

BRIEF REPORT

An Immunodeficiency Disease with RAG Mutations and Granulomas

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SUMMARY

We describe three unrelated girls who had an immunodeficiency disease with granulomas in the skin, mucous membranes, and internal organs. All three girls had severe complications after viral infections, including B-cell lymphoma associated with Epstein–Barr virus (EBV). Other findings were hypogammaglobulinemia, a diminished number of T and B cells, and sparse thymic tissue on ultrasonography. Molecular analysis revealed that the patients were compound heterozygotes for mutations in recombination activating gene 1 or 2 (*RAG1* or *RAG2*). In each case, both parents were heterozygous carriers of a *RAG* mutation. The mutations were associated with reduced function of *RAG* in vitro (3 to 30% of normal activity). The parents and one sibling in the three families were healthy.

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SEVERE COMBINED IMMUNODEFICIENCY DISEASE (SCID) COMPRISES A heterogeneous group of immunodeficiency diseases, one of which is caused by a null mutation in both alleles of *RAG1* or *RAG2*.^{1,2} This form of SCID accounts for approximately 20% of cases. The heterodimeric *RAG* enzyme recombines subgenic elements at the immunoglobulin and T-cell-receptor loci, thereby generating the variable part of antigen-binding receptor genes, a process termed V(D)J recombination. The *RAG* complex is active in precursor T cells and B cells, which cannot develop without *RAG* activity. Null mutations of both alleles of *RAG1* or *RAG2* cause the classic SCID phenotype, with absent T cells and B cells. Hypomorphic missense mutations of *RAG1* or *RAG2*, which allow residual *RAG* activity, occur in the Omenn syndrome, in which the main features are hepatosplenomegaly, lymphadenopathy, eosinophilia, elevated serum IgE levels, and infiltration of various tissues, notably the skin and the gastrointestinal tract, by oligoclonal populations of T cells.³ The Omenn syndrome can have a fatal outcome despite stem-cell transplantation.³

We describe a type of primary immunodeficiency disease with *RAG1* or *RAG2* mutations that diminish *RAG* activity and allow the maturation of a limited number of T and B cells. Three girls under the age of 10 years from three different families presented with extensive granulomatous disease involving the skin, mucous membranes, and internal organs.

CASE REPORTS

PATIENT 1

At the age of 2.5 years, Patient 1, the daughter of nonconsanguineous parents, was referred to the University Children's Hospital Düsseldorf because of papulonodular skin lesions on the face and limbs. During the previous year, the lesions had failed to respond to treatment with topical corticosteroids (Table 1 and Fig. 1A). The medical history was otherwise unremarkable, and the patient had received standard immunizations without complications.

A skin biopsy showed parakeratosis, epidermal hyperplasia, and lichenoid lymphocytic infiltrates with few neutrophils in the upper dermis. In the middle and lower dermis were nodular collections of epithelioid cells (Fig. 1B) surrounded by moderately dense lymphocytic infiltrates and some neutrophils (Fig. 1C). Some of the epithelioid cells were multinucleated, and some of the lymphocytes had atypical nuclei. Some of the epithelioid histiocyte collections had a central zone of necrosis (Fig. 1D).

In immunohistochemical studies, the epithelioid cells were positive for CD68, and the lymphocytes were positive for CD3 and CD8 and negative for CD79a and CD4. Paraffin-fixed skin-biopsy samples, examined by seminested, multiplex polymerase-chain-reaction (PCR) assay, showed clonal rearrangements of the gene encoding T-cell receptor γ . These findings supported a diagnosis of small-cell pleomorphic T-cell lymphoma. Biopsies of bone marrow and liver did not reveal clear evidence of systemic involvement. Immunologic abnormalities were considered to be secondary to the presumed lymphoma.

The child was treated with chemotherapy for T-cell lymphoma, but numerous new papulonodular efflorescences developed, and the existing lesions progressed. After the lesions failed to improve after 4 months of chemotherapy, the treatment was stopped and the diagnosis of T-cell lymphoma was reconsidered. A primary immunodeficiency was suspected, since there was profound hypogammaglobulinemia (Table 2), and tissue infiltrates of clonal CD8+ T cells had been described in a patient with common variable idiopathic immunodeficiency.⁷ Periodic acid–Schiff (PAS), Giemsa, and Ziehl–Neelsen staining of skin-biopsy specimens showed no evidence of fungi,

leishmania, or mycobacteria, and PCR assays for mycobacterium tuberculosis and Whipple's bacilli were negative (Table 2 of the Supplementary Appendix, available with the full text of this article at www.nejm.org).

