the infection rate was 21% during the first 28 days following CAR-T infusion in this study, and thus prophylaxis of infection applies here as it does in other immunosuppressed patients. It is possible that prophylaxis might eventually include other immune-modulating therapies, such as tocilizumab, steroids, or agents yet to be learned. However, the key to prevention of CRS, like that of irreversible sepsis, will require a better understanding of its pathogenesis. We suggest that management will require interventions based on control of proinflammatory and anti-inflammatory processes. For example, research such as that shown in a recent report on the effects of human resistin[6] on the early events of sepsis could be extended to the search for improved prevention and management of CRS.

Given the short follow-up of this study, it is unknown if these CAR–T-treated patients retain an immunodeficiency similar to that of patients post–autologous transplant, requiring revaccination. Future studies incorporating evaluation of the host cellular immunity late after CAR-T treatment will shed light on this issue. A limitation of this study is that it is a single-center study. All patients received treatment using a defined CD19 CAR–T-cell product derived from the patient's own T cells. Therefore, future studies examining the incidence of infection in patients receiving different CD19 CAR–T-cell products will be needed to determine if the findings in this study are generalizable. Until then, the devil we know is better than the devil we do not, and improvement in prevention and management of CSR after CAR–T-cell therapy will likely emerge side by side with improvements in control of sepsis.

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MYELOID NEOPLASIA

Comment on Gillissen et al, page 131

AML: exposed and exploited?

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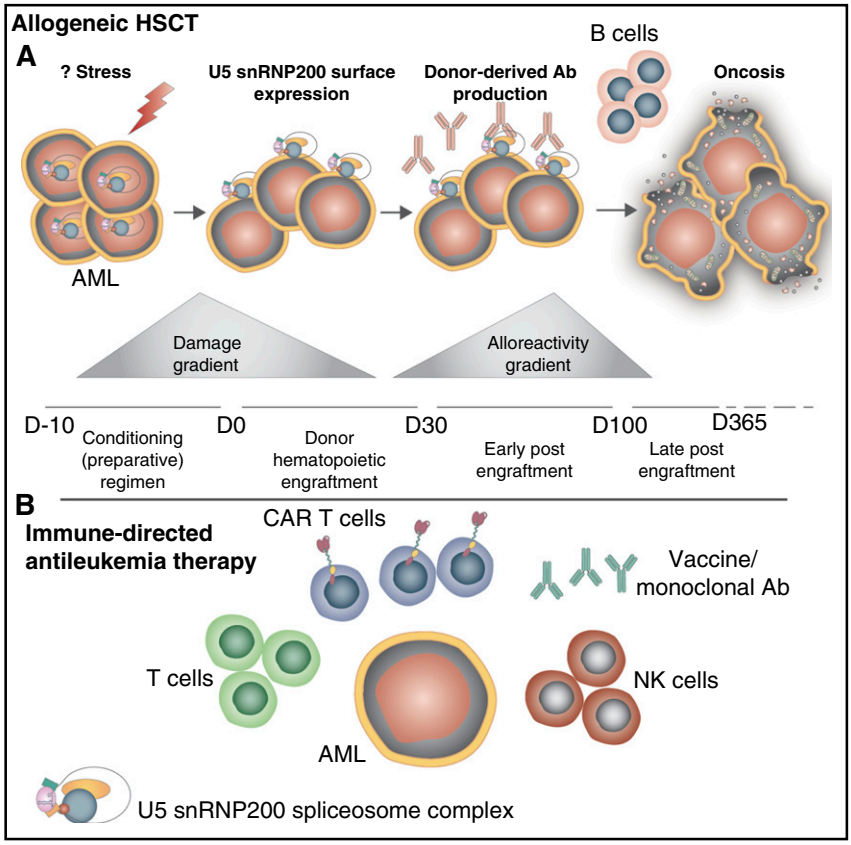
In this issue of *Blood*, Gillissen and colleagues characterize donor-derived cytotoxic antibodies, isolated from allogeneic hematopoietic cell transplant (HSCT) patients with acute myelogenous leukemia (AML) in sustained remission, that targeted the spliceosome U5 snRNP200 complex expressed on the cell membrane of AML blasts. Mechanistically, in vitro antibody-dependent cytotoxicity did not cause leukemia cell apoptosis, but rather destabilization of the cell membrane cytoskeleton and subsequent pore formation, resulting in cellular swelling and extravasation of intracellular contents (oncosis). In addition, in vivo reduction in AML burden using a U5 snRNP200–specific antibody was demonstrated in a murine SCID xenograft model. Collectively, the authors' work suggests a potential role for donor-derived antibodies in mediating graft-versus-leukemia (GVL) activity following allogeneic HSCT.[1]

