A systematic analysis of recombination activity and genotype-phenotype correlation in human recombination-activating gene 1 deficiency

Yu Nee Lee, PhD,[a] Francesco Frugoni, PhD,[a] Kerry Dobbs, BS,[a] Jolan E. Walter, MD, PhD,[a,b] Silvia Giliani, PhD,[c] Andrew R. Gennery, MD,[d] Waleed Al-Herz, MD,[e] Elie Haddad, MD, PhD,[f] Francoise LeDeist, MD, PhD,[f] Jack H. Bleesing, MD, PhD,[g] Lauren A. Henderson, MD,[a] Sung-Yun Pai, MD,[h] Robert P. Nelson, MD,[i] Dalia H. El-Ghoneimy, MD,[j] Reem A. El-Feky, MD,[j] Shereen M. Reda, MD, PhD,[j] Elham Hossny, MD, PhD,[j] Pere Soler-Palacin, MD,[k] Ramsay L. Fuleihan, MD,[l] Niraj C. Patel, MD,[m] Michel J. Massaad, PhD,[a] Raif S. Geha, MD,[a] Jennifer M. Puck, MD,[n] Paolo Palma, MD,[o] Caterina Cancrini, MD,[o] Karin Chen, MD,[p] Mauno Vihinen, PhD,[q] Frederick W. Alt, PhD,[r] and Luigi D. Notarangelo, MD[a] *Boston, Mass, Brescia and Rome, Italy, Newcastle upon Tyne, United Kingdom, Kuwait City, Kuwait, Montreal, Quebec, Canada, Cincinnati, Ohio, Indianapolis, Ind, Cairo, Egypt, Barcelona, Spain, Chicago, Ill, Charlotte, NC, San Francisco, Calif, Salt Lake City, Utah, and Lund, Sweden*

Background: The recombination-activating gene (RAG) 1/2 proteins play a critical role in the development of T and B cells by initiating the VDJ recombination process that leads to generation of a broad T-cell receptor (TCR) and B-cell receptor repertoire. Pathogenic mutations in the *RAG1/2* genes result in various forms of primary immunodeficiency, ranging from T^-B^- severe combined immune deficiency to delayed-onset disease with granuloma formation, autoimmunity, or both. It is not clear what contributes to such heterogeneity of phenotypes.

Objective: We sought to investigate the molecular basis for phenotypic diversity presented in patients with various *RAG1* mutations.

Methods: We have developed a flow cytometry–based assay that allows analysis of RAG recombination activity based on green fluorescent protein expression and have assessed the induction of the *Igh*c locus rearrangements in mouse *Rag1*$^{-/-}$ pro-B cells reconstituted with wild-type or mutant human RAG1 (hRAG1) using deep sequencing technology.

Results: Here we demonstrate correlation between defective recombination activity of hRAG1 mutant proteins and severity of the clinical and immunologic phenotype and provide insights on the molecular mechanisms accounting for such phenotypic diversity.

Conclusions: Using a sensitive assay to measure the *RAG1* activity level of 79 mutations in a physiologic setting, we demonstrate correlation between recombination activity of RAG1 mutants and the severity of clinical presentation and show that RAG1 mutants can induce specific abnormalities of

From [a]the Division of Immunology and Manton Center for Orphan Disease Research, Children's Hospital, Harvard Medical School, Boston; [b]the Division of Pediatric Allergy/Immunology, Massachusetts General Hospital for Children, Boston; [c]A. Nocivelli Institute for Molecular Medicine, Pediatric Clinic, University of Brescia, and the Section of Genetics, Department of Pathology Spedali Civili, Brescia; [d]the Department of Paediatric Immunology, Newcastle Upon Tyne Hospital, NHS Foundation Trust, United Kingdom and Institute of Cellular Medicine, Newcastle University, Newcastle upon Tyne; [e]the Department of Pediatrics, Faculty of Medicine, Kuwait University, Kuwait City; [f]the Department of Pediatrics and Department of Microbiology, Infectiology and Immunology, University of Montreal, CHU Sainte-Justine Research Center, Montreal; [g]the Division of Hematology/Oncology, Cincinnati Children's Hospital Medical Center, Cincinnati; [h]the Division of Hematology-Oncology, Boston Children's Hospital, Boston; [i]the Divisions of Hematology and Oncology, Indiana University School of Medicine, Indianapolis; [j]the Department of Pediatric Allergy and Immunology, Children's Hospital, Faculty of Medicine, Ain Shams University, Cairo; [k]the Paediatric Infectious Diseases and Immunodeficiencies Unit, Vall d'Hebron University Hospital, Barcelona; [l]the Division of Allergy and Immunology, Ann & Robert H. Lurie Children's Hospital of Chicago, Northwestern University Feinberg School of Medicine, Chicago; [m]the Immunology Clinic, Levine Children's Hospital, Carolinas Medical Center, Charlotte; [n]the Department of Pediatrics, University of California San Francisco and UCSF Benioff Children's Hospital, San Francisco; [o]DPUO, University Department of Pediatrics, Bambino Gesù Children's Hospital and University of Tor Vergata School of Medicine, Rome; [p]the Division of Allergy, Immunology & Rheumatology, Department of Pediatrics, University of Utah, Salt Lake City; [q]the Department of Experimental Medical Science, Lund University; and [r]the Howard Hughes Medical Institute, Program in Cellular and Molecular Medicine, Boston Children's Hospital, and the Department of Genetics, Harvard Medical School, Boston.

