

β-Arrestin2 expressed in mast cells regulates ciprofloxacin-induced pseudoallergy and IgE-mediated anaphylaxis



To the Editor:

Anaphylaxis is a life-threatening allergic reaction that results from rapid mast cell (MC) degranulation through aggregation of the high-affinity IgE receptor FcεRI. MCs also undergo degranulation in response to drugs approved by the US Food and Drug Administration, resulting in pseudoallergic reactions.^{1,2} These reactions are mediated by opioids, neuromuscular blocking agents, and the fluoroquinolone group of antibiotics, such as ciprofloxacin, which activate human MCs through Mas-related G protein-coupled receptor (GPCR) X2 (MRGPRX2) and mouse MCs through Mrgprb2.^{1,2}

β-Arrestin2 is a well-established adapter protein that contributes to GPCR desensitization,³ but the possibility that it regulates MC-mediated pseudoallergy has not been tested.⁴ Although FcεRI signaling requires tyrosine phosphorylation of Syk, the role of GPCRs in IgE-mediated MC degranulation and anaphylaxis is well documented. Trans-activation of sphingosine-1-phosphate receptor 2 contributes to antigen/IgE-mediated MC degranulation and anaphylaxis.^{5,6} Furthermore, the anaphylatoxin C3a induces robust degranulation in human MCs, and FcεRI-mediated cutaneous anaphylaxis is substantially reduced in C3a receptor knockout mice.^{4,7} Based on these findings, we hypothesized that β-arrestin2 expressed in MCs negatively regulates both Mrgprb2-mediated pseudoallergy and FcεRI-mediated allergy.

Using quantitative RT-PCR and Western blotting, we first confirmed the absence of β-arrestin2 mRNA and protein in bone marrow-derived mast cells (BMMCs) generated from global β-arr2^{-/-} mice (see Figs E1 and E2 in this article's Online Repository at www.jacionline.org). BMMCs from both wild-type (WT) and β-arr2^{-/-} mice showed comparable levels of surface c-Kit and FcεRI expression and similar level of toluidine blue and alcian/safranin staining (see Figs E3 and E4 in this article's Online Repository at www.jacionline.org).

To determine the effect of β-arrestin2 on *in vivo* differentiation of peritoneal mast cells (PMCs), a mixed cell population obtained from peritoneal lavage (PTL) fluid was stained with toluidine blue and alcian/safranin and counted. As shown in Figs E5 and E6 in this article's Online Repository at www.jacionline.org, the absence of β-arrestin2 had no effect on the staining properties and numbers of PMCs. Furthermore, transmission electron microscopy did not reveal any difference in the granule content of WT and β-arr2^{-/-} PMCs (see Fig E7 in this article's Online Repository at www.jacionline.org). Toluidine blue staining of ear tissue showed no change in dermal MC numbers and immunofluorescence staining with Texas red avidin (MCs), and Alexa Fluor-conjugated CD31 (endothelial cells) showed similar compartmentalization of MCs adjacent to blood vessels in WT and β-arr2^{-/-} mice (see Figs E8 and E9 in this article's Online Repository at www.jacionline.org). We also generated mice with MC-specific deletion of β-arrestin2 by crossing *Cpa3-cre* mice with β-arr2^{fl/fl} mice. MCs from these mice (*Cpa-3Cre*⁺/β-arr2^{fl/fl}) and control mice (*Cpa-3Cre*⁻/β-arr2^{fl/fl}, see Fig E10 in this article's Online Repository at www.jacionline.org) exhibited similar FcεRI/c-Kit expression

(see Fig E11 in this article's Online Repository at www.jacionline.org), staining properties (see Figs E12 and E13 in this article's Online Repository at www.jacionline.org), and MC numbers and distribution (see Fig E14 and E15 in this article's Online Repository at www.jacionline.org). These findings demonstrate that β-arrestin2 has no effect on the development and maturation of MCs.

