

Plasma and Platelet HLA in Normal Individuals: Quantitation by Competitive Enzyme-Linked Immunoassay

By K.J. Kao

Recent studies on platelet HLA indicate that >50% of platelet HLA antigens are adsorbed on the platelet surface and may be derived from plasma. It has been speculated that platelet HLA may be directly proportional to plasma HLA concentration. To determine the quantitative correlation between plasma and platelet HLA, a precise competitive enzyme-linked immunoassay (ELISA) for measurements of soluble and cellular HLA antigens was developed by using purified HLA antigens and W6/32 anti-HLA monoclonal antibody. The useful range of the standard curve for the assay was 0.01 to 5.0 $\mu\text{g}/\text{mL}$. The intraassay and interassay variations were 7% and 14%, respectively. The plasma HLA concentrations measured in 61 healthy adults ranged from 0.25 to 4.1 $\mu\text{g}/\text{mL}$, and the mean plasma HLA concentration was $1.47 \pm 0.87 \mu\text{g}/\text{mL}$ (\pm SD). Platelet HLA concentrations determined in the same 61 persons ranged

from 4.7 to 17.33 $\text{fg}/\text{platelet}$, and the mean concentration was $9.3 \pm 2.9 \text{ fg}/\text{platelet}$ (\pm SD). Chloroquine-elutable platelet HLA concentrations were also determined in 42 of the 61 persons, with the mean value of $5.7 \pm 2.1 \text{ fg}/\text{platelet}$ (\pm SD). The plasma HLA concentration of each individual was then correlated with the same person's total or chloroquine-elutable platelet HLA concentration. Linear regression analyses of the results revealed no significant correlation between platelet and plasma HLA concentrations. Thus, it is unlikely that chloroquine-elutable HLAs are derived from plasma. The developed solid-phase assay for HLA will be useful for further study of the quantitative significance of plasma HLA antigens in allosensitization of transfused individuals.

© 1987 by Grune & Stratton, Inc.

CLASS I MOLECULES of the human major histocompatibility complex (HLA) are the most important antigenic system in determining the survival of transfused platelets in alloimmunized recipients.¹⁻³ Recently, on the average of 50,000 to 120,000 molecules of HLA antigens per platelet were demonstrated to exist among different individuals,⁴ and >50% of platelet HLA antigens can be selectively removed by acid-hypertonic chloroquine without affecting platelet membrane integrity⁵ and other platelet membrane glycoproteins.^{5,6} The platelet-surface HLA antigens removed by chloroquine could also be detected in the eluate.^{4,5} These results suggest that most platelet HLA antigens are adsorbed on the platelet surface and do not span the platelet plasma membrane.

The existence of soluble HLA antigens in serum^{7,8} was first reported in 1970. Subsequent studies on a few individuals suggested that quantitative expression of HLA-B12 antigen on platelets was proportional to the serum content of that antigen.⁹ Later, Lalezari and Driscoll¹⁰ qualitatively demonstrated that platelets have the ability to acquire HLA specificity from plasma. These results suggest that adsorbed platelet HLA antigens may be derived from plasma and that platelet HLA antigens may be quantitatively proportional to plasma HLA antigens. In addition, Pellegrino and associates¹¹ reported that anti-HLA antibodies were elicited in individuals who had received intravenous (IV) infusion of

human plasma. Their finding indicates that plasma HLA is immunogenic. Nevertheless, concentrations of soluble plasma HLA antigen have only been measured semiquantitatively by inhibition of a lymphocytotoxicity test^{12,13} or by neutralization of a platelet cytotoxicity test.^{9,14}

We report the development of a competitive enzyme-linked immunoassay (ELISA) for precise quantitation of both plasma soluble and platelet cellular HLA antigens. The assay uses purified HLA antigens and W6/32 anti-HLA monoclonal antibody, and was applied to study the quantitative correlation between platelet and plasma HLA concentrations. The potential application of the assay to study plasma HLA immunogenicity is discussed.