Molecular studies identified compound heterozygous mutations in *RAG1* (Table 3). When the patient was 5.7 years of age, a tumor of the right tonsil developed. On histologic examination, the tumor was a diffuse large B-cell lymphoma with clonal IgH genes and expression of CD20, CD79a, and CD30. In situ hybridization of the tumor for EBV-encoded small RNA (EBER) was strongly positive. She was treated with rituximab, which led to partial remission. At 6 years of age, the patient underwent hematopoietic stem-cell transplantation from an HLA-matched unrelated donor. More than 3 years after transplantation, the patient is not receiving any medication, the skin lesions have disappeared, and immune functions are normal.

PATIENT 2

At the age of 7.5 years, Patient 2, the daughter of nonconsanguineous parents, was referred to the University Children's Hospital Ulm for evaluation of an immunodeficiency. Her 6-year-old brother was healthy. At the age of 9 months, she had severe varicella with hepatitis and bacterial superinfection. At 27 months of age, multiple, treatment-resistant ulcerative skin lesions developed on her limbs and face (Fig. 1 of the Supplementary Appendix). Skin-biopsy specimens showed changes similar to those in Patient 1: nodular collections of epithelioid cells surrounded by moderately dense lymphocytic infiltrates with some neutrophils. However, no necrosis was observed. Extensive testing failed to conclusively reveal microbiologic agents (Table 2 of the Supplementary Appendix). In an attempt to treat the lesions, regular subcutaneous immunoglobulin injections were started when the patient was 4 years old. At 6.5 years of age, nodular lesions developed on her tongue; these lesions had a histologic similarity to the cutaneous lesions (Fig. 1 of the Supplementary Appendix). PAS, Giemsa, and Ziehl–Neelsen staining showed no pathogens in either skin or tongue. PCR assays for mycobacterium tuberculosis and Whipple's bacilli that were performed on a tongue-biopsy specimen were negative. Similar granulomatous changes were also noted in biopsy specimens of the

Characteristic	Patient 1	Patient 2	Patient 3
Patients			
Age at diagnosis (yr)	3	7.8	10.7
Leading symptom	Skin lesions	Skin lesions, recurrent bronchopneumonias	Splenomegaly
Infectious disease history	No severe or recurrent infections	Severe varicella infection in infancy, vaccine-induced measles, recurrent bronchopneumonias	Severe varicella infection at 8 yr of age, recurrent bronchopneumonias
Thymus visible on ultrasonography	No	No	No
Stem-cell transplantation from a matched unrelated donor	Yes	Yes	No
Outcome	Donor chimerism of 100%, alive and well, attending school	Donor chimerism of 100%, alive, chronic graft-versus-host disease	Evaluation for possible stem-cell transplantation
Granulomas			
Age at first manifestation (mo)	17	24	24
Localization	Skin	Skin, lungs, tongue, adenoids	Spleen, lungs
Histology	Epithelioid granuloma with lymphocyte and neutrophil infiltration	Granulomatous folliculitis (skin), interstitial pneumonia with epithelioid-cell granuloma (lungs), epithelioid granuloma (tongue)	Noncaseating epithelioid-cell granulomas
Therapeutic approach	Combination chemotherapy	Cyclosporine, corticosteroids, thalidomide, dapsone, clofazimine	None
Efficacy of therapy	Worsening	Ineffective, worsening on cyclosporine	NA

* All three patients had compound heterozygous RAG mutations; in Patients 1 and 2 the mutations were in RAG1, and in Patient 3 the mutation was in RAG2. NA denotes not applicable.

adenoids and the lung, which were obtained because of respiratory complications (pneumonias, bronchiectasis, and atelectasis).

