

cause of thrombocytopenia in ITP. In the very first issue of *Blood*, published in 1946, William Dameshek summarized his extensive study of marrow morphology in patients with ITP and agreed with Frank that "... the fundamental defect leading to thrombocytopenia is a dysfunction of the megakaryocytes ..."⁴ In the subsequent 20 years, it was shown that ITP is associated with platelet-specific autoantibodies.^{5,6} Moreover, technical advances made it possible to measure the lifespan of transfused and autologous platelets, and studies using the new tools showed that platelet survival is markedly shortened in almost all cases of ITP.⁷ Accordingly, Baldini concluded in an authoritative review published in 1966 that "... the old hypothesis ... of a toxic depression of megakaryocytes and their activity resulting in thrombocytopenia (in ITP) has (now) been disproved ..."⁸

The idea that platelet-reactive antibodies might be cytotoxic for megakaryocytes would not die, however. In the subsequent 4 decades, in vivo and in vitro studies provided evidence that antibodies, and perhaps cellular immune mechanisms as well, can in fact act on megakaryocytes to suppress platelet production.^{9,10} Patients with immune thrombocytopenia appear to be heterogeneous in respect to which of the 2 mechanisms predominates, making it likely that platelet antibodies differ from patient to patient in their ability to adversely affect megakaryocyte viability and maturation. The antibody studied by Greinacher et al provides a particularly striking example of an immunoglobulin that not only caused acute destruction of peripheral platelets, but also depleted the bone marrow of mature megakaryocytes expressing GPIIb/IIIa. This causes more severe and prolonged

thrombocytopenia than is usually the case in patients sensitive to the platelet inhibitors eptifibatide or tirofiban.¹¹ Antibodies associated with this condition appear to recognize a restricted domain in the vicinity of the RGD recognition site of GPIIb/IIIa. Studies to determine whether other immunoglobulins of this type are selectively cytotoxic to megakaryocytes could be rewarding.

Conflict-of-interest disclosure: The author declares no competing financial interests. ■

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Becker and colleagues address this question.² Starting from earlier reports on anti-CD4-mediated tolerance and Treg activation and their own report on activation of human Tregs through CD4,³ the authors take advantage of the HIV gp120 protein being a high-affinity ligand for CD4 and report that gp120-mediated activation of Tregs through CD4 is sufficient to turn on the suppressive activity of naturally occurring Tregs. They find that the CD4-mediated activation depends on Lck and cyclic adenosine monophosphate (cAMP) production and can be blocked by Src family kinase inhibitors and adenylyl cyclase inhibitors. Furthermore, functional analysis of the effect of gp120-mediated activation of Treg in vivo in a graft-versus-host-disease model demonstrates that the Treg activation by gp120 through adenylyl cyclase and cAMP can abolish the rejection. The data on gp120 are highly interesting in the context of how Tregs may be engaged in bystander suppression in vivo and exciting as a starting point for potential new therapies using gp120-derived biologicals to harness Treg-mediated dampening of autoimmunity and tissue rejection. Furthermore, it is interesting to speculate that the observed Treg-mediated suppression of HIV-specific immunity in HIV-infected patients⁴ may be elicited by gp120-mediated activation of patient Tregs.

In terms of the link between Lck and the adenylyl cyclase leading to increased cAMP levels in Tregs, earlier reports point to the possibility of TCR-dependent and Lck-dependent recruitment and activation of the α subunit of heterotrimeric G proteins Gs and Gq in effector T cells.^{5,6} As Gs activates the cyclase, this could (if mechanisms are similar in Tregs) explain how CD4 ligation and subsequent Lck activation could increase cAMP (see figure), although it remains to be shown mechanistically how Lck may activate G proteins. Elevated cAMP levels inside Tregs contribute to their anergic state, but more importantly, may directly suppress effector T cells in a contact-dependent manner by Tregs forming GAP junctions with effector T cells. This establishes a concentration gradient that allows diffusion of cAMP from inside Tregs to inside effector T cells as elegantly shown by some of the same authors in an earlier report (see figure).⁷ A large body of work has established how cAMP suppresses effector functions through the cAMP-protein kinase A (PKA) type I-C-terminal Src kinase (Csk)

● ● ● TRANSPLANTATION

Comment on Becker et al, page 1263

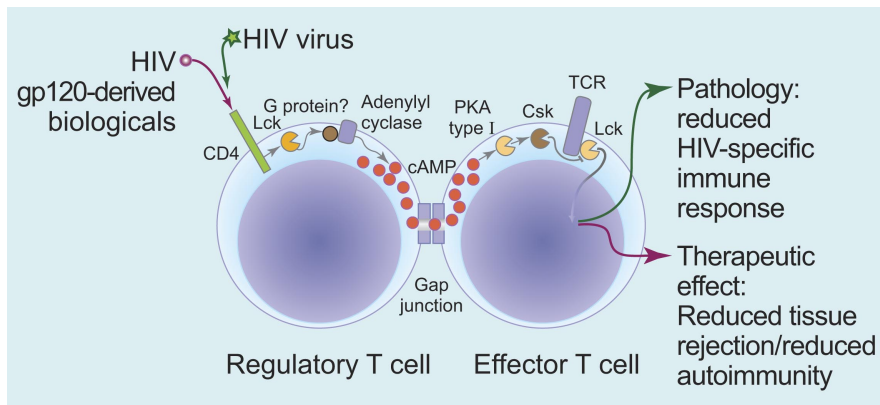
Waking up regulatory T cells

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Using the HIV gp120 protein as a ligand for CD4, regulatory T cells (Tregs) can be "woken up" from a dormant state and activated to suppress surrounding T cells.