Ciprofloxacin and compound 48/80 induce MC degranulation through Mrgprb2.^{1,8} Because PMCs express Mrgprb2, we initially used mixed PTL cells as a source of PMCs for degranulation studies. Both ciprofloxacin and compound 48/80 induced β-hexosaminidase release in PTL cells, but these responses were significantly enhanced in cells obtained from β-arr2^{-/-} mice (Fig 1, A). We used a short-term culture to enrich PMCs greater than 90%⁸ and showed that these MCs generated from β-arr2^{-/-} mice also display greater degranulation in response to ciprofloxacin and compound 48/80 when compared with cells obtained from WT mice (Fig 1, B).

To determine the *in vivo* role of β-arrestin2 on pseudoallergy, we intradermally injected ciprofloxacin or vehicle (PBS) into the ears of WT and β-arr2^{-/-} mice just after intravenous injection of Evans blue dye. Consistent with PMC degranulation, there was a significant enhancement of vascular permeability, as measured by means of dye extravasation, in response to ciprofloxacin in β-arr2^{-/-} mice when compared with WT mice (Fig 1, C and D). We also found that ciprofloxacin induced significantly enhanced dye extravasation in *Cpa-3Cre*⁺/β-arr2^{fl/fl} mice when compared with control *Cpa-3Cre*⁻/β-arr2^{fl/fl} mice (Fig 1, E and F). These findings suggest that β-arrestin2 expressed in MCs contributes to Mrgprb2 desensitization and that its absence enhances both ciprofloxacin-induced degranulation *in vitro* and pseudoallergy *in vivo*.

To determine the effects of β-arrestin2 on FcεRI signaling and mediator release, we used BMMCs and PMCs generated from WT and β-arr2^{-/-} mice. The absence of β-arrestin2 in BMMCs had no effect on early FcεRI signaling, Syk phosphorylation, or Ca²⁺ mobilization (Fig 2, A and B). However, antigen (2,4-dinitrophenylated BSA [DNP-BSA])-induced FcεRI-mediated degranulation, as measured based on β-hexosaminidase release, was significantly enhanced in β-arr2^{-/-} BMMCs when compared with WT-BMMCs (Fig 2, C). Antigen-induced β-hexosaminidase release was also significantly enhanced in β-arr2^{-/-} PMCs when compared with WT cells (Fig 2, D). The fusion of lysosomal-associated membrane protein 1 with the plasma membrane on activation is indicative of MC degranulation and can be measured by using flow cytometry.⁹ As shown in Fig 2, E, antigen triggered an increase in surface expression of lysosomal-associated membrane protein 1 in WT BMMCs, but this response was significantly enhanced in β-arr2^{-/-} cells.

We then sought to determine whether enhanced antigen-induced MC degranulation in the absence of β-arrestin2 *in vitro* correlates with increased passive cutaneous anaphylaxis (PCA) *in vivo*. For this, ears were intradermally injected with dinitrophenol-specific IgE or vehicle, 24 hours later mice were subjected to an intravenous injection of Evans blue and antigen (dinitrophenylated BSA), and dye extravasation was determined. As shown in Fig 2, F, antigen caused an approximately 2-fold increase in dye extravasation when compared with PBS control

