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Modulation of Platelet Surface Adhesion Receptors during Cardiopulmonary Bypass

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Alterations in platelet receptors critical to adhesion may play a role in the pathogenesis of the qualitative platelet defect associated with cardiopulmonary bypass. Using flow cytometry, we measured changes in the following platelet surface adhesive proteins: the von Willebrand factor receptor, glycoprotein Ib; the fibrinogen receptor, glycoprotein IIb/IIIa; the thrombospondin receptor, glycoprotein IV; the adhesive glycoprotein granule membrane protein 140, whose expression also reflects platelet activation and α-granule release; and, as a control, the nonreceptor protein HLA A,B,C. Glycoprotein Ib decreased during cardiopulmonary bypass ($P < 0.05$) and reached a nadir at 72% ($P < 0.05$) of its baseline value at 2–4 h after bypass. This decrease correlated ($r = 0.76$) with the magnitude of platelet activation (α-granule release) in any given patient, but even platelets that were not activated demonstrated a decrease in glycoprotein Ib expression. Glycoprotein IIb/IIIa also decreased in both the activated (47% of baseline, $P < 0.01$) and unactivated (63% of baseline, $P < 0.01$) subsets of platelets at the end of cardiopulmonary bypass. Glycoprotein IV and HLA A,B,C did not decrease, but instead increased 2–4 h after cardiopulmonary bypass ($P < 0.05$). We conclude that cardiopulmonary bypass produces selective decreases in surface glycoproteins Ib and IIb/IIIa as well as in platelet activation; that these two alterations are temporally but not necessarily mechanistically linked; and that these changes have the potential to adversely affect platelet function. (Key words: Cardiopulmonary bypass, coagulation: platelet activation; platelet glycoproteins. Measurement techniques, flow cytometry: platelet adhesion.)

CARDIOPULMONARY BYPASS (CPB) produces a hemostatic defect out of proportion to the simultaneously occurring thrombocytopenia.[1,2] A qualitative platelet defect during CPB has been demonstrated by decreased aggregation to adenosine diphosphate and epinephrine *in vitro*[3,4]; the nature of this defect, however, is not fully understood. Possibly responsible are two major alterations in platelet physiology: changes in quantitative or qualitative expression of major platelet surface receptors participating in adhesion, and/or the generation of "activated" platelets, *i.e.*, platelets that have undergone α-granule release. Other investigators[5,6] have demonstrated that the von Willebrand factor receptor (glycoprotein [GP] Ib) and the fibrinogen receptor (GPIIb/IIIa) are decreased during CPB. GPIV, a receptor for thrombospondin stabilization of the platelet aggregate[7] and a possible receptor for collagen,[8] has not been studied in the setting of CPB.

Platelet activation, as manifested by α-granule release, has been demonstrated in our laboratory to occur during CPB, as indicated by the appearance on the platelet surface of the α-granule glycoprotein responsible for platelet adhesion to monocytes and granulocytes—granule membrane protein (GMP)-140.[9] Platelet activation during CPB is supported by findings of increased serum concentrations of thromboxane B2 and β-thromboglobulin.[10–12] However, except for changes in GMP-140, the relationship of platelet activation to changes in platelet adhesive glycoprotein receptors in the setting of CPB has not been studied. Moreover, it is unknown whether alterations in surface adhesive receptors occur nonspecifically, perhaps through nonselective membrane shearing, or specifically, through receptor interaction with soluble factors.

Flow cytometry techniques allow the examination of large numbers of individual cells in whole blood fixed immediately after blood drawing, permitting the detection of changes in surface adhesive proteins of platelets "frozen in time."[13] It also allows the detection and quantitation of these changes in activated *versus* unactivated platelet subsets.[14] In the current study we used these techniques to ask 1) whether quantitative changes in platelet adhesive molecules occur specifically, through certain receptors, rather than nonspecifically, through global membrane alterations and 2) whether these receptor changes occur solely as a result of "activation" or whether unactivated platelets are also affected.

