.** Blood was obtained from a single animal at monthly intervals and stored at 4°C in Alsevers solution. For radiolabeling, 100 μl of thrice washed, packed cells were mixed with 200–300 μCi of 51Cr (NaCrO4, 1 mCi/ml; New England Nuclear, Boston, MA) and incubated in a shaking water bath for 1 h. They were washed (three times) with saline and resuspended in 9.9 ml of veronal buffered saline containing 0.1% gelatin (VBS.G). For sensitization, 1 ml of this 1% suspension of cells was mixed with an equal volume of antiserum or solution of monoclonal antibody, and the mixture was brought to a volume of 10 ml, making a final concentration of SRBC 0.1%. The cells and antibody were incubated at room temperature for 15 min and then stored at 4°C.

**Blood Fractions.** Whole blood was obtained from the retro-orbital sinus of mice in heparinized microhematocrit tubes or siliconized pasteur pipettes that had been freshly rinsed with acid citrate dextrose (ACD) (Becton, Dickinson & Co., Rutherford, NJ). The blood was transferred directly to microtiter trays for dilution and assay, or, when blood from several animals was to be pooled, to a 13 × 100-mm siliconized tube containing anticoagulant (heparin or ACD). Platelet-rich plasma (prp) was obtained by centrifuging whole blood at 200 g for 10 min at room temperature and carefully aspirating the supernatant material. The fractions so obtained contained 4 × 10^5–10^6 platelets/mm^3 and were virtually free of leukocytes. Platelet-poor plasma (ppp) was obtained by centrifugation of the blood remaining after removal of prp at 1,000 g for 20 min. These fractions contained 20,000–40,000 platelets/mm^3.

**Test System.** Doubling dilutions of whole blood, prp, or ppp were made up in 25-μl vol using VBS.G as a diluent in round-bottomed microtiter plates (Dynatech Laboratories, Dynatech Corp., Alexandria, VA). To each dilution was added 25 μl of 0.1% SRBC; the plates were placed on a microshaker (Micro-Shaker II, Dynatech Laboratories Inc.) for 15 s, speed setting 6, and then put at 37°C. At 15-min intervals the plates were again placed on the microshaker for 15 s. At the end of the incubation period, 150 μl of VBS.G was added to each well and the plate was agitated and then centrifuged at 2,000 rpm (650 g) for 5 min in a refrigerated centrifuge; 100 μl of supernatant fluid was removed and assayed for radioactivity in a Gamma Counter (Packard Instrument Co., Inc., Downers Grove, IL). Maximum available cpm was determined by counting 25 μl of SRBC and dividing the resulting cpm by 2.

**Lymphoid Cells.** Suspensions of cells were prepared from spleens, lymph nodes, and thymi by perfusion with Tris-buffered NH4Cl or by gentle teasing with 22-gauge hypodermic needles. Large clumps of cells and tissue debris were allowed to settle, and the suspended cells were removed, centrifuged at 200 g for 5 min, and then resuspended in L 15 containing 0.25% gelatin. When lymphoid cells were used as effector cells, they were treated exactly as whole blood or fractions thereof. They were made up in doubling dilutions, mixed with 51Cr-SRBC, and incubated, with frequent shaking for 2–3 h, at 37°C. When used as targets, they were sensitized with RAMTS or alloantiserum, added to doubling dilutions of prp, and incubated at 37°C with frequent shaking. The plates containing the mixtures were then centrifuged, the supernatant fluid was removed, and the cells were resuspended in 0.2% trypan blue in saline. A sample from each well was examined microscopically to determine the percentage of stained cells.

**Treatment of Mice with Cobra Venom Factor (Cof).** A purified preparation of the anti-complementary factor present in cobra venom was obtained from Cordis Laboratories Inc., Miami, FL. The lyophilized material was reconstituted to contain 20 U/ml, just before use. Mice received two injections (intraperitoneally) of 0.25 ml (5 U) given 18 h apart. After the second injection, blood was drawn and assayed for lytic activity. This schedule of treatment invariably reduced hemolytic complement in the serum of C5^+ mice to <5% of pretreatment levels.

