

# Human RIPK1 deficiency causes combined immunodeficiency and inflammatory bowel diseases

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Receptor-interacting serine/threonine-protein kinase 1 (RIPK1) is a critical regulator of cell death and inflammation, but its relevance for human disease pathogenesis remains elusive. Studies of monogenic disorders might provide critical insights into disease mechanisms and therapeutic targeting of RIPK1 for common diseases. Here, we report on eight patients from six unrelated pedigrees with biallelic loss-of-function mutations in *RIPK1* presenting with primary immunodeficiency and/or intestinal inflammation. Mutations in *RIPK1* were associated with reduced NF- $\kappa$ B activity, defective differentiation of T and B cells, increased inflammasome activity, and impaired response to TNFR1-mediated cell death in intestinal epithelial cells. The characterization of RIPK1-deficient patients highlights the essential role of RIPK1 in controlling human immune and intestinal homeostasis, and might have critical implications for therapies targeting RIPK1.

primary immunodeficiency | inflammatory bowel diseases | rare diseases

Single-gene inborn errors of immunity underlie diverse pathologies such as infection, allergy, autoimmunity, autoinflammation, and malignancy. Until now, the discovery of more than 350 monogenic immune disorders has opened unprecedented insights into genes and pathways orchestrating differentiation and function of the human immune system (1). Very early onset inflammatory bowel diseases (VEO-IBDs) may also result from inborn errors of immunity, as evidenced by IL-10R deficiency (2). Although the spectrum of monogenic disorders affecting the intestinal immune homeostasis has recently expanded, most patients with VEO-IBDs lack a genetic diagnosis. It is of great therapeutic relevance to define underlying genetic defects: Whereas disorders of the hematopoietic system can be cured by allogeneic hematopoietic stem cell transplantation (HSCT), intrinsic defects in epithelial or stromal cells require other therapeutic strategies. The discovery of patients with monogenic diseases highlights the functional relevance of genes and pathways, provides a basis for the development of targeted therapies for both rare and common diseases, and may add to a critical appraisal of anticipated effects or side effects of therapies (3).

The receptor-interacting serine/threonine-protein kinase 1 (RIPK1) is a key signaling molecule controlling inflammation and cell death responses through both scaffolding- and kinase-specific

## Significance

RIPK1 is a key signaling molecule controlling inflammation and cell death. Molecular targeting of RIPK1 is considered to be an attractive therapeutic strategy for inflammatory diseases or autoimmunity. However, the precise function of RIPK1 in human health and disease remains a matter of debate. Here, we report that human RIPK1 deficiency results in both immune and intestinal epithelial cell dysfunctions. Our studies provide insights into the pleiotropic functions of human RIPK1 and warrant awareness about potential toxicities of therapeutic strategies targeting RIPK1.

Author contributions: Y. Li, M. Führer, E.B., K.S., C.K., and D.K. designed research; Y. Liu, M.M.G., and V.H. contributed new reagents/analytic tools; Y. Li, M. Führer, and E.B. conducted experiments and analyzed the data; Y. Li, C.K., and D.K. wrote the paper; P.S., M.K.-D., A.B., S.M., E.S., D.T., R. Boukari, R. Belbouab, E.A.I., H.A.A., F.E.S., M.G., G.d.S.B., M.H., S.K., A.M.M., and S.B.S. recruited patients with RIPK1 deficiency; P.S., M.K.-D., A.B., S.M., E.S., D.T., R. Boukari, R. Belbouab, E.A.I., H.A.A., F.E.S., M.G., G.d.S.B., M.H., S.K., A.M.M., and S.B.S. were critical in the interpretation of the clinical data; Y. Liu assisted in CRISPR/Cas9-mediated genetic engineering; A.S.L. supported analysis of inflammasome activity; T.M. conducted T cell proliferation assays; M.R. performed whole exome sequencing; S.H., M. Field, N.W., U.P., and K.S. conducted the bioinformatics analysis of sequencing data; R.C. and M.H. supported immunophenotypic analysis; B.B. conducted the electrophoretic mobility-shift assays; C.W. performed pathological analysis; M.M.G. and V.H. provided the RIPK1-deficient BLaER1 cell model and experimental expertise; K.S., C.K., and D.K. conceived the study design and supervised Y. Li, M. Führer, and E.B.; and K.S., C.K., and D.K. recruited study participants.

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Data deposition: The identified *RIPK1* mutations reported in this paper have been deposited in the ClinVar database, <https://www.ncbi.nlm.nih.gov/clinvar/> (accession nos.: SCV000854770–SCV000854774). Information on the raw whole-exome sequencing data is not published to protect research participant privacy.