MATERIALS AND METHODS

Isolation of W6/32 anti-HLA monoclonal antibody. The W6/32 hybridoma, obtained from the American Type Culture Collection (Rockville, MD), was originally developed by Barnstable, and co-workers¹⁵ and produces an IgG_{2a} monoclonal antibody that recognizes a common monomorphic determinant on HLA-A, B, and C molecules. W6/32 anti-HLA monoclonal antibody (W6/32 McAb) was isolated from culture medium by protein A-affinity column chromatography as described previously.⁴

Purification of HLA antigens. Sixty units of outdated platelet concentrates were obtained from the Civitan Regional Blood Center (Gainesville, FL). Platelets from each unit of platelet concentrate were washed twice with 50 mL of 0.02 mol/L of Tris-HCl, pH 7.4, containing 0.15 mol/L of NaCl and 2 mmol/L of EDTA (Tris-saline-EDTA). Washed platelets were pooled, centrifuged at 10,000 g for 10 minutes, and solubilized by resuspension in an equal volume of 2% NP-40 made in 0.02 mol/L of Tris-HCl, pH 7.4, containing 1 mmol/L of phenyl-methyl-sulfonyl fluoride (PMSF) and 1 mmol/L of ϵ -amino-*n*-caproic acid (EACA). The resuspended platelets were incubated on ice for 60 minutes. Solubilized platelets were then centrifuged at 10,000 g for 10 minutes at 4°C. The supernatant was collected and centrifuged again at 50,000 g for 60 minutes at 4°C. Solubilized HLA antigens were then isolated from the final supernatant by column chromatography on the following three sequentially linked columns: (a) a Sepharose-4B column, (b) a normal mouse IgG-Sepharose-4B column, and (c) a W6/32 McAb-Sepharose 4B column. Details of preparing the columns have been described previously.¹⁶ The size of each column was 1.6 \times 6 cm. After

From the Department of Pathology, College of Medicine University of Florida, Gainesville.

Submitted November 24, 1986; accepted March 13, 1987.

Supported by Grant No. ROI-HL35124 from the National Institutes of Health, Bethesda, MD.

Address reprint requests to Dr K. J. Kao, Box J-275, Department of Pathology, University of Florida, 1600 SW Archer Rd, Gainesville, FL 32610.

The publication costs of this article were defrayed in part by page charge payment. This article must therefore be hereby marked "advertisement" in accordance with 18 U.S.C. §1734 solely to indicate this fact.

© 1987 by Grune & Stratton, Inc.
0006-4971/87/7001-0045\$3.00/0

extensive washing, HLA antigens were eluted by 0.05 mol/L of diethylamine, pH 11.5, containing 0.1% NP-40 and collected in 1-mL fractions. The eluted protein peak was pooled and dialyzed extensively against 0.01 mol/L of phosphate-buffered saline (PBS), pH 7.2, containing 0.05% NaN_3 and 0.1% NP-40. The concentration of HLA antigens was determined by a modified Lowry assay¹⁷ using bovine serum albumin (BSA) as the standard. Homogeneity of purified HLA antigens was evaluated by sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE).¹⁸

Preparation of platelet samples for HLA assay. Platelets were isolated from freshly drawn venous blood by differential centrifugation and washed as described.⁴ Routinely, the venous blood was drawn into a 7-mL vacutainer containing EDTA as an anticoagulant. The washed platelets were suspended in 3 mL of Tris-saline-EDTA buffer and enumerated by a S-plus Coulter counter (Coulter Electronics, Hialeah, FL). The contamination of WBCs is always <0.01%. Ten to 40 million washed platelets were pipetted into a 1-mL conical polystyrene tube and pelleted by a Beckman Microfuge B (Beckman Instruments, Palo Alto, CA) at room temperature for 2 minutes. The supernatant was aspirated and discarded. Platelets were then resuspended in 100 μL 0.02 mol/L of Tris-HCl, pH 7.4, containing 0.05% NP-40, 5 mmol/L of EDTA and 1 mmol/L of PMSF, and left on ice for 60 minutes with occasional vortexing. After solubilization, all samples were stored at -70°C until assayed. The frozen samples were stable for at least 12 months at -70°C . Chloroquine-treated platelets were prepared as reported previously.⁴

