NIH Public Access
Author Manuscript
J Allergy Clin Immunol. Author manuscript; available in PMC 2015 October 01.

Published in final edited form as:
J Allergy Clin Immunol. 2014 October ; 134(4): 983–985.e1. doi:10.1016/j.jaci.2014.04.037.

NIH-PA Author Manuscript NIH-PA Author Manuscript NIH-PA Author Manuscript

Lessons in gene hunting: a RAG1 mutation presenting with agammaglobulinemia and absence of B cells

Mona Hedayat, MD[a,^], **Michel J. Massaad, PhD**[a,^], **Yu Nee Lee, PhD**[a,^], **Mary Ellen Conley, MD**[b], **Jordan S. Orange, MD, PhD**[c], **Toshiro K. Ohsumi, PhD**[d], **Waleed Al-Herz, MD**[e], **Luigi D. Notarangelo, MD**[a,*], **Raif S. Geha, MD**[a,*], and **Janet Chou, MD**[a,*]

[a]Division of Immunology, Children's Hospital and Department of Pediatrics, Harvard Medical School, Boston, MA

[b]St. Giles Laboratory of Human Genetics of Infectious Diseases, The Rockefeller University, New York NY

[c]Immunology, Allergy, and Rheumatology, Baylor College of Medicine and the Texas Children's Hospital

[d]Department of Molecular Biology, Massachusetts General Hospital, Boston, MA

[e]Department of Pediatrics, Faculty of Medicine, Kuwait University, Kuwait City, Kuwait

Keywords

RAG1; combined immune deficiency; agammaglobulinemia

To the Editor

Recombination activating genes (*RAG*) 1 and 2 are critical for V(D)J recombination and lymphocyte development.[1] Mutations in *RAG1/2* associated with <1% of wild-type recombination activity typically result in severe combined immunodeficiency (SCID) lacking T and B cells.[2] Hypomorphic mutations with residual RAG activity can result in Omenn syndrome, characterized by erythroderma, lymphadenopathy, and the presence of oligoclonal T cells. More recently, studies have broadened the phenotypic variability associated with *RAG1/2* mutations, which now includes granulomatous disease, γδ T-cell expansion, $CD4^+$ lymphopenia with intact T cell function, and hyper-IgM syndrome.[1, 3] We report a patient with a homozygous missense mutation in *RAG1*, who presented initially with agammaglobulinemia, absent B cells, and normal numbers of T cells, followed by evolution into a $T^-B^-NK^+$ SCID phenotype with opportunistic infections and granulomas.

© 2014 American Academy of Allergy, Asthma and Immunology. Published by Mosby, Inc. All rights reserved.

Corresponding author: Janet Chou, MD, Division of Immunology, Children's Hospital, Karp building 10th floor, 1 Blackfan Circle, Boston, MA, 02115, USA. Tel: (617) 919-2478; Fax: (617) 730-0528; Janet.Chou@childrens.harvard.edu.
^M.H., M.J.M. and Y.L. contributed equally to this work
*L.D.N., R.S.G. and J.C. contributed equally to this work

Publisher's Disclaimer: This is a PDF file of an unedited manuscript that has been accepted for publication. As a service to our customers we are providing this early version of the manuscript. The manuscript will undergo copyediting, typesetting, and review of the resulting proof before it is published in its final citable form. Please note that during the production process errors may be discovered which could affect the content, and all legal disclaimers that apply to the journal pertain.

NIH-PA Author Manuscript

NIH-PA Author Manuscript

NIH-PA Author Manuscript

The patient was born to Kuwaiti first-degree cousin parents and presented at 6 months of age with pneumonia and failure to thrive The physical examination was notable for the absence of tonsils. Computerized tomography of the chest revealed a prominent thymus. He had undetectable IgG and IgA. IgM was 30 mg/dL. The absolute lymphocyte count was 5010 cells/μL with normal numbers of total $CD3^+$ cells, $CD4^+$ and $CD8^+$ T cells, $CD16^+/CD56^+$ NK cells, but virtually no $CD19^+$ B cells (Table 1). Genetic testing did not reveal any mutations in the genes encoding BTK, μ heavy chain, Igα, Igβ, λ5, and VpreB. He was given a diagnosis of agammaglobulinemia with absent B cells and was treated with IVIG.