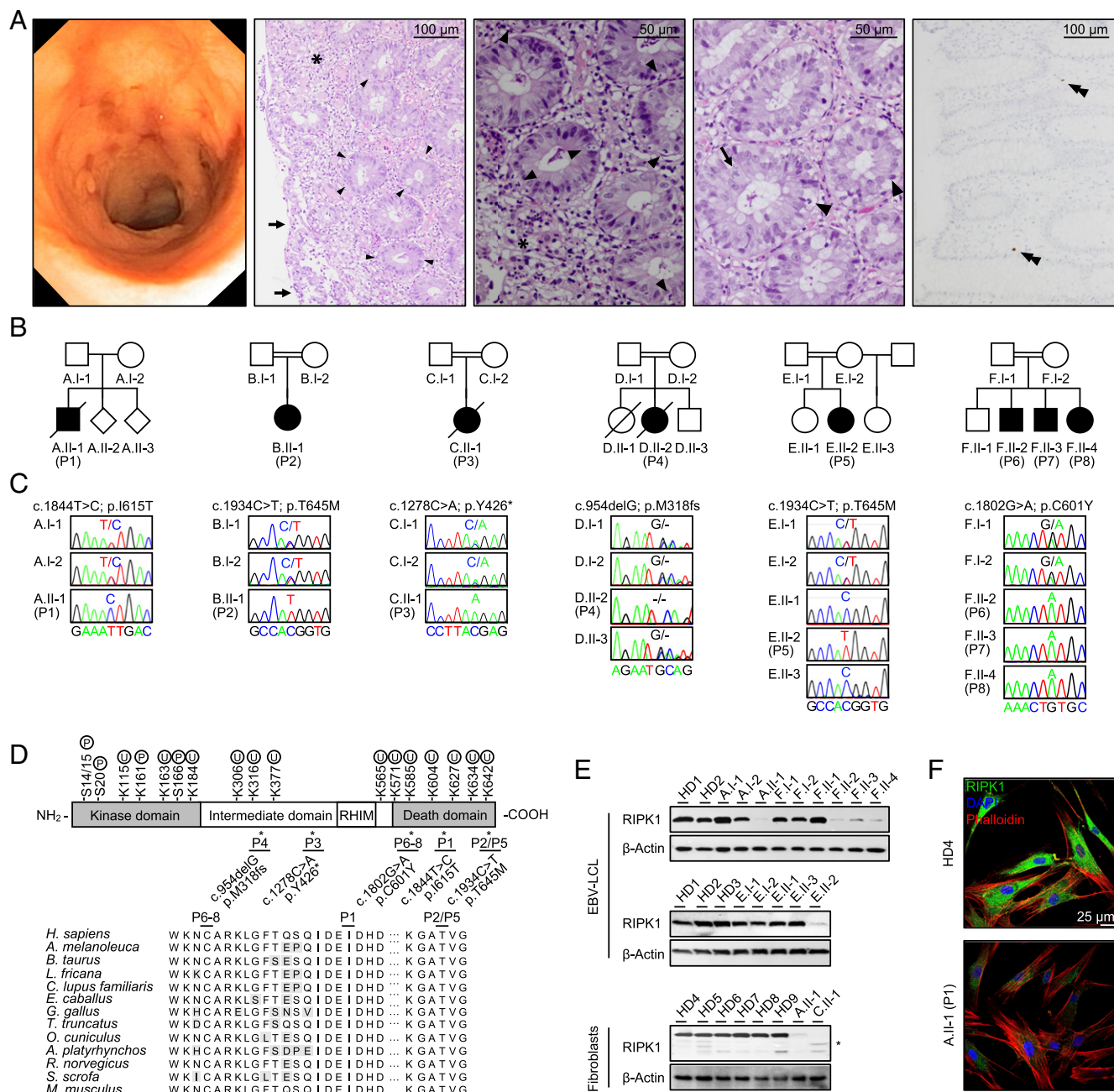
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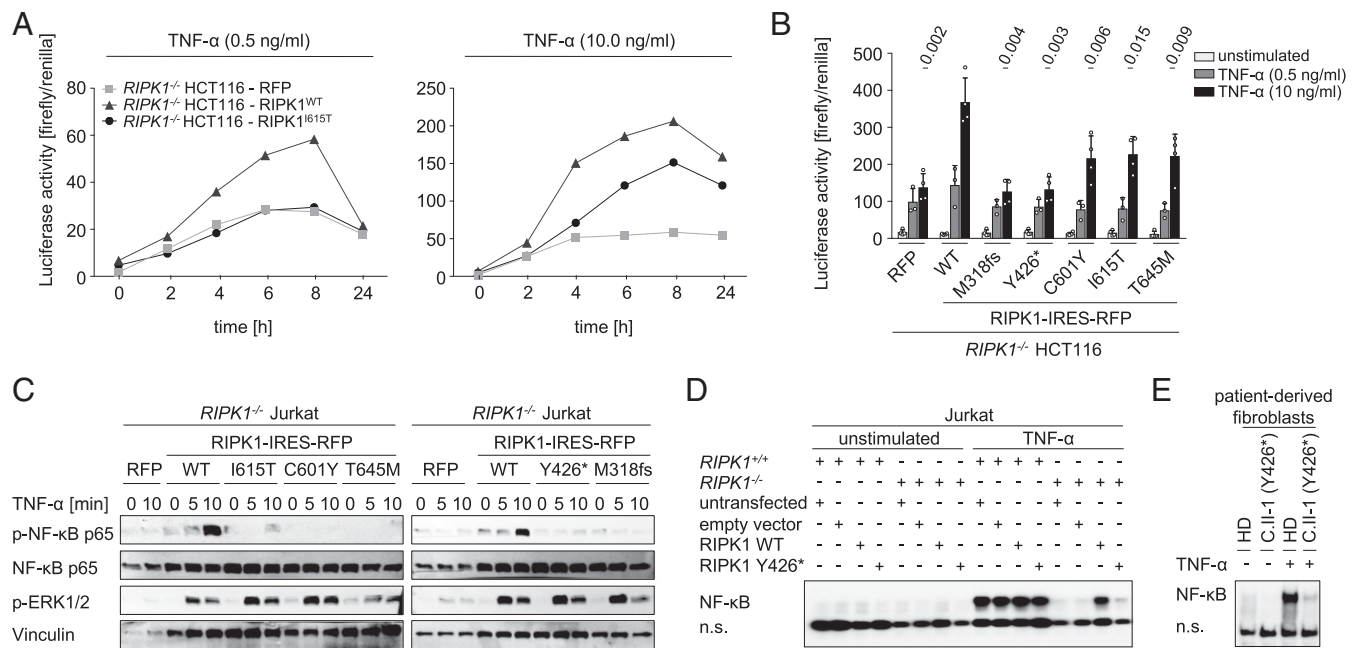