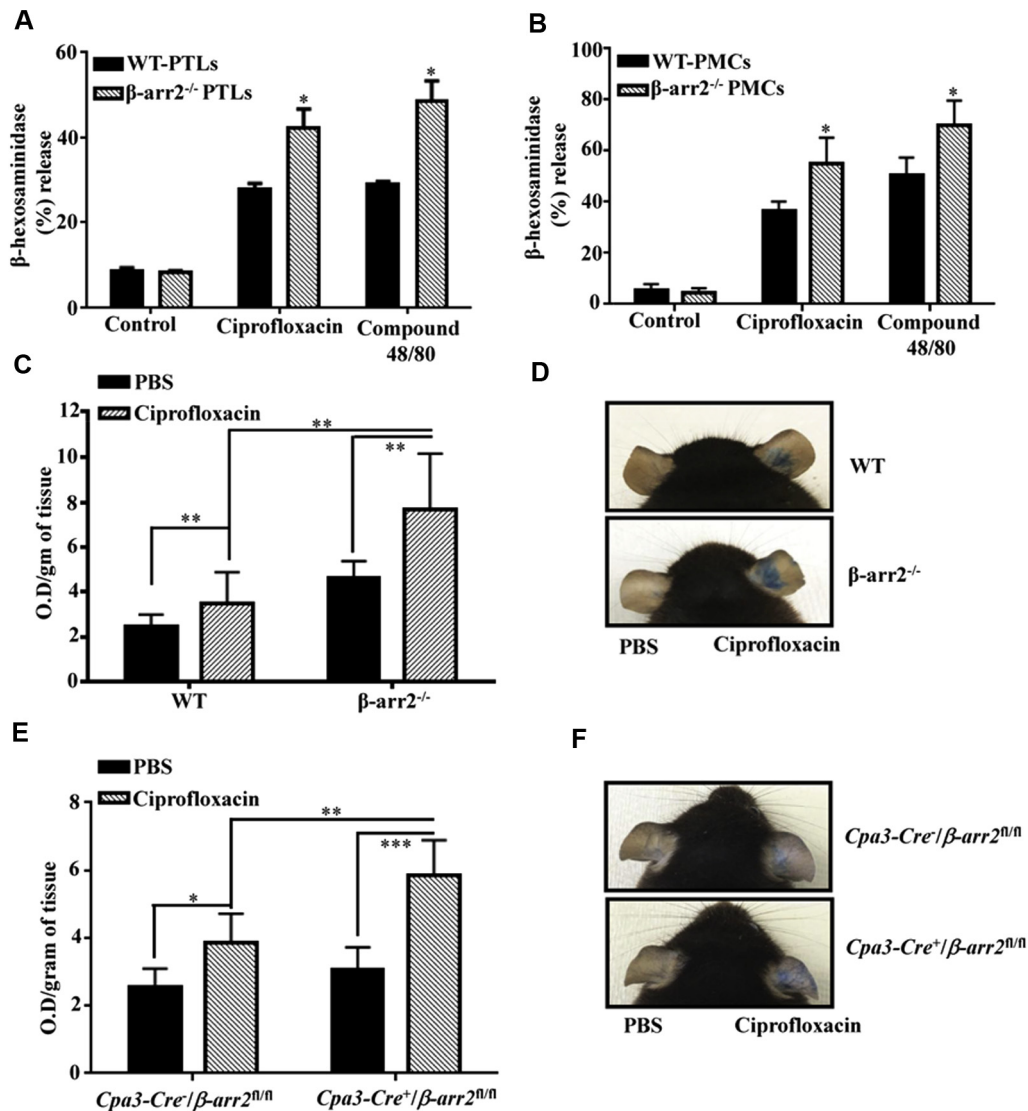


FIG 1. β -Arrestin2 regulates ciprofloxacin-induced MC degranulation *in vitro* and pseudoallergy *in vivo*. **A** and **B**, Degranulation in WT and β -arr2^{-/-} PTLs (Fig 1, **A**) and PMCs (Fig 1, **B**) on stimulation with ciprofloxacin (200 μ g/mL) or compound 48/80 (10 μ g/mL). **C**, WT and β -arr2^{-/-} mice were intradermally injected with ciprofloxacin (400 μ g/mL) or PBS, and Evan's blue dye extravasation was determined (n = 5-6). **D**, A representative image is shown. **E**, Evan blue extravasation in *Cpa3-Cre*⁺/*β-arr2*^{fl/fl} and *Cpa3-Cre*⁺/*β-arr2*^{N/n} mice (n = 5-6). **F**, A representative image is shown. Error bars are SDs. **P* < .05, ***P* < .01, and ****P* < .001, nonparametric *t* test.

in WT mice. However, this response was significantly enhanced in β -arr2^{-/-} mice. Antigen-induced PCA was also enhanced in *Cpa3-Cre*⁺/*β-arr2*^{fl/fl} mice when compared with control *Cpa3-Cre*⁻/*β-arr2*^{fl/fl} mice (Fig 2, **G**). These findings demonstrate that β -arrestin2 inhibits antigen-induced MC degranulation *in vitro* and PCA *in vivo* through modification of signaling components downstream of Syk phosphorylation and Ca²⁺ mobilization.