Competitive ELISA for HLA antigens. Each well of a 96-well Immunoplate (Nunc Inter-Med, Kamstrup, Denmark) was incubated overnight with 100 μL of purified HLA antigens (2 $\mu\text{g}/\text{ml}$) diluted in 0.01 mol/L of sodium phosphate buffer, pH 7.2, containing 0.15 mol/L of NaCl and 0.02% NaN_3 (PBS-azide) at 4°C . Subsequently, each well was washed three times with PBS-azide containing 0.5% Tween-20 (PBS-T) and then blocked with 300 μL 1% BSA in PBS-T for 30 minutes at room temperature. After the wells were washed three times, 50 μL of different concentrations of purified HLA standard (0.01 to 5 $\mu\text{g}/\text{mL}$), diluted plasma or solubilized platelet samples were added to wells in duplicate, followed by the addition of 50 μL W6/32 anti-HLA McAb (0.02 $\mu\text{g}/\text{mL}$). The incubations were mixed by placing plates on a Micro-Shaker II (Dynatech Laboratories, Alexandria, VA) for 15 seconds, and incubated at room temperature for 90 minutes. The plate was washed three times again, followed by addition of 100 μL of rabbit antimouse IgG alkaline phosphatase conjugate (1,000 \times dilution (Sigma Chemical, St Louis) into each well and incubated for 30 minutes.

Finally, 200 μL *p*-nitrophenyl phosphate disodium (1 mg/mL prepared in 0.01 mol/L of sodium bicarbonate buffer, pH 9.6, containing 2 mmol/L of MgCl_2) was added to each well, and the plates were incubated at room temperature for 90 to 120 minutes in the dark. Fifty microliters of 3N NaOH was then added to each incubation to stop the reaction. Absorbance of each well at a wave length of 405 nm was measured in a BioRad ELISA plate reader (BioRad Laboratories Richmond, CA). All samples were diluted as needed in PBS-T containing 1% BSA and 5 mmol/L of EDTA and were assayed in duplicate. Plasma samples were also assayed at two different dilutions (5 \times and 10 \times) to ensure absence of interfering factors.

RESULTS

Isolation of platelet HLA antigens. HLA antigens isolated from solubilized platelets appeared to be homogeneous as judged by SDS-PAGE (Fig 1). The purified HLA antigens consist of a 44-kd heavy chain and a 12-kd noncoval-

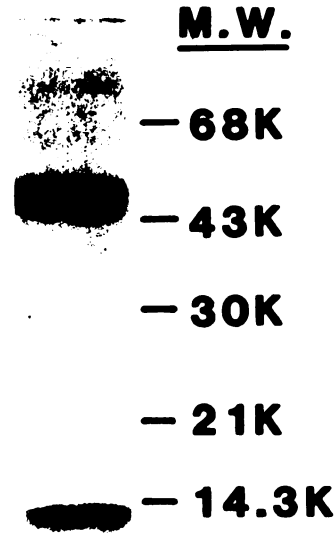


Fig 1. Sodiumdodecyl sulfate-polyacrylamide gel (12.5%) electrophoresis of purified HLA antigens. The purified HLA antigens consist of a 44-kd heavy chain and a 12-kd noncovalently associated β_2 -microglobulin. The same two polypeptide chains were also noted after reduction by 5% mercaptoethanol.

ently associated β_2 -microglobulin. Routinely, 2 to 3 mg of HLA antigens were recovered from 60 U of outdated platelet concentrates. These purified HLA antigens were used to coat the wells and served as standards for the assay.

Competitive ELISA for HLA antigens. The quantitative assay for HLA antigens was developed based on the principle of competition for the binding of W6/32 anti-HLA McAb between plate-bound HLA antigens and unbound HLA

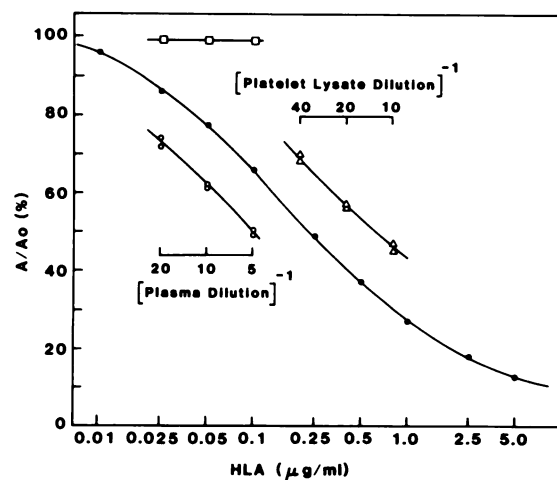


Fig 2. Standard curve of the competitive enzyme-linked immunoassay for HLA antigens (—●—). (A) absorbance of each well at 405nm; (Ao) mean absorbance of duplicated wells in which no purified HLA antigen was added. Open circles represent 5 \times , 10 \times , and 20 \times dilutions of pooled normal plasma (—○—); squares, same dilutions of HLA-depleted pooled plasma (—□—). Dilutions of 4 \times 10⁷ platelets solubilized in 100 μL of lysing buffer (—▲—).

antigens in the standards or unknown samples. The concentrations of the HLA antigens used to coat the wells (2.0 $\mu\text{g}/\text{mL}$) and W6/32 anti-HLA McAb (0.02 $\mu\text{g}/\text{mL}$) used in the assay were found to be optimal to gain greater sensitivity and precision. As shown in Fig 2, the useful range of the standard curve was between 0.01 and 5.0 $\mu\text{g}/\text{mL}$.

