Clinical Communications

Partial RAG deficiency in a patient with varicella infection, autoimmune cytopenia, and anticytokine antibodies

Vera Goda, MD[a], Aniko Malik, MD[b], Tibor Kalmar, PhD[c], Zoltan Maroti, PhD[c], Bhumika Patel, MD[d], Boglarka Ujhazi, MS[d], Krisztian Csomos, PhD[d], Jaime E. Hale, MS[e], Karin Chen, MD[f], Jacob Bleesing, MD[g], Paolo Palma, MD[h], Caterina Cancrini, MD, PhD[h], Anne M. Comeau, PhD[e], Gergely Krivan, MD, PhD[a], and Jolan E. Walter, MD PhD[d,i]

Clinical Implications

- Combined immunodeficiencies may initially present with herpes virus infections, autoimmune cytopenia, and antibodies to cytokines. Beyond the assessment of naive T cells, screen for anticytokine antibodies is useful to identify an underlying combined immunodeficiency ultimately confirmed by genetic testing.

TO THE EDITOR:

Autoimmune cytopenia (AIC) can occur after viral infection as a comorbid condition with primary immunodeficiencies (PIDs). Patients with PIDs may develop severe complications with acute viral infections and AIC.[1,2] Early detection of an underlying PID may promote definitive treatment, such as hematopoietic stem cell transplantation (HSCT).

Patients with partial defects in severe combined immunodeficiency (SCID)-associated genes, such as recombination activating gene (RAG), are increasingly reported with autoimmunity, such as AIC beyond infections.[3,4] The RAG1/2 proteins are essential components of the V(D)J recombination process that diversifies the repertoires of the T- and B-cell receptors. RAG1/2 deficiency has a broad phenotypic spectrum including combined immunodeficiency with granulomatous disease and/or autoimmunity (CID-G/AI) where milder impairment of RAG1/2 recombinase activity results in relatively preserved T- and B-lymphocyte counts and immunoglobulin levels; however, naive T-cell counts progressively decrease.[5] Therefore, measurement of T-cell receptor excision circles (TRECs), reflecting naive T-cell count, may identify these patients at birth in asymptomatic stage (via newborn screening) or later in life in the midst of infectious or autoimmune complications. However, in countries where newborn screening for SCID is not available, serological testing with a panel of anticytokine autoantibodies targeting IFN-α, IFN-ω, and IL-12 may serve as a complementary tool to raise suspicion for an underlying RAG deficiency with CID-G/AI phenotype in a child with a history of refractory AIC with severe viral infections.[3]

Hereby we report a 26-month-old female with partial RAG deficiency, nonvaccine strain varicella infection, and severe refractory AIC and discuss our diagnostic approach in Hungary where newborn screening for SCID is not available.

TABLE I. Lymphocyte subsets and immunoglobulin levels at first assessment of the child

	Results	Normal value
CD3	152/μL	900-4,500/μL
CD4+	92.8/μL	500-2,400/μL
CD8+	32.5/μL	300-1,600/μL
CD4+CD45RA+	19%	>60%
CD19+	213.8/μL	200-2,100/μL
CD16+ CD 56+	444.7/μL	100-1,000/μL
IgG	1,997 mg/dL	453-916 mg/dL
IgA	0.01 mg/dL	20-100 mg/dL
IgM	128 mg/dL	19-146 mg/dL

A previously healthy 26-month-old white female was referred to our immunology group for immune evaluation of prolonged varicella infection. The patient had extensive vesicular rash on the entire body and continued to develop new vesicular eruptions up to 2 weeks after onset of symptoms. No other organ involvement was noted. Two months after the onset of varicella, she developed immune thrombocytopenia (platelet count, 2×10^9/L), recalcitrant to high-dose intravenous immunoglobulin and pulse glucocorticosteroid treatment. The family history was negative for consanguinity, PID, and autoimmunity.

Immune phenotyping was notable for decreased total and naive T-cell count, but preserved B- and natural killer—cell compartments. Polyclonal gammopathy, low IgA level, and normal IgM level were also noted (Table I). Lymphocyte proliferation assay revealed low response to PHA (Systéme Internationale [SI], 3) and concanavalin A (SI, 8), and a low normal response to pokeweed mitogen (SI, 11) (SI normal value, >10.) Infection with HIV, EBV, and cytomegalovirus was ruled out by serologic assay. The patient generated appropriate varicella zoster antibody response with seroconversion on day 8. (Testing with Virotech ELISA anti—varicella zoster virus IgM positive OD/CO 3.396, IgG positive: OD/CO 1.384, IgA negative.)

Retrospective testing of TRECs from newborn screening blood spot specimen was undetectable (<252 copies/μL) in collaboration with the New England Newborn Screening Program.