To determine whether the effects of β -arrestin2 on MC degranulation are specific for Mrgprb2 and Fc ϵ RI, BMBCs and PMCs from WT and β -arr2^{-/-} mice were stimulated with phorbol 12-myristate 13-acetate/Ca²⁺ ionophore, and β -hexosaminidase release was determined. Surprisingly, β -arrestin2^{-/-} MCs exhibited enhanced degranulation when

compared with WT cells (see Figs E16 and E17 in this article's Online Repository at www.jacionline.org). These findings suggest that β -arrestin2 regulates Mrgprb2- and Fc ϵ RI-mediated MC degranulation through modification of a shared target downstream of Ca²⁺ mobilization.

The novel finding of the present study is that the same adapter protein, β -arrestin2, negatively regulates both Mrgprb2- and Fc ϵ RI-mediated MC degranulation to attenuate drug-induced pseudoallergy and IgE-mediated anaphylaxis.^{1,2,4-7} We also demonstrated that the absence of β -arrestin2 results in enhanced degranulation in response to Ca²⁺ ionophore. This suggests that β -arrestin2 plays a global role in regulating MC responsiveness to any stimuli that induce degranulation. Thus, a detailed understanding of the molecular mechanism through which

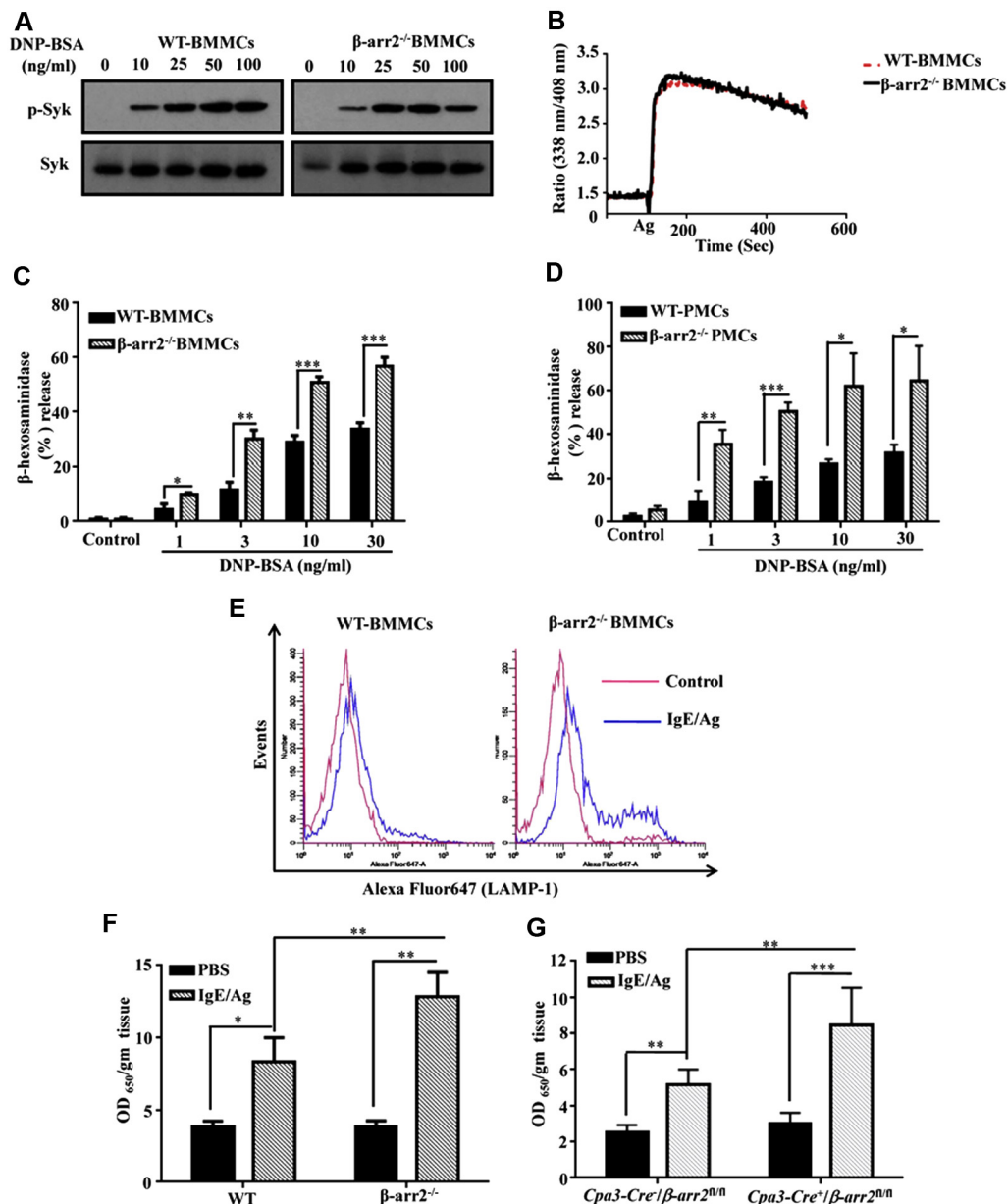


FIG 2. β -Arrestin2 regulates Ag/IgE-mediated MC degranulation *in vitro* and cutaneous anaphylaxis *in vivo*. **A** and **B**, IgE (1 μ g/mL)-primed WT and β -arr2^{-/-} BMMCs were stimulated with antigen, DNP-BSA, and Syk phosphorylation (Fig 2, A) and Ca²⁺ mobilization (Fig 2, B) were determined. **C** and **D**, Antigen-induced