When plasma pooled from six different healthy adults without history of previous blood transfusion or pregnancy was assayed at different dilutions, the values were in parallel with the standard curve (Fig 2). The result indicates that there was no factor(s) in plasma interfering with the assay. Next, the specificity of the assay was evaluated by assaying a HLA-depleted pooled plasma prepared by incubating pooled plasma with equal volumes of PA2.6 anti-HLA McAb-Sepharose 4B beads at 4°C for 4 hours. PA2.6 anti-HLA antibody was an IgG₁ monoclonal antibody developed by Brodsky and Parham.¹⁹ As expected, no significant amount of HLA could be measured in the sample after absorption (Fig 2).

Next, both plasma and serum samples were prepared from each of six different volunteer donors and assayed for HLA concentrations. No significant difference was observed between plasma and serum HLA concentrations ($P > .5$). The intraassay and the interassay assay variations were evaluated according to the method described by Bauer and Kennedy.²⁰ Pooled normal plasma was divided into aliquots and frozen at -70°C . On each of the ten different dates, one aliquot was thawed and assayed in duplicate. The intraassay and interassay variations were 7% and 14%, respectively.

Quantitation of platelet HLA concentrations. To measure cellular HLA concentration with the developed competitive ELISA, washed platelets (4×10^7 cells) were solubilized with 0.5% NP-40. The solubilized platelet sample was serially diluted and assayed. As shown in Fig 2, the results

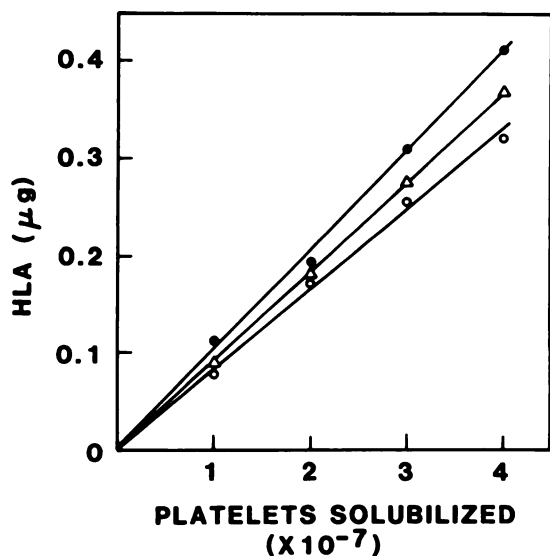


Fig 3. HLA contents of solubilized platelets. Different numbers of platelets were solubilized with 100 μl of lysing buffer. Amount of HLA antigen was measured by the enzyme-linked immunoassay. Each line represents platelets prepared from one individual.

were in parallel with the standard curve of the assay, which excluded presence of any interfering factor(s) in the solubilized platelet samples.

To determine whether the amount of detergent used was sufficient to solubilize platelets, 1×10^7 to 4×10^7 washed platelets from each of three different individuals were lysed by 100 μL of solubilization buffer and then assayed. If 100 μL of 0.5% NP-40 is capable of solubilizing all the platelets and releasing all cellular HLA antigens, a linear correlation should be expected between HLA contents and the number of platelets solubilized. As shown in Fig 3, the amount of HLA indeed was linearly proportional to the number of platelets solubilized. Thus, 100 μL of 0.5% NP-40 was sufficient to release HLA from $\leq 4 \times 10^7$ platelets.

The competitive ELISA was further evaluated by comparing this method with the surface binding assay in which ^{125}I -labeled W6/32 Fab fragments were used. Concentrations of platelet HLA of four different individuals were determined by these two separate methods. As shown in Table 1, platelet HLA concentrations determined by either method yielded similar results.

