Original Article

Outcomes and Treatment Strategies for Autoimmunity and Hyperinflammation in Patients with RAG Deficiency

Jocelyn R. Farmer, MD, PhD[a,*], Zsofia Foldvari, MD[b,*], Boglarka Ujhazi, MS[c], Suk See De Ravin, MD, PhD[d], Karin Chen, MD[e], Jack J.H. Bleesing, MD, PhD[f], Catharina Schuetz, MD[g], Waleed Al-Herz, MD[h,i], Roshini S. Abraham, PhD[j,k], Avni Y. Joshi, MD[l], Beatriz T. Costa-Carvalho, MD[m], David Buchbinder, MD[n], Claire Booth, MD, PhD[o], Andreas Reiff, MD[p], Polly J. Ferguson, MD[q], Asghar Aghamohammadi, MD, PhD[r], Hassan Abolhassani, MD, PhD[r], Jennifer M. Puck, MD[s], Mehdi Adeli, MD[t], Caterina Cancrini, MD, PhD[u,v], Paolo Palma, MD, PhD[w], Alice Bertaina, MD, PhD[x], Franco Locatelli, MD, PhD[x,y], Gigliola Di Matteo, BS, PhD[u,v], Raif S. Geha, MD[z], Maria G. Kanariou, MD, PhD[aa], Lilia Lycopoulou, MD[bb], Marianna Tzanoudaki, MD, PhD[aa], John W. Sleasman, MD[cc], Suhag Parikh, MD[dd], Gloria Pinero, CCRC[cc], Bernard M. Fischer, DVM, PhD[cc], Ghassan Dbaibo, MD, FAAP[ee], Ekrem Unal, MD[ff], Turkan Patiroglu, MD[ff,gg], Musa Karakukcu, MD[ff], Khulood Khalifa Al-Saad, MD[hh], Meredith A. Dilley, MD, MPH[ii], Sung-Yun Pai, MD[jj,kk,ll], Cullen M. Dutmer, MD[mm], Erwin W. Gelfand, MD[nn], Christoph B. Geier, BSC[oo], Martha M. Eibl, MD[oo,pp], Hermann M. Wolf, MD[oo,qq], Lauren A. Henderson, MD, MMSc[rr], Melissa M. Hazen, MD[rr], Carmem Bonfim, MD, PhD[ss], Beata Wolska-Kuśnierz, MD, PhD[tt], Manish J. Butte, MD, PhD[uu], Joseph D. Hernandez, MD, PhD[vv], Sarah K. Nicholas, MD[ww], Polina Stepensky, MD[xx], Shanmuganathan Chandrakasan, MD[yy], Maurizio Miano, MD[zz], Emma Westermann-Clark, MD[aaa], Vera Goda, MD[bbb], Gergely Kriván, MD, PhD[bbb], Steven M. Holland, MD[ccc], Olajumoke Fadugba, MD[ddd], Sarah E. Henrickson, MD, PhD[eee,fff], Ahmet Ozen, MD[ggg], Elif Karakoc-Aydiner, MD[ggg], Safa Baris, MD[ggg], Ayca Kiykim, MD[hhh], Robbert Bredius, MD, PhD[iii], Birgit Hoeger, PhD[jjj,kkk], Kaan Boztug, MD[jjj,kkk,lll,mmm], Olga Pashchenko, MD, PhD[nnn], Benedicte Neven, MD, PhD[ooo,ppp,qqq], Despina Moshous, MD, PhD[ooo,ppp,rrr], Jean-Pierre de Villartay, PhD[rrr], Ahmed Aziz Bousfiha, MD[sss,ttt], Harry R. Hill, MD[uuu], Luigi D. Notarangelo, MD[zz,‡], and Jolan E. Walter, MD, PhD[c,vvv,‡]

Boston, Mass; Radiumhospitalet, Norway; Saint Petersburg and Tampa, Fla; Bethesda, Md; Salt Lake City, Utah; Cincinnati and Columbus, Ohio; Ulm, Germany; Kuwait City, Kuwait; Rochester, Minn; São Paulo, Brazil; Irvine, Los Angeles, San Francisco, and Stanford, Calif; London, United Kingdom; Iowa City, Iowa; Tehran, Iran; Doha, Qatar; Rome, Italy; Athens, Greece; Durham, NC; Beirut, Lebanon; Kayseri, Turkey; Kingdom of Bahrain; Aurora and Denver, Colo; Vienna, Austria; Curitiba, Brazil; Warsaw, Poland; Houston, Texas; Jerusalem, Israel; Atlanta, Ga; Genova, Italy; Budapest, Hungary; Philadelphia, Pa; Istanbul, Turkey; Leiden, the Netherlands; Moscow, Russia; Paris, France; and Casablanca, Morocco

What is already known about this topic? Knowledge of autoimmunity in recombination activating gene (RAG) deficiency has been limited to small case series; herein, we introduce the largest international database of RAG-deficient cases with prominent autoimmune and hyperinflammatory disease, facilitating detailed outcomes and treatment analysis.

What does this article add to our knowledge? RAG diagnosis is delayed in the setting of autoimmunity or hyperinflammation (median, 5 years); autoimmune cytopenias are prevalent (84.1%), have early onset (median, 1.9-2.6 years), and lack of first-line treatment response correlates strongly with multilineage disease.

How does this study impact current management guidelines? RAG deficiency can present with autoimmunity/hyperinflammation; low naive ($CD45RA^+$) T-cell counts are a useful diagnostic tool; and multilineage cytopenias are refractory to immunosuppressive treatment in most cases and should prompt expedited hematopoietic cell transplantation evaluation.

[a]Massachusetts General Hospital, Harvard Medical School, Boston, Mass
[b]Department of Cancer Immunology, Institute for Cancer Research, Oslo University Hospital, Radiumhospitalet, Oslo, Norway
[c]University of South Florida and Johns Hopkins All Children's Hospital, Saint Petersburg, Fla
[d]Laboratory of Host Defenses, National Institutes of Allergy and Infectious Diseases, NIH, Bethesda, Md

[e]Division of Allergy and Immunology, Department of Pediatrics, University of Utah School of Medicine, Salt Lake City, Utah
[f]Division of Bone Marrow Transplantation and Immune Deficiency, Cancer and Blood Diseases Institute, Cincinnati Children's Hospital Medical Center, Cincinnati, Ohio
[g]Department of Pediatrics, Medizinische Fakultät Carl Gustav Carus, Technische Universität Dresden, Dresden, Germany
[h]Faculty of Medicine, Pediatrics Department, Kuwait University, Kuwait City, Kuwait
[i]Allergy and Clinical Immunology Unit, Pediatrics Department, Alsabah Hospital, Kuwait City, Kuwait
[j]Department of Pathology and Laboratory Medicine, Nationwide Children's Hospital, Columbus, Ohio
[k]Department of Laboratory Medicine and Pathology, Mayo Clinic, Rochester, Minn
[l]Division of Pediatric Allergy/Immunology, Mayo Clinic Children's Center Rochester, Rochester, Minn
[m]Department of Pediatrics, UNIFESP-EPM, São Paulo, Brazil
[n]Pediatrics/Hematology, CHOC Children's Hospital - UC Irvine, Irvine, Calif
[o]Department of Paediatric Immunology, Great Ormond Street Hospital, London, United Kingdom
[p]Division of Rheumatology, Children's Hospital Los Angeles, Keck School of Medicine, USC, Los Angeles, Calif
[q]Department of Pediatrics, University of Iowa Carver College of Medicine, Iowa City, Iowa
[r]Research Center for Immunodeficiencies, Pediatrics Center of Excellence, Children's Medical Center, Tehran University of Medical Sciences, Tehran, Iran
[s]Department of Pediatrics, University of California San Francisco and UCSF Benioff Children's Hospital, San Francisco, Calif
[t]Sidra Medicine, Weill Cornell Medicine, and Hamad Medical Corporation, Doha, Qatar
[u]Academic Department of Pediatrics (DPUO), Unit of Immune and Infectious Diseases, Childrens' Hospital Bambino Gesù, Rome, Italy
[v]Department of Systems Medicine, University of Rome Tor Vergata, Rome, Italy
[w]Academic Department of Pediatrics (DPUO), Research Unit in Congenital and Perinatal Infection, Children's Hospital Bambino Gesù, Rome, Italy
[x]Department of Pediatric Hematology/Oncology and Cell and Gene Therapy, IRCCS, Ospedale Bambino Gesù, Rome, Italy
[y]Department of Pediatrics, Sapienza, University of Rome, Rome, Italy
[z]Immunology Division, Department of Pediatrics, Boston Children's Hospital, Harvard Medical School, Boston, Mass
[aa]Department of Immunology - Histocompatibility, Specialized Center & Referral Center for Primary Immunodeficiencies - Paediatric Immunology, "Aghia Sophia" Children's Hospital, Athens, Greece
[bb]1st Department of Pediatrics, University of Athens, Aghia Sofia Children's Hospital, Athens, Greece
[cc]Division of Allergy, Immunology and Pulmonary Medicine, Duke University School of Medicine, Durham, NC
[dd]Division of Pediatric Blood and Marrow Transplantation, Duke University School of Medicine, Durham, NC
[ee]Department of Pediatrics and Adolescent Medicine, Center for Infectious Diseases Research, American University of Beirut, Beirut, Lebanon
[ff]Division of Pediatric Hematology and Oncology & HCST Unit, Department of Pediatrics, Faculty of Medicine, Erciyes University, Kayseri, Turkey
[gg]Division of Pediatric Immunology, Department of Pediatrics, Faculty of Medicine, Erciyes University, Kayseri, Turkey
[hh]Salmanyia Medical Complex, Department of Pediatrics, Division of Pediatric Hematology and Oncology, Manama, Bahrain
[ii]Department of Immunology, Boston Children's Hospital, Harvard Medical School, Boston, Mass
[jj]Division of Hematology-Oncology, Boston Children's Hospital, Boston, Mass
[kk]Department of Pediatric Oncology, Dana-Farber Cancer Institute, Boston, Mass
[ll]Harvard Medical School, Boston, Mass
[mm]Division of Allergy & Immunology, Children's Hospital Colorado, University of Colorado School of Medicine, Aurora, Colo
[nn]Department of Pediatrics, National Jewish Health, Denver, Colo
[oo]Immunology Outpatient Clinic, Vienna, Austria
[pp]Biomedizinische Forschungs GmbH, Vienna, Austria
[qq]Sigmund Freud Private University-Medical School, Vienna, Austria
[rr]Division of Immunology, Boston Children's Hospital, Harvard Medical School, Boston, Mass
[ss]Hospital Infantil Pequeno Principe, Curitiba, Brazil
[tt]Immunology Department, Children's Memorial Health Institute, Warsaw, Poland
[uu]Division of Immunology, Allergy, and Rheumatology, Department of Pediatrics and Jeffrey Modell Diagnostic and Research Center, University of California, Los Angeles, Los Angeles, Calif
[vv]Department of Pediatrics, Division of Allergy, Immunology and Rheumatology, Stanford University, Stanford, Calif
[ww]Section of Immunology, Allergy, and Rheumatology, Baylor College of Medicine, Texas Children's Hospital, Houston, Texas
[xx]Department of Bone Marrow Transplantation, Hadassah Hebrew University Hospital, Jerusalem, Israel
[yy]Division of Bone Marrow Transplant, Aflac Cancer and Blood Disorders Center, Children's Healthcare of Atlanta, Emory University School of Medicine, Atlanta, Ga
[zz]Haematology Unit, Department of Pediatric Haematology-Oncology, IRCCS Istituto Giannina Gaslini, Genova, Italy
[aaa]Department of Internal Medicine, Division of Allergy/Immunology, University of South Florida Morsani College of Medicine, Tampa, Fla
[bbb]Department for Pediatric Hematology and Hemopoietic Stem Cell Transplantation, Central Hospital of Southern Pest- National Institute of Hematology and Infectious Diseases, Budapest, Hungary
[ccc]Laboratory of Clinical Immunology and Microbiology, National Institute of Allergy and Infectious Diseases, NIH, Bethesda, Md
[ddd]Division of Pulmonary, Allergy and Critical Care, Perelman School of Medicine, University of Pennsylvania, Philadelphia, Pa
[eee]Allergy Immunology Division, Department of Pediatrics, The Children's Hospital of Philadelphia, Philadelphia, Pa
[fff]Institute for Immunology, the University of Pennsylvania, Philadelphia, Pa
[ggg]Marmara University School of Medicine, Division of Pediatric Allergy and Immunology, Istanbul, Turkey
[hhh]Ministry of Health, Marmara University Pendik Training and Research Hospital, Istanbul, Turkey
[iii]Department of Pediatrics, Section Pediatric Immunology, Infections and Stem Cell Transplantation, Leiden University Medical Center, Leiden, the Netherlands
[jjj]Ludwig Boltzmann Institute for Rare and Undiagnosed Diseases, Vienna, Austria
[kkk]CeMM Research Center for Molecular Medicine of the Austrian Academy of Sciences, Vienna, Austria
[lll]St Anna Kinderspital and Children's Cancer Research Institute, Department of Pediatrics, Medical University of Vienna, Vienna, Austria
[mmm]Department of Pediatrics and Adolescent Medicine, Medical University of Vienna, Vienna, Austria
[nnn]Department of Immunology, Pirogov Russian National Research Medical University, Russian Clinical Children's Hospital, Moscow, Russia
[ooo]Paris Descartes Sorbonne Paris Cité University, Imagine Institute, Paris, France
[ppp]Pediatric Hematology-Immunology and Rheumatology Unit, Necker-Enfants Malades University Hospital, Assistance Publique-Hôpitaux de Paris, Paris, France
[qqq]Laboratory "Immunogenetics of Pediatric Autoimmune Diseases", INSERM UMR1163, Université Paris Descartes Sorbonne Paris Cité, Institut Imagine, Paris, France
[rrr]Laboratory "Genome Dynamics in The Immune System", INSERM UMR1163, Université Paris Descartes Sorbonne Paris Cité, Institut Imagine, Paris, France
[sss]Laboratoire d'Immunologie Clinique, d'Inflammation et d'Allergie LICIA, Faculty of Medicine and Pharmacy, Hassan II University, Casablanca, Morocco
[ttt]Clinical Immunology Unit, Casablanca Children's Hospital, Ibn Rochd Medical School, Hassan II University, Casablanca, Morocco
[uuu]Division of Clinical Immunology, Departments of Pathology, Pediatrics and Medicine, University of Utah, Salt Lake City, Utah
[vvv]Division of Allergy and Immunology, Massachusetts General Hospital for Children, Boston, Mass