Results of immunohistochemical analysis of the tongue lesion were similar to findings in Patient 1. Studies of the tongue-biopsy specimens with seminested, multiplex PCR showed a polyclonal population of T cells (Table 1 of the Supplementary Appendix). Therapy with corticosteroids, dapsone, and clofazimine was ineffective. Cyclosporine treatment led to deterioration of skin lesions. Repeated skin grafting was necessary after partial destruction of the Achilles tendon by progressive ulceration. Immunophenotyping of blood cells revealed low numbers of T and B cells but a normal number of natural killer cells (Table 2). Thymic tissue was undetectable on ultrasonography. Molecular studies revealed compound heterozygous RAG1 mutations.

Hematopoietic stem-cell transplantation from an HLA-matched unrelated donor was performed when the patient was 8.5 years old. More than a year after transplantation, no new skin lesions have developed, and the patient is receiving immunosuppressive therapy for chronic graft-versus-host disease.

PATIENT 3

At the age of 9.9 years, Patient 3, the only child of nonconsanguineous parents, was referred to the University Children's Hospital Düsseldorf with a history of recurrent pneumonia beginning at the age of 2 years. Otherwise, she had an uneventful early childhood. At 8.5 years of age, she had a severe varicella infection complicated by encephalitis and the acute respiratory distress syndrome. An immunologic evaluation that was performed when she was 9.9 years old showed profound hypogam-

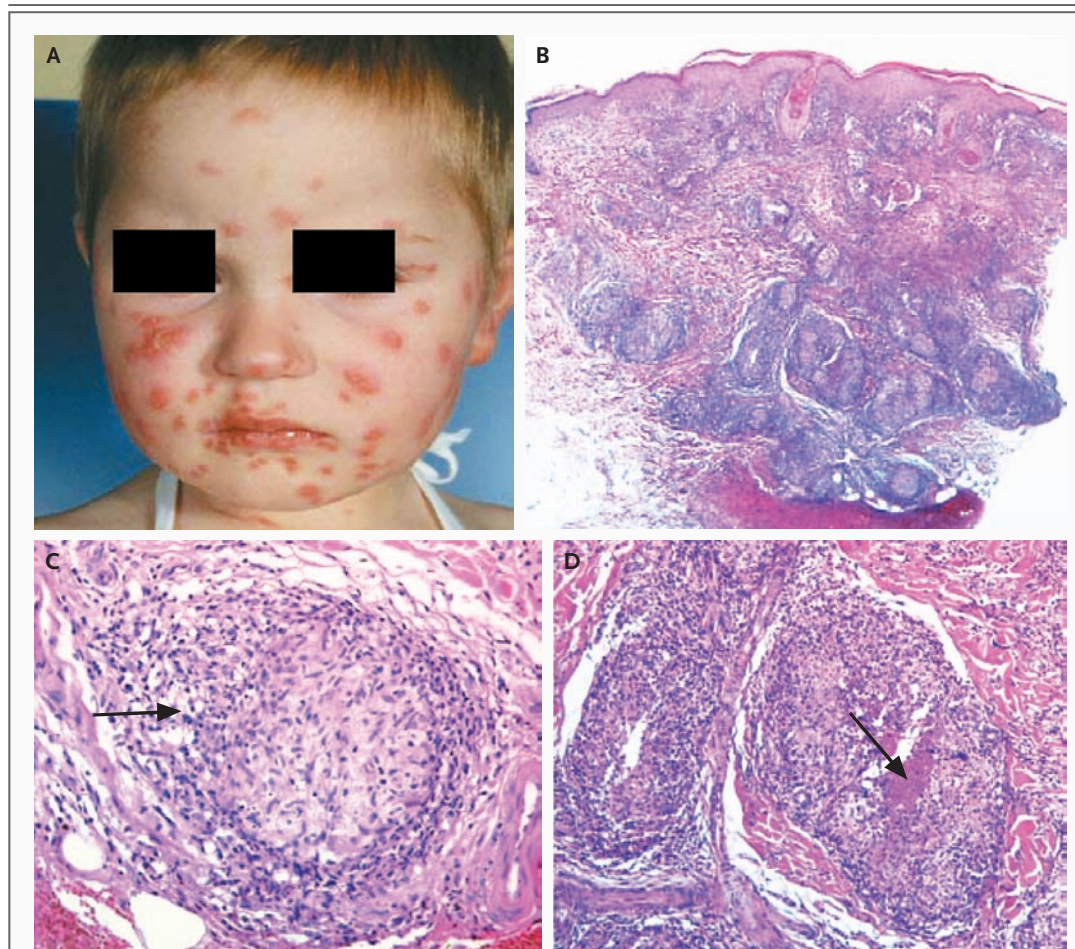


Figure 1. Facial Lesions and Histopathological Specimens.

