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Expanding the spectrum of recombination-activating gene 1 deficiency: A family with early-onset autoimmunity

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To the Editor:

Lymphocyte receptor diversity is generated by recombining variable, diversity, and joining (VDJ) gene segments of the immunoglobulin and T-cell receptor (TCR) loci. V(D)J recombination requires DNA breakage, a process mediated by recombination-activating gene (*RAG*) 1 and 2. *RAG* deficiency was initially described in patients with the T⁺B⁺ severe combined immunodeficiency (SCID) phenotype¹; however, the spectrum of the disease has expanded to include Omenn syndrome, cytomegalovirus infection with $\gamma\delta$ T-cell expansion, combined immunodeficiency with granuloma, and isolated CD4⁺ lymphopenia.²⁻⁶ The pleomorphic manifestations of *RAG* deficiency are partially explained by residual *RAG* activity, with null mutations producing an SCID phenotype and hypomorphic mutations presenting more variably.^{2,7} Although autoimmunity is a known feature of aberrant *RAG* function, it has never been described as the primary manifestation of the disease in an infant. We describe a novel presentation of *RAG* deficiency characterized by the presence of B cells and early-onset autoimmunity.

A full-term female infant (patient B) with undetectable T-cell receptor excision circles was identified by means of newborn screening, indicating a concern for SCID. The baby was

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born to nonconsanguineous parents and vigorous at birth. Further immunologic evaluation revealed T-cell lymphopenia with poor lymphocyte proliferation to mitogens (Table I).

Of concern, the couple's first daughter (patient A) had died at 2 years of age after a prolonged disease course characterized by autoimmunity and recurrent infections. Initially, patient A was healthy, aside from recurrent urinary tract infections and a respiratory syncytial viral infection that required an intensive care unit admission. At 6 months, she had fleeting rashes and digital necrosis. Angiography demonstrated multifocal irregularities in vessels of the hand suggestive of thrombotic disease and vasculitis. Shortly thereafter, patient A had hypotonia with increased muscle enzyme levels (aspartate aminotransferase, 566 U/L; alanine aminotransferase, 1041 U/L; and aldolase, 34 U/L), although the creatinine kinase level was normal. A muscle biopsy specimen was suggestive of an inflammatory myopathy, showing foci of mononuclear cells, whereas a liver biopsy specimen demonstrated mild lobular hepatitis with no signs of infection. Progressive cytopenias were noted with a direct Coombs-positive hemolytic anemia, anti-platelet antibody-mediated thrombocytopenia, lymphopenia, and neutropenia. Other auto antibodies with increased levels included anti-nuclear antibody at a titer of 1:40, cardiolipin IgG (31.2 IgG phospholipid units [GPL]; normal value, <19 GPL) and IgM (13.7 IgM phospholipid units [MPL]; normal value, <10 MPL), thyroid peroxidase (42.2 IU/mL; normal value, <14.9 IU/mL), and thyroglobulin (37.0 IU/mL; normal value, <24.9 IU/mL). Inflammatory markers were consistently increased (erythrocyte sedimentation ratio, 80-100 mm/h). The C4 level was decreased, with a nadir of 6 mg/dL (normal value, 14-42 mg/dL), whereas the C3 level and total complement activity were normal. Malignancy was excluded based on results of a bone marrow biopsy and imaging of the chest, abdomen, and pelvis.

Immune dysregulation secondary to an underlying immunodeficiency was suspected, and an immune evaluation was performed at 1 year of age, while patient A was receiving glucocorticoids. There was severe T-cell lymphopenia with poor lymphocyte proliferation to mitogens, normal natural killer cell numbers, partially preserved B-lymphocyte numbers, and normal to increased levels of serum immunoglobulins (Table I). Tetanus and *Haemophilus influenzae* b titers measured at 11 months of age were nonprotective: tetanus antibody level of less than 0.10 IU/mL (protective titer, >0.15 IU/mL) and *H influenzae* b antibody level of 0.31 µg/mL (protective titer, >1.0 µg/mL). Test results for mutations in genes responsible for T⁺B⁺ SCID (*IL7R*, *JAK3*, *CD3D*, and *CD3E*) were negative, and no mutations were identified in the *ADA* and *PNP* genes. Results of a comparative genomic hybridization array for copy number variation was normal.