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Corresponding author: Jolan E. Walter, MD, PhD, 601 4th St CRI 4008, St Petersburg, FL 33701. E-mail: jolanwalter@health.usf.edu.
* Co-first authors.
‡ Co-senior authors.
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Abbreviations used
AIHA- Autoimmune hemolytic anemia
AN- Autoimmune neutropenia
AS- Atypical SCID
CID-G/AI- Combined immunodeficiency with granulomatous disease and/or autoimmunity
CVID- Common variable immunodeficiency
HIM- Hyper-IgM syndrome
ICL- Idiopathic CD4+ T-cell lymphopenia
ITP- Immune thrombocytopenia
IQR- Interquartile range
IVIG- *Intravenous immunoglobulin*
HSCT- Hematopoietic stem cell transplantation
NR- *Nonresponder*
RAG- Recombination activating gene
SCID- Severe combined immunodeficiency

BACKGROUND: Although autoimmunity and hyperinflammation secondary to recombination activating gene (RAG) deficiency have been associated with delayed diagnosis and even death, our current understanding is limited primarily to small case series.

OBJECTIVE: Understand the frequency, severity, and treatment responsiveness of autoimmunity and hyperinflammation in RAG deficiency.

METHODS: In reviewing the literature and our own database, we identified 85 patients with RAG deficiency, reported between 2001 and 2016, and compiled the largest case series to date of 63 patients with prominent autoimmune and/or hyperinflammatory pathology.

RESULTS: Diagnosis of RAG deficiency was delayed a median of 5 years from the first clinical signs of immune dysregulation. Most patients (55.6%) presented with more than 1 autoimmune or hyperinflammatory complication, with the most common etiologies being cytopenias (84.1%), granulomas (23.8%), and inflammatory skin disorders (19.0%). Infections, including live viral vaccinations, closely preceded the onset of autoimmunity in 28.6% of cases. Autoimmune cytopenias had early onset (median, 1.9, 2.1, and 2.6 years for autoimmune hemolytic anemia, immune thrombocytopenia, and autoimmune neutropenia, respectively) and were refractory to intravenous immunoglobulin, steroids, and rituximab in most cases (64.7%, 73.7%, and 71.4% for autoimmune hemolytic anemia, immune thrombocytopenia, and autoimmune neutropenia, respectively). Evans syndrome specifically was associated with lack of response to first-line therapy. Treatment-refractory autoimmunity/hyperinflammation prompted hematopoietic stem cell transplantation in 20 patients.

CONCLUSIONS: Autoimmunity/hyperinflammation can be a presenting sign of RAG deficiency and should prompt further evaluation. Multilineage cytopenias are often refractory to immunosuppressive treatment and may require hematopoietic cell transplantation for definitive management. **(J Allergy Clin Immunol Pract 2019;7:1970-85)**

Key words: *Recombination activating gene* (RAG)*; Severe combined immunodeficiency (SCID); Immune dysregulation; Autoimmune cytopenias; Hematopoietic stem cell transplantation (HSCT)*

INTRODUCTION

Recombination activating genes (*RAG1* and *RAG2*) initiate the V(D)J recombination process, supporting the development of a diverse repertoire of T and B lymphocytes.[1] Mutations in *RAG* were first described in patients with severe combined immunodeficiency (SCID) with lack of T and B cells (T^- B^- SCID).[2] Subsequently, the clinical presentation of RAG deficiency was expanded to include Omenn syndrome, in which partial V(D)J recombination activity accounts for the generation of autologous oligoclonal T lymphocytes that infiltrate and damage end-organs.[3,4] More recently, hypomorphic *RAG* mutations have been associated with a broad clinical spectrum of atypical SCID (AS), including a phenotype with predominance of T-cell receptor γδ+ T cells (γδ AS),[5,6] and a phenotype of combined immunodeficiency with granulomatous disease and/or autoimmunity (CID-G/AI), where frequent autoimmunity, granulomatous lesions, and occasionally EBV-driven lymphoproliferation are the predominant clinical features.[6-10] Finally, RAG deficiency has been case reported to clinically mimic "idiopathic" $CD4^+$ T-cell lymphopenia (ICL),[11] hyper-IgM syndrome (HIM),[12] common variable immunodeficiency (CVID),[13] and even refractory autoimmune entities such as chronic multifocal osteomyelitis and demyelinating neuropathy.[9]

The development of autoimmunity in RAG deficiency has been linked to checkpoint breaks in both T- and B-cell tolerance, including abnormal thymic negative selection of autoreactive T cells (central T-cell tolerance), impaired development and dysfunction of regulatory T cells (peripheral T-cell tolerance), impaired B-cell receptor editing in the bone marrow (central B-cell tolerance), and elevated levels of B-cell activating factor allowing survival of immature B cells with self-reactive specificity (peripheral B-cell tolerance).[4,14,15] Environmental factors such as composition of the host intestinal microbiota may play an additional role in sustaining intestinal T-cell infiltration and autoimmune/hyperinflammatory pathology.[16]

To date, however, our understanding of the clinical spectrum of autoimmunity and hyperinflammatory pathology that can occur in RAG deficiency has been limited to small case series and a single review of the literature.[17,18] Moreover, there have been no larger studies addressing treatment outcomes for autoimmune and hyperinflammatory disease in the background of RAG deficiency. To address this deficit, we herein present the results of a literature search and review of our internal database and report on the largest case series of annotated and curated cases of RAG deficiency with prominent autoimmune and hyperinflammatory disease.

METHODS

Literature search

We reviewed all RAG-deficient cases in PubMed published between September 2001 and 2016. We excluded reports that did not detail the presence or absence of autoimmune/hyperinflammatory complications. Data were extracted regarding *RAG* mutation, sex, clinical phenotype including autoimmune/hyperinflammatory complications, and age of hematopoietic stem cell transplantation (HSCT), if used. We assigned clinical phenotypes according to

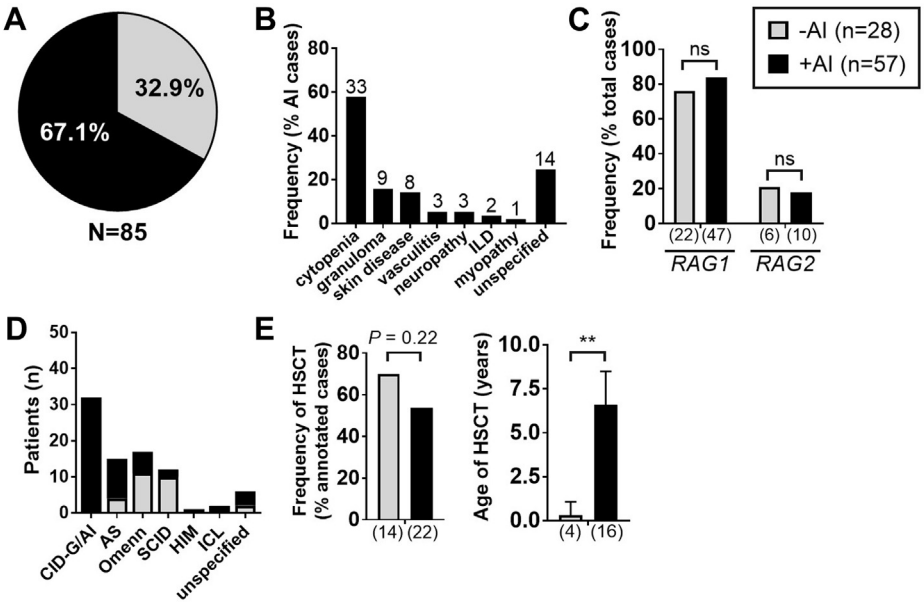

FIGURE 1. Autoimmunity and hyperinflammation are frequent complications in published cases of RAG deficiency. A total of 85 published cases of RAG deficiency were reviewed for the presence (+AI: n = 57, shown in black) or absence (−AI: n = 28, shown in gray) of autoimmune and/or hyperinflammatory complications, with results shown as prevalence of (**A**) +AI vs −AI (frequency as % total cases, n = 85), (**B**) individual autoimmune and hyperinflammatory complications (frequency as % AI subtype, n = 57), (**C**) genotype (frequency as % total cases, n = 85), (**D**) clinical phenotype (as absolute patient count), (**D**) occurrence of HSCT (frequency as % annotated total cases, n = 36), and (**E**) age of HSCT (median ± 95% CI). Exact patient counts as shown, with statistical difference indicated. $^{**}P < .005$. *ILD*, Interstitial lung disease; *ns*, not significant.

criteria from the Primary Immune Deficiency Treatment Consortium.[19] The CID-G/AI phenotype was defined by a clinical history of recurrent infections and immune dysregulation (autoimmunity and/or granulomas).[6-10]

Patient database

On the basis of literature search above and our data repository of unpublished cases, we generated a highly annotated and curated patient database that included 63 cases. Information was collected as follows: sex, age (current as of November 2017, at clinical diagnosis of immunodeficiency and/or autoimmunity, at molecular diagnosis of RAG deficiency, and at death or HSCT where applicable), genotype (specific *RAG1* or *RAG2* mutations), immune phenotype (lymphocyte counts and function, immunoglobulin levels, and autoantibodies), autoimmune/hyperinflammatory complications (type, age at onset, preceding infections if available, length, and severity), and therapies trialed (including response and complications). Predicted V(D)J recombination activity was recorded as previously described.[20,21] The study was approved by the Institutional Review Board of the University of South Florida (protocol no. Pro00025693).