Histopathological analysis of erythematous papulonodular lesions covering the face of Patient 1 (Panel A) show nodular collections of epithelioid cells (Panel B) surrounded by moderately dense lymphocytic infiltrate with some neutrophils (Panel C, arrow). In some of these collections, a central zone of necrosis is visible (Panel D, arrow).

maglobulinemia with low numbers of T and B cells (Table 2). At the age of 10.5 years, she was found to have massive splenomegaly with hypodense infiltrates on ultrasonography. Splenic biopsy revealed granulomas with epithelioid cells. Staining with PAS, Giemsa, and Ziehl–Neelsen was negative. PCR assays for mycobacterium tuberculosis and Whipple's bacilli that were performed on the splenic specimen were negative (Fig. 2 of the Supplementary Appendix).

Immunophenotyping of the lesion showed a polyclonal population of T cells (Table 2). Small nodular infiltrates consistent with granulomatous disease were seen on computed tomography of the lungs. The results of bronchoalveolar lavage were inconclusive. Molecular analysis, which was

performed when the patient was 10.7 years old, revealed compound heterozygous mutations in *RAG2*. The patient's condition is stable on regular IgG infusions, and she is being evaluated for stem-cell transplantation.

MOLECULAR FINDINGS

The parents of each of the three children gave informed consent to carry out the investigations described below.

Compound heterozygous mutations in *RAG1* or *RAG2* were identified in all three patients (Fig. 2 and Table 3). None of the mutations were detected in 105 healthy control subjects who were matched for white race (data not shown). The functional

Table 2. Immunologic Analysis.*

Variable	Patient 1	Patient 2	Patient 3
	Value	Value	Value
	Reference Range	Reference Range	Reference Range
Age range (yr)	2.6–3.1	7.5–8.5	9.8–12.2
Immunoglobulin			
IgG (mg/dl)	92–209	890†	146
IgA (mg/dl)	<6	14	<6
IgM (mg/dl)	<5	67	11.3
IgE (IU/ml)	<4.4	ND	<5.0
Specific antibodies			
Tetanus toxoid	Positive	Positive	Positive
Diphtheria toxoid	Negative	Negative	Negative
Isohemagglutinins	Negative	Negative	Negative
White-cell count (cells per mm ³)			
Leukocytes	1100–2700	2500–5700	2900–7000
Lymphocytes	320–721	1000–1200	769–1554
Granulocytes	570–1310	4420–4800	1700–5200
Monocytes	55–260	220–260	200–520
Eosinophils	0–21	112–200	0–44
Lymphocyte subgroup‡:			
CD3+ T (cells per mm ³)	120–315	592–606	538–1057
CD3+/CD4+ T (cells per mm ³)	52–204	108–184	323–668
CD3+/CD8+ T (cells per mm ³)	48–122	360–420	177–326
CD20+ B (cells per mm ³)	0–30	58–132	54–202
CD56+/CD3– natural killer (cells per mm ³)	130–548	370–504	131–355
CD3+/HLA-DR+ (%)	11–40	3–13	15–33
TCRαβ+/CD4–/CD8– (%)	0–0.2	NA	1.4–1.7
TCRγδ+/CD3+ (%)	2.5–3.7	NA	7.3–11.2
CD4+/CD45RA+ (%)	1–12	50–85	9–27
CD4+/CD45RO+ (%)	98–100	9–26	75–96

