(page 1261) provide the first in vivo demonstration of elimination of T cells expressing an inducible Fas molecule. Their construct consists of a chimeric human protein expressing the Fas intracellular domain with 2 copies of an FK506-binding protein, which should not be immunogenic as it contains only human components.[4] Transduced cells rapidly undergo apoptosis in vitro with the addition of subnanomolar concentrations of AP1903, a bivalent "dimerizer" drug that binds FK506 binding protein and induces Fas cross-linking and that has proved safe in testing in healthy volunteers. In the current study, Berger et al evaluate this approach in a primate model and show administration of AP1903 results in elimination of autologous T cells transduced with this construct.

One potential drawback is that a small number of transduced cells were unresponsive to CIDs and survived. While it is unclear what the clinical significance of this will be, the authors make a compelling argument that the surviving cells are resting T cells with a low level of transgene expression and are as such less likely to be alloreactive. Alternatively, it may reflect an effect of cellular inhibitors of apoptosis and could potentially be overcome by using a downstream-acting molecule in the caspase family.[3,5] A second issue is that a transgene-specific immune response was generated, but epitope mapping showed that it was directed at sequences in the Fas suicide construct that differed between human and macaque, so it is unlikely to be a problem in human clinical studies. The results described in Berger et al are therefore encouraging that CIDs can be used to induce death if necessary in adoptively transferred T cells transduced with human apoptosis molecules.

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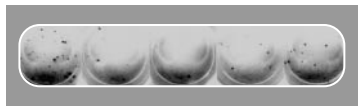
Anti-CD40L: biology and therapy in ITP

Immune thrombocytopenic purpura (ITP) is an autoantibody-mediated disease. Platelet-reactive T cells have been found in the blood of patients with this disorder, with the major target antigen being platelet membrane glycoprotein IIb/IIIa (GPIIb-IIIa); autoreactive $CD4^+$ T cells are found in lesser numbers in the blood of healthy controls. Kuwana, Ikeda, et al in a series of studies have demonstrated that B-cell production of antiplatelet antibody requires antigen-specific $CD4^+$ T-cell help. Thus, T cells are related to the pathogenic process in chronic ITP.[1]

CD40L (CD154, gp39), a transmembrane protein and member of the tumor necrosis factor (TNF) family, is expressed on activated $CD4^+$ T cells, mast cells, basophils, eosinophils, natural killer (NK) cells, and activated platelets. CD40L is important for T-cell–dependent B-cell responses; a prominent function of CD40L, isotype switching, is demonstrated by the hyper–immunoglobulin M (IgM) syndrome in which CD40L is congenitally deficient. The interaction of CD40L-CD40 (on antigen-presenting cells such as dendritic cells) is essential for T-cell priming and the T-cell–dependent humoral immune response.[2] Therefore, interruption of the CD40-CD40L interaction with an anti-CD40L monoclonal antibody (mAb) has been considered to be a possible therapeutic strategy in human autoimmune disease, based upon the above information and on studies in animals.

In this issue, Kuwana and colleagues (page 1229) report on a phase 1 study of anti-CD40L humanized mAb (IDEC-131/E6040) in patients with refractory ITP that allows them to link pathobiology with treatment effect. The investigators explored the in vivo effects of a cohort dose escalation (1 to 10 mg/kg) single infusion study of the effects of anti-CD40L mAb on 3 types of autoreactive T- and B-cell responses to GPIIb/IIIa at 3 time points (before treatment at day 0; after treatment at days 7 and 42) and compared them with the platelet responses. A platelet response was achieved only in 3 of the 5 patients treated at the highest dose (10 mg/kg), even though all 5 patients at this dose level had decreased numbers of B cells producing anti-GPIIb/IIIa antibodies by enzyme-linked immunospot (ELISPOT), reduced GPIIb/IIIa-induced T-cell proliferation, and decreased anti-GPIIb/IIIa antibody in vitro. No platelet increases were seen at the 3 lower doses, even though patients treated at both the 5 and 10 mg/kg doses showed these autoimmune responses to be decreased. T-cell response to an irrelevant antigen (to which



there was no ongoing stimulation) was not affected by anti-CD40L. The authors speculate that failure to achieve a platelet effect at 5 mg/kg, despite suppression of the autoimmune responses, may have been related to the shorter duration of the effect. It is also likely that extravascular effects (ie, in the spleen or other parts of the reticuloendothelial system) are critical and require a higher dose. In addition, there may be a heterogeneity in the pathophysiology of ITP since the responders and nonresponders at 10 mg/kg could not be distinguished on the basis of their in vitro responses.

