Letters to the Editor

Impaired control of multiple viral infections in a family with complete IRF9 deficiency

To the Editor:

Interferon regulatory factor (IRF) 9 contributes to IFN- $\alpha\beta$ -induced gene expression by binding interferon-stimulated response element sequences located in the promoters of interferon-stimulated genes (ISGs),¹ whereas a C-terminal IRF association domain mediates interactions with signal transducer and activator of transcription (STAT) 1 and STAT2 to increase ISG transcription.^{2,3} Recently, a child who presented with a severe respiratory tract infection caused by influenza A was identified as IRF9 deficient because of a c.991G>A mutation in IRF9 that led to skipping of exon 7 and a shortened IRF9 protein with reduced function.⁴ Here we report a family in which several members showed a striking susceptibility to infection by a wide range of viruses (Fig 1, A, and see the Methods section in this article's Online Repository at www.jacionline.org) and also proved to be IRF9 deficient.

The index patient is a 10-year-old boy born at term to healthy consanguineous parents (first cousins of Portuguese origin and residents of Venezuela). From the first year of life, the child had severe viral infections and diseases, including 4 prolonged stays in the pediatric intensive care unit (PICU), that resulted in persistent neurological impairment and bronchiectasis (see Table E1 in this article's Online Repository at www.jacionline.org). Genetic analysis using a next-generation sequencing (NGS)-customized panel (detailed methods are provided in the Methods section in this article's Online Repository) revealed a homozygous splicing mutation in the *IRF9* gene. The mutation, c.577+1G>T (NM_006084), which is located in the donor splice site of introns 5 and 6, was confirmed in homozygosity in the proband and his sister and in heterozygosity in both parents (Fig 1, B). PCR and sequencing analyses showed that the patients' mRNAs were slightly smaller than those in healthy donors (see Fig E1 in this article's Online Repository at www.jacionline.org) because they lacked exon 5. Skipping of this exon generated a frameshift and a premature stop codon (p. Glu166LeufsTer80; Fig 1, C). No expression of IRF9 was observed in either unstimulated or IFN- α -stimulated PBMCs from patients (Fig 1, D). IRF9 protein was also undetectable in 293T cells transfected with a c.577+1G>T IRF9 construct (see Fig E2 in this article's Online Repository at www.jacionline.org), suggesting that either this mRNA is not translated or the protein is rapidly degraded. These data strongly suggest that these subjects are effectively IRF9 null.

Because STAT1 was phosphorylated after IFN- α stimulation of primary fibroblasts from healthy control subjects and patients, the initial steps of the signaling cascade were conserved in the setting of IRF9 deficiency, but no induction of ISGs, including *MX1*, *IFIT3*, or *ISG15*, was observed (Fig 1, E). Similar observations were made in fibroblasts from control subjects and patients after infection with respiratory syncytial virus (RSV; Fig 1, F). Importantly, although IFN- α pretreatment markedly reduced RSV infection in control cells, no inhibition of RSV replication was noted in IRF9-deficient cells (Fig 1, G). Similar data were obtained in experiments studying herpes simplex virus (HSV) 1 infection (data not shown).

Finally, PCR analysis of freshly isolated PBMCs treated or not with IFN- $\alpha\beta$ revealed that IRF9-deficient cells were profoundly defective in induction of multiple ISGs (Fig 1, H). Because IFN- α induction of ISG expression in the mother's PBMCs was comparable with the average of 4 healthy donors, IRF9 p.Glu166LeufsTer80 does not exert any dominant negative effect on IFN- $\alpha\beta$ stimulation. PCR experiments analyzing macrophages derived from primary monocytes of patient II.2 (IRF9^{-/-}) produced similar results (see Fig E3, A, in this article's Online Repository at www.jacionline.org), and GM-CSF cultured macrophages from patients secreted much less CXCL10 than control macrophages, validating the PCR data (see Fig E3, B). Macrophages from both patients and control subjects released TNF- α after LPS stimulation (see Fig E3, C), showing that nuclear factor κ B activity was intact in these subjects.

Cell lines in which IRF9 expression was targeted by using CRISPR/Cas9 technology were prepared to establish a direct relationship between IRF9 deficiency and susceptibility to virus infection. Impaired induction of ISGs after IFN- α treatment was noted in cells in which IRF9 expression was reduced by 2 independent guide RNAs (Fig 2, A). IRF9 silencing decreased cellular control of viral replication (Fig 2, B), especially when cells were pretreated with IFN- α (Fig 2, C). In contrast, transfection of patients' cells with wild-type, but not mutant, IRF9 enhanced their ability to control the replication of various viruses (Fig 2, D). Therefore IRF9 deficiency is sufficient to explain the susceptibility to viral infection.

