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Adult-onset manifestation of idiopathic T-cell lymphopenia due to a heterozygous *RAG1* mutation

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To the Editor:

We would like to share a relevant and interesting immunodeficiency case that would be of interest to the readers of the *Journal of Allergy and Clinical Immunology*.

This report describes an adult-onset idiopathic T-cell lymphopenia due to recombina-
se activating gene 1 (*RAG1*) deficiency in an HIV-negative male patient with no recurrent or
opportunistic infections presenting at the age of 38 years with chronic dermatitis, pruritus,
and hyperkeratosis. Clinical and immunologic examination revealed eosinophilia with
modestly elevated IgE (747 kU/L), normal IgG, IgA, and IgM, profound pan-T-cell
lymphopenia, high normal total B cells, and slightly reduced natural killer cells. Genetic
analysis revealed a heterozygous frameshift mutation in the *RAG1* gene, resulting in a
truncated RAG1 protein. This is a late clinical presentation of an idiopathic T-cell
lymphopenia secondary to a heterozygous hypomorphic *RAG1* mutation and has important
implications for the considerable phenotypic variability related to molecular defects in genes
typically associated with severe combined immunodeficiency or Omenn syndrome (OS).

A 38-year-old man presented with significant pruritic skin rash on his legs and eosinophilia
that had been present for 2 years with poor resolution using over-the-counter topical
treatments. Medical history revealed a healthy childhood and adulthood until 2 years earlier
when the skin rash first appeared on the lower extremities. Gross examination of the skin
revealed a generalized dermatitis with hyperkeratosis without histologic evidence of
granulomas (Fig 1, A). There was also keratoderma of the feet and onychodystrophy. Skin

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biopsy of the lesions on the leg revealed a chronic dermatitis with eosinophilia, which was negative for IgG, IgM, or IgA with weak, discontinuous granular deposition of C3 along the basement membrane zone. Complete blood cell count with differential revealed significant lymphopenia, which on detailed lymphocyte subset evaluation by flow cytometry revealed profound pan-T-cell lymphopenia (Table I). There was a relative increase in the frequency of activated T cells (CD4⁺CD25⁺ and HLA-DR⁺ CD4 and CD8 T cells). Thymic function was significantly impaired for age, with almost absent CD4 recent thymic emigrants and TREC. Genetic analysis revealed the following heterozygous frameshift mutation (c. 256_257delAA, K86VfsX33) in exon 2 of the gene encoding *RAG1* that has been previously reported to be associated with SCID and OS, when present as a homozygous or compound heterozygous mutation.¹ No other pathogenic genetic variations were found in either the known coding or promoter regions of the *RAG1* gene or in any of the following SCID-associated genes: *RAG2*, *JAK3*, *IL7R*, *ADA*, *CD3D*, *CD3E*, *DCLRE1C*, and *IL2RG*. Genetic analysis for the above *RAG1* mutation was performed not only on DNA extracted from whole blood but also from PBMCs and buccal brushing. In all 3 sample types, the same heterozygous mutation was identified, indicating that this was not a somatic mutation but a germline variation in the *RAG1* gene. Genetic testing for this specific *RAG1* mutation was performed on PBMCs isolated from whole blood of both parents of this patient, and the father was shown to have the same heterozygous mutation as the patient; however, the father was clinically, phenotypically, and immunologically normal with normal T, B, and natural killer cell counts. A second mutation was not identified in the patient or the mother. The patient has a single older male sibling who was not evaluated but was negative for relevant clinical history.

Further immunologic analysis revealed robust antibody responses to vaccine antigens such as tetanus and diphtheria toxoids, but significantly abnormal lymphocyte proliferation to mitogens (PHA, PWM) and antigens (*Candida* and tetanus toxoid) and stimulation with soluble anti-CD3. RAG1 function was determined by recombinase activity (Fig 1, B), and the 256–257delAA mutant had only 2.67% ± 0.58% activity as compared with wild-type RAG1. Although overexpression experiments have shown that this mutation can induce the production of an N-terminal-truncated protein by using a second internal ATG¹ (which might explain the residual recombination activity), we failed to detect the expression of the mutant RAG1 on retrovirus-mediated transduction of *Rag1*^{-/-} pro-B cells. Detailed B-cell subset analysis revealed low total memory B cells with low class-switched memory and marginal zone B cells with a significant increase in naive B cells (CD27⁻) and transitional B cells (CD19⁺CD24⁺⁺CD38⁺⁺). Functional B-cell analysis revealed that the patient's B cells were capable of plasmablast differentiation on stimulation with CD40L and IL-21 or CpG, though it was decreased than that in a healthy control (data not shown). B-cell proliferation in response to CD40L + IL-21 was lower than in control B cells, but almost comparable to that in control with CpG. Also, the distribution of immunoglobulin light chains (kappa/lambda) was normal, arguing against significant defects of receptor editing of B cells. Therefore, it would appear that patient's B cells are capable of responding to helper T-cell signals, and the reduced frequency of memory B cells is likely related to the low numbers of such T cells. There was evidence of autoantibody production with high titer of ANA, double-stranded DNA and SS-A antigens in addition to antibodies to 14 other autoantigens. Although there was no clinical evidence of specific autoimmune disease or granulomatous disease and renal function was preserved,^{2,3} the diffuse skin rash, which was responsive to steroids, suggests that a clear inflammatory process was present (prior to treatment). Molecular T-cell receptor repertoire analysis (T-cell receptor Vb spectratyping) revealed an overall polyclonal T-cell repertoire for most T-cell receptor Vb families with oligoclonality of a few (family 2 was absent and families 3–1, 6–4, 13, and 16 were oligoclonal with limited diversity). There was a significant increase in peripheral levels of IL-7, which is related to the presence of T-cell lymphopenia. Clinically, the patient responded well to wet

dressings with ammonium lactate topical (AmLactin), a keratolytic, and treatment with triamcinolone. He was treated with prophylactic antibiotics with no evidence of infection.

