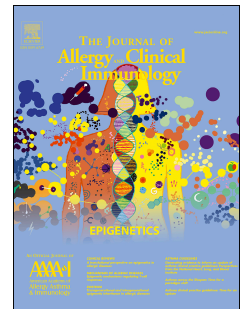


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Intra-thymic delivery a new route for adenoviral associated vector gene therapy

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SCID and standard therapy

Severe combined immunodeficiency (SCID) is a heterogeneous group of rare disorders caused by mutations in different genes involved in the development and function of immune cells. SCID patients are usually highly susceptible to severe opportunistic infection, and, if untreated, die within one year. Since the first successful bone marrow transplants in X-linked SCID (SCID-X1) and Wiskott-Aldrich syndrome (WAS), hematopoietic stem cell (HSC) transplantation has become the standard treatment for most SCID patients. Survival is excellent in the HLA identical donor setting and is progressively increasing in other settings thanks to the improvements of HSCT methodologies and technologies, including conditioning regimens, prophylaxis and treatment of infectious complications, graft-versus-host disease (GvHD) prevention, and stem cell selection.

HSC-based gene therapy in SCID patients

HSC gene therapy has been successfully implemented for the treatment of SCID patients who lack a suitable donor (Figure 1)¹. The autologous HSC gene therapy approach avoids the risk of GvHD and, in case of preconditioning of the patient, required to make space for engrafting HSC cells, reduces myelosuppressive and immunosuppressive¹. The selective advantage of and the expansion of the corrected cells facilitated the success of gene therapy approaches and indicates that only a limited number of gene corrected progenitors are needed to reconstitute the T cell compartment and eliminate SCID associated symptoms. Despite these results, in some cases, efficacy of HSC gene therapy has been counterbalanced by the occurrence of insertional oncogenesis. Nonetheless, the understanding of the molecular events promoting oncogenesis, the development of safer vectors, and the introduction of lentiviral(LV), allowed the progress of gene therapy approaches for SCID patients. In the last decade, gene therapy trials based on the infusion of autologous gene-corrected HSCs performed in SCID-X1 and ADA-SCID as well as in Wiskott-Aldrich syndrome demonstrated the safety and efficacy of this strategy²⁻⁴.

Alternative gene delivery approaches

T cell differentiation occurs in the thymus; thus, it has been hypothesized that intra-thymic injection of HSCs or progenitors would improve T cell output and reconstitution in SCID and CID patients (Figure 1). In pre-clinical models of SCID and ZAP-70 deficiency, it was indeed shown that intra-thymic injection of bone marrow progenitors is a safe procedure and represents an effective strategy to generate protective T cell immunity⁵⁻⁷. Upon intra-thymic HSC injection, a rapid T cell differentiation, with mature thymocytes detected by 4 weeks after transplantation occurred. A significantly high number of peripheral T cells, increased percentages of naive T cells, and diverse T cell receptor repertoires was also achieved⁵. The increased number of thymocytes was associated with the generation of a normal medullary structure, which sustained long-term T cell differentiation⁶. These results, together with evidences that gene correction of a limited number of T cell progenitors are sufficient to reconstitute the T cell compartment and that thymus can be targeted by antigen or vectors lead to employ *in vivo* intra-thymic gene therapy as an alternative approach for treating ZAP-70 deficiency. This strategy was explored in a preclinical model of the disease using intra-

thymic delivery of a T-cell specific LV encoding for ZAP-70 (Figure 1). ZAP-70 deficiency is a rare subtype of CID in which T cell development is blocked at the stage of double-positive cells, leading to complete absence of peripheral CD8⁺ T cells and the presence of CD4⁺ T cells with defective function. Intra-thymic LV-ZAP-70 gene delivery promoted partial immune reconstitution and transgene expression in peripheral T cells. Despite the low percentage of transduced thymocytes, gene corrected cells were observed long-term, indicating ZAP-70 integration in progenitor cells⁸. However, the approach was effective in few treated mice.

In the current issue, Pouzolles et al.⁸ employed an alternative intra-thymic ZAP-70 delivery based on adeno-associated virus (AAV). In accordance with the higher efficacy of AAV compared to LV in transducing thymocytes⁹, intra-thymic injection of AAV-ZAP-70 lead to up of 5% of thymocyte transduction. Interestingly, AAV-ZAP-70 intra-thymic injection promoted a rapid (10 days) development of gene-corrected mature thymocytes. This effective gene delivery was associated with the generation of a thymic medulla that sustained the differentiation of effector/memory and regulatory T cells. Moreover, this gene transfer delivery allowed the modification of the thymic architecture with reconstitution of AIRE expression, known to mediate tolerance. The mechanisms underlying this effect is not clear, and it warrants further investigation. Finally, in AAV-ZAP70 treated mice low levels of anti-AAV capsid, anti-transgene, and autoantibodies were induced at early time points after gene therapy, and antibodies levels decreased overtime. Despite the transient thymocyte differentiation, ZAP-70-corrected T cells persisted in the periphery of treated mice up to 40 weeks after gene therapy. In conclusion, a single wave of T cell differentiation, associated with the selective advantage of gene-corrected cells in ZAP-70 deficient mice allowed to the long-term maintenance of functional peripheral T cells.

