Chromium-51 Release from Human Blood Platelets by Anti A Antibody

PAUL ZAKOWICH* AND EDWARD E. MORSE, M.D.

*University of Connecticut at Storrs, Storrs, CT 06268
and
Department of Laboratory Medicine, University of Connecticut School of Medicine, Farmington, CT 06032

ABSTRACT

Platelets were obtained by differential centrifugation from Group A donors and were incubated with Na\textsubscript{251}CrO\textsubscript{4}. After washing to remove excess chromium, the platelets were exposed for one hour to Anti A antibody which showed agglutinating activity only. Chromium release was no greater than that of controls. Hemolytic Anti A in the presence of rabbit complement released 15 percent more chromium than controls. Anti PIA\textsubscript{1}, a platelet specific antibody, when incubated with platelets in the presence of rabbit complement released 45 percent more chromium than controls.

It appears that Anti A antibody is not as destructive to platelets as a platelet specific antibody is, even when the Anti A antibody shows hemolytic activity with red cells. These observations are in agreement with previous in vivo studies of ABO incompatible platelets.

Introduction

Platelet transfusions are, in many areas of the country, performed without regard to the ABO compatibility of the donor and recipient. Most platelet transfusions are given to patients with leukemia or other neoplastic conditions under treatment with cytotoxic agents which are also immunosuppressive. The success of random donor platelet transfusion may be due to a relatively poor immunologic response in such patients or to some other factor such as a lesser concentration of ABO antigen sites on platelets.

Recently, Lohrmann et al\textsuperscript{10} have indicated that the HL-A antigen system is important to the success of repeated platelet transfusion and that the ABO system has only a small role to play. Nevertheless, two careful studies by Aster and by Pfisterer\textsuperscript{2,13} cannot be ignored. Both of these investigators showed that ABO incompatible platelets, particularly Group A platelets, have a reduced recovery in the circulation after transfusion to Group O recipients who are not immunosuppressed.

In an effort to elucidate the effects of anti A, on Group A platelets, the release of
Chromium-51 from Group A platelets exposed to antibodies has been measured in our laboratory. This technique is well established for detecting immunologic damage to lymphocytes and tissue culture cells, but few investigators have applied the technique to platelets.

**Methods**

Platelet concentrates were prepared by differential centrifugation of citrate-phosphate-dextrose (CPD) blood from normal donors. The platelets were freed of contaminating red blood cells by slow centrifugation (50 g) and washed twice in saline. The platelets were incubated for one hour at room temperature with 100 μCi $^{51}$Cr or for 30 minutes at 37° with 50 μCi $^{51}$Cr as Na$_2$ CrO$_4$ and washed twice in 10 volumes of saline.

The platelet preparations were frequently checked macroscopically and microscopically for contamination and were found to be virtually free of red cells and white cells. Platelet concentrations were adjusted to levels between 100,000 and one million per cu mm and were then incubated with the antibody to one part in 10 of platelet suspension.

Anti A$_1$ was obtained from six normal donors. Four showed agglutinating activity only in titers up to 1/64 and two showed hemolytic activity for red cells at 1/64 and 1/128, respectively.

**Results**

Preliminary experiments showed no difference between the activity of the anti-

<table>
<thead>
<tr>
<th>Incubation</th>
<th>Release</th>
<th>S.D.</th>
<th>P</th>
</tr>
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<tbody>
<tr>
<td>1 hour</td>
<td>6.5% ±</td>
<td>6%</td>
<td>&lt;0.02</td>
</tr>
<tr>
<td>24 hours</td>
<td>13% ±</td>
<td>3.5%</td>
<td>&lt;0.01</td>
</tr>
</tbody>
</table>

Platelet concentration 1 X 10$^6$ per cu mm

bodies tested whether collected as serum or as CPD plasma. Thus, for convenience of quantity as well as stability, the CPD plasma antibodies were used for this work.

Agglutinating anti A antibodies did not consistently produce release of $^{51}$Cr from platelets, but the hemolytic anti A$_1$ antibodies released small amounts of $^{51}$Cr regularly (table I). $^{51}$Cr tagged platelets were incubated in 2.7 ml buffered saline with the addition of 0.3 ml hemolytic anti A$_1$ plasma. After incubation, control and antibody treated platelets were washed twice and the chromium release compared between them. These results indicate the mean release of $^{51}$Cr in 12 experiments and are statistically different from control release by paired t test with a P value of less than 0.02.

To determine the effects of anti A$_1$ in comparison to a known anti-platelet antibody (anti PIA$_1$),* the antibodies in the same dilutions were incubated with platelets at 100,000 per cu mm, both with and without added rabbit complement† (table II).

$^{51}$Cr tagged platelets were washed twice, incubated at 37° in 1/10 volume of antibody for one hour and washed once before the proportion of $^{51}$Cr remaining was determined. The values shown indicate net release above controls.

