Quantitative Analysis of Platelet Surface HLA by W6/32 Anti-HLA Monoclonal Antibody

By K.J. Kao, Daniel J. Cook, and Juan C. Scornik

Class I molecules of human major histocompatibility complex (HLA) are the most important antigenic system in determining the survival of transfused platelets in alloimmunized patients. Platelets with reduced expression of a specific type of HLA antigen may escape specific anti-HLA antibody-mediated destruction. By using 125I-labeled Fab fragments of W6/32 anti-HLA monoclonal antibody and competitive protein binding assays, we measured the range of total HLA concentrations on platelets. In 12 individuals examined, the mean number of HLA-A, B, and C molecules per platelet was 81.58 ± 20.016 (mean ± SD); its range was between 54.782 to 116.185 molecules per platelet. After treatment with chloroquine, 79.9 ± 7.0% (mean ± SD, n = 6) of HLA antigens were removed from platelets as determined by binding of 125I-W6/32 Fab. A similar result was obtained when HLA antigens on chloroquine-treated platelets were evaluated with immunofluorescence flow cytometry. In contrast, chloroquine treatment did not remove integral membrane protein such as P141 antigens on platelets. The presence of HLA antigens in the chloroquine eluate of platelets could be demonstrated to contain HLA antigens similar in mol wts to intact class I molecules by an immunoblotting technique. These data suggest that 70% to 80% of platelet HLA antigens are adsorbed and that such HLA antigens are not proteolytic products of integral membrane class I molecules. The origin of the adsorbed platelet HLA-antigens remains to be determined.

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THE EXISTENCE OF HLA antigens on platelets was first demonstrated with platelet complement-fixation tests in the 1960s1-5 and was later confirmed with the purification of HLA antigens from platelets.6-7 Clinically, HLA is the most important antigen system involved in determining survival of transfused platelets in alloimmunized patients.8-13 Liebert and Aster and co-workers14-18 used an inhibition of lymphocytotoxicity test to show that several B-locus HLA antigens of platelets are variably expressed among different individuals. A threefold variation of HLA-A2 on platelets was also reported by Svejgaard.19 HLA-C antigens are only weakly expressed on human platelets20 and do not seem to play any significant role in the alloimmunization to platelets.21 In addition, platelets with reduced expression of HLA-B12 may escape destruction and survive normally in recipients whose plasma contains antibodies against HLA-B12.22,23 These observations suggest that quantitative difference of HLA antigen expression may have a potential clinical importance and stress the need for more rigorous studies to improve methods of quantitation over serological techniques relied upon by previous investigations.14-19 Recently, Blumberg and colleagues24 reported that acid hypertonic chloroquine can elute a portion of HLA antigens without affecting P141 antigens on the platelet surface. The findings indicated that certain amounts of HLA antigens on the platelet surface were adsorbed. However, the exact quantity was not determined.

In this report, we describe methods of quantitation of platelet surface HLA antigens and show the instability of these antigens and variations of HLA concentrations on platelets of normal individuals.

MATERIALS AND METHODS

Materials. RPMI 1640 medium, l-cysteine, antibiotic-antimycotic solution, and fetal calf serum (FCS) were purchased from GIBCO Laboratory (Grand Island, NY). Protein A-Sepharose CL 4B, mercuripapain, bovine serum albumin (BSA), nitroblue tetrazolium (NBT), 5-Bromo-4-chloro 3-indolyl phosphate-p-toluidine salt (BCIP), rabbit anti-mouse IgG alkaline phosphatase conjugate, and chloroquine diphosphate were obtained from Sigma (St Louis). Na125I and solid-phase lactoperoxidase were the products of Amersham Co (Arlington Heights, Ill) and P-L. Biochemicals (Milwaukee), respectively. Fluorescein-labeled sheep anti-mouse IgG F(ab')2 fragments and mouse IgG were obtained from CooperBiomedical, Inc (Malvern, Pa). Goat anti-human IgG F(ab')2 fragments were from Kallestad Laboratory (Austin, Tex). Diethylaminoethoxy DEAE) affigel2 and nitrocelullose paper with 0.45-m pore size were purchased from Biorad Co (Richmond, Calif). All other chemical reagents were analytical grade. Mouse anti-influenza virus type A monoclonal antibody was a generous gift from Dr P.A. Klein, Department of Pathology, University of Florida.

