ORIGINAL ARTICLE

IRF8 Mutations and Human Dendritic-Cell Immunodeficiency

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ABSTRACT

BACKGROUND

The genetic analysis of human primary immunodeficiencies has defined the contribution of specific cell populations and molecular pathways in the host defense against infection. Disseminated infection caused by bacille Calmette–Guérin (BCG) vaccines is an early manifestation of primary immunodeficiencies, such as severe combined immunodeficiency. In many affected persons, the cause of disseminated BCG disease is unexplained.

METHODS

We evaluated an infant presenting with features of severe immunodeficiency, including early-onset disseminated BCG disease, who required hematopoietic stem-cell transplantation. We also studied two otherwise healthy subjects with a history of disseminated but curable BCG disease in childhood. We characterized the monocyte and dendritic-cell compartments in these three subjects and sequenced candidate genes in which mutations could plausibly confer susceptibility to BCG disease.

RESULTS

We detected two distinct disease-causing mutations affecting interferon regulatory factor 8 (IRF8). Both K108E and T80A mutations impair IRF8 transcriptional activity by disrupting the interaction between IRF8 and DNA. The K108E variant was associated with an autosomal recessive severe immunodeficiency with a complete lack of circulating monocytes and dendritic cells. The T80A variant was associated with an autosomal dominant, milder immunodeficiency and a selective depletion of CD11c+CD1c+ circulating dendritic cells.

CONCLUSIONS

These findings define a class of human primary immunodeficiencies that affect the differentiation of mononuclear phagocytes. They also show that human IRF8 is critical for the development of monocytes and dendritic cells and for antimycobacterial immunity. (Funded by the Medical Research Council and others.)

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The discovery of human primary immunodeficiencies that affect the development of granulocytes, B cells, and T cells has been instrumental in defining the contribution of these cell types to protective immunity.[1,2] Monocytes, macrophages, and dendritic cells — all mononuclear phagocytes — have essential functions in both innate and acquired immunity. These cells initially recognize and engulf invading microbes, produce proinflammatory cytokines (e.g., interleukin-12), and process antigens for presentation to naive T cells, which consequently secrete various lymphokines (e.g., interferon-γ).[3,4] On activation by cytokines secreted by T cells, mononuclear phagocytes destroy ingested microorganisms. There are no known genetic causes of primary immunodeficiencies affecting the development of the mononuclear phagocyte.

Bacille Calmette–Guérin (BCG) vaccines and environmental mycobacteria are efficiently destroyed by T-cell–activated macrophages.[5] However, disseminated mycobacterial disease after BCG vaccination is a sign of immunodeficiency.[6] It occurs in children with severe combined immunodeficiency (i.e., an absence of T cells) or with chronic granulomatous disease. Although such children are vulnerable to multiple infections, persons with mendelian susceptibility to mycobacterial disease (MSMD)[7] have a narrow vulnerability to poorly virulent mycobacteria, including BCG. Some persons with MSMD harbor genetic defects in the circuit involving interleukin-12 and interferon-γ, with mutations in *IL12B*, the gene encoding interleukin-12B (also called interleukin-12p40), along with the interleukin-12 receptor β1 (IL12RB1), both subunits of the receptor for interferon-γ and its signaling partner STAT1, the nuclear factor-κB regulator IKBKG (NEMO), and the effector CYBB (GP91-PHOX).[7]

We hypothesized that certain persons may be vulnerable to BCG because of mutations impairing the development of mononuclear phagocytes. Among the genes encoding transcription factors that are essential to the development of mononuclear phagocytes in mice, *IRF8*, encoding interferon regulatory factor 8, stood out as a strong candidate.[8] It is expressed at very high levels in mononuclear phagocytes[9] and regulates both the differentiation of granulocytes and macrophages[10,11] and the development of dendritic cells.[12-15] Acting in heterodimeric complexes with other transcription factors, IRF8 also controls the transcriptional response of mature myeloid cells to interferons and toll-like receptor agonists, a response in which IRF8 binds and transactivates the promoters of *IL12B* and *NOS2*, which encodes inducible nitric oxide synthase.[8] Mice carrying the R294C hypomorphic variant (a mutation that partially compromises protein function) in *Irf8* have a specific dendritic-cell phenotype with loss of CD8α+ lymphoid dendritic cells and CD103+ tissue myeloid dendritic cells, whereas *Irf8* knockout mice (which are devoid of Irf8) also lack plasmacytoid dendritic cells.[13-15] Mice with mutant *Irf8* are susceptible to infection with intramacrophagic pathogens and are hypersusceptible to *Mycobacterium bovis* BCG and *M. tuberculosis* infections.[16,17]

METHODS

STUDY SUBJECTS

A 10-week-old female infant (Subject 1) presenting with disseminated BCG infection after vaccination, oral candidiasis, and cachexia was admitted to the hospital for evaluation of suspected immunodeficiency. (Details about the case are provided in the Supplementary Appendix, available with the full text of this article at NEJM.org.) The infant underwent multiple rounds of aggressive antibiotic treatment that were only partially effective in improving her health. She was successfully treated by means of transplantation with cord-blood stem cells. She was the second-born child of healthy, unrelated parents of Irish ancestry (kindred A); the elder sibling was well, and all four family members had received BCG vaccination.