The patient subsequently developed recurrent otitis media and cytomegalovirus-associated retinitis at 4 years of age. Immunologic evaluation performed at 5 years of age revealed virtually absent B cells, $CD3^+$ and $CD4^+$ T cell lymphopenia, and normal numbers of NK cells (Table I). Lymphocyte proliferation to PHA was severely decreased (Table I). NK cell cytotoxic function was normal (data not shown). At 8 years of age, he developed fever and diarrhea due to Epstein-Barr virus viremia and *Salmonella enteritidis*. At 10 years of age, the patient developed persistent colitis; endoscopy and colonoscopy revealed esophageal candidiasis and colonic granulomas. At that time, he had progressive T cell lymphopenia with a predominantly $CD45RO^+$ activated phenotype, and nearly absent $CD4^+CD45RA^+CD31^+$ recent thymic emigrants (Table I). HLA typing of the patient and his mother revealed no evidence of maternal engraftment. T cell proliferation to PHA and anti-CD3 stimulation was minimal. Proliferation to anti-CD3 stimulation increased with the addition of anti-CD28, suggesting residual signaling through the CD3 pathway. The combination of absent B cells, agammaglobulinemia, and progressive T cell lymphopenia with poor T cell function led to a diagnosis of combined immunodeficiency. The patient died at the age of 12 years from sepsis.

Whole genome sequencing was performed on DNA obtained from the patient prior to his death, his father, and his healthy sibling. Thirteen candidate genes had novel mutations that were homozygous in the patient, heterozygous in the father, and heterozygous or absent in the sibling (Table E1). Only the c.1211G>A mutation in *RAG1* correlated with the patient's phenotype of combined immunodeficiency. This mutation resulted in the substitution of arginine to glutamine at position 404 (p.R404Q) in the nonamer-binding region (NBR) of the protein. The NBR of RAG1 binds to recombination signal sequences that flank the Variable (V), Diversity (D) and Joining (J) elements of the immunoglobulin and T cell receptor genes, and is critical for V(D)J recombination activity.[4] Using a flow cytometry-based assay that permits analysis of RAG1 recombination activity on an intrachromosomal substrate,[5] we have demonstrated that the R404Q RAG1 mutant protein is normally expressed, but has markedly reduced recombination activity ($1.18 \pm 0.14\%$ of wild-type) (Fig. 1). This mutation has been previously reported in patients with T^-B^- SCID or Omenn syndrome.[6] In contrast to previously published patients with this mutation, our patient presented with clinical and laboratory features of agammaglobulinemia with absent B cells, a prominent thymus, normal numbers of circulating T cells, and no features of Omenn syndrome such as erythroderma or eosinophilia. Normal numbers of T cells with absence of B cells have been associated with another *RAG1* mutation (c.T2686C, p.W896A) in a patient who presented with isolated culture-negative pneumonitis at 6 months of age.[7] This patient,

NIH-PA Author Manuscript

NIH-PA Author Manuscript

NIH-PA Author Manuscript

like ours, had a normal thymic shadow and normal T cell numbers. The W896A mutation resulted in impaired proliferation to mitogens and a highly restricted TCRβ repertoire that was not of maternal origin. We have previously demonstrated that the VDJ recombination activity of the W896A mutant is associated with minimally preserved RAG1 function (0.9 ± 0.1% of wild-type)[5], similar to the R404Q mutation in our patient,

This case broadens the spectrum of disease associated with a *RAG1* mutation that nearly abolishes recombination activity. This phenomenon has been seen in families where the same mutation gives rise to different clinical and immunological phenotypes, suggesting that the manifestations of *RAG1/2* mutations may reflect interactions with other uncharacterized genetic modifiers, epigenetic factors, or environmental exposures. The progression of the patient's disease also underscores how a single mutation can have variable time-dependent effects in the same individual. Although he had normal numbers of T cells at 6 months of age, T cell function may have been abnormal at that time as suggested by his history of pneumonia and failure to thrive. He developed progressively worsening T cell numbers and function, opportunistic infections, granulomas, and ultimately died of sepsis, which, in retrospect, are consistent with an underlying *RAG1/2* mutation. The diagnostic delay in this case underscores the importance of SCID newborn screening, particularly in areas of the world where lymphocyte proliferation or TCR repertoire studies are not available. Quantification of T cell receptor excision circles (TRECs) can identify poor thymic output, even in the case of normal T cell numbers due to maternal engraftment or oligoclonality.[8] Additionally, cases of SCID which present with absent B cell numbers, as in our patient and others[7], support the inclusion of quantification of κ-deleting recombination excision circles, which can detect absent B cells, as part of newborn screening for SCID for improved early detection.[9]