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Materials and Methods

All patient studies were approved by the Yale University School of Medicine Human Investigations Committee. After informed consent, 24 consecutive patients undergoing elective procedures requiring CPB were studied. Twenty of these patients underwent coronary artery bypass grafting, and four received aortic valve replacement. Patients having a prolonged bleeding time were excluded. Six patients had received aspirin or a nonsteroidal antiinflammatory agent in the 5 days before surgery but were included because of a normal template bleeding time. No other patients were receiving antiplatelet agents at the time of surgery. All patients had a platelet count within the normal range before surgery.

Patients received an initial heparin dose of 4 mg/kg; additional heparin was added as necessary to increase the activated clotting time to greater than 400 seconds. Patients were cooled to 28° C by a CPB circuit with a membrane oxygenator (COBE CML EXCEL). Whole-blood samples were drawn from the radial artery catheter at the following time points: immediately before incision, after heparin, 10 min after the start of bypass, just before separation from bypass, 2–4 h after termination of bypass, and 18 h after bypass. Protamine was given at 1.3 mg protamine per 1 mg heparin, for which the heparin was calculated by the heparin *versus* activated clotting time response curve and confirmed by a postprotamine activated clotting time within 5% of the patient's baseline activated clotting time.

A 50-μl aliquot of whole blood was immediately placed in 2% paraformaldehyde in phosphate-buffered saline; fixed samples were stored at 4° C for 1 h and then prepared as described below. Whole blood samples were also analyzed for hematocrit, hemoglobin, and platelet count by an automated blood cell counter (Coulter Electronics, Hialeah, FL), and platelet counts less of than 100×10^9 per liter were confirmed by manual counting. Blood loss was assessed by measuring chest tube drainage in the first 12 h after bypass. The number of platelet transfusions received in the same time period was recorded.

Monoclonal Antibodies

A murine monoclonal antibody, 1E3, recognizes a 140-kD glycoprotein that becomes associated with the platelet surface during α granule secretion.[15] This glycoprotein has previously been designated GMP-140[16,17] or platelet-activation–dependent granule external membrane protein.[18] Commercially obtained monoclonal antibodies included the following: anti-GPIb and anti-GPIIb/IIIa, both binding to epitopes at the ligand binding site[19,20] (AMAC, Westbrook, ME); anti-GPIV (Ortho Diagnostic Systems, Raritan, NJ); and anti-HLA A,B,C backbone (Accurate Chemical and Scientific Corporation, Westbury, NY). Antibodies were conjugated with fluorescein isothiocyanate (FITC), biotin, or phycoerythrin (PE) to allow dual fluorescence staining of platelet antigens.[21] For experiments in which biotin-conjugated antibody was used, secondary labeling with PE–avidin or FITC–avidin (Becton-Dickinson, Mountainview, CA) was carried out. Control labeling was performed with FITC-, PE-, or biotin-conjugated irrelevant murine monoclonal antibodies (Becton-Dickinson). Monoclonal antibodies directed at the following pairs of platelet surface glycoproteins were used for simultaneous staining:

Group I: 8 patients
- GPIb and HLA A,B,C
- GPIV and HLA A,B,C
- GPIb and GMP-140

Group 2: 16 patients
- GPIIb/IIIa and HLA A,B,C
- GPIIb/IIIa and GMP-140

Flow Cytometric Analysis

After incubating for at least 1 h at 4° C, fixed samples were washed and incubated with saturating concentrations of the antibody pairs as described previously.[14] The sample was then washed and analyzed in a FACScan flow cytometer (Becton Dickinson Immunocytometry Systems). The FACScan was set to measure forward light scatter, which is a relative index of particle size, and FITC and PE fluorescence, for expression of platelet glycoproteins, HLA, and GMP-140. Platelets were identified in whole-blood samples by triggering the cytometer on size and FITC/PE fluorescence.[21] Preliminary experiments and analysis of the 24 patients did not demonstrate any platelet population that had completely lost either GPIb, GPIIb/IIIa, or HLA. A gate was used to exclude cell fragments from the analysis, thereby also excluding any platelet microparticles that might have been present.