AML is the most common adult and second most common childhood acute leukemia, but associates with compromised patient survival due to its high incidence of refractory and relapsed disease. Allogeneic HSCT remains the definitive cure for high-risk and relapsed AML in adult[2] and pediatric[3] patients, as allogeneic HSCT uses donor-derived immunity to eradicate leukemic cells in the transplant recipient (GVL). Natural killer (NK) and T cells have conventionally been regarded as the primary cell mediators of the GVL response, and their ex vivo expansion and engineering have proven useful in reducing malignant disease relapse in the context of allogeneic HSCT.[4] However, the contribution of B cells to GVL activity is less established. Therefore, the work by Gillissen and colleagues is intriguing and begs the question: how can we exploit antibodies targeting neoantigens expressed by AML to reduce disease relapse and to improve disease cure following allogeneic HSCT?

The authors identified AML-specific antibodies using conventional laboratory techniques, including B-cell isolation and cell line immortalization, cell lysate immunoprecipitation, and mass spectroscopy and then confirmed target specificity using enzyme-linked immunosorbent assay and flow cytometry. The authors identified the spliceosome component, U5 snRNP200 complex, as the antibody-specific target for inducing oncosis in AML cells. Spliceosomes are responsible for removing introns from primary messenger RNA (mRNA) precursors for mature mRNA to be translated into protein.[5] Interestingly, aberrant RNA splicing and spliceosome mutations may contribute to chemotherapy drug resistance and leukemogenesis in AML.[6] So how did a large, multiprotein intracellular component of



(A) Potential schematic for antibody-specific induction of oncosis in AML following allogeneic HSCT. Normally located within the cytoplasm and nucleus, the spliceosome complex U5 snRNP200 becomes expressed on the cell surface of AML blasts via an undefined cell stress–inducible factor and mechanism for antigen processing and presentation. Damage and alloreactivity gradients associated with allogeneic HSCT may provide the necessary cellular stress in leukemic blasts to instigate this process. Cell surface expression of the spliceosome complex enables donor-derived antibody production specific to the U5 snRNP200 complex, resulting in leukemia cell injury characterized by cytoskeleton disruption and pore formation and subsequent extravasation of intracellular contents and ultimate leukemia cell demise (oncosis). (B) Emerging immune-directed therapy targeting AML. CAR T cells[9] as well as other alternative forms of T-cell therapies[10] could be engineered and/or expanded ex vivo to target preferential antigen expression on AML blasts like the U5 snRNP200 complex. NK cells used in the setting of killer cell immunoglobulin-like receptor mismatch allogeneic HSCT can be expanded and potentially engineered to target AML-specific receptors. Monoclonal antibodies, including gemtuzumab ozogamicin (anti-CD33), target AML blasts, and vaccination strategies incorporating AML-specific antibodies could also be considered. Ab, antibody.

the spliceosome end up on the cell surface of AML blasts?

Given that cytotoxic antibodies were derived from allogeneic HSCT patients who experienced graft-versus-host disease (GVHD), GVHD itself could have induced cellular stress and damage to expose the U5 snRNP200 spliceosome complex, enabling subsequent donor-derived antibody formation via donor B-cell priming (see figure). HSCT-associated damage is well described and has been shown to instigate alloimmune reactions in transplant recipient, including acute GVHD. Specifically, conditioning regimen–induced damage-associated molecular patterns are recognized by pathogen recognition receptors like Toll-like receptors expressed on recipient antigen-presenting cells (APCs).[7] Subsequent APC activation induces donor T-cell activation, which results in target tissue cytotoxicity in the HSCT recipient. However, the fact that not all AML patients who experienced GVHD developed anti-spliceosome complex antibodies suggests that GVHD itself may not be necessary to induce AML cellular stress and subsequent spliceosome complex surface expression. Therefore, other cellular stress–inducible factors as well as the process by which spliceosome complex surface expression occurs remain unknown.

Importantly, antibody specificity was confined to AML blasts expressing surface U5 snRNP200 complex, as the antibody did not bind to other hematopoietic (bone marrow–derived CD34$^+$ hematopoietic progenitor cells, peripheral blood mononuclear cells, and monocytes) or nonhematopoietic cells (endothelial cells and fibroblasts). Therefore, subsequent antibody-mediated oncosis only affected AML blasts. Furthermore, the U5 snRNP200 complex–specific antibody was not found in healthy patients or in multiple myeloma patients undergoing allogeneic HSCT. Such target specificity is essential for using either vaccine- or cell-based immunotherapies to ensure the desired anti-tumor effects and to reduce off-target complications. Given these study findings, chimeric antigen receptor (CAR) T cells and NK cells could potentially be engineered to target AML cells expressing U5 snRNP200 complex. Furthermore, combining immune-directed cellular and pharmaceutical-based products like monoclonal antibodies might also be possible[8] (see figure).