In a mouse model of a hypomorphic *Rag1* variant, progressive T-cell lymphopenia with age was demonstrated,⁴ suggesting that heterozygosity for RAG defects may lead to premature immune senescence and T-cell dysfunction.

This case is unique and exemplifies the magnitude of phenotypic variations that can be seen in molecular defects typically associated with SCID, or hypomorphic mutations in genes that are associated with OS,² but in this case represents an idiopathic T-cell lymphopenia, adding to the spectrum of previously reported mutations.⁵ What is remarkable is the apparent adult-onset presentation of eosinophilia, skin rash, and low T-cell counts in this patient with only a single copy of a frameshift variation in the 5' open reading frame of the *RAG1* gene that usually causes an autosomal-recessive disorder. There is evidence that frameshift mutations in the 5' end of *RAG1* can maintain V(D)J recombination activity,¹ and this may partially explain the late onset of the phenotype and normal B-cell numbers in the context of T-cell lymphopenia. As seen in the mouse model of hypomorphic *Rag1* mutation,⁴ it is possible that the T-cell lymphopenia is due to an age-related decline in immunocompensatory mechanisms that permit the phenotype to be revealed later than typical.

There are several differences between OS and idiopathic T-cell lymphopenia, with the former typically being clinically severe and characterized by elevated eosinophils and IgE along with erythrodermia, hepatosplenomegaly, lymphadenopathy, T-cell counts that are variable but generally oligoclonal in diversity, absent B cells, and normal natural killer cells.⁶ Patients with OS usually present early in life with recurrent and opportunistic infections, and the clinical course is fulminant and typically managed with hematopoietic cell transplantation. Adult-onset patients with idiopathic T-cell lymphopenia may have an overall milder clinical phenotype and are unlikely to require hematopoietic cell transplantation; nonetheless, these patients require careful monitoring to control significant infections as well as the development of autoimmunity and subsequent end-organ damage.

Adult-onset ADA deficiency is the only other SCID-associated defect that has been reported with delayed manifestations in the context of heterozygous mutations in the ADA gene,⁷ resulting in partial ADA deficiency and a milder phenotype. In the partial ADA deficiency, immune function has been shown to decline over time, resulting in an immunologic and clinical phenotype. This phenomenon appears to be reproduced in this patient with the heterozygous *RAG1* mutation.

The presence of severe T-cell lymphopenia with dysregulated B-cell responses raises intriguing questions about the impact of hypomorphic mutations on immune function, and further the lack of an immunologic or clinical phenotype in the parent who is a carrier for this mutation suggests that there is an underlying additional genetic, epigenetic, or environmental modulation of RAG1 function in this patient.

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FIG 1.

A, Photograph showing skin rash. Diffuse skin rash was present on the extremities and, to a lesser extent, on the trunk of this patient with a heterozygous *RAG1* mutation. **B**, Determination of recombinase activity level of wild-type and mutant *RAG1*. Stable v-abl-transformed pro-B-cell lines (pro-B Abelson lines) were generated from the bone marrow of *Rag1*^{-/-} and Bcl2 transgenic mice. *Rag1*^{-/-} pro-B Abelson lines (10⁶/mL) were infected with an inverted GFP cassette/recombination substrate, pMX- INV, and single integration was selected. These cells were further infected with virus containing pBMN-mock/*RAG1*/*RAG1* (256-7delAA)-IRES-hCD2, which were prepared in a similar manner as has been

described previously. Cells with pMX-INV and pBMN-mock/RAG1/RAG1(256-7delAA)-IRES-hCD2 proviral integrants were isolated by magnetic bead sorting by human CD4 and human CD2 microbeads, respectively (Miltenyi Biotec GmbH, Germany). Finally, cells were treated with 3 mM STI-571 (Novartis), an abl kinase inhibitor, for 96 hours to promote recombination and subsequently analyzed by flow cytometry for recombination-dependent expression of GFP.

TABLE I

Lymphocyte subset quantitation by flow cytometry

Lymphocyte subset	Percent	Normal range (%)	Absolute count (cells/uL)	Normal range (cells/uL)
CD45 lymph count			793	990–3150
CD3 ⁺	6	59–83	43	677–2383
CD3 ⁺ 4 ⁺	4	31–59	30	424–1509
CD3 ⁺ 8 ⁺	2	12–38	12	169–955
CD19 ⁺	78	6–22	567	99–527
CD16 ⁺ 56 ⁺	16	6–27	117	101–678

Boldface text defines abnormal values relative to the reference range provided in the table.