β -hexosaminidase release in WT and β -arr2^{-/-} BMMCs (Fig 2, C) and PMCs (Fig 2, D). **E**, Antigen (10 ng/mL)-induced lysosomal-associated membrane protein 1 expression in WT and β -arr2^{-/-} BMMCs. **F** and **G**, Mice were sensitized with intradermal injection of IgE (20 ng), and Evan's blue extravasation was determined after intravenous injection of DNP-BSA (100 μ g) in WT and β -arr2^{-/-} (Fig 2, F) and *Cpa3-Cre⁻/β-arr2^{fl/fl}* and *Cpa3-Cre⁺/β-arr2^{fl/fl}* (Fig 2, G) mice (n = 5-6). Error bars are SDs. *P < .05, **P < .01, and ***P < .001, nonparametric t test.

β -arrestin2 regulates MC function might provide a novel strategy for the modulation of diseases mediated by MCs.

Saptarshi Roy, PhD
Kshitij Gupta, PhD
Anirban Ganguly, BA
Hydar Ali, PhD

From the Department of Pathology, School of Dental Medicine, University of Pennsylvania, Philadelphia, Pa. E-mail: alih@upenn.edu.

Supported by National Institutes of Health grants R21-AI115688 and R01-AI124182 (to H.A.).

Disclosure of potential conflict of interest: The authors declare that they have no relevant conflicts of interest.

REFERENCES

- McNeil BD, Pundir P, Meeker S, Han L, Undem BJ, Kulka M, et al. Identification of a mast-cell-specific receptor crucial for pseudo-allergic drug reactions. *Nature* 2015; 519:237-41.
- Subramanian H, Gupta K, Ali H. Roles of Mas-related G protein-coupled receptor X2 on mast cell-mediated host defense, pseudoallergic drug reactions, and chronic inflammatory diseases. *J Allergy Clin Immunol* 2016;138:700-10.