Therapeutic response score

Therapeutic response was scored for all annotated cases of autoimmune cytopenias and granulomas using the following criteria: "no" = no clinical response to the intervention was seen or side effects were limiting; "partial" = some clinical improvement to the intervention was seen but therapeutic escalation was ultimately required for stabilization; or "full" = clinical improvement to the intervention was seen and no subsequent escalation has been required for stabilization to date. Across all centers, the term "treatment-refractory" was applied only in those cases in which "no clinical response to intervention" was specifically documented by the managing clinical care team.

Statistical analysis

All data were assembled and analyzed using GraphPad Prism software version 7.01 (GraphPad Software Inc., San Diego, Calif). Groups were compared using a 2-tailed Student's *t* test. Kaplan-Meier curves were compared using a log-rank (Mantel-Cox) test. Significance was defined as a *P* value of less than .05.

RESULTS

RAG-deficient cases based on literature search (n = 85)

We performed a literature search of published cases of RAG deficiency between 2001 and 2016 and identified 134 cases, of which 85 met criteria for further analysis. In review of these 85 published cases, autoimmune and/or hyperinflammatory complications were identified in 57 patients (67.1% of total cases) (Figure 1, *A*), and included autoimmune cytopenias (n = 33 [57.9%]), granulomas (n = 9 [15.8%]), skin disease (n = 8 [14.0%]), vasculitis (n = 3 [5.3%]), neuropathy (n = 3 [5.3%]), interstitial lung disease (n = 2 [3.5%]), and myopathy (n = 1 [1.8%]) (Figure 1, *B*).

We next compared the RAG-deficient patients without versus with autoimmune and hyperinflammatory clinical manifestations. Sex and genotype were evenly distributed, and *RAG1* mutations accounted for most patients in both groups (Figure 1, *C*). In review of the clinical phenotype, 32 patients with CID-G/AI accounted for most of the autoimmune/hyperinflammatory subset (32 of 57 patients [56.1%]). In addition, autoimmune

TABLE I. Patient characteristics of curated RAG deficiency database (n = 63)

Case	Sex	Clinical phenotype	Gene	Mutation	Recombination activity (% wild-type protein)	Age, current (y)	Age, clinical Dx (y)	Age, molecular Dx (y)	HSCT	Age, HSCT (y)	AI indication for HSCT?	Cytopenia	Granuloma	AI other	AI preceded by infection? (etiology; timing)	Autoantibody
1[17,22]	M	CID-G/AI	*RAG1*	a. W522C; b. L541Cfs*30	a. 41.6; b. 1.0	20*	10	17	+	19	+	—	Midline (oropharynx, skin)	Myasthenia gravis	–	AChR, IFN-α/β/ω, IL-12p70, IL-22
2[17,23]	F	AS	*RAG1*	a. R474C; b. K983Nfs*9	a. 125.4; b. 0.1	8	NA	2	+	2 & 4	–	AN, ITP, AIHA	—	Eczematous rash, nodular splenomegaly	+ AN (vaccine-strain VZV; 1 mo)	Coombs, neutrophil
3[23]	F	AS	*RAG1*	a. R474C; b. K983Nfs*9	a. 125.4; b. 0.1	14	NA	7.9	+	8	–	AIHA	—	Nephrotic syndrome, splenomegaly	+ AIHA (MMR/DTaP/HBV/PC vaccines; 2 weeks)	Neutrophil, ANA, APLA, thyroid (TG/TPO)
4[17]	F	CID-G/AI	*RAG2*	a. G451A; b. M459L	a. 60; b. 30.8	7	NA	NA	+	1.5	–	AN	—	—	–	Coombs, neutrophil, ANA, IFN-α/ω
5[7,17]	F	CID-G/AI	*RAG1*	a. R778Q; b. R975W	a. 8.58; b. 0.1	9.5*	7.8	7.8	+	8.5	+	—	Skin, oropharynx, lung	—	NA	—
6[7,17]	F	CID-G/AI	*RAG1*	a. R314W; b. R507W/R737H	a. 24.3; b. 0.09	18	3	3	+	6	NA	—	Skin	EBV-driven B-cell lymphoma	NA	—
7[7,17]	F	CID-G/AI	*RAG2*	a. T77N; b. G451A	a. 0.73; b. 0.75	22	9.9	10.7	+	14	NA	ITP, AN	Spleen, lung, bone	—	NA	Coombs
8[17]	F	AS	*RAG2*	a./b. G35A	a./b. 22.1	10.83*	1.33	9	– (eval)			AIHA, AN	—	Psoriasis, splenomegaly	–	Coombs
9[17]	F	CID-G/AI	*RAG1*	a./b. C176F	NA	16	3.5	11	+	12.5	NA	—	Skin	—	–	—
10[17]	M	CID-G/AI	*RAG1*	a. I102Sfe*15; b. P118Lfs*21	NA	11	7	7.5	+	8	–	AIHA	Skin	—	–	Coombs
11[17,24]	F	CID-G/AI	*RAG1*	a. K86fs*33; b. H612R	a. 2.7; b. 121.6	20	3	15	+	18	+	ITP, AIHA	Lung	GLILD, duodenitis, vitiligo	–	Coombs, thyroid (TPO)
12[17]	F	CID-G/AI	*RAG2*	a./b. T215I	a./b. 48.4	7.33*	6.5	6.5	+	7.25	NA	AN	—	—	NA	Neutrophil
13[25]	M	CID-G/AI	*RAG1*	a./b. S480G	NA	10.25*	6	8	+	9.5	NA	AIHA, AN	—	—	–	Coombs, neutrophil
14[25]	M	ICL	*RAG1*	a./b. S480G	NA	19†	15	15	–			—	—	Vitiligo	NA	—
15[25]	M	CID-G/AI	*RAG1*	a./b. H612R	a./b. 121.6	18†	7	9	+	7.5	NA	AN, AIHA, ITP	Skin	—	–	Coombs
16[9]	M	CID-G/AI	*RAG1*	a./b. R699W	a./b. 19.3	11*	9	11‡	–			AIHA	Skin, lung, liver, bone, pancreas, testes	Vitiligo	–	Coombs, ANA, dsDNA
17[13]	M	CID-G/AI	*RAG1*	a./b. C358Y	a./b. 48.8	14*	10	10	– (died)			AN	Liver	MALT lymphoma, splenomegaly	+ AN (Leishmania; 3 mo)	Neutrophil (ANCA)
18[17]	M	AS	*RAG2*	a./b. E407*	a./b. 2.9	25	0.1	0.1	+	19	+	AIHA	—	Partial alopecia, IBD	–	—
19[17,26]	M	AS	*RAG1*	a. R699W; b. M435V	a. 19.3; b. 23.6	17	NA	NA	+	6.5	NA	AIHA, ITP, AN	—	Vitiligo, psoriasis, Guillain-Barré syndrome	–	Coombs, neutrophil, platelet
20[17]	F	AS	*RAG1*	a./b. R108*	a./b. 1.8	5.5	0.25	0.33	+	0.5	–	AN	—	—	–	Coombs

21[17]	M	AS	*RAG1*	a./b. K86Vfs*33	a./b. 2.7	6	0.91	1.08	+	1.5	−	AIHA	—	Miller Fisher syndrome	+ MFS (CMV reactivation; 96 h)	Coombs
22[17]	F	AS	*RAG1*	a. H612R; b. A857V	a. 121.6; b. 121.6	6	1.91	2.5	+	5	NA	AIHA	—	Thyroiditis	−	Neutrophil (ANCA), B2GPI, microsomal
23 —	M	AS	*RAG1*	a. W522C; b. M435V/ M1006V	a. 41.6; b. 23.6/105.6	4.8*	2	NA	+	3	+	AIHA, ITP	—	—	+ AIHA (VZV vaccine; 10 mo)	Coombs, platelet
24 —	F	CID-G/AI	*RAG1*	a. R474C; b. R975W	a. 125.4; b. 0.1	21*	9	NA	+	20	+	AIHA, ITP	Skin	—	−	Coombs, platelet
25 —	M	CID-G/AI	*RAG1*	a. W522C; b. H994R	a. 41.6; b. NA	6*	2.5	3	+	5	+	AIHA	—	Vasculitis	+ vasculitis (vaccine-strain VZV; coincident)	—
26[12,17]	F	Omenn	*RAG2*	a./b.M459L	a./b. 30.8	NA*	1.33	2.17	+	1.58	NA	AIHA	—	—	NA	Coombs, C3
27[12]	M	HIM	*RAG2*	a./b. M459L	a./b. 30.8	NA*	2	5.17	−			AIHA	—	—	NA	APLA
28[27]	F	CID-G/AI	*RAG1*	a./b. R764C	NA	20.5	8	11	− (eval)			AIHA, ITP	Skin, bone	—	−	—
29 —	M	AS	*RAG1*	a. R396C; b. M435V	a. 0.6; b. 23.6	2.67*	1.42	1.5	+	1.75 & 2.5	+	AIHA	—	Vasculitis	−	Coombs, IFN-α
30[28]	F	AS	*RAG2*	a. P180H; b. R73H	a. 31.3; b.11.0	1.25*	1.08	1.25‡	− (died)			AIHA, ITP	—	—	+ AIHA (VZV/ MMR vaccines; 3 wk)	Coombs
31[29]	F	CID-G/AI	*RAG1*	a. M1V; b. R737H	a. NA; b. 0.2	48*	20	46	−			-	—	Vasculitis	NA	ANA, dsDNA, APLA, RF, thyroid (TG/TPO/ TSHR)
32[8]	F	CID-G/AI	*RAG1*	a. R841Q; b. F974L	a. 0; b. 56.5	2*	1	2‡	− (died)			AIHA, ITP, AN	—	Vasculitis, myopathy, central demyelinating neuropathy	−	Coombs, platelet
33 —	M	AS	*RAG1*	a./b. R841W	a./b. 10	1.75*	0.5	0.75	+	0.83	+	AIHA	—	—	+ AIHA (acute viral URI; coincident)	Coombs
34 —	M	SCID	*RAG1*	a. N766I; b. K86VfsX33	a. NA; b. 2.7	19.17	NA	13	+	0.42 & 0.67	NA	AIHA, AN	—	Thyroiditis, hepatitis, urticaria	−	Coombs
35[17]	F	CID-G/AI	*RAG2*	a./b. F62L	a./b. 19.6	31	5	27	−			ITP	Lung	—	−	—
36 —	M	AS	*RAG2*	a. G35A; b. E437K	a. 22.1; b. 0.9	7*	0.37	0.46	− (died)			AIHA	—	—	+ AIHA (CMV; coincident)	Coombs
37 —	F	AS	*RAG1*	a./b. C335R	NA	16*	5	15	+	16	−	ITP	—	T-cell cutaneous lymphoma, uveitis	+ ITP (VZV; coincident)	—
38 —	F	AS	*RAG1*	a. K86VfsX33; b. R108X	a. 2.7; b. 1.8	16	14	14	+	15	+	AN	—	—	−	—
39 —	F	CID-G/AI	*RAG1*	a./b. H612R	a./b. 121.6	18	13.6	15.6	+	17	NA	AIHA, AN	—	Alopecia areata, thyroiditis	−	Coombs, IFN-α, thyroid (TPO/TG)
40[30]	M	CID-G/AI	*RAG1*	a./b. R507G	a./b. 19.2	8	2.5	5	+	5.25	+	AIHA, AN	—	Hepatosplenomegaly	+ AIHA (CMV; coincident)	Coombs, neutrophil

(continued)