**Fig. 1.** Identification of biallelic *RIPK1* mutations in patients with combined immunodeficiency and pediatric inflammatory bowel disease. (A) Colonoscopy showing pancolitis with ulcers and granuloma in P1 (Left). Histology of colonic biopsies from P1 revealed chronic-active inflammation (asterisk) with erosions of the mucosal surface (arrow) (second image from Left) and epithelial degeneration. Higher magnification displays epithelial regeneration with increased mitotic activity (arrow) and apoptotic bodies (arrowhead) within the crypt epithelium (third and fourth images from the Left). Multiple myeloma oncogene 1 immunohistochemistry indicated subtotal depletion of plasma cells (Right, double arrowhead). (B) Pedigrees of six families (A to F) with patients (P1 to P8) presenting with primary immunodeficiencies and/or VEO-IBDs. (C) Sanger sequencing confirmed segregation of the biallelic *RIPK1* mutations with the disease phenotype in available first-degree relatives. (D) Schematic illustration of the *RIPK1* protein domain architecture (NM\_003804.3, NP\_003795.2). Alignment of the human *RIPK1* protein sequence showed that the mutated amino acids are conserved in orthologs. RHIM, RIP homotypic interaction motif. (E) Immunoblotting of three independent experiments revealing reduced protein expression of *RIPK1* in patient-derived EBV-transformed B cells (P1, P5, P6, P7, P8) or fibroblast cell lines (P1, P3) in contrast to healthy donors (HDs) or patients' relatives. Truncated *RIPK1* protein expression in P3 is indicated by an asterisk. (F) Representative confocal immunofluorescence microscopy images confirming reduced expression of *RIPK1* in fibroblasts from P1, compared with HDs.

functions. In particular, *RIPK1* is known to mediate multimodal signaling downstream of *TNFR1* depending on cell type and biological context (4). While  $\text{TNF-}\alpha$ -induced  $\text{NF-}\kappa\text{B}$  nuclear translocation promotes cell survival and inflammatory signaling, modulation of intracellular signaling cascades can also induce caspase-8 (*CASP8*)-mediated apoptosis or *RIPK3*-dependent necroptosis in the

absence of *CASP8* (4). The exact mechanisms controlling the multimodal transition switches from *RIPK1*-mediated cell survival and inflammation to cell death remain largely unknown.

Mice with constitutive deletion of *Ripk1* die perinatally due to hyperinflammation and increased sensitivity to  $\text{TNF-}\alpha$ -induced cell death and *RIPK3*-mediated necroptosis (5, 6). Depending on



**Fig. 2.** Biallelic loss-of-function *RIPK1* mutations lead to impaired NF- $\kappa$ B-mediated signaling. (**A** and **B**) Representative luciferase reporter assays showed reduced NF- $\kappa$ B activation upon TNF- $\alpha$  stimulation in HCT-116 cells with *RIPK1* KO or lentiviral-mediated overexpression of *RIPK1* mutants, compared with WT *RIPK1*. Data shown represent the mean  $\pm$  SD. (**C**) Representative immunoblotting ( $n = 3$ ) of serum-starved *RIPK1*<sup>-/-</sup> Jurkat cells with transgenic expression of mutant *RIPK1* variants revealed decreased phosphorylation of the NF- $\kappa$ B p65 subunit (Ser536) in response to TNF- $\alpha$  (50 ng/mL), whereas phosphorylation of ERK1/2 was normal. (**D** and **E**) Representative EMSA ( $n = 3$ ) showed reduced DNA-binding activity of the NF- $\kappa$ B p65 subunit in Jurkat cells overexpressing the *RIPK1* mutation Y426\* (**D**) or fibroblasts derived from P3 (**E**) after TNF- $\alpha$  stimulation (50 ng/mL) for 30 min. n.s., nonspecific bands.

