Molldrem and colleagues have previously reported absence of recognition of normal cells by PR1-specific CTLs, suggesting that presentation of the antigen on normal cells may be absent.[8] The complexity of studying detailed, tissue-specific expression and recognition of the PR1 epitope using T cells has complicated these analyses.

The development of an anti–PR1/HLA-2 complex-specific antibody by Sergeeva et al as reported in this issue of *Blood* has strongly facilitated the detailed characterization of the PR1/HLA-A2 complex as potential target for immunotherapy.[1] The authors have used an elegant approach to generate a monoclonal murine antibody directed against the human PR1/HLA complex, and demonstrated specific recognition of this T-cell epitope. Sergeeva and colleagues have used this antibody to specifically map the PR1/HLA-A2 epitope within the hematopoietic compartment.

Preferential recognition of AML (precuror) cells by both direct staining and cytotoxicity assays, including a complement-depended cytotoxicity, was demonstrated, indicating that PR1 may be a potentially relevant target antigen. However, clear, significant expression on normal hematopoietic stem cells, myeloblasts, and monocytes was also demonstrated, illustrating expression of the antigen under normal circumstances. This constitutive expression may explain the absence of high-avidity T cells under normal conditions, preventing a significant clinical immune responses after vaccination in most cases. The results demonstrate that there may be a limited therapeutic window to target PR1. Low-avidity antibodies or T cells may suppress AML or CML maturating cells without eliminating clonogenic leukemic stem cells, whereas high-avidity T cells and antibodies may successfully target the leukemic stem cells but at the cost of potent hematopoietic toxicity. Whether such temporary toxicity would still allow therapeutic applications of the PR1/HLA-A2–specific antibody needs to be determined. Antibodies and T cells make use of different effector mechanisms to kill target cells. Sergeeva et al have generated a great tool to further study not only the potential benefits and risks of using PR1 as a target for immunotherapy, but their findings also allow further elucidation and comparison of the mechanism of action of humoral and cellular immunotherapeutic strategies.

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

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● ● ● LYMPHOID NEOPLASIA

Comment on Ouyang et al, page 4315

A less sour sweet; blocking galectin

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In this issue of *Blood*, Ouyang et al describe both an important tumor immune evasion strategy and a means by which it can be overcome.[1] They show that EBV proteins LMP1 and 2A induce expression of galectin-1 (Gal1) by the B lymphoblasts of EBV$^+$ posttransplantation lymphoproliferative disease (PTLD), and that they could block the apoptosis this sugar-binding molecule would otherwise induce in effector cytotoxic T lymphocytes using a Gal1 directed monoclonal antibody.

Both tumor cells and virus-infected cells have devised multiple strategies to evade the immune system. These include the failure to present tumor or viral antigens appropriately to the immune system, secretion of factors, such as TGFβ, that diminish T-cell survival and function, or the secretion of chemokines that attract regulatory or inhibitory T-cell subsets rather than antitumor effectors.[2] An additional inhibitory mechanism mediated by a family of carbohydrate-binding proteins known as galectins is attracting increasing interest for their immunosuppressive activities in the tumor microenvironment.[3] Gal1 is an endogenous glycan-binding protein that is expressed by a number of malignancies and at sites of inflammation. Gal1 has broad effects on both the innate and adaptive immune system through its interaction with specific cell-surface glycans on receptors such as CD45, CD43, and CD7 expressed by immune system cells.[3] Gal1 induces tolerogenic dendritic cells, regulates the suppressive function of regulatory cells, and induces apoptosis of several T-cell subtypes including antigen-specific T cells. Several groups have shown that Reed-Sternberg cells in classic Hodgkin lymphoma overexpress Gal1, skewing the immune response toward a T_H2-type cytokine profile with consequent expansion of regulatory T cells and inhibition of EBV-specific T-cell immune responses.[4,5]

Because Gal1 has a role in viral infections[6] and EBV is detected in Reed-Sternberg cells in a significant percentage of patients with Hodgkin lymphoma, Ouyang et al evaluated whether Gal1 was also expressed in EBV-driven PTLD. They found that Gal1 is expressed in 76% of primary PTLD samples as well as in EBV-transformed B lymphoblastoid cell lines (LCLs) and that expression is driven by the viral LMP1 and 2 genes through AP-1 and P13K/AKT signaling. Taken together these observations suggest that Gal1 expression induced by EBV-encoded proteins may be a means by which the virus can evade an EBV-specific immune response.