Analysis of 5,000 platelets was performed on each sample, and the parameters measured were 1) the mean total fluorescence of GPIb, GPIIb/IIIa, GPIV, and HLA A,B,C expression and 2) the fluorescence of GPIb and GPIIb/IIIa in platelets expressing GMP-140, *i.e.*, activated platelets, and in GMP-140-negative platelets. For purposes of comparison, absolute fluorescence values reflecting quantitative receptor surface expression were normalized to a percentage of baseline values in some experiments. There was no significant change in platelet size over time, thus eliminating formation of platelet aggregates or appearance of "giant" platelets as a source of apparent changes in glycoprotein density on the platelet surface.

Thiazole orange (Becton Dickinson Immunocytometry Systems), was used for ribonucleic acid (RNA) labeling of platelets in five patients of the second group. Thiazole

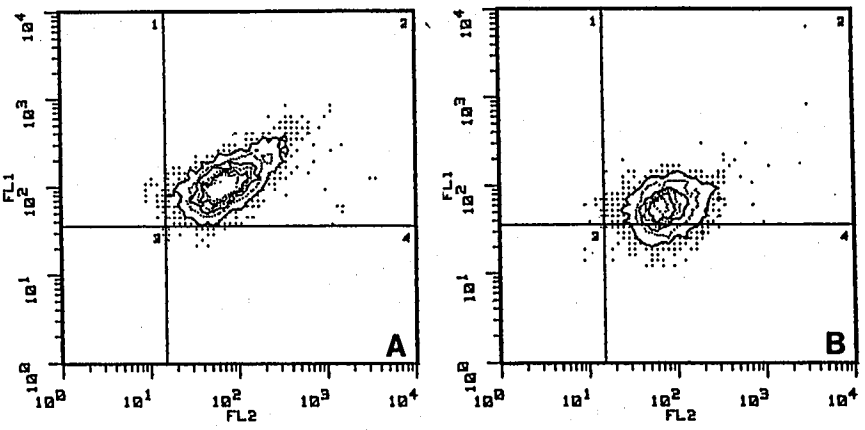

FIG. 1. Contour graphs of platelets, with the fluorescence (FL2) of the anti-HLA A, B, C antibody in a log scale on the x-axis and the anti-GPIb antibody fluorescence (FL1) in a log scale on the y-axis. *A*: A sample taken from a patient 5 min after heparinization. *B*: A similar sample taken 2–4 h after termination of CPB. Compared with plot *A*, GPIb decreased by 51% in plot *B*, whereas HLA A, B, C is unchanged.

fluorescence has been demonstrated to allow detection of very young platelets[22] (high RNA content) in the circulation, usually under conditions of extremely short platelet survival, such as occurs in immune thrombocytopenic purpura. Samples for thiazole orange staining first were incubated with PE–anti-GPIIb/IIIa antibody as above, were washed, and then were incubated with a saturating concentration of thiazole orange for 1 h at room temperature and then analyzed immediately on the FACScan.

All patient values for each time point were pooled and subjected to analysis of variance for repeated measurements, with significance accepted at $P < 0.05$. Values were compared with one another and with chest tube drainage using a two-tailed *t* test. Patients receiving platelet transfusions and the six patients who had received aspirin or a nonsteroidal antiinflammatory agent before surgery were analyzed separately for differences in platelet activation, chest tube drainage, and changes in platelet surface receptors. The sample size did not permit multivariate analysis to be performed.

Results

Surface expression of immunoreactive GPIb on platelets decreased to 84% of its baseline value by the end of CPB and continued to decrease to 72% of baseline at 2–4 h after CPB ($P < 0.05$). Representative contour graphs of the change in GPIb in a single patient are shown in figure 1. In the total patient population, by 18 h after termination of CPB, surface GPIb expression had returned to a value not significantly different from baseline (fig. 2). Distribution of GPIb expression on platelets remained monomodal in all studies; that is, no distinct subsets of platelets with regard to GPIb expression were identified. Activation of platelets, as measured by the percentage of platelets expressing GMP-140, increased to 15% at termination of bypass, peaked at 18% 2–4 h after bypass, and then returned to baseline at 18 h after CPB (fig. 3). As seen in fig. 4, for each patient, the change in percent activation (percentage of platelets expressing GMP-140) at 2–4 h after CPB showed a positive correlation with loss of GPIb ($r = 0.76$); that is, patients showing the greatest activation also showed the greatest loss in surface GPIb expression. For each patient sample, taken at 2–4 h after CPB (the point of maximum change), the expression of GPIb on platelets expressing GMP-140 ("activated") was compared to GPIb expression on GMP-140–negative ("resting") platelets. Both fractions showed loss of GPIb, but the activated fraction of platelets (GMP-140–positive) showed a greater average loss of surface GPIb (44%) than did the unactivated (GMP-140–negative) fraction (26%).

