Establish ROR1 as a novel target for CAR

Antigen specificity is imparted by the genetic transfer of a single antigen receptor, consisting of either physiologic, HLA-restricted T-cell receptors (TCRs) or artificial, non-HLA-restricted receptors, which vary in the molecular composition and are broadly referred to as chimeric antigen receptor (CARs). Most CARs use an antibody-derived antigen-binding motif to recognize antigen, and comprise activating and costimulatory signaling domains in their cytoplasmic portion. CARs are thus targeted to cell-surface antigens and do not have to be matched to the patient’s HLA.

In the hematologic malignancies, CD19, which is relevant to chronic and acute leukemias as well as non-Hodgkin lymphomas, has emerged as a pivotal target antigen. It is the focus of more than a dozen active protocols in the United States, all of which are based on the infusion of CD19-targeted T cells. Over 15 centers are planning trials based on this approach in the United States, Europe, and Japan. At a recent meeting of the BMT CTN Network held in May 2010, it was determined that 19 patients had already been treated with CD19-targeted T cells in the United States. CD19 is normally expressed in the B-cell lineage from the pro-B-cell stage on albeit not in plasma cells. Importantly, it is not found in hematopoietic stem cells and in other hematopoietic lineages. The targeting of CD19 is thus expected to induce B-cell aplasia, which has been verified in animal models. The duration and consequences of this induced B-cell deficit are yet to be fully investigated, but there is ample precedent for the successful clinical management of similar conditions after bone marrow transplantation or monoclonal antibody therapy. The broad relevance of CD19 to the majority of leukemias and lymphomas notwithstanding, the availability of a highly tumor-specific target antigen would avert the risk of a sustained B-cell deficit.

In this issue of Blood, Hudecek et al establish ROR1 as a novel target for CAR-mediated T-cell therapy. ROR1 is a cell-surface antigen receptor with an extra-
cellular domain that contains Ig-like, Frizzled and Kringle domains, which may act in Wnt signaling and promote tumor cell survival. It is expressed during fetal development and not known to be expressed post-natally elsewhere than in early maturing B cells, the pancreas, and adipose tissue. Significantly, it is expressed at a high level in chronic lymphocytic leukemia (B-CLL), a subset of acute lymphoblastic leukemia (B-ALL), and, as shown by Hudecek et al, in mantle cell lymphoma (MCL). In their study, Hudecek and colleagues describe a novel ROR1-specific CAR and demonstrate its ability to redirect patient T cells against CLL and MCL tumor cells, resulting in efficient in vitro cytotoxicity and other key functional T-cell responses including cytokine secretion and proliferation. While these studies stop short of demonstrating T-cell efficacy in mice bearing systemic leukemia or lymphoma—and a much-anticipated comparison to CD19 targeting—this report conclusively brings ROR1 to the fore as an attractive target for immunotherapeutic strategies in selected leukemias and lymphomas.

As the potency of genetically enhanced T lymphocytes gains strength, so does the concern over toxicity, including on-target effects (whereby T cells attack tissues that normally express the targeted antigen). Several reports underscore the ability of genetically targeted T cells to react against normal tissues, for example, carbonic anhydrase IX in biliary epithelium and MART-1 in the inner ear, and in patients with renal cancer or melanoma who were given T cells targeted against these antigens. These undesirable effects may, however, be manageable and do not constitute grounds for not investigating differentiation antigens such as CD19 (which is highly restricted to the B-cell lineage) or an oncofetal antigen-like receptor such as ROR1. The consequences of low-level ROR1 expression in adipocytes and possibly some pancreatic cells will have to be closely investigated.

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REFERENCES

Comment on Bao et al, page 4639

IPT: Tregs come to the rescue

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In this issue of Blood, Bao et al report an increase in regulatory T-cell activity in patients with ITP treated with thrombopoietin receptor (TPO-R) agonists. This finding implies that TPO-R agonists may have an unexpected immune-regulatory activity. If this is indeed the case, the mechanism by which TPO-R agonists could perform such a function is currently unclear.
Treg suppression of pathogenic immune cells in ITP. (Professional illustration by Marie Dauenheimer.)