While it has been clear for a long time that CD⁺CD25⁺ Tregs need to be activated to exert their suppressive function on bystander effector T cells,¹ it has remained elusive how

activation of Tregs may occur effectively, as their suppression is not restricted and their antigen specificity may be different from the cells they suppress. In this issue of *Blood*,



A schematic representation of regulatory T cell (Treg) immunosuppression by cAMP following gp120 ligation of CD4. Upon triggering of CD4 on Tregs by gp120 protein or possibly by gp120-derived agonists, Lck becomes active and turns on cAMP production by adenylyl cyclase possibly through interaction with a G protein. cAMP is transferred from Treg to effector T cells through cell-to-cell contacts called GAP junctions that allow diffusion of small molecules down the concentration gradient and into effector T cells. Once inside effector cells, cAMP inhibits immune function through PKA–Csk inhibitory pathway that turns off T-cell activation proximally under the T-cell receptor. This could be damaging in HIV, leading to defective anti-HIV immune responses, but a wanted effect when using gp120-derived agonists to modulate the unwanted immunity in tissue rejection or excessive autoimmune responses. Professional illustration by Paulette Dennis.

inhibitory pathway,⁸ which is anchored by a scaffold consisting of Ezrin, Ezrin binding protein 50, and the Csk binding protein PAG in T-cell lipid rafts, and completely turns off proximal T-cell receptor mediated signaling by inhibition of Lck (see figure). As a consequence, the HIV virus may, through gp120 ligation of CD4, elicit Treg-mediated suppression of effector T cells, through the cAMP inhibitory pathway, leading to T-cell dysfunction and reduced HIV-specific immune responses. Furthermore, gp120-derived mimics may be used to exploit this mechanism favorably to turn off unwanted immunity (see figure).

Conflict-of-interest disclosure: The author declares no competing financial interests. ■

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Iron is the 6th most abundant element in the universe comprising most of the constituents of the Earth (35%).² Going from the big picture of the universe to the smaller theater of human life, iron is important in many biologic processes. Iron biology and potential implications of iron overload have not received much attention in hematopoietic stem cell transplantation (HSCT), largely due to the lack of practical ways to address excess body iron during the peritransplantation period. Consequently, there has been a discrepancy between the explosion of biologic and genetic knowledge and clinical application as it pertains to iron overload significance and treatment in HSCT.

Ferritin keeps iron in a nonreactive state, preventing the Fenton chemical reaction that leads to the formation of oxygen radicals. Although most ferritin is kept intracellularly, the circulating fraction is used as a surrogate for iron stores. Interestingly, little is known about the role of serum ferritin compared to transferrin.³ Moreover, ferritin is an acute phase reactant and may be elevated for a variety of reasons.

Maradei et al show that a pretransplant ferritin level above 1000 ng/dL is an independent risk factor for decreased 5-year survival and is associated with a high incidence of hepatic sinusoidal obstruction syndrome (SOS, also referred to as veno-occlusive disease or VOD), a poorly understood but often deadly complication of HSCT. Allogeneic transplantation, busulfan-based conditioning regimen, and use of imatinib before HSCT completed the list of SOS-associated risk factors identified by multivariate analysis.

The authors had pretransplant ferritin levels from most of the patients from their institution and used a standard clinical definition of SOS. No transfusion information was provided, and there were no other measurements of iron stores, such as MRI of the liver.⁴ The rate of SOS was high, a phenomenon frequently seen when busulfan is given orally without dose adjustments. The high rates of SOS likely allowed the statistical detection of the ferritin effect. The limitations in estimating the actual iron stores here raise an issue: If the ferritin elevation does not reflect iron stores, what does this association tell us? In addition, the increased risk of SOS with pre-SCT imatinib observation is potentially important (although based on a small subgroup), and deserves evaluation in larger cohorts.

● ● ● TRANSPLANTATION

Comment on Maradei et al, page 1270

SOS: too many irons in the fire!

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In this issue of *Blood*, Maradei and colleagues investigate the role of prehematopoietic stem cell transplantation hyperferritinemia as a risk factor for sinusoidal obstruction syndrome.¹ They show that increased levels of ferritin (presumptively reflecting increased iron stores) increases the incidence of this complication after allogeneic transplantation.