The investigators also showed that a newly developed gal1-directed neutralizing monoclonal antibody selectively inhibited galectin-induced apoptosis of EBV-specific CD8 T cells, thereby limiting the development of an immunosuppressive Th2/Treg-skewed tumor microenvironment. Further evidence to support the value of such a neutralizing Gal1 antibody comes from studies in a melanoma model, in which targeted inhibition of Gal1 expression in tumor cells potentiated anti-tumor effector T cells.[7]

An alternative approach to bypassing Gal1-mediated immunosuppression is suggested by Khanna and colleagues, who showed that inhibition of LMP-specific T cells could be overcome by ex vivo stimulation with an effective antigen presenting cell—in this case mononuclear cells incubated with an adenoviral vector encoding multiple LMP-derived epitopes.[8] Indeed, LMP2-specific cytotoxic T lymphocytes (CTLs) made in a similar manner have had clinical activity against EBV$^+$ Hodgkin lymphoma,[9] although it is unclear whether infused cells retain their activity long term or if instead they ultimately become susceptible to Gal1 or other inhibitory molecules such as TGFβ.

How clinically valuable will it be to overcome Gal1-mediated inhibition? In Hodgkin lymphoma, evidence is strong that the presence of an immunosuppressive environment inhibits specific CTL responses. In this disease, a neutralizing Gal1 monoclonal antibody either as monotherapy or in combination with other immunotherapies targeting EBV[9] may therefore be of benefit. In PTLD, by contrast, the issue is less the presence of a tumor-associated immunosuppressive environment and more the deficiency in an EBV-specific immune response that can be restored by adoptive transfer of EBV-specific T cells.[10] Hence the likely benefits of blocking Gal1 expression may be less apparent.

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

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● ● ● PHAGOCYTES & GRANULOCYTES

Comment on Köhler et al, page 4349

CXCR2 is the Tpo of the iceberg

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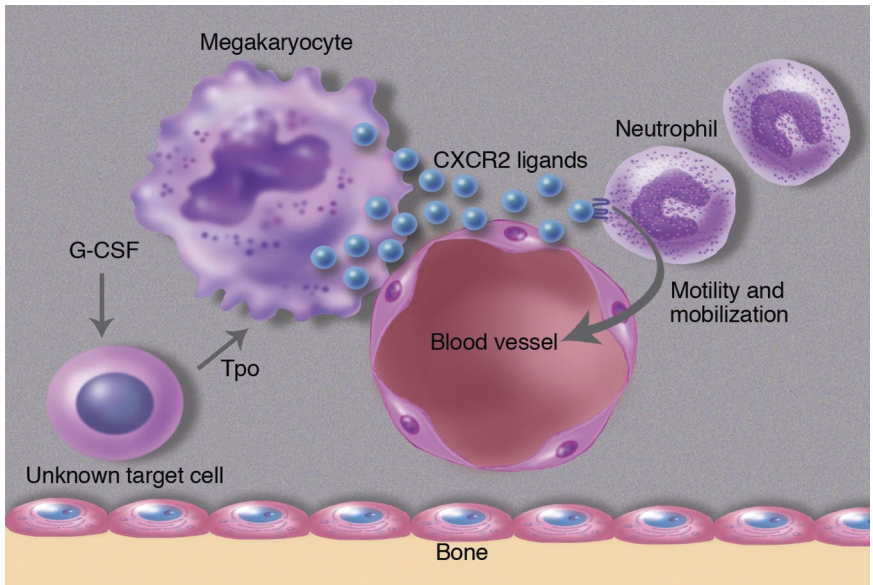
In this issue of *Blood*, Köhler and colleagues show that thrombopoietin, a hematopoietic cytokine best known for its regulation of platelet production, may also control neutrophil release from the bone marrow by regulating expression of CXCR2 ligands in endothelial cells and megakaryocytes.[1]

Neutrophils are an essential component of the innate immune response and a major contributor to inflammation. Accordingly, the number of neutrophils in the blood is tightly controlled through a balance of neutrophil production, release from the bone marrow, and clearance from the circulation. At baseline, only a small fraction of total body neutrophils circulate in the peripheral blood, while the majority remains in reserve in the bone marrow. However, in response to certain infections, neutrophils are rapidly mobilized from the bone marrow to blood.

Granulocyte colony-stimulating factor (G-CSF) is the principal cytokine regulating neutrophil homeostasis and is widely used in the clinical setting to treat neutropenia. The primary mechanism by which G-CSF increases



G-CSF acts on an unknown cell population to produce thrombopoietin, which in turn induces megakaryocytes and endothelial cells to secrete the CXCR2 ligands KC and MIP-2. These CXCR2 ligands stimulate neutrophil migration in the bone marrow, ultimately leading to their release into the blood. Professional illustration by Marie Dauenheimer.