TABLE I. *(Continued)*

Case	Sex	Clinical phenotype	Gene	Mutation	Recombination activity (% wild-type protein)	Age, current (y)	Age, clinical Dx (y)	Age, molecular Dx (y)	HSCT	Age, HSCT (y)	AI indication for HSCT?	Cytopenia	Granuloma	AI other	AI preceded by infection? (etiology; timing)	Autoantibody
41 —	F	CID-G/AI	*RAG1*	a. A472V; b. H612R	a. NA; b. 121.6	8	2	2	+	4.33	NA	—	—	Aseptic encephalitis	–	AChR, GAD, CV2/CRMP5
42 —	F	SCID	*RAG1*	a./b. K86VfsX33	a./b. 2.7	10.42	0.08	1.33	+	1.25	–	AIHA	—	—	–	—
43 —	M	CID-G/AI	*RAG1*	a./b. K86VfsX33	a./b. 2.7	7.67	3	4	+	4	+	AIHA, AN, ITP	—	—	–	Coombs
44 —	M	SCID	*RAG1*	a./b. K86VfsX33	a./b. 2.7	8.67	0.5	0.58	+	0.75	+	ITP	—	—	–	—
45 —	M	Omenn	*RAG2*	NA	NA	16.58	0.08	0.17	+	0.75	–	—	—	Dermatitis, hepatitis, & severe diarrhea	–	—
46[31]	F	SCID	*RAG1*	a. K992E; b. A444V	a. 9.1; b.1.4	2.5	2.17	NA	+	2.5	+	ITP	—	Polyclonal gammopathy, isolated ALP elevation	+ ITP (VZV; 2 mo)	ANA, IFN-α/ω, IL-12
47[26]	NA	CID-G/AI	*RAG1*	a. R396C; b. R975Q	a. 0.6; b. 57.9	5*	NA	NA	–			ITP	Skin	—	–	—
48 —	M	CID-G/AI	*RAG2*	a. N173S; b. E437K	a. NA; b. 0.9	36	31	36	+	NA	NA	—	—	Myopathy	–	—
49 —	F	AS	*RAG2*	a./b. G35A	a./b. 22.1	2.67*	1.33	2.67‡	+	2.67	+	ITP, AN, AIHA	—	—	+ AIC relapses (viral infections; ~1 wk)	Coombs
50 —	M	CID-G/AI	*RAG1*	a./b. N855S	NA	11*	5	11‡	– (died)			AIHA	—	Enteropathy	+ enteropathy & AIHA (Salmonella; 2 & 2.5 mo)	Coombs, enterocyte/goblet cell
51[29]	F	CID-G/AI	*RAG2*	a. S381*; b. G95R	a. NA; b. 0	48	35	46	–			AIHA	—	—	–	Coombs
52 —	F	CID-G/AI	*RAG1*	a. R314W; b. R396C	a. 24.3; b. 0.6	NA	5	9	+	9	–	—	—	Thyroiditis, vitiligo, diabetes, nail dystrophy	–	GAD, ICA, thyroid (TG)
53 —	F	AS	*RAG1*	a./b. R474H	NA	12	10	11	NA			AIHA, ITP	—	Amyloidosis	+ AIHA relapses (severe URIs; ~1 wk)	Coombs
54 —	M	AS	*RAG1*	a. R561H; b. R778Q	a. 2.0; b. 8.6	17	11	17	– (eval)			AIHA	—	—	+ AIHA (VZV; coincident)	—
55 —	M	AS	*RAG1*	a. N855S; b. K992E	a. NA; b. 9.1	8.58	2.5	2.75	+	3	–	AIHA	—	Hepatitis	–	—
56 —	F	AS	*RAG1*	a. R112H; b. K86Vfs33*	a. NA; b. 2.7	5	NA	NA	NA			AN	—	—	–	—
57 —	F	AS	*RAG1*	a. R142*; b. T477S	a. 9.0; b. NA	3.33*	2.5	2.83	+	3.25	+	AIHA, ITP, AN	—	—	–	Coombs, platelet
58 —	M	CID-G/AI	*RAG1*	a./b. G816R	NA	9.5	1.5	7.5	–			AIHA	—	Sclerosing cholangitis	+ AIHA (CMV; 2 wk)	—
59 —	F	AS	*RAG1*	a. R112L; b. H735Q	NA	3.74	0.92	NA	+	1.33	+	ITP, AIHA	—	—	+ ITP (VZV; several weeks)	Coombs, thyroid (TPO)

60	—	F	AS	*RAG2*	a. G35A; b. A456D	a. 22.1; b. NA	3.44	0.25	1.67	+	0.5	+	ITP	—	—	—	—
61	—	F	AS	*RAG1*	a. T708A; b. E669K	NA	7.25	2.25	2.5	+	2.67	–	AN	—	—	–	Neutrophil
62[32]		M	CID-G/AI	*RAG1*	a. H375D; b. Y562C	NA	18.67	9	15	+	15.83	+	ITP	Skin, liver, spleen	—	–	—
63	—	M	AS	*RAG1*	NA	NA	14.5*	NA	14.5‡	+	14	+	AN, ITP, AIHA	—	Vitiligo, IBD	–	—

+, Yes; –, no; *AChR*, acetylcholine receptor; *AI*, autoimmune; *AIC*, autoimmune cytopenia; *AIHA*, autoimmune hemolytic anemia; *ALP*, alkaline phosphatase; *AMA*, antimitochondrial antibody; *AN*, autoimmune neutropenia; *ANA*, antinuclear antibody; *APLA*, antiphospholipid antibody; *ANCA*, antineutrophil cytoplasmic antibody; *AS*, atypical SCID; *B2GP1*, beta-2-glycoprotein I; *CID-G/AI*, combined immunodeficiency with granulomatous disease and/or autoimmunity; *CMV*, cytomegalovirus; *dsDNA*, double-stranded DNA; *DTap*, diphtheria, tetanus, pertussis; *dx*, diagnosis; *EBV*, Epstein-Barr virus; *eval*, evaluated; *F*, female; *GAD*, glutamic acid decarboxylase; *GLILD*, granulomatous and lymphointerstitial lung disease; *HBV*, hepatitis B virus; *HIM*, hyper-IgM syndrome; *IBD*, inflammatory bowel disease; *ICA*, islet cell antibody; *ICL*, idiopathic $CD4^+$ T-cell lymphopenia; *IFN*, interferon; *IL*, interleukin; *ITP*, immune thrombocytopenia; *M*, male; *MALT*, mucosa-associated lymphoid tissue; *MFS*, Miller Fisher syndrome; *MMR*, measles-mumps-rubella; *NA*, not available; *Omenn*, Omenn syndrome; *PC*, pneumococcal; *SCID*, severe combined immunodeficiency; *RF*, rheumatoid factor; *TG*, thyroglobulin; *TPO*, thyroid peroxidase; *TSHR*, thyroid stimulating hormone receptor; *URI*, upper respiratory tract infection; *VZV*, varicella zoster virus.
*Deceased.
†Lost to follow-up.
‡Postmortem.

and/or hyperinflammatory complications were prominent among patients with AS (11 of 15 patients [73.3%]), but rare among patients with Omenn syndrome (6 of 17 [35.3%]) and SCID (2 of 12 [16.7%]) (Figure 1, *D*). Finally, no significant difference was observed in the proportion of patients who received HSCT among patients without versus with immune dysregulation, but the latter group received HSCT at a significantly older age (median, 0.3 vs 6.6 years in patients without vs with immune dysregulation; $P = .0003$) (Figure 1, *E*). To gain more insights into the natural history of patients with RAG deficiency complicated by immune dysregulation, we created a curated longitudinal database and analyzed the data.

Annotated and curated patient database (n = 63)

On the basis of literature search and our own database of unpublished cases, we identified 63 total cases of RAG deficiency with prominent autoimmune and/or hyperinflammatory manifestations. The characteristics of this patient cohort are described in Table I. There was a slight predominance of female patients (54.8% females; 45.2% males). The clinical phenotype was predominantly CID-G/AI (30 cases, 47.6%), followed by AS (25 cases, 39.7%), SCID (4 cases, 6.3%), Omenn syndrome (2 cases, 3.2%), and single cases of HIM and ICL (1.6% each) (Figure 2, *A*). *RAG1* and *RAG2* mutations were present in 48 (76.2%) and 15 (23.8%) patients, respectively. Functional data of *in vitro* recombination activity were available for 63 of 96 *RAG1* and 23 of 30 *RAG2* alleles. On dividing patients into 3 groups (CID-G/AI, AS/Other, and SCID), the average recombination activity, expressed as percentage of wild-type protein, was 38.1, 25.5, and 3.4, respectively (Figure 2, *B*). Thirty-nine patients (61.9%) were alive at the time of review at a median age of 10.6 years (Figure 2, *C*). The median age at clinical diagnosis (immunodeficiency and/or autoimmunity) was 2.5 years. In comparison, the median age of genetic diagnosis of RAG deficiency was 7.5 years, with 6 cases identified postmortem. In total, 45 patients (71.4%) had received HSCT at the time of review at a median age of 5.0 years. In addition, 8 patients (12.7%) were either being evaluated for HSCT or had passed away before anticipated HSCT at the time of review. There were no occurrences of solid organ transplantation. Twenty-four patients (38.1%) were deceased at the time of review at a median age of 8.4 years, which was statistically coincident with the age of genetic diagnosis ($P = .70$) (Figure 2, *D*). Multiorgan failure and/or sepsis were the leading cause of death in 10 cases (41.7%) (see Figure E1, *A*, in this article's Online Repository at www.jaci-inpractice.org). Median patient survival was 14 versus 21 years in untransplanted compared with hematopoietic cell transplanted patients; however, these Kaplan-Meier curves failed to reach statistical difference (Figure E1, *B*; $P = .42$). Information on patient condition at the time of HSCT was unavailable in most cases.

Next, we reviewed the immunologic phenotype. Immunoglobulin serum levels were highly variable, with a median native IgG level of 890 mg/dL (25%-75% interquartile range [IQR], 296-1770 mg/dL), IgA level of 25.5 mg/dL (25%-75% IQR, 6-73.3 mg/dL), IgM level of 87.3 mg/dL (25%-75% IQR, 28.8-162.8 mg/dL), and IgE level of of 5 IU/mL (25%-75% IQR, 3.3-51.3 IU/mL) (Figure 3, *A*). Interestingly, 26.3% of patients with CID-G/AI and AS manifested hypergammaglobulinemia. Increased serum IgE levels were present in the 2 patients with Omenn syndrome (IgE, 427 and 2448 IU/mL, respectively). T- and B-lymphocyte counts were decreased overall in the

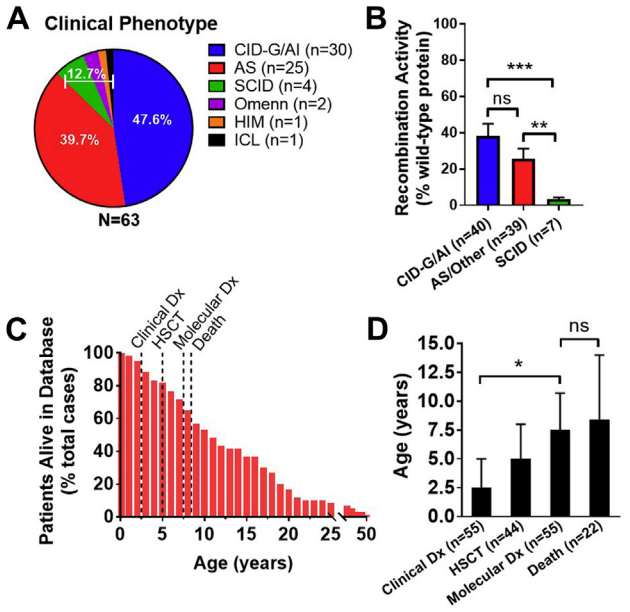

FIGURE 2. Demographic characteristics of curated RAG deficiency database (n = 63). **A,** Clinical diagnosis (frequency as % total cases). **B,** Recombination activity from all available *RAG1* (n = 63) and *RAG2* (n = 23) alleles (average ± SEM as % wild-type protein and in color by clinical phenotype). **C,** Patients alive in database (% by age with clinical milestones annotated). **D,** Age of clinical milestones (median ± 95% CI). Exact patient counts (Figure 2, *A*, *C*, and *D*) and allele counts (Figure 2, *B*) as shown, with statistical difference indicated. **P* < .05, ***P* < .005, ****P* < .0001. *dx*, Diagnosis; *ns*, not significant.

curated patient database (median $CD3^+$, 599 cells/μL; median $CD19^+$, 102.5 cells/μL), whereas natural killer cells were in the normal range (median, 279.5 cells/μL) (see Table E1 in this article's Online Repository at www.jaci-inpractice.org). As expected by clinical phenotype, loss of T and B lymphocytes was most pronounced for patients with SCID versus CID-G/AI and AS (Figure 3, *B*). Within the $CD4^+$ T-cell compartment, $CD45RA^+/RO^+$ subtyping was available for 26 and 31 patients with CID-G/AI and AS, respectively, and demonstrated a predominance of memory ($CD45RO^+$) $CD4^+$ T cells in circulation for both groups (Figure 3, *C*). Expansion of T-cell receptor $\gamma\delta^+$ T cells was documented in 3 patients with AS and in the single patient with HIM (data not shown). Data on T-cell proliferation to PHA were available in 33 cases and were low to severely low in most (26 patients) (Table E1).

