Efficacy of lentivirus-mediated gene therapy in an Omenn syndrome recombination-activating gene 2 mouse model is not hindered by inflammation and immune dysregulation

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Background: Omenn syndrome (OS) is a rare severe combined immunodeficiency associated with autoimmunity and caused by defects in lymphoid-specific V(D)J recombination. Most patients carry hypomorphic mutations in recombination-activating gene (*RAG*) 1 or 2. Hematopoietic stem cell transplantation is the standard treatment; however, gene therapy (GT) might represent a valid alternative, especially for patients lacking a matched donor.

Objective: We sought to determine the efficacy of lentiviral vector (LV)–mediated GT in the murine model of OS (*Rag2*$^{R229Q/R229Q}$) in correcting immunodeficiency and autoimmunity.

Methods: Lineage-negative cells from mice with OS were transduced with an LV encoding the human *RAG2* gene and injected into irradiated recipients with OS. Control mice underwent transplantation with wild-type or OS-untransduced lineage-negative cells. Immunophenotyping, T-dependent and T-independent antigen challenge, immune spectratyping, autoantibody detection, and detailed tissue immunohistochemical analyses were performed.

Results: LV-mediated GT allowed immunologic reconstitution, although it was suboptimal compared with that seen in wild-type bone marrow (BM)−transplanted OS mice in peripheral blood and hematopoietic organs, such as the BM, thymus, and spleen. We observed *in vivo* variability in the efficacy of GT correlating with the levels of transduction achieved. Immunoglobulin levels and T-cell repertoire normalized, and gene-corrected mice responded properly to challenges *in vivo*. Autoimmune manifestations, such as skin infiltration and autoantibodies, dramatically improved in GT mice with a vector copy number/genome higher than 1 in the BM and 2 in the thymus.

Conclusions: Our data show that LV-mediated GT for patients with OS significantly ameliorates the immunodeficiency, even in an inflammatory environment. (J Allergy Clin Immunol 2017;■■■:■■■-■■■.)

Key words: *Gene therapy, Omenn syndrome, autoimmunity, lentiviral vector,* Rag *genes*

Genetic defects in recombination-activating gene (*RAG*) 1 or 2 result in a spectrum of severe immune defects ranging from a profound block in T- and B-cell differentiation (T^-B^- severe combined immunodeficiency [SCID] forms) to the presence of oligoclonal lymphocytes, a condition referred to as "atypical" or leaky SCID.[1] In recent years, whole-exome sequencing has identified *RAG* mutations in late childhood or young adulthood, presenting a broad spectrum of clinical manifestations, including combined immunodeficiency associated with granulomas and/or autoimmunity or expansion of T-cell receptor (TCR) γδ T cells and variable antibody deficiency.[1-5] Hypomorphic mutations also cause a distinct clinical phenotype, Omenn syndrome (OS), in which immunodeficiency associates with the presence of eosinophilia and early-onset erythroderma caused by infiltration of oligoclonal activated T cells.[6-8] Although circulating B cells are

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Supported by the Italian Telethon foundation (Telethon Core TGT E2 Grant to A.V.); by the European Commission's 5th, 6th, and 7th and Horizon 2020 Framework Programs (contracts QLK3-CT-2001-00427-INHERINET, LSHB-CT-2004-005242-CONSERT, grant agreement 261387 CELL-PID to A.V. and grant 666908-SCIDNET to A.V.); and by The Netherlands Health Research and Development Organization ZonMw, Translational Gene Therapy Program (project 43100016 to G.W.) and by Italian Ministry of Health (grant GR-2011-02349759 to B.C.).

Disclosure of potential conflict of interest: L. D. Notarangelo is Editor of *Frontiers in Immunology*, Associate Editor for the *Journal of Clinical Immunology*, and Associate Editor for *Clinical Immunology*; is employed by the National Institutes of Health and the Department of Health and Human Services; and has received royalties from UpToDate. G. Wagemaker has received grants and travel support from The Netherlands Organization for Health Research ZonMw and the European Commission. A. Villa has received a grant from the European Commission. The rest of the authors declare that they have no relevant conflict of interest.

Received for publication May 15, 2017; revised October 2, 2017; accepted for publication November 1, 2017.

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0091-6749/$36.00

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https://doi.org/10.1016/j.jaci.2017.11.015

Abbreviations used
AIRE: Autoimmune regulator
BAFF: B cell–activating factor
BM: Bone marrow
GT: Gene therapy
HSCT: Hematopoietic stem cell transplantation
Lin^-: Lineage negative
LV: Lentiviral vector
OS: Omenn syndrome
PID: Primary immunodeficiency
RAG: Recombination-activating gene
SCID: Severe combined immunodeficiency
TCR: T-cell receptor
UCOE: Ubiquitous chromatin opening element
VCN: Vector copy number/genome
WT: Wild-type

virtually absent, plasmablasts might be present in peripheral tissues and contribute to the pathogenesis of immune dysregulation, as revealed by the presence of autoantibodies.[9-11] Because all these forms are fatal if untreated, hematopoietic stem cell transplantation (HSCT) is the standard of care, even in SCID diagnosed late.