Isolation of W6/32 anti-HLA monoclonal antibody. The W6/32 hybridoma, obtained from the American Type Culture Collection (Rockville, Md), was originally developed and had been shown to recognize a common monomorphic determinant of HLA-A, B, and C molecules by Barnstable and colleagues.25 The W6/32 hybridoma was maintained in RPMI 1640 culture medium supplemented with 10% FCS, gentamycin (0.5 mg/mL), penicillin (100 U/mL), streptomycin (100 mg/mL), and fungizone (0.25 mg/mL). W6/32 anti-HLA monoclonal antibody was purified from culture medium by protein A-Sepharose CL-4B affinity column chromatography.26 Protein concentration and homogeneity of the purified W6/32 monoclonal IgG antibody were determined by the assay of Lowry and co-workers27 and sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS-PAGE)28 respectively.

Preparation of Fab fragments of W6/32 monoclonal antibody. The Fab fragments of W6/32 anti-HLA monoclonal antibody (W6/32-Fab) were prepared by papain digestion as described by Porter.29 In brief, 20 mg of W6/32 antibody in 5 mL of 0.01 mol/L of potassium phosphate buffer, pH 6.5, containing 2 mmol/L of EDTA and 0.01 mol/L of L-cysteine were incubated with 0.4 mg mercuripapain at 37 °C for 4 hours. The papain digest of W6/32 antibody was dialyzed with 0.02 mol/L of Tris-HCl, pH 8.0, containing 0.025 mol/L of NaCl at 4°C for 10 hours and then...
loaded on a DEAE affi-gel blue column (1.5 x 10 cm) to separate Fab fragments from Fc fragments. The Fab fragments were recovered from the protein peak in the flowthrough and further purified with a protein A-Sepharose CL 4B column chromatography (1.5 x 3 cm) described above.

Preparation of washed platelets. Only freshly drawn venous blood from healthy volunteer donors was used. Blood was anticoagulated with 1/4 vol of 3.8% sodium citrate. Platelets were isolated from 18 mL of the blood by differential centrifugation. The isolated platelets were washed twice with 0.02 mol/L of Tris-HCl, pH 7.4, containing 0.15 mol/L of NaCl and 1 mmol/L of EDTA (Tris-saline-EDTA) as described, and finally suspended in 0.02 mol/L of Tris-HCl, pH 7.4, containing 0.15 mol/L of NaCl and 0.2% bovine serum albumin (BSA) (Tris-saline BSA). The platelet concentrations were enumerated by an S-plus Coulter counter. The contamination of WBCs was always <0.03%.

Chloroquine elution of platelet HLA. The chloroquine solution was prepared as reported by Blumberg and co-workers.

Quantitative assay for platelet HLA-A, B, and C antigens. The concentration of total HLA-A, B, and C antigens on platelets was determined by using 125I-labeled Fab fragments of W6/32 anti-HLA monoclonal antibody (125I-W6/32 Fab) and a competitive protein binding assay. The isolated W6/32 Fab was labeled with 125I by solid-phase lactoperoxidase method as described previously. The specific radioactivity of 125I-W6/32 Fab was ~0.6 µCi/µg. The specific bindings of 125I-W6/32 Fab to washed platelets were determined by incubating 5 x 10^5 platelets and specified amounts of 125I-W6/32 Fab in 250 µL of Tris-saline-BSA with or without a 160-fold excess of unlabeled W6/32 Fab. After 2 hours of incubation at room temperature, the incubations were terminated by adding 0.5 mL of ice-cold Tris-saline-BSA, and they were centrifuged at 2,200 g for 10 minutes at 4 °C. The addition of 0.5 mL of ice-cold Tris-saline-BSA reduced the nonspecific binding without any significant effect on the specific binding. The supernatant of each incubation was aspirated, and the platelet pellet was counted in an LKB gamma counter. The 125I-W6/32 bound on platelets which could be competed only by an excess of unlabeled W6/32 Fab was defined as specific binding. All experiments were performed in duplicate. The variation between duplicates was <7%. The specific binding of 125I-W6/32 to platelets was not affected by the presence or absence of 0.05% sodium azide in the incubation mixtures.