T-cell function (mitogen and antigen stimulation)§	500	44,000	73,000	367,622	21,000	59,000
Phytohemagglutinin (2 µg/ml)	500	44,000	73,000	367,622	21,000	59,000
Pokeweed mitogen (10 µg/ml)	2600	42,000	ND		23,000	50,000
Anti-CD3 (OKT3 antibody) (50 ng/ml)	1000	27,000	119,053	139,297	34,000	63,000
<i>Staphylococcus aureus</i> Cowan (1:1000)	100	10,000	ND		1,000	8,000
Tetanus toxoid (1:1000)	1300	34,200	Negative		18,000	2,000
Purified protein derivative (10 µg/ml)	300	30,800	ND		2,000	6,000
Candida (1 µg/ml)	300	21,100	319	8,412	1,000	10,000
αβ T-cell-receptor repertoire¶	Diversified repertoire, oligoclonal expansions in CD4 and CD8 T cells		Diversified repertoire		Diversified repertoire, oligoclonal expansions in CD4 and CD8 T cells	
Maternal T cells	No		No		No	

* Listed are reference ranges or laboratory values for the patient's age group. For some lymphocyte subgroups, the reference range is either a percentage or the 10th and 90th percentiles, for the age groups of 2 to 6 years and 6 to 12 years⁴; for CD3+/HLA-DR+, the reference range is the 5th and 95th percentiles for the age groups of 2 to 5 years and 5 to 10 years.⁵ NA denotes not applicable, and ND not determined.

† Since the patient was undergoing intravenous immunoglobulin substitution, serum immunoglobulin levels could not be assessed before the initiation of therapy.

‡ Analyses of lymphocytes were performed with the use of a fluorescence-activated cell sorter (FACS).

§ Counts per minute of incorporated [³H]thymidine for patients and control subjects (which are used as the reference range) are expressed as a mean of triple measurements. (Values were obtained for the patients and the control subjects on the same day.) Medium values were subtracted in all measurements. Medium values for the patients and control subjects were below 500 counts per minute in all three experiments.

¶ Results were obtained with the use of spectratyping⁶ in Patients 1 and 3 and the use of a FACS with monoclonal Vβ antibodies in Patient 2.

|| Analyses were performed with the use of HLA typing of peripheral-blood mononuclear cells from the patients and their mothers.

significance of the mutations was evaluated in primary human dermal fibroblasts that were cotransfected with substrate vectors that allow scoring for V(D)J recombination efficiency and expression plasmids coding for wild-type or mutant RAG1 or RAG2 proteins (Table 4). The percentages of recombination-positive cells in the subpopulation of transfected fibroblasts varied from less than 5% to approximately 30% of the positive control. Lacking suitable monoclonal antibodies against human RAG proteins, we measured RNA stability of the in vitro transfectants as a surrogate. All RNAs were equally well expressed, as assessed by semiquantitative reverse-transcriptase PCR.

Mutations in the nucleotide-binding oligomerization domain-containing 2 (*NOD2*) gene have been implicated in various granulomatous diseases (e.g., Crohn's disease, Blau syndrome, and early-onset sarcoidosis). Since our patients had granulomas in various organs, we checked their *NOD2* genotypes by genomic sequencing. No changes in the predicted amino acid sequences, as compared with those of wild-type alleles, were detected (data not shown).

DISCUSSION

We describe three girls with a primary immunodeficiency disease associated with hypomorphic mutations in one of the two recombinase activating genes (*RAG1* and *RAG2*). Two of the three patients (Patients 1 and 3) had either no or insignificant infections in infancy or early childhood.

The histologic findings in various organs were notable for granulomas containing CD68+ epithelioid cells and CD8+ T cells, which were polyclonal in two of the three patients. In peripheral blood, there were diminished numbers of T and B cells but a normal number of natural killer cells. The proportion of activated T cells (HLA-DR+) was increased, and most T cells were of the memory type (CD45RO+).

The clinical findings in the three children were clearly distinct from those of classical SCID (null mutations of both *RAG* alleles and no *RAG* activity), the Omenn syndrome (a missense mutation of at least one *RAG* allele with retention of some *RAG* activity), and atypical SCID (a missense mutation of at least one *RAG* allele but with features that are not typical of the Omenn syndrome).^{8,9} In contrast to our patients, those with atypical