The thrombospondin binding protein, GPIV, showed no change during CPB. At 2–4 h after CPB it increased significantly, and then at 18 h after CPB it returned to baseline. Similarly, the non–adhesion-receptor protein HLA A,B,C did not change significantly over the course of CPB, increased 2–4 h after bypass, then returned to a

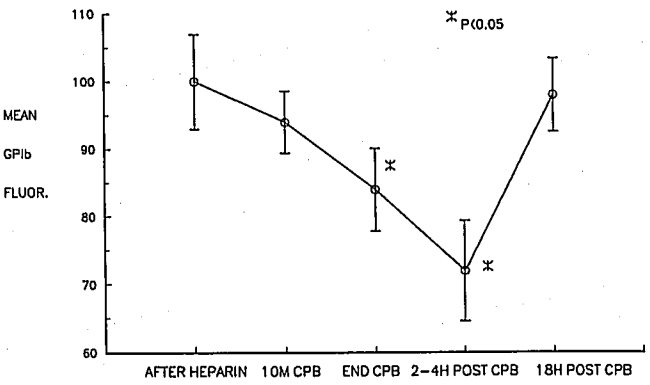

FIG. 2. Fluorescence of the anti-GPIb antibody expressed as a percentage of baseline beginning 5 min after heparinization ("AFTER HEPARIN"), 10 min after start of CPB ("10 M CPB"), just before separation from CPB ("END CPB"), 2–4 h after termination of CPB ("2–4 H POST CPB"), and approximately 18 h after CPB ("18 H POST CPB"). Values are mean ± standard error of the mean.

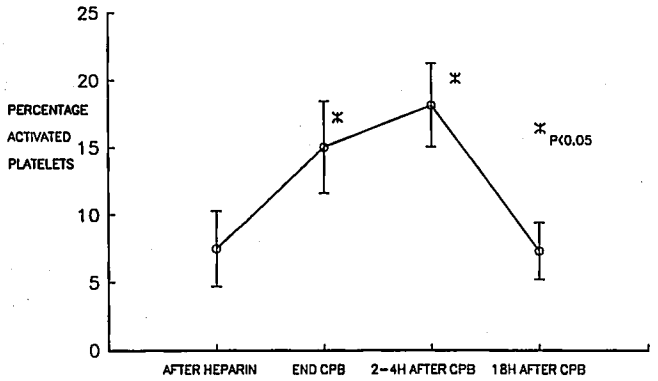

FIG. 3. Percentage of activated platelets, indicated by the percentage of platelets binding anti-GMP-140 at the different time points described in figure 1. Values are mean ± standard error of the mean.

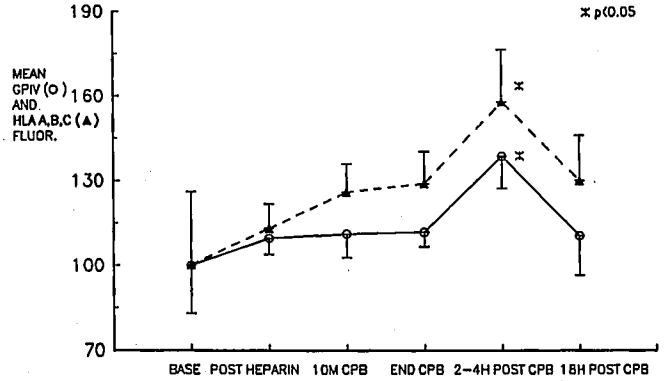

FIG. 5. Fluorescence of anti-GPIV (circles), expressed as a percentage of the baseline value, and anti-HLA A, B, C (triangles), also expressed as a percentage of baseline. Baseline for these receptors represents a sample taken after induction of anesthesia and before the start of surgery. Both are means ± standard error of the mean.

