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GENE THERAPY

Comment on Brault et al, page 2768

Is this a cure for XMEN?

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In this issue of *Blood*, Brault et al present exciting new data suggesting that a novel gene editing approach to the treatment of X-linked MAGT1 deficiency with increased susceptibility to Epstein-Barr virus (EBV) infection and N-linked glycosylation defect (XMEN) can restore magnesium transporter expression and natural killer (NK) group 2 member D (NKG2D) expression on $CD8^+$ T cells and NK cells, thereby restoring their function.[1]

Patients with XMEN experience a combined immunodeficiency that leads to significant morbidity and early mortality. The syndrome is caused by mutations in the *MAGT1* gene, located on the X chromosome at Xq21.1. Inherited in an X-linked recessive pattern, the syndrome is characterized by CD4 T-cell lymphopenia and associated humoral immune defects caused by poor T-cell help and viral infections, of which chronic EBV infection is the most problematic, leading to EBV lymphoproliferative disease.[2] Consequently, patients often survive only into their third or fourth decade of life. Molecular defects in the magnesium transporter encoded by *MAGT1* leads to a range of immunologic defects, including decreased expression of the NKG2D receptor on NK and $CD8^+$ cytotoxic T cells. Abnormal NKG2D expression is thought to be a major contributor to the poor antiviral responses that are characteristic of the syndrome.[3]

A variety of approaches have been tried to control disease, enhance immune function, and improve outcomes, with only modest success. In patients with EBV lymphoproliferative disease, B-cell depletion therapy with rituximab has shown temporary efficacy, typically as a preparative step before hematopoietic cell transplantation (HCT). Immunoglobulin replacement therapy has been used to compensate for the hypogammaglobulinemia intrinsic to the disorder or induced by rituximab to decrease sinopulmonary infections, but this has had no effect on chronic EBV. Magnesium supplementation, particularly with magnesium threonate, seems to be a safe way to increase lymphocyte Mg^{++} levels, but NKG2D expression on $CD8^+$ cytotoxic T cells and NK cells was not significantly improved and EBV viremia was not decreased.[4] HCT has also been tried in some patients, particularly those with EBV lymphoproliferative disease, but survival has not been encouraging. At present, patients are left with few effective therapeutic options.[5]

Brault et al now present data on a novel gene editing approach that can restore magnesium transporter expression and NKG2D expression on $CD8^+$ T cells and NK cells, restoring their function. The approach utilizes CRISPR to create a double-stranded DNA break within the first coding exon of the endogenous *MAGT1* gene followed by insertion of a spliced, codon-optimized *MAGT1* complementary DNA (cDNA) via homologous recombination using a repair template delivered by recombinant adeno-associated virus (rAAV). By placing the spliced cDNA near the initiation codon, expression of the transcript is controlled by the endogenous *MAGT1* promoter, allowing appropriate tissue-specific expression. Cleverly, this approach also makes the therapy universal to virtually all patients with XMEN, regardless of the location of their mutation within the gene (excluding mutations that might affect the integrity of the gene promoter itself). The methodology also offers the potential of a 2-stage therapeutic approach where patient T cells could be edited to provide temporary control of EBV before administration of edited hematopoietic stem cells (HSCs), which will almost certainly require the use of a conditioning regimen to achieve significant levels of engraftment.

Two other advancements used by the study team enhance this approach, making it more innovative and likely to be successful therapeutically. The first improves gene editing efficiency by skewing double-strand break-repair mechanisms toward homology-directed repair. This is accomplished by transient expression of i53 to block 53BP1 accumulation at DNA breaks, thus inhibiting nonhomologous end joining. The second addresses a major problem encountered by virtually all laboratories working on gene editing in human HSCs, namely the poor engraftability of edited cells. The authors note that treatment of HSCs with the rAAV vector carrying the repair template by itself led to significant activation of the DNA damage response, reflected by increased phosphorylation of H2AX and decreased cell viability. By transiently expressing a humanized genetic suppressor element that inhibits TP53 activity during the gene editing process, they limited the DNA damage response, improved the viability of the edited cells, and achieved substantial improvements in edited HSC engraftment in a humanized murine model.

This unique combination of approaches may offer a viable opportunity for an XMEN cure that could prevent the severe outcomes and early death associated with this combined immunodeficiency syndrome.

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HEMATOPOIESIS AND STEM CELLS

Comment on Pagliuca et al, page 2781, and Zaimoku et al, page 2799

HLA in AA: innocent bystander or culprit?

