viral infections following allogeneic stem cell transplantation.

Is this the future of cell therapy for viral infections? It might well be, if a large-enough cell-line bank could be created. The different strategies to obtain cells with antiviral activity and their potential problems have been reviewed.[8] The 2 competing options are separating the preexisting (and low-frequency) VSTs from donors by a variety of methods[4,5] or generating them in vitro.[6] The advantage of the first method is that it may be fast; the disadvantage is that sometimes it may impossible for either logistical reasons (donor not available) or technical reasons (VSTs too low to detect or separate). The advantages of the VSTs generated in vitro include obtaining the desired antiviral specificity regardless of preexisting immunity and potentially treating several infections (and maybe even the malignant disease[9]) simultaneously. The disadvantage is time. Even with improved rapid expansion methods, at least 10 days separate the time it is decided that a cell line is needed and the infusion of the VSTs, usually 2 weeks or more.[10] Depending on the clinical situation, time may not be available.

Prebanked, partially HLA-matched VSTs seem to offer significant advantages. If the lack of GVHD is confirmed in further studies, this approach will likely become the preferred first choice because it provides immediate efficacy and does not preclude obtaining cells from the stem cell donors by any of the other 2 methods, although the efficacy of the third-party VSTs seems already very promising.

The only caveat (which may be significant) is whether these VSTs will perform as effectively in the presence of more immunosuppression. The patients included in this study represent a highly selected subset of all of those who went on to have viral infections: patients were excluded if they had active, acute GVHD grades II to IV; had received T-cell–depleting monoclonal agents like anti-thymocyte globulin or alemtuzumab; or were receiving more than 0.5 mg/kg/day of prednisone. Even under these relatively favorable immunologic circumstances, 10 of the 50 patients died of viral infections. In practice, it is common for viral reactivation to occur in the midst of GVHD actively treated with higher doses of steroids and other immunosuppressive agents, which may affect both the efficacy and safety of the VSTs. These concerns, as well as the myriad of immunologic topics brought up by these data (eg, differential effects of the same VSTs on different viruses in different patients, antiviral activity in the absence of detectable circulating antiviral T cells) will be addressed in future trials. After this study, the promise of effective cellular therapy for refractory viral infections seems closer than ever.

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Comment on Covens et al, page 5176

To B1 or not to B1: that really is still the question!

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In this issue of *Blood*, Covens et al revisit the contentious area of human B1 cells and provide evidence that human $CD20^+CD27^+CD43^+$ cells are preplasmablasts, rather than committed to the B1 lineage.[1]

It is well-established that 2 populations of B cells—B1 and B2—exist in mice. Decades of research have demonstrated that these subsets can be distinguished from one another according to phenotype, ontogeny, anatomical location, and function.[2-4] Thus, B2 cells are continually generated from bone marrow precursors and circulate throughout the blood and secondary lymphoid tissues. Follicular and marginal zone B cells both arise from the B2 lineage and can respond to a broad range of T-dependent (TD) and T-independent antigens. B2 cells can undergo class switching, somatic hypermutation, and generate memory cells, thereby potentially providing long-lived antibody-mediated protection against many structurally diverse pathogens.[3,4]

B1 cells, on the other hand, predominate during fetal and neonatal development, self-renew following their generation from stem cells present at these early stages of life, and predominantly localize to peritoneal and pleural cavities.[2-4] B1 cells are considered innate immune cells that produce the majority of "natural" immunoglobulin M (IgM) and IgA, which is largely encoded by germline immunoglobulin genes. Because of its polyreactivity and ability to recognize large repetitive structures, this natural IgM acts as a first line of defense against pathogens such as encapsulated polysaccharide-expressing bacteria.[3,4] Subsets of B1 cells also exist that can be delineated by differential expression of CD5.[3] These subsets are also functionally

distinct, with $CD5^+$ B1a cells producing broadly reactive natural IgM, whereas $CD5^-$ B1b cells can generate T-independent, long-lasting memory-type IgM responses to some infectious pathogens. These distinctions between B1 and B2 cells led the Herzenbergs to presciently propose that the immune system develops in "layers," such that B cells appearing early in ontogeny (ie, B1) provide low-affinity IgM-mediated polyreactive humoral immunity against a broad spectrum of infections, whereas B cells generated later in life (ie, B2) generate high-affinity, monospecific class–switched immunoglobulin that provides long-lived serological memory.[2]