Polyclonal gammopathy prompted screening for autoantibodies. There was a borderline positive antinuclear antibody titer at 1:40. Antiplatelet antibodies were not tested by clinical laboratory testing because this laboratory assay is not available for routine testing for immune thrombocytopenia in Hungary. Antibodies against double-stranded deoxyribonucleic acid, anti—smooth-muscle antibody, antimitochondrial antibody, antineutrophil cytoplasmic antibody, and antibodies against cytoplasmic antigens, centromere antigens, and parietal cells were all negative by clinical laboratory testing. Autoantibodies targeting cytokines IFN-α, IFN-ω, and IL-12 were detected by ELISA (Figure 1).

The patient's history of prolonged varicella infection with AIC, reduced naive T-cell compartment, and impaired T-cell proliferation was concerning for a severe form of PID and

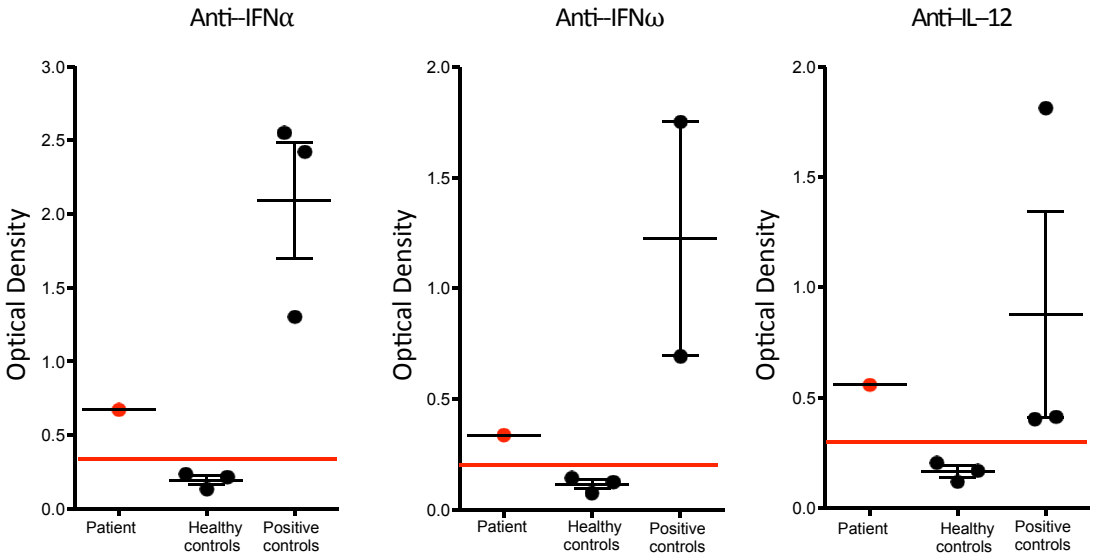

FIGURE 1. Anticytokine autoantibodies targeting IFN-α, IFN-ω, and IL-12 were detected by ELISA. Our patient had higher levels of anticytokine autoantibodies than the normal healthy controls. Positive controls are patients (aged 30, 18, and 6 years) with hypomorphic RAG deficiency and persistence of anticytokine antibodies, as previously described.[3]

prompted preparation for emergent allogeneic HSCT. The combination of clinical and laboratory features with naive T-cell lymphopenia and the presence of autoantibodies targeting IFN-α, IFN-ω, and IL-12 raised the possibility of RAG deficiency.[3]

A search for an underlying genetic mutation was initiated concomitantly with preparation for HSCT. Genomic DNA separated from peripheral blood samples from the patient and parents was used for library preparation and sequenced with Trusight One clinical exome kit (Illumina) on Illumina MiSeq platform for next-generation sequencing. This clinical exome kit covers the coding region of 4813 clinically relevant, disease-associated genes.

Three missense variants in the RAG1 gene were identified. We excluded NM_000448.2:c.746A>G (rs3740955) as a possible causative mutation because it has high minor allele frequency (MAF, 44.70) in the Exome Aggregation Consortium database (http://exac.broadinstitute.org). Two rare missense variants in exon 2 (NM_000448.2:c.1331C>T, rs199474685 with an MAF of 0.0008 and NM_000448.2:c.2974A>G, rs539590514 with an MAF of 0.000007) were identified by next-generation sequencing and confirmed by targeted Sanger sequencing. Patient was confirmed to have a compound heterozygous RAG1 mutation by inheriting RAG1 c.2974A>G p.Lys992Glu from the mother and RAG1 c.1331C>T p.Ala444Val from the father. Residual Rag1 recombination activity was 9.2% (p.Lys992Glu) and 1.4% (p.Ala444Val), as measured by an *in vitro* recombinase assay.[6] No mutations were identified in the RAG2 gene.