In general, patients with genetic defects that compromise IFN- $\alpha\beta$ signaling have problems with viral infections,⁵⁻⁹ and IRF9 deficiency fits squarely into this paradigm. The index patient had severe infections from a range of DNA and RNA viruses, several of which required admission to the PICU. Similarly, although the history of the other IRF9-deficient patient was dominated by a life-threatening influenza A virus infection, this child also had multiple other viral infections (bronchiolitis, gingivostomatitis, adenitis, prolonged gastroenteritis, and admission to the PICU because of idiopathic biliary perforation 14 days after measles, mumps, rubella vaccination), as well as recurrent fevers associated with digestive features, transient cutaneous rash, and intermittent urticaria, which was initially suggestive of an autoinflammatory syndrome.⁴ Different mutations in IRF9 might also influence the evolution of disease in these patients. The c.991G>A mutation led to expression of an IRF9 protein with an internal deletion affecting the IRF association domain, but that could still translocate to the nucleus.⁴ RNA sequencing analysis showed that although ISG expression after IFN- α 2b treatment was severely compromised, it was not abolished.⁴ In contrast, expression of the c.577+1G>T IRF9 mutant in cells from patients was undetectable, and essentially no ISGs were induced. Thus although the changes in cell biology observed in the different patients' cells showed a similar tendency, these data suggest that although the c.991G>A mutant of IRF9 is severely disabled, the c.577+1G>T mutation produces a complete loss of IRF9 function. We cannot exclude that some of these differences might also be due to differences between the cells studied: SV40-immortalized fibroblasts and B-lymphoblastoid cell lines⁴ compared with PBMCs and primary fibroblasts (this article).

FIG 1. A, Family tree of the IRF9-deficient patients. Circles, Female subjects; squares, male subject; triangles, spontaneous miscarriage; black figures, affected subjects; gray figures, suspected affected subjects; black/white figures, healthy carriers. B, Sanger sequencing of the c.577+1G>T mutation in patient II.2 and a heterozygous parent. C, Schematic representation of wild-type and mutant IRF9. D, No IRF9 protein is detected in patients' PBMCs. E, No induction of ISGs after IFN-α treatment of patients' fibroblasts. F, No induction of ISGs after RSV infection of patients' fibroblasts. G, IFN-α pretreatment does not establish an antiviral state in patients' fibroblasts. H, ISG induction is strongly impaired in PBMCs from patient II.4 compared with those from patient I.1 and healthy control subjects untreated or stimulated with IFN-α.

These possible differences in residual IRF9 activity can also contribute to differences in immunologic phenotypes observed (see Table E2 in this article's Online Repository at www.jacionline.org). Although the patient with the c.991G>A mutation had numbers and proportions of lymphocytes very close to normal values, the patients with the c.577+1G>T mutation who were available for study showed a mild CD4⁺ T and B lymphopenia and IgG hypogammaglobulinemia (II.2) and a transient natural killer and B lymphopenia (II.4). Because IgG levels in patient II.2 were normal until 6 years of age but had decreased by 8 years of age (IgG: 8 years old, 402 mg/dL; 9 years old, 252 mg/dL), it is possible that IgG hypogammaglobulinemia

could be a primary defect in IRF9 deficiency. Finally, that the previous patient started intravenous immunoglobulin (IVIG) therapy at 2 years and 8 months of age⁴ whereas patient II.2 was 9 years old when IVIG treatment began likely also influenced the clinical history of these patients.

Indeed, since the initiation of treatment in patient II.2 with nonspecific gammaglobulin, no severe infectious diseases have occurred. His sister, who has received nonspecific gammaglobulin since 3 weeks of age, has never experienced such diseases. Thus this therapy appears sufficient to avoid severe complications from viral infection. Nevertheless, given the current lack of knowledge about the prognosis of this new entity, the clinical

FIG 2. A, Reduced ISG induction in cells with reduced IRF9 expression. B, IRF9 deficient fibroblasts are less able to restrict RSV infection (1 and 2 are cell lines in which IRF9 has been targeted using CRISPR7Cas9 and 2 independent guide RNAs). C, IFN-α pretreatment does not establish an effective antiviral state in IRF9 CRISPR'd fibroblasts. D, Reconstitution of IRF9 expression in patients' cells restores the ability to restrict replication of a several viruses. IAV, Influenza A virus.

evolution of these patients will need to be followed closely. Another remarkable feature of these IRF9-deficient patients is the rapidity with which septic shock episodes develop, even in the absence of detectable pathogens. Whether this is only due to their impaired response to viral infections or whether IRF9 deficiency also produces dysregulation of inflammatory responses is as yet unknown.

