T cells is sufficient to acquire effector function is not clear. Thus, recognition of self by T cells outside of the thymus in both physiologic and pathologic conditions such as lymphopenia plays a critical role in T-cell maintenance and function.

The Wilms tumor 1 (WT1) antigen is over-expressed in a number of malignancies and has been validated as a tumor antigen in preclinical models and in patients. Importantly, the WT1 sequence is highly conserved in mammals with shared epitopes that can be appropriately processed and recognized by T cells in both mice and humans. WT1-specific T cells can be identified in healthy individuals with evidence for increased frequency of WT1-specific T cells in patients with malignancy. Because the WT1 gene shows restricted expression in normal adult tissues, including renal podocytes and some CD34+ hematopoietic stem cells, identification of T cells that can recognize WT1 in healthy individuals indicates that central tolerance to WT1 is incomplete.

Pospori et al use a lentiviral vector to transduce murine HLA-A02 transgenic stem cells with a human TCR that recognizes a dominant HLA-A0201–restricted epitope derived from WT1 (see figure). Importantly, this TCR was generated from the repertoire of an HLA-A02 negative individual, an approach that is used to generate higher affinity TCRs because T cells in these individuals will not be negatively selected in an HLA-A02–expressing thymus. The transduced murine stem cells were then transplanted into HLA-A02 transgenic mice. Thus, T cells expressing the human WT1–specific TCR can be positively selected in the thymus on HLA-A02 with the possibility that presentation of WT1 peptide derived from the murine protein (with an identical TCR recognition sequence to humans in peptide derived from the murine protein (with an A02 with the possibility that presentation of WT1 can be positively selected in the thymus on HLA-A02 transduced murine stem cells were then transduced in an HLA-02-expressing thymus.9 The negative individual, an approach that is used to generate murine HLA-A02 transgenic stem cells expresses in a number of malignancies and has a critical role in T-cell maintenance and function.

REFERENCES

Comment on Agar et al, page 6939

A new fish for the β2GPI hook: LPS!

Jacob H. Rand MONTEFIORE MEDICAL CENTER

In this issue of Blood, Agar and colleagues provide evidence that the plasma protein β2-glycoprotein I (β2GPI) changes conformation to a fish hook structure to bind to bacterial LPS via the protein’s carboxyterminal domain, and that this binding promotes the clearance of LPS from the blood circulation.1

B eta2GPI is a plasma protein that is familiar to hematologists as the primary antigenic target of autoantibodies in the antiphospholipid syndrome (APS). Although a range of properties and affinities have been described, some of which are reviewed in the article by Agar et al,1 the protein’s biologic functions have not been established. The mystery is compounded by the observation that deficiency of β2GPI in humans has not been associated with any obvious disease or alterations.2

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null mice to heterozygous parents. Of the births of fewer than expected homozygous phenotypes other than the interesting finding of thrombin generation but the absence of any obvious thrombotic events, investigators hypothesized to trigger an autoimmune response leading to antiphospholipid syndrome in susceptible patients. The β2GPI-LPS complex is taken up by LRP, endocytosed, and degraded within the cell. (4) As previously described, β2GPI can also change conformation to bind to membranes that display anionic phospholipids. This binding similarly exposes the epitope on domain 1 that may also trigger an autoimmune response leading to antiphospholipid syndrome. Author’s adaptation of Figure 7 of the article by Agar et al executed by Paulette Dennis.

Furthermore, investigation of transgenic β2GPI-null mice reported evidence for impaired in vitro thrombin generation but the absence of any obvious thrombotic events other than the interesting finding of the births of fewer than expected homozygous null mice to heterozygous parents. Last year, Agar et al reported that plasma β2GPI has a circular conformation that is maintained by affinity of its carboxyterminal domain V for amino-terminal domain I and that the protein opens into a fish hook conformation when it becomes bound to phospholipid via its hydrophobic “barb” on domain V. They showed that this alteration exposes an epitope on domain I that is cryptic in the circular conformation and proposed that immunologic recognition of this epitope was part of the APECED disease process. In the current paper, Agar et al demonstrate that β2GPI also binds LPS through domain V and that this binding is associated with a similar conformational change (see figure). An interesting aspect of their results was the finding that β2GPI binds to LPS at sites other than the lipid A core, where the molecule’s endotoxin activity resides. In addition, β2GPI does not neutralize the endotoxin activity of LPS directly. Rather, the β2GPI-LPS complexes are bound and endocytosed via low density lipoprotein-related protein receptors on cell surfaces—monocyte-macrophages in their experiments—but they propose that vascular endothelial cells can have a similar function. They also show that this uptake occurs via affinity of receptors for the open, but not the circular, conformation of β2GPI.

Agar and colleagues provide convincing evidence for the biologic significance of this β2GPI-LPS affinity in humans. Under a protocol approved by their institutional scientific and ethics committees, healthy human volunteers were intravenously infused with low doses of LPS. A rapid decline of plasma β2GPI levels was observed. In addition, febrile responses and serum levels of inflammatory cytokines correlated inversely with the subjects’ baseline β2GPI levels. Also, plasma β2GPI levels of hospital patients with Gram-negative sepsis were reduced during the acute event and increased after recovery.

In addition to shedding new light on the biologic function of β2GPI and extending knowledge about host responses to infection, these results by Agar et al also fuel consideration of potential clinical implications. For example, might β2GPI levels be helpful in defining prognostic factors in Gram-negative sepsis? Agar and colleagues also suggest the possibility of novel biopharmaceutical approaches for this often fatal disorder.

To “close the circle,” these results regarding LPS also return our attention to the APS disease process because clinical studies indicate that infections may be one of the triggers for APS. This is particularly true for a severe form of the disorder, catastrophic APS, that is marked by disseminated small vessel occlusions and multiorgan failure. It is possible, as Agar and colleagues suggest, that in susceptible patients, the binding of β2GPI to Gram-negative bacteria leads to a conformational change in β2GPI with exposure of former cryptic epitopes that in turn lead to the immunologic response and the devastating thromboembolic sequelae.

This article by Agar et al represents a significant advance in the biology of β2GPI and in the elucidation of innate host defenses against microorganisms. We can hope for even more solid confirmation of the hypothesis with additional clinical studies of the β2GPI-deficient patients to learn whether they might be more susceptible to sepsis and when investigators return to the β2GPI-null animal model to determine whether these mice might have an altered response to LPS challenge.

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

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**TRANSPANTATION**

Comment on Soiffer et al, page 6963

**ATG and RIC: not such a good match?**

Sergio Giralt MEMORIAL SLOAN-KETTERING CANCER CENTER

It has been more than 50 years since Professor George Mathé explored the use of non-specific immunotherapy to treat secondary disease after allogeneic transplantation. Weden and colleagues then began exploring the use of antithymocyte globulin (ATG) as prophylaxis of acute GVHD. Bacigalupo et al have reported a series of randomized trials in which 109 patients with hematologic malignancies undergoing myeloablative bone marrow
A new fish for the β2GPI hook: LPS!

Jacob H. Rand