Ability of Thrombocytes to Acquire HLA Specificity From Plasma
By Parviz Lalezari and Ann M. Driscoll

Thrombocytes from HLA-A1 and HLA-A2 negative donors were incubated with plasma obtained from respective HLA-positive donors. The plasma-treated thrombocytes were shown to acquire HLA specificity of the plasma as demonstrated by their ability to absorb lymphocytotoxic activity of monospecific anti-HLA sera. The data are consistent with the hypothesis that the expression of HLA antigens in cells of hematopoietic origin diminishes in the course of differentiation and maturation and that the expression of HLA antigens on thrombocytes is primarily due to absorption of these antigens from plasma.

THE HLA ANTIGENS are known to be expressed on precursors of hematopoietic cells.1 It is also known that during the process of differentiation and maturation the expression of these antigens is reduced to a considerable extent on erythrocytes2 and granulocytes.1,3 We have suggested that the HLA content of blood-forming cells may be a reflection of their state of maturation, and that the loss of HLA antigens from the terminal blood cells may be a general phenomenon in this tissue.4,5 It was recognized, however, that the presence of HLA antigens on thrombocytes was inconsistent with this generalization. To explain this inconsistency, it was suggested that thrombocytes may lose most of their native HLA antigens upon maturation, but may adsorb these antigens from plasma while in circulation.4,5 In this report, data are presented that demonstrate the ability of thrombocytes to acquire HLA antigens from plasma in vitro.

MATERIALS AND METHODS
Monospecific anti-HLA-A1 and anti-HLA-A2 antisera (Raymond, Cat. no. 00010 and Wroten, Cat. no. 00700B) were kindly supplied by the NIH Serum Bank. Thrombocytes were isolated by centrifugation at 500 g for 10 min from freshly drawn EDTA-anticoagulated blood. Thrombocyte-rich supernates were examined microscopically, and if necessary, centrifugation was repeated until no erythrocyte or lymphocyte contaminants were seen. The separated thrombocytes were washed twice in phosphate-buffered saline containing 0.1% disodium EDTA and 0.3% bovine serum albumin (PBS/BSA/EDTA, pH 7.4) and were packed by centrifugation at 3500 g for 4 min in a Fisher model 59 centrifuge. Plasma was separated free of thrombocytes from fasting blood by centrifugation. To assure removal of particulate matter, plasma was re-centrifuged for 10 min at 15000 g before use. For transfer of HLA-A1 or HLA-A2 antigens from plasma to thrombocytes, 30-μl aliquots of packed thrombocytes (approximately 1.5 x 10⁶ cells) isolated from HLA-negative donors were resuspended in 1 ml of plasma obtained from donors known to be positive for these antigens. The cell suspensions were continuously mixed for 1.5 hr at 37°C, sedimented by centrifugation (3500 g for 4 min), and resuspended in another 1 ml aliquot of the HLA-positive plasma. The mixtures were incubated for an additional 1.5 hr at 37°C. In some experiments, the mixtures were incubated for 48 hr at 24°C instead of 37°C. Controls consisted of HLA-negative thrombocytes incubated with HLA-negative plasma and HLA-positive thrombocytes incubated with plasma obtained from either HLA-positive or negative donors. Transfer of HLA activity from plasma was investigated by determining the ability of the plasma-treated thrombocytes to reduce lymphocytotoxicity titers of the known HLA antisera. For this purpose, thrombocytes incubated with plasma were washed 3 times in 1 ml aliquots of PBS/BSA/EDTA, packed, and then resuspended in 25 μl of a heat-inactivated (30 min x 56°C) anti-HLA antiserum appropriately diluted in Hanks balanced salt solution without Ca²⁺ or Mg²⁺. Antibody absorption was carried out by 3-hr incubation at 37°C, after which the thrombocytes were sedimented by 5-min centrifugation at 5000 g and the supernates were isolated and titrated for lymphocytotoxicity activity using a two-stage dye exclusion assay.6

RESULTS
Figure 1 shows the results of an experiment in HLA-A1 specificity. It may be seen that HLA-A1-negative thrombocytes, after incubation with HLA-A1-positive plasma, acquire ability to remove cytotoxicity of the test serum comparable to the control HLA-A1-positive thrombocytes. In this experiment, no antibody was absorbed by the control HLA-A1-negative thrombocytes incubated with HLA-A1-negative plasma. These experiments were repeated twice, giving identical results. Figure 2 shows the results of six experiments with HLA-A2 specificity, using different thrombocyte donors. It may be seen that in all cases HLA-A2-negative thrombocytes, after incubation with HLA-A2-positive plasma, behaved like the HLA-A2-positive control cells. The stability of the acquired antigen was tested in an additional experiment as follows: The HLA-A2-negative thrombocytes that had been preincubated with HLA-A2-positive plasma were resuspended in 5 volumes of the original HLA-A2-negative plasma and incubated for 48 hr at 24°C. The cells were then washed 3 times and tested for ability to absorb anti-HLA-A2 antibody. Lymphocytotoxicity titer of the control antibody was 32 (after

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167
absorption with the unmodified HLA-A2-negative thrombocytes), and was reduced only to 16 after absorption by the modified thrombocytes. By contrast, HLA-A2-positive thrombocytes that had been incubated for 48 hr with HLA-A2-negative plasma, did not lose the ability to remove the antibody.