The marked susceptibility to viral infections associated with IRF9 deficiency emphasizes that children presenting with unexpectedly severe consequences after either viral infection or vaccination with live attenuated vaccines should be evaluated, bearing in mind the possibility of immune defects affecting the interferon system. This case also reinforces that attenuated viral vaccines should never be administered to patients known to be deficient for interferon responses.

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METHODS

Case report

Patient II.2. Patient II.2 was a 10-year-old boy born at term to healthy consanguineous parents (first cousins of Portuguese origin and residents of Venezuela). From the first year of life, the child displayed a marked susceptibility to viral infections with moderate-to-severe symptoms of disease that resulted in persistent neurological impairment and bronchiectasis.

Immunologic studies carried out in Venezuela when the patient was 8 years old revealed diminished levels of IgG (402 mg/dL) with normal levels of IgA (75 mg/dL), IgM (45 mg/dL), and IgE (3 kU/mL) and a mild lymphopenia of CD4⁺ T cells leading to inversion of the CD4/CD8 ratio, for which he was receiving prophylactic treatment with cotrimoxazole.

At 9 years of age and after the family had moved to Madrid, the patient was admitted to the PICU after diagnosis of purpura fulminans and suspected septic shock with negative microbiologic results accompanied by increased reactive protein C levels, lymphopenia, thrombocytopenia, and reduced prothrombin activity. While in the PICU, the patient required mechanical ventilation, broad-spectrum antibiotics, and inotropic therapy. At presentation, the patient was profoundly lymphopenic, with an inverted CD4/CD8 ratio and diminished lymphocyte proliferation in response to mitogens. Because immunologic studies also revealed IgG and IgM hypogammaglobulinemia (IgG, 252 mg/dL; IgM, 24 mg/dL) with undetectable antibodies to tetanus and diphtheria toxins or pneumococci, treatment with IVIG replacement therapy was instituted. Despite the best efforts of the multidisciplinary care team, amputation of the legs and fingers proved necessary. On discharge, the patient's lymphocyte proliferative response had recovered partially, but T- and B-cell lymphopenia with inversion of the normal CD4/CD8 ratio was still present (see Table E2).

Since initiation of IVIG therapy, this patient has been hospitalized twice: once for a respiratory tract infection with influenza B virus and a second time for acute bronchitis. Admission to the PICU was not required on either of these occasions.

Genetic analysis in patient II.2 using an NGS-customized panel allowed us to exclude mutations in all previously reported primary immunodeficiency-related genes but revealed a homozygous splicing mutation in the *IRF9* gene, which had been included as a candidate gene because of its implication in interferon-dependent immunity responses.

The mutation c.577+1G>T (NM_006084), which is located in the donor splice site of introns 5 and 6, was confirmed in homozygosity in the proband and in heterozygosity in both parents (Fig 1, B).

Patient II.4. Patient II.4 was a 6-month-old girl, the sibling of patient II.2, who was born at 38 weeks of an uncomplicated pregnancy weighing 2.56 kg and measuring 46 cm. At birth, she was given a diagnosis of clubfoot (left) and congenital (left) hip dysplasia. Because of her family history, immunologic analyses were carried out when she was 3 days old, and these studies revealed normal levels of IgG and IgM and normal lymphocyte proliferation in response to PHA but a B-cell and natural killer cell lymphopenia. Genetic analyses revealed the presence of the c.577+1G>T mutation in homozygosity. Palivizumab was administered prophylactically during the RSV season, and treatment with IVIG and cotrimoxazole were begun at 3 and 6 weeks of age, respectively. To date, the patient has not presented with infections.

Patient II.1. Patient II.1 was female and the eldest sibling of patients II.2 and II.4, with a history of severe viral infections and late-onset neonatal sepsis. At 9 months of age, she was hospitalized in the PICU because of meningoencephalitis associated with HSV infection, and at the age of 11 months, she was hospitalized again with pneumonia. She died at 14 months of age after having an enterohemorrhagic fever subsequent to vaccination for yellow fever virus.

Subject II.3. Subject II.3 had a miscarriage at 36 weeks of gestation (hydrops fetalis, no microbiological confirmation) that coincided with infection of the mother and patient II.2 with dengue and Zika virus.