Quantitative correlation between plasma and platelet HLA concentrations. After validation of the assay, plasma and platelet samples were collected from 61 healthy volunteer donors and assayed for their HLA concentrations. The range of plasma HLA concentrations was from 0.25 to 4.1 $\mu\text{g}/\text{mL}$, and the mean plasma HLA concentration was $1.47 \pm 0.87 \mu\text{g}/\text{mL}$ (mean \pm SD). Platelet HLA concentrations of the same 61 persons ranged from 4.7 to 17.33 fg/platelet, and the mean concentration was $9.3 \pm 2.9 \text{ fg}/\text{platelet}$ (mean \pm SD). The plasma HLA concentration of each individual was then correlated with his or her own platelet HLA concentration. Linear regression analysis²¹ showed no significant correlation between the two parameters ($r = .06$ and $P > 0.5$) (Fig 4).

HLA concentrations of chloroquine-treated platelets were also determined in 42 of the 61 volunteer donors. The quantities of chloroquine-elutable platelet HLA antigens were then calculated as the difference between HLA concentration of platelets before and after the treatment of chloroquine. The range of chloroquine-elutable platelet HLA antigens among the 42 individuals was 2.5 to 10.3 fg/platelet. The mean chloroquine-elutable platelet HLA was 5.7 ± 2.1

Table 1. Platelet HLA Concentrations Determined by EIA and SBA

Case No.	Platelet HLA Concentration (fg/platelet)	
	EIA	SBA*
1	11.5	10.7
2	7.7	8.1
3	7.9	8.6
4	6.7	6.5

EIA, enzyme-linked immunoassay; SBA, surface binding assay.

*SBA was performed by using ^{125}I -labeled W6/32 monoclonal Fab fragments and Scatchard analysis.⁴ The concentrations of platelets HLA were converted from molecules per platelet to femtograms per platelet. The mol wt of 56 kd for HLA antigens was used for calculation. There was no significant difference between HLA concentrations determined by the two assays ($P > .5$).

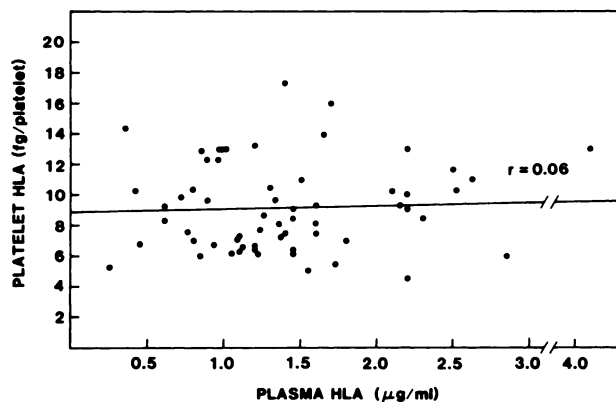


Fig 4. Quantitative correlation between plasma and total platelet HLA concentrations. Sixty-one normal adults were included in the study. Linear regression analysis revealed no significant correlation between the two parameters ($r = .06$, $P > .5$).

fg/platelet (mean \pm SD). Again, no significant correlation was observed between chloroquine-elutable platelet HLA and the same person's plasma HLA concentration ($r = .03$ and $P > .8$). (Fig 5).

DISCUSSION

Although quantitation of plasma and platelet HLA antigens has been reported,^{9,12-14} these previous studies used inhibition of lymphocytotoxicity or platelet cytotoxicity as tests to measure relative rather than exact quantities of the HLA antigens. Theoretically, the degree of inhibition in those cytotoxicity assays is proportional to the actual quantity of HLA antigens. The HLA antigen concentrations determined can be affected, however, by microheterogeneity of the same serologically defined HLA antigen, by presence of undefined HLA antigens, and by cross-reactivity between different specific HLA antigens. Furthermore, the inhibition

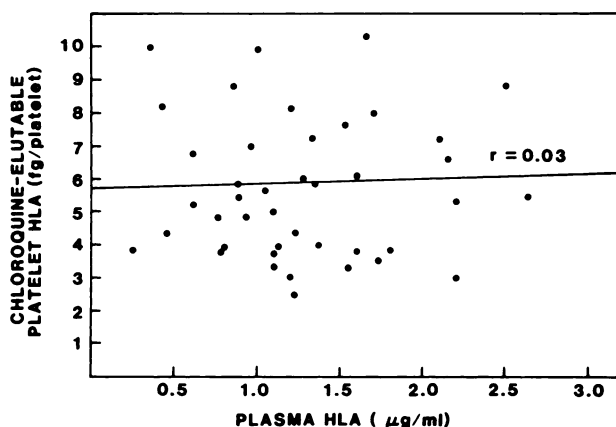


Fig 5. Quantitative correlation between plasma and chloroquine-elutable platelet HLA concentrations. Chloroquine-elutable platelet HLA was determined as the difference of HLA concentration between platelets before and after chloroquine treatment. Forty-two subjects were available for the study. Linear regression analysis did not reveal any significant correlation between the two parameters ($r = .03$, $P > .8$).

of cytotoxicity assay measured only concentration of one specific type of HLA antigens at one time.