**Results**

The phenomenon that we report on here is illustrated in Fig. 1, in which the hemolytic titration curves for plasma and whole blood of C5^+ (C3H/HeJ) and C5^- (B10.D2 old) mice are shown. Attention was first drawn to the phenomenon through the observation (Fig. 1A) that whole blood from C5^+ mice was invariably more active in lysing sensitized SRBC than was anticipated on the basis of the activity of its
LYSIS OF ANTIBODY-COATED CELLS BY PLATELETS

isolated plasma. The early plateau in the titration curve shown here for plasma is probably due to the anti-C activity of the heparin used for anticoagulation. The effect, noted also when citrate was used, was not seen with serum or with plasma that contained high levels of hemolytic C. Nevertheless, in all of >100 titrations, whole blood was decisively more active than plasma. The ratio of the activity of whole blood to that of plasma varied widely among individual mice and was markedly dependent on the nature of the antiserum or monoclonal antibody used for sensitization of the SRBC, suggesting that a mechanism other than the classical pathway of C-mediated lysis was involved. This suggestion was strongly supported by the finding that C5− whole blood had high levels of lytic activity for sensitized SRBC, whereas C5− plasma had little or no activity (Fig. 1B). Low levels of hemolysis observed with some samples of C5− plasma are, as shown below, probably because of the presence of small numbers of platelets. These titrations were carried out at 37°C, and on the basis of preliminary studies, the mixtures were incubated for 3 h with brief (15-s) periods of shaking at 15-min intervals. The time course of the reactions is described in more detail below.

The Role of Antibody in the Lysis of SRBC by C5− Whole Blood. Labeled SRBC suspended in saline or in various concentrations of A/J anti-SRBC was mixed with C5− (AKD2F1) whole blood serially diluted with VBS.G, and the mixtures were incubated at 37°C for 2 h. They were then centrifuged at 4°C, and aliquots of the supernatant fluids were analyzed for 51Cr. The results of these tests are illustrated in Fig. 2. Clearly, the degree of lysis caused by C5− whole blood is directly related to the amounts of anti-SRBC serum used for sensitization, and in the absence of such serum, there is no detectable lysis.

Cells Prepared from Spleens, Lymph Nodes, and Thymi as Effectors of Lysis of Sensitized SRBC. Because the phenomenon described here resembles other forms of antibody-dependent cytotoxicity that are mediated by lymphoreticular cells, we have examined

![Graph](image-url)
WILLIAM D. SOPER, SCOTT P. BARTLETT, AND HENRY J. WINN

Fig. 2. (Left) The role of antibody in the lysis of $^{51}$Cr-SRBC by C5$^-$ whole blood. Cells were sensitized with varying concentrations of A/J (C5$^-$) anti-SRBC serum and incubated with doubling dilutions of B10.D2 whole blood for 2 h at 37°C. Maximum available cpm = 4,750; spontaneous release = 130 cpm. Values in parenthesis represent dilutions of whole blood.

Fig. 3. (Right) Comparison of the effects of whole blood (○) and lymphoid (spleen) cells (□) on sensitized $^{51}$Cr-SRBC. Open symbols, cells diluted in VBS.G; closed symbols, cells diluted in 20% fresh C5$^-$ plasma. Starting concentration of spleen cells = $1.7 \times 10^7$/ml. Maximum cpm = 3,620; spontaneous release = 140 cpm.

It should be noted that the starting concentrations of lymphoid cells used in these tests were from 4 to 45 times as great as the concentrations of leukocytes in mouse blood. Thus, the very low hemolytic activity of these preparations suggests that the lymphoid cells of whole blood are not the principle effectors of the lytic reactions described here.