Supported in part by National Institutes of Health grants U54AI082973 (to L.D.N.) and 1P01AI076210-01A1 (to L.D.N., F.W.A., and R.S.G.). F.W.A is an investigator of the Howard Hughes Medical Institute. This work received additionally contributions from the March of Dimes (grant 1-FY-13-500 to L.D.N.), the Jeffrey Modell Foundation (to L.D.N.), the Translational Research Program grant TRP 2009 042809 (to L.D.N.), the Dubai Harvard Foundation for Medical Research (to L.D.N. and R.S.G.), and the Manton Foundation (to Y.N.L. and L.D.N.).

Disclosure of potential conflict of interest: Y. N. Lee has received research support from the Manton Foundation and the March of Dimes and is employed by Boston Children's Hospital. F. Frugoni, J. Puck, and F. Alt have received research support from the National Institutes of Health (NIH). J. E. Walter is employed by Massachusetts General Hospital and receives research support from the National Institute of Allergy and Infectious Disease. L. A. Henderson has received travel support from the American College of Rheumatology and the American Academy of Pediatrics. S.-Y. Pai is employed by Boston Children's Hospital and receives research support from the Translational Investigator Service Award from Boston Children's Hospital. P. Soler-Palacin has consultant arrangements with CSL Behring; has provided expert testimony on behalf of CSL Behring; has received research support from Baxter, CSL Behring, and Octapharma; has received payment for lectures from CSL Behring; and has received travel support from the European Society of Immunodeficiencies. R. S. Geha has received research support from the NIH and the Dubai Harvard Foundation for Medical Research. L. D. Notarangelo has received research support from the NIH, the Jeffrey Modell Foundation, the March of Dimes, the Dubai Harvard Foundation for Medical Research, and the Manton Foundation; has board memberships with the Program in Molecular and Cellular Medicine and Pediatric University Hospital "Meyer" in Florence, Italy; is employed by Boston Children's Hospital; and has received royalties from UpToDate. The rest of the authors declare that they have no relevant conflicts of interest.

Received for publication August 22, 2013; revised October 3, 2013; accepted for publication October 4, 2013.

Corresponding author: Frederick W. Alt, PhD, Program in Molecular and Cellular Medicine, Boston Children's Hospital, Karp Research Building, 9th Floor, One Blackfan Circle, Boston, MA 02115. E-mail: alt@enders.tch.harvard.edu. Or: Luigi D. Notarangelo, MD, Division of Immunology, Boston Children's Hospital, Karp Research Building, Rm 20117, One Blackfan Circle, Boston, MA 02115. E-mail: luigi.notarangelo@childrens.harvard.edu.

0091-6749/$36.00

© 2013 American Academy of Allergy, Asthma & Immunology

http://dx.doi.org/10.1016/j.jaci.2013.10.007

the VDJ recombination process. (J Allergy Clin Immunol 2013;■■■:■■■-■■■.)

Key words: *Recombination-activating gene 1, V(D)J recombination, severe combined immune deficiency, Omenn syndrome, autoimmunity, genotype-phenotype correlation, immune repertoire*

The recombination-activating gene (RAG) 1 and RAG2 proteins initiate the VDJ recombination process by generating DNA double-strand breaks at the recombination signal sequences (RSSs) that flank the variable (V), diversity (D), and joining (J) gene segments of the immunoglobulin and T-cell receptor (TCR) genes.[1] These DNA double-strand breaks are then joined by the ubiquitous mechanism of nonhomologous end-joining machinery.[2] As a result of VDJ recombination, a polyclonal pool of functional T and B lymphocytes is generated, expressing a diverse repertoire of productive TCR and B-cell receptor rearrangements.

