The past 10 years have seen a rapid expansion of our understanding of TRALI and the first tentative steps to protect patients. TRALI is a "2 hit" process, requiring a clinical setting of inflammation with activation of neutrophils and/or vascular endothelium, with sequestration of neutrophils in the lungs. Transfusion provides a second insult that triggers neutrophil attack, leading to destruction of the alveolar vascular integrity and the flooding of airspaces with protein-rich fluid.[1] Transfusion-derived "second hits" include HNA antibodies or biologic response modifiers (that accumulate on blood storage, eg, bioactive lipids, cytokines, CD40 ligand, etc) that directly activate neutrophils; HLA class I antibodies that bind to neutrophils, endothelium and other somatic cells; and finally, HLA class II antibodies that act indirectly by stimulating class II antigen-bearing cells such as endothelium or macrophages. TRALI, therefore, has multiple possible etiologies; consequently, prevention requires several different approaches, with each intervention engendering its own cost-benefit decision.

Some decisions are simple and low-cost: avoidance of unnecessary transfusion prevents TRALI and saves resources, while deferral of donors implicated in TRALI reactions may prevent future cases. Alternatively, most severe cases of TRALI are associated with plasma-rich transfusions from female donors containing alloreactive HLA class I and/or II antibodies elicited during pregnancy. After the observation in the United Kingdom that conversion from 50% to > 90% male plasma for transfusion was associated with a substantial drop in TRALI cases,[1] the American Association of Blood Banks (AABB) recommended a similar practice in the US, starting in November 2007. Fatal TRALI cases reported to the FDA (see figure) now document a ~ 60% reduction in 2008-2009 compared with 2006-2007, and a similar reduction in nonfatal cases is reported by the American Red Cross Hemovigilance Program.[8] Absolute exclusion of female blood donors is not feasible for red cell, platelet, or blood group AB plasma donors in the US due to blood availability issues. The alternative approach of screening donors for HLA antibodies has been proposed and adopted for plasma and platelet donors by some blood centers. Approximately 17% of all female blood donors harbor HLA antibodies, with prevalence increasing with the number of prior pregnancies.[9] HLA antibody screening may have less impact on blood availability but increases the cost of all tested components. An effective method to screen donor blood for HNA antibodies is not yet available to blood centers. Likewise, avoidance or elimination of biologic response modifiers may require the use of fresher blood products and/or further processing (washing) before use. None of these interventions are required by FDA regulations or AABB standards, nor are they reimbursed by Medicare or private payors.

Mitigation strategies to further reduce the risk of TRALI will affect the cost and availability of blood products. A more systematic approach promises to identify at-risk populations, design interventions, and perform clinical trials that allow proper cost-benefit analyses. Vlaar et al demonstrate such a need in cardiac surgery and provide a suitable model for randomized controlled studies to test the efficacy of interventions.[7] A commitment to evidence-based transfusion medicine demands no less.

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

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● ● ● IMMUNOBIOLOGY

Comment on Sergeeva et al, page 4262

PR1 on the edge of humoral immunotherapy

J. H. Frederik Falkenburg LEIDEN UNIVERSITY MEDICAL CENTER

The development of a monoclonal antibody against the PR1/HLA-A2 complex that is aberrantly expressed on myeloid malignancies gives the opportunity to better define the potential benefits and risks of targeting the tumor-associated proteinase 3 and neutrophil elastase-specific PR1 antigen by both cellular and humoral immunotherapeutic approaches.[1]

Previously, Molldrem et al have identified PR1 as a potential target for cellular immunotherapy.[2,3] They and others[3,4] reported the presence of cytotoxic T lymphocytes (CTLs) directed against the PR1 epitope in patients with chronic myeloid leukemia (CML) and acute myeloid leukemia (AML), and demonstrated a correlation with disease outcome. Vaccination studies using synthetic PR1 peptides were able to show the development of biologically relevant immune responses to PR1 in some patients.[4,5] Unfortunately, sustained clinical responses were rare, and ex vivo clonal expansion of high avidity T cells from responding patients directly documenting in vivo expansion of T cells has not been reported.[6] This may indicate that endogenous presentation of PR1 peptide in HLA-A2 molecules could have prevented the in vivo development of high avidity T cells by natural negative selection, thereby impairing successful vaccination strategies.[7] However,

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.

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● ● ● LYMPHOID NEOPLASIA

Comment on Ouyang et al, page 4315

A less sour sweet; blocking galectin

Helen E. Heslop BAYLOR COLLEGE OF MEDICINE

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.