The Role of Platelets in the Lysis of SRBC by C5$^-$ Whole Blood. A major role for leukocytes in the phenomenon considered here is further contraindicated on the basis of the ratio of the numbers of leukocytes in mouse blood to the numbers of target cells...
used in the test system. Mouse blood contains \( \sim 10^7 \) leukocytes/ml, whereas the concentration of target cells is \( 2 \times 10^7/ml \). It is commonly found that whole blood diluted 1:10 causes 100% lysis, and in some cases 100% lysis is seen when blood is diluted 1:25. In those circumstances, the ratio of target cells to leukocytes is 20:1 or 50:1. Obviously, the ratios are higher for individual classes of leukocytes, e.g., 100-250:1 for neutrophils, and 1,200-3,000:1 for monocytes.

These considerations have led us to examine the role of platelets in the lysis of SRBC by C5- blood. prp, ppp, and whole blood were serially diluted and mixed with sensitized, radiolabeled SRBC. Release of \( ^{51} \)Cr was determined after the mixtures had been incubated at 37°C for 3 h, and the results are summarized in Fig. 4. Platelets are, clearly, the major effectors of the reaction under consideration, and indeed they could account for all of the hemolysis observed with whole blood. The numbers of leukocytes contained in prp are so small (see Materials and Methods) that such cells could not have played a measurable role in these tests. Furthermore, we have examined, microscopically, samples of mixtures of prp and sensitized SRBC, and invariably have observed adherence of platelets to these erythrocytes before the time that lysis occurs. The adherence, which is illustrated in Fig. 5, appears to be firm as judged by its resistance to mechanical shaking, but was not observed when unsensitized SRBC were used.

The Role of Nonantibody Plasma Constituents in the Lysis of Sensitized SRBC by Platelets. Anti-SRBC serum and platelets play essential roles in the lytic phenomena considered here. However, fresh plasma was also present in the test system, and it remains to be determined whether plasma components also play roles in lysis. The involvement of C calls for special consideration, as it is known that mouse platelets bear receptors for C3b and can by means of these receptors attach to sensitized SRBC that have reacted with C (1, 2).

The importance of C3 was examined by injecting C5- mice with purified Cof according to the schedule described above and summarized in Fig. 6. Whole blood and prp from treated or control mice were tested for lytic activity with sensitized SRBC, but data are presented for whole blood only, as they are essentially indistinguishable from those obtained with prp. Treatment with Cof greatly reduced, and in
Fig. 5. Adherence of platelets to sensitized SRBC. The SRBC were mixed with A/J anti-SRBC (A) or normal A/J serum (B) and then added to C5⁺ prp. These mixtures were incubated at 37°C for 30 min with intermittent shaking, and then photographed using phase microscopy at × 266. Numerous platelets were attached to each of the sensitized erythrocytes (A); no association between platelets and unsensitized SRBC was observed (B).

Fig. 6. (Left) Depression of lytic activity of C5⁺ whole blood by treatment of donors with purified Cof. B10.D2 old mice received 5 U of Cof at 18 h and again at 2 h before blood was drawn. Whole blood from: normal, control, mice (■); Cof-treated mice (○); Cof-treated mice diluted in 20% fresh C5⁺ plasma (●). Maximum cpm = 3,025; spontaneous release = 135 cpm. Fig. 7. (Right) The effect of mechanical shaking at 37°C on lysis of sensitized ⁵¹Cr-SRBC by C5⁺ whole blood. All assays were incubated for 180 min. Shaking at 15-min intervals was discontinued after: 30 min (■); 60 min (●); 120 min (●); or 180 min (○). Maximum cpm = 3,420; spontaneous release = 65, 130, 145, and 190 cpm.

In many cases completely abrogated, the lytic activity of whole blood and platelet rich plasma. Lytic activity was, however, restored by the addition of fresh C5⁺ plasma to the test system. Thus, it appears that constituents of fresh plasma, probably early
acting components of C (C1,2, 4,3), are essential for the lysis of SRBC by platelets. Failure of fresh C5- plasma to restore full lytic activity to high concentrations of whole blood or prp is probably because of residual effects of Cof.