The Gillissen et al study does have apparent limitations. First, the mechanism by which the U5 snRNP200 complex becomes exposed on the surface of AML cells is unknown. Second, the Gillissen et al study does not prove that antibody development results from direct, donor-derived B-cell priming by leukemia cells themselves. Third, in vivo spliceosome complex antibody–mediated GVL activity was not demonstrated using a preclinical allogeneic HSCT model. Finally, clinical use of spliceosome complex–specific antibodies has yet to be studied and may even be restricted to certain AML subtypes in certain patients. However, these limitations do not necessarily diminish the study results, but rather open the door for further investigation in this area. As such, the work by Gillissen and colleagues exemplifies how screening for antibody repertoires in allogeneic HSCT patients may identify novel targets needed to exploit a potential cure for AML using immune-directed therapies.

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TRANSFUSION MEDICINE

Comment on Saris et al, page 144

The delectability of platelets to a phagocyte

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In this issue of *Blood*, Saris et al show that some HLA antigens, namely, B8, B12, and B35, vary in expression on the platelets of some individuals and that this is a constant variant in these people.[1]

In this study, a unique opsonification assay is used to support the authors' hypothesis of antigen density as an important predictor of phagocytosis and measure of whether platelet transfusion refractoriness may occur. These laboratory studies were prompted by careful platelet transfusion studies done several decades ago where alloimmunized recipients to these antigens had good transfusion outcomes.[2,3]

An opsonin (from the Greek *opsōneîn*, "to prepare for eating and improving delectability") is any molecule that enhances phagocytosis by marking an antigen on a cell for an immune response (ie, causes the phagocyte to "relish" the marked cell). Classic immunohematology methodology shows that the determinants of the opsonification process and subsequent antigen clearance include antigen density, qualitative and quantitative features of the antibody, Fc receptor features, and activity of the effector cell, as well as most likely physical determinants such as surface tension and contact area.[4] Immunohematologists have taken advantage of these factors in both alloimmune and autoimmune destruction of cells to develop strategies to circumvent these pathologic processes.

The importance of HLA antigen density is, first, that these platelets with low antigen expression can be transfused into individuals and not be destroyed by alloantibodies with these HLA specificities and, secondly, the antigens may be less immunogenic if repeatedly transfused if there is no antigen presentation process that occurs. The advantage to the patient and to blood resource management would be increasing the number of potential platelet donors for all alloimmunized patients, who would ordinarily be excluded if one just relied on HLA typing results. To support their hypothesis, they first typed donors and determined the relative expression of these antigens. Those that showed little to no expression by monoclonal antibody typing of the platelets were used to test their delectability to phagocytic monocytes after opsonification with HLA antibody. Indeed, they showed that compared with opsonized controls, internalization of platelets obtained from donors with high HLA class I expression antigen density was significantly increased, in contrast to no observed internalization obtained from donors with low class I expression. Furthermore, they showed that the expression of HLA class I B locus antigens paralleled the expression on white cells and was a constant feature of these donors, which did not seem to vary with possible environmental conditions such as inflammation.

These studies support earlier transfusion outcome studies, where HLA-B12 mismatched platelets to refractory, alloimmunized patients with satisfactory increments in 69% of transfusions.[2] In this study, no immunohematologic studies were done; measure of antigen density was done to account for the failed transfusions. In many populations, HLA-B7, -B8, -B12, and -B35 are the most frequently expressed. Identifying donors with natural low expression of these frequent class I antigens could improve the availability of HLA matched donors. Clearly, before adopting such an approach to transfusions in patient populations, we will need to determine the efficacy of this approach. Further consideration of other factors may need to be given such as antibody titer, avidity, and isotype. It is possible that the threshold for expression and clearance may vary with specific patients. It is also possible that although a limited number of transfusion exposures of the donor may not provoke an immune response, eventually the patient will become immunized and refractory to the HLA type.

The strategies to provide platelet transfusions to alloimmunized patients include (1) HLA matching of donor recipient HLA class I A, B antigens, with or without identification of HLA antibody specificities,[5,6] and (2) cross matching platelets.[7,8] Of course, the HLA matching of donor and recipients is operationally complicated and expensive because the matching requires the availability of several thousand typed donors to provide the number of transfusions that may be necessary for a patient. The use of donors with acceptable antigen mismatches has