value not significantly different from baseline by 18 h after CPB (fig. 5). Analysis of the mean "size" of platelets as assessed by forward-angle scatter measurement on the flow cytometer[21] revealed no change in the mean forward scatter during or after CPB, which varied less than 5% from baseline in all cases. Similarly, there was no evidence of microaggregate formation.[21]

The expression of GPIIb/IIIa, as with GPIb, GPIV, and HLA A,B,C, was always monomodal. GPIIb/IIIa decreased to an average of 79% of baseline by the end of CPB, persisting at this value 2–4 h after CPB. Neither decrease reached statistical significance, despite the larger sample size (n = 16). GPIIb/IIIa expression at 18 h was therefore not measured. Unlike GPIb, the percentage decrease in GPIIb/IIIa was inversely correlated with the change in activation at the end of CPB ($P < 0.05$); that is, patients showing the largest percentage increase in activated platelets showed the least decline in surface GPIIb/IIIa (fig. 6). Both activated and unactivated platelet populations (defined by GMP-140 expression) showed a decrease in GPIIb/IIIa, although for each time point, the activated platelets demonstrated a higher average surface expression of GPIIb/IIIa than did unactivated platelets (fig. 7). Because of the possibility that this differential behavior of activated *versus* resting platelets might mask significant changes in GPIIb/IIIa induced by the CPB circuit, analysis of the changes in GPIIb/IIIa in the activated and unactivated populations was carried out separately. Indeed, when analyzed separately, receptor loss in each platelet subset at termination of CPB and 2–4 h after CPB reached significance at $P < 0.01$.

Decreased detection of immunoreactive antigen might be explained by occupation of the receptor by its ligand

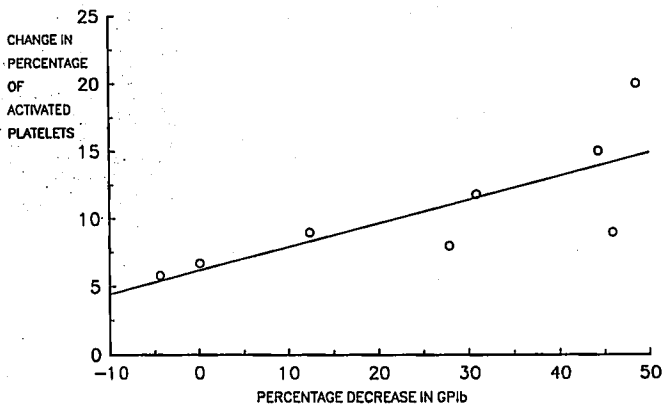

FIG. 4. Correlation between platelet activation and the percentage decrease in GPIb. The x-axis is the percentage decrease in GPIb in samples of whole blood taken after heparinization and 2–4 h after the end of CPB. The y-axis is the increase in the numbers of circulating activated platelets between these same time points. r = 0.76; $P < 0.05$.

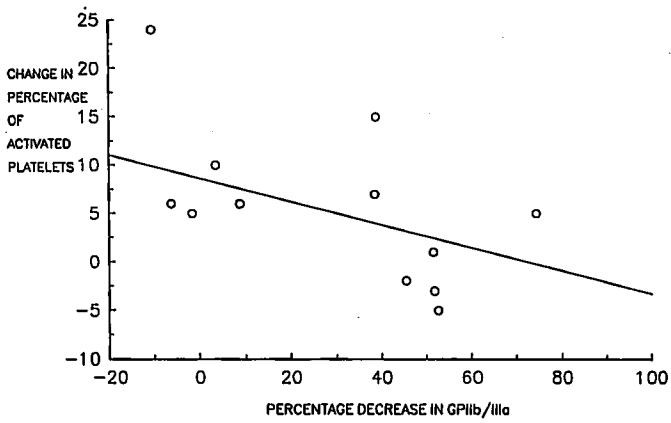

FIG. 6. Correlation between the change in the percentage of activated platelets and the percentage decrease in GPIIb/IIIa in samples of whole blood taken after heparinization and at the end of CPB. r = 0.6; $P < 0.05$.