- Sente A, Peer R, Srivastava A, Baidya M, Lesk AM, Balaji S, et al. Molecular mechanism of modulating arrestin conformation by GPCR phosphorylation. *Nat Struct Mol Biol* 2018;25:538-45.
- Vibhuti A, Gupta K, Subramanian H, Guo Q, Ali H. Distinct and shared roles of beta-arrestin-1 and beta-arrestin-2 on the regulation of C3a receptor signaling in human mast cells. *PLoS One* 2011;6:e19585.
- Jolly PS, Bektas M, Olivera A, Gonzalez-Espinosa C, Proia RL, Rivera J, et al. Transactivation of sphingosine-1-phosphate receptors by FcεRI triggering is required for normal mast cell degranulation and chemotaxis. *J Exp Med* 2004;199: 959-70.
- Oskeritzián CA, Hait NC, Wedman P, Chumanevich A, Kolawole EM, Price MM, et al. The sphingosine-1-phosphate/sphingosine-1-phosphate receptor 2 axis regulates early airway T-cell infiltration in murine mast cell-dependent allergic responses. *J Allergy Clin Immunol* 2015;135:1008-18.e1.
- Schafer B, Piliposky AM, Oka T, Song CH, Gerard NP, Gerard C, et al. Mast cell anaphylatoxin receptor expression can enhance IgE-dependent skin inflammation in mice. *J Allergy Clin Immunol* 2013;131:541-8, e1-9.
- Takamori A, Izawa K, Kaitani A, Ando T, Okamoto Y, Maehara A, et al. Identification of inhibitory mechanisms in pseudo-allergy involving Mrgprb2/MRGPRX2-mediated mast cell activation. *J Allergy Clin Immunol* 2019;143: 1231-5.e12.
- Galand C, Leyva-Castillo JM, Yoon J, Han A, Lee MS, McKenzie ANJ, et al. IL-33 promotes food anaphylaxis in epicutaneously sensitized mice by targeting mast cells. *J Allergy Clin Immunol* 2016;138:1356-66.

Available online May 9, 2019.

<https://doi.org/10.1016/j.jaci.2019.04.024>

Combined immunodeficiency in a patient with c-Rel deficiency



To the Editor:

c-Rel is a transcription factor in the nuclear factor κB (NF-κB) family that is critical for T- and B-cell function. c-Rel binds to the promoters of genes that encode cytokines important for immunity against infectious pathogens, including IL-2, IFN-γ, IL-12, IL-21, and IL-23.¹ Rel-deficient mice have impaired T- and B-cell proliferation, hypogammaglobulinemia, and cytokine secretion.²⁻⁵ Human patients with mutations in c-Rel have not been previously published. Here we describe a homozygous mutation abrogating c-Rel expression in a patient with impaired T- and B-cell activation, susceptibility to opportunistic infections, and *Mycobacterium tuberculosis*.

The patient is the son of first-degree cousins of Arabic descent. He received live vaccinations against poliovirus, measles, and BCG without clinical sequelae. At 30 months of age, he had chronic diarrhea associated with multiple infections, including *Salmonella enterica*, cytomegalovirus, and *Cryptosporidium* species. In the setting of chronic cryptosporidiosis, he had hepatomegaly and sclerosing cholangitis. Immunologic evaluation at 6 years of age was notable for leukocytosis, lymphocytosis, and thrombocytosis (Table I). He had increased numbers of CD4⁺ and CD8⁺ T cells with decreased memory CD4⁺CD45RO⁺ T-cell counts and reduced proliferation to PHA (Table I). He had B-cell lymphopenia, impaired B-cell proliferation on stimulation with CD40 ligand plus IL-21, reduced IgG levels, undetectable IgA levels, and non-protective titers to the tetanus and diphtheria vaccines despite booster vaccinations (Table I). Despite vaccination with BCG, he had femoral *M tuberculosis* osteomyelitis at 7 years of age that was successfully treated with isoniazid, rifampin, pyrazinamide, and ethambutol. He also had 1-lobe pneumonia confirmed by chest radiography. Additional immunophenotyping done at 9 years of age revealed a predominance of naive CD19⁺IgD⁺CD27⁻ B cells (85%; normal, 47.3% to 77%) and reduced percentages

