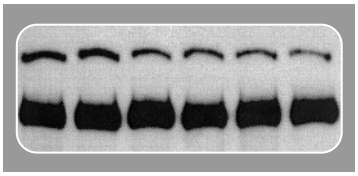
inside blood

GPIb, filamin, and platelet activation: a view from within

The platelet glycoprotein Ib (GPIb) receptor, once thought to have a straight-forward hemostatic function by mediating high-shear–dependent platelet adhesion to von Willebrand factor (VWF), is increasingly recognized as having a potentially diverse range of cellular functions. Over the last few years, an impressive number of potential ligands (VWF, thrombin, factors XI and XII), counter-receptors (Mac-1 [CD11b/CD18], P-selectin), and binding partners (filamin A, 14.3.3) for GPIb have emerged, and functional roles of this receptor now



extend to the regulation of the platelet cytoskeleton, coagulation, and potentially inflammation. GPIb also has the capability of transmitting signals linked to integrin $\alpha_{IIb}\beta_3$ activation, a fundamental step in the normal hemostatic and thrombotic processes. However, a clear understanding of how this receptor transduces signals has yet to emerge. One possibility, examined by Feng and colleagues in this issue (page 2122), is that the membrane skeletal anchorage of GPIb, as a consequence of its binding to filamin A, is necessary for cytoskeletal signaling events linked to integrin $\alpha_{IIb}\beta_3$ activation.

Feng and colleagues delve deeper into the molecular basis and functional importance of the interaction between the cytoplasmic tail of GPIbα and filamin A. In initial studies, they provide further definition to the specific region in the GPIbα tail that is required for filamin A binding, suggesting that the primary binding site may reside within residues 557-575, a finding consistent with previous observations.[1,2] To elucidate the functional consequences of disrupting the GPIb-filamin interaction in intact platelets, Feng et al employed a novel approach using a cell-penetrating peptide incorporating residues 557-575. The peptide specifically inhibited VWF-dependent aggregation and, under pathologic shear conditions, inhibited GPIb association with filamin. Moreover, the reduction in filamin binding to GPIb correlated with the inhibition of shear-dependent tyrosine phosphorylation suggests a role for this interaction in the regulation of GPIb signaling and, potentially, integrin $\alpha_{IIb}\beta_3$ activation.

These results are interesting and potentially important and, although they fall short of definitively establishing an essential role for the GPIb-filamin interaction for integrin $\alpha_{IIb}\beta_3$ activation, they nonetheless provide additional evidence that this interaction is likely to play a fundamental role in regulating the adhesive and signaling function of GPIb. These studies are timely as the recent development of transgenic mouse models expressing mutant forms of GPIb, combined with an improved understanding of the molecular basis for the GPIb-filamin interaction, sets the scene for more definitive studies on the importance of this interaction for normal platelet physiology. Perhaps some clarity to this long-standing vexing issue of platelet function will soon be realized.

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Good-bye to GVHD

Bone marrow transplantation would be even more widely used if the complications of graft-versus-host disease (GVHD) could be routinely and simply avoided. GVHD is induced by T cells in the bone marrow (BM) graft recognizing and reacting against the host. The disease tends to be much more severe when host and donor differ at the major histocompatibility complex (MHC) but can also occur for MHC-identical transplants. The simple solution of removing all T cells from the graft prevents GVHD induction but then the graft, for reasons that are not totally clear, often fails. Residual T cells not only promote engraftment but also may provide some immune (particularly antitumor) protection. A solution to this problem might be a procedure that specifically removes only those T cells in the graft that can recognize and react against the host. Bachar-Lustig and colleagues (page 1943) describe such a protocol using "veto cells" for the successful transplantation of fully allogeneic BM containing large numbers of allogeneic T cells. They have worked with a mouse model but the design is such that the protocol should be readily transferable to the clinic. More important, they also show that when the immunosuppressive drug rapamycin is included in the protocol, it synergizes with rather than blocks the action of the veto cells.

Veto cells have been defined as cells that can delete T cells that recognize them.[1] As reviewed by Bachar-Lustig and colleagues, a number of different cells appear to have this ability, among them being $CD8^+$ cytotoxic T-lymphocyte (CTL) lines. Let A, B, C, and D represent 4 MHC-different inbred mouse strains. When an A anti-B mixed-lymphocyte reaction (MLR) is set up, strain-A origin CTLs reactive against strain B are generated. If $CD8^+$ CTLs syngeneic to the strain-B stimulator but not reactive against the A-strain responder (eg, from a B anti-D MLR) are included in the culture, they act as veto cells.[2] A anti-B CTL precursors in the culture that recognize strain-B antigens on the added CTLs are now known to undergo apoptosis in a process involving both CD8 and Fas-L on the veto cell (Figure 1 in Bachar-Lustig et al). When the same B anti-D CTLs are added to an A anti-C MLR they do

not affect the response because the A anti-C–activated CTL precursors cannot recognize them. Bachar-Lustig et al have effectively done the same series of experiments in vivo. Lethally irradiated strain-B mice received strain-A BM transplants containing large numbers of strain-A T cells. Rapid death ensued unless cells from a B anti-D CTL line were also included. Addition of rapamycin could make these cells more effective. In principle, the problem of GVHD has been solved!