Immunofluorescent flow cytometry. Immunofluorescent flow cytometry was conducted on a Becton-Dickinson FACS-II fluorescence-activated cell sorter. Routinely, 1 x 10^6 washed platelets were incubated with saturable amounts of primary antibody, either 10 µg/mL of W6/32 monoclonal antibody or 20 µL of anti-P14 antibody in a final volume of 100 µL of Tris-saline at room temperature for 30 minutes. Thereafter, the platelets were washed three times with 0.5 mL of Tris-saline-EDTA and reincubated with 50 µL of fluorescein-labeled F(ab')2 of anti-human or mouse IgG at 1 to 20 dilution for 20 minutes at room temperature. Finally, the platelets were washed twice and resuspended in 1 mL of 0.01 mol/L of sodium phosphate buffer, pH 7.4, containing 0.15 mol/L of NaCl (phosphate buffer saline, PBS). The fluorescent intensity of 10,000 platelets was measured. The histogram was depicted, and the mean fluorescence intensity per platelet was computed with a Hewlett-Packard 9845T computer.

RESULTS

**SDS-PAGE and Western blot.** SDS-PAGE was performed according to the procedure of Laemmli. The supernatant of washed platelet suspension (1 x 10^9 cells/mL) that was treated with 4 vol of chloroquine was collected by centrifugation at 4,000 g for 10 minutes, and dialyzed extensively with Tris-saline. The supernatant was then concentrated 75-fold with Aquacide II and Amicon B15 concentrator and was mixed with an equal volume of sample buffer for SDS-PAGE. Control washed platelets and chloroquine-treated washed platelets were solubilized by incubating 1 x 10^6 platelets with 0.3 mL of 0.5% Nonidet-P40 in PBS and containing 1 mmol/L of phenylmethylsulfonyl-fluoride and 1% epsilon-amino-n-caproic acid for 15 minutes on ice. Insoluble cellular debris was removed by centrifugation at 10,000 g for 5 minutes. The solubilized platelets were concentrated threefold with an Amicon B15 concentrator and mixed with an equal volume of sample buffer for SDS-PAGE.

Western blot was performed by following the procedure of Tobin and co-workers in a Trans-Blot cell (Bio-Rad, Richmond, Calif). The blotting process was conducted at constant voltage of 40 V for 4.5 hours. The nitrocellulose blot was blocked with 5% nonfat milk made in PBS containing 0.05% sodium azide and incubated sequentially with W6/32 anti-HLA antibody (20 µg/mL), alkaline phosphatase conjugate of rabbit anti-mouse IgG (1:1,000 dilution), and alkaline phosphatase substrate, which contains 0.1 mg/mL of NBT and 0.05 mg/mL of BCIP as described.

**Immunofluorescent flow cytometry.** Immunofluorescent flow cytometry was conducted on a Becton-Dickinson FACS-II fluorescence-activated cell sorter. Routinely, 1 x 10^6 washed platelets were incubated with saturable amounts of primary antibody, either 10 µg/mL of W6/32 monoclonal antibody or 20 µL of anti-P14 antibody in a final volume of 100 µL of Tris-saline at room temperature for 30 minutes. Thereafter, the platelets were washed three times with 0.5 mL of Tris-saline-EDTA and reincubated with 50 µL of fluorescein-labeled F(ab')2 of anti-human or mouse IgG at 1 to 20 dilution for 20 minutes at room temperature. Finally, the platelets were washed twice and resuspended in 1 mL of 0.01 mol/L of sodium phosphate buffer, pH 7.4, containing 0.15 mol/L of NaCl (phosphate buffer saline, PBS). The fluorescent intensity of 10,000 platelets was measured. The histogram was depicted, and the mean fluorescence intensity per platelet was computed with a Hewlett-Packard 9845T computer.

**Fig 1.** Sodium dodecyl sulfate-polyacrylamide gel electrophoretic analysis of purified W6/32 anti-HLA monoclonal antibody and its Fab fragments. The polyacrylamide gel was 10%. Lane A, reduced W6/32 antibody (12 µg); lane B, reduced W6/32 Fab (10 µg); lane C, nonreduced W6/32 antibody (10 µg); lane D, nonreduced W6/32 Fab (10 µg). Left lane, mol wt standards: phosphorylase B, bovine serum albumin, ovalbumin, carbonic anhydrase, and soybean trypsin inhibitor.
equal amounts of two intact light chains. The findings suggest that only 50% of W6/32 Fab may be functional and will react with HLA-A, B, and C molecules. This was experimentally tested by incubating $^{125}$I-W6/32 Fab (1 μg/mL) and platelets (2.0 × 10^8 cells/mL) that contained an excess of HLA antigens in the presence or the absence of 150 μg/mL of unlabelled W6/32 Fab at 25 °C for 3 hours. All the incubations were performed in duplicate. The results showed that 47% of W6/32 Fab specifically bound to platelets, thereby confirming that ~50% of the Fab fragments have HLA binding activity.