Two additional persons in whom MSMD was diagnosed were also enrolled in the study. The first of these persons (Subject 2 in kindred B) was a 40-year-old man born to nonconsanguineous parents of Italian descent living in Brazil. He was vaccinated with BCG at birth and had recurrent episodes of lymphadenopathies at the age of 15 months, 20 years, and 30 years. During two such episodes, analyses of lymph-node–biopsy samples revealed the presence of acid-fast bacilli. All such episodes were successfully treated with antimycobacterial drug regimens. His mother, father, and sister had been vaccinated with BCG and did not present with clinical infectious disease suggesting immunodeficiency.

The second affected person with MSMD (Subject 3 in kindred C) was a 14-year-old girl born

to a nonconsanguineous family of Italian descent living in Chile. She was also vaccinated with BCG at birth. At 1 year of age, lymphadenopathy with chronic granulomatous tuberculoid lesions developed. At 2 years of age, she presented with multiple lymphadenopathies and fever, requiring hospital admission. Histologic analysis of a lymph-node–biopsy sample and bacterial culture identified pyrazinamide-resistant *M. bovis,* and the child was successfully treated with adjusted antibiotic treatment administered for 12 months; no subsequent clinical episodes were reported.

For all three subjects, we obtained written informed consent from the subjects or their parents. The studies were approved by the institutional review board at each study center. The Italian ancestry of the two subjects living in South America was determined in physicians' interviews with either the subjects or their parents.

GENETIC AND TRANSCRIPTIONAL ANALYSES

We sequenced the exons and selected noncoding sequences of *IRF8* from the subjects' genomic DNA after standard polymerase-chain-reaction amplification with sequence-specific primers (Table S3 in the Supplementary Appendix). We assayed the transcriptional activity of mutant and nonmutant *IRF8* through activation of the *IL12B* and *NOS2* promoters, using reporter constructs that were transiently transfected into RAW 264.7 macrophages (a mouse macrophage cell line), as described previously.[16] Data regarding the methods that were used in biochemical assays, molecular characterization, and statistical analysis are provided in the Supplementary Appendix.

RESULTS

AUTOSOMAL RECESSIVE IRF8 DEFICIENCY

In Subject 1, the 10-week-old infant, the blood count revealed a strikingly abnormal myeloid compartment with an absence of monocytes and a very high neutrophil count (Fig. 1, and Table S1 in the Supplementary Appendix). Analysis of peripheral-blood mononuclear cells (PBMCs) by means of flow cytometry confirmed severe depletion of the nonlymphoid (CD3−CD19−CD56−) HLA-DR+ compartment and in particular a total absence of both CD14+ and CD16+ monocytes (Fig. 1A, 1B, and 1C). Furthermore, we could not detect any dendritic cells in the blood, including both CD11c+ myeloid cells (CD1c+ or CD141+) and CD123+ plasmacytoid cells (Fig. 1A through 1D). The only HLA-DR+ and lineage-negative cells proved to be circulating CD34+ progenitor cells, which were present in elevated numbers (Fig. S1A in the Supplementary Appendix) and correlated with elevated serum levels of FMS-like tyrosine kinase 3 ligand (Fig. S1B in the Supplementary Appendix). In contrast, B cells and natural killer cells were present in normal numbers, thus ruling out the recently described syndrome of a deficiency in dendritic cells, monocytes, and B and natural killer cells[18,19] (Table S2 and Fig. S2 in the Supplementary Appendix).

Assays of whole blood showed that the production of interleukin-12 in response to BCG, phytohemagglutinin (PHA), and lipopolysaccharide was completely absent and interferon-γ production was poor, with similarly poor production of tumor necrosis factor α, interleukin-10, and interleukin-6 (Fig. 1F, 1G, and 1H). Preincubation of the infant's cells with interleukin-12 partially restored interferon-γ production in response to the same stimuli (Fig. 1G). Biopsy samples of the infant's bone marrow and axillary lymph node showed striking myeloid hyperplasia, as well as the presence of acid-fast bacilli within granulomata in the lymph node (Fig. S3 in the Supplementary Appendix). Despite the subject's profound peripheral monocytopenia, the diseased node was positive for histiocytic markers CD68 and CD163, and bone trabeculae showed evidence of normal osteoclast activity (Fig. S4 in the Supplementary Appendix). In dermal tissues, the density of CD1a+ and CD14+ dendritic cells was remarkably low (Fig. 1C and 1D). In contrast, we observed epidermal Langerhans' cells that were of normal density (Fig. 1E) and detected the occasional Langerin+CD1a+ dendritic cell in the lymph node (Fig. S4 in the Supplementary Appendix). We concluded that the infant had a profound deficit of tissue dendritic cells and blood dendritic cells and monocytes, along with a variable deficit of tissue macrophages and normal numbers of Langerhans' cells.

This combination of immunodeficiency,[10,11] dendritic-cell deficiency,[12-15] and myeloproliferation[20] is strikingly similar to the phenotype of mice with loss-of-function mutations in *Irf8*. On sequencing of *IRF8* in the infant, we observed a homozygous missense variant predicted to cause the substitution of glutamic acid for lysine at position 108 (K108E) (Fig. 2A). Both parents were

