now provided us several novel agents to augment both the effenter and effenter phases of the immune response following therapeutic vaccination. The effenter phase of the immune response could be enhanced by using more potent vaccines with novel adjuvants, such as Toll-like receptor ligands, or by vaccinating donors of transplant recipients who have a healthy immune system as opposed to patients who may be immunocompromised either from the cancer or from therapy. Alternatively, vaccines could be used in combination with agents that inhibit the immunosuppressive mechanisms such as coinhibitory receptors/ligands and/or deplete regulatory T cells to augment the effector phase of the immune response.

The natural history of development of a novel therapeutic modality is often marked by initial enthusiasm (up) followed by periods of discouragement (down), as obstacles are encountered. The promising results reported by Di Nicola et al, the recent approval of a heat shock shock shock protein vaccine (vitespren) in Russia for the adjuvant treatment of patients with renal cell carcinoma at intermediate risk of disease recurrence, and advances in understanding of immune tolerance suggest that we may be very close to overcoming these barriers to success for therapeutic cancer vaccines.

**Conflict-of-interest disclosure:** S.S.N. declares no competing financial interests. L.W.K. is a consultant for Antigenics Inc.

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**Comment on Alexander et al, page 214**

**Resetting the clock**

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In this issue of *Blood*, Alexander and colleagues describe the reversal of the abnormalities in adaptive immunity following ASCT for SLE. These much needed data provide mechanistic support to immunoablative therapeutic approaches in SLE.

The rationale for autologous hematopoietic stem cell transplantation (ASCT) in systemic autoimmune diseases, such as systemic lupus erythematosus (SLE), is based on 2 major assumptions. The first is that the immunodepleting regimen will lead to deletion of autoreactive cells of the adaptive immune system and, second, that the regenerating immune system will be (more) tolerant to self-antigens; in effect, “resetting the immunologic clock” to a pre-autoimmune state.

Although over 100 patients with severe, treatment-resistant lupus were reported to have undergone ASCT, there are very scarce data about the impact of ASCT on the underlying pathologic processes. The key question is whether ASCT fundamentally changes the abnormal immune response observed in SLE. Alexander et al address this question by performing a detailed phenotypic analysis of T and B lymphocytes and autoantibody responses in 7 patients before and after ASCT. At baseline, patients exhibited abnormalities characteristic of active lupus, such as lymphopenia, restricted T-cell repertoire, dominance of memory versus naive T and B cells, expansion of plasmablasts and high-titer autoantibodies. Conditioning with cyclophosphamide and rabbit antithymocyte globulin (ATG) achieved the expected lymphodepleting effect. The novelty of the paper is the careful analysis of the regenerating adaptive immune system showing the reversal of all, and normalization of most, baseline abnormalities, albeit with different kinetics. The authors confirmed the previously described normalization of the restricted T-cell repertoire by 1 year after transplantation but also provided a description of the kinetics of this normalization. They observed an initial expansion of memory T cells immediately after transplantation (driven by exogenous antigens), followed by an increased output of recent thymic emigrants starting around 6 months after transplantation that led to a diverse, normal-looking T-cell repertoire.

Similarly, there was a dramatic shift in B-cell subpopulations from memory to a naive B-cell dominance after transplantation with disappearance of circulating plasmablasts, a hallmark of active lupus. Accordingly, anti-dsDNA antibodies, which correlate with disease activity in lupus and are thought to be secreted primarily by plasmablasts, disappeared in all patients. The disappearance of protective vaccine-specific antibodies suggested an effect on long-lived antibody-secreting cells, which are thought to also secrete other autoantibodies, such as antinuclear antibodies and anti-Ro/SSA and anti-La/SSB. Similar to vaccine-specific antibodies, antinuclear antibodies either disappeared or decreased significantly. Interestingly, anti-Ro/SSA and anti-La/SSB levels persisted in the 2 patients who had these antibodies at baseline, which is especially intriguing because 1 of these patients flared 18 months after transplantation. The reason for the persistence of these antibodies is unclear but may reflect the resistance of some long-lived plasma cells or a difference in the availability or presentation of various autoantigens after transplantation. The clinical significance of this observation remains to be determined. There are a few limitations to the study. First, the number of patients is relatively low, but the long follow-up and the consistency of findings among the 5 patients with lasting remissions strengthen the results. The observation that CD4 CD25brightFoxP3 regulatory T cells return to the range seen in healthy controls...
and inactive lupus patients is limited by the lack of pretransplantation data and functional analysis demonstrating the suppressive capacity of these cells. The demonstration of thymic regeneration of the T-cell repertoire is exciting but its applicability to older populations has yet to be determined because all patients but 1 in the study were younger than 40 years and there are some concerns that older patients may lose their thymic function and may regenerate a more restricted T-cell repertoire. How this would impact lupus is unknown. Moreover, the study focused exclusively on the adaptive immune system, and it would be important to include analysis of the innate immune system as well. For example, it would be very instructive to know what happened with the interferon signature after transplantation in patients who maintained remission and those who flared.