Open questions and future directions

Intra-thymic gene delivery is technically challenging in human subjects suffering from SCID and CID, since in these patients the thymus is small and lacks thymocytes. Although the introduction of endoscopic-mediated guidance for the intra-thymic injection, optimized in large animal models¹⁰, improved the success rate of the treatment, it might be difficult to translate this approach into clinical practice. Moreover, the transient effect of intra-thymic AAV-based gene therapy and the poor transduction efficiency are likely to limit the clinical application of this strategy. Only up to 5% of thymocytes were transduced⁸, thus, it is questionable whether this transient and limited level of gene correction will be sufficient to reach the therapeutic levels in patients. Intra-thymic gene therapy in infant deficient mice showed an improved success rate of T cell output⁵, suggesting that also intra-thymic AAV-based gene therapy might result in a more durable effects if applied early in life.

Intra-thymic AAV gene therapy lead to a rapid modification of the thymic architecture associated with a single wave of thymocyte differentiation that proceeded peripheral differentiation of T cells with a memory/effector phenotype. These interesting observations lead to some further considerations. Specifically, while memory T cells can persist several years, generation of naïve T cells is needed overtime, and it requires correction of HSCs with self-renewing capacity. Thus, a combined therapy with intra-thymic AAV gene delivery associated with HSC transplantation, which will allow long-term T cell differentiation in a reconstituted thymus might be considered. However, which type of HSCs will be

transplanted: autologous gene corrected cells or allogeneic cells; in the former case how HSCs will be *ex vivo* genetically modified? Based on the above considerations, additional studies in small and large animal models are required for understanding whether efficacy of gene correction in immunodeficient setting is possible using a non-HSC based approach alone or in combination with HSCs.

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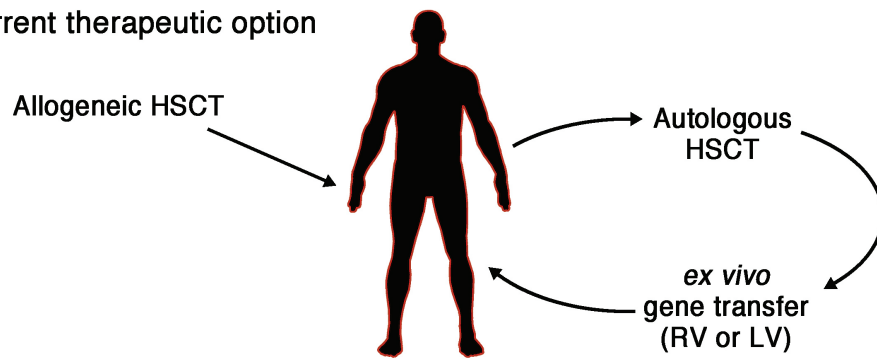
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Figure Legend

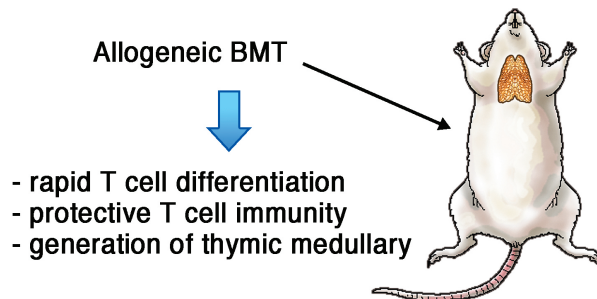
Figure 1. Therapeutic approaches for SCID patients. **A.** *Current therapeutic option for SCID patients.* Allogeneic HSCT is the current therapeutic choice for SCID and CID patients. For patients who lacked a suitable donor, *ex vivo* gene therapy is an alternative treatment. In the latter, bone marrow is harvested from patients and CD34⁺ cells are selected, *in vitro* stimulated with a cocktail of cytokines, and transduced with retroviral (RV) or lentiviral (LV) vector encoding for the therapeutic gene. Transduced cells are reinfused into the patients with conditioning regimen aim at making space for engrafting HSCs. **B.** *Intra-thymic HSC delivery.* In pre-clinical models of SCID and CID, allogeneic bone marrow cells have been injected intra-thymically, with or without pre-conditioning⁵⁻⁷. **C.** *Intra-thymic gene therapy.* In pre-clinical models of ZAP-70 immunodeficiency LV encoding for ZAP-70 have been intra-thymically injected⁸. In this issue of the *JACI*, Pouzolles et al⁸ injected adeno-associated viral vector (AAV) encoding for ZAP-70 in the thymi of ZAP-70 deficient mice.

A. Current therapeutic option



Systemic delivery of allogeneic or autologous gene corrected cells

B. Intra-thymic HSC delivery



C. Intra-thymic gene therapy