The Effects of Temperature and Mixing on the Lysis of SRBC by Platelets. In the experiments described thus far, the test mixtures were incubated at 37°C with mixing for brief periods at 15-min intervals. These conditions were selected on the basis of preliminary experiments carried out with whole blood, and their appropriateness is here examined in more detail. Whole blood or prp was mixed with sensitized 51Cr-SRBC and incubated with frequent mixing at 4°C, room temperature, or 37°C for various periods of time up to 3 h. There was no detectable release of 51Cr at 4°C or room temperature, whereas up to 100% release was observed at 37°C.

With respect to the importance of mixing, we have found repeatedly that there is no detectable release of 51Cr in mixtures that are incubated without shaking. Furthermore, as shown in the following experiments, shaking must be practiced throughout the period of incubation to achieve optimal levels of 51Cr release. Multiple assays were prepared and incubated at 37°C for 3 h. Mixing was carried out at the start of the incubation period and at 15-min intervals thereafter, for periods of time varying, for individual assays, from 30 to 180 min. It is apparent from the results of these tests (Fig. 7) that under the conditions of incubation, release of 51Cr approaches completion only after ~3 h. Moreover, there is a direct relationship between the number of times that shaking was practiced and the amount of 51Cr released. However, these data do not exclude the possibility that only the shaking at the end of the incubation period is essential for optimal 51Cr release. This point was examined in an experiment in which multiple assays were incubated at 37°C for 3 h with shaking of the mixtures carried out according to one of the following schedules: (a) every 15 min throughout the course of the reaction; (b) only once, at 165 min, i.e., at 15 min before the end of the incubation period; (c) once, at 15 min after the beginning of the incubation period; (d) not at all. The results of these tests, shown in Fig. 8, indicate that shaking must be practiced throughout the course of the reaction to achieve optimal release of 51Cr.

Fig. 8. The effect of mechanical shaking at 37°C on lysis of sensitized 51Cr-SRBC by C5- (AKD2F1) whole blood. All assays were incubated for 180 min. Shaking was carried out as follows: at 15-min intervals throughout the period of incubation (○); once, 15 min after the start of incubation (□); once, 15 min before the end of the incubation period (■); not at all (○). Maximum cpm = 2,560; spontaneous release = 96, 75, 74, and 32 cpm.
Platelet-mediated Lysis of Cells other than SRBC

Erythrocytes from Other Species. RRBC, GRBC, and CRBC were labeled with $^{51}$Cr and sensitized with mouse antisera of appropriate specificity as described above for SRBC. Sensitized erythrocytes were mixed with doubling dilution of C5− whole blood or prp, and incubated at 37°C for 3 h with intermittent shaking, after which the release of $^{51}$Cr was determined. There was no significant release of $^{51}$Cr from cells incubated in platelet-free, C5− plasma, but as shown in Table I, all three types of sensitized erythrocytes were lysed in dose-dependent form by whole blood or prp. Sensitized cells that had been incubated in prp were also examined for evidence of immune adherence. Without exception, lysis was preceded by firm attachment of platelets to cell surfaces. Such attachment was never observed in the absence of antibody or subsequent lysis.

Nucleated Cells from Mice, Rats, and Guinea Pigs. Suspensions of cells prepared from spleens, lymph nodes, or thymi were sensitized with either alloantisera (anti-major histocompatibility complex or anti-Thy-1.2) or xenantisera (RAMTS) and then incubated at 37°C with C5− prp. At intervals up to 4 h, samples of the suspensions were removed and examined for cell viability after addition of trypan blue. There was no detectable killing of the target cells by platelets, though all three types of sensitized cells were killed using fresh rabbit serum as a source of C and there was no evident association of platelets and target cells. Similar tests were carried out with tumor cells obtained from mice, rats, and guinea pigs, and again we failed to observe platelet-mediated killing or evidence of immune adherence. It is not clear whether failure to observe lysis of the nucleated cells used here is attributable to their resistance to the platelet-associated lysin or to their inability to bind platelets under the conditions in which the tests were carried out. However, we report elsewhere (S. P. Bartlett, K. S. Stenger, and H. J. Winn, manuscript in preparation) a platelet-derived lysin that acts on nonsensitized nucleated cells as well as on erythrocytes.