Unfortunately, neither necropsies nor genetic studies were carried out for either patient II.1 or II.3, and DNA samples are not available. Nevertheless, given the history of recurrent and severe infections in both cases, it seems likely that these subjects were also homozygous for the *IRF9* mutation.

Patients

The study was conducted according to the principles expressed in the Declaration of Helsinki and approved by the Institutional Research Ethics Committee of La Paz University Hospital. All participants provided informed consent for collection of samples and subsequent analyses.

NGS sequencing panel

DNA extracted from peripheral blood was screened for mutations by using a customized NGS gene panel containing 453 genes associated with immunopathologies. This panel was designed with NimbleDesign software (<https://design.nimblegen.com>). For each sample, paired-end libraries were created with the help of KAPA HTP Library Preparation Kit for Illumina platforms (Roche NimbleGen, Mannheim, Germany), SeqCap EZ Library SR (Roche NimbleGen), and the NEXTflex-96 PreCapture Combo Kit for indexing (Bioo Scientific, Austin, Tex). Sequencing was conducted on a MiSeq system (Illumina, San Diego, Calif), according to the standard operating protocol.

Cells

PBMCs were purified by means of centrifugation on Ficoll-Hypaque (GE Healthcare, Fairfield, Conn) and cultured in RPMI 1640. Dermal fibroblasts from patients and control subjects were isolated by outgrowth of fibroblasts from explanted tissue pieces. 293T, Vero, and Madin-Darby Canine Kidney (MDCK) cells were purchased from the European Collection of Authenticated Cell Cultures (ECACC). PBMCs were cultured in RPMI with 10% FCS, whereas primary fibroblasts and the 293T, Vero, and MDCK cell lines were cultivated in Dulbecco modified Eagle medium with 10% FCS. Media were supplemented with 2 mmol/L L-glutamine, 0.1 mmol/L sodium pyruvate, 100 U/mL penicillin, 100 U/mL streptomycin, 50 μ mol/L β -mercaptoethanol, and, where indicated, either IFN- α or IFN- β (1000 U/mL).

Immortalized cell lines from patients and control subjects were prepared by using lentiviral transduction to overexpress human telomerase. pLV-hTERT-IRES-hygro was a gift from Tobias Meyer (plasmid no. 85140; Addgene, Watertown, Mass).^{E1}

For some experiments, human monocytes were purified from PBMCs by means of magnetic cell sorting with anti-CD14 microbeads (>95% CD14⁺ cells; Miltenyi Biotech, Bergisch Gladbach, Germany) and cultured at 0.5×10^6 cells/mL for 7 days in RPMI/10% FCS. To generate macrophages (GM macrophages), 1000 U/mL GM-CSF (Immuno Tools GmbH, Friesoythe, Germany) was added every 2 days.^{E2} When indicated, macrophages were activated with *Escherichia coli* 055:B5 LPS (10 ng/mL) or IFN- β (1000 U/mL) for 18 hours.

Cell-surface staining

Peripheral blood samples, PBMCs, monocytes, and dendritic cells were usually incubated with mAbs (20–30 minutes at room temperature in the dark). For whole blood, erythrocytes were lysed in FACS Lysing solution (BD Biosciences, San Diego, Calif) and washed. For B cells, samples were stained according to the EUROclass trial protocols.^{E3} Cells were stained with the conjugated anti-human antibodies listed in Table E3 (all from BD Biosciences).

Lymphocyte proliferation assays

Lymphocyte proliferation assays were performed with mitogens (PHA, concanavalin A, pokeweed mitogen, and OKT3) and analyzed based on tritiated thymidine (Amersham Biosciences, Piscataway, NJ) uptake after 3 days.

Viral infections

Subconfluent monolayers of fibroblasts were infected with either green fluorescent protein-expressing RSV A2 (a gift of Dr J. A. Melero, ISCIII, Madrid, Spain),^{E4} HSV-1 strain 17 (a gift of Dr Helena Browne, University of Cambridge), or influenza virus A/PR8/8/34 (H1N1; PR8, a kind gift of Dr

Amelia Nieto, CNB-CSIC, Madrid, Spain) at multiplicities of infection of 3 plaque-forming units per cell. After incubation with the virus for 90 minutes at 37°C, inoculum was removed, and fresh medium was added. After 24 hours of infection, cells were lysed and analyzed by means of Western blotting.

