the 6 conserved aspartate residues (designated "D" in amino acid notation) in the molecule (see figure) are permissive to change, but the other 2 (D109 and D156) are critical for function with resulting marked impairment or total loss of function when mutated. Why aspartate? This amino acid is more highly conserved than others because of several attributes that include a short side chain, a high charge density, strong polar interactions, and molecular rigidity.[2]

Recently, the same laboratory were the first to identify that PCFT plays a critical role in folate absorption from the relatively acidic milieu of the upper small intestine and transports folate into the brain through the blood, choroid plexus, cerebrospinal fluid conduit.[3] Unlike the reduced folate receptor, PCFT has a similar affinity for reduced folate and folic acid, at pH 5.5, ambient in the upper small intestine. A loss-of-function mutation affecting the *pcft* gene coding for this transmembrane protein was identified as the cause of hereditary folate malabsorption,[3,4] an uncommon cause of folate deficiency of previously obscure etiology that presents within the first few months of life and leads to death during early infancy if not treated. Since then, several mutations affecting various other critical amino acid residues have been described in individuals with hereditary folate malabsorption (HFM) and these have been reviewed recently.[5,6] Using a HELA cell subclone that lacks membrane folate transporters including PCFT, Shin and coworkers transiently transfected the cells with site-directed mutants of PCFTs and monitored functionality using tritiated methotrexate as surrogate cargo for the transporter.[1] The overall conclusions from these studies were that D156, located in the fourth transmembrane domain, is critical for PCFT protein stability. In addition, D109, in the first intracellular loop (between the second and third transmembrane domains) is absolutely essential for PCFT function, perhaps to maintain flexibility at what may be a critical hinge point in the molecule allowing for inward or outward flip-flop during the transport cycle.

Recommended treatment for HFM consists of parenteral injection of folate, preferably with folinic acid (5-formyl tetrahydrofolate), for correction of the anemia and central nervous system problems.[6,7] As emphasized in the recent comprehensive review of this topic,[6] the fact that folinic acid (leucovorin) has a much higher affinity than folic acid for the reduced folate carrier proves important in the management of HFM. In addition, as further pointed out by the same group, folic acid can bind irreversibly to the folate receptor alpha in the choroid plexus, also required for transport across this organ, putatively blocking transport of reduced folate forms including leucovorin that provide rescue to a folate-deprived brain.[8]

More than just a tour de force of molecular exploration, the present publication has, at core, a happy ending. All too often, by the time that the diagnosis of HFM is made, the patient has sustained irreversible damage to the nervous system. The efficacy of oral folate (as occurred in the patient who formed the basis of the current study) is curious and suggests either passive diffusion or hobbled participation by the reduced folate carrier located on the apical brush border membrane of the small intestine that functions poorly at low pH. Sufficiently high blood folate concentrations presumably then could be attained to clear the choroid plexus hurdle. In the currently described patient, after treatment first with oral folic acid and then only later with supplementary folinic acid (1.6 mg/kg/d), the outcome was fortunately excellent. At age 18 months, she fell above the 95th percentile for weight, having attained normal developmental milestones—a satisfying, though seemingly fortuitous, example of translational success.

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

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IMMUNOBIOLOGY

Comment on Mei et al, page 5181

A gut feeling about plasmablasts

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Detailed characterization of the plasma cell precursors that persist in patients' blood after rituximab treatment suggests a mucosal origin, potentially identifying a subset of B cells refractory to anti-CD20 depletion and capable of continued differentiation.

Rituximab, the CD20-specific antibody introduced more than 10 years ago as a therapy for non-Hodgkin B-cell lymphoma,[1] has since had its use extended with success into the treatment of several forms of autoimmune disease thought to have a B-cell component, including rheumatoid arthritis (RA) and systemic lupus erythematosus (SLE).[2,3] Although in some diseases there is a near complete ablation of autoantibodies after anti-CD20 therapy, others—including RA and SLE—show a persistence of autoimmune disease markers. That is, despite almost complete B-cell deletion as measured in peripheral blood, aspects of autoimmune disease such as rheumatoid factor persist. While there is agreement that long-lived plasma cells resist CD20-mediated depletion due to their sessile lifestyle and low-level expression of CD20, the persistence of other B-cell types is less easily

understood. In particular, the plasmablasts in the blood of rituximab-treated RA patients is of interest as, almost by definition, their presence indicates continued immunologic activity, which in turn requires treatment-resistant B-cell precursors capable of continued differentiation. Specifically, it would be of interest to know the nature of B cells that resist rituximab depletion and how these cells continue to produce plasmablasts.