Platelet-mediated Lysis of SRBC Sensitized With IgG1 and IgM Monoclonal Antibodies. We shall report separately on a systematic study of the biological properties of various classes of mouse immunoglobulins (S. P. Bartlett, K. S. Stenger, and H. J. Winn, manuscript in preparation). However, it is pertinent to consider here the abilities of some so-called non-lytic immunoglobulins to prepare SRBC for platelet-mediated

<table>
<thead>
<tr>
<th>Dilution of whole blood</th>
<th>RRBC + aRRBC</th>
<th>GRBC + aGRBC</th>
<th>CRBC + aCRBC</th>
<th>SRBC + McIgM©</th>
<th>SRBC + McIgG1</th>
</tr>
</thead>
<tbody>
<tr>
<td>1:4</td>
<td>4170</td>
<td>2460</td>
<td>962</td>
<td>1930</td>
<td>2620</td>
</tr>
<tr>
<td>1:8</td>
<td>2730</td>
<td>1380</td>
<td>313</td>
<td>930</td>
<td>1725</td>
</tr>
<tr>
<td>1:16</td>
<td>1570</td>
<td>590</td>
<td>155</td>
<td>625</td>
<td>530</td>
</tr>
<tr>
<td>1:32</td>
<td>680</td>
<td>490</td>
<td>90</td>
<td>480</td>
<td>70</td>
</tr>
<tr>
<td>1:64</td>
<td>340</td>
<td>200</td>
<td>24</td>
<td>110</td>
<td>45</td>
</tr>
<tr>
<td>1:128</td>
<td>0</td>
<td>0</td>
<td>48</td>
<td>65</td>
<td>105</td>
</tr>
<tr>
<td>Spontaneous cpm</td>
<td>300</td>
<td>260</td>
<td>5</td>
<td>117</td>
<td>205</td>
</tr>
<tr>
<td>Maximum cpm</td>
<td>4800</td>
<td>4460</td>
<td>2140</td>
<td>3150</td>
<td>3740</td>
</tr>
</tbody>
</table>

* Individual tests were carried out on different dates with different preparations of whole blood.
© Mc, Monoclonal anti-SRBC.
LYSIS OF ANTIBODY-COATED CELLS BY PLATELETS

Cells sensitized with either monoclonal IgG1 or IgM antibodies were incubated at 37°C for 3 h with C5+ plasma, C5− plasma, or C5− whole blood and examined for release of 51Cr as described above. In confirmation of an earlier study by Klaus et al. (3), we found no significant release during incubation with either C5+ or C5− plasma, but as shown in Table I, there was readily detectable lysis of cells incubated with C5− whole blood. Similar results were obtained in related studies with IgG2a, IgG2b, and IgG3 monoclonal anti-SRBC reagents. The initiation of platelet-mediated damage by antibodies that do not cause lysis through activation of the late-acting components of C is not inconsistent with the observation that early acting components of C may be required for platelet activity (3).