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Monosomy 7 and the myeloid malignancies

Cytogenetics has made an enormous contribution to our understanding of the pathophysiology and prognosis of childhood leukemia. In most cases of acute lymphocytic leukemia (ALL) various translocations within a pre-B cell that probably occurred in utero set the stage for another event or events that trigger unbridled growth. The individual translocations, though not solely responsible for the leukemia, have enormous prognostic significance. The tel–acute myeloid leukemia 1 (AML1) fusion associated with a (12:21) translocation has a standard chemotherapy-induced cure rate of between 90% and 100%. In contrast, ALL with the Philadelphia chromosome is only effectively treated with massive chemoradiation therapy and stem cell transplantation.

In this issue of *Blood,* Kardos and colleagues (page 1997) review a large European experience of refractory anemia in childhood. This heterogeneous collection of premyeloid and virtual myeloid leukemias is characterized by several different cytogenetic abnormalities, the most glaring of which is monosomy 7, a disorder characterized by an extremely poor prognosis. It must be treated with stem cell transplantation. Even a matched unrelated donor transplant offers a better chance for survival than watchful waiting or chemotherapy. Adults with monosomy 7 usually have multiple cytogenetic abnormalities, whereas the disorder is usually unadulterated in children. The haploinsufficiency associated with loss of the short arm of the chromosome is probably responsible for the development of malignancy that occurs at a variable rate even in patients with familial loss of the chromosome. The collection of monosomy 7 cells established by Kardos et al provides an important opportunity to learn much more about this disorder. It is vital to study gene expression in monosomy 7 to learn how the chromosome disorder leads to malignancy. It is believed that loss of a key suppressor is responsible, but does this permit the unbridled expression of a tyrosine kinase that finally drives the leukemia? This is the next step of inquiry in this instructive disease. For now, Kardos and her colleagues have given us important information on the best treatment approaches.

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Challenges and progress in gene therapy for hemophilia A

In this issue, Powell and colleagues (page 2038) report the results of a phase 1 gene therapy clinical trial for hemophilia A, based on intravenous injection of a retroviral vector encoding B-domain–deleted factor VIII (FVIIIΔB). This phase 1 trial was based on encouraging preclinical studies, mostly in rabbits,[1] and essentially confirms the safety of this approach in patients. The vectors could be detected in the peripheral blood mononuclear cells for at least a year. Although some participants had detectable circulating FVIII levels (>1%) on repeated occasions, experienced fewer bleeding episodes, and required fewer FVIII protein infusions compared with historic rates, the clinical benefits were overall rather limited and a dose response was lacking. It appears therefore that the preclinical studies that constituted the basis of this trial may not have accurately predicted the vector doses required to achieve therapeutic FVIII levels. However, the limited efficacy of this particular gene therapy approach in adult patients was not entirely unexpected, due to the inability of retroviral vectors to transduce nondividing cells.

Previous studies had shown that stable therapeutic levels of FVIII or FIX could only be obtained in neonatal hemophilic mouse and dog models or in adult mice that received hepatocyte growth factor to stimulate hepatocyte cell division.[2,3] This inherent limitation of retroviral vectors justifies the development of vectors that can also transduce nondividing cells, such as lentiviral[4] adeno-associated viral vectors (AAV). In this same issue, Scallan and colleagues (page 2031) report stable expression (> 14 months) of therapeutic levels of FVIII (2%-4%) in 2 dogs with hemophilia A following liver-directed gene therapy using an AAV-based vector encoding FVIIIΔB. Although AAV has successfully been used for gene therapy in hemophilia B dogs[5] and results from clinical trials for hemophilia B are encouraging, progress in hemophilia A gene therapy has been hampered by the inherent, limited packaging capacity of AAV and the relatively large size of the B-domain–deleted FVIIIΔB cDNA. Scallan and colleagues showed that this limitation could be overcome by using small regulatory elements to drive FVIII expression, in accordance with previous reports.[6] Although recent studies had shown that therapeutic levels of FVIII could be achieved in hemophilia A dogs with no apparent toxicity following gene therapy,[7] the work by Scallan and colleagues is an important step forward since it is the first demonstration that long-term phenotypic correction of the bleeding diathesis could be achieved, albeit partial, in a clinically relevant, large animal model of hemophilia A. However, the reason for the lack of a dose response is not clear and warrants further studies in larger cohorts. Additional improvements in vector design and increased gene transfer efficiencies will be required to further increase FVIII expression