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Taming the ABO barrier in transplantation

Thomas Wekerle

In this issue of Blood, Tazawa et al demonstrate that invariant natural killer T (iNKT) cells play a critical role in the production of antibodies against the blood group A antigen. Interrupting iNKT cell function with an anti-CD1d antibody prevents anti-A antibody formation, revealing a potential therapeutic target for ABO-incompatible transplantations.

Donor shortage remains the single most important factor limiting the success of transplantation medicine. In face of this unrelenting pressure, otherwise prohibitive immunological barriers are increasingly accepted, such as ABO incompatibility of a kidney from an available living donor. Treatment protocols for such situations involve varying combinations of B-cell depletion, removal of isohemagglutinins through plasmapheresis/immunoadsorption, and augmented immunosuppression. Outcome in terms of patient and graft survival is excellent both in pediatric and adult recipients. The rate of graft loss in the first weeks after transplant is increased, however, in ABO-incompatible transplants, and concerns regarding the long-term infection risk persist. Moreover, indirect signs of antibody deposition are commonly found on graft biopsies even though their clinical significance remains unclear. Also, treatment protocols need to start before the transplant, making them unsuitable for the deceased donor setting and thus restricting their use. Although ABO compatibility is less important in hematopoietic stem cell transplantation, clinical outcome is nevertheless inferior in recipients of an ABO-incompatible graft. Thus, therapies better controlling ABO-antibody production could improve outcome in ABO-incompatible organ and stem cell transplantation.

In this issue of Blood, Tazawa et al find that iNKT cells regulate the production of antibodies against the blood group A antigen. Previously, the authors had established that in mice B cells (of the B-1a type) exist with surface IgM receptors for group A antigen, that natural anti-A antibodies are present, and that anti-A antibody levels are further increased on immunization. They now demonstrate that 2 strains of mice deficient in iNKT cells (CD1d−/− and Jα18−/− mice) fail to develop increased anti-A levels on immunization with blood group A red blood cells. In the reverse experiment, activation of iNKT cells with α-galactosyl ceramide boosts anti-A levels. Importantly, the authors then manage to block the induced anti-A antibody production by administering an anti-CD1d monoclonal antibody (mAb) at the time of immunization. They conclude that CD1d-restricted iNKT cells are required to induce anti-A antibody production, presumably through interacting with CD1d expressed on B cells.

To dissect the mechanism of how iNKT cells stimulate anti-A production, the authors measured cytokine serum levels and found a transient elevation of interleukin (IL)
5 (but not of any other tested cytokine) after immunization. No elevation was present in CD1d<sup>-/-</sup> or anti-CD1d treated mice. Anti-IL5 diminished anti-A antibody production, although the effect was less pronounced than with anti-CD1d mAb. Using distinct cell populations sorted from liver mononuclear cells (known to be rich in iNKT cells) in enzyme-linked immunospot assays, the authors identify iNKT cells as the main source of IL5. Thus, IL5 secreted by iNKT regulates anti-A production by B cells.

Finally, to test whether their murine findings are relevant for the human situation, the investigators used a humanized mouse model in which they engrafted NOD/SCID/γc<sup>-null</sup> mice with human blood group O peripheral blood mononuclear cells. Injection of anti-human CD1d mAb prior to immunization with blood group A red blood cells inhibited anti-A antibody production, indicating that iNKT cells are also regulating anti-A antibodies in humans.

iNKT comprise a small population of thymus-derived lymphocytes that express an invariant T-cell receptor-α chain (Vα14Jα18 in mice and Vα24Jα28 in humans; for recent reviews on iNKT cells, see refs. 6 and 7). iNKT recognize glycolipids presented by the nonclassical major histocompatibility complex I antigen presenting molecule CD1d. On activation, iNKT can rapidly secrete a wide range of cytokines. Numerous—sometimes seemingly contradictory—functions have been ascribed to iNKT cells, including a role in promoting protective immunity against tumors and pathogens and in suppressing autoimmunity. Likewise, both protolerogenic and antitolerogenic effects have been reported in alloimmune organ and hematopoietic stem cell transplants. 8,10

The major contribution of this work is to establish a novel role for iNKT cells in regulating the antibody response to blood group antigens. The results from the humanized mouse experiments strengthen the confidence that this mechanism occurs not only in mice but also in humans. Therefore, the report raises the hope of therapeutically manipulating antibodies against blood group antigens. Modulation of iNKT cells is being explored for several clinical indications, but is still in its infancy. 7 With this study identifying an additional function of iNKT cells, inhibitory approaches might become of interest also in the ABO-incompatible transplant setting. It needs to be awaited, however, whether anti-CD1d mAb can be developed for clinical use, and in face of the pleiotropic functions of iNKT cells, the safety of such a drug is unclear.

Notably, iNKT-deficient mice had similar levels of natural anti-A antibody levels as wild-type mice. Thus, iNKT cells are not required for natural anti-A antibodies (mainly of the IgM isotype) but only for the induced de novo antibody production (of both IgM and IgG isotype) on immunization with antigen. What factor distinguishes these 2 situations, making one iNKT dependent and the other not? This finding also implies that anti-CD1d mAb could become useful in the prevention/treatment of antibody-mediated rejection after an ABO-incompatible kidney transplant but that preexisting (natural) anti-A/anti-B isoforms still need to be removed by other means before the transplant. Initially, the authors set out to investigate the role of iNKT cells in the antibody response against a range of transplant-relevant antigens. As they demonstrate, production against xenogeneic carbohydrates (<em>α1,3Gal</em> and <em>N-glycolylneuraminic acid</em>) and allo-major histocompatibility complex C antigens is not dependent on iNKT cells. The question why anti-A, but not anti-gal or anti-NeuGc, antibodies are iNKT dependent despite structural similarities remains to be answered.

Interestingly, cognate (ie, invariant T-cell receptor–CD1d) and noncognate (ie, IL5) mechanisms were found to act in concert in stimulating anti-A antibody production by iNKT cells. The current experiments do not yet determine, however, which CD1d<sup>+</sup> cell the iNKT cell needs to bind to. Although B cells are the likely candidate, it cannot be ruled out that iNKT cells bind to another CD1d<sup>+</sup> population that indirectly exerts a stimulating effect on B cells.

The work by this group deepens our understanding of the biology of antibody production against blood group antigens. It opens the door to future research that might lead to drugs that better control antibodies in the clinical setting of ABO-incompatible transplants.

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REFERENCES


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Genotype of proband and thrombophilia screening

Per Morten Sandset1 • OSLO UNIVERSITY HOSPITAL RIKSHOSPITALET

In this issue of Blood, Bucciarelli et al<sup>1</sup> report on the risk for venous thrombosis (VT) among family members of heterozygous and homozygous carriers of 2
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