We describe competitive EIA for precise quantitation of total plasma soluble and platelet cellular HLA antigens. As shown in Table 1, the concentrations of platelet cellular HLA antigens that were determined by the competitive EIA were comparable with those determined by the surface binding assay using I^{125} -labelled Fab fragments of W6/32 anti-HLA McAb and Scatchard analysis. The results indicate that all the platelet HLA antigens measured by the competitive EIA were on the platelet surface, and there was no significant amount of HLA antigens in platelet cytoplasm. This finding is consistent with the absence of active protein synthesis in platelets. In contrast, the concentration of lymphocyte HLA antigens measured by the described competitive ELISA, was two to three times higher than that measured by the surface binding assay (data not shown). These differences are most likely due to the release of cytoplasmic HLA antigens from lymphocytes during solubilization. Thus, the HLA antigens measured in nucleated cells by the ELISA represent total cellular HLA antigens, which includes cytoplasmic and surface HLA antigens. The active synthesis of HLA antigens and the relatively fast turnover of membrane HLA antigens in lymphocytes²² are most likely responsible for the presence of high levels of HLA antigens in cytoplasm.

To test if the presence of anti-HLA antibodies in plasma samples from individuals with previous pregnancies or multiple blood transfusions might interfere with the assay, five of such plasma samples were assayed at 5 \times , 10 \times , and 20 \times dilutions. Due to the presence of anti-HLA antibodies that interfered with the assay, the values obtained were not in parallel with the standard curve of the assay (data not shown). Therefore, to obtain a valid measurement of plasma or serum HLA concentration, it is imperative to assay all plasma samples at least at two different dilutions to exclude those samples containing interfering factor(s). All the plasma HLA concentrations assayed in this report were measured at 5 \times and 10 \times dilutions. The results were all in parallel with the standard curve.

According to the present study, the average plasma HLA concentration in normal adults is 1.47 μ g/mL. This number indicates that a significant amount of HLA antigens is present in the plasma of various blood components. Although the potential immunogenicity of plasma HLA antigens was reported,¹¹ the clinical significance of the quantitative variation of plasma HLA in allosensitization of recipients of plasma containing blood products remains unclear. The precise quantitation of plasma HLA antigens by using the developed assay will facilitate the study of plasma HLA immunogenicity.

Because previously platelet HLA-B12 antigen concentrations was shown to be proportional to plasma HLA-B12 content,⁹ that platelets can acquire plasma HLA antigens¹⁰ and that most platelet HLA is probably adsorbed on platelet surface,^{4,5} it has been speculated that the amounts of platelet-surface HLA would be directly proportional to plasma HLA levels. Results of the present study showed that there was no significant quantitative correlation between total platelet

and plasma HLA (Fig 4). Because it is possible that only the adsorbed platelet HLA may derive from plasma and may correlate to plasma HLA, quantities of adsorbed platelet HLA were then determined in 42 individuals by subtracting HLA concentration left on chloroquine-treated platelets from total platelet HLA content. Nevertheless, no quantitative correlation between plasma and adsorbed platelet HLA was observed (Fig 5). The results of the present study differed from the earlier observation made by Liebert and Aster.⁹ They reported that serum HLA-B12 antigens were roughly proportional to HLA-B12 on platelets. The small number of subjects (N = 5) included in their study and the assay method used may account for the differences.

In conclusion, the results we report highly suggest that the chloroquine-elutable platelet HLA antigens may not be derived from plasma and that platelet HLA concentration is not in dynamic equilibrium with plasma HLA. Therefore,

the quantitative expression of specific HLA antigens and the immunogenicity of platelets most likely could not be altered by incubating platelets with selected plasma. Although plasma HLA may not be the source of adsorbed platelet HLA, our results do not exclude the possibility that most platelet HLAs indeed may be adsorptive in nature. Further studies on the surface orientation and the physicochemical properties of chloroquine-elutable HLA antigens are needed to identify the origin and the adsorptive nature of platelet HLA antigens.