**Quantitative Assay for Platelet HLA-A, B, and C Antigens.** The purified W6/32 Fab was labeled with $^{125}$I and used to quantitate platelet HLA concentrations in a competitive protein binding assay. Fab fragments were used to avoid binding to platelet Fc receptors. First, the specific binding of $^{125}$I-W6/32 Fab to 5 × 10^6 platelets was studied as a function of duration of incubation. The results showed that 2 hours of incubation is required for the binding to reach a steady state (Fig 2). Thereafter, specific binding was determined as a function of increasing concentrations of $^{125}$I-W6/32 Fab after a 2-hour incubation time. As shown in Fig 3, the specific binding of $^{125}$I-W6/32 Fab to a constant number of platelets was saturable. As expected, the nonspecific binding increased linearly with increasing concentrations of $^{125}$I-W6/32 Fab and was not saturable. The binding data of Fig 3 were then analyzed by a Scatchard plot to determine the steady-state dissociation constant (Kd) and the concentration of total platelet HLA in the incubations. The results showed that the Kd was 6.1 nmol/L and total platelet HLA concentration was 3.35 nmol/L or 100,835 molecules per platelet (Fig 4).

To demonstrate the specificity of ligand binding, human fibrinogen and IgG from human and mouse were used to compete with $^{125}$I-W6/32 Fab for binding. As shown in Fig 5, only unlabelled W6/32 Fab can compete for the binding of labeled W6/32 Fab. The results in Fig 5 were also analyzed by Scatchard plot. Similar binding affinity (Kd) and concentration of platelet HLA molecules to those determined from the binding data of Fig 3 were obtained. In these studies (Figs 3 and 5), washed platelets from the same individual were used. The results indicate that both methods are precise quantitations of platelet HLA concentration.

The specific bindings of $^{125}$I-W6/32 Fab to human lymphoblastoid cells, platelets, Daudi cells (a Burkitt's lymphoma cell line), and RBCs were also determined to demonstrate that the binding of $^{125}$I-W6/32 Fab is specific for class I HLA molecules. Specific binding per 5 million cells was observed in a lymphoblastoid cell line (28,035 cpm) and platelets (11,836 cpm) but not in RBCs (500 cpm) and Daudi cells (191 cpm). The results agree with earlier studies demonstrating that lymphoblastoid cells are rich in HLA antigens, while HLA antigens are low or absent in Daudi cells and RBCs.

The intersay variation for quantitating platelet HLA concentration by using $^{125}$I-W6/32 Fab and Scatchard analysis was also evaluated. HLA concentrations on platelets

![Fig 2](image2.png) **Fig 2.** Time course study of binding of $^{125}$I-labeled W6/32 Fab to platelets. Each incubation contained 5 × 10^6 platelets and 0.075 μg $^{125}$I-W6/32 Fab (1.08 × 10^4 cpm/μg) with or without a 150-fold excess of unlabelled W6/32 Fab in a final volume of 250 μL. The bound $^{125}$I-W6/32 Fab that could not be unlabelled W6/32 Fab represented nonspecific binding.

![Fig 3](image3.png) **Fig 3.** Saturable binding of $^{125}$I-W6/32 Fab to platelets. The specific binding and the nonspecific binding of $^{125}$I-W6/32 Fab to a constant number of platelets (5 × 10^6 cells) were determined as a function of increasing concentrations on $^{125}$I-W6/32 Fab.

![Fig 4](image4.png) **Fig 4.** Scatchard plots of $^{125}$I-W6/32 Fab binding to platelets. The binding data obtained from the studies of Fig 3 (——) and Fig 5 (----) were analyzed. The lines were drawn by linear regression analysis. The assumption that only 50% of added W6/32 Fab are functional was used for Scatchard analyses. The mol wt of 46 kd for W6/32 Fab was used for calculation. Kd, steady-state dissociation constant; r, correlation coefficient.
harvested from three different persons were determined individually after their immediate collection and on the second and the third day were stored in Tris-saline containing 0.05% sodium azide at 4 °C. The interassay variations for each individual were 6.3%, 6.7%, and 6.8%, respectively.