the context, murine *RIPK1* deficiency might be associated with increased sensitivity to both *RIPK3*-dependent necroptosis and TNF- $\alpha$ - and/or *CASP8*-dependent apoptosis (5–7). Studies on conditional *Ripk1* knockout (KO) mice have demonstrated that *RIPK1* plays a critical role in controlling skin and intestinal inflammation, autoimmunity, and tissue fibrosis (8–11). *RIPK3*–*MLKL*-dependent necroptosis has been described as a common pathomechanism. However, the triggers and ligands relevant for activation of the necroptotic pathway in vivo remain poorly understood. Furthermore, *RIPK1* has also been implicated in murine hematopoiesis (12), T and B cell homeostasis (13, 14), and inflammasome activity (5).

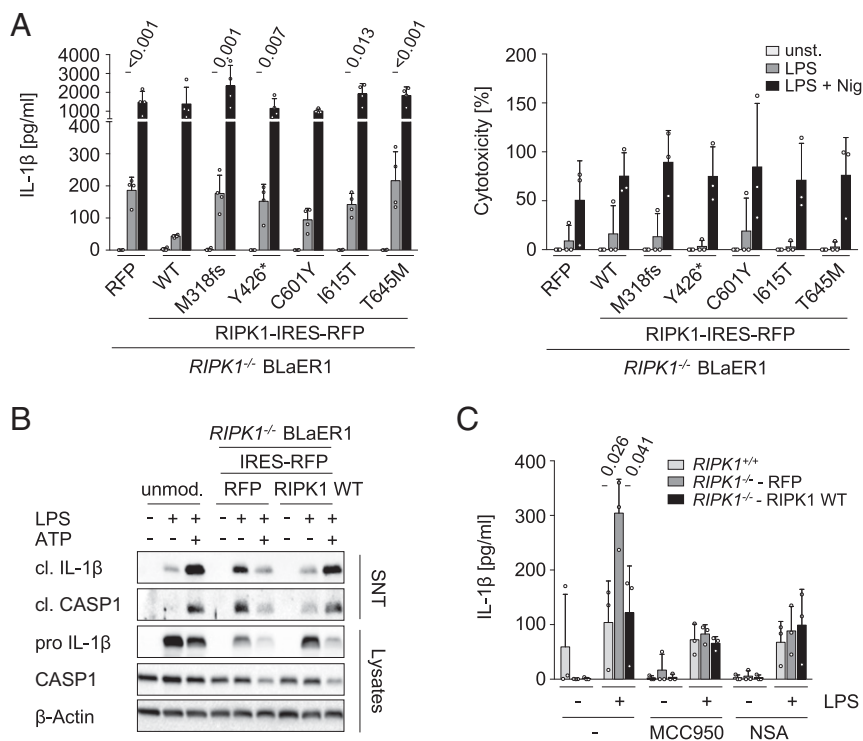
A pathogenic role of *RIPK1* has been previously linked to multiple mouse models of disease, including colitis, skin inflammation, myocardial infarction, atherosclerosis, pancreatitis, and viral infections, as well as liver, retinal, and renal injury (15). Pharmacological inhibition of *RIPK1* has been shown to block necroptosis and protect from ischemic organ damage (16). Small-molecule inhibitors of *RIPK1* activity are currently being evaluated for patients with psoriasis, rheumatoid arthritis, and ulcerative colitis (17). Recently, *RIPK1* has also been implicated in tumorigenesis and proposed as a therapeutic target in melanoma (18), colon cancer (19), and leukemia (20). However, the relevance of *RIPK1* for human pathogenesis and the balance of anticipated effects and side effects of targeting *RIPK1* are still discussed controversially.

Here, we report that biallelic loss-of-function mutations in human *RIPK1* cause impaired innate and adaptive immunity and predispose to VEO-IBD.

## Results

**Identification of Patients with Biallelic Mutations in *RIPK1*.** Our index patient (P1 (A.II-1)) born to Caucasian parents from Poland presented with VEO-IBD characterized by growth failure, abdominal pain, chronic mucous and bloody diarrhea, oral aphthous lesions, and perianal lesions at the age of 6 mo. Endoscopy