ACKNOWLEDGMENT

I am grateful to Drs Paul A. Klein and Juan C. Scornik for their valuable criticisms, to Claudia McQueen for her excellent technical assistance, to Cathy Smith for the preparation of the manuscript, and to the Civitan Regional Blood Center for the collection of blood specimens.

REFERENCES

1. Yankee RA, Grumet FC, Rogentine GN: Platelet transfusion therapy: The selection of compatible donors for refractory patients by lymphocyte HLA typing. *N Engl J Med* 27:1208, 1969
2. Mittal KK, Ruder EA, Green D: Matching of histocompatibility antigens for platelet transfusion. *Blood* 47:31, 1976
3. Thomasulo PA: Management of the alloimmunized patient with HLA-matched platelets, in Schiffer J (ed): *Platelet Physiology and Transfusion*. Arlington, VA, American Association of Blood Banks, 1978, p 69
4. Kao KJ, Cook DJ, Scornik JC: Quantitative analysis of platelet surface HLA by W6/32 anti-HLA monodonal antibody. *Blood* 68:627, 1986
5. Kao KJ: Selective elution of HLA antigens and β_2 -microglobulin from human platelets by chloroquine diphosphate. *Transfusion* (in press)
6. Blumberg N, Masel D, Mayer T, Horan P, Heal J: Removal of HLA-A, B antigens from platelets. *Blood* 63:448, 1984
7. Van Rood JJ, van Leeuwen A, van Santen MC: Anti-HL-A 9 inhibition in normal human serum. *Nature* 226:336, 1970
8. Charlton RK, Zmijewski CM: Soluble HL-A7 antigen: Localization in the B-lipoprotein fraction of human serum. *Science* 170:636, 1970
9. Liebert M, Aster RH: Expression of HLA-B12 on platelets; on lymphocytes and in serum: A quantitative study. *Tissue Antigens* 9:199, 1977
10. Lalezari P, Driscoll AM: Ability of thrombocytes to acquire HLA specificity from plasma. *Blood* 59:167, 1982
11. Pellegrino MA, Indiveri F, Fagiolo V, Antonello A, Ferrone S: Immunogenicity of serum HLA in allogenic combinations. *Transplantation* 33:530, 1982
12. Pellegrino MA, Ferrone S, Pellegrino A: Seislogic detection of soluble HL-A antigens, in Kahay BD, Reisfeld RA (eds): *Transplantation Antigens: Markers of Biological Individuality*. Orlando, FL, Academic Press, 1972, p 433
13. Pellegrino MA, Ferrone S, Pellegrino AG, Oh Sk, Reisfeld RA: Evaluation of two sources of soluble HL-A antigens: Platelets and serum. *Eur J Immunol* 4:246, 1974
14. Aster RH, Miskovich BH and Rodey GE: Histocompatibility antigens of human plasma. *Transplantation* 16:205, 1973
15. Barnstable CJ, Bodmer JF, Brown G, Galfrie G, Milstein C, William AF, Zeigler A: Production of monoclonal antibodies to group A erythrocytes, HLA and other human cell surface antigens—New tools for genetic studies. *Cell* 14:9, 1978
16. Parham P: Purification of immunologically active HLA-A and -B antigens by a series of monoclonal antibody columns. *J Biol Chem* 254:8709, 1979
17. Markwell MAK, Haas SM, Biebe LL, Tolbert NE: A modification of the Lowry procedure to simplify protein determination in membrane and lipoprotein samples. *Anal Biochem* 87:206, 1978
18. Laemmli UK: Cleavage of structural proteins during the assembly of the head of bacteria phage T₄. *Nature* 227:680, 1970
19. Brodsky FM, Parham P: Monomorphic anti-HLA-A,B,C monoclonal antibodies detecting molecular subunits and combinatorial determinants. *J Immunol* 128:129, 1982
20. Bauer S, Kennedy JW: Applied statistics for the clinical laboratory: III. Variability. *J Clin Lab Auto* 2:35, 1982
21. Arkin H and Colton RR: Correlation and regression analysis—linear, in *Statistical Methods*. New York, Barnes and Noble 1970, p 82
22. Monos DS, Cooper HL: Rapid turnover of HLA proteins in quiescent lymphocytes: Proposed connection with immunologic surveillance. *J Immunol* 131:341, 1983