Medical treatment was initiated with glucocorticoids, intravenous immunoglobulin, enoxaparin, and rituximab (375 mg/m²), administered weekly for a total of 4 doses. There was significant clinical improvement. The child remained stable for the next year until prednisolone was decreased to 1.5 mg/d and the Raynaud symptoms returned, along with recurrence of increased inflammatory markers. New-onset choreiform movements in the upper extremities led to magnetic resonance imaging/magnetic resonance angiography of the brain, which showed white matter lesions. The clinical worsening and persistent immunologic abnormalities (Table I) were thought to be consistent with a novel immunodeficiency, and plans were made for a hematopoietic stem cell transplantation. Unfortunately, the child died from an *Enterobacter* species central line infection before the transplantation could be performed.

Because of patient A's death, the younger sister (patient B) was immediately started on sulfamethoxazole/trimethoprim (Bactrim) prophylaxis and intravenous immunoglobulin and never had infections or clinical manifestations of autoimmunity. Similar to her sister, she was also found to have severe T-cell lymphopenia, with normal natural killer lymphocyte

numbers, partially preserved B-cell numbers, and normal immunoglobulin levels (Table I). The patient underwent a 10/10 HLA-matched unrelated bone marrow transplantation at 3 months of age. Conditioning consisted of intravenous busulfan (adjusted to achieve an area under the curve of 1026 $\mu\text{mol}\cdot\text{min}$), cyclophosphamide, and antithymocyte globulin. Neutrophil engraftment was achieved on day +30, with no complications. Fourteen months after transplantation, the patient is in excellent condition with full immune reconstitution (Table I), and she remains off immunosuppression with no evidence of graft-versus-host disease.

We sought to further evaluate the cause of early-onset autoimmunity and T-lymphocyte immunodeficiency in this family. Genetic testing in patient B revealed compound heterozygous missense mutations in *RAG1* (c.2522 G>A, p.R841Q; c.2920 T>C, p.F974L). Analysis of frozen genomic DNA from patient A confirmed identical *RAG1* mutations, and the father and mother were both carriers (paternal allele: p.R841Q; maternal allele: p.F974L; see the Methods section and Fig E1 in this article's Online Repository at www.jacionline.org). Interestingly, a patient homozygous for R841W mutations in *RAG1* has been described with preserved B cells, although this patient presented with Omenn syndrome.⁸

RAG1 expression and function were assessed by using Abelson-immortalized murine *RAG1*^{-/-} pro-B cells transduced with vectors encoding either wild-type or mutant *RAG1* alleles (see the Methods section in this article's Online Repository). The R841Q mutant showed lack of protein expression and recombinase activity; in contrast, protein expression was preserved in the F974L mutant, and recombinase activity was reduced (Fig 1). Patient B's TCR repertoire was moderately oligoclonal, with only 8 of 24 TCR β variable region families falling in the normal range (Fig 1 and see the Methods section in this article's Online Repository). The TCR diversity corrected after transplantation (Fig 1).

The cases presented here demonstrate that the clinical spectrum of RAG deficiency must expand to encompass early-onset autoimmunity with preserved B lymphocytes. The clinical and immunologic phenotype of this condition is expected to diversify through early recognition by the universal newborn screening for SCID. In this family the younger sister was asymptomatic, likely because of the lack of environmental exposures triggering immune activation, coupled with early initiation of definitive treatment. The pathogenesis of autoimmunity in patients with RAG deficiency remains unclear, although potential mechanisms based on mouse models include dysfunctional B-cell receptor editing, autoreactive B-cell survival promoted by increased B-cell activating factor levels, and an inflammatory milieu with chronic innate immune system activation.⁹ Regardless of the cause, patients who present with early-onset autoimmunity should be evaluated for an underlying immunodeficiency, particularly *RAG* mutations, even in the presence of B cells.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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REFERENCES