confirmed the diagnosis of pancolitis with ulcers and granuloma (Fig. 1A, *Left*), esophagitis, and gastric ulcers. Histology of gastric and colonic biopsies revealed chronic-active inflammation with erosions (Fig. 1A, second panel from *Left*), increased apoptotic bodies within the cryptic bases (Fig. 1A, third and fourth panels from *Left*), and subtotal depletion of lamina propria plasma cells (Fig. 1A, *Right*). Extraintestinal manifestations included hepatosplenomegaly, maculopapular skin and transient atopic skin lesions, recurrent fever, and infections (pneumonia, conjunctivitis), including episodes of deep-seated infections and sepsis in the neonatal phase. He showed a refractory course despite amino acid-based formula, parenteral nutrition, antibiotics, steroids, azathioprine, and ileostomy and succumbed to septicemia at the age of 4 y. To decipher the molecular cause of disease in this patient, we have conducted whole-exome sequencing and found a rare homozygous missense mutation in the *RIPK1* gene (NM\_003804.3, c.1844T>C; NP\_003795.2, p.I615T) (Fig. 1B and C). Further screening for biallelic *RIPK1* mutations in more than 1,942 patients with VEO-IBDs and/or primary immunodeficiencies identified another seven patients from five unrelated pedigrees with homozygous germ-line mutations in *RIPK1* (Fig. 1B and C). The sequence variants in *RIPK1* have been deposited in the ClinVar database (21) (accession nos.: SCV000854770–SCV000854774). While P3 (c.1278C>A, p.Y426\*) and P4 (c.954delG, p.M318fsTer194) primarily manifested with combined immunodeficiency associated with lymphopenia, P2 (c.1934C>T, p.T645M), P5 (c.1934C>T, p.T645M), P6 (c.1802G>A, p.C601Y), P7 (c.1802G>A, p.C601Y), and P8 (c.1802G>A, p.C601Y) were primarily referred for genetic testing due to signs of VEO-IBD. All patients suffered from recurrent bacterial and/or viral infections and had episodes of diarrhea. Perianal disease was reported in all patients except for P3. Further clinical details for the patients are summarized in *SI Appendix, Table S1*. Segregation of the *RIPK1* mutations with the disease phenotype could be confirmed by Sanger sequencing of available first-degree family members (Fig. 1C). In silico analysis using PolyPhen (22) and SIFT (23) predicted that the identified missense mutants in *RIPK1* are deleterious. These homozygous mutations have not been previously reported in the



**Fig. 3.** RIPK1 deficiency is associated with increased inflammasome activity upon LPS priming. (A) ELISA showed increased release of IL-1 $\beta$  upon LPS priming (20 ng/mL, 3 h) in conditioned media from heterologous BLaER1 cells with *RIPK1* KO or overexpression of *RIPK1* mutant variants. *P* values are analyzed in comparison with cells expressing WT *RIPK1* (Left). No difference in inflammasome activation of *RIPK1*-deficient macrophages has been observed upon LPS + nigericin in contrast to WT *RIPK1*. Data are representative of four independent experiments. LDH release assays of three independent experiments showed no difference of cytotoxicity between *RIPK1* WT and mutant BLaER1 cells upon stimulation with LPS  $\pm$  nigericin (Right). (B) Representative immunoblotting ( $n = 4$ ) of heterologous *RIPK1*-deficient BLaER1 cells confirmed increased secretion of the mature IL-1 $\beta$  and cleavage of CASP1 in comparison with unmodified or WT *RIPK1*-expressing cells (LPS, 200 ng/mL, 14 h). (C) Analysis of IL-1 $\beta$  release in *RIPK1*<sup>-/-</sup> BLaER1 cells overexpressing RFP or WT *RIPK1* upon treatment with LPS  $\pm$  small-molecule inhibitors for NLRP3 (MCC950) or MLKL (NSA) (three independent experiments). Data shown in A and C represent the means  $\pm$  SD. cl., cleaved; SNT, supernatant.

