type II transmembrane serine protease, matriptase-2, that plays an essential role in erythropoiesis because its inactivation causes iron refractoriness. It was previously demonstrated that genetic loss of Tmprss6 reduces systemic iron overload in Hfe−/− mice by increasing hepcidin through activation of the BMP-SMAD signaling. Accordingly, pharmacologic activation of hepcidin, achieved by Tmprss6 silencing in Hbbth3/H9252 adult mice, not only corrects iron overload but also ameliorates anemia, as demonstrated by the increased hemoglobin levels, RBC count, and RBC survival, and by the decreased spleen size and serum erythropoietin. Trying to explain the mechanism of improved erythropoiesis, Schmidt et al demonstrate that the amount of membrane-associated globin is decreased in the double mutant mice compared with the thalassemic mice reliving the erythroid damage and death, likely as a result of low iron availability for single erythroid cell.

The effects of Tmprss6 inactivation strengthen and extend previous findings on the beneficial role of limiting iron supply in thalassemia by increasing circulating transferrin or directly enhancing hepcidin levels by transplantation of β-thalassemia bone marrow in Hmp transgenic mice. However, despite the advantage obtained in β-thalassemia, the LNP–Tmprss6 siRNA treatment impaired erythropoiesis, thus causing hypochromic microcytic iron deficiency anemia in hemochromatosis mice, as shown in Hfe−/− mice with genetic inactivation of Tmprss6.

Tmprss6 appears to be a key molecule in the negative regulation of hepcidin, attenuating the BMP6 signaling effect in mice; as such, it could become an important therapeutic target also in humans. In the perspective of applying a similar therapeutic approach in patients, the challenge will be to develop protocols that precisely titrate hepcidin expression to avoid the unwanted side-effect of iron deficiency, as observed in human and murine models with chronic hepcidin activation. Conflict-of-interest disclosure: The author declares no competing financial interests.

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Comment on Ophir et al, page 1220

Location, location, location: advancing veto cell therapies

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In this issue of Blood, Ophir et al have identified and characterized refined conditions in which the immunoregulatory promise of veto cells, as a cellular therapy, may finally be realized.
Veto cells have long been a somewhat obscure immunoregulatory cell type, with seemingly mysterious properties. This is due in part to the fact that there is no distinct cell type or cellular lineage that defines a “veto cell.” It is more accurate to state that experimental “veto activity” is observed in a variety of settings and can be displayed by several cell types. Veto activity was first described, per se, in 1979 as cells that “can prevent generation of cytotoxic lymphocytes by normal spleen cells against self-antigens.”2 This description came at a time when the modern notion of T-cell biology and antigen-presenting cells was in its infancy. However, translating this definition into the current paradigms and concepts, veto cells can be simply defined as dele- tional antigen-presenting cells. In other words, veto cells delete T cells that recognize the veto cells (the responding T cells are “vetoed” and thus removed from the system).

The most potent known veto cells are activated CD8+ T cells, thus CD8+ T cells delete CD8+ T cells. The mechanism(s) of vetoing responding CD8+ T cells (that recognize antigens on the veto cell) involve ligation of MHC-I on the deleted cell by CD8 on the veto cell and also Fas/FasL pathways. More recent elucidations have revealed a role for perforin as well.3 What is particular to veto cell biology is that while the veto cell is a T cell itself, with its own specific TCR, the specificity of the veto activity is not determined by the TCR but rather by antigens presented on MHC-I that are recognized by the deleted CD8+ T cell. However, a CD8+ veto cell maintains its own function as a T cell (through TCR ligation) while still possessing veto activity when recognized as a target by a separate T cell. Thus, a polyclonal population of CD8+ T cells with a wide repertoire of specificities because of a wide distribution of TCR recombinations can simultaneously be monospecific with regard to veto activity, as each cell expresses the same MHC-I and likely presents similar peptides (peptides derived from the TCR notwithstanding).

Veto cells have shown considerable promise as a cellular therapeutic in the context of bone marrow transplantation (BMT), due to both their immunoregulatory potential and the ability to be expanded into large numbers of CD8+ veto cells. For example, veto cells of donor origin have the capacity to delete recipient anti-donor T cells, and thus mitigate graft rejection. In contrast, veto cells of recipient origin have the capacity to delete donor T cells that recognize recipient MHC-I, and thus mitigate graft-versus-host disease (GVHD). Although CD8+ T cells are the most potent veto cells described, there is a potentially very serious complication to their use. In addition to their desired veto activity, CD8+ veto cells maintain their function as T cells through their TCR (typically cytolytic T-cell activity). Thus, CD8+ veto cells have a potential coincident toxicity, if their TCR recognizes an antigen in the system (recognition of donor antigens could cause graft rejection whereas recognition of recipient antigens could lead to GVHD). This problem has been circumvented by generating CD8+ veto cells for which the TCR is focused against a third-party antigen not present in either donor or recipient.4 Although risk of cross-reactivity remains, as the TCR does not undergo affinity maturation, a properly isolated veto cell population should be safe in this context. As noted above, because the specificity of a CD8+ veto cell is determined not by its TCR, but rather by its MHC, diverting the TCR to an irrelevant antigen does not affect the veto function.

An additional problem with implementing veto cell–based therapies is that the source of veto cells has traditionally been optimized using tissue culture systems (eg, mixed lymphocyte reactions). As is always the case with reductionist experimentation, a gain in experimental control comes at the cost of deviation from the authentic setting one is modeling. In a tissue culture dish, responding T cells and veto cells are in close juxtaposition; thus, the cells being deleted by veto cells have no physical means of escaping. In contrast, in vivo, a T cell that is anatomically sequestered from a veto cell is not susceptible to deletion. It appears that this was precisely the reason that early veto cell preparations worked in tissue culture, but failed to have the same effects in vivo (ie, the veto cells were not homing to the lymph node tissues where responding CD8+ T cells were located). This limitation was elegantly circumvented by differentiating CD8+ veto cells into a central memory phenotype, which allowed them to home to the lymph nodes.5

Here, Ophir et al present a detailed analysis of an in vivo model, in which the phenotypic properties of expanded CD8+ veto cells (central memory phenotype) are studied. In addition, veto cell conjugates with anti-donor T cells are visualized by 2-photon microscopy and the modified cells now show efficacy both by confining anti-donor T cells to the lymph nodes and also inducing their deletion, whereas previous preparations that did not promote lymph node homing failed to do so. In aggregate, these studies elegantly demonstrate a substantial advance in cellular therapy engineering of veto cells in an in vivo preclinical model. Translation of this approach into the human setting has the potential to have a substantial impact on immunoregulation by veto cell–based cellular therapies in general and on BMT medicine in particular.

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