inside blood commentary

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CLINICAL TRIALS AND OBSERVATIONS

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To B or not to B maintained?

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In this issue of *Blood*, Bhoj et al explore the longevity of plasma cells (PCs) and humoral immunity in the absence of B cells. This is done by studying subjects experiencing B-cell aplasia after CD19-directed chimeric antigen receptor (CAR) therapy.[1]

Rapid immune protection against previously encountered antigens depends on the presence of circulating antibodies. These antibodies are secreted by antigen-specific PCs, a product of the germinal center reaction after infection or vaccination. Despite a maximum half-life of ~1 month for IgG, this humoral protection can last for the entire lifetime of the host. Thus, PCs must be either particularly long-lived or constantly replenished from the B-cell pool, or both.

It has been >40 years since McMillan and colleagues first suggested that long-lived PCs residing in the bone marrow are the primary source of circulating IgG in humans.[2] Understanding the PC compartment in humans is essential to fully appreciate the mechanisms of how long-lasting humoral immunity is formed and maintained. Still, this population remains largely elusive in humans. Conversely, in mice, the concept of long-lived PCs is widely accepted. There is evidence that long-lived PCs are maintained independently of both antigen and replenishment from the B-cell pool.[3,4] To date, the best evidence supporting a long-lived PC population in humans is the demonstration that PCs persist for at least 6 months after B-cell depletion therapy.[5,6] Recent reports of bone marrow–resident $CD19^{-}CD38^{hi}CD138^{+}$ PCs with a prosurvival phenotype and specificity for historic antigens[6,7] also provide evidentiary support.

In this issue, Bhoj et al provide evidence for the persistence of long-lived $CD19^{-}$ PCs in the complete absence of $CD19^{+}CD20^{+}$ B cells in humans. The authors studied 16 subjects (4 adults and 12 pediatric patients) included in clinical trials for a CD19-directed CAR-based adopted T-cell therapy (CTL019) for the treatment of several B-cell malignancies. CD19 is expressed not only on tumor cells, but also on all normal B cells from the early precursor stages through maturation until terminal differentiation. Therefore, profound B-cell aplasia and hypogammaglobulinemia[8] were expected adverse effects of CTL019 treatment. Consequently, patients undergoing this treatment offer a unique opportunity to study the long-term maintenance of $CD19^{-}$ PCs and preexisting humoral immunity in humans without the interference of recruitment from the B-cell pool.

Bhoj et al confirm previous findings of a $CD19^{-}CD38^{hi}CD138^{+}$ subset of PCs[6,7] in the bone marrow. Using flow cytometry, they further show that, as expected, $CD19^{-}$, but not $CD19^{+}$, PCs are spared after CTL019 treatment. Immunohistochemical analysis of bone marrow biopsies taken post–CTL019 treatment confirmed the results from flow cytometry. By both of these techniques, they showed complete loss of $CD19^{+}$ and $CD20^{+}$ cells in all patients except one. In several of the subjects, there was a $CD19^{-}CD138^{+}$ PC population preserved for as long as 25 months after CTL019 treatment. These results demonstrate that $CD19^{+}CD20^{+}$ B cells are not required for maintenance of the long-lived PC population. It is worth noting that although $CD138^{+}$ PCs were present in the bone marrow biopsies from all 4 adult subjects after CTL109 treatment, these cells were detectable in only 4 of the 12 pediatric patients. Interestingly, these 4 pediatric patients were between 17 and 21 years old, and thus were among the oldest included in the pediatric cohort, indicating that it takes time to form a detectable $CD19^{-}$ PC population. Mei et al previously showed that $CD19^{-}$ PCs are completely lacking in infants for up to at least 7 months.[6] Thus, the long-lived $CD19^{-}$ PC subset appears to be formed throughout childhood and adolescence, not reaching numbers above a detectable limit in bone marrow biopsies until between 15 and 20 years of age. This model emphasizes the importance of immune priming by vaccination during these years for making up a pool of protective circulating antibodies. In fact, Bhoj et al show that, even in a state of chronic B-cell aplasia, the continuous secretion of antigen-specific IgG and IgA remains. Although the cohort was limited in size, these data nevertheless convincingly demonstrate the existence of a long-lived PC population important to maintaining humoral immunity to antigens encountered early in life.