The average overall survival for patients with SCID treated early in infancy is greater than 90% at 3 years after transplantation.[12-17] Poor overall survival has been reported after haploidentical HSCT with no or limited conditioning, clearly indicating that myeloablative conditioning is required to achieve long-term T- and B-cell recovery.[18]

Given the encouraging results of gene therapy (GT) studies obtained in the setting of X-linked SCID[19,20] and in adenosine deaminase deficiency,[21,22] the GT approach represents an attractive therapeutic option for patients with OS. Preclinical studies in the *Rag1*$^{-/-}$ model indicated that low RAG1 expression resulted in incomplete thymic reconstitution and consequent development of OS manifestations[23] or in very low T- and B-cell counts.[24] The preclinical model of *Rag2*$^{-/-}$ GT provided more encouraging data, showing amelioration of the immunodeficiency in the absence of side effects with both retroviral and lentiviral vectors (LVs).[25,26] Significant improvement in T- and B-cell reconstitution was observed by using an LV carrying codon-optimized human *RAG2* cDNA under the control of ubiquitous chromatin opening element (UCOE) promoter (UCOE-RAG2co). Nonetheless, it remains unclear whether the same therapeutic strategy could be effective in the presence of hypomorphic *RAG2* mutations, in which gene-corrected lymphoid progenitors compete with endogenous uncorrected lymphocytes in an inflammatory and autoimmune environment.

Here we tested the UCOE-RAG2co LV in the OS preclinical model (*Rag2*$^{R229Q/R229Q}$ mouse)[27] to evaluate the efficacy of a lentivirus-mediated GT approach. We demonstrated that adequate transgene expression is required to overcome T- and B-cell differentiation block and restore thymic epithelial structure. GT-treated mice showed dramatic amelioration in peripheral tissue infiltration, particularly in the skin, and antigen-specific antibody production on *in vivo* challenges. In summary, our data demonstrated the feasibility of the lentiviral GT approach, even in the context of residual RAG2 expression and, more importantly, in an inflammatory environment predisposing to autoimmunity.

METHODS

LV production

The 2.6kbUCOE-RAG2co LV, in which the human codon-optimized *RAG2* cDNA was driven by a 2.6kbUCOE, was produced as previously described.[26] For the 2.2kb UCOE-RAG2co LV, the 2.6kbUCOE was replaced by a shorter 2.2kbUCOE form.[28]

Animals

Animal experimental procedures were approved by the Institutional Animal Care and Use Committee of San Raffaele Hospital and Italian Ministry of Health (Institutional Animal Care and Use Committee no. 710). C57Bl/6 wild-type (WT) mice were obtained from Charles River (Bar Harbor, Me). The knock-in C57BL/6 *Rag2*$^{R229Q/R229Q}$ colony was maintained onsite with heterozygous breeders.[27]

Lineage-negative transduction and transplantation

Six- to 10-week-old donor WT or *Rag2*$^{R229Q/R229Q}$ (OS) mice were euthanized, and femurs and tibias were flushed to collect bone marrow (BM). Lineage-negative (Lin^-) cells were enriched from total BM with the Lineage Cell Depletion Kit and autoMACS separator (Miltenyi Biotec, Bergisch Gladbach, Germany), according to the manufacturer's instructions. OS Lin^- cells were transduced overnight with LVs at a multiplicity of infection of 5 to 10 (vector titer, 1.3-3.8 $\times$ 10^8 transducing units per mL) in StemSpan SFEM medium (STEMCELL Technologies, Vancouver, British Columbia, Canada) supplemented with 2% FCS (Euroclone, Milan, Italy), 1% penicillin/streptomycin, 1% glutamine (Gibco, Carlsbad, Calif), and the following cytokines (PeproTech, Rocky Hills, NJ): recombinant murine thromobopoietin, 20 ng/mL; recombinant murine stem cell factor, 50 ng/mL; rhFLT3L, 10 ng/mL; rmIL-3, 10 ng/mL; and rhIL-6, 20 ng/mL. Untransduced Lin^- cells from mice with OS and WT mice were cultured in parallel in the same medium. Recipient mice were conditioned by using lethal total-body irradiation (900 rad, split dose) at least 2 hours before transplantation and were then injected in the caudal vein with 0.5 $\times$ 10^6 Lin^- cells. Donors and recipients were mismatched by sex or CD45 alleles to follow-up chimerism. Gentamicin sulfate (Italfarmaco, Milan, Italy) was administered in drinking water (8 μg/mL) for the first 2 weeks after transplantation to prevent infections.

Flow cytometric analysis

Single-cell suspensions from tissues were obtained, as previously described.[29] Cells were stained with the following antibodies (BD PharMingen, San Jose, Calif; Miltenyi Biotec; BioLegend, San Diego, Calif; or eBioscience, San Diego, Calif): CD3, CD4, CD8, CD11b, CD19, CD21, CD23, CD24, CD25, CD43, CD44, CD45.1/2, CD62 ligand, CD69, CD117, B220, IgM, IgD, NKp46, NK1.1, Lin^+ cocktail, and Sca1. Viability was determined by using the Live/Dead Fixable Dead Cell Stain Kit (Thermo Fisher Scientific, Waltham, Mass). Samples were acquired on a FACSCanto II (BD) and analyzed with FlowJo software (TreeStar, Ashland, Ore).

Real-time quantitative PCR

Genomic DNA was extracted with the QIAamp DNA Blood Mini Kit (Qiagen, Hilden, Germany), according to the manufacturer's instructions. Vector copy number/genome (VCN) was quantified by using quantitative real-time PCR on the Viia7 PCR System (Applied Biosystems, Foster City, Calif), as previously described.[30] For digital droplet PCR, 20 ng of genomic DNA was analyzed with the QX200 Droplet Digital PCR System (Bio-Rad Laboratories, Hercules, Calif), according to the manufacturer's instructions, with the same set of primers and probes used for real-time PCR and the *Rpp30* gene (Bio-Rad Laboratories) as a normalizer. Chimerism in sex-mismatched transplants was evaluated by means of quantification of cells carrying the Y chromosome, as previously described.[31]

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