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

Supported by: NIH P01AI-076210, AI-094017, a grant from the Dubai Harvard Foundation for Medical Research (RSG), a Manton Foundation Pilot Award (JC), and Kuwait Foundation for Advancement of Sciences grant 2010-1302-05 (WA).

References

1. Niehues T, Perez-Becker R, Schuetz C. More than just SCID--the phenotypic range of combined immunodeficiencies associated with mutations in the recombinase activating genes (RAG) 1 and 2. Clinical Immunology. 2010; 135:183–92. [PubMed: 20172764]
2. Notarangelo LD, Villa A, Schwarz K. RAG and RAG defects. Current Opinion in Immunology. 1999; 11:435–42. [PubMed: 10448134]
3. Chou J, Hanna-Wakim R, Tirosh I, Kane J, Fraulino D, Lee YN, et al. A novel homozygous mutation in recombination activating gene 2 in 2 relatives with different clinical phenotypes: Omenn syndrome and hyper-IgM syndrome. Journal of Allergy and Clinical Immunology. 2012; 130:1414–6. [PubMed: 22841008]
4. Kirch SA, Sudarsanam P, Oettinger MA. Regions of RAG1 protein critical for V(D)J recombination. European Journal of Immunology. 1996; 26:886–91. [PubMed: 8625984]

NIH-PA Author Manuscript
NIH-PA Author Manuscript
NIH-PA Author Manuscript

5. Lee Y, Frugoni F, Dobbs K, Walter JE, Giliani S, Gennery AR, et al. A systematic analysis of recombination activity and genotype-phenotype correlation in human RAG1-deficiency. Journal of Allergy and Clinical Immunology. 2013 in press.
6. Sobacchi C, Marrella V, Rucci F, Vezzoni P, Villa A. RAG-dependent primary immunodeficiencies. Human Mutation. 2006; 27:1174–84. [PubMed: 16960852]
7. Zhang J, Quintal L, Atkinson A, Williams B, Grunebaum E, Roifman CM. Novel RAG1 mutation in a case of severe combined immunodeficiency. Pediatrics. 2005; 116:e445–9. [PubMed: 16061569]
8. Puck JM. Laboratory technology for population-based screening for severe combined immunodeficiency in neonates: the winner is T-cell receptor excision circles. Journal of Allergy and Clinical Immunology. 2012; 129:607–16. [PubMed: 22285280]
9. Borte S, von Dobeln U, Fasth A, Wang N, Janzi M, Winiarski J, et al. Neonatal screening for severe primary immunodeficiency diseases using high-throughput triplex real-time PCR. Blood. 2012; 119:2552–5. [PubMed: 22130802]