Western blotting

Cells were washed once with ice-cold PBS and lysed in RIPA lysis buffer before analysis by means of Western blotting after separation on SDS-PAGE.^{E5} The primary antibodies used are shown in Table E4. Bound antibodies were visualized by using highly cross-adsorbed goat anti-mouse or goat anti-rabbit secondary reagents coupled to Alexa Fluor Plus 680 (Invitrogen, Carlsbad, Calif) and the Odyssey Western Blot Detection System (LI-COR, Lincoln, Neb). Quantitative analysis of these data were carried out with the Image Studio Software application (LI-COR).

Plasmids

The guide RNAs IRF9_1 (CACCGGCACCCGAAAACCTCCGGAAC and AAACGTTCCGGAGTTTTCGGGTGCC) and IRF9_2 (CACCGAGACC ATGTTCCGGATTCCC and AAACGGGAATCCGGAACATGGTCTC) for inactivation of IRF9 were annealed and cloned into the vector lentiCRISPR (version 2; a gift from Feng Zhang [Addgene]; plasmid no. 52961).^{E6}

For reconstitution experiments, full-length wild-type or mutant IRF9 was amplified by using oligos (GCGGATCCGCCACCATGGCATCAGGCAGG GCACG and TCGCGGCCGCTACACCAGGGACAGAATGGC) and cloned into the lentiviral vector pHRSIN-C56W-UbEM (a gift from Professor Paul Lehner, Cambridge Institute for Medical Research, Cambridge).

Lentiviral gene transduction

Lentiviruses were generated and used to infect cells, as previously described.^{E7}

RNA extraction, cDNA synthesis, and quantitative PCR

Total RNA was isolated, cDNA was synthesized, and quantitative PCR analysis was carried out, as previously described.^{E5} Sequences of primers used for each target gene are shown in Table E3. These reactions produced single amplicons of the expected length and melting temperature, as assessed by using double-sided DNA melting curve analysis. Data were analyzed with SDS2.2 sequence detection systems. Data are expressed as relative fold change in expression compared with untreated PBMCs. The control data correspond to means $\pm$ SDs for the 4 healthy control subjects.

ELISA

Culture supernatants from untreated, LPS-treated (18 hours), or IFN- β -treated (18 hours) human macrophages were assayed for the presence of cytokines by using commercially available ELISA for human TNF- α (BD

PharMingen) and CXCL10 (BioLegend, San Diego, Calif) performed according to the manufacturers' protocols.

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FIG E1. A, IRF9 RT-PCR comparing cDNA prepared from PBMCs of patient II.2, a healthy control subject (*HC*), patient II.4, and patient I.1 (father). **B,** Sanger sequencing of an IRF9 RT-PCR of patient II.2's PBMCs showing in-frame splicing of exon 4 to exon 6.

FIG E2. IRF9 expression is not detectable in cells expressing the c.577+1G>T transcript. 293T cells, untransfected or transfected to over-express either *IRF9* wild-type or *IRF9* E166LfsTer80, were lysed, and IRF9 expression was analyzed by means of Western blotting. *GAPDH*, Glyceraldehyde-3-phosphate dehydrogenase.

FIG E3. **A**, Relative expression of the indicated genes in untreated and IFN- β -treated macrophages from patient II.2 (IRF9^{-/-}) and healthy donors (father; IRF9^{+/-}; healthy control donor, IRF9^{+/+}). Relative mRNA levels indicate expression of each marker relative to expression of the *HPRT* and *TBP* genes. Results show expression of each gene in interferon-treated macrophages and relative to its expression in untreated macrophages. **B**, Production of CXCL10 by IFN- β -stimulated macrophages (18 hours) derived from a healthy father (IRF9^{+/-}) and patient II.2 (IRF9^{-/-}) and from a healthy control subject (IRF9^{+/+}) and the patient II.2 (IRF9^{-/-}). **C**, Production of TNF by LPS-stimulated macrophages from indicated donors treated for 18 hours. Each determination was performed in triplicates.

TABLE E1. Infections in patient II.2

Infections	Age	Clinical Severity*
Bronchiolitis RSV (+)	6 mo	Moderate
Disseminated chickenpox with pneumonitis after vaccination (neurological impairment was detected after admission)	12 mo	Severe
H1N1 influenza with pneumonia	18 mo	Moderate
Dengue fever	2 y	Mild to moderate
Pneumonia	2 y	Moderate
Bronchitis	3 y	Moderate
Pneumonia	3 y	Moderate
Enterovirus encephalitis (subsequent EEG abnormalities)	5 y	Severe
Bronchitis and sinusitis	5 y	Moderate
Severe dengue fever and Zika virus disease	8 y	Severe
Pneumonia	8 y	Moderate
Septic shock without microbiological confirmation, purpura fulminans	9 y	Severe
B influenza	9 y	Mild to moderate
Pneumonia	9 y	Mild to moderate

EEG, Electroencephalogram.

