Molecular Therapy
Editorial

ERT Degrades Gene Therapy for Storage Disorder

Lysosomal storage diseases (LSDs) comprise a rare subset of inherited metabolic diseases that impact the function of the lysosome. Patients can present with organ-specific or multisystemic disease, depending on the normal function of the mutated gene, owing to toxicity caused by the accumulation of unprocessed macromolecules within the cell. Some LSDs can be treated with enzyme replacement therapy (ERT), where mannose-6-phosphate (M6P) present in the enzymes allows for cellular uptake by the M6P receptor, leading to trafficking of the therapeutic protein to the lysosome. However, the efficacy of ERT is limited when treating LSDs characterized by CNS involvement because the therapeutic protein is unable to cross the blood-brain barrier. Furthermore, patients who do not have residual enzyme expression are at risk of developing anti-drug antibodies to the infused therapeutic. Hence, treatment of LSDs with hematopoietic stem cell (HSC) gene therapy using lentiviral (LV) vectors is increasingly being explored as a potential solution to these problems.[1–3] However, Squeri et al.[4] now report, in this issue of *Molecular Therapy*, the surprising finding that exposure to a therapeutic antigen during ERT may not only activate B cells but also $CD8^+$ T cells, which may effectively wipe out transplanted LV vector-transduced cells.

LV transduction of autologous HSCs and reintroduction of the gene-modified cells back into the patient is now widely used to treat LSDs.[1,2] However, therapeutic efficacy is dependent on engraftment efficiency. Pretransplant conditioning, which is often a chemotherapeutic drug or radiation, is used to eliminate endogenous HSCs and provide a niche for the engraftment of the gene-modified HSCs. The choice of non-myeloablative or myeloablative conditioning depends upon several factors such as whether the transgene provides a selective growth advantage over nonmodified HSCs, the desired engraftment level of the gene modified HSCs, and transgene expression levels needed for therapeutic efficacy.

In the present study, mucopolysaccharidosis type I (MPS-I) mice immunized with IDUA protein in adjuvant and pre-conditioned with total body irradiation rejected engraftment of LV-IDUA-transduced HSCs.[4] Interestingly, the authors found that radioresistant $CD8^+$ T cells, and not anti-IDUA antibodies, were responsible for the impaired engraftment. Engraftment was partially rescued with adjunct immune suppression with a moderate T cell-depleting agent, fludarabine, whereas nearly all treated mice receiving a T cell-depleting anti-CD3 antibody exhibited successful engraftment. These findings are interesting for several reasons. Immune responses that occur during ERT for LSD and other diseases such as hemophilia often focus on $CD4^+$ T helper cell-dependent anti-drug antibodies, whereas $CD8^+$ T cell responses are not considered relevant for restoring the therapeutic benefit of ERT. However, in the context of LV-IDUA HSC gene therapy, $CD8^+$ T cells are revealed to play a significant in role in determining therapeutic efficacy. Interestingly, others have reported a similar impairment in the context of HSC gene therapy for factor VIII expression in platelets when cells were adoptively transferred into hemophilia A mice with pre-existing factor VIII protein immunity.[5] Additional immune modulatory therapy (either low-dose total body irradiation or anti-thymocyte globulin) to supplement busulfan pre-conditioning enhanced LV-2bF8 (FVIII expressed from a platelet specific promoter) HSC engraftment. However, unlike the present study, the authors did not determine the reason for impaired HSC engraftment.

There are several limitations worth mentioning about this study in terms of relevance to human patients undergoing ERT. While the authors mention the identification of $CD8^+$ T cell responses to GAA ERT in Pompe disease patients, it is unclear how frequently patients receiving ERT for LSD actually develop a $CD8^+$ T cell response. The authors required the use of an adjuvant to not only provoke a uniform antibody response to human IDUA, but to also obtain a measurable T cell response in their MPS-I mouse model, which does not mimic ERT in humans. Second, the authors selected total body irradiation for pre-conditioning despite using busulfan in an ongoing clinical study of MPS-I patients. While it is known that certain subsets of lymphocytes are resistant to radiation, busulfan is reported to have minimal impact on lymphocytes in various animal models. Thus, it is unclear how effective adjunct fludarabine immune suppression would be compared to the more robust anti-CD3 T cell-depleting antibody. Third, it is not clear what promoter is used to drive IDUA expression and what are the critical cell lineages needed to express IDUA for therapeutic efficacy. If the authors could prevent expression of IDUA in HSCs, such as through the use of a HSC-specific microRNA (miRNA) target sequence, would that have a positive impact on engraftment in the presence of IDUA $CD8^+$ T cells?

Nonetheless, this study highlights that pre-existing $CD8^+$ T immunity developed during ERT may impact the efficacy of LV-transduced HSC gene therapies for LSD and other diseases amendable to correction with HSC gene therapy. Any future design of clinical protocols for LV-HSC gene therapy for diseases with ERT should consider assays to test for transgene $CD8^+$ T cell frequencies and include adjunct immune suppression to provide the best therapeutic outcome for patients.

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