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In this issue of *Blood*, 2 papers deal with HLA in patients with acquired idiopathic aplastic anemia (AA). Pagliuca et al[1] show that patients with AA have a reduced structural divergence of homologous HLA alleles, possibly contributing to reduced T-cell receptor repertoire diversity, cross-reactivity, and emergence of autoreactive T-cell clones. In parallel, Zaimoku et al[2] document that somatic mutations in HLA genes leading to functional loss are frequent and correlate with clinical manifestations of AA, such as age onset and risk of clonal evolution.

These 2 independent experimental works highlight that HLA plays a major role in AA pathophysiology and clinical course. Both papers ultimately confirm the well-established immune-mediated pathophysiology of acquired AA.[3] The 2 papers describe different aspects of HLA involvement and are complementary in shaping a fuller story for HLA in AA. The full story will only be known when the key player, the target antigen in AA, is identified. That acquired idiopathic AA is a T cell–mediated disorder is clearly proven by the clinical efficacy of antithymocyte globulin[4] and by a number of experimental observations.[3] In particular, autoreactive T-cell clones have been identified as the key pathogenic effector in patients with AA.[5] Even if the identification of the target antigen(s) remains elusive, it is plausible that these auto-reactive T cells recognize specific epitopes presented on hematopoietic progenitor/stem cells (HSCs) through HLA molecules. Thus, HLA is necessarily involved in the immune-mediated damage of hematopoietic progenitors, either as an innocent bystander or as a culprit. The 2 manuscripts both support a scenario where HLA is not an innocent bystander, but rather, it appears to be a vicious culprit involved during the whole course of the disease.

In their work, Pagliuca et al show that evolutionary divergence in class II alleles is lower in patients with AA. This is associated with a smaller size than predicted of the immunopeptidomic spectrum, thus accounting for decreased T-cell receptor (TCR) diversity. In addition to the evolutionary divergence assessed through metric quantification of the Grantham distance, the reduced diversity of homologous class II HLA alleles is also caused by a higher frequency of homozygosity at these loci. In evolutionary biology, this condition is supposed to confer a disadvantage because of a less efficient T-cell response against tumors and infectious agents. Thus, in humans, the HLA loci have evolved, developing the greatest diversity. Therefore, in individual subjects with reduced HLA divergence, the immune response remains globally efficient, but it may resort to molecular mimicry and cross-reactivity, which may favor autoimmune phenomena. The work of Pagliuca et al is a very elegant attempt to investigate immunogenetic risk in the development of AA, with the caveat of the understanding of the metric quantification of divergence (variations are quite small, with large overlap between patients with AA and a control population). Nevertheless, they could not make further meaningful steps toward the Holy Grail of AA: namely the target antigen. Pagliuca et al exploit an in silico approach trying to identify recurrent amino acid structures in the peptide binding site of HLA and trying to model antigen interaction and subsequent TCR binding. However, even looking at the HSC-specific proteomic reference, they could only find a lower peptide binding capability, without identifying putative target peptides bound by recurrent HLA binding site structures. Finally, high-throughput analysis of the TCR repertoire confirmed that TCR diversity is lower in patients with AA, but it did not correlate with class II HLA evolutionary divergence. However, patients with AA showed increased frequency of T-cell clones harboring TCR CDR3 sequences with known autoreactive specificity, supporting the authors' hypothesis that molecular mimicry and cross-reactivity may result in pathogenic events.

In the other paper, Zaimoku et al point out that HLA molecules do not remain passive during the clinical course of AA, because somatic genetic lesions ranging from locus deletion (ie, 6p loss of heterozygosity) to single nucleotide mutations may affect about half of patients with AA, leading to functional HLA loss (seen as lack of surface expression). The occurrence of these mutations seems consistent with the normal somatic mutation rate of rapidly replicating cells, but their emergence over normally polyclonal hematopoiesis can be caused by a specific immune privilege of HSCs harboring HLA loss (ie, multiple mutant clones are possible), similar to *PIGA*-mutated cells.[6] Nevertheless, in contrast with paroxysmal nosturnal hemoglobinuria (PNH), in the case of HLA loss, mutant clones usually are unable to effectively replace normal hematopoiesis (variant allele frequency is relatively low and tends to decrease after immunosuppressive treatment). Zaimoku et al also demonstrate that HLA loss and the presence of specific HLA alleles frequently found with HLA loss (irrespective of their loss, such as *HLA-B*1402*) are associated with a high risk of clonal evolution. Thus,