and platelet clearance by other antibodies, including the unmodified antibody from which it is derived. In addition, they have shown that the antibody interacts normally with FcRn so that it can be transported across the placenta.

In this paper, the investigators report for the first time the use of the modified antibody in human studies. Using dual-radiolabeled platelet survival techniques in normal volunteers, they show that autologous HPA-1a/b platelets coated with the modified anti–HPA-1a blocking antibody have a near-normal in vivo survival (188 hours) and contrast this with survival of platelets coated with the unmodified anti–HPA-1a antibody, which is dramatically shortened (2 hours). Simulating what would occur in therapeutic use for FNAIT, they also show that sensitizing autologous platelets with both antibodies prolongs platelet survival over that achieved using the unmodified anti–HPA-1a antibody alone. The selected portion of the figure from the article by Ghevaert et al dealing with this last point shows that there appears to be a small effect on the clearance of labeled platelets resulting in the slowing of destruction of antibody coated platelets when there is a 3:1 and 9:1 mixture of their modified antibody to the active antibody from which it was derived. The authors appropriately point out that this is not a dramatic effect and may or may not be sufficient to be useful clinically. If the glass is half full, then the effect will be enough to increase the fetal platelet count. If the glass is half empty, then the effect will be insufficient to prevent in utero complications from thrombocytopenia (ie, intracranial hemorrhage). Even if the effect is sufficient, as would be hoped, this study does not address what dose of modified antibody would have to be given or how often to achieve this effect. Unfortunately, it may not be possible to be sure of this strategy's clinical usefulness until the modified antibody is tested in a situation like the one for which it is intended.

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● ● ● GENE THERAPY

Comment on Schmitt et al, page 348

Trick to treat: tricking the thymus to treat cancer

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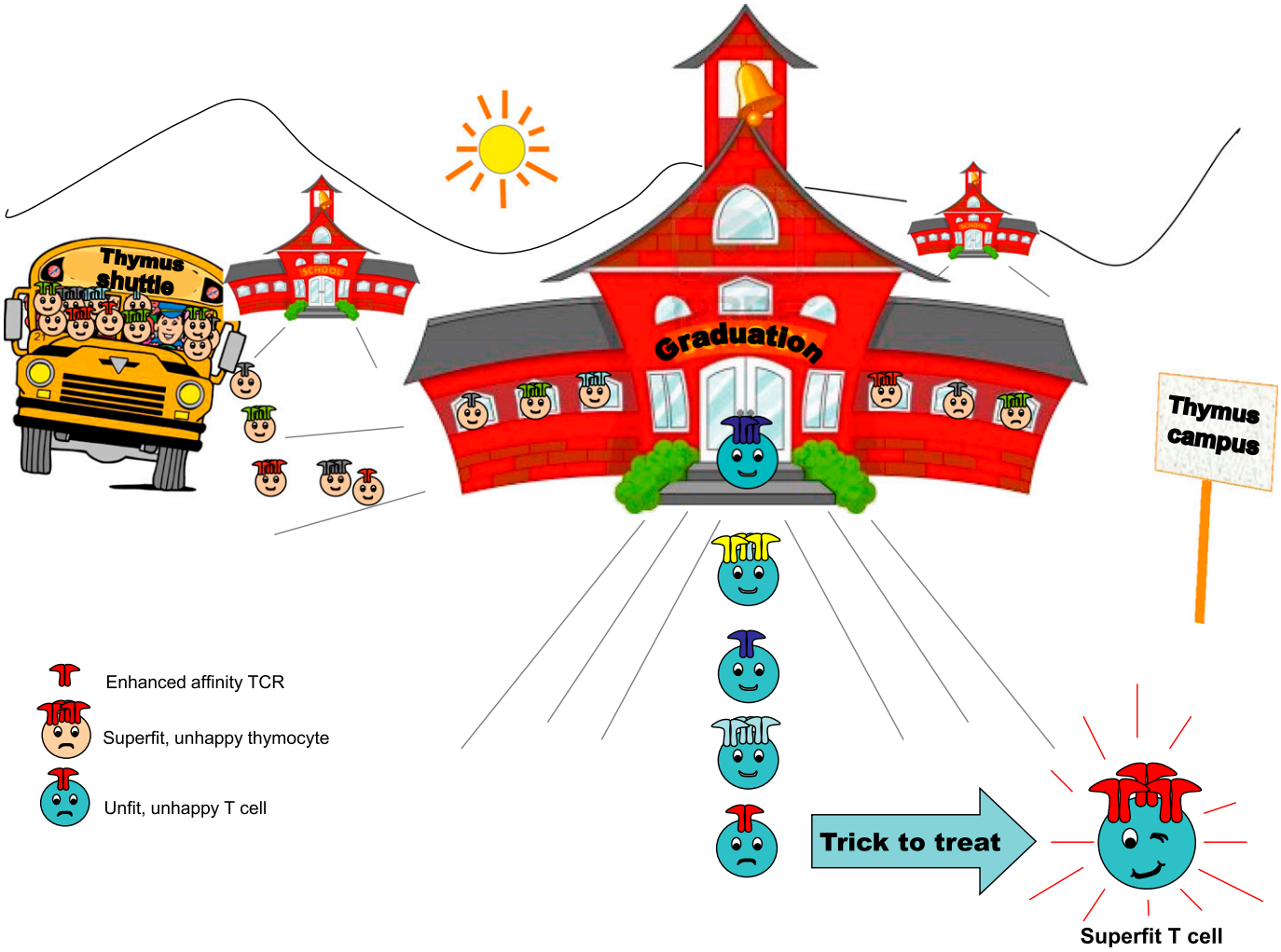
In this issue of *Blood*, Schmitt et al address the biology and safety of T cells engineered to express T-cell receptor (TCR) variants endowed with enhanced affinity for tumor-associated antigens.[1]