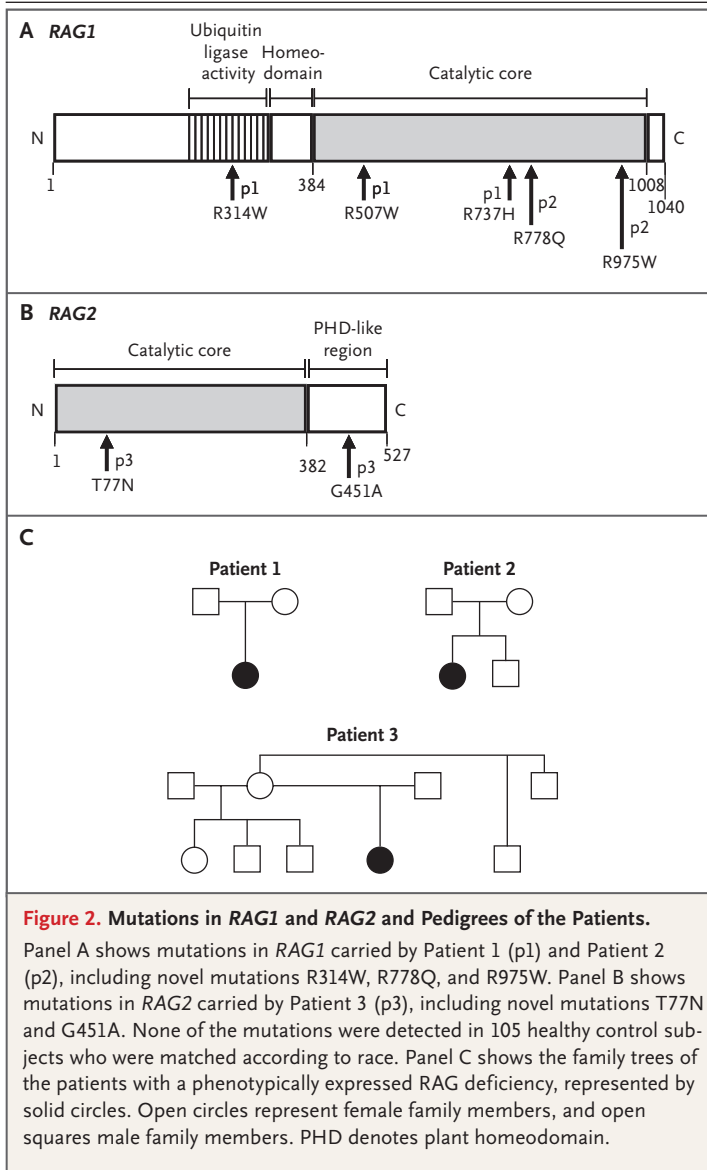
Table 3. Missense RAG Mutations.

Patient No.	Gene	Allele 1	Allele 2
1	RAG1	R314W*	R507W/R737H†
2‡	RAG1	R778Q	R975W
3	RAG2	T77 N†	G451A*

* The allele is of maternal origin.

† The allele is of paternal origin.

‡ The parents of Patient 2 did not undergo genetic analysis.



SCID are children of consanguineous parents, present with threatening infections during infancy, and do not have granulomatous disease. Of five

such patients who underwent hematopoietic stem-cell transplantation in infancy or early childhood, two survived and three died of severe cytomegalovirus infection.

The mutations we found in RAG genes have been detected in other patients with a different phenotype.¹⁰ The R507W mutation in Patient 1 has also been detected in an infant who had a skin rash, eosinophilia, slight lymphopenia, and normal levels of IgG but decreased IgA and IgM. Another child with a heterozygous R737H mutation (found in Patient 1) presented at the age of 1 month with typical Omenn syndrome. One of the mutations found in Patient 2 (R975W, RAG1) has been reported in a patient with the Omenn syndrome but with a different amino acid exchange (R975Q).³ The other mutation in Patient 2 and both mutations in Patient 3 (RAG2) have not been reported previously.

Functional analysis of RAG alleles showed V(D)J recombination activity varying between less than 5% in RAG mutants of Patients 1 and 2 and approximately 30% in RAG mutants of Patients 1 and 3, making it highly likely that the RAG mutations caused the impaired V(D)J recombination. Residual functional RAG1 or RAG2 allows a low level of T-cell production.^{3,10,11} The presence of CD4+/CD45RA+ cells in all three of our patients may reflect production of naive T cells by the thymus. Spectratyping of T-cell receptors in Patients 1 and 3 revealed a diverse repertoire. This finding was unexpected, since patients with SCID or the Omenn syndrome have no T cells or a population of T cells with a very restricted receptor repertoire.¹² The number of T cells in our patients is decreased, but the late onset and low incidence of repeated or life-threatening infections may indicate that the T-cell repertoire is sufficiently broad and competent for protection against microbes, at least for a limited period.