Platelet HLA concentrations in normal adults. Platelet HLA concentrations in 12 healthy adults were determined by the established binding assay. All except 1 of the 12 adults were HLA typed. Among these 12 individuals, the average number of HLA-A, B, and C molecules per platelet varied between 54,782 to 113,068 molecules per platelet (Table 1). The mean number of HLA molecules on a platelet was 81,587 ± 20,016 (mean ± SD). The results indicate that there is at least a twofold variation in platelet HLA concentration among different individuals. Because the interassay variation is 7%, the observed twofold difference most likely reflects true individual variation.

Quantitation of HLA molecules on chloroquine-treated platelets. The specific bindings of 125I-W6/32 Fab to platelets pretreated with 4 vol of 20% chloroquine for 15 to 60 minutes were determined. It was noted that loss of HLA antigens reached its maximum after 45 minutes of incubation with chloroquine. The elution of platelet HLA antigens was then studied as a function of chloroquine concentration, and all platelets were incubated with chloroquine for 45 minutes. The results of these studies indicated that maximal elution was attained after incubating platelets with 4 vol of 20% chloroquine for 45 minutes. Subsequently, we performed experiments in six healthy individuals whose platelets were pretreated with 4 vol of 20% chloroquine for 60 minutes. Losses of 71.5% to 90.0% of HLA-A, B, and C molecules from the platelets were observed (Table 2).

Although chloroquine treatment reduced HLA antigens, P1A antigens remained unchanged on platelets, as determined by the binding of anti-P1A antisera and quantitative immunofluorescence flow cytometry. The mean fluorescence intensity for HLA antigen on each of the chloroquine-treated platelets was only 25.3 ± 9.3% (mean ± SD) of the control.

DISCUSSION

Although the presence of HLA antigens1-5 and variable expression of some specific types of HLA antigens on human platelets6-19 are known, the exact quantity of HLA molecules on platelets and its variability among different individuals has not been demonstrated. By using Fab fragments of W6/32 anti-HLA monoclonal antibody and competitive protein binding, a quantitative assay for determining concentration of platelet HLA-A, B, and C molecules was developed.

Table 1. Concentrations of Platelet HLA in Normal Adults

<table>
<thead>
<tr>
<th>Case No.</th>
<th>Sex</th>
<th>HLA Phenotype</th>
<th>HLA-A, B, C (Molecules/per Platelet)*</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>M</td>
<td>2.24,62,39</td>
<td>100,835</td>
</tr>
<tr>
<td>2</td>
<td>M</td>
<td>24, —, 7—</td>
<td>66,220</td>
</tr>
<tr>
<td>3</td>
<td>M</td>
<td>26,28,14,53</td>
<td>60,200</td>
</tr>
<tr>
<td>4</td>
<td>M</td>
<td>1,3,7,8</td>
<td>90,300</td>
</tr>
<tr>
<td>5</td>
<td>M</td>
<td>1,2,7,12</td>
<td>65,317</td>
</tr>
<tr>
<td>6</td>
<td>M</td>
<td>2,28,17,40</td>
<td>116,185</td>
</tr>
<tr>
<td>7</td>
<td>M</td>
<td>24,30,7,14</td>
<td>79,765</td>
</tr>
<tr>
<td>8</td>
<td>F</td>
<td>ND†</td>
<td>54,782</td>
</tr>
<tr>
<td>9</td>
<td>F</td>
<td>2,23,8,44</td>
<td>72,240</td>
</tr>
<tr>
<td>10</td>
<td>F</td>
<td>24,30,18,44</td>
<td>79,463</td>
</tr>
<tr>
<td>11</td>
<td>F</td>
<td>2,32,4,37</td>
<td>113,068</td>
</tr>
<tr>
<td>12</td>
<td>F</td>
<td>3,24,7,8</td>
<td>80,667</td>
</tr>
</tbody>
</table>

*The concentrations of HLA-A, B, and C molecules were determined from Scatchard analyses of the binding studies.
†HLA phenotype was not done on case no. 8.