**DISCUSSION**

Thrombocytes are known to adsorb soluble substances from plasma. An example is adsorption of P1^A^ antigens released from transfused thrombocytes, a phenomenon implicated in the pathogenesis of post-transfusion purpura in immunized P1^A^-negative patients. Moreover, thrombocytes have been shown to have the capacity to adsorb A and B blood substances in vitro. The data presented here demonstrate the ability of thrombocytes to acquire HLA activity from plasma and suggest that this reaction could also occur in vivo. The mechanism of this transfer has not been explored. Aster et al. have reported that 73% of the total HLA content of blood reside in thrombocytes. This observation suggests a possible accumulation against a concentration gradient. Thus, the possibility that this accumulation is facilitated by the presence of specific membrane receptors cannot be excluded. Megakaryocytes are known to have HLA antigens. Accordingly, the HLA antigens on thrombocytes may have both native and acquired origins. Concentration of the “native” component is probably a function of the rate of HLA loss in the course of maturation, and the proportion of the native component may be higher.
in young thrombocytes released from the bone marrow in response to stress. Concentration of the acquired form, on the other hand, would probably be influenced by the concentration of HLA substances in plasma. At present, no method exists that can distinguish between these two suggested types of antigenic expressions. If a larger proportion of HLA antigens on thrombocytes is acquired, then a direct relationship should exist between the plasma concentration and the expression of the HLA antigens on thrombocytes. Data available in literature support such a relationship.

Miyajima et al.\(^\text{10}\) and Billings et al.\(^\text{11}\) found the concentrations of HLA-B8, B13, and B14 to be low in plasma, while those of B5 and B7 were high. Szatkowski and Aster\(^\text{12}\) independently reported that B8, B13, and B14 were poorly expressed on thrombocytes, but B5, B7 were fully expressed. Several investigators have noticed marked variability in the expression of HLA-B12 and HLA-B8 on human thrombocytes and not on human lymphocytes.\(^\text{13,14}\) It has also been reported that thrombocytes with poor expression of HLA-B12 survive and function normally in recipients whose plasma contain antibody to HLA-B12.\(^\text{15,16}\) In a separate study,\(^\text{17}\) Liebert and Aster noted a direct relationship between the expression of HLA-B12 on thrombocytes and its plasma concentration, while no such relationship could be demonstrated between plasma and the circulating lymphocytes. Based on these relationships, and concluding from their observations on the in vitro release of HLA antigens from thrombocytes into plasma, Szatkowski and Aster have suggested that platelets may be the major source of soluble HLA substance in plasma.\(^\text{13}\) This interpretation, although it is consistent with the hypothesis that the maturing hematopoietic cells release their HLA substance into the plasma, does not consider the mechanism proposed here, namely the antigen acquisition from plasma. The poor expression of HLA-B13, B14 on thrombocytes suggests that the "native" component of HLA antigens on these cells very low and that the expression of other HLA antigens would have been similarly poor if their plasma concentrations were also low. It thus may be concluded that most of the HLA antigens on thrombocytes are acquired. Experimental data must be obtained, however, for each specificity. For thrombocytes to adsorb HLA antigens, these substances must be present in plasma in soluble form. This property varies for various specificities and is probably under genetic control, as in ABH blood group substances. The HLA antigens absorbed on thrombocytes in vitro appear to dissociate more readily than those presumed to be acquired in vivo. This difference may be attributed to the in vitro conditions employed for the transfer of antigen that may not have been optimal. The continuous exposure of thrombocytes to a large volume of plasma over several days at physiologic temperature may serve to stabilize the antigens acquired in vivo. Even these antigens have been found to be partially released at 4°C if incubated with plasma or PBS.\(^\text{18}\)

Acquisition of plasma antigens may not be limited to HLA, and thrombocytes may adsorb antigenic structures arising from unrelated tissues. Demonstration of such antigens on thrombocytes may give misleading information on the tissue distribution of various antigens. This distribution has been proposed to be determined by cell ontogeny.\(^\text{19,20}\) Ability of soluble antigens in plasma to modify antigenic expression on blood cells is not limited to thrombocytes. Swanson has shown conversion to Bg (a +) of Bg (a -) erythrocytes, if the latter cells are incubated with plasma obtained from Bg (a +) donors.\(^\text{18}\)

It has been previously observed that thrombocytes are not effective immunogens for the production of HLA antibodies\(^\text{19}\) and that a direct relationship between the number of thrombocyte transfusions and the incidence of HLA immunization does not exist.\(^\text{20}\) These observations suggest that HLA antigens adsorbed on thrombocytes may be poor immunogens, and if the contaminating lymphocytes are removed, thrombocytes may be transfused for a longer time without causing HLA immunization.\(^\text{21}\) The HLA antigens on thrombocytes, however, appear to be suitable targets for immunologic damage mediated by HLA antibodies. This is indicated by the occurrence of refractoriness to thrombocyte transfusion in HLA immunized patients. The data presented suggest that HLA-B13 and B14 donors, and HLA-B8 and B12 individuals who have low thrombocyte HLA content, may be considered as "universal donors" for patients who have become refractory to thrombocyte transfusion because of immunization to HLA-B antigens. From a serologic point of view, the data suggest that without consideration of the HLA content of the plasma, the use of thrombocytes would not provide reliable HLA typing data. Also, incubation of thrombocytes with plasmas obtained from multiple donors may provide "polyvalent" cells useful for absorption of HLA antibodies, which frequently contaminate other typing reagents.

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