Despite there being general consensus regarding the distinct origins and functions of B1 and B2 cells, B1 cells have suffered an identity crisis. Debate has raged as to whether B1 cells represent a distinct B-cell lineage or correspond to a stage of activation that all B cells experience.[2-4] This has not been helped by the ambiguities associated with identifying B1 cells in humans that have arisen from logistical difficulties in isolating B cells from peritoneal and pleural cavities of humans at all, let alone healthy individuals, and the assumption that human B cells expressing CD5—such as those in cord blood—are the counterpart to murine B1 cells.[4]

A recent study concluded that human B1 cells can be identified and resolved from other human B-cell subsets by a distinct surface phenotype, specifically $CD20^+CD27^+CD43^+$. These cells, comprising ~5% to 10% of B cells in cord or adult blood, were defined as B1 cells based on shared key features with murine B1 cells: the ability to spontaneously secrete IgM, to induce proliferation of allogeneic T cells, and to exhibit tonic signaling in the absence of direct B-cell receptor engagement.[5] The population of human "B1" cells consisted of both $CD5^-$ and $CD5^+$ subsets, with the $CD5^-$ subset preferentially producing antibody against polysaccharide antigens.[5,6] Although such a discovery may have been expected to be welcomed by human B-cell biologists, in keeping with the historical controversies that have dogged murine B1 cells, the veracity of this initial study has been vigorously debated.[7,8] For example, an alternative explanation for some of these data was that human $CD20^+CD27^+CD43^+$ B cells actually correspond to plasmablast-type cells.[8]

This possibility has now been addressed.[1] By employing a phenotypic, functional, gene-profiling, and in vitro culture approach, it was concluded that $CD20^+CD27^+CD43^+$ B cells are more likely to correspond to activated B2 cells undergoing the early stages of commitment to the plasma cell lineage—and thus are termed preplasmablasts—rather than belonging to a distinct B-cell lineage (that of B1 cells). Direct evidence for this was that $CD20^+CD27^+CD43^+$ B cells spontaneously secreted IgM, IgG, and IgA, including IgG specific for the TD immunogen tetanus toxoid following booster vaccination, and that gene expression profiling revealed them to be more similar to plasmablasts than naive or memory B cells.[1] If $CD20^+CD27^+CD43^+$ B cells were exclusively B1 cells, they would not be predicted to produce class-switched immunoglobulin nor to elicit enhanced recall responses following booster immunization with TD antigen (ie, tetanus).[2-4] The concept that $CD20^+CD27^+CD43^+$ B cells are preplasmablasts is also consistent with these cells exhibiting additional features of plasma cells, such as a $CD38^{hi}CD20^{lo}$ phenotype,[7,8] and greater expression of *PRDM1* (encoding the transcription factor Blimp-1) and *BCMA*[1] compared with naive and memory B-cell subsets. Additional evidence that $CD20^+CD27^+CD43^+$ B cells may not be B1 cells is that cells with this phenotype could be generated in vitro following stimulation of memory cell precursors, and that $CD20^+CD27^+CD43^+$ B cells themselves could undergo further differentiation in vitro, acquiring a phenotype consistent with plasma cells.

Do these findings definitively resolve the uncertainty surrounding the nature of human B1 cells? Probably not. An underappreciated finding was that only ~5% to 10% of $CD20^+CD27^+CD43^+$ B cells spontaneously secreted immunoglobulin in vitro.[1,5,6] Although this may reflect limitations of the in vitro assay, it may actually suggest that $CD20^+CD27^+CD43^+$ B cells are a mixed population comprising both preplasmablasts and putative B1 cells. It is also worth noting that murine B1 cells constitutively express low but detectable levels of Blimp-1.[9] Thus, the detection of *PRDM1* transcripts in human $CD20^+CD27^+CD43^+$ B cells does not necessarily preclude them from being B1-type cells. Furthermore, it was recently reported that serum IgM levels correlated with frequencies of $CD20^+CD27^+CD43^+$ B cells and that these cells are reduced in individuals with common variable immunodeficiency.[10] The reduction in IgM in these individuals could reflect the preplasmablast nature of $CD20^+CD27^+CD43^+$ B cells or the ability of B1 cells to constitutively produce IgM. Despite these uncertainties, the current study by Coven et al[1] has provided insight into the origins and functions of at least a subset of $CD20^+CD27^+CD43^+$ B cells. Studies such as these will ensure that discussion, analysis, and debate of human B cells, including putative B1 cells, will continue in earnest for years to come.

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