During the course of the study, the authors also had the opportunity to analyze postmortem tissue samples from a CTL019-treated subject. They reported that during chronic B-cell aplasia, $CD19^{-}$ PCs are not restricted to the bone marrow, as previously thought,[6,7] but are also present in lymph nodes and in the mucosa of the gastrointestinal tract. Supporting this observation was a study of human gut biopsies,[9] as well as a recent study in mice showing that there is indeed a survival niche in the intestinal lamina propria

for long-lived PCs generated in mucosal responses.[10]

With this study, Bhoj et al provide evidence for a long-lived CD19$^-$ PC population that is maintained independently of B cells. These findings may also be of direct clinical importance when B-cell depletion is used to treat autoimmune diseases or any disease with antibody-mediated pathology. That is, the pathogenic autoantibodies may be a product of long-lived PCs and will consequently persist despite the ensuing B-cell aplasia. Future studies on larger cohorts will likely provide firmer conclusions and greater insight into PC biology. These studies could demonstrate whether loss of CD19 expression reflects commitment to the long-lived PC population, or if CD19$^+$ PCs can also be long-lived. Finally, little is known about the biological triggers that drive long- vs short-lived PC differentiation or development as a B-memory cell vs as a PC.

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

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Comment on Krejcik et al, page 384

A new era of immune therapy in multiple myeloma

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In this issue of *Blood*, Krejcik et al provide the first clinical data that describe unexpected immune stimulatory activity of the monoclonal antibody (mAb) daratumumab. By targeting CD38-expressing immune suppressive cells, clonal memory T-cell function is induced in heavily pretreated patients with relapsed and refractory multiple myeloma (MM).[1]

Despite early disappointments, mAb's have now entered the clinical armamentarium for MM. They act via mechanisms distinct from currently available therapies and could complement other treatments at all stages of treatment. In particular, the development of immunotherapies targeting CD38 is based on its overexpression on malignant plasma cells (PCs) in all stages of MM.[2] More than 2 decades ago, 2 preclinical studies reported a chimeric mAb or immunotoxin, providing evidence for CD38 as a promising target in MM. However, because of concerns about adverse effects related to CD38 expression on immune effector, endothelial, and committed hematopoietic progenitor cells, clinical development of anti-CD38 mAb therapy was delayed. Of note, CD38 is not expressed on primitive hematopoietic precursors (CD34$^+$CD38$^-$), suggesting that hematopoietic recovery would occur following CD38-targeted cytotoxic agents. Indeed, growth of burst-forming unit erythroid and granulocyte-macrophage colony-forming unit was unaltered or only moderately affected in these 2 early preclinical studies.[3,4]

Promising preclinical data showing multiple Fc-dependent and immune-mediated mechanisms of MM cytotoxicity,[5] coupled with single-agent activity in patients with heavily pretreated relapsed and refractory MM (RR MM),[6,7] provided the framework for the anti-CD38 mAb daratumumab to be approved by the U.S. Food and Drug Administration in 2015. A second anti-CD38 mAb, isatuximab, also shows single-agent activity in patients with RR MM. Both anti-CD38 mAb's trigger antibody-dependent cellular cytotoxicity, complement-dependent cytotoxicity, and antibody-dependent phagocytosis, as well as inhibition of the enzymatic activity of CD38. Moreover, even in the absence of Fc-receptor–expressing effector cells, both mAb's can induce direct apoptosis and lysosome-mediated cell death in MM cells harboring p53 mutations.[8] Most importantly, this preclinical activity has translated to clinical utility as monotherapy even in high-risk, multiply relapsed MM.

What are the most important mechanisms underlying this impressive single-agent clinical activity of daratumumab? In particular, given that CD38 can be expressed on activated immune effector cells, what is the effect of daratumumab treatment on immune mechanisms of MM patients in vivo? In elegant correlative science studies, Krejcik et al collected peripheral blood mononuclear cells and bone marrow mononuclear cells pre- and postdaratumumab treatment from patients enrolled in 2 seminal trials to characterize immune inhibitory and stimulatory cells known to express CD38. They showed that CD38 expression is highest on MM cells, natural killer cells, and regulatory B cells (Bregs), followed by regulatory T cells (Tregs), B cells, and T cells, in both MM patients and healthy donors. CD38 expression on effector T cells is lower in MM patients compared with healthy donors. Myeloid-derived suppressor cells (MDSCs) were detectable at only low levels in fresh samples but highly express CD38 following expansion in cocultures with MM cell lines. Importantly, daratumumab depletes immunosuppressive CD38$^+$Bregs, MDSCs, and Tregs in patient samples at 1 week after treatment. Those Tregs