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/<i>γc</i><sup>-/-</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 prototolerogenic and antitolerogenic effects have been reported in allogeneic 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 isohemagglutinins 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 (α1,3Gal and N-glycolylneuraminic acid) 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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**Genotype of proband and thrombophilia screening**

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In this issue of <i>Blood</i>, Bucciarelli et al<sup>1</sup> report on the risk for venous thrombosis (VT) among family members of heterozygous and homozygous carriers of 2
common polymorphisms, $F_5$ rs6025 and $F_2$ rs1799963, commonly known as the factor V Leiden (FVL) and the prothrombin (FII) gene G20210A (PT20210A) polymorphisms, respectively. $F_5$ rs6025 yields an arginine to glutamine shift at position 506 of the mature factor V molecule, an important cleavage site for its inactivation by activated protein C, leading to slower inactivation of factor V and prolonged activation of coagulation, whereas $F_2$ rs1799963 occurs in a regulatory region of the prothrombin gene that results in higher levels of prothrombin in plasma. As they lead to increased clotting factor activity or level, both are labeled “gain-of-function” polymorphisms to differentiate them from the mutations or polymorphisms that occur in the coagulation inhibitor genes, such as the antithrombin, protein C, and protein S genes, resulting in reduced activity or deficiency or “loss-of-function”.

The FVL and PT20210A polymorphisms are prevalent in Caucasians and are found in 2% to 8% and 1% to 3% of European populations, respectively, but are rare among individuals of African and Asian descent. FVL increases the risk for a first VT by 3% to 5% and PT20210A by 2% to 3%, but they only marginally increase the risk for recurrent VT. Family members are at increased risk, and a recent study suggested that the risk was higher if the proband presented with VT, rather than other clinical presentations. The genotype of the proband could also be of importance, as homozygotes have a several-fold higher risk for VT compared with heterozygotes.

Bucciarelli et al also addressed this issue and investigated 192 kindreds with a total of 886 relatives with at least a single member being homozygous for either of the polymorphisms. The incidence of VT among family members was higher when the proband was heterozygous compared with being homozygous. The researchers confirmed that family members of probands presenting with VT had increased risk compared with other clinical presentations. Overall, family members of probands with heterozygous polymorphisms and who presented with VT had a 4-fold increased risk compared with family members of homozygous probands without VT. Using population statistics, Bucciarelli et al found that family members of homozygous probands without VT had an incidence rate of VT similar to that of the general population, but that the incidence was 4.5-fold higher compared with the general population among family members of heterozygous probands with VT.

The implication of these findings is that screening of family members may be limited to family members with a proband who is heterozygous for either the FVL or the PT20210A polymorphisms and who presents with VT, but this should be tested in prospective studies.

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

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LYMPHOID NEOPLASIA

Comment on Dubovsky et al, page 2539

Two targets for the price of one

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In this issue of Blood, Dubovsky et al show that ibrutinib inhibits interleukin-2 (IL-2) inducible kinase (ITK) and thereby modulates T-cell signaling and function. Ibrutinib is an effective inhibitor of the B-cell receptor (BCR) signaling pathway and has demonstrated clinical efficacy in the treatment of various B-cell malignancies. Data reported by Dubovsky et al1 show that ibrutinib also inhibits IL-2 ITK and thereby modulates T-cell signaling and function.

Many B-cell malignancies, particularly chronic lymphocytic leukemia (CLL), mantle cell lymphoma, and activated B cell–type diffuse large B-cell lymphoma, have constitutive activation of the BCR signaling pathway and are particularly susceptible to inhibition of this pathway.1,2 Bruton’s tyrosine kinase (BTK) is a mediator of the BCR signaling pathway and has been implicated in the pathogenesis of B-cell malignancies. Ibrutinib, a BTK inhibitor, has been used to treat various B-cell malignancies and has shown particularly significant activity in treating patients with relapsed or refractory mantle cell lymphoma, relapsed or refractory CLL, or activated B cell–type diffuse large B-cell lymphoma.3,4 The agent also has significant promise in Waldenstrom macroglobulinemia. Although ibrutinib has been found to be a potent inhibitor of BTK and downstream signaling of the BCR pathway, data have also been generated that suggest that ibrutinib can inhibit other important pathways. Studies have shown that ibrutinib is able to inhibit activation and function of human basophils. BTK is also able to inhibit the secretion of tumor necrosis factor–α, IL-1β, and IL-6 from primary monocytes, particularly those present in autoimmune arthritis.5 Furthermore, BTK inhibition inhibits the
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