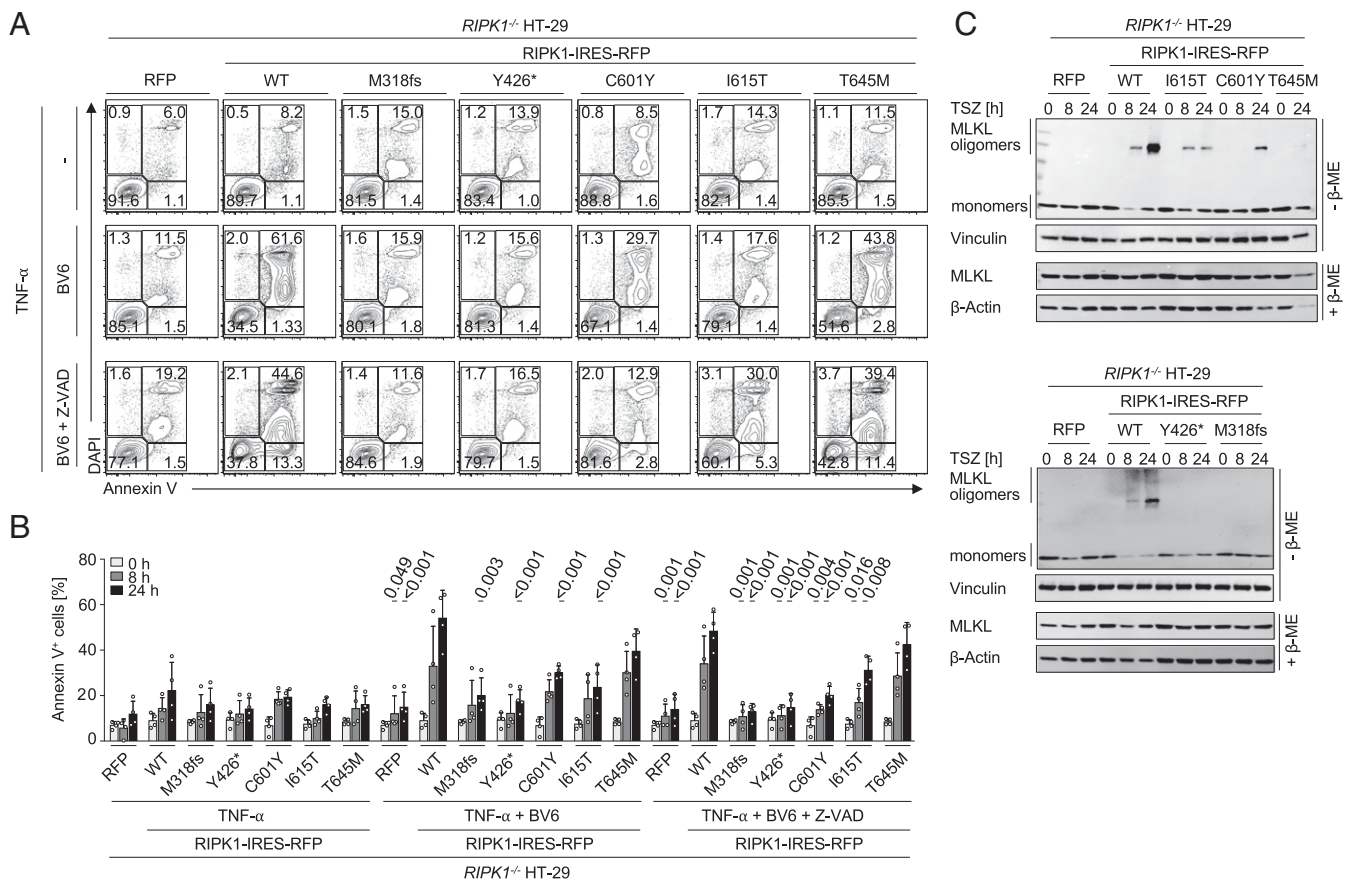
Genome Aggregation Database (24). Sequence homology analysis revealed that the mutated amino acids in the death domain of *RIPK1* are evolutionarily conserved (Fig. 1D). Immunoblotting of EBV-transformed lymphoblastoid cell lines (EBV-LCLs) from pedigrees A (P1), E (P5), and F (P6, P7, P8) and primary fibroblasts from pedigrees A (P1) and C (P3) (Fig. 1E), as well as confocal immunofluorescence microscopy of fibroblasts from pedigree A (P1) (Fig. 1F), demonstrated a reduced protein expression of mutated *RIPK1*. P3 carrying a frameshift mutation in *RIPK1* showed reduced expression of a truncated protein.

P3 and P4 presented with lymphopenia affecting T and B cells (SI Appendix, Table S2). Immunophenotyping of peripheral blood mononuclear cells from P1, P6, P7, and P8 showed a decreased frequency of CD45RO<sup>+</sup>CCR7<sup>+</sup> central memory and CD45RO<sup>+</sup>CCR7<sup>-</sup> effector memory CD4<sup>+</sup> and CD8<sup>+</sup> T cells (SI Appendix, Fig. S1A and B), CD45RO<sup>+</sup>HLA-DR<sup>+</sup> memory activated regulatory T cells (SI Appendix, Fig. S1C), and CXCR3<sup>+</sup>CCR6<sup>-</sup> T-helper 1 (Th1) and CXCR3<sup>-</sup>CCR6<sup>+</sup> T-helper 17 (Th17) populations (SI Appendix, Fig. S1D), as well as IgD<sup>+</sup>CD27<sup>+</sup> class-switched B cells (SI Appendix, Fig. S1E), whereas P5 exhibited no measurable changes in these parameters (SI Appendix, Table S3). These data suggest that *RIPK1* deficiency may lead to combined T and B cell dysfunction. However, T cell proliferation, activation, and cell death in response to anti-CD3, anti-CD3/CD28, or anti-PMA/ionomycin were normal. In addition, we could not observe a significant difference in cell death in *RIPK1*-deficient Jurkat cells upon treatment with FAS ligand, TNF- $\alpha$   $\pm$  BV6 (the second mitochondrial activator of apoptosis mimetic), or TNF- $\alpha$   $\pm$  cycloheximide in comparison with *RIPK1* wild-type (WT) reconstituted cells (SI Appendix, Fig. S2).