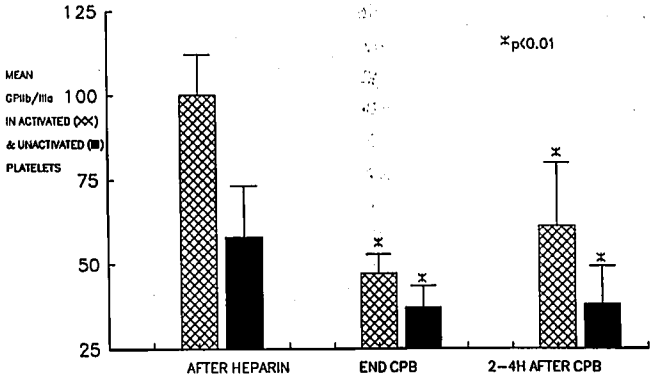

FIG. 7. Fluorescence of the anti-GPIIb/IIIa antibody in the activated platelets (platelets binding anti-GMP-140), shown in the hatched bars, and the unactivated platelet subset in solid bars at the same time points. Values are expressed as a percentage of baseline ± standard error of the mean. ANOVA analysis was performed to compare changes in GPIIb/IIIa within each subset.

(*i.e.*, competitive inhibition of the anti-GP monoclonal antibody by the ligand) rather than by actual receptor protein loss from the platelet surface. GPIb, however, has been demonstrated to bind only von Willebrand factor that has been prebound to subendothelium exposed to high shear stresses,[23] such that this scenario is highly unlikely for GPIb. To examine this possibility for GPIIb/IIIa, platelets from a patient after heparinization and at termination of CPB were gel-filtered, and total platelet bound fibrinogen was cytometrically assayed using polyclonal anti-fibrinogen antibodies[24] (Tago Laboratories). Platelets at the end of CPB showed no increase in bound fibrinogen as compared to the postheparin sample and demonstrated a decrease in immunoreactive GPIIb/IIIa typical for this interval. Thus, the change in detectable GPIIb/IIIa was not due to competitive inhibition of the monoclonal antibody by fibrinogen ligand.

The average duration of CPB was 110 min, with a standard deviation of 45 min, and did not correlate with the change in GPIb or the change in GPIIb/IIIa in either the activated or unactivated platelet subsets. The platelet count decreased from an average of 198,000 per microliter immediately before the onset of CPB, to 143,000 per microliter at the termination of CPB; this decrease was significant (at $P < 0.05$), and 2–4 h after bypass, at 151,000 per microliter, was essentially unchanged. These counts are in the range that has been reported by other studies.[1] One patient received platelets at the discretion of the surgeon shortly after termination of CPB and differed only from the rest of the patients in having a higher platelet count at the 2–4-h time point. One patient in the first group of 8 patients and 5 in the group of 16 patients received aspirin in the 5 days before surgery. Analyzed separately, their glycoprotein changes and chest tube drainage did not differ significantly from patients not receiving aspirin. Likewise, considered separately, the patients undergoing aortic valve replacement had a longer average CPB duration (150 *vs.* 110 min for coronary artery grafting) but did not differ in terms of chest tube drainage or glycoprotein changes from the rest of the study patients.

The average chest tube drainage was 90 ml during the first 2 h and 645 ml during the first 12 h. When analyzed with respect to changes in surface protein expression, neither parameter correlated with changes in GPIb, GPIIb/IIIa, or time on CPB. There was a weak correlation ($r^2 = 0.25$) with the platelet count at 2–4 h.

The percentage of platelets containing increased RNA, as measured by thiazole orange staining, did not change significantly over the course of CPB, suggesting that there is no significant influx of relatively immature platelets into the circulation from marrow sources over this early postoperative period.

Discussion

Surface expression of glycoprotein Ib, the binding site for von Willebrand factor in the initial adhesion of the platelet to the subendothelium,[25] decreased significantly over the course of CPB. This loss of GPIb continued in the first 2–4 h after CPB, and GPIb levels returned to baseline by 18 h after bypass. These findings are similar to those of George *et al.*[5] In addition, however, our findings, using simultaneous measurement of platelet activation, as indicated by α granule release resulting in surface expression of GMP-140, showed significant positive correlation of GMP-140 expression with GPIb receptor loss at the 2–4-h time point, demonstrating that patients with the greater extent of platelet activation showed the highest quantitative loss of GPIb.