The Fate of Nonsensitized Bystander Cells During the Lysis of Sensitized Cells by Platelets. Mouse platelets are known to adhere to cells or antigen-coated particles that have reacted with antibody and early acting components of C, a phenomenon that has been designated immune adherence (4). As stated above, we have observed such adherence in our test system and it seems likely that it is an essential forerunner of lysis. The question arises as to whether platelets lyse only the cells to which they have become attached, or whether they release a lytic agent that may act on other, nonsensitized cells. In an effort to resolve this issue, whole blood or prp was mixed with sensitized but unlabeled GRBC and unsensitized 51Cr-labeled SRBC and incubated for 3 h with intermittent shaking. Release of 51Cr from the bystander cells was then determined. There was no significant release from unsensitized cells that had been intimately mixed with sensitized unlabeled cells and platelets (Table II), although in control mixtures, both GRBC and SRBC labeled and sensitized with antibody released readily detectable amounts of 51Cr. Lysis is evidently confined to those cells that are coated with antibody and C, and have, thereby, interacted with platelets.

Discussion

Platelets have long been known to interact with immune aggregates in vitro, and their tendency to adhere to sensitized cells, first described by Nelson (4), is now widely recognized and occasionally used for the detection of antibodies reactive with cell-

**Table II**

<table>
<thead>
<tr>
<th>51Cr-labeled cells:</th>
<th>SRBC</th>
<th>GRBC</th>
<th>SRBC</th>
<th>GRBC</th>
<th>SRBC</th>
<th>GRBC</th>
</tr>
</thead>
<tbody>
<tr>
<td>Unlabeled cells: aGRBC</td>
<td>aGRBC</td>
<td>aGRBC</td>
<td>aGRBC</td>
<td>aGRBC</td>
<td>aGRBC</td>
<td>aGRBC</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Dilution of effectors</th>
<th>WB*</th>
<th>prp</th>
<th>WB</th>
<th>prp</th>
<th>WB</th>
<th>prp</th>
</tr>
</thead>
<tbody>
<tr>
<td>1:2</td>
<td>25</td>
<td>60</td>
<td>400</td>
<td>790</td>
<td>730</td>
<td>675</td>
</tr>
<tr>
<td>1:4</td>
<td>40</td>
<td>50</td>
<td>510</td>
<td>290</td>
<td>505</td>
<td>250</td>
</tr>
<tr>
<td>1:8</td>
<td>35</td>
<td>40</td>
<td>410</td>
<td>220</td>
<td>275</td>
<td>82</td>
</tr>
<tr>
<td>1:16</td>
<td>30</td>
<td>30</td>
<td>140</td>
<td>40</td>
<td>70</td>
<td>35</td>
</tr>
<tr>
<td>1:32</td>
<td>20</td>
<td>50</td>
<td>50</td>
<td>0</td>
<td>30</td>
<td>10</td>
</tr>
</tbody>
</table>

Spontaneous cpm: 22 96 64 64
Maximum cpm: 2,700 2,650 2,370 2,370

* Results are expressed as net cpm, i.e., observed cpm - spontaneous release cpm.
surface antigens. These reactions, which depend on membrane receptors for C3b or for the Fc portion of antibody molecules, have been shown to lead to the release of platelet granules and, in turn, to the liberation of substances with diverse biological properties—vasoactive, chemotactic, thrombogenic, or microbicidal (5–7). There are, however, no reports of the direct cytotoxicity of platelets for cells to which they have become attached. It may seem surprising that such activity has not been previously observed, but it has probably been masked by the lytic properties of plasma complement or obscured by the unusual test conditions required for its optimal expression.

Our observations have depended on the use of C5− mice, whose blood contains early acting components of C but not a complete lytic system, and on the use of whole blood rather than serum, a feature that inadvertently introduced platelets into the test system. It is our view that platelets adhere to cells that have bound antibody and early acting components of C, and are induced thereby to release, among other biologically active substances, a cytolysin. Our data do not provide direct evidence for the release of a cytotoxic substance by platelets, but we favor this view on the basis of observations that the clotting of whole blood and prp leads to the appearance of cytolysic activity in the resulting serum, whereas no such activity appears in serum derived from platelet-free plasma (S. P. Bartlett, K. S. Stenger, and H. J. Winn, manuscript in preparation). Similar findings have been reported for a platelet-derived bactericidal agent (8), and it is likely that this substance is also released by platelets that adhere to antibody-coated cells including bacterial cells. Indeed, the cytolysic agent that we have described here may be identical with the bactericidin, and a major function of the antigen-antibody-induced release reaction may be to provide protection against microbial infection or other parasitic infestation.