The late onset and the presence of functional T cells could be related to somatic mosaicism in T cells or engraftment of maternal T cells. Somatic mosaicism has been observed repeatedly in patients with SCID that is caused by adenosine deaminase deficiency or with X-linked SCID (a deficiency in the interleukin-2-receptor common γ chain). The mosaicism results from a mutation that reverts the mutation in adenosine deaminase or X-linked SCID to a wild-type mutant.¹³⁻¹⁵ In our three patients, revertants were excluded by genomic sequencing of sorted CD3, CD19, and CD14

Table 4. Percentage of Cells with V(D)J Recombination.*

Variable	Experiment 1	Experiment 2	Experiment 3	Average (% of wild-type activity)†
	<i>percent</i>			
RAG1				
Positive control	70.01	68.38	52.40	
–RAG1	0.06	0.05	0.05	
+RAG1	2.91	2.31	4.40	3.2 (100)
Patient 1				
+R314W	0.91	1.05	0.93	0.96 (30)
+R507W/R737H	0.12	0.09	0.06	0.09 (3)
Patient 2				
+R778Q	0.14	0.12	0.14	0.13 (4)
+R975W	0.08	0.10	0.13	0.10 (3)
RAG2				
Positive control	67.43	73.08	70.62	
–RAG2	0.05	0.06	0.04	
+RAG2	2.28	2.73	2.40	2.47 (100)
Patient 3				
+T77N	0.96	0.68	0.57	0.73 (30)
+G451A	0.83	0.79	0.63	0.75 (30)

* V(D)J recombination activity was assessed with the use of assays testing RAG mutants in normal human primary dermal fibroblasts, cotransfected with V(D)J recombination substrate vector and expression plasmids coding for wild-type RAG1 (+RAG1) or for mutant RAG1 proteins (+R778Q, +R975W, +R314W, and +R507W/R737H) and for RAG2 (+RAG2) or for mutant RAG2 proteins (+T77N and +G451A), respectively. The average recombination activity indicates residual activity of the mutant RAG1 or RAG2 protein, as compared with wild-type proteins, on the basis of three experiments. As a negative control, the same cotransfection without the expression plasmids coding for RAG1 or RAG2 (–RAG1 and –RAG2) was performed. As a positive control, a prerecombined substrate vector was transfected. In each assay, 5×10^4 fibroblasts were analyzed with the use of a fluorescence-activated cell sorter.

† The average V(D)J recombination activity refers to the proportion of activity as compared with that of the wild-type protein.

cells in which we found only the disease-causing mutations. Engraftment of maternal T cells, which can also lead to partial immunologic reconstitution resulting in a milder phenotype, was excluded by HLA typing of CD3 T cells in all three patients.

It is unclear whether the granulomas in our patients are the reactive sarcoid-like granulomas found in other primary immunodeficiency disorders, especially in granulomatous common variable immunodeficiency disease.^{7,16-19} In a cohort of 19 adults with this disease, we found no functionally relevant RAG1 or RAG2 mutations (data not shown). Furthermore, we did not see resolution of granulomas after treatment with intravenous immunoglobulin, as has been noted in some patients with common variable immunodeficiency.

^{18,19} Extensive and repeated tests revealed no pathogen in the granulomas, but it is possible that the lesions were a reaction to an undetected low-virulence pathogen, comparable to “sterile” granuloma formation seen in patients with chronic granulomatous disease. It is also possible that, apart from the RAG mutations, other mutations in modifier genes may be responsible for the granulomas.

The late onset and atypical clinical picture of the forms of RAG1 or RAG2 deficiency we describe here may prompt the inappropriate use of chemotherapy or immunosuppression. RAG deficiency should be a consideration in older patients with evidence of combined humoral and cellular immunodeficiency and otherwise unexplained granulomatous lesions.

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