**Defective TNF- $\alpha$ -Mediated NF- $\kappa$ B Signaling in *RIPK1*-Deficient Cells.** *RIPK1* regulates multimodal signaling downstream of TNFR1 in a cell- and context-dependent manner (25). To assess the consequences of identified mutations for *RIPK1* downstream signaling, we engineered colon carcinoma-derived HCT-116 cells with a CRISPR/Cas9-mediated *RIPK1* KO and subsequent lentiviral overexpression of WT or mutant *RIPK1* variants. NF- $\kappa$ B luciferase reporter assays showed that cells expressing the *RIPK1* variant I615T (identified in P1) exhibited impaired NF- $\kappa$ B activity in

response to TNF- $\alpha$ , compared with cells with WT *RIPK1* (Fig. 2A). Similarly, we could detect reduced luciferase activity for all five identified *RIPK1* mutants after TNF- $\alpha$  stimulation (Fig. 2B). Correspondingly, immunoblotting revealed reduced phosphorylation of the NF- $\kappa$ B p65 subunit (Ser536) in Jurkat cells with *RIPK1* KO or expression of mutant *RIPK1* (Fig. 2C), whereas phosphorylation of ERK1/2 (Thr202/Tyr204) was normal. Electrophoretic mobility-shift assays confirmed reduced NF- $\kappa$ B DNA-binding activity in Jurkat cells expressing the *RIPK1* mutant Y426\* (Fig. 2D) and fibroblasts of P3 (Fig. 2E) in response to TNF- $\alpha$ , compared with WT *RIPK1* reconstituted Jurkat cells and healthy donor fibroblasts, respectively. These data indicate that the identified mutations in *RIPK1* are associated with impaired TNF- $\alpha$ -induced NF- $\kappa$ B signaling.

**Altered Inflammasome Activity in *RIPK1*-Deficient Macrophages.** Previous studies have documented an altered inflammasome activity in conditional *Ripk1* KO mice (5, 26). To examine effects of the identified *RIPK1* mutations on inflammasome activity, we have adapted a BLaER1 monocyte cell model with KO of *CASP4* and *RIPK1* (27) and reconstituted the patients' mutations by lentiviral gene transfer. In contrast to cells with reconstitution of WT *RIPK1*, cells with KO of *RIPK1* or overexpression of the *RIPK1* mutants (M318fs, Y426\*, I615T, and T645M) showed increased IL-1 $\beta$  secretion without the requirement of a secondary stimulus for the processing of mature IL-1 $\beta$  (Fig. 3A). Increased inflammasome activity in *RIPK1*-deficient macrophages was not associated with increased cytotoxicity upon LPS priming for 3 h, as indicated by the LDH assay (Fig. 3A). Of note, no difference of IL-1 $\beta$  secretion could be observed upon addition of nigericin between cells with overexpression of WT and *RIPK1* mutants (Fig. 3A). Immunoblotting confirmed increased release of mature IL-1 $\beta$  upon treatment with LPS in *RIPK1*-deficient macrophages (Fig. 3B). To test whether the altered IL-1 $\beta$  release is associated with increased NLRP3 activity and/or MLKL-dependent necroptosis in human *RIPK1* deficiency, we assessed the inflammasome activation upon treatment with small-molecule inhibitors of NLRP3 (MCC950) and MLKL (NSA) (Fig. 3C). The inhibitors reduced IL-1 $\beta$  secretion in LPS-stimulated *RIPK1*-deficient macrophages, suggesting that both



**Fig. 4.** RIPK1-deficient intestinal epithelial cells show altered cell death responses. (A) Representative FACS analysis of Annexin V/DAPI staining ( $n = 4$ ) in HT-29 cells expressing mutant RIPK1 upon 24 h of treatment with TNF- $\alpha$   $\pm$  BV6  $\pm$  Z-VAD-FMK. (B) Graphical representation ( $n = 4$ ) showing decreased frequencies of Annexin V<sup>+</sup> RIPK1-deficient cells compared with cells with WT RIPK1 after stimulation with TNF- $\alpha$  + BV6  $\pm$  Z-VAD-FMK. (C) Representative SDS/PAGE under nonreducing conditions ( $n = 3$ ) revealed reduced MLKL oligomerization upon treatment with TNF- $\alpha$  + BV6 + Z-VAD-FMK in RIPK1-deficient cells. Data shown represent the means  $\pm$  SD. ME, mercaptoethanol; TSZ, TNF- $\alpha$  + BV6 (SMAC mimetic) + Z-VAD-FMK.

pathways might be implicated in dysregulation of proinflammatory responses. Taken together, our results suggest that human RIPK1 plays a critical role in regulating LPS-mediated inflammasome activation.

**Impaired TNF- $\alpha$ -Mediated Cell Death Responses in RIPK1-Deficient Epithelial Cells.** RIPK1 and RIPK3 are critical regulators of cell death (28). To study the effect of patients' mutations on TNF- $\alpha$ -mediated cell death responses in epithelial cells, we engineered HT-29 colon carcinoma cells with KO of *RIPK1* and lentiviral reconstitution of WT or mutant RIPK1 variants. No alteration of cell death could be observed upon treatment with TNF- $\alpha$  in RIPK1-deficient HT-29 cells (Fig. 4 A and B). However, cell death responses were impaired upon treatment with TNF- $\alpha$  and BV6  $\pm$  the pan caspase inhibitor Z-VAD-FMK in cells expressing mutated RIPK1 variants (M318fs, Y426\*, C601Y, and I615T) compared with cells overexpressing WT RIPK1 (Fig. 4 A and B). Correspondingly, immunoblotting showed reduced MLKL oligomerization in RIPK1-deficient HT-29 cells in response to TNF- $\alpha$ , BV6, and Z-VAD-FMK, suggesting impaired necroptosis under conditions of RIPK1 deficiency (Fig. 4C).