It has recently been shown that platelet activation by thrombin *in vitro* results in decreased surface expression of GPIb,[26] and thus platelet activation during CPB may have contributed in part to the decrease in GPIb measured here. However, comparison of the activated with the unactivated platelet populations revealed that both demonstrated a decrease in GPIb; this suggests that activation is not the sole cause of decreased GPIb, even on platelets that have not undergone α granule release. At least two processes may be postulated to account for these platelet changes. Some work *in vitro* has suggested that plasmin formation can result both in platelet activation and in cleavage of the glycocalicin portion of GPIb[27,28] (although this observation has not been uniformly reproducible[29]). Plasmin, in combination with other as yet unidentified proteases activated by CPB,[30,31] may therefore contribute to both the platelet activation and decrease in surface GPIb measured here. Alternatively, the complement

fragment C5b-9, which has been demonstrated to increase significantly in plasma during CPB,[32] has been shown *in vitro* both to activate platelets and to cause the budding-off of platelet microparticles that are rich in GPIb.[33]

Either or both of these processes might operate during and after CPB to cause the loss of surface expression of GPIb demonstrated here. The recovery of surface expression of GPIb by 18 h postoperatively may be due to a combination of platelet release from marrow or spleen and relocation of GPIb to the platelet surface from intracellular pools. Intracellular GPIb has been demonstrated to move to the surface of GPIb-depleted platelets in a process that appears to require hours or even days,[28,34] similar to the time course suggested here.

Total platelet glycoprotein IIb/IIIa has previously been shown to be decreased in patients undergoing CPB[6]; this decrease may contribute to the abnormal adenosine diphosphate–induced aggregation in these patients. In our studies, surface expression rather than total platelet IIb/IIIa was measured. This decreased in the 16 patients studied, although proportionally the decrease was not as dramatic as that seen for GPIb. Also, unlike GPIb, the percentage loss of surface GPIIb/IIIa was inversely correlated with platelet activation. This finding is consistent with the known movement of as much as 30% of the total platelet GPIIb/IIIa from internal membrane stores to the surface of the platelet almost instantaneously when the platelet is activated to release its α granules.[35] At all time points measured, the GPIIb/IIIa density was an average of 13% higher in the activated platelets than in the unactivated fraction. When the surface GPIIb/IIIa expression was examined separately in the activated and unactivated platelet populations, both demonstrated a significant decrease. We therefore postulate that mechanisms distinct from platelet activation alone lead to a decrease in GPIIb/IIIa expression.

In addition, we hypothesize that the loss of average surface GPIIb/IIIa during CPB is less than that seen with GPIb because the simultaneously occurring platelet activation results in the movement of intracellular IIb/IIIa to the surface, thereby partly compensating for the loss of surface GPIIb/IIIa. Possible sources of the IIb/IIIa receptor loss include the effects of plasmin, which has been shown *in vitro* to cleave GPIIb/IIIa[36] as well as GPIb, and other fibrinolytic proteases. Microparticles formed by complement activation of platelets also appear to contain GPIIb/IIIa[33] and therefore may contribute to the decrease in GPIIb/IIIa.

In contrast to GPIb and IIb/IIIa, both GPIV and HLA A,B,C maintain their surface density during CPB and exhibit a slight increase at 2–4 h after CPB. The preservation of these glycoproteins suggests that the decreases measured in GPIb and IIb/IIIa are selective, rather than nonspecific, as for a shearing effect of the CPB circuit. The increases in GPIV and HLA A,B,C at 2–4 h after CPB may be the result of an influx of younger platelets with a higher surface expression of both of these glycoproteins. The average size of the platelets did not change, nor was there evidence of microaggregate formation that might artifactually increase surface expression of these proteins. The percentage of platelets containing increased RNA, which have been referred to as "stress platelets" or "reticulated platelets,"[22] did not change significantly after CPB, perhaps indicating that any new platelets in the circulation are recruited from sources other than the marrow, such as the spleen. *In vitro* activation of platelets does not increase expression of either GPIV or HLA A,B,C (data not shown), so the increase after CPB does not appear to be an artifact of the concurrent platelet activation that peaks at this time point.