Null mutations of *RAG1* and *RAG2* genes result in the T^-B^- severe combined immune deficiency (SCID) phenotype.[3] However, hypomorphic *RAG* mutations have been associated with a spectrum of clinical and immunologic phenotypes that include Omenn syndrome (OS),[4-10] with erythroderma, lymphadenopathy, eosinophilia, increased serum IgE levels, and the presence of autologous, oligoclonal, and activated T lymphocytes; leaky/atypical SCID,[10] with varying numbers of T and B cells but without the typical features of OS; SCID with expansion of γδ T lymphocytes (γδ-T),[11,12] which us often associated with cytomegalovirus infection; delayed-onset combined immune deficiency with granuloma and/or autoimmunity (CID-G/A)[13-15]; and in a single case of idiopathic $CD4^+$ T cell lymphopenia (ICL), presenting with extensive chickenpox and recurrent pneumonia.[16]

Attempts to correlate the phenotypic diversity of RAG-related disorders in human subjects with functional activity of the mutant proteins were largely based on a transient transfection assay in nonlymphoid adherent cells.[17] In this assay cells are cotransfected with plasmids encoding for wild-type (or mutant) human RAG1 (hRAG1) and hRAG2 and a third plasmid containing a suitable recombination substrate that would allow expression of an antibiotic resistance gene upon recognition and cleavage by hRAG1 and hRAG2 and nonhomologous end joining–mediated ligation. However, with this assay, the recombination activity of RAG proteins is analyzed on an extrachromosomal substrate (ie, a nonphysiologic setting), and functional impairment of mutants that specifically affect nuclear translocation of the hRAG proteins might be missed. Furthermore, it has been shown that stability and posttranslational modifications of the RAG proteins differ significantly in lymphoid versus nonlymphoid cells.[18]

Recently, Abelson murine leukemia virus (A-MuLV)–transformed pro-B cells containing an inverted green fluorescent protein (GFP) cassette flanked by RSS (pMX-INV) have been used to measure VDJ recombination activity on an intrachromosomal substrate by using flow cytometry with GFP expression as a read-out.[19] On the basis of this platform, we have analyzed the expression and recombination activity of 79 naturally occurring hRAG1 mutant proteins and thereby performed the largest comprehensive analysis of genotype-proteotype-phenotype correlation for hRAG1 deficiency. Our results provide novel insights into the molecular mechanisms underlying phenotypic diversity in patients with this disease.

Abbreviations used

A-MuLV:	Abelson murine leukemia virus
CDR:	Complementarity-determining region
CID-G/A:	Combined immune deficiency with granuloma and/or autoimmunity
γδ-T:	SCID with expansion of γδ T lymphocytes
GFP:	Green fluorescent protein
HBR:	Heptamer-binding region
hRAG1:	Human RAG1
ICL:	Idiopathic $CD4^+$ T-cell lymphopenia
mRag1:	Mouse Rag1
NBR:	Nonamer-binding region
OS:	Omenn syndrome
PON-P:	Pathogenic Or Not Pipeline
RAG:	Recombination-activating gene
RF:	Reading frame
RSS:	Recombination signal sequence
SCID:	Severe combined immune deficiency
TCR:	T-cell receptor

METHODS

Patient selection and assignment to phenotypic subgroups

Deidentified patients' clinical, immunologic, and molecular data were provided by an international network of physicians in Europe, the Middle East, South America, and the United States, according to protocols approved by the local institutional review boards. On the basis of phenotype, each patient was assigned to one of the following subgroups: T^-B^- SCID, OS, γδ-T, atypical/leaky SCID-G/A, and ICL.

Determination of recombinase activity level of wild-type and mutant *RAG1*

Analysis of recombination activity of wild-type and mutant RAG1 constructs was performed, as previously described.[14] For detailed methods, see the Methods section in this article's Online Repository at www.jacionline.org.

Immune repertoire analysis

Analysis of rearrangements at the endogenous *Ighc* locus was performed by using as a template the mRNA extracted from A-MuLV pro-B cells transduced with retroviral vectors encoding for wild-type or mutant hRAG1. A set of nested primers specific for various V_H and C_H elements of the *Ighc* locus were used for the first amplification cycles, followed by amplification with communal primers according to the manufacturer's protocol for MBCR (iRepertoire, Huntsville, Ala). By using this protocol, amplicon-rescued multiplex PCR allows semiquantitative amplification of the immune repertoire.[20] Purified PCR products were sequenced with the GS Junior 454 platform (Roche, Mannheim, Germany).

Raw sequences were filtered for PCR errors, and a tree map and 2-dimensional map were generated from the total complementarity-determining region (CDR)–H3 sequences to analyze V_H-J_H pairing and the relative distribution of distinct rearrangements (iRepertoire). The filtered sequences, excluding duplicates, were further analyzed for V_H, D_H, and J_H gene usage; composition; CDR-H3 length; reading frame (RF) determination; and the diversity index of Shannon entropy by using IMGT HighV-QUEST output files[21] and IgAT software.[22] Rarefaction curves were generated by using the VDJ statistics file from IgAT analysis and the PAST program.[23]

Download English Version:

https://daneshyari.com/en/article/6064417

Download Persian Version:

https://daneshyari.com/article/6064417

Daneshyari.com