As the authors indicate, there are 3 reports[3-5] on clinical trials using another anti-CD40L mAb (hu5c8) in patients whose ITP was substantially more refractory than those included in this study (only 7 of 20 reported here had undergone splenectomy). From these reports and unpublished data (J. B. B., January 2004) for IDEC-131/E6040, it appears that 25% to 50% of patients with refractory ITP will have platelet responses to doses of 10

to 20 mg/kg of anti-CD40L administered every 2 to 4 weeks although the responses are rarely durable. These abstracts,[3-5] together with the study reported here, suggest that CD40/CD40L blockade with IDEC-131/E6040 is a potentially effective therapy for refractory ITP through selective suppression of autoreactive T cells to platelet antigens.

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And a final message from antithrombin

Much is now made of the finding that the genome of the human is only somewhat larger than that of the simplest of organisms. Essentially then, all species are composed of a similar array of constituent proteins. What makes us special is not the number of these proteins but the way evolution has adapted them to allow their function to vary from tissue to tissue. As hematologists, we have been able to see exactly how this happens through the advances over the last decade in our understanding of the molecular mechanisms of coagulation. It turns out that thrombin is not just a passive protease but an allosterically controlled protein that changes its function from initiator of coagulation in the arterial circulation to anticoagulant when it binds to the endothelium of the capillaries. Similarly, the plasminogen activator inhibitor PAI-1, which has a prime function in controlling fibrinolysis, has evolved a series of complex interactions that also affect tissue growth and differentiation. PAI-1, as with other serpins, traps its target protease with a springlike movement of its reactive site, which shifts from the exterior to the interior of the molecule. This mechanism normally occurs in serpins following proteolytic cleavage of the reactive site, but with PAI-1 it can also occur spontaneously, without cleavage of the loop, to give an inactive latent form. The maintenance of PAI-1 in its active form is due to its allosteric interaction with a plasma protein vitronectin, but vitronectin also competitively binds to a range of cell surface integrins and activators. This leads to a series of complex molecular and cellular interactions that are well described as cross-talking. An increasingly recognized contributor to such interactions, the mobile phones, so to speak, of cross-talking, are the heparan proteoglycans, epitomized in hematology by the heparins.

Evolution has added yet another layer to this complexity in the utilization of spent coagulation factors as signals for a variety of tissue responses. An example is the adaptation of a fragment of plasminogen to yield endostatin, an inhibitor of angiogenesis. But the most spectacular example of antiangiogenesis came with the finding by O'Reilly and others[1] that spent forms of antithrombin blocked angiogenesis in the mouse, with an accompanying induction of tumor regression. Antithrombin can, like PAI-1, undergo a conformational transition to latent and cleaved forms, but what has puzzled the field is how such minor rearrangements could lead to such a remarkable suppression of angiogenesis. An answer is provided here in the paper of Zhang and colleagues (page 1185). They show that the latent and cleaved, but not the native, forms of antithrombin produce a down-regulation of the gene for the proangiogenic proteoglycan, perlecan. The effect is to decrease the cell surface receptors for the growth factors that stimulate angiogenesis. The importance of this paper is in the completeness and credence it adds to the earlier findings of O'Reilly et al. It opens up intriguing prospects for biochemical, structural, and cellular research. We really are beginning to listen in to, as well as observe, the cross-talk that underlies our biologic complexity.

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Thrombosis down to the vessel wall

Protein C is the major natural anticoagulant and a complete deficiency leads to a severe thrombotic tendency shortly after birth. It is activated by thrombin when it is bound to thrombomodulin on the endothelial surface. Since thrombin is the central procoagulant, this constitutes a strong regulatory system to keep coagulation limited and localized. Activated protein C (APC) inhibits coagulation, in the presence of its cofactor protein S, by proteolytic cleavage of procoagulant factors Va and VIIIa. Reduced performance of this system, such as in partial (heterozygous) deficiencies of protein C or protein S, or in an amino acid change at one of the cleavage sites of factor V, as in factor V Leiden, results in thrombophilia (ie, an increased risk of venous thrombosis).

The endothelial protein C receptor (EPCR), discovered in 1994, has been reported to enhance the activation of protein C by thrombin bound to thrombomodulin.[1,2] It is logical to postulate that abnormalities in this receptor play a role in the etiology of venous thrombosis. Because the receptor is bound to the endothelial cells of the blood vessels, its function cannot be readily assessed in vivo. However, a soluble form of EPCR (sEPCR) can be measured in plasma, which is probably a degradation product of EPCR, but still has some of the functions of EPCR, such as binding to protein C. Interestingly, levels of sEPCR have a strikingly bimodal distribution in plasma, suggestive of single locus genetic control.[3]

Poor function of EPCR could cause