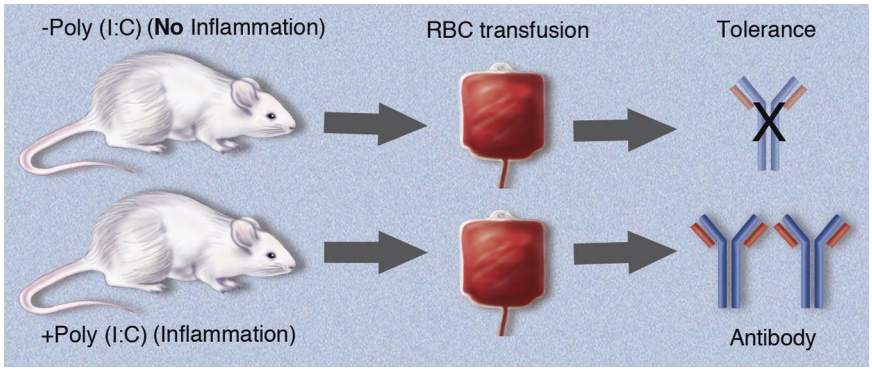

Transfusion in the absence of an inflammatory stimulus induces tolerance to future transfusions while transfusion in the absence of inflammation often induces an immune response, not tolerance, as reported by Smith et al.[1] Professional illustration by Marie Dauenheimer.

blood banks transfuse RhD− RBCs for RhD− patients and some blood banks provide RBCs lacking Kell and sometimes a few other antigens for select patient populations. The other approach to prevent antibody development, administering Rh immune globulin to prevent the development of anti-RhD antibodies, is a passive immunization used to prevent an active immune response and is frequently used for pregnant women. These approaches are limited by the fact that supplies of RBC units lacking a specific antigen may be in short supply and Rh immune globulin is only effective in preventing development of antibodies to RhD.

An alternate approach suggested by Smith et al would be to tolerize patients to specific antigens by transfusing RBCs with those antigens.[1] They have found that mice transfused with RBCs expressing human glycophorin A (hGPA) or membrane-bound hen egg lysozyme (mHEL) become tolerant of the transfused antigens if the initial transfusions occurred in the absence of inflammation (see figure).[1] This is the first model in which the investigators have been able to modify transfusion procedures to determine whether a transfusion induces tolerance or an antibody response. If such an approach were to work in humans, then transfusion with a small number of RBC units could protect against alloimmunization from future transfusions. This could be especially beneficial for patients with hemoglobinopathies who are frequently transfused.

While the report of Smith et al is promising, several issues need to be studied before this approach can be used clinically. It is not clear whether this approach will work for humans and if it does, it is not clear if all patients can be tolerized and, if they can be tolerized, for how long.

Part of the difficulty in predicting the answers to these questions lies in the fact that the mechanism by which tolerance is induced is not known. Under somewhat different conditions, murine tolerance to hGPA is known to be associated with increased functioning of regulatory T cells (Tregs).[2] However, it is not known whether tolerance induced by transfusion in the absence of inflammation augments Treg functioning.

One finding in humans suggests that RBC transfusion may tolerize patients to RBC antigens. Some reports have found that the younger thalassemia major patients are when they start their chronic transfusion programs, the lower their chances of developing antibodies to RBC antigens.[3,4] Taken together, these reports suggest that patients first transfused before the age of 1 year had the lowest chance of alloimmunization, patients first transfused when they were less than 3 years old had an intermediate alloimmunization risk, and patients first transfused at older ages had the highest alloimmunization risk. These findings suggest that tolerance may be inducible in humans but that tolerance induction is more likely to occur at younger ages.

Other human studies have found induction of tolerance to ABO antigens. If an infant is transplanted with a heart expressing foreign ABO antigens, that child will become tolerant to those ABO antigens.[5] Indeed, that tolerant patient will have no B cells producing antibodies against the heart's ABO antigens. However, such antibodies will develop if the patient is re-transplanted with a heart that does not express the foreign ABO antigens.

While studies of human thalassemia and heart transplant patients suggest that tolerance to antigens expressed on RBCs can be induced in humans, they also suggest some limitations to the process. Tolerance may only be inducible at young ages, may depend on continued presence of the foreign antigen, and may not last indefinately. Indeed, clinical experience would suggest that human tolerance to RBC antigens is neither complete nor permanent because chronically transfused patients sometimes make an antibody to an antigen that they would have been exposed to from multiple prior transfusions.

In addition, to translate to clinical use, one would need to know how to determine whether a person is in an inflamed state making him or her more likely to mount an immune response or in a noninflamed state in which he or she would be likely to become tolerant. Future studies using the murine transfusion model used by Smith et al could help identify characteristics of the noninflamed state conducive to transfusion induced tolerance.