*Clinical severity: *Severe*, admission to PICU; *Moderate*, hospital admission >1 week; and *Mild to moderate*, admission <1 week.

TABLE E2. Immunophenotype of patients II.2 and II.4

	Patient II.2			Patient II.4		
	9 y old (PICU)	9 y old (scGG)	Normal values	3 d old	Six mo old (scGG)	Normal values
Humoral						
IgG levels (mg/dL)	252	1040	462-1682	708	581	690-1532
IgA levels (mg/dL)	37	87	34-274	<8	<8	0-32
IgM levels (mg/dL)	24	84	38-251	13	19	7-28
IgE levels (kU/L)	14.8		<180			
Pneumococcus, tetanus, and diphtheria toxoid-specific antibody	Absent					
Cellular						
Total lymphocytes/ μ L	190	2880	1900-3700	3590	5510	3400-7600
CD3 ⁺ cells/ μ L	36	1240	1200-1600	3340	4730	2500-5500
CD3 ⁺ TCR $\alpha\beta$ ⁺ cells (of total CD3 ⁺ cells)	98.3%	65%	39% to 94%	99%		39% to 94%
CD3 ⁺ TCR $\gamma\delta$ ⁺ cells (of total CD3 ⁺ cells)	1.4%	35%	0.82% to 10%	0.5%		0.82% to 10%
TCR $\alpha\beta$ ⁺ CD3 ⁺ CD4 ⁻ CD8 ⁻ cells	3.2%	3%	0.39% to 4%	0.2%		0.39% to 4%
CD3 ⁺ DR ⁺ cells	8.3%	53.4%	0.5% to 4.2%			
CD4 ⁺ cells/ μ L	25	290	650-1500]	2480	3270	1600-4000
CD4 ⁺ CD45RA ⁺ CD31 ⁺ cells (of total CD4 ⁺ cells)	66%	39%	43.9% to 66.4%	78%	82.4%	56.3% to 76.4%
CD4 ⁺ CD45RA ⁺ cells (of total CD4 ⁺ cells)	65%	38%	46% to 77%	84%	83.3%	64% to 95%
CD4 ⁺ CD45RO ⁺ cells (of total CD4 ⁺ cells)	14%	45%	13% to 30%	1.8%	3.7%	2% to 22%
CD4 ⁺ CD25 ⁺ CD127 ⁻ cells	4.3%	17.9%	2.3% to 7.7%			
CD4 ⁺ CXCR3 ⁺ CCR6 ⁻ cells (T _H 1 [of total CD4 ⁺ cells])		22.9%	12.1% to 29.5%	2.2%	5.8%	12.1% to 29.5%
CD4 ⁺ CCR4 ⁺ CXCR3 ⁻ cells (T _H 2 [of total CD4 ⁺ cells])		9.8%	45.1% to 64.1%	2.7%	5.4%	45.1% to 64.1%
CD4 ⁺ CCR6 ⁺ CXCR3 ⁻ cells (T _H 17 [of total CD4 ⁺ cells])		8.7%	12.4% to 23.6%	1.1%	2.7%	12.4% to 23.6%
CD4 ⁺ CXCR5 ⁺ CD45Ra ⁻ cells (T _{FH} [of total CD4 ⁺ cells])		6.5%	11%*	0.4%	2.6%	11%*
CD8 ⁺ cells/ μ L	15	350	370-1100	860	1310	560-1700
CD8 ⁺ CD45RA ⁺ (of total CD8 ⁺ cells)	83%	46%	63% to 92%	81%	84.1%	80% to 99%
CD8 ⁺ CD45RO ⁺ (of total CD8 ⁺ cells)	0.4%	17%	4% to 21%	2.7%	1.7%	1% to 9%
CD19 ⁺ cells/ μ L	133	10	270-860	160	520	430-2000
B naive cells (of total CD19 ⁺ cells)	81.9%	84.5%	62% to 94%		94.6%	88% to 100%
Plasmablasts (of total CD19 ⁺ cells)	0.1%	0.8%	0.1% to 3%		0.1%	0% to 6%
CD21 ^{low} cells (of total CD19 ⁺ cells)	18.1%	4.6%	1% to 17%		0.6%	0.3% to 10%
B transitional cells (of total CD19 ⁺ cells)	4.3%	14.7%	2% to 30%		10.3%	4% to 52%
Unswitched memory B cells (of total CD19 ⁺ cells)	2.3%	5.5%	4% to 24%		2.4%	0.9% to 11%
Switched memory B cells (of total CD19 ⁺ cells)	3.7%	5.5%	3% to 18%		1.1%	0.1% to 6%
CD16 ⁺ CD56 ⁺ cells/ μ L	14	1530	100-480	80	240	170-1100
Proliferation assays (PHA)	Diminished	Normal	Normal	Normal		Normal
Proliferation assays (ConA)	Diminished	Normal	Normal			
Proliferation assays (PWM, anti-CD3)	Diminished	Diminished	Normal			
CD4 ⁺ T-cell activation markers (CD69, ICOS, and CD25) after anti-CD3/CD28	Diminished		Normal			