The most frequent autoimmune and/or hyperinflammatory complications were autoimmune cytopenias (n = 53 [84.1%]), granulomas (n = 15 [23.8%]), and skin manifestations including vitiligo, psoriasis, and alopecia (n = 12 [19.0%]) (Figure 4, *A*). A total of 55.6% of patients had more than 1 autoimmune or hyperinflammatory complication; specifically, 60.4% of cytopenia cases presented with an additional autoimmune/hyperinflammatory manifestation (Figure 4, *B*). Infections closely preceded the onset of autoimmunity in 28.6% of cases (Figure 4, *C*). Viruses were the most frequent etiology in 16 cases and included both live vaccinations and natural infections (Table I). Infections due to *Leishmania* and *Salmonella* preceded the development of autoimmunity in a single patient each. The burden of treating autoimmune and hyperinflammatory complications was substantial as measured by the use of steroids, biological agents, and HSCT (Figure 4, *D*). Treatment-refractory autoimmunity and/or hyperinflammation were an indication to HSCT in 20 cases (44.4% of total HSCT) and included autoimmune cytopenias (n = 12), inflammatory bowel disease/enteropathy (n = 4), granulomas (n = 3), vasculitis (n = 3), and progressive pulmonary disease (n = 1). Finally, type of immune dysregulation (cytopenia, granuloma, or "other") did not correlate with the average predicted level of patient recombination activity (29.9%, 36.5%, and 34.8% of wild-type protein, respectively) (see Figure E2 in this article's Online Repository at www.jaci-inpractice.org), perhaps due to the high co-occurrence of these conditions (Figure 4, *B*). However, the cumulative number of autoimmune/hyperinflammatory complications per patient did correlate both positively and linearly with the average predicted level of patient recombination activity (17.3%, 36.0%, and 49.6% of wild-type protein for patients with 1, 2, or 3 autoimmune/hyperinflammatory complications, respectively) (Figure 4, *E*).

Autoimmune cytopenias: Occurrence, outcomes, and treatment

Autoimmune hemolytic anemia (AIHA) was the most frequent autoimmune complication identified in the curated patient database (n = 38 [60.3%]), followed by immune thrombocytopenia (ITP) (n = 23 [36.5%]) and autoimmune neutropenia (AN) (n = 21 [33.3%]). Evans syndrome was observed in 13 cases (20.6%), and pancytopenia was observed in 8 cases (12.7%) (Figure 5, *A*). The median age at onset was 1.9 years for AIHA, 2.1 years for ITP, and 2.6 years for AN, which coincided with the clinical diagnosis of immunodeficiency/autoimmunity, but statistically preceded the molecular diagnosis of RAG deficiency by a median of 5.5 years (Figure 5, *B*). Moreover, the cytopenias were often severe. The median cell nadir during disease flare was hemoglobin level of 5.5 g/dL for AIHA, platelet count of 20,000 cells/μL for ITP, and absolute neutrophil count of 200 cells/μL for AN (Figure 5, *C*). In addition, median duration of relapsing/remitting cytopenia disease course in total was 1.5 years for AIHA and 1 year for ITP and AN (Figure 5, *D*). Finally, most patients with cytopenias had positive autoantibodies to at least 1 cell lineage, including Coombs (n = 30 [55.6%]), antigranulocyte (n = 10 [18.5%]), and antiplatelet (n = 5 [9.3%]) antibodies (Figure 5, *E*). All cytopenias occurred in the pretransplant period apart from one patient who developed AIHA at age 23 months (5 months post-HSCT) and another patient who underwent 2 consecutive HSCTs and developed AIHA at age 26 months (18 months after the final HSCT) and AN at age 13 years.

Treatment outcomes as available were reviewed in detail for cases of AIHA (n = 34), ITP (n = 19), and AN (n = 14) (Figure 6, *A-C*). Intravenous immunoglobulin (IVIG), steroids, and granulocyte-colony stimulating factor (G-CSF) in the context of AN specifically were frequently used as first-line agents. However, definitive control to first-line therapy was achieved in only a subset of patients (23.5% in AIHA, 21.1% in ITP, and 21.4% in AN). Most patients progressed to second-line therapy, which most frequently included B-cell depletion using rituximab (AIHA: n = 14 [41%]; ITP: n = 4 [21%]; AN: n = 5 [35%]). Even this approach often failed to control the disease.

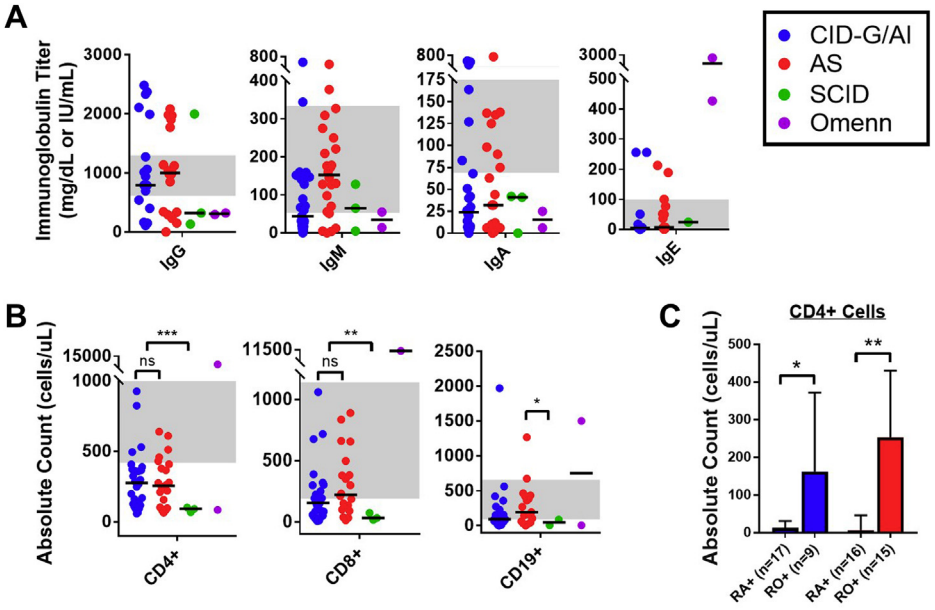

FIGURE 3. Immunophenotype of curated RAG deficiency database (n = 63). **A,** Immunoglobulin titers (shown in color by clinical phenotype, with symbols representing individual patients and bars representing clinical subset medians). **B,** Lymphocyte counts (shown in color by clinical phenotype, with symbols representing individual patients and bars representing clinical subset medians). **C,** $CD4^+$ T-cell subsets, $CD45RA^+$ "RA^+" and $CD45RO^+$ "RO^+" (median ± 95% CI, shown in color by clinical phenotype). Gray background indicates normal adult reference ranges from the Massachusetts General Hospital. Exact patient counts as shown, with statistical difference indicated. *$P < .05$, **$P < .005$, ***$P < .0001$. *ns*, Not significant.

Specifically, complete remission after use of rituximab was observed in only 28.9%, 16.7%, and 20.0% of patients with AIHA, ITP, and AN, respectively. Sirolimus was used only in 2 patients, leading to full remission of AIHA and AN in 1 of them. At the time of review, 64.7% of AIHA cases, 73.7% of ITP cases, and 71.4% of AN cases had no or only partial disease control to all first- and second-line therapeutics trialed. Among patients who received HSCT because of treatment-refractory autoimmune cytopenias, complete remission was observed in 76.9% of AIHA, 71.4% of ITP, and 77.8% of AN cases, respectively.

To further investigate clinical features that correlate with response to treatment for cytopenias, we analyzed patients who had definitive control at first-line therapy (R-first-line) versus patients who had definitive control following rituximab (R-rituximab) versus patients with incomplete response ("no" or "partial") to all first- and/or second-line therapies trialed to date (NR). For AIHA, in comparison to R-first-line, we observed lower hemoglobin nadirs in the NR (median, 4.3 vs 7.0 g/dL; $P = .035$) and the R-rituximab (median, 5.0 g/dL vs 7.0 g/dL; $P = .0047$) groups. In addition, we observed more frequent occurrence of multilineage cytopenias in the NR group (median 2 vs 1 cell lineage affected; $P = .015$). There was also a trend toward earlier age at onset of cytopenias in the NR and R-rituximab groups that did not meet statistical significance (Figure 6, *D*). For ITP and AN, we had only a single patient who met criteria for R-rituximab, precluding further subset analysis. However, a similar observation of multilineage cytopenias in the NR group versus the R-first-line group was seen for ITP (median 2 vs 1 cell lineage affected; $P = .018$), with a trend toward significance for AN (median 2 vs 1 cell lineage affected; $P = .097$) (Figure 6, *E* and *F*). Finally, for AN we observed a later age at onset in the NR group versus the R-first-line group (0.75 vs 4 years; $P = .0099$) (Figure 6, *F*). Together, these data suggest that several factors correlate with lack of response to first-line therapy in autoimmune cytopenias, in particular: (1) Evans syndrome (≥2 affected lineages); (2) low hemoglobin nadir (≤5.0 g/dL) in patients with AIHA; and (3) delayed age at onset (≥4 years) in patients developing AN.

Other autoimmune and hyperinflammatory complications: Occurrence, outcomes, and treatment

In total, 42 patients (66.7%) presented with other autoimmune or hyperinflammatory complications either alternatively (15.9%) or additionally (50.7%) to cytopenias. Granulomas were the most common, occurring in 15 patients (23.8%). Most granulomas were confined to a single organ (60.0%), with a subset of patients who developed multiorgan disease (40.0%). Single-organ granulomas were predominantly limited to the skin (n = 6), with the exception of 2 patients with lung granulomas and 1 patient with liver granulomas. However, various organs may be affected by granulomas, including skin (n = 11), lungs (n = 5), liver (n = 3), bone (n = 3), oropharynx (n = 2), spleen (n = 2), pancreas (n = 1), and testes (n = 1) (Figure 7, *A*). Inflammatory skin disorders were also prominent in the curated patient database, occurring in 12 patients (19.0%), and included combinations of vitiligo (n = 6), psoriasis (n = 2), alopecia (n = 2), eczema/dermatitis (n = 2), urticaria (n = 1), and noninfectious nail dystrophy (n = 1). Vasculitis occurred in 5 patients (7.9%), and when further annotated, was complicated by digital necrosis (n = 2), stroke and Henoch-Schönlein purpura (n = 1), and skin manifestations only (n = 1). Enteropathy occurred in 5

