Removal of HLA-A,B Antigens From Platelets

By Neil Blumberg, Debra Masel, Theodor Mayer, Paul Horan, and Joanna Heal

Recent evidence suggests that HLA-A,B antigens, present on human platelets, are acquired from plasma. Using hypertonic acid chloroquine (200 mg/ml, pH 5.0, 1 hr, 4°C), we demonstrate elution of most or all HLA-A2 and HLA-B7,40 immunoreactive material from platelets. Corresponding antigens on human lymphocytes were not affected by this treatment. These findings are consistent with the hypothesis that HLA-A,B antigens are adsorbed onto platelet surfaces.

A N UNDERSTANDING of the structure of human platelet membrane antigens is of both biologic and clinical interest. HLA-A,B antigens and those of the ABH system are present on platelets. However, adsorption of exogenous soluble HLA-A,B antigens and ABH substances onto platelets has been demonstrated in vitro. This raises the question of whether these antigens are integral membrane proteins, as has been shown for the HLA-A,B antigens of lymphocytes, or are adsorbed onto the platelet surface.

Nondestructive removal of HLA-A,B antigens from platelets should be feasible if they are indeed adsorbed from plasma. The antimalarial drug, chloroquine, is known to dissociate immune complexes and to remove antibody from red cell surfaces. Because unpublished data from our laboratory demonstrated that chloroquine treatment removed passively adsorbed Lewis blood group antigens from red cells, we attempted to similarly dissociate HLA-A,B antigens from platelets.

MATERIALS AND METHODS

Detection of HLA-A,B Antigens on Platelets

Human platelets from normal blood donors of known HLA-A,B phenotype were collected in EDTA vacuum tubes (Sherwood, St. Louis, MO). These were washed three times (2,500 g/10 min) with phosphate-buffered saline EDTA solution (PBS-EDTA: 3.0 g/liter Na2 EDTA, 3.75 g/liter NaHPO4, and 8.2 g/liter NaCl at pH 7.0) and resuspended at a count of 350,000/µl. Mouse monoclonal anti-A2 or anti-B7,40 antibodies (Atlantic Antibodies, Scarborough, ME) were used to detect these HLA antigens on platelets by employing an enzyme-linked antiglobulin test. Affinity-purified anti-mouse lgG coupled to alkaline phosphatase (Sigma, St. Louis, MO) was used to detect monoclonal anti-HLA antibody bound to platelets. Bound anti-HLA was measured as endpoint optical density at 405 nm (OD405). Measurements were made in microtiter plates (Immulon II) using a through-the-plate spectrophotometer (micro ELISA Reader, Dynatech, Alexandria, VA).

Chloroquine Treatment of Platelets and Lymphocytes

To determine if chloroquine removed HLA-A,B antigens, washed platelets obtained from 7 ml of EDTA whole blood were incubated (4°C, 1 hr) either with 4 vol of chloroquine diphosphate (200 mg/ml, pH 5.0, osmolarity 980 mosmole; Gamma Biologicals, Houston, TX) dissolved in PBS-EDTA, or with PBS-EDTA alone. Resultant pH is 5.1 and osmolarity is 840 mosmole. After washing and adjusting platelet counts to 350,000/µl, 0.2 ml of platelets was incubated with 50 µl of murine anti-A2 or anti-B7,40 (1:100 dilution with PBS in 1% bovine serum albumin [BSA;Sigma]) for 1 hr at 37°C. Platelets were washed, resuspended in 0.2 ml PBS-BSA, and then incubated for 1 hr at 37°C with 0.2 ml of anti-mouse IgG coupled to alkaline phosphatase (1:500 in PBS-BSA). After further washing, 0.2 ml of substrate solution (paranitrophenyl phosphate: 1 mg/ml in 5.3 g/liter Na2CO3, 0.2 g/liter MgCl2 · 6H2O, pH 9.8; Sigma) was added to the platelet pellets and color development was allowed to proceed for 1 hr. Reactions were stopped by addition of 0.1 ml of 1 M NaOH. After centrifugation, 0.2 ml of each reaction product was transferred to a well of a microtiter plate and OD405 was determined.

Detection of HLA-A2 Eluted From Platelets by Chloroquine

 Supernatants from platelets treated with 200 mg/ml chloroquine were tested for the presence of eluted HLA-A2 antigen by a solid-phase enzyme-immunoassay. To remove chloroquine, supernatant eluates (1.0 ml) from chloroquine-treated A2-positive and A2-negative platelets were passed through a 1 x 25 cm G-25 Sephadex (Pharmacia) column, equilibrated with microtiter plate coating buffer (1.6 g/liter Na2CO3, 2.93 g/liter NaHCO3, pH 9.8). After the void volume was collected, 5 0.5-ml fractions were collected and incubated in microtiter plate wells (Immulon II), each in 5 0.1-ml aliquots (37°C for 2 hr, then 4°C for 14 hr). Plates were washed (MIniwash, Dynatech) (PBS-EDTA-1% BSA), incubated with 0.2 ml of 1:200 mouse monoclonal anti-A2 for 30 min at 37°C, washed, and incubated for 1 hr at room temperature with 0.2 ml of anti-mouse IgG coupled to alkaline phosphatase (1:500). After a final wash, 0.2 ml of substrate solution was added and the OD405 measured at 1–1.5 hr. Mean results of the 5 wells were used for each column fraction. A ratio of the OD405 for the A2-positive eluate to
Table 2. Anti-HLA-B7.40 Binding to B40-Positive and Negative Platelets Before and After Chloroquine Treatment

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<tr>
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<th>Before Chloroquine</th>
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<td>OD (nm) ± SD</td>
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<td>B40-positive</td>
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<tr>
<td>A2-positive</td>
<td>8 0.516 ± 0.061</td>
<td>9 0.404 ± 0.052</td>
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<tr>
<td>A2-negative</td>
<td>8 0.273 ± 0.107</td>
<td>9 0.368 ± 0.087</td>
</tr>
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*Not significantly different (p > 0.2).
†p < 0.001.
All data are OD_{405}nm ± 1 SD.
Statistical significance by unpaired Student’s t test.

DISCUSSION

Our data suggest that hypertonic acid chloroquine treatment rapidly removed HLA-A,B immunoreactive material from human platelets, but not from lymphocytes. It is theoretically possible that the monoclonal antibodies we used preferentially recognized adsorbed HLA-A,B antigens compared to intrinsic ones. However, the demonstration that HLA-A,B antigens are detectable on lymphocytes before and after chloroquine treatment suggests that this is unlikely to be the case. We have not performed similar studies using polyclonal anti-HLA antisera to see if similar results are obtained.

That our results indicate elution of HLA-A,B antigens, rather than destruction, is suggested by our ability to recover antigen in the eluates from HLA-
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A2-positive platelets. These data are consistent with the hypothesis that HLA-A,B antigens are associated with the platelet surface in a manner different from that of lymphocytes. A possible explanation is that they are adsorbed onto the surface of platelets, rather than being integral membrane components. It seems likely that HLA-A,B antigens are acquired from soluble plasma sources rather than from endogenous synthesis during thrombopoiesis. Proof of this hypothesis awaits detailed structural characterization of HLA-A,B antigens from platelets. We cannot rule out the possibility that HLA-A,B antigens are identical in structure on both platelets and lymphocytes, but that chloroquine has different effects on the molecule in the two cell types. Given other findings on the human platelet's ability to adsorb soluble plasma antigens,35,5 it appears that the platelet is an immunobiologic "sponge." The platelet's role in clearing immune comple-

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