ConA, Concanavalin A; ICOS, inducible costimulator; PWM, pokeweed mitogen; TCR, T-cell receptor; T_{FH}, follicular helper T.

*Normal range defined "in-house".

TABLE E3. Antibodies for flow cytometry

Antibody	Clone	Supplier
CD45-V500	HI30	BD Biosciences, San Jose, Calif
CD45-PerCP	2D1	BD Biosciences
CD3-APC	SK7	BD Biosciences
CD3-FITC	SK7	BD Biosciences
CD4-V450	RPAT4	BD Biosciences
CD4-PerCP	SK3	BD Biosciences
anti-TCR $\alpha\beta$ -FITC	WT31	BD Biosciences
anti-TCR $\gamma\delta$ -PE-Cy7	11F2	BD Biosciences
CD8-PerCP	SK1	BD Biosciences
CD8-APC	SK1	BD Biosciences
CD19-PE-Cy7	SJ25C1	BD Biosciences
CD19-PerCP	SJ25C1	BD Biosciences
CD19-PerCP-Cy5.5	SJ25C1	BD Biosciences
CD16-PE	B73.1	BD Biosciences
HLA-I-PE	EMR8-5	BD Biosciences
HLA-DR-APC	L243	BD Biosciences
CD27-FITC	L128	BD Biosciences
CD38-FITC	HIT2	BD Biosciences
CD21-PE	B-ly4	BD Biosciences
CD127-FITC	HIL-7R-M21	BD Biosciences
CD25-APC	2A3	BD Biosciences
CXCR5-FITC	RF8B2	BD Biosciences
CXCR3-PE	1C6	BD Biosciences
CCR6-APC	11A9	BD Biosciences
CCR4-PerCP-Cy5.5	IG1	BD Biosciences
CD31-PE	WM59	BD Biosciences
CD45RA-APC	HI100	BD Biosciences
CD45RA-APC-H7	HI100	BD Biosciences
CD45Ro-PE-Cy7	UCHL-1	BD Biosciences
CD14-V450	MOP9	BD Biosciences
CD69-PE	L78	BD Biosciences
anti-ICOS-PE	G22	BD Biosciences
Isotype control IgG $_{1\kappa}$ -FITC	MOPC-21	BD Biosciences
Isotype control IgG $_{1\kappa}$ -APC	MOPC-21	BD Biosciences
Isotype control IgG $_1$ -PE	X40	BD Biosciences
Isotype control IgG $_1$ -PerCP	X40	BD Biosciences

APC, Allophycocyanin; FITC, fluorescein isothiocyanate; PE, phycoerythrin, PerCP, peridinin-chlorophyll-protein complex.

TABLE E4. Antibodies for Western blotting

Antibody	Source
Rabbit mAb anti-IRF9 (D87GH)	Cell Signaling technology, Danvers, Mass
Rabbit mAb anti-pSTAT1 (58D6)	Cell Signaling technology
Rabbit polyclonal anti-STAT1	Cell Signaling technology
Rabbit mAb anti GAPDH (14C10)	Cell Signaling technology
Rabbit polyclonal anti-IRF9	Proteintech Group, Rosemont, Ill
Rabbit polyclonal anti-STAT2	Proteintech Group
Rabbit polyclonal anti-MX1	Proteintech Group
Rabbit polyclonal anti IFIT3	Proteintech Group
Rabbit polyclonal anti-ISG15	Proteintech Group
Mouse mAb anti- β -actin (AC-15)	Sigma-Aldrich, St Louis, Mo
Mouse mAb anti-glycoprotein F of RSV	Gift of J. A. Melero ²⁴
Mouse mAb anti-influenza A NS1 (NS1-23-1)	Santa Cruz Biotechnology, Dallas, Tex
Mouse mAb anti-HSV UL42 (13C9)	Santa Cruz Biotechnology

GAPDH, Glyceraldehyde-3-phosphate dehydrogenase; *pSTAT*, phosphorylated signal transducer and activator of transcription.