1. Schwarz K, Gauss GH, Ludwig L, Pannicke U, Li Z, Lindner D, et al. RAG mutations in human B cell-negative SCID. *Science*. 1996; 274:97–9. [PubMed: 8810255]
2. Villa A, Santagata S, Bozzi F, Giliani S, Frattini A, Imberti L, et al. Partial V(D)J recombination activity leads to Omenn syndrome. *Cell*. 1998; 93:885–96. [PubMed: 9630231]
3. Schuetz C, Huck K, Gudowius S, Megahed M, Feyen O, Hubner B, et al. An immunodeficiency disease with RAG mutations and granulomas. *N Engl J Med*. 2008; 358:2030–8. [PubMed: 18463379]
4. de Villartay JP, Lim A, Al-Mousa H, Dupont S, Dechanet-Merville J, Coumau-Gatbois E, et al. A novel immunodeficiency associated with hypomorphic RAG1 mutations and CMV infection. *J Clin Invest*. 2005; 115:3291–9. [PubMed: 16276422]
5. Ehl S, Schwarz K, Enders A, Duffner U, Pannicke U, Kuhr J, et al. A variant of SCID with specific immune responses and predominance of gamma delta T cells. *J Clin Invest*. 2005; 115:3140–8. [PubMed: 16211094]
6. Kuijpers TW, Ijspeert H, van Leeuwen EM, Jansen MH, Hazenberg MD, Weijer KC, et al. Idiopathic CD41 T lymphopenia without autoimmunity or granulomatous disease in the slipstream of RAG mutations. *Blood*. 2011; 117:5892–6. [PubMed: 21502542]
7. Villa A, Sobacchi C, Notarangelo LD, Bozzi F, Abinun M, Abrahamsen TG, et al. V(D)J recombination defects in lymphocytes due to RAG mutations: severe immunodeficiency with a spectrum of clinical presentations. *Blood*. 2001; 97:81–8. [PubMed: 11133745]
8. Ohm-Laursen L, Nielsen C, Fisker N, Lillevang ST, Barington T. Lack of nonfunctional B-cell receptor rearrangements in a patient with normal B cell numbers despite partial RAG1 deficiency and atypical SCID/Omenn syndrome. *J Clin Immunol*. 2008; 28:588–92. [PubMed: 18592361]
9. Walter JE, Rucci F, Patrizi L, Recher M, Regenass S, Paganini T, et al. Expansion of immunoglobulin-secreting cells and defects in B cell tolerance in Rag-dependent immunodeficiency. *J Exp Med*. 2010; 207:1541–54. [PubMed: 20547827]

FIG 1. Immunologic characteristics of patients A and B. **A**, Expression of RAG1 protein in the Abelson-immortalized lines transfected with the R841Q mutation, the F947L mutation, mock vector, and wild-type *RAG1*. Actin is a loading control. **B**, Recombinase activity of the *RAG1* mutations as measured based on green fluorescent protein expression in the Abelson-immortalized lines transfected with the R841Q and F947L mutations. **C** and **D**, Expression of TCRVβ families in patient B's CD3⁺ lymphocytes before (Fig 1, **C**) and after (Fig 1, **D**) bone marrow transplantation. *Open circles*, Less than normal values, *closed squares*, within range; *open triangles*, greater than normal values.

TABLE I

Hematologic and immunologic characteristics of patient B and her older sister (patient A)

	Patient A (12 mo)	Patient A (24 mo)	Patient B (6 wk)	Patient B, 1 y s/p BMT
WBC (cells $\times 10^{-3}/\mu\text{L}$)	6.37	9.63	6.09	8.81
IgG (mg/dL)	1,570	1,480 *	371	736
IgA (mg/dL)	77	187	22	50
IgM (mg/dL)	230	133	67	127
IgE (IU/mL)	227	—	38	—
CD3 ⁺ (cells/ μL [%])	89 (11)	160 (25)	34 (5)	3,584 (68)
CD4 ⁺ (cells/ μL [%])	59 (8)	108 (17)	30 (4)	2,372 (45)
CD8 ⁺ (cells/ μL [%])	7 (1)	10 (2)	4 (0)	1,055 (20)
CD16 ⁺ CD56 ⁺ (cells/ μL [%])	320 (40)	340 (55)	395 (56)	655 (13)
CD19 ⁺ (cells/ μL [%])	359 (45)	81 (13) *	265 (38)	908 (17)
CD3 ⁺ CD4 ⁺ CD45RA ⁺ (%)	—	—	13.9	73.1
CD3 ⁺ CD4 ⁺ CD45RO ⁺ (%)	—	—	86.1	26.5
CD3 ⁺ CD8 ⁺ CD45RA ⁺ (%)	—	—	63.2	43.3
CD3 ⁺ CD8 ⁺ CD45RO ⁺ (%)	—	—	36.8	42.7
PHA (cpm [% control])	21,254 (15.0)	32,916 (25.3)	6,408 (14.5)	108,861 (70.2)
ConA (cpm [% control])	6,097 (6.1)	2,243 (1.9)	10,730 (53.4)	102,455 (100)
TREC (copies/ μL)	—	—	Undetectable	658
RNase P (copies/ μL)	—	—	11,876	21,819

BMT, Bone marrow transplantation; *ConA*, concanavalin A; *cpm*, counts per minute; *s/p*, status post; *TREC*, T-cell receptor excision circles (measured as described from dried blood spots with RNase P control gene determination).

* Laboratory tests performed after intravenous immunoglobulin and rituximab were administered.