To better understand the results of Mei and colleagues,[4] it is important to review some aspects of plasma cell biology.[5] The term plasma cell is used to define nondividing, sessile cells secreting large quantities of antibody that are the terminal stage of B-cell differentiation. In this state many of the characteristics of B cells are lost due in large part to the loss of expression of the B-cell identity factor, Pax5. While the precise means by which an activated, proliferating B cell is transformed into a nondividing plasma cell remain somewhat uncertain, there is an early stage in which the proliferating B cell begins secreting antibody. This cell, retaining characteristics of the B cell yet having gained some aspects of plasma cells, is called a plasmablast. The means by which a plasmablast converts to a plasma cell remain shrouded in uncertainty, but it is thought to have to be completed relatively quickly as plasmablasts do not have intrinsically long lifespans. In the human, it is estimated in days. Plasma cell survival, which is better defined, is entirely context-dependent, requiring continuous receipt of external signals including the chemokine CXCL12, the cytokine IL-6, the tumor necrosis factor family member APRIL, and ligands to a variety of adhesion molecules. Thus, plasmablasts present in the blood are transient, destined either to die or to become long-lived plasma cells by gaining access to a survival niche in a lymphoid organ. The persistence of plasmablasts in the blood of rituximab-treated RA patients, observed by Mei and colleagues in this issue of *Blood*, almost by definition requires these cells to be continuously released into the blood from a store of B lymphocytes that survived the depletion therapy.

The insight provided by Mei and colleagues arises from using the detailed characterization of the plasmablasts present in the blood of RA patients after rituximab therapy to fit these cells into a program of plasma cell development.[4] They conclude that the majority of these cells have a mucosal origin. The rationale underpinning this suggestion arises in large part from the previous work of this group in which they observed how the circumstances surrounding the development of plasma cells are reflected in the molecules expressed by the plasma cell and in the response of the plasma cell to external stimuli.[6] That is, plasma cells that develop in, for example, inflammatory circumstances characterized by high levels of interferon γ, reflect that origin by expressing the chemokine receptor CXCR3 and responding to its ligand, CXCL9.[5] In this case, the persisting plasmablasts predominantly secrete immunoglobulin A (IgA) and express the mucosal cell adhesion molecule b7 integrin and mucosal chemokine receptor CCR10 that permit migration toward the mucosal-expressed chemokine, CCL28. These attributes, previously documented by this group, essentially define a mucosal-derived plasmablast population. Furthermore, these presumptive gut-derived plasmablasts displayed somatic mutations within their Ig heavy-chain variable-region genes (an indicator of T cell–dependent immune responses), were mostly positive for the marker of proliferation detected by the antibody Ki-67, and secreted antibodies apparently enriched for reactivity for gut microbes. Collectively, these clues led the authors to conclude that these circulating IgA plasmablasts were recently produced in mucosal tissues by T cell–dependent immune responses to intestinal antigens. Although the persistence of circulating IgA plasmablasts in splenectomized patients supports this conclusion by not providing an alternate source of such cells, the best support comes from the direct examination of the lamina propria of rituximab-treated patients.

Mei et al were able to examine biopsies taken from the gastrointestinal tract of 3 patients being treated with rituximab for diffuse large B-cell lymphoma. While not an ideal match for the previous blood analysis, it was a remarkable opportunity to examine the tissue under consideration. The authors found that rituximab was very efficient at deleting $CD20^+$ cells from the lamina propria; there was clear evidence for IgA^+ plasma cells and for IgA^+ plasmablasts, identified as such by expression of Ki67. It is curious that these plasmablasts were present at low frequency, as they presumably have to resupply those previously detected in the blood. When and where T cells are involved in this process remains an open question; their involvement is indicated by the somatic mutation of the plasmablast V genes.

The results of this study, while not providing a handle on the cells that continue to produce autoreactive antibodies in RA patients after rituximab therapy, do provide an interesting insight into the immunology underlying the production of a distinct population of plasma cell precursors. It shows, as the authors point out, that these cells can be continuously produced from a reservoir that does not require replenishment from a $CD20^+$ compartment either in the lamina propria, the spleen or the bone marrow, which is efficiently depleted of B cells by rituximab therapy. This clearly leaves open the questions of how these plasmablasts are produced, what cell types are involved, and what pathways are engaged. For example, the microbial specificity may suggest the involvement of pathogen associated molecular pattern receptors, including Toll-like receptors.[7]

The examination of patients undergoing immunomodulatory therapies afford opportunities not only to gain potentially important insights into diseases and their treatment, but also to examine immunologic processes that may be fundamental to continuing good health.

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

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