Anti PIA$_1$ released twice as much chromium as anti A$_1$ without added complement, and three times as much in the

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* Kindly supplied by Dr. Richard Aster, Director of Milwaukee Blood Center, Milwaukee, WI.
† Pel-Freez, Biologicals, Rogers, AR.
RELEASE OF CHROMIUM-51 BY ANTI A ANTIBODY

presence of added complement. Rabbit complement alone released 20 to 40 percent of the \(_{51}^{Cr}\) in control platelets which increased the variability of the results.

The maximum proportion of chromium that could be released from platelets was determined by suspending tagged platelets in distilled water. After centrifugation of the stroma, about 80 percent of the chromium was detected in the supernatant. This figure agrees with that recently reported by Aster.²

To determine the degree of immunologic lysis of platelets, as measured by release of chromium in experiments in which a substantial amount of chromium is released in control platelets, Aster² developed a formula to correct spontaneous release in controls. To use this formula, it is necessary to assume that immunologic release and spontaneous release of chromium are independent processes. With this provision, a measured release of chromium of 45 percent over and above controls measures immunologic lysis of 80 percent (table III).

The percentages (probabilities) shown in table III are calculated thus: \(y\) is used to express the probability (as a percentage) of immunologic lysis and \(b\) is the probability of lysis owing to some other reaction in control platelets: i.e., "spontaneous lysis" or passive leakage of chromium from control platelets. \(F\) is the total fraction of chromium actually released divided by 0.8, the fraction that could maximally be released. Then, \((1-y)\) is the fraction of platelets remaining after immunologic lysis, \((1-b)\) is the fraction remaining after spontaneous lysis and \((1-y) (1-b) = (1-F)\), the fraction of platelets not lysed by either process and, hence, remaining in the sample to be counted. Solving for \(y\), \(y = F - b/1.8\). Thus, \(y\), the fraction of platelets lysed by immunologic reaction, equals the fraction lysed in the antibody sample \((F)\) minus the fraction lysed in control tube \((b)\), divided by 1 minus the fraction lysed in the control tube.

The effect of anti PLA\(_{1}\) reported here is in substantial agreement with that previously reported by Aster.² Our results indicate that anti A\(_{1}\), in concentrations usually found, has relatively less damaging effect on A\(_{1}\) platelets, even in the presence of added complement, than does anti PLA\(_{1}\), a specific platelet antibody.

Discussion

The ABO antigens, particularly the A\(_{1}\) antigen, have been demonstrated on platelets from individuals with A\(_{1}\) red blood cells.⁵⁻⁸,¹⁷ Relatively little in vitro work has been done to measure the immunologic damage to platelets caused by ABO incompatibility. The lack of chromium release by agglutinating anti A\(_{1}\) and the relatively mild damage produced by hemolytic anti A\(_{1}\) in the usual concentrations is in agreement with the published in vivo studies.¹,²,⁴,⁶,⁸,¹³,¹⁷

Pfästerer and Aster,²,¹³ in separate studies, both reported reduced post-transfusion recovery of A\(_{1}\) platelets in recipients who were ABO incompatible. Recovery of A\(_{1}\) platelets averaged 20 percent in patients with anti A\(_{1}\) antibody while autologous or ABO compatible platelets had an average recovery of 65 percent.²

In Aster's report, the recovery of A\(_{1}\) platelets correlated to some degree with the concentration of anti A\(_{1}\).² Titers greater than 1/64 produced the lower recoveries. Thus, it seems clear that the concentration of antibody to A substance is important to

<table>
<thead>
<tr>
<th>Table III</th>
<th>Chromium Release from Platelets as a Measure of Immunologic Lysis by Aster's Formula</th>
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<tbody>
<tr>
<td>Saline</td>
<td>complement</td>
</tr>
<tr>
<td>Anti Pl A(_{1}) 1:10</td>
<td>28%</td>
</tr>
<tr>
<td>Anti A(_{1}) 1:10</td>
<td>13%</td>
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the effects on platelets. Our studies suggest, in addition, that the anti A must have hemolytic activity in the presence of complement to be effective in damaging platelets and releasing chromium. Whether or not this property is merely a concentration dependent effect of anti A1 or is a phenomenon related to antibody configuration remains to be proven. Agglutinating antibodies probably do not release chromium or lyse platelets.

Platelets may be less susceptible to anti A1 than to anti P1A1, either because of differences in the antibodies or, more likely, because there may be fewer A1 sites than P1A1 sites on the platelet surface. Similarly, platelets may be less susceptible to anti A1 than are red blood cells because of a lower antigen density on the platelet surface. These speculations await further work, requiring radioactive antibodies and radioautography or iron labelled antibodies and the electron microscope.

Summary

What recommendations can be made for the transfusion of ABO compatible platelets? In the non-immunosuppressed patient, ABO compatible platelets probably should be used routinely. In those patients exposed to chemotherapy or radiation, ABO compatible platelets should be used when: (1) there is a high concentration of anti A1, particularly if it is demonstrably hemolytic; (2) there is poor post transfusion recovery demonstrated following use of random donor platelets; (3) there are reactions associated with random donor platelets; and (4) platelets stored longer than 48 hours are being used, since such platelets also have a reduced recovery.

References