## Discussion

The functional relevance of RIPK1 in human disease has been controversially discussed. We report RIPK1 deficiency as a Mendelian disorder predisposing to immunodeficiency and severe colitis. Whereas it may appear counterintuitive at first sight to associate immunodeficiency and hyperinflammatory

responses, several monogenic diseases have a poorly understood Janus-faced appearance, for example autoimmune lymphoproliferative syndrome caused by TNFRSF6 (29) and CASP10 (30) deficiency or lymphoproliferation and autoimmunity caused by *IL2RA* null mutations (31).

Constitutive *Ripk1* KO mice appear to exhibit no developmental defects but show perinatal mortality associated with systemic multiorgan inflammation and apoptosis in lymphoid and adipose tissues (32). A potential role of RIPK1 in pathogenesis has been documented in several models of inflammation and tissue damage (16). In particular, conditional ablation of *Ripk1* has been reported to result in severe intestinal and skin inflammation associated with FADD-CASP8-dependent apoptosis of intestinal epithelial cells and ZBP1-RIPK3-MLKL-dependent necroptosis of keratinocytes, respectively (8, 11). Our patients with homozygous mutations in *RIPK1* showed no obvious developmental defects, and predominantly presented with immunodeficiency and diarrhea or colitis. Whereas children with complete loss of function of RIPK1 (P3, stop-gain mutation; P4, frameshift mutation) primarily manifested with combined immunodeficiency and diarrhea, patients with missense mutations in the death domain of RIPK1 were referred for genetic testing due to IBD-like conditions. Differences in clinical manifestation might be reflective of genotype-phenotype correlations and incomplete penetrance, or may be due to secondary factors such as genetic modifiers, infections, and treatment. Emerging evidence suggests that kinase-independent RIPK1 functions are critical in controlling intestinal epithelial homeostasis (5, 6, 8, 11). None of our identified patients had mutations directly affecting the

kinase domain of RIPK1, but the identified mutations perturbed total protein expression. Therefore, our study cannot unequivocally define whether the abrogated kinase activity is critical in mediating intestinal inflammation in our patients.

Mice with *Ripk1* KO in intestinal epithelial cells develop colitis accompanied by disrupted tissue architecture and increased apoptosis (8, 11). In parallel investigations, Cuchet-Lourenço et al. (33) identified four patients with loss-of-function mutations in *RIPK1* causing combined immunodeficiency and intestinal inflammation due to altered cytokine secretion and necroptosis of immune cells. Whereas these authors concluded that allogeneic HSCT may constitute a curative therapy, our studies suggest that RIPK1 plays a critical role in controlling cell death of the intestinal epithelium, and thus warrant awareness that HSCT might dampen intestinal inflammation but not rescue intrinsic intestinal phenotypes of human RIPK1 deficiency, similar to NF- $\kappa$ B essential modulator deficiency (34). The exact triggers perturbing epithelial integrity in RIPK1 deficiency could not be fully determined in our studies or mouse models yet. Further studies are required to shed light on cell- and context-dependent functions of RIPK1 in controlling intestinal inflammation *in vivo*.

Necroptosis has been previously linked to the pathogenesis in various disease models such as atherosclerosis, myocardial infarction, ischemic brain injury, systemic inflammation, liver injury, and neurodegeneration (16). Targeting RIPK1 and RIPK3 represents an attractive therapeutic strategy for diseases with increased necroptotic activity. Necrostatin-1 allosterically inhibits RIPK1 activity and has been shown to block necroptosis in mouse models of ischemia (16). Recently, a small molecule (GSK2982772) has been developed as an inhibitor of RIPK1 to treat plaque-type psoriasis, rheumatoid arthritis, and ulcerative colitis in phase 2a clinical studies (17). The beneficial effects of this therapeutic strategy in patients still remain unclear. Inhibition of RIPK1 activity might be considered in patients with severe or refractory inflammatory or autoinflammatory diseases. Our study on RIPK1-deficient patients highlights that human RIPK1 has

pleiotropic cell- and context-specific functions and thus warrants awareness about potential toxicities of targeting RIPK1.

Taken together, we report that patients with biallelic RIPK1 deficiency present with life-threatening combined immunodeficiency and/or intestinal inflammation associated with impaired lymphocyte functions, increased inflammasome activity, and altered TNF- $\alpha$ -mediated epithelial cell death responses. Thus, our study highlights the central role of RIPK1 in controlling human immunity and intestinal homeostasis.

## Materials and Methods

**Patients.** Peripheral blood and skin biopsies from patients, first-degree family members, and healthy donors were acquired upon written consent. The study was approved by the respective institutional review boards of the University of Ulm, Necker Medical School, and University Hospital, LMU Munich and conducted in accordance with current ethical and legal frameworks.

**Genetic, Immunologic, and Biochemical Analyses.** Methods of genetic analyses, immunological studies, and biochemical and cell biological assays as well as statistics are described in *SI Appendix*.

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