responded within 90 days from the last dose of daclizumab, although only 2 showed normal blood counts. One patient progressed under treatment; however, he did respond to subsequent treatment with antithymocyte globulin (ATG)/cyclosporine (CsA). This finding, together with available data reported in patients treated with ATG, suggests that the immunosuppressive/immunoregulatory effects induced by daclizumab may be inferior to ATG, the conventional modality for AA. Indeed, the incomplete response in many of the patients and the relapse already observed in one patient suggests that more effective immunosuppression may still be indicated for effective treatment of AA. The option of continuous treatment to prolong the beneficial effects of daclizumab may improve the long-term outlook, but is less preferable than achieving better results up front. Unfortunately, the results in the table summarizing the actual blood counts appear to be somewhat less impressive than the general conclusions, since 2 of 16 patients considered in complete remission (CR) did not show normal platelet counts or any dramatic increases in other values. Therefore, it appears that the authors' conclusions should be adopted with great caution, since the severity of the disease of the patients selected was moderate and the success rate of treatment, compared with conventional immunosuppressive modalities such as ATG, was less than optimal. However, it should be remembered that for patients, especially the young ones, with severe AA, allogeneic stem cell transplantation (SCT) still remains the treatment of choice. Also, since procedure-related toxicity and mortality are currently extremely low due to available reduced-intensity conditioning, SCT should be considered for patients with a fully matched donor available, even those who do not meet the full criteria of severe AA for patients. This possibility must be weighed against long-term immunosuppressive treatment, which is time consuming and frequently not effective. A most interesting observation noted by the authors was the relationship of responsiveness to daclizumab and the presence of interferon-γ: 5 of 6 tested positive and responded to daclizumab, whereas no response was observed in any of the 5 patients testing negative. Since similar observations were reported in another study in AA, such a test may be used to predict responsiveness, and may possibly serve as a guideline for choosing the proper protocol for patients who do not qualify for SCT. Also of interest is the implied role of IL-2 receptor activation, which could shed more light on the mechanisms controlling autoimmunity. Taken together, it appears that AA is a complicated multifactorial syndrome caused, perhaps, in patients who do respond to immunosuppressive treatment, by an autoimmune syndrome, and caused by other etiologies in patients who do not. A better understanding of the mechanisms of AA, including the role of lymphocyte subsets and their cytokines, will certainly help elucidate the etiology and thus improve treatment strategies.

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TCR gene therapy of leukemia

The power of adoptive immunotherapy with antigen-specific cytotoxic T lymphocytes (CTLs) was recently demonstrated in advanced-stage melanoma patients.[1] Antigen-specific immunotherapy of leukemia can be directed toward leukemia-specific antigens such as the products arising from translocations (eg, BCR/ABL) and mutations (eg, RAS), or against leukemia-associated self-antigens (eg, Wilms tumor antigen 1, proteinase 3, murine-double-minute 2 [MDM-2]) that are expressed at high levels in leukemic cells but also present in normal tissues. Following allogeneic stem cell transplantation, CTLs can also recognize leukemia-associated alloantigens, such as minor histocompatibility antigens (mHags) expressed in cells of the hematopoietic lineage (eg, HA-1, HA-2). While tolerance mechanisms are likely to blunt CTL responses against leukemia-associated self-antigens, they do not interfere with CTL responses against lineage-specific mHags. High-avidity donor CTLs can directly recognize and attack leukemia cells and patient hematopoietic cells, but spare patient nonhematopoietic tissues and donor hematopoietic cells. However, some mHags, such as the CTL-recognized HA-2 allele, are expressed in most humans, limiting immunotherapy options to rare HA-2–mismatched patient-donor combinations. In this issue, Heemskerk and colleagues (page 3530) demonstrate an elegant strategy that can, paradoxically, result in HA-2–specific CTL therapy in HA-2–matched patient-donor combinations.

This strategy takes advantage of *TCR* gene transfer and of the fact that T-cell receptor (TCR) recognition of HA-2 is HLA-A2 restricted. The genes encoding the TCR α and β chains were cloned from CTL clones isolated from a patient who received a rare HA-2–mismatched, HLA-identical transplant. Heemskerk et al demonstrated that retroviral vectors readily transferred the *TCR* genes and the HA-2 specificity to T cells of HLA-A2–positive individuals and, importantly, to T cells of HLA-A2–negative individuals. The *TCR*-transduced HLA-A2–negative CTLs specifically recognized the HA-2 peptide epitope presented by HLA-A2 class I molecules, indicating that the CTLs were HLA-A2 restricted. This provides a rationale for HA-2–directed immunotherapy of patients undergoing HA-2–matched, HLA-A2–mismatched stem cell transplantation. HA-2 specificity is created by *TCR* gene transfer into A2-negative donor T cells, and selective attack of patient hematopoietic cells, including leukemia cells, is achieved by the HLA-A2 restriction of the transferred TCR.