**C** D4+ T cells are commonly divided into T helper (Th) cells and regulatory T cells (Tregs). Tregs are usually identified by markers such as CD25, CTLA-4, CD127, and Foxp3. The main function of Tregs is the prevention of autoimmune diseases by maintaining self-tolerance. They suppress immune response by a mechanism based on a 3-way interaction between Tregs, Th cells, and antigen-presenting cells (APCs) (see figure). There is recent evidence that Tregs also suppress B cells. These multicellular immunosuppressive activities result in Tregs maintaining self-tolerance and preventing the emergence of autoimmune diseases such as immune primary thrombocytopenia (ITP).

ITP is an autoimmune “bleeding” disorder characterized by low platelet counts. Similar to other autoimmune diseases, in ITP, APCs, Th cells, and B cells play a crucial role in its pathogenesis. Autoreactive Th cells and B cells escape Treg surveillance and thus are allowed to become activated and proliferate. B cells produce auto-antibodies against platelet antigens (GP Ib/IIa and/or GP Ib-IX) on the surface of platelets. Antibody-coated platelets are phagocytosed by splenic and hepatic macrophages. As these antigens are also present on megakaryocytes, the autoantibodies bind to platelet antigens and antibody-coated platelets are phagocytosed by splenic and hepatic macrophages, and thus the pathogenic loop is maintained in chronic ITP. These pathogenic immune reactions probably persist because of a deficiency in Treg function. If ITP treatment can restore Treg function and suppress autoimmune pathogenic processes, sustained remission of the disease could be expected.

ITP is usually treated medically with immunosuppressive/immunomodulatory agents (such as glucocorticosteroids, intravenous immunoglobulin, anti-CD 20 antibody [rituximab], danazol, azathioprine, and cyclosporine) or surgically with splenectomy. Splenectomy gives a long-term remission rate of 66%-70%. With medical treatment, sustained response was until recently uncommon; however, dexamethasone and rituximab treatment in ITP have been reported to produce sustained responses in excess of 40%. Interestingly, improved Treg activity has been observed in ITP patients successfully treated with these 2 drugs. It is possible that the increased Treg activity may be responsible for the sustained response with these therapies. As these are immunomodulatory drugs, these findings are not totally unexpected.

With the knowledge that the thrombocytopenia in ITP is caused in part by impaired platelet production, TPO-R agonists have recently been used successfully to treat ITP. Response rates of 70% or higher have been achieved, but only while patients continue on treatment is the effect on their platelets maintained. As platelet response is driven by TPO-R agonist stimulation of thrombopoiesis, it is not unexpected that drug-induced platelet production thus ceases when the drug is stopped. In this context, the first demonstration of improved Treg activity with TPO-R agonists by Bao and colleagues is surprising and highly significant. However, this small cross-sectional study requires confirmation by a much larger longitudinal study, in particular a study which also shows antigen-specific increase in Treg activity (as immunosuppression by ‘Tregs is antigen-dependent’). Nevertheless, the finding of Bao et al is still interesting and may explain the sustained platelet response experienced by 7% of ITP patients treated with TPO-R agonists in clinical trials. The unexpected remissions were initially attributed to spontaneous remission which can occur occasionally in chronic ITP, although all these patients had failed several ITP treatments before receiving TPO-R agonist therapy.