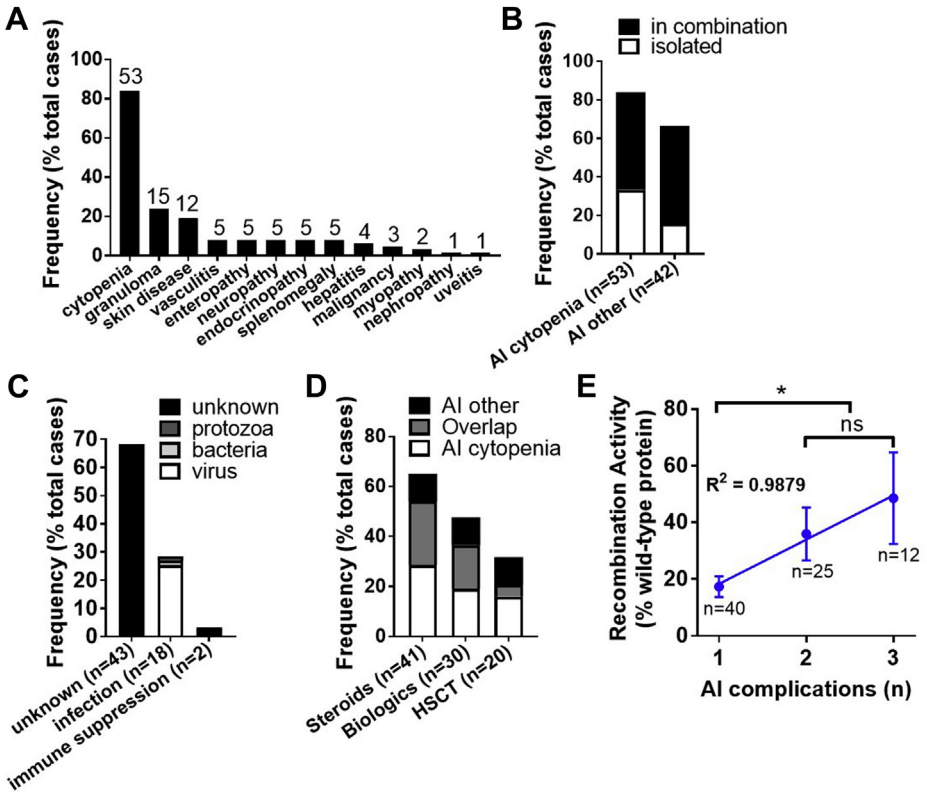

FIGURE 4. Autoimmune and hyperinflammatory outcomes of curated RAG deficiency database (n = 63). **A,** Prevalence of individual autoimmune and hyperinflammatory complications (frequency as % total cases). **B,** Occurrence of autoimmune and hyperinflammatory complications in isolation or combination (frequency as % total cases). **C,** Clinician-annotated triggers for autoimmune and hyperinflammatory disease development (frequency as % total cases). **D,** Burden of treatment for autoimmune and hyperinflammatory complications (frequency as % total cases). **E,** Correlation between number of autoimmune complications (cumulative per patient) and recombination activity (average ± SEM as % wild-type protein); linear regression of mean *Y* values with R^2 shown. Exact patient counts (Figure 4, *A-D*) and allele counts (Figure 4, *E*) as shown, with statistical difference indicated. *$P < .05$. *AI*, Autoimmune; *ns*, not significant.

patients (7.9%) and was annotated as inflammatory bowel disease (n = 2), autoimmune enteropathy (n = 1), duodenitis (n = 1), and severe noninfectious diarrhea (n = 1). Autoimmune neuropathy occurred in 5 patients (7.9%) and was recorded as Guillain-Barré syndrome, Miller Fisher syndrome, myasthenia gravis, central demyelinating neuropathy, and aseptic encephalitis in 1 patient each. Endocrinopathies occurred in 5 patients (7.9%) and included autoimmune thyroiditis (n = 4) and type I diabetes mellitus (n = 1). Hepatitis occurred in 4 patients (6.3%) and included autoimmune hepatitis (n = 3) and sclerosing cholangitis (n = 1). Malignancy occurred in 3 patients (4.8%) and was exclusively lymphoma (1 cutaneous T-cell lymphoma, 1 mucosa-associated lymphoid tissue lymphoma, and 1 EBV-driven B-cell lymphoma of the tonsil). Finally, there were rare cases of inflammatory myopathy (n = 2), minimal change nephropathy (n = 1), and uveitis (n = 1).

Despite wide patient-to-patient variability, the median age of onset of vasculitis (1.6 years), nephropathy (1.6 years), thyroiditis (1.75 years), hepatitis (2.0 years), and neuropathy (2.0 years) indicated that these were among the earliest immune dysregulatory complications (Figure 7, *B*). In contrast to the autoimmune cytopenias, however, none of these complications statistically preceded the timing of genetic diagnosis, suggesting lower yield benefit in terms of facilitating the diagnosis of RAG deficiency. Autoantibody production was prominent, with antinuclear, anticytokine, and antithyroid antibodies being most common (Figure 7, *C*).

Treatment outcomes were well annotated in 10 of the 15 patients who developed granulomas (Figure 7, *D*). Spontaneous granuloma resolution was seen in 2 patients with skin manifestations only, while the remainder of patients (80.0%) did not respond to first-line IVIG and/or steroids. Of the second-line agent trialed, only infliximab resulted in full response in 1 patient with multiorgan disease as well as partial response (temporizing for years) in 1 patient with isolated lung granulomas. Ultimately, HSCT was required for definitive management in 5 cases (50.0%) without granuloma recurrence to date.

Among the 5 patients with vasculitis, topical and systemic steroids were sufficient to induce remission in 1 case of late-onset (8 years) disease limited to skin manifestations. In contrast, first- and second-line treatment with steroids, IVIG, cyclophosphamide, alemtuzumab, and/or rituximab failed to achieve a sustained response in the remaining 4 cases of early-onset (median, 1.0 years) and severe disease (complicated by digital necrosis,

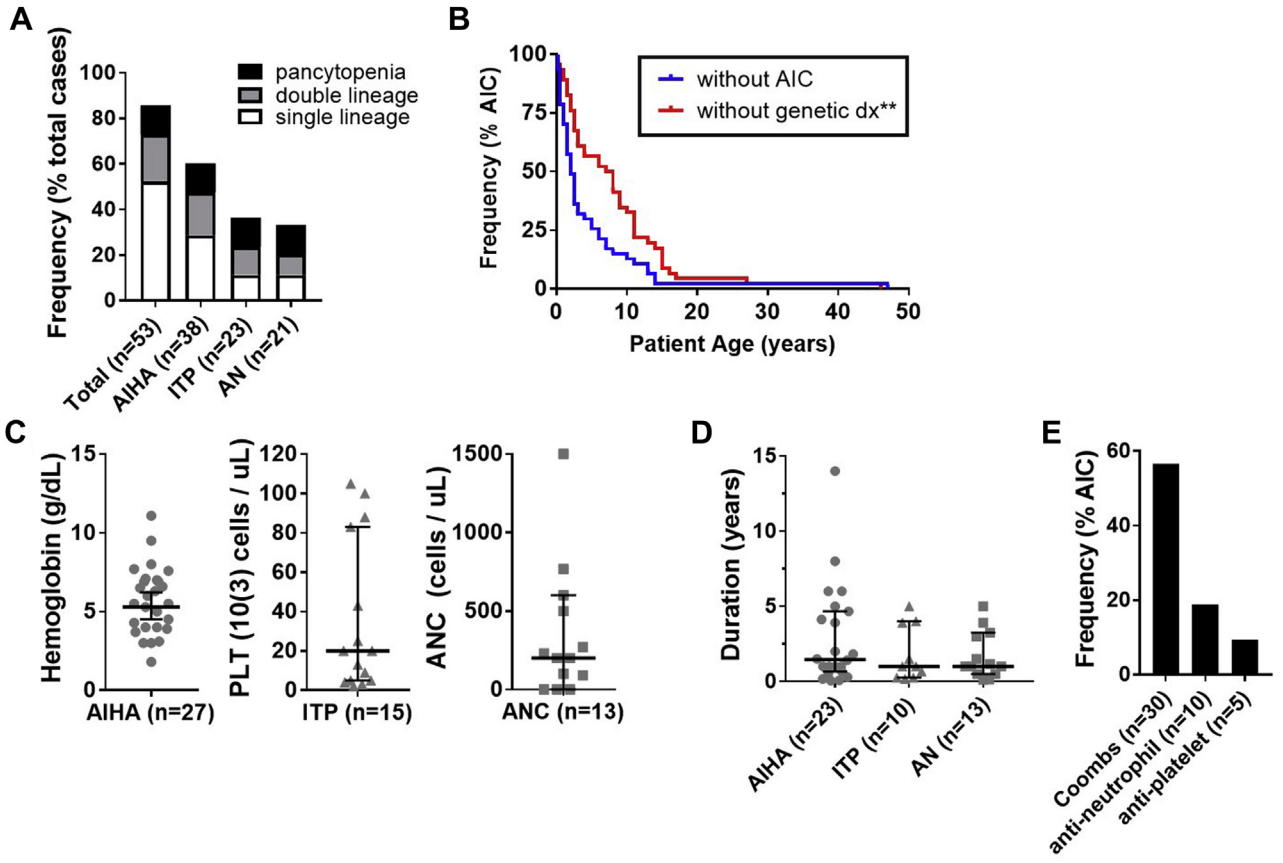

FIGURE 5. Autoimmune cytopenias are a frequent and early-onset complication in patients with RAG deficiency. **A,** Prevalence of single- and multilineage cytopenias (frequency as % total cases). **B,** Kaplan-Meier curves of RAG-deficient patients with autoimmune cytopenias (n = 53), showing difference in timing of cytopenia onset (blue line) and genetic diagnosis of RAG deficiency (red line). Severity of autoimmune cytopenias by (**C**) cell nadir and (**D**) duration (symbols representing individual patients; median ± 95% CI shown). **E,** Prevalence of positive autoimmune cytopenia autoantibodies (frequency as % AIC cases). Exact patient counts as shown, with statistical difference indicated. $^{**}P < .005$. *AIC*, Autoimmune cytopenia; *ANC*, absolute neutrophil count; *dx*, diagnosis; *PLT*, platelet.

stroke, and Henoch-Schönlein purpura). Ultimately, 3 of these patients were stabilized with HSCT while the final patient passed away before anticipated HSCT.

Three of the 5 cases of enteropathy had well-annotated treatment outcomes. There was limited response to first- and/or second-line therapy with steroids, nonsteroidal anti-inflammatories, cyclosporine, and sirolimus in all 3 cases. Adalimumab (Humira) was temporizing for a year in 1 case of duodenitis; however, all 3 cases ultimately required progression to transplant for definitive management.

DISCUSSION

Herein, we present the largest assembled case series of RAG deficiency with prominent autoimmune and/or hyperinflammatory complications. The compilation of this patient database allowed for the first systematic analysis of autoimmune and hyperinflammatory complications secondary to RAG deficiency in terms of frequency, outcome, and response to therapeutic intervention. We observed a high prevalence of autoimmune and hyperinflammatory complications in published cases of RAG deficiency (67.1%). However, we do acknowledge a potential publication bias toward unusual clinical presentations of RAG deficiency that may skew toward an overrepresentation of autoimmune and/or hyperinflammatory comorbidities in the literature.[33]

In our curated patient database, we observed a median 5-year delay between the clinical recognition of immune dysregulation (immunodeficiency and/or autoimmunity) and the final diagnosis of RAG deficiency. This diagnostic delay likely reflects lack of recognition that hypomorphic *RAG* mutations are often associated with severe manifestations of immune dysregulation and with normal to elevated IgG serum levels, in contrast to what has been observed in patients with T^- B^- SCID due to null *RAG* mutations.[2] However, because of the retrospective nature of this study, it included many patients whose clinical manifestations of immune dysregulation occurred before the clinical phenotype of CID-G/AI was reported in 2008.[7] A prospective collection of clinical, immunologic, and molecular data will help to assess whether improved awareness of the phenotypic spectrum of the disease may lead to more prompt recognition of cases with hypomorphic mutations and more prevalent autoimmune and hyperinflammatory manifestations. Experience with newborn screening for SCID and related disorders has highlighted that *RAG* mutations are more often associated with AS