The mutation p.Lys992Glu has previously been reported in a homozygous and compound heterozygous form in Omenn syndrome at age 5 to 6 months.[7] The mutation p.Ala444Val has been reported in both homozygous and compound heterozygous forms in young infants with the leaky SCID, Omenn syndrome, and other SCID phenotypes at age 0 to 4 months.[7,8] All patients had B-cell lymphopenia and no history of autoimmunity. This is in contrast to our previously healthy patient presenting at age 26 months with varicella infection followed by severe AIC and laboratory phenotype of preserved B-cell compartment and polyclonal gammopathy. We postulate that our patient had polyclonal B-cell activation and AIC secondary to viral infection. The low naive T-cell compartment, undetectable TRECs, and poor T-cell proliferation are consistent with definition of leaky SCID; however, preserved B-cell lymphocyte count, polyclonal gammopathy, and autoimmune complications suggest a shift in phenotype to CID-G/AI. Our patient underwent HSCT with a 10/10 matched unrelated donor. Thrombocytes engrafted day 43 and CD3+/CD4+ reached the normal range on day 129 posttransplant. Patient is currently 13 months after transplantation with no evidence of cytopenia.

Regarding the clinical history, our case had some similarities with 3 additional cases that we previously published.[3] The comparison of cases is listed in Table E1, available in this article's Online Repository at www.jaci-inpractice.org. There is close timing of 2 to 4 months between the onset of viral infection and AIC with emergence of anticytokine antibodies. These antibodies were absent in young infants with hypomorphic RAG and absence of infection.[3,9]

Evaluation of a child with autoimmune cytopenia for CID, such as our case, can be difficult if lymphocyte immunophenotyping is atypical and immunoglobulin levels normal or elevated. The low number of naive T cells is a key finding and can be expedited by using the TREC assay. However, in countries where TREC screening is not available, such as Hungary, using autoantibody profiling with anticytokine antibody testing may help to identify those with an underlying combined immunodeficiency, such as partial RAG deficiency, and expedite early

recognition and confirmation by genetic testing, which is essential for timely preparation for stem cell transplantation and favorable outcomes.

[a]Department of Pediatric Hematology and Stem Cell Transplantation, United Saint Istvan and Saint Laszlo Hospital, Budapest, Hungary
[b]Department of Pediatrics, Semmelweis University, Budapest, Hungary
[c]Genetic Diagnostic Laboratory, Department of Pediatrics, Albert Szent-Györgyi Medical Center, Faculty of Medicine, University of Szeged, Szeged, Hungary
[d]Division of Pediatric Allergy & Immunology, Department of Pediatrics, University of South Florida, Tampa, Fla
[e]New England Newborn Screening Program, Department of Pediatrics, University of Massachusetts Medical School, Jamaica Plain, Mass
[f]Division of Allergy and Immunology, Department of Pediatrics, University of Utah School of Medicine, Salt Lake City, Utah
[g]Division of Bone Marrow Transplantation and Immunodeficiency, Cincinnati Children's Hospital Medical Center, Cincinnati, Ohio
[h]University Department of Pediatrics, Bambino Gesù Children's Hospital IRCCS, Rome, Italy
[i]Division of Pediatric Allergy Immunology, Massachusetts General Hospital for Children, Boston, Mass

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Corresponding author: Jolan E. Walter, MD, PhD, University of South Florida at Johns Hopkins All Children's Hospital, Children's Research Institute, 601 4th St South (CRI 4008), St Petersburg, FL 33701. E-mail: jolanwalter@health.usf.edu. Or: vera.goda@freemail.hu.

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TABLE E1. Clinical history and laboratory features of patients with partial RAG deficiency and AIC with herpes virus infection

Pt	Phenotype	Mutation	Live vaccination	Onset of VZV or CMV infection	Onset of autoimmune cytopenia	Time diffence between infection and AIC	Age of diagnosis of RAG deficiency	Time difference between AIC and diagnosis of RAG deficiency	Immune modulation	HCT	Age at testing for autoantibodies	Anticytokine antibodies	Reference
1	Leaky SCID	RAG 1 a. R474C b. K983NfsX9	None	VZV—20 mo	Coombs+ AIHA— 21 mo	1 mo	24 mo	3 mo	Nonresponsive to GCSF. Given high-dose IVIG 1 g/kg/d × 2 d without response. Rituximab starting at ∼22 mo × 4 doses	2 and 4 y	2 y	IFN-α, IFN-ω	E1,E2
2	CID-G/AI	RAG 2 a. G451A b. M459L	VZV	VA-VZV— 13 mo	Antineutrophil antibody+ AN— 13 mo	0 m	16 mo	3 mo	NA	18 mo	13 mo	IFN-α	E1
3	Leaky SCID	RAG 1 a-b. K86fsX33	BCG, MR	CMV—7 mo	Coombs+ AIHA— 11 mo	4 mo	13 mo	2 mo	High-dose steroid, high-dose IVIG, rituximab, plasmapheresis	18 mo	11 mo	IFN-α, IFN-ω	E1
4	CID-G/AI	RAG 2 a. A444V b. L992G	None	VZV—24 mo	ITP—26 mo	2 mo	30 mo	4 mo	High-dose steroid, high-dose IVIG	30 mo	28 mo	IFN-α, IFN-ω, IL-12	Current case

GCSF, Granulocyte colony stimulating factor; *IFN-α*, interferon-alfa; *IFN-ω*, interferon omega; *ITP*, immune thrombocytopenia; *IVIG*, intravenous immunoglobulin; *NA*, not available; *VZV*, varicella zoster virus.

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