Recombination-activating gene 1 (*Rag1*)–deficient mice with severe combined immunodeficiency treated with lentiviral gene therapy demonstrate autoimmune Omenn-like syndrome

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Background: Recombination-activating gene 1 (RAG1) deficiency results in severe combined immunodeficiency (SCID) caused by a complete lack of T and B lymphocytes. If untreated, patients succumb to recurrent infections.

Objectives: We sought to develop lentiviral gene therapy for RAG1-induced SCID and to test its safety.

Methods: Constructs containing the viral spleen-focus-forming virus (SF), ubiquitous promoters, or cell type-restricted promoters driving sequence-optimized *RAG1* were compared for efficacy and safety in sublethally preconditioned *Rag1*$^{-/-}$ mice undergoing transplantation with transduced bone marrow progenitors.

Results: Peripheral blood CD3$^+$ T-cell reconstitution was achieved with SF, ubiquitous promoters, and cell type-restricted promoters but 3- to 18-fold lower than that seen in wild-type mice, and with a compromised CD4$^+$/CD8$^+$ ratio. Mitogen-mediated T-cell responses and T cell–dependent and T cell–independent B-cell responses were not restored, and T-cell receptor patterns were skewed. Reconstitution of mature peripheral blood B cells was approximately 20-fold less for the SF vector than in wild-type mice and often not detectable with the other promoters, and plasma immunoglobulin levels were abnormal. Two months after transplantation, gene therapy–treated mice had rashes with cellular tissue infiltrates, activated peripheral blood CD44$^+$CD69$^+$ T cells, high plasma IgE levels, antibodies against double-stranded DNA, and increased B cell–activating factor levels. Only rather high SF vector copy numbers could boost T- and B-cell reconstitution, but mRNA expression levels during T- and B-cell progenitor stages consistently remained less than wild-type levels.

Conclusions: These results underline that further development is required for improved expression to successfully treat patients with RAG1-induced SCID while maintaining low vector copy numbers and minimizing potential risks, including autoimmune reactions resembling Omenn syndrome. (J Allergy Clin Immunol 2013;■■■:■■■-■■■.)

Key words: *Severe combined immunodeficiency, lentiviral gene therapy, autoimmune reactions*

Recombination-activating gene 1 (RAG1) deficiency is an autosomal recessive disorder that results in severe combined immunodeficiency (SCID) because of complete lack of mature T and B lymphocytes. The function of RAG1 is to exert variable, diverse, and joining (V[D]J) recombination of T-cell receptors (TCRs) and immunoglobulins in developing lymphocytes, and to function properly, it requires recombination-activating gene 2 (RAG2).[1]

Hypomorphic RAG1 mutations, which impair but do not abolish V(D)J recombination, can result in Omenn syndrome, a severe autoimmune disease presenting with erythroderma, lymphadenopathy, eosinophilia, and profound immunodeficiency.[2]

Because of the relative lack of HLA-identical hematopoietic stem cell (HSC) donors, the overall success rate of HSC transplantation is limited,[3,4] and intervention before 3.5 months of age seems to be a critical parameter for successful reconstitution.[3]

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Abbreviations used
AIRE: Autoimmune regulator
BAFF: B cell–activating factor
CMD: Corticomedullary differentiation
CP: Cell type-restricted promoter
DP: Double positive
dsDNA: Double-stranded DNA
Foxp3: Forkhead box protein 3
Gy: Gamma ray
HSC: Hematopoietic stem cell
MOI: Multiplicity of infection
mTEC: Medullary thymic epithelial cell
RAG1: Recombination-activating gene 1
RAG2: Recombination-activating gene 2
SCID: Severe combined immunodeficiency
SF: Spleen focus-forming virus
TCR: T-cell receptor
TdT: Terminal deoxynucleotidyl transferase
Treg: Regulatory T
UCOE: Ubiquitous chromatin opening element
UP: Ubiquitous promoter
VCN: Lentiviral copy number per cell
WT: Wild-type

Gammaretroviral vector gene therapy is an effective treatment for X-linked SCID and adenosine deaminase–SCID, with sustained correction of the phenotype.[5] Adverse events were observed several years after therapy caused by gammaretroviral vector–derived insertional oncogenesis in the X-linked SCID trial,[5] but no other serious effects have been reported. Improved self-inactivating gammaretroviral vector with an internal cassette driving elongation factor 1α-short to reduce genotoxicity are currently being tested in a multicenter international phase I/II trial.[6]

More recently, initiated gene therapy trials for adrenoleukodystrophy,[7] β-thalassemia,[8] Wiskott-Aldrich syndrome,[9] and metachromatic leukodystrophy[10] have improved the vector type and substituted the early-design gammaretroviral vectors with third-generation HIV-based lentiviral vectors,[11,12] which transfer their genetic cargo to HSCs more efficiently without the requirement for growth factor stimulation before transduction. Lentiviral vectors might be further improved by reducing aberrant splicing,[13] an event that caused clonal dominance in a patient with β-thalassemia.[8] Both gammaretroviral[14] and lentiviral gene therapy have been investigated for their potential to treat RAG1-induced SCID,[15,16] with the lentiviral vector partially restoring the SCID phenotype.