Abbreviations

RAG Recombination activating genes

SCID severe combined immunodeficiency

NIH-PA Author Manuscript
NIH-PA Author Manuscript
NIH-PA Author Manuscript

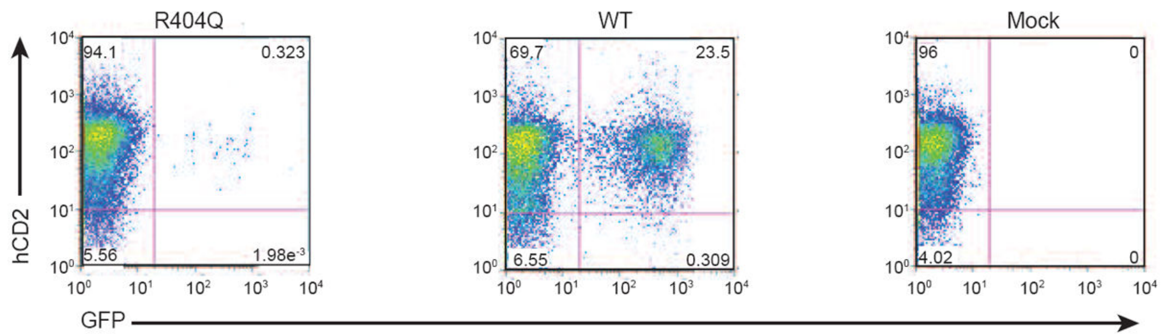

Figure 1.
Recombination activity of the RAG1 R404Q mutant protein. Abelson (Abl) virus-transformed *Rag1*$^{-/-}$ mouse pro-B cells engineered to contain a single integrant of an inverted green fluorescent protein (GFP) cassette flanked by recombination signal sequences (RSS) were transduced with retroviral vectors allowing expression of wild-type (WT) human RAG1 or the mutant R404Q RAG1 protein, and human CD2 (hCD2) as a reporter. Mock-transduced cells served as a negative control. Following MACS-purification of hCD2-expressing cells and treatment *in vitro* with imatinib, recombination activity of the R404Q mutant protein was measured by normalizing GFP expression to levels observed in the presence of wild-type RAG1, as previously described.[5] One representative experiment of three is shown.

NIH-PA Author Manuscript

NIH-PA Author Manuscript

NIH-PA Author Manuscript

Table 1

Immune profiles

	6 months	Proband 5 years	11 years
Lymphocytes, cells/μL (normal range)[i]	5010 (3400-9000)	***1550*** (2300-5400)	***1620*** (1900-3700)
$CD3^+$	4520 (1900-5900)	***1150*** (1400-3700)	***541*** (1200-2600)
$CD3^+CD4^+$	3300 (1400-4300)	***388*** (700-2200)	***260*** (650-1500)
$CD3^+CD8^+$	1220 (500-1700)	679 (490-1300)	***279*** (370-1100)
$CD4^+/CD8^+$ ratio	2.7 (1.6-3.8)	***0.51*** (0.9-2.6)	0.93 (0.9 – 3.4)
$CD4^+CD45RA^+$	ND	ND	***3.7*** (46-77%)
$CD4^+CD45RO^+$	ND	ND	***95*** (13-30%)
$CD4^+CD45RA^+CD31^+$	ND	ND	***0.96*** (45.7%)[iv]
$CD19^+$	***2*** (610-2600)	***4*** (390-1400)	***61*** (270-860)
$CD16^+/CD56^+$	340 (160-950)	349 (130-720)	***1004*** (100-480)
Eosinophils, cells/μL (normal range)[ii]	184 (200-300)	95 (0-600)	210 (0-600)
Immunoglobulins, mg/dL (normal range)[iii]			
IgG	***Undetectable*** (215-704)	ND	ND
IgA	***Undetectable*** (8.1-68)	ND	ND
IgM	30 (35-102)	ND	ND
Proliferation, cpm (normal control)[iv]			
Phytohemagglutinin	ND	***1578*** (16,485)	***541*** (65,369)
Anti-CD3	ND	ND	***232*** (3,814)
Anti-CD3+CD28	ND	ND	***6,797*** (19,170)
Background	ND	32 (192)	131 (221.5)

ND, Not Determined

[i] Shearer WT, Rosenblatt HM, Gelman RS, Oyomopito R, Plaeger S, Stiehm ER, Wara DW, Douglas SD, Luzuriaga K, McFarland EJ, Yogev R, Rathore MH, Levy W, Graham BL, Spector SA; Pediatric AIDS Clinical Trial Group. Lymphocyte subsets in healthy children from birth through 18 years of age: the Pediatric AIDS Clinical Trials Group P1009 study. J Allergy Clin Immunol 2003; 112(5):973-80.

[ii] Tietz NW (Ed): Clinical Guide to Laboratory Tests, 3rd ed. W. B. Saunders, Philadelphia, PA, 1995

[iii] Jolliff CR Cost KM, Stivrins PC, Grossman PP, Nolte CR, Franco SM, Fijan KJ, Fletcher LL, Shriner HC. Clin Chem. Reference intervals for serum IgG, IgA, IgM, C3, and C4 as determined by rate nephelometry. 1982; 28:126-128.

[iv] Value in parenthesis for lymphocyte proliferation are derived from studies done on a healthy control the same day.