It is tempting to speculate that TPO-R agonists may have immune-modulating activity and thus induce sustained remission in ITP patients if given to appropriate patients for an adequate duration. However, immune cells do not have TPO-Rs. If this activity exists, TPO-R agonists must exert this effect via a mechanism independent of TPO-R. Tregs and other less well-studied suppressive T cells are probably the key to this activity. ITP occurs because APCs, macrophages, stimulatory Th cells, and B cells (which perpetuate the disease) escape the immune surveillance by Tregs. Tregs exert immune control by modifying the functions and numbers of these cells, and consequently return the immune system to homeostasis and health. For example, Tregs can induce apoptosis of the effector cells or can inhibit their activation and functions. These Treg actions are mediated by soluble factors such as transforming growth factor-β (TGF-β), interleukin-10, perforins, etc and cell-associated molecules (such as cytokotoxic T lymphocyte antigen 4, lymphocyte activation gene-3, LFA-1/CD11a, CD18, CD39, etc). However, how
TPO-R agonists improve Treg activity is still unknown. It may be via a sustained increase in antigen load that induces tolerance or via an increase in anti-inflammatory cytokines such as TGF-β as both antigen and TGF-β can induce Tregs. There is no good evidence to support either hypothesis and hence further studies are clearly needed.

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Comment on Zhang et al, page 4684

Safe(r) anticoagulation

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In this issue of Blood, Zhang and colleagues demonstrate that targeting factor XI expression by antisense oligonucleotides prevents arterial and venous thrombosis in treated mice without increasing the risk of bleeding.1

Function of FXI in hemostasis and thrombosis. Hemostasis: At sites of injury, fibrin formation is initiated by the tissue factor (TF)/factor VIIa complex. FXI is activated by thrombin and contributes to sustained fibrin production when TF activity is reduced by reaction with tissue-factor pathway inhibitor (TFPI). Thrombosis: On activated platelet surfaces, released polyphosphates (polyP) initiate contact activation of FXII which, in turn, activates FXI. Further FXI activity is generated by feedback activation. Targeting FXI with ASO abolishes pathologic thrombosis but has minor impact on hemostasis. (Professional illustration by Kenneth X. Probst.)

Coagulation is a complex process vital to hemostasis—the cessation of blood loss from an injured vessel—but, under pathologic conditions, otherwise life-saving mechanisms can precipitate life-threatening occlusive thrombotic events, collectively the most common causes of disability and death in the developed world. A major goal in anticoagulation therapy is to identify targets for blocking thrombosis without increasing the risk of dangerous bleeding; unfortunately, anticoagulant drugs currently in use (such as heparins, vitamin K antagonists, or direct inhibitors of factor Xa and thrombin) target molecules that are also essential for hemostasis. Therefore, therapeutic and prophylactic use of anticoagulant agents for thromboprophylaxis will be associated with potentially severe and fatal bleeding complications.

In the classical cascade model, fibrin formation is initiated by the intrinsic and extrinsic pathways of coagulation. The intrinsic pathway is activated by “contact” of factor XII (FXII, Hageman factor) to negatively charged surfaces in a reaction involving plasma kaliko-rein and high-molecular-weight kininogen (contact activation system). Activated FXII (FXIIa) triggers coagulation via activating its substrate, coagulation factor XI (FXI), that in turn contributes to fibrin formation by activating factor IX. Deficiency in factor IX results in severe hemorrhage in patients (hemophilia B), whereas FXI-deficient humans suffer from minor/relatively mild bleeding (hemophilia C), which is characterized by trauma or soft tissue–related hemorrhage, primarily involving tissues with high fibrinolytic activity. Bleeding tendencies vary substantially between patients with similar FXI plasma levels and are not directly related to FXI antigen levels. In contrast, FXII deficiency is not associated with any increased bleeding risk, indicating the existence of FXII-independent pathways for FXI activation.2

Thrombin has been shown to convert FXI to the active protease FXIa, and anti-FXI antibodies were found to interfere with sustained fibrin production by thrombin-driven FXI feedback activation in plasma.3

The role of FXI in hemostasis and thrombosis has been extensively studied in animal models.3,4 In contrast to patients with hereditary FXI deficiency, FXI-null mice do not bleed excessively when challenged by surgical procedures. FXI-null mice have not been systematically analyzed by injury to tissues with high fibrinolytic activity, so it is not known whether the protease is required for normal hemostasis in mice in some situations. Challenging the dogma of a coagulation balance, FXI-deficient mice have severely reduced thrombus formation in response to various
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Beng H. Chong