In the present study we found that *RAG1* lentiviral vector gene therapy in the murine model was associated with the development of an Omenn-like phenotype in $Rag1^{-/-}$ mice, revealing a potentially serious adverse effect compromising clinical application.

METHODS

Animals

Normal C57BL/6 (wild-type [WT]) and congenic $Rag1^{-/-}$ knockout mice were obtained, as previously described.[17] The $Rag1^{-/-}$ mice completely lack mature T and B lymphocytes. The animal experiments were reviewed and approved by an ethics committee of Erasmus MC, Rotterdam, The Netherlands, in accordance with legislation in The Netherlands.

Lentiviral vector construction and production

The human *RAG1* cDNA was codon optimized *(RAG1co)* with GeneOptimizer software (GeneArt, Regensburg, Germany) to improve transcription and translation.[16] *RAG1co* expression was driven by the spleen focus-forming virus (SF) promoter in the previously described third-generation self-inactivating pRRL.PPT.SF.*RAG1co*.bPRE4*.SIN vector (SF-*RAG1co*.)[11,12,16] Therapeutic promoter cassettes were divided into ubiquitous promoters (UPs) and cell type-restricted promoters (CPs), which are described in the Methods section and Table E1 in this article's Online Repository at www.jacionline.org.

Lentiviral vectors were produced by using standard calcium phosphate transfection of HEK 293T cells with the plasmids pMDL-g/pRRE, pMD2-VSVg, and pRSV-Rev.[18] Transducing units per milliliter of concentrated vector batches were determined by means of serial dilution on HeLa cells, followed by quantitative PCR on genomic DNA.[18]

Lentiviral HSC transduction

Hematopoietic progenitors were purified by means of lineage depletion (Lin^-; BD Biosciences, San Jose, Calif) from donor bone marrow extracted from 8- to 12-week-old male $Rag1^{-/-}$ or WT mice. Overnight lentiviral transduction of $Rag1^{-/-}$ Lin^- cells at a cell density of 10^6 cells/mL and a multiplicity of infection (MOI) of 2 to 10 or 100 was performed, as previously described.[18] The following day, 5×10^5 transduced Lin^- cells were injected in the tail veins of 6 gamma ray (Gy) sublethally irradiated 8- to 12-week-old female $Rag1^{-/-}$ recipients (recipients of WT cells are designated as WT Tx–treated mice). Follow-up with immunologic phenotyping and histopathology were performed, as previously described[18] and detailed in the Methods section this article's Online Repository.

Immunoglobulin levels, humoral immune responses, and mitogen responses

Baseline IgM, IgG_1, IgG_{2a}, IgG_{2b}, IgG_3, and IgA levels were measured in mouse plasma by using a multiplex assay kit (Beadlyte Mouse Immunoglobulin Isotyping Kit; Millipore, Billerica, Mass) on a Bio-Plex reader (Bio-Rad Laboratories, Hercules, Calif). Mice were immunized with *Streptococcus pneumoniae* (PNEUMO 23; Sanofi Pasteur MSD, Lyon, France) and tetanus toxoid, and specific antibodies were detected by means of ELISA.[18] Plasma mouse IgM-and IgG-specific antibodies to double-stranded DNA (dsDNA; Alpha Diagnostic, San Antonio, Tex) and Quantikine immunoassay to mouse B cell–activating factor (BAFF; R&D Systems, Minneapolis, Minn) ELISAs were also performed. Spleen cell proliferation assays were described previously.[18]

TCR rearrangement, lentiviral copy number analysis, *RAG1* mRNA expression, and recombination activity

GeneScan analysis of the murine TCRβ repertoire was performed on total RNA purified from fresh spleen cells, as previously described.[16,18,19] The lentiviral copy number per cell (VCN) value was determined on genomic DNA of bone marrow and spleen, myeloid cells selected for CD11b, and T and B lymphocyte–enriched populations (BD Imag, BD Biosciences) by means of quantification of the PCR product in an ABI PRISM 7900 HT sequence detection system (Applied Biosystems, Foster City, Calif), as previously described.[20,21] For quantification of *Rag1* and *RAG1co* mRNA expression in early T- and B-cell progenitors, $CD44^-CD25^+CD4^-CD8^-$ (double negative; DN3 stage), $CD4^+CD8^+$ double-positive (DP) thymic progenitors, and early B-cell progenitors ($B220^+CD43^-IgM^-IgD^-$) were cell sorted with a FACSAria cell sorter (BD Biosciences). RNA was extracted with the RNeasy microkit (Qiagen, Hilden, Germany), and cDNA synthesis was performed with the QuantiTect reverse transcription kit (Qiagen). Amplification was performed with *Rag1*- and *RAG1co*-specific primers, and cDNA transcripts were determined relative to glyceraldehyde-3-phosphate dehydrogenase. All primer sequences are provided in Table E2 in this article's Online Repository at www.jacionline.org. Recombination assays to assess the