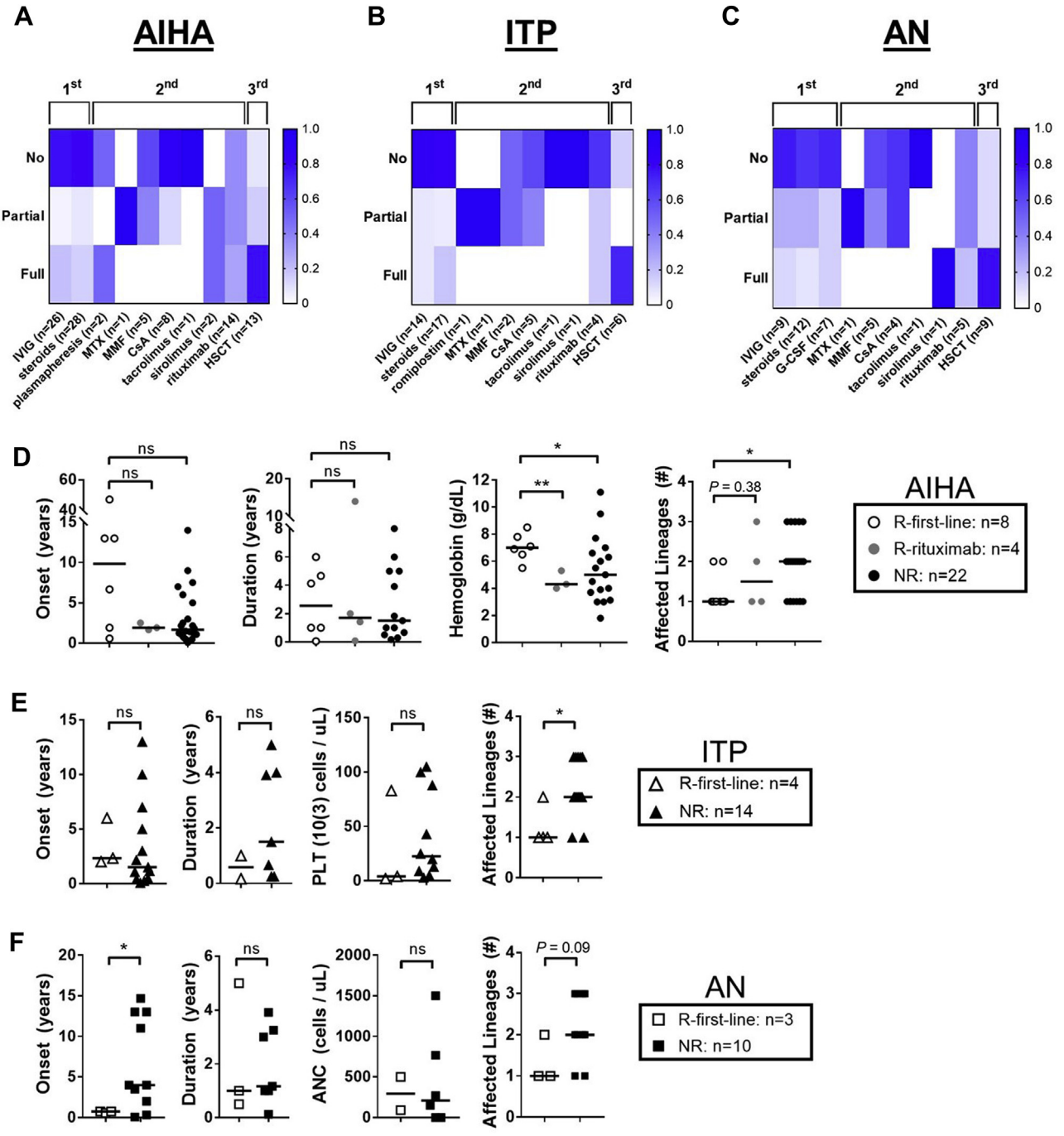

FIGURE 6. Autoimmune cytopenias in RAG deficiency are refractory to first- and second-line therapy. Autoimmune cytopenia treatment response, scored by individual treatment modality for each incidence of (**A**) AIHA, (**B**) ITP, (**C**) and AN (% response per trialed therapeutic shown by color gradation as indicated; therapeutic grouping by first-line [IVIG, steroids, and/or G-CSF], second-line [all biologics], and third-line [HSCT] agents as shown; number of annotated therapeutic trials shown). Clinical response at first-line therapy (R-first-line) vs at rituximab therapy (R-rituximab) vs nonresponders to all first- and second-line therapies trialed to date (NR) is compared for (**D**) AIHA, (**E**) ITP, and (**F**) AN according to cytopenia onset, cytopenia duration, cell line nadir, and number of cell lineages involved (symbols representing individual patients, median shown, exact patient counts shown, and statistical difference indicated. $*P < .05$, $**P < .005$. *ANC*, Absolute neutrophil count; *CsA*, cyclosporine A; *MMF*, mycophenolate mofetil; *MTX*, methotrexate; *ns*, not significant; *PLT*, platelet.

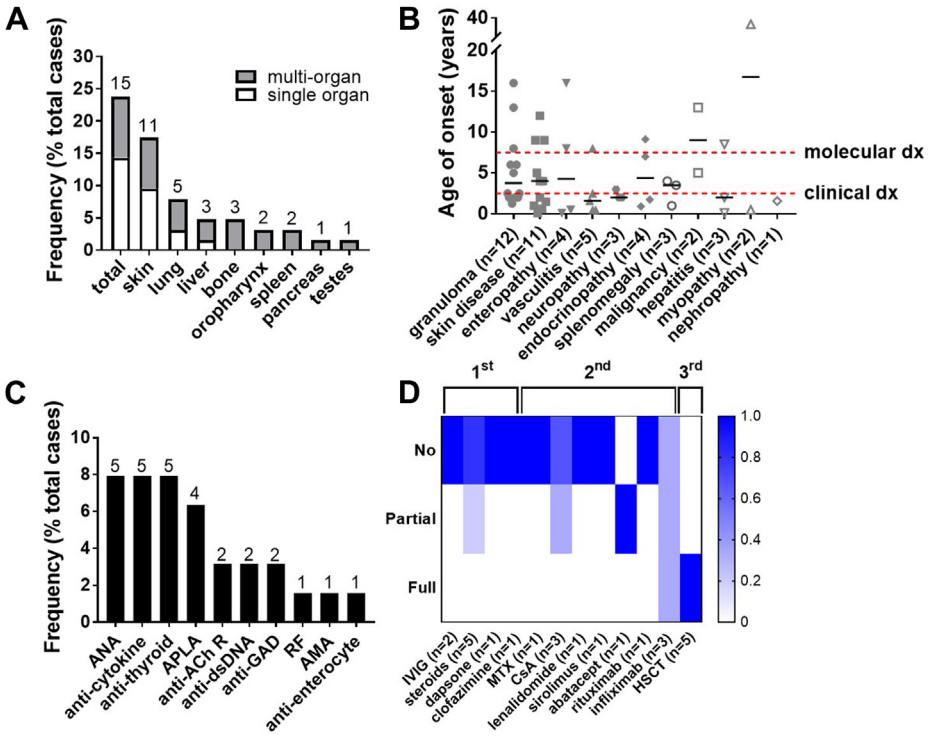

FIGURE 7. A spectrum of other autoimmune and hyperinflammatory diseases occurs in RAG deficiency. **A,** Prevalence of single- and multiorgan granulomas listed by anatomic location (frequency as % total cases). **B,** Age of onset for the other autoimmune and hyperinflammatory complications (symbols representing individual patients, median for each complication shown, clinical milestones annotated). **C,** Prevalence of positive autoantibodies (frequency as % total cases). **D,** Granuloma treatment response, scored by individual treatment modality for each incidence of granulomatous disease (% response per trialed therapeutic shown by color gradation as indicated; therapeutic grouping by first-line [IVIG, steroids, and/or antiinfectives], second-line [all biologics], and third-line [HSCT] agents as shown; number of annotated therapeutic trials shown). *AChR*, Acetylcholine receptor; *AMA*, antimitochondrial antibody; *ANA*, antinuclear antibody; *APLA*, antiphospholipid antibody; *CsA*, cyclosporin A; *dsDNA*, double-stranded DNA; *GAD*, glutamic acid decarboxylase; *MTX*, methotrexate; *RF*, rheumatoid factor.

and Omenn syndrome than with T^- B^- SCID.[34] Whether newborn screening is also capable of identifying patients who will manifest a CID-G/AI phenotype remains to be studied. Alternative screening approaches such as analysis of T-cell receptor α bias using the PROMIDISα biomarker may additionally prove clinically beneficial.[35] Finally, as we demonstrated reduced T-cell counts and diminished proportion of peripheral naive $CD4^+$ cells across multiple RAG-deficient clinical phenotypes, including CID-G/AI specifically, detailed $CD4^+$ immunophenotyping may be of particular utility in suspecting RAG deficiency in those patients manifesting primarily with features of immune dysregulation.

Infections frequently preceded the onset of autoimmunity/hyperinflammation in the patient database by a temporal association of days to months, with most naturally acquired viral infections and live viral vaccinations. These data highlight the clinical importance of diagnosing RAG deficiency before administering live viral vaccines. However, how viral infections may precipitate immune dysregulation in patients with RAG deficiency remains unclear.

Cytopenias were the most frequent autoimmune/hyperinflammatory manifestation in our series and presented early in life (median onset, 1.9 years for AIHA, 2.1 years for ITP, and 2.6 years for AN). A lack of response to first-line therapy (predominantly IVIG and steroids) and second-line therapy (predominantly rituximab) was observed in most cases. In particular, complete remission after use of rituximab was achieved in only 28.9% of AIHA cases, 16.7% of ITP cases, and 20.0% of AN cases. These data are in contrast to the benefit of rituximab that has been reported in the literature previously in patients with CVID who develop autoimmune cytopenias (85% initial complete patient response rate for AIHA and/or ITP),[36] and more closely resemble the intermittent rituximab responsiveness for autoimmune cytopenias reported in patients with combined T-cell dysfunction syndromes, including autoimmune lymphoproliferative syndrome (see Table E2 in this article's Online Repository at www.jaci-inpractice.org). However, we acknowledge the limitation of our retrospective, international, multicenter study, which relied on physician annotation to score therapeutic response as compared with the more objective measure of cell counts used in CVID previously.[36] In our case series, multilineage cytopenias, a low nadir of hemoglobin (≤5.0 g/dL) during AIHA episodes, and later age of onset (≥4 years) for AN were associated with lack of response to first-line treatment of autoimmune cytopenias. Sirolimus has been shown to be beneficial in the management of refractory cytopenias in patients

with autoimmune lymphoproliferative syndrome and CVID[37]; however, it was used in only 2 patients in the present case series, and additional experience must be collected to document its efficacy in RAG deficiency. Definitive therapy with HSCT was successful in most RAG-deficient patients with severe autoimmune cytopenias in this series. Thus, although RAG deficiency is a small contributor to the overall incidence of autoimmune cytopenias in the general population, these data suggest that consideration of RAG deficiency in the differential diagnosis of treatment-refractory multilineage disease may have potential therapeutic benefit, specifically early consideration of HSCT for definitive management.

Granulomas were the second most prevalent autoimmune/hyperinflammatory complication identified (23.8%) in this series. Single-organ disease was more frequent and often limited to the skin. TNF inhibitors were used in 3 patients in this series and led to full remission in one patient with multiorgan disease and partial and transient response in another patient with lung granulomas. Additional clinical experience must be collected to evaluate the efficacy of this treatment. However, 50% of the patients with treatment-refractory granulomas ultimately required HSCT for definitive management in this series.

Finally, vasculitis occurred early in the course of RAG deficiency (median, 1.6 years), was often complicated by significant end-organ involvement, and in most cases was not responsive to first- or second-line therapy but required HSCT for definitive management in this series. Similarly, most patients with severe gastrointestinal manifestations required HSCT for definitive management in this series. One patient experienced initial benefit from adalimumab.

Overall, our data demonstrate that immune dysregulation is a common feature of RAG deficiency and is often refractory to conventional medical management. Characterization of factors associated with lack of response to first- and second-line treatment may help to identify patients in which HSCT should be considered early in the course of the disease, before development of severe organ damage.

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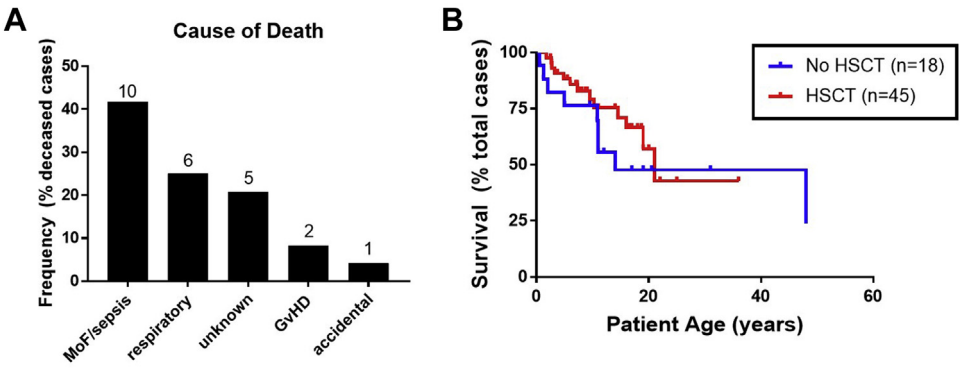

FIGURE E1. Mortality in the curated RAG deficiency database (n = 63). **A,** Cause of death (frequency as % deceased cohort, n = 24). **B,** Kaplan-Meier curves showing percent survival by patient age in RAG-deficient patients who received HSCT (n = 45, red line) or not (n = 18, blue line). *GvHD*, Graft vs host disease; *MoF*, multiorgan failure.

