The repertoire is dominated by (oligo)clonal expansions early after BMT, characteristic of the fetal repertoire. In addition, because the B-cell lower frequencies during fetal development. For instance, an estimation of V_H family expression levels may not be the most suitable marker to distinguish fetal- or adult-type immune reconstitution. This pattern of diversity is consistent with the fact that it is conserved through evolution—i.e., it has been described in species as diverse as frogs, rabbits, mice, and humans and holds true for both B- and T-cell receptors. Prime determinants of CDR3 size are the usage pattern of diversity (D_Q) elements and the length and frequency of N-regions—stretches of DNA that are added by the enzyme terminal deoxynucleotidyl-transferase during formation of the antigen receptor gene. As compared with the situation in the adult, N-regions are expressed at lower frequencies during fetal development. For instance, an estimated 20% of CDR3 regions in 12- to 14-week-old fetal livers lack N-regions altogether. In addition, up to 50% of fetal B-cell receptor CDR3 segments use the DQ52 D_H element (which is relatively small). By contrast to this fetal pattern of CDR3 diversity, CDR3 regions in reconstituting B cells exhibit none of the characteristics that define a fetal repertoire: they rarely encode DQ52, they exhibit adult patterns of N-region addition, and their general size is indistinguishable from that in adult peripheral blood. This pattern of diversity is identical to that in adult bone marrow pre-B cells (which also produce adult-type CDR3 regions). In other words, there is no reason to expect a recapitulation of fetal development after BMT, because the graft, consisting of adult lymphoid progenitors, is placed in the adult environment of the recipient.

In conclusion, immune reconstitution after BMT follows many established ontogenetic patterns relating to the appearance of particular membrane markers, Ig subclasses, and onset of antigen receptor rearrangements. The sequence of events that occur during successful BMT can be regarded as a blueprint for immune reconstitution in other clinical settings as well. However, in the description and interpretation of these events, it is important to realize that immune reconstitution does not appear to recapitulate human fetal ontogeny.

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REFERENCES

To the Editor:

Several laboratories have proposed that glycoprotein (GP) Ib, the receptor for von Willebrand factor (vWF) on platelet external surfaces, is rapidly redistributed to internal membranes during cardiopulmonary bypass surgery, induction of bleeding time wounds, or exposure to thrombin in vitro (for review, see Nurden). The recent article by van Zanten et al confirms the observation that GP Ib decreases on the surfaces of activated platelets, but shows that a 50% reduction in the vWF receptor does not affect platelet adhesion under flow conditions. The findings contrast with those of earlier investigators who presumed that clearance of GP Ib resulted in decreased adhesive capacity, but agree with reports showing that carriers of the Bernard-Soulier syndrome (BSS) with half the normal number of GP Ib copies on their platelets do not bleed. Yet, following the logic of previous investigations, BSS carrier platelets should also lose 60% to 80% of the half normal number of GP Ib receptors present on their resting cells after activation in vitro or in vivo. Thus, the assumption that the data obtained in this study explain why carriers of BSS syndrome do not bleed seems open to question. A more inviting rationale regarding the lack of bleeding symptoms in carriers of BSS might be that the half normal number of GP Ib receptors remain on exposed membranes of their activated platelets for interaction with damaged vascular surfaces.

Results of the ultrastructural studies performed by van Zanten et al are pertinent to that concern. Normal platelets treated with thrombin receptor activation peptide (TRAP) in suspension showed a marked reduction in immunogold particles on external membranes and increased frequency of particles associated with membranes of the open canalicular system (OCS) when compared with resting control cells. However, when TRAP-treated platelets were allowed to interact with collagen under conditions of high shear, the frequency of immunogold particles bound to GP Ib on external and internal membrane surfaces was the same as that on resting control platelets. The investigators suggested that GP Ib receptors cleared to the OCS after exposure to TRAP in suspension were rapidly returned to the exterior membrane during spreading on collagen.

An alternative explanation for the results obtained by van Zanten et al deserves consideration. There appears to be considerably more internal membrane in the TRAP-treated platelet shown in Fig 4B of their report than in the control cell shown in Fig 4A. The number of OCS channels does not increase after platelet activation in suspension, but due to shape change and internal transformation, OCS channels become dilated and indistinguishable from the surface membranes with which they are continuous.

Our experience indicates that it is always easier to label the receptors of dilated channels than those on membranes of narrow canalici in well-preserved, discoid platelets. Others are beginning to make similar observations. As an example, we have noted the same distribution of GP Ib on external and internal OCS membranes of platelets from patients with giant platelet disorders, other than the BSS, whose OCS channels are usually dilated (Figs 1 and 2). Another confirmation is based on studies with cytochalasin B (CB), an agent that inhibits new actin filament assembly and is reputed to prevent translocation of GP Ib. CB causes dilation of the OCS of resting cells. Immunogold staining for GP Ib is as prominent on membranes of the OCS in CB-treated discoid platelets as on the exposed surface (Figs 3 and 4).

Accepting the possibility that immunocytochemical techniques have limitations in their ability to quantitate antigens on narrow internal channels would lead to a second view of the morphometric data presented in Table 2 of the report by van Zanten et al. According to the data, gold labeling for GP Ib in resting platelets is distributed 70% versus 25% (external v internal membranes). After TRAP activation, the distribution is modified to 35% versus 58% (external v internal membranes).

Studies of giant platelets and CB-treated normal platelets described above would lead to the assumption that the frequency of GP Ib/IX receptors on external and internal membranes of resting discoid platelets should be the same. Thus, a starting point of 47.5% versus 47.5% (external v internal membranes) would be expected. Current literature suggests that activation with TRAP should cause disappearance of 60% to 80% of GP Ib/IX receptors from the platelet surface as a result of translocation to internal membranes. If that was the case, immunolabeling on TRAP-activated platelets would show immunolabeling distributions close to 20% versus 75% (external v internal membranes). This would represent a real internalization of GP Ib receptors. Interestingly, the results in Table 2 of van Zanten et al show that the immunolabeling on the internal membranes of TRAP-activated platelets is still below that on external membranes of resting cells (57.9% v 69.0%; activated v resting). We believe that the proposed increase in the percentage of gold particles identifying GP Ib on internal membranes of TRAP-treated platelets may be due to their increased availability for staining in dilated channels rather than to translocation.

Restoration of the percentages of gold particles marking GP Ib on internal and external membranes of TRAP-treated platelets after spreading on collagen to values found on resting platelets in suspension is also of interest. During the course of surface interaction, channels of the OCS are evaginated and become part of the exposed surface, accounting for a greater than 400% increase in surface area on spread platelets. This should result in a decreased percentage of gold particles on internal membranes, because few OCS channels remain inside. It should also cause a marked increase in the relative percentage of particles on the spread cell surface for the same reason. It is unlikely that fully spread cells would have the same percentages of GP Ib on internal and external membranes as found in suspended control cells. Evidence has been presented to support that point of view. Perhaps van Zanten et al could respond to these concerns.

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Distribution of GPIb on and in Resting Platelets
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