We have been unable to show that sensitized nucleated cells, other than CRBC, are lysed by platelets. However, this may be traced to the failure of these cells to bind platelets in the circumstances in which our experiments have been conducted. As mentioned above, we have evidence that platelets can be induced to release a substance that lyses nucleated cells as well as erythrocytes. Furthermore, it has been reported (9) that the addition of platelets to mixtures of tumor cells and anti-tumor cell sera enhances the neutralizing effects of the serum that occurs when the mixtures are injected into hosts that are susceptible to the tumor.

Thus, the lytic reaction that we describe may play roles in the destruction of viral infected cells, tumor cells, or grafts of foreign cells, and its significance will be understood only after a great deal more study. It is already evident, however, that it is a remarkably effective reaction in the sense that virtually complete lysis is observed even when approximately equal numbers of platelets and target cells are present; in view of the relatively large numbers of platelets in circulating blood, it looms as a major effector mechanism of the immune response. From this point of view, it is particularly important to note that most, and perhaps all, classes of mouse immunoglobulins can initiate the platelet-mediated lytic reaction.

With respect to the detailed mechanism of the lytic reaction and the optimal conditions under which it occurs in vitro, there are several aspects of the phenomenon that call for further study. That nonsensitized bystander cells are unaffected by the reaction of sensitized cells with platelets suggests the need for close contact between effector and target cells, but that does not exclude the involvement of a soluble mediator released by platelets. Indeed, as mentioned above, we have discovered a
potential mediator and the isolation and characterization of this substance should be helpful in elucidating the mechanism of lysis.

Among the experimental conditions found essential to the expression of the lytic reaction, the requirement for frequent shaking is puzzling, especially in so far as it contrasts sharply with observations made on other forms of cell-mediated cytotoxicity. It is difficult to evaluate the significance of this finding because of the complexity of the overall lytic phenomenon, which involves the interactions of antibody, C, and platelets with target cells. The optimal concentrations of these constituents and the conditions under which they contribute maximally to the lytic reaction remain to be determined. Nevertheless, our data establish the general circumstances in which platelet-mediated lysis of sensitized cells can be observed routinely and in a highly reproducible manner, and they provide the means for probing further this intriguing and potentially important phenomenon.

Summary

Antibody-coated erythrocytes are lysed by murine C5− whole blood but not by plasma separated from such blood. The lytic activity has been shown to derive from platelets that attach to sensitized cells probably through membrane receptors for C3b. Whole blood or platelet-rich plasma (prp) obtained from mice that have been treated with purified cobra venom factor has little or no activity unless it is fortified with fresh C5− plasma. Lysis is observed only if the reactants are incubated at 37°C and mechanical shaking is practiced, at least intermittently, throughout the period of incubation. Adherence of platelets and subsequent lysis are mediated by antibodies of a variety of immunoglobulin classes, including those that fail to mediate complement-dependent lysis. Platelet-mediated lysis is limited to cells to which the platelets adhere; 51Cr labeled, unsensitized cells that are mixed with prp and sensitized, unlabeled cells do not release 51Cr. Normal murine lymphoid cells and ascites tumor cells of mice, rats, and guinea pigs were apparently unaffected by sensitization and incubation with prp. However, because adherence of platelets to these sensitized cells was not observed, it is not clear whether the cells are resistant to the lytic action of platelets or whether the conditions of incubation were unfavorable for the attachment of platelets to the surfaces of nucleated cells. The significance of the lytic reaction described here is not known but may lie in antibody mediated release of microbicidal substances from platelets.

We gratefully acknowledge the excellent technical assistance of Karla Stenger.

Received for publication 3 May 1982 and in revised form 14 July 1982.

References