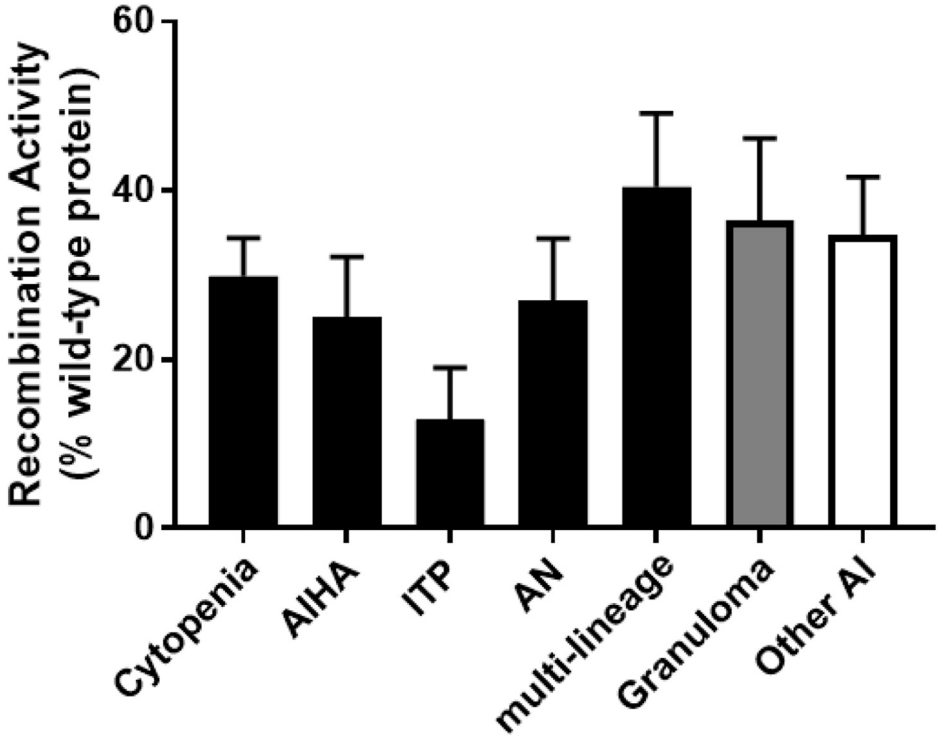

FIGURE E2. Recombination activity by immune dysregulatory complication in the curated RAG deficiency database (n = 63). Recombination activity from all available *RAG1* (n = 63) and *RAG2* (n = 23) alleles (average ± SEM as % wild-type protein by clinical immune dysregulatory complication as shown). *AI*, Autoimmune.

TABLE E1. Immune phenotype of curated RAG deficiency database (n = 63)

Case	Time of sample (y)	Lymphocytes (cells/μL)	Eosinophils (cells/μL)	CD3+ (cells/μL)	$CD3^+$ (% lymphocytes)	$CD4^+$ (cells/μL)	$CD4^+$ (% lymphocytes)	$CD8^+$ (cells/μL)	$CD8^+$ (% lymphocytes)	$CD56^+$ (cells/μL)	$CD56^+$ (% lymphocytes)	$CD20^+$ (cells/μL)	$CD20^+$ (% lymphocytes)	$CD19^+$ (cells/μL)	$CD19^+$ (% lymphocytes)	IgG (mg/dL)	IgA (mg/dL)	IgM (mg/dL)	IgE (IU/mL)	PHA Proliferation (CPM)
1				1,404	70.4	927	46.5	233	11.7	98		485		562	28.2	1,270	512	749	<5	H
2	2	1,700	0	972	62	380	24	232	15	213	14			360	23	973	44	70	1	N
3	3.5	1,600	0	691	47	458	31	187	13	278	19			464	32	1,135	90	98	2	L
4		480		138		95		40		86				12		1,060*	30	44	4	N
5	7.5-8.5	1,000-1,200	112-200	592-606		108-184		360-420		370-504		58-132				890	14	67		L
6	2.6-3.5	300-721	0-21	120-315		52-204		48-122		130-548		0-30				92-209	<6	<5	<4.4	L
7	9.8-12.2	769-1554	0-44	538-1,070		323-668		177-326		131-355		54-202				146	<6		<5	L
8	3			716	67.9	277	26.8	102	9.9	209	20.3			105	10.3	229	75	327		L
9	4	1,080	120	388	68	163	48	11	13			2.4		274	3	793	68	159	<2	L
10	8	6,000	126	225	41.9	118	19.5	137	22.4	311	51.1	106	8	42	7	2,106	82.9	128		
11	6	1,340	238	426	32	326	245	59	4	598	52			228	17	402	<7	<2	51	
12	5.5			650	52	390	31	160	13					100	8	2,370	<6	147	<2	
13		1,160-1,820		234-552		121		126		98										L
14		312-552								37-82				3-143		1,250	<6	85		N
15		510-1160		245-570		200-400		110-190		110-280				0-290		146	<6	11.3	<5	L
16	9	L		250	29	109	13	26	5	369	62	71	9	96	11	2480	381	N	N	N
17	10	312-1920		157-1,501	50-78.2	825	55.2	300	20	134-105	6.9-34			50-85	5.7-16	450-580*	<20	<40	2-5	
18	13	1,310		878		367		498						0		0	0	0	0	
19																				
20	0.25	2,300	300	2,550	58.1	212	4.6	1,731	38.3	1,121	25.6			193	4.5	2,080	98.1	275	213	L
21	1		590	1,380	50	77	2.8	890	32.3	287	10.4	1,045				1,981	137	209		L
22	2	4,391	223	2,256	51.3	280	6.38	837	19.52	1249	24.54			1,265	23.16	1,900	6	127	1.88	L
23		735	0	132	18	88	12	27	3.7					146	19.9	1,770	138	176	5	25,061
24		470	60	453	96.3	95	20.3	322	68.5					0	0.1	110	0	0	5	43,600
25		680	240	295	43.4	171	25.1	94	13.8					48	7	1,410*	40	31	6	72,939
26	1.33	520	43	149	30	85	17	61	12	279	58			4	1	320	<24.9	54.9	427	
27	1.33	1,728	90	691	40	311	18	449	26	588	34	190		173	10	<152	0	171		
28	8	710	10	639	90	249	38.9	304	47.6	50	0.7			59	8.3	1,990	551	89.6	17	
29	1.42	30	50	65	17	65	8	31	8	275	73			31	8	1,036	<7	145	77	2,955
30	1.08	5,100	1,900	788	15	621	12	167	3					1,970	39	505*	32	309	50	L
31	47			454	63	367	51	194	27	130	18	10		58	8	697	127	60	256	51,486
32	0.92			89	11	59	8	7	1	320	40			359	45	2,330		152	256	
33	0.83	3,494		2,678	76.65	157.2	4.5	380.8	10.9	382.6	10.95			433.3	12.4	982	<12	221	3.7	
34	0.92			179	21.5	26	3.3	151	19	383	48.8			16	2.1	321	5	16	<2	
35	30-31			422	62	200	30	191	27	159	23			89	13	614*	<8	16.9	<2	
36	0.29			152	40.8	13	3.5	74	19.8				0.2	7	1.8	549*				
37		2,000-2,500					24		40						18	1,000	135	250		L
38			400	600	84	513		39									<8	<5	6.9	L
39	13.6	1,017	204	726	71.4	409	40.2	225	22	128	10.9			157	13.9	542	26	28	4.17	N
40					83.1		31.7		27.4		13.7				1.1					
41	4.33				85.6		54		7.8		12				0	164	<4	344		31
42	1			197	27.6	102	14.3	74	10.3	418	58.5			88	12.3	134	42	5		
43	3			211	35.5	66	11	45	7.6	225	37.7			153	25.7	788	164	71	N	
44	0.58			110	18.4	68	11.4	18	2.9	461	76.9			4	0.6	1,600*	41	65	24.2	

(continued)

TABLE E1. *(Continued)*

Case	Time of sample (y)	Lymphocytes (cells/μL)	Eosinophils (cells/μL)	CD3+ (cells/ μL)	$CD3^+$ (% lymphocytes)	$CD4^+$ (cells/ μL)	$CD4^+$ (% lymphocytes)	$CD8^+$ (cells/ μL)	$CD8^+$ (% lymphocytes)	$CD56^+$ (cells/ μL)	$CD56^+$ (% lymphocytes)	$CD20^+$ (cells/ μL)	$CD20^+$ (% lymphocytes)	$CD19^+$ (cells/ μL)	$CD19^+$ (% lymphocytes)	IgG (mg/ dL)	IgA (mg/ dL)	IgM (mg/ dL)	IgE (IU/ mL)	PHA Proliferation (CPM)
45	0.17			25,410	42	9,575	38	11,090	44	3,075	12			1,499	6	296	<6	14	2448	
46	2.17	800	450	152.2	19.03	92.8	11.6	32.5	4.07	420.8	52.6			213.8	26.7	1,997	0.01	128	<2.2	L
47																				
48		560	160	449	79	360	63	86	15	103	18			11	2	843*	22	19		L
49		1,090	0	774	71	98	12.7	383	49.5	86	11.1			157	14.4	1,970	6	130	52	
50		1,340-3,800		840-1,520	40-63	268-456	12-20	562-874	23-41.8	108-513	8-13.5			84-760	19.6-6.3	1,014	26	141	0	
51	48			1,279	82	374	24	1061	68	477	15	1		0	0	940	<6	<7	<2.2	208,010
52		700	0	406	58	287	41	126	18	224	32			7	1	1,470*	51	29	2.5	L
53		2,700	100	1,323	49	640	23.7	662	24.5					675	25	1,120	6	12	1	
54		900	0	682	75.8	252	28	300	33.4					25	2.8	1,970	740	730	6.6	
55		1,000		458	46	222	22	212	21			270		270	27	290	0	377		
56		1,310		590	45	410	31	90	7	280	21			420	32	344	1.56	51.1		L
57		1,870	670-1,400	269	24	94	8.4	135	12	393	3			381	34	337	10	178	<100	
58		2,200	1,710	1,107	54	267	13	677	33	636	31			82	4		3	160		
59				106		78		14		141		431				851	63	128		
60				727		611		34		918		68					125	160	189	
61				630		261		351		90		153				1,510	113	172		L
62				647		531		51		22		58					42	32		
63		900		783	87	432	48	153	17					45	5	1,180*	<6	<4		L

CPM, Counts per minute; *H*, high; *L*, low; *N*, normal.

Note: Case 47 had no immune phenotypic data available.

*On IVIG.

TABLE E2. Response rates to rituximab as second-line therapy for autoimmune cytopenias in patients with primary immune deficiency

Primary immune deficiency	Cytopenia type	Initial complete response rate	Sustained complete response rate	Reference
CVID	AIHA and/or ITP (n = 34)	85% (total cases)	59% (total cases)	E1
			Mean follow-up 39 mo	
ALPS	AIHA (n = 3) or ITP (n = 9)	77.8% (ITP cases); 0% (AIHA cases)	77.8% (ITP cases); 0% (AIHA cases)	E2
			Median follow-up 21 mo	
Any (CVID, WAS, ALPS, CID)	AIHA, ITP, and/or AN (n = 8)	90% (total treatments)	19.8% (total treatments)	E3
			Median follow-up 53 wk	
RAG deficiency	AIHA, ITP, and/or AN (n = 53)	28.9% (AIHA cases); 16.7% (ITP cases); 20.0% (AN cases)		

ALPS, Autoimmune lymphoproliferative syndrome; *CID*, combined immunodeficiency; *WAS*, Wiskott-Aldrich syndrome.

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