TABLE I. Immunologic profile of the proband at 6 years of age

Hemogram (10 ³ cells/μL)	6 y
White blood cells	12,600 (5,700-9,900)
Neutrophils	5,800 (2,800-6,300)
Lymphocytes	5,900 (1,200-4,700)
Platelets	447,000 (227,000-350,000)
Lymphocyte subpopulations	
CD3 ⁺ , 10 ³ cells/μL	4,969 (1,200-2,600)
CD3 ⁺ CD45RO ⁺ , % CD3 ⁺	10.3% (15-41)
CD3 ⁺ CD4 ⁺ , 10 ³ cells/μL	1,843 (650-1,500)
CD3 ⁺ CD8 ⁺ , 10 ³ cells/μL	2,483 (370-1,100)
CD19 ⁺ , 10 ³ cells/μL	103 (270-860)
CD3 ⁻ CD56 ⁺ , 10 ³ cells/μL	70 (100-480)
T-cell proliferation (% control)	
PHA	47.3%
PMA + ionomycin	108%
B-cell proliferation (% control)	
CD40L + IL-21	6.4%
Serum immunoglobulins (normal)	
IgG (mg/dL)	150 (650-1,150)
IgM (mg/dL)	150 (50-150)
IgA (mg/dL)	Undetectable (40-160)
Anti-diphtheria (IU/mL)	0.015 (>0.1)
Tetanus (IU/mL)	0.041 (>0.1)

Boldface values are outside the normal range. Age-matched normal ranges are shown in parentheses.

of switched memory CD19⁺CD27⁺IgD⁻ B cells (0.3%; reference range, 10.0% to 30.4%). He is currently being treated with intravenous immunoglobulin and prophylactic antibiotics while undergoing evaluation for hematopoietic stem cell transplantation.

Whole-exome sequencing of genomic DNA from the proband identified 80 rare variants in the proband, which were absent from the gnomAD and 1000 Genomes databases (see Table E1 in this article's Online Repository at www.jacionline.org). Of these, a homozygous mutation in the canonical donor splice site after exon 5 of *REL* (NM_002908.3: c.535+1G>A) was considered the most likely pathogenic variant because of the known contribution of c-Rel in T- and B-cell activation and cytokine secretion. Sanger sequencing confirmed that the mutation is homozygous in the proband and heterozygous in his parents and healthy brother (see Fig E1 in this article's Online Repository at www.jacionline.org). Amplification of the patient's *REL* cDNA using primers specific to sequences in exons 2 and 7 identified an alternatively spliced transcript with lower expression than that of wild-type *REL* from control cDNA (Fig 1). Sanger sequencing of the patient's mutant *REL* transcript indicated that the mutant uses a cryptic donor splice site in exon 5 (NM_002908.3: c.483) and a cryptic acceptor splice site near the start of exon 6 (NM_002908.3: c.538; Fig 1, B, top). The mutant transcript lacks 54 nucleotides encoding 18 residues within the Rel homology domain (Fig 1, B, bottom). If expressed, the molecular mass of the mutant c-Rel protein is predicted to be 66 kDa. However, immunoblotting using an antibody specific to the protein's N-terminus demonstrated the absence of c-Rel protein in the patient's PBMCs with no detectable truncation products, indicating that the mutation abrogates protein expression (Fig 1, C).

The patient's history of infections with cytomegalovirus and cryptosporidiosis with secondary sclerosing cholangitis indicated defective T-cell function. Mice deficient in c-Rel have impaired