Despite the few limitations, this is the most comprehensive study so far in this area and together the data strongly suggest that the immunologic clock has been reset to a preautoimmune state after ASCT and provides mechanistic support for continued exploration of ASCT in lupus. What is not clear is if these changes are specific to this approach and whether resetting the clock is sufficient to prevent the recurrence of lupus. To address the first question, it is of utmost importance to include this type of analysis in clinical studies using other immunodepleting strategies of various intensity (eg, B-cell depletion or high-dose cyclophosphamide without H SCT) to identify the changes that are crucial for success. Only time will tell if resetting the clock has a curative potential for some. But the fact that disease-free survival was around 50% in the 2 largest published cohorts suggests that this approach may not be equally effective for all lupus patients. Therefore, it is very important to identify prognostic factors that may predict response before transplantation or the reason for nonresponse or relapse after transplantation. Long-term monitoring of the innate and adaptive immune system of patients who responded to ASCT may also identify potential targets for therapies to prevent the reemergence of autoimmunity.

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The transcobalamin receptor, redux

Donald W. Jacobsen and Alla V. Glushchenko CLEVELAND CLINIC

In this issue of Blood, Quadros and colleagues have identified a novel high-affinity cellular receptor for transcobalamin, a serum B12-binding protein that escorts the micronutrient to sites of cellular uptake. Importantly, they also identify the gene for the transcobalamin receptor.

Cobalamin (Cbl; B12) is an essential micro-nutrient required by all cells in the body. For several decades, we have been aware that Cbl-binding proteins escort B12 to sites of absorption and cellular uptake. Thus, intrinsic factor (IF), with its high-binding specificity for Cbl, mediates the absorption of B12 in the gut. The distribution and delivery of Cbl to cellular uptake sites is mediated by transcobalamin (TC, transcobalamin II), a nonglycosylated serum Cbl-binding protein. In contrast, the mechanics of Cbl absorption and cellular uptake, both receptor driven, are only now being understood.

Dietary Cbl binds to IF in the duodenum and then escorts it to sites of uptake in the ileum. Recent work on the IF-Cbl receptor has identified cubilin as the protein that binds IF-Cbl and amnionless as the protein that tethers cubilin to the plasma membrane of the ileal enterocyte. The IF-Cbl-cubilin-amnionless complex undergoes endocytosis followed by proteolytic degradation of IF in lysosomes and release of Cbl to the cytoplasm. Newly absorbed Cbl enters portal circulation as TC-Cbl and undergoes systemic distribution. Cellular requirements for B12 are satisfied by expression of cell surface receptors for TC-Cbl.

Functional aspects of TC receptor dynamics and regulation of its expression were characterized in early studies using cultured mammalian cells. High-affinity TC receptors capture TC-Cbl and internalize the complex by endocytosis involving clathrin-coated pits and vesicles, as shown in the figure. In acidic endosomes, TC-Cbl dissociates from the TC receptor, which then recycles back to the cell surface. The entire cycle takes approximately 20 minutes. Endosomes carrying TC-Cbl fuse to form lysosomes where TC is degraded by proteolysis, and Cbl is exported to the cytoplasm. Cobalamins are processed in the cytoplasm by mechanisms that remain largely unknown. However, intracellular escorts or chaperones are likely to be involved. In mitochondria, 5- deoxyadenosyl-Cbl is synthesized from processed Cbl, and it serves as the coenzyme for the conversion of methylmalonyl-CoA to succinyl-CoA by methylenyl-CoA mutase. In the cytoplasm, methyl-Cbl is formed, probably on methionine synthase, and serves as a coenzyme for the remethylation of homocysteine to methionine using N5-methyltetrahydrofolate as the methyl group donor.

Although Quadros et al6 purified and characterized human TC in 1986 and later cloned its gene, complete characterization, including cloning of the TC receptor, has been more challenging and unfulfilled. However, in this issue of Blood, Quadros and colleagues appear to have met the challenge using an elaborate 3-tier affinity purification protocol. This technique has provided them with enough pure protein from human placenta to determine the amino acid sequences of TC receptor peptides and to identify a protein in the data bank. With this information in hand, it was a relatively simple matter to identify the TC receptor gene.
Resetting the clock

Gabor G. Illei