TCR gene transfer is a powerful new technology capable of producing antigen-specific CTLs without the need for prior antigen-specific immunization. In fact, as shown by Heemskerk et al, *TCR* gene transfer can be exploited to endow CTLs with novel specificities that are not present in the natural TCR repertoire. In the future, it will be possible to develop *TCR* gene therapy options that do not require allogeneic transplantation. For example, several technologies have been developed to bypass immunologic tolerance allowing isolation of

high-affinity TCRs specific for leukemia-associated self-antigens.[2,3] The transfer of such TCRs into human CTLs has been reported,[4] and experiments in mice have shown that *TCR*-transduced CTLs can eliminate tumor cells in vivo.[5] It will be exciting to move, after appropriate risk assessment, to clinical studies with *TCR*-transduced CTLs in leukemia patients.

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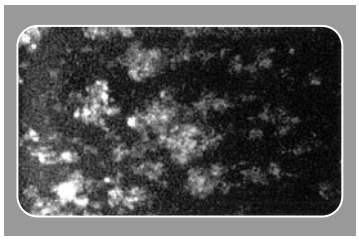
A tale of 2 (or more) tails: PECAM-1 regulation of GPIb-IX-V signaling

A defining characteristic of many blood cells is their ability to attach to the vessel wall from flowing blood. Of all blood cells, platelets are uniquely able to attach efficiently at shear stresses exceeding 10 dynes/cm^2, a property that reflects their preeminent role in stanching blood loss in areas of high flow velocity and high shear stress. This specialized function is carried out by the glycoprotein Ib-IX-V (GPIb-IX-V) complex, a peculiarly adapted agglomeration of 4 polypeptides that binds its vessel wall ligand, von Willebrand factor (VWF), through a ligand-binding domain situated atop a long mucin stalk. This interaction is insufficient to firmly arrest the platelets, allowing them only to roll on the surface until an integrin (either $\alpha 2\beta 1$ or $\alpha_{IIb}\beta_3$) is engaged. Integrin activation and other prohemostatic reactions are facilitated by intracellular signals generated by VWF binding. Although the 4 GPIb-IX-V polypeptides lack kinase activity and are not tyrosine phosphorylated, VWF binding activates a number of signaling pathways involving tyrosine and phosphatidylinositide phosphorylation,[1] facilitated by localization of a portion of the complex to lipid rafts.[2] Among the phosphorylated substrates in human platelets are 2 membrane proteins containing immunoreceptor tyrosine-based activation motifs (ITAMs),



the Fc receptor FcγRIIA, and the Fcγ chain.

In this issue of *Blood,* Rathore and colleagues (page 3658) demonstrate that in addition to activating stimulatory pathways, the GPIb-IX-V–VWF interaction produces signals that lead to phosphorylation of platelet endothelial cell adhesion molecule-1 (PECAM-1), a transmembrane protein containing 2 immunoreceptor tyrosine-based inhibitory motifs (ITIMs) and previously shown capable of negatively regulating the ITAM-containing receptors by engaging the hematopoietic phosphatase Src homology 2–containing tyrosine phosphatase 2 (SHP-2), which dephosphorylates ITAMs. This study extends the inhibitory role for PECAM-1 in regulating postadhesion signaling, a role previously described for collagen-induced signaling.[3,4] Using platelets from mice lacking PECAM-1, these investigators demonstrate an increased rate, but not extent, of Fcγ chain phosphorylation upon VWF binding. This correlates with increased platelet spreading on a VWF surface, enhanced aggregation to VWF/botrocetin, and enhanced thrombus formation on a VWF surface under flow.

A major question raised by these studies is teleologic: why should platelets possess such an inhibitory pathway, particularly given that most platelet stimuli positively feedback to activate other platelets? Activated platelets, for example, produce the potent platelet agonist thromboxane A2, release granules containing the agonists adenosine diphosphate (ADP) and serotonin, and externalize negatively charged phospholipids that facilitate coagulation and generation of the most potent agonist, thrombin. One clue is provided in the study of thrombus formation, where the inhibitory effect of PECAM-1 on thrombus accumulation was not seen at the highest shear stress. Perhaps the stimulatory pathway protects against one potentially deleterious aspect of the GPIb-VWF interaction: it is induced in the fluid phase by high shear stress and is largely responsible for shear-induced platelet aggregation and the production of embolic microthrombi. In this situation, despite the high shear stresses required, the strain applied to the bond is low because the ligand is not fixed to a surface stationary with respect to the flowing blood. Thus, fluid-phase association at high shear stress is similar to the attachment of the platelets to a stationary surface at low shear stress—a situation in which PECAM-1 markedly attenuates the activation signals. This possibility will have to be tested experimentally. Nevertheless, the data presented by Rathore et al provide tantalizing insights into the potential physiologic relevance of adhesion-induced inhibitory pathways.

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