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

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● ● ● TRANSPLANTATION

Comment on Srinivasan et al, page 1570

Exciting new murine model of cGVHD

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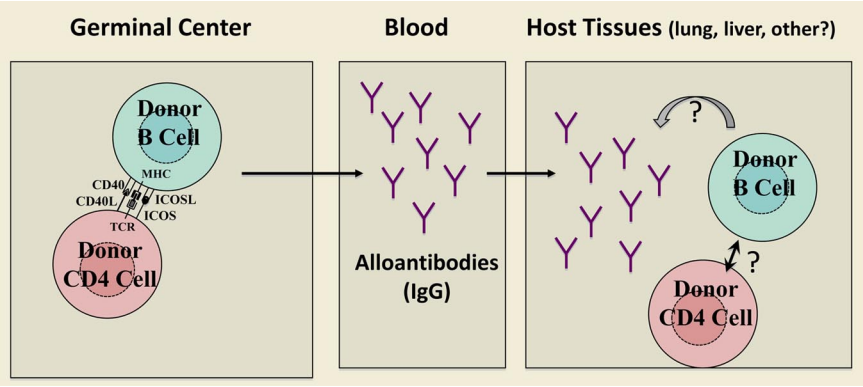
In this issue of *Blood*, Srinivasan et al report that mice conditioned for allogeneic hematopoietic stem cell transplantation with cyclophosphamide and total body irradiation develop wide-spectrum manifestations of chronic graft-versus-host disease (cGVHD).[1] This represents an exciting new preclinical model that can be used to uncover mechanisms of cGVHD and test interventional therapies.



Srinivasan et al show that donor alloantibody production is required for murine cGVHD. This figure highlights several questions raised by their interesting results.

While preclinical murine animal models of human cGVHD have provided insights to clinical disease, each model usually only simulates a limited number of the complex manifestations that are observed in patients (reviewed in Chu and Gress[2]). In addition, some of the models do not use any pretransplantation conditioning. When the model used by Srinivasan et al here was introduced in 2007, where mice preconditioned with cyclophosphamide and total body irradiation were transplanted with small numbers of T cells and bone marrow, the only manifestations reported were pathology and tissue fibrosis in the lungs.[3] Lung tissue damage in these mice resulted in the development of bronchiolitis obliterans, which is pathopneumonic for cGVHD.[4] On further examination, Srinivasan et al now report that these mice also develop pathologic manifestations in several other organs including liver, tongue, thymus, colon, and spleen.[1] This model provides a new preclinical system to study cGVHD pathogenesis and treatment strategies.

The most interesting observations in this paper are that donor-derived alloantibody (IgG) is required for the development of cGVHD (and bronchiolitis obliterans) and that disruption of germinal center formation, by inhibiting lymphotoxin-β (LTβ) signaling, is capable of treating "established" cGVHD. Because cGVHD is associated with the presence of host-reactive antibodies,[5] and some cGVHD patients have responded to treatment with anti-CD20 monoclonal antibodies,[6] the murine model further characterized in this issue could prove to be of value for assessing various therapies that directly or indirectly target antibody production.

Srinivasan et al focused much of their work on examining two of the organs most severely affected in this model, the lungs and liver, where they observed IgG deposition as well as the co-infiltration of CD4 T cells and B cells. These observations prompt several follow-up questions regarding this model (please refer to figure). First, is IgG deposition limited to the lungs and liver or does it occur in other organs? Second, what is the mechanism of IgG-mediated pathology? Is the tissue damage because of complement fixation or through Fc receptor–expressing immune effector cells? Third, is the tissue-reactive IgG specific for alloantigens, autoantigens, or both? Autoantibodies have been observed in cGVHD patients and in other cGVHD mouse models[2,5] but the role of alloantigen-specific Ig is unclear. Fourth, because co-localization of CD4 T cells and B cells was observed, is it possible that tertiary lymphoid tissues develop in the affected tissues, contributing to the alloantibody production? The formation of tertiary lymphoid tissues has been observed at sites of inflammation in mice and humans,[7,8] and LTβ is also important for the formation and maintenance of these tissues. Finally, because CD40/CD40L and ICOS/ICOSL interactions are also known to be important for germinal center formation,[9,10] is it possible that these pathways could also be targeted to treat cGVHD in these mice?

In summary, while there are no perfect models of clinical cGVHD, murine models such as the one reported by Srinivasan et al in this issue can provide important insights to clinical disease. This exciting model could prove to be an ideal system for testing new cGVHD treatment strategies.

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

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