TABLE E5. Oligonucleotides used for quantitative RT-PCR

Gene	Primers	References
<i>IFI27</i>	For, GCCTCTGGCTCTGCCGTAGTT Rev, ATGGAGGACGAGGCGATTCC	E8
<i>BST2</i>	For, ACGCGTCTGCAGAGGTGGAG Rev, GCAGCGGAGCTGGAGTCCT	E8
<i>OAS1</i>	For, TGAGGTCCAGGCTCCACGCT Rev, GCAGGTCCGTGCACTCCTCG	E8
<i>OAS2</i>	For, AGGTGGCTCCTATGACGGAA Rev, GGCTTCTCTTCTGATCCTGGAATTG	E8
<i>MX1</i>	For, CTTTCCAGTCCAGCTCGGCA Rev, AGCTGCTGGCCGTACGTCTG	E8
<i>IFIT1</i>	For, TCTCAGAGGAGCCTGGCTAA Rev, CCAGACTATCCTTGACCTGATGA	E8
<i>IFIT3</i>	For, CAGAACTGCAGGGAAACAGC Rev, TGAATAAGTTCCAGGTGAAATGGC	E8
<i>MYD88</i>	For, GTCTGACCGCGATGTCCTGCC Rev, ACAACCACCACTCCGGCG	E8
<i>IRF1</i>	For, CATGAGACCCTGGCTAGAGATG Rev, TCCGGAACAAACAGGCATCC	E8
<i>IFIT2</i>	For, GACACGGTTAAAGTGTGGAGG Rev, TCCAGACGGTAGCTTGCTATT	E9
<i>IRF7</i>	For, GCTGGACGTGACCATCATGTA Rev, GGGCCGTATAGGAACGTGC	E9
<i>ISG15</i>	For, CACAGCCATGGGCTGGGACCTG Rev, GCACGCCGATCTTCTGGGTGA	E10
<i>USP18</i>	For, GAAAACGAAAGCTGGGCGGGG Rev, GCCAGGCACGATGGAATCTCTCAA	E10
<i>RIG-I</i>	For, GACTGGACGTGGCAAAACAA Rev, TTGAATGCATCCAATATACACTTCTG	E10
<i>TNFSF10</i>	For, AGACCTGCGTGCTGATCGTG Rev, CAAGCAATGCCACTTTTGGA	E11
<i>SOCS1</i>	For, AGAGCTTCGACTGCCTCTTC Rev, AGGGGAAGGAGCTCAGGTAG	E12
<i>STING</i>	For, GAGCAGGCCAAACTCTTCTG Rev, TGCCCACAGTAACCTCTTC	E13
<i>IP10</i>	For, CTGCTTTGGGGTTATCAGA Rev, CCACTGAAAGAATTGGGGC	This article
<i>IFNA</i>	For, GAAACCACTGACTGTATATTGTGTGAAA Rev, CAGCGTCACTAAAAACACTGCTTT	E14
<i>PKR</i>	For, TACGTGTGAGTCCCAAAGCA Rev, ATGCCAAACCTCTTGTCAC	This article
<i>STAT1</i>	For, ACCGCACCTTCAGTCTTTTCC Rev, TGAAGTGGACCCCTGTCTTCA	E15
<i>Sirt1</i>	For, CAGTGGCTGGAACAGTGAGA Rev, AGCGCCATGGAATGTAAAC	E16
<i>MDA5</i>	For, GCTGAAGTAGGAGTCAAAGCCC Rev, CCACTGTGGTAGCGATAAGCAG	This article
<i>CCL5</i>	For, GCTGTCATCCTCATTGCTACTG Rev, TGGTGTAGAAATACTCCTTGATGTG	E17
<i>STAT2</i>	For, CAGGTCACAGAGTTGCTACAGC Rev, CGGTGAACTTGCTGCCAGTCTT	This article
<i>p53</i>	For, CCTCAGCATCTTATCCGAGTGG Rev, TGGATGGTGGTACAGTCAGAGC	This article

For, Forward; Rev, reverse.