The authors hypothesize that the negative selection of high-affinity self-reactive thymocytes might overprotect against self-reactivity and result in the maturation of T cells with low-affinity TCR and suboptimal recognition of tumor-expressing, nonmutated self-antigens. This notion is supported by the finding that TCRs that bind viral nonself antigens fall within higher affinity ranges when compared with those that bind tumor-related antigens.[2] As high-avidity T cells bear the potential to respond to minute antigen expression, efforts have been focused on the retrieval and/or the engineering of high-avidity tumor-specific T cells for adoptive cellular immunotherapy. Thanks to increasing knowledge of TCR engineering, it is now possible to design increased-affinity variants of any given TCR and to engineer patient T cells to recreate potent high-avidity tumor-directed T-cell responses.[3] The potential to artificially increase the affinity of any given TCRs and express them on patients' autologous lymphocytes, however, raises important questions: Could thymic mechanisms of negative selection be tricked into improving tumor recognition and, thus, the efficacy of cancer immunotherapy? And could T cells expressing an enhanced-affinity TCR bypass the affinity limits imposed by thymic selection and be safe, or are they potentially at risk to mediate autoimmune manifestations?

To begin addressing these questions, Schmitt et al engineered mature T cells with TCR variants with enhanced affinity for cognate peptides derived from WT1 and Mesothelin, two highly relevant unmutated self-antigens that are overexpressed in several tumors and are linked to oncogenesis and tumor progression.[4,5] The results described show that such engineered T lymphocytes can be infused into mice without causing autoimmune tissue infiltration or damage, even after their terminal differentiation into cytolytic effectors in response to pathogenic antigen-expressing *Listeria* monocytogenes. Thus, limits imposed by negative selection in the thymus could be safely overcome by the genetic manipulation of mature cells (at least in the case or WT1 and Mesothelin) (see Figure). Of note, when expressed in developing precursors, using the retrogenic technique, high-affinity TCR resulted in negative selection of cells with



Happy thymocytes enter the thymus. Super-fit ones will be retained. Only the fit ones will graduate. Some of the unfit ones will survive too, but would not be functional in the periphery. TCR gene transfer might now be adopted to make the fit, superfit.

abundant TCR levels, as well as in positive selection of low-expressing cells. In spite of surviving negative selection, however, cells expressing the enhanced-affinity TCR at low levels revealed attenuated antigen sensitivity in the periphery, supporting that the thymus imposes a given affinity threshold that cannot be overcome.[6] Thus, results obtained by Phil Greenberg and collaborators indicate that although central tolerance in the thymus cannot be overruled, it can be tricked by targeting mature peripheral T cells while still avoiding autoimmunity.

This study has implications about the biology underlying negative selection in the thymus and direct consequences for adoptive T-cell immunotherapy. It elegantly demonstrates that central tolerance mechanisms can indeed be overprotective, thus limiting protective immunity (and also self-reactivity) against given nonmutated self antigens and providing an explanation for the failure to retrieve high-avidity self-reactive T cells in mice or humans. It also paves the way for more efficacious adoptive immunotherapy, providing an interesting window of opportunity for the design of better TCRs. Finally, it provocatively suggests that naive T cells with low TCR surface levels might be the ideal source for the identification of naturally occurring TCRs with an intrinsically higher affinity for tumor/self-antigens, which could then be expressed at high levels in T cells and exploited for adoptive TCR gene therapy (see Figure).