One of the limitations of the present study is that it was not designed to examine the clinical correlates of these platelet changes. While the decreases in GPIb and GPIIb/IIIa and the increases in platelet activation examined individually showed no correlation with blood loss as measured by chest tube drainage, the ability to detect such a correlation is limited by the small number of patients studied here. Similarly, the occurrence of these changes together may result in a more profound qualitative platelet defect; however, the numbers also did not permit multivariate analysis to be performed. Inclusion of patients receiving aspirin and nonsteroidal antiinflammatory agents also might be faulted. Inhibitors of the arachidonic acid pathway, while impairing the ability to undergo irreversible aggregation, have not been demonstrated to affect platelet receptor density, and when analyzed separately, did not differ in any regard from those of the routine patient. Moreover, the normal preoperative bleeding time suggests that in the patients included in this study, the clinical impact of these drugs was minor. Data from the single patient who received a platelet transfusion might have biased the results toward earlier recovery of GPIb and GPIIb/IIIa; however, exclusion of that patient did not alter the findings for either glycoprotein.

Another possible limitation is that monoclonal antibodies as used in this study measure only the presence of an epitope on the receptor. Hypothetically, the antigenic site might be lost while receptor function was preserved. We believe that this is unlikely in the present study. The monoclonal antibodies used against GPIb and GPIIb/IIIa block ligand binding, implying proximity of the antigenic site to the ligand binding site on the receptor. Receptor occupancy by its adhesive ligand does not explain the decrease in GPIIb/IIIa, since platelets showed no increase in bound fibrinogen while still exhibiting a decrease in GPIIb/IIIa. GPIb binds von Willebrand factor only under

very specific circumstances, so although it was not possible to eliminate this hypothesis completely as a possibility, its probability remained very low.

GPIb functions in the initial adhesion of platelets to the subendothelium in vessels with high shear stresses such as the microcirculation.[37] The clinical impact of decreased expression of GPIb can be ascertained from Bernard-Soulier syndrome, a congenital GPIb-deficiency disease, in which GPIb levels below 40% of normal can produce clinically significant bleeding.[38] The average decrease in GPIb in this study was to 72% of baseline; however, individual patients demonstrated surface density as low as 41% of their baseline. In addition to defects related to platelet adhesion *via* the GPIb–von Willebrand factor receptor–ligand pair, this study has demonstrated decreases in the surface expression of the fibrinogen receptor GPIIb/IIIa. Loss of GPIIb/IIIa has the potential to impair platelet–platelet binding,[39] and abnormal platelet aggregation has previously been demonstrated in patients undergoing CPB.[3,4] The combination of GPIb and IIb/IIIa loss from the surface may be synergistic in decreasing platelet function.

Persistent circulation of activated platelets has been described by us in earlier work.[9] Such degranulated platelets can become trapped in the platelet aggregate but are unable to contribute clot promoters such as thrombospondin,[7] platelet adenosine diphosphate,[40] and activated factor V[41] and may therefore be particularly vulnerable to fibrinolysis. The present investigation has demonstrated a correlation between platelet activation and decreases in GPIb and GPIIb/IIIa, suggesting that the two processes may be at least temporally linked. Platelet activation alone, however, does not fully explain changes in surface expression of adhesive receptors.

In conclusion, CPB produces changes in platelet surface adhesion receptors, and these changes have the potential to affect adhesive function at multiple levels. Loss of GPIb may result in a defect in platelet adhesion to damaged endothelium. Loss of GPIIb/IIIa may impair platelet–platelet interactions, and continued circulation of a significant number of activated platelets may result in platelet aggregates that are particularly vulnerable to the actions of fibrinolytic enzymes. These adhesion receptor changes are specific and are not the consequence of generalized platelet membrane loss. The use of flow cytometry to measure simultaneously multiple platelet features may prove invaluable in the evaluation of interventions designed to block CPB-induced changes.

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