Table 2. Effect of Chloroquine Treatment on Platelet HLA Concentration

<table>
<thead>
<tr>
<th>Case No.</th>
<th>HLA Concentration (Molecules/per Platelet)*</th>
<th>HLA Eluted (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>72,240</td>
<td>16,555</td>
</tr>
<tr>
<td>2</td>
<td>54,782</td>
<td>9,933</td>
</tr>
<tr>
<td>3</td>
<td>65,317</td>
<td>19,264</td>
</tr>
<tr>
<td>4</td>
<td>79,765</td>
<td>12,130</td>
</tr>
<tr>
<td>5</td>
<td>113,068</td>
<td>29,623</td>
</tr>
<tr>
<td>6</td>
<td>80,667</td>
<td>8,066</td>
</tr>
</tbody>
</table>

Mean ± SD 77,639 ± 19,861 15,928 ± 7,889 79.9 ± 7.0

*The HLA-antigen concentration on platelets were determined by 125I-W6/32 Fab binding studies and Scatchard analyses.
on HLA-A, B, and C molecules. One of the light chains is contains equal amounts of two different light chains and this assay.

The saturability of binding, ligand and cellular specificity, and high binding affinity substantiate the validity of this assay.

As reported previously,25 the W6/32 monoclonal antibody contains equal amounts of two different light chains and reacts with a common monomorphic antigenic determinant on HLA-A, B, and C molecules. One of the light chains is parental myeloma type.23 Therefore, it is expected that only 50% of Fab fragments will react with HLA antigens, a fact that we confirmed experimentally. This was taken into consideration when the binding data were analyzed by Scatchard plot. The linear Scatchard plots indicated that only one class of binding sites on platelets and Fab fragments was apparently homogeneous in binding.32,33

The results of studying platelets from 12 healthy individuals revealed that at least a twofold variation exists between the lowest and highest platelet HLA concentrations. This finding is in parallel with the reported variability for platelet HLA-A2, B5, B7, B27, or B1316,18,19 but not for B12 and B8.14 A 35-fold and an eightfold variation was reported for platelet HLA-B12 and B8, respectively.15 Most of these previous studies using inhibition of lymphocytotoxicity measured the relative rather than the exact quantity of platelet HLA antigen. Although the degree of inhibition can be proportional to the actual quantity of HLA antigens, other factors, such as presence of other HLA antibodies and cross-reactivity with other HLA antigens, may affect the results.

Although there was no apparent association between platelet HLA concentration and specific HLA phenotype, the number of individuals (n = 12) involved in the present study was small. Further study on a larger number of individuals will provide a more definite conclusion. The mean number of HLA-A, B, and C molecules on a platelet as determined in our studies is almost the same as that on a peripheral blood T lymphocyte29 and about one-third of that on a peripheral blood B lymphocyte.29 Considering the size of platelets (2 to 3 μm in diameter) and lymphocytes (6 to 14 μm in diameter), it appears that the density of HLA antigen on the platelet surface is greater than that on peripheral blood lymphocytes.

Recently, Blumberg and co-workers30 reported a reduction of platelet surface HLA antigens after acid hypertonic chloroquine treatment of platelets. However, the exact quantity of HLA antigens reduced by chloroquine was not clear. By using 125I-W6/32 Fab and competitive protein binding assay, we found that 70% to 80% of platelet HLA antigens could be removed by chloroquine (Table 2). Because the presence of HLA antigens was identified in the chloroquine eluate by immunoblotting (Fig 6) and because treatment of chloroquine did not significantly affect platelet P1 antigens which are part of the integral membrane glycoprotein IIa.43 These results strongly suggest that most platelet HLA antigens are adsorptive in nature. Moreover, the elutable HLA antigens appear to have the same mol wts as do intact HLA molecules (Fig 6). Thus, it is unlikely that they are proteolytic products of intact membrane HLA molecules. Previously, Lalezari and Driscoll44 demonstrated that platelets may acquire HLA antigens from plasma. The possible contribution of plasma HLA antigens to those adsorbed on platelets remains to be determined.

In conclusion, the results of our studies indicated that human platelets contain high concentrations of HLA antigens. More important, most HLA antigens are adsorbed on platelets. Thus, further studies on the origin and the binding mechanism of adsorbed platelet HLA antigens may lead to the development of ways to manipulate platelet HLA antigens quantitatively and qualitatively. By such manipulation, one may be able to reduce the platelet immunogenicity for their use in patients with alloimmunization against HLA antigens in the future.

ACKNOWLEDGMENT

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