The study also leaves open questions. For instance, TCR variants tested by Schmitt et al were obtained by genetic engineering of the α chain CDR3 region. Would this be applicable to TCRs engineered in CDR1 or CDR2 loops? Would this approach be extendible to the large majority of nonmutated self-antigen? Would T cells expressing enhanced-affinity TCRs be resistant to intratumoral tolerance[7,8] and result in better antitumor protection? Would a similar approach be applicable to CD4, or would this result in cells with regulatory phenotypes? Further work will certainly follow, but the results of the study indicate that it is indeed possible to trick the thymus to further the goal of treating cancer.

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LYMPHOID NEOPLASIA

Comment on Liapis et al, page 424

Scouting out the neighborhood in AIDS-related lymphoma

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In this issue of *Blood*, Liapis et al investigate the characteristics of the tumor microenvironment as well as the role of viral components in AIDS-related diffuse large B-cell lymphoma (AR-DLBCL) and compare these findings with sporadic cases of DLBCL.[1]

There is now a burgeoning recognition that components of the local environment of tumor cells play important roles in cancer biology. Within the "tumoral microenvironment," stromal and immune-related cells as well as angiogenic vascular cells are likely to engage in "cross-talk" with tumor cells and this may provide a critically important milieu for tumor growth and invasion. The assorted roles of these components and the communication they engage in within the tumor microenvironment have not been well elucidated, and fresh insights are needed to identify new targets for drug discovery and development.

In DLBCL, gene expression profiling has amply established the existence of molecular subtypes with distinct genetic signatures and clinical outcomes.[2,3] As to the role that the tumor microenvironment plays, 1 survival model, in HIV-negative cases, examined 2 signatures derived from the nonmalignant cells in DLBCL and determined that these could independently predict outcome.[4] The first of these signatures, termed "stromal-1," was prognostically favorable in patients treated with both cyclophosphamide, doxorubicin, vincristine, and prednisone (CHOP) and rituximab plus CHOP (R-CHOP) and included genes that are coequally expressed in many normal mesenchymal tissues and mostly associated with the extracellular matrix. Infiltration by myeloid lineage cells was a characteristic attribute. In contrast, DLBCL tumors with the prognostically unfavorable "stromal-2" signature included genes involved in endothelial cell biology and adipocyte function and correlated with increased blood vessel density.

The composition of the nonmalignant tumor environment and the roles of these various components in AIDS-related lymphoma have not been well studied. In this issue, Liapis and colleagues set out to characterize the tumor microenvironment in AR-DLBCL and directly compare its features with those of sporadic DLBCL cases.[1] Using paraffin-embedded tissue, their study revealed a number of interesting findings. First, they found that the microenvironment of AR-DLBCL was characterized by much higher angiogenic activity than sporadic cases of DLBCL and this correlated with immunoblastic and plasmablastic morphology as well as with Epstein-Barr virus (EBV) positivity, suggesting an association between viral lymphomagenesis and angiogenesis. The principal EBV oncoprotein, latent membrane protein 1 (LMP1), increases production of vascular endothelial growth factor (VEGF) in nasopharyngeal carcinoma; this is a likely mechanism in AR-DLBCL.[5] Additionally, HIV-1 may directly promote angiogenesis.[6] Second, they found that a much higher proportion of AR-DLBCL cases (23.5% vs 5.6% of sporadic cases) were characterized by a MYC rearrangement. Third, compared with sporadic cases, they found reduced T-helper (CD4) and T-regulatory ($FOXP3^+$) cells but markedly higher numbers of $CD8^+$ T cells in cases of AR-DLBCL. Cases with viral antigen expression (LMP1 and/or p24) had much higher numbers of $CD8^+$ T cells implicating differences in immunosurveillance in virally driven tumors.

The study provides interesting insights into the pathophysiology of AR-DLBCL thus providing a rationale for the consideration of novel therapeutics targeting the tumor microenvironment and angiogenesis (see figure). For example, future efforts could focus on characterizing the function of the tumor-infiltrating $CD8^+$ T cells, especially with the advent of EBV-directed cytotoxic T-cell therapies targeting LMP antigens as a potential therapeutic for EBV-associated lymphomas.[7,8] Additionally, it would be compelling to assess gene-expression signatures from the host microenvironment cells in AR-DLBCL as has been done in sporadic cases. Regulating angiogenesis involves a complicated interplay of cells and cytokines and studies that better elucidate the mechanics of this are needed; inhibiting angiogenesis by monoclonal antibodies to VEGF, or by using small-molecule inhibitors, may be interesting future strategies to investigate in AR-DLBCL.[9,10]

Conflict-of-interest disclosure: The authors declare no competing financial interests. ■

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