which have been previously shown to be an adverse prognostic factor in ovarian cancer.[6] It is known that inducible Tregs and Th17 cells share a reciprocal differentiation pathway from uncommitted CD4+ precursors.[4] In the current study, the authors show that macrophages isolated from ovarian tumors biased the in vitro differentiation of uncommitted CD4+ T cells toward Th17 cells. Therefore, the observed inverse correlation between Th17 cells and Tregs in tumors may reflect differing conditions within different tumors, favoring either inflammation (Th17) or suppression (Tregs). Furthermore, the Th17 and Treg lineages have recently been shown to be rather more plastic than previously thought, with several studies suggesting the possibility of interconversion between the 2 lineages.[7] Thus, the inverse relationship between Th17 cells and Tregs observed in ovarian cancers may be more than simply a descriptive association. It may reflect fundamental differences in the nature of the spontaneous antitumor immune response—differences that appear to have significant impact on patient survival.

Finally, the Th17 cells in ovarian cancers were found to simultaneously express high levels of multiple other proinflammatory effector cytokines (IL-2, TNF-α, IFN-γ) in addition to IL-17 (panel B in the figure). In other settings, this so-called polyfunctional pattern of effector cytokine production has been associated with robust CD4+ response to infection and vaccination.[8] Other investigators, including myself, have reported similar polyfunctional cytokine response by Th17-like cells in mouse tumor models as well.[9] Thus, taken together, Kryczek et al have described a functionally important and hitherto unrecognized population of CD4+ T cells in ovarian cancers that favor enhanced inflammatory responses and reduced Treg-mediated suppression. The presence of these polyfunctional Th17 cells was statistically associated with better clinical outcome. This raises the question of whether a similar population could be therapeutically induced or expanded (eg, by vaccines or other active immunotherapy), and if this would likewise result in improved patient outcome.

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

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● ● ● PLATELETS & THROMBOPOIESIS

Comment on Greinacher et al, page 1250

Double jeopardy

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Patients treated with the widely used "RGD-mimetic" platelet inhibitors sometimes experience severe but self-limited thrombocytopenia after exposure to one of these agents. Since thrombocytopenia occurs within a few hours, it is generally thought that the responsible drug-dependent antibodies affect only circulating platelets.

In this issue of *Blood*, Greinacher and colleagues describe the case of a 67-year-old man who experienced severe thrombocytopenia and profuse bleeding after a second exposure to the platelet function inhibitor eptifibatide.[1] An eptifibatide-dependent, platelet-reactive antibody specific for the platelet glycoprotein GPIIb/IIIa complex was detected in the patient's serum, providing a likely explanation for the acute drop in platelet levels. Unlike most patients with eptifibatide-induced immune thrombocytopenia, however, the platelet count remained profoundly low (< 5000/uL) for 4 days. A normal count was not achieved until more than a week later. Four days after the acute episode, the overall numbers of megakaryocytes in the marrow was reduced, and they had a "young" morphology. This suggested that the drug-dependent antibody might have injured mature megakaryocytes in addition to causing destruction of circulating platelets, and provided a likely explanation for the 4- to 6-day delay before platelet levels began to recover. To test this possibility, CD34+ cord blood stem cells were cultured under conditions favoring differentiation into megakaryocytes and were then treated with patient Immunoglobulin G (IgG) in the presence and absence of eptifibatide. Loss of cell viability (trypan blue exclusion) was significantly greater under these conditions than after treatment with drug alone or IgG alone. Cytologic studies showed that drug-dependent cytotoxicity preferentially affected megakaryocytes that had a high surface density of GPIIb/IIIa, presumably a population of relative mature cells. From these findings, the authors conclude that prolonged thrombocytopenia observed in this patient was caused by the cytoxic effect of a GPIIb/IIIa-specific, drug-dependent antibody on mature megakaryocytes.

The issue of whether platelet-specific antibodies can damage megakaryocytes and impair platelet production has a long and interesting history. Almost a century ago, Frank observed that, although normal or increased numbers megakarocytes were present in the bone marrow of patients with "essential (idiopathic, autoimmune) thrombocytopenic purpura" (ITP), many of the cells were small, agranular, and devoid of free platelets clustered about their periphery.[2] From these findings, he concluded that low platelet counts in this condition were the result of insufficient platelet production by defective megakaryocytes. However, a contemporary, George Minot, concluded from his examinations of bone marrow that, in such cases, platelets were destroyed "as fast as they were formed."[3] Thereafter followed a lively and long controversy over which of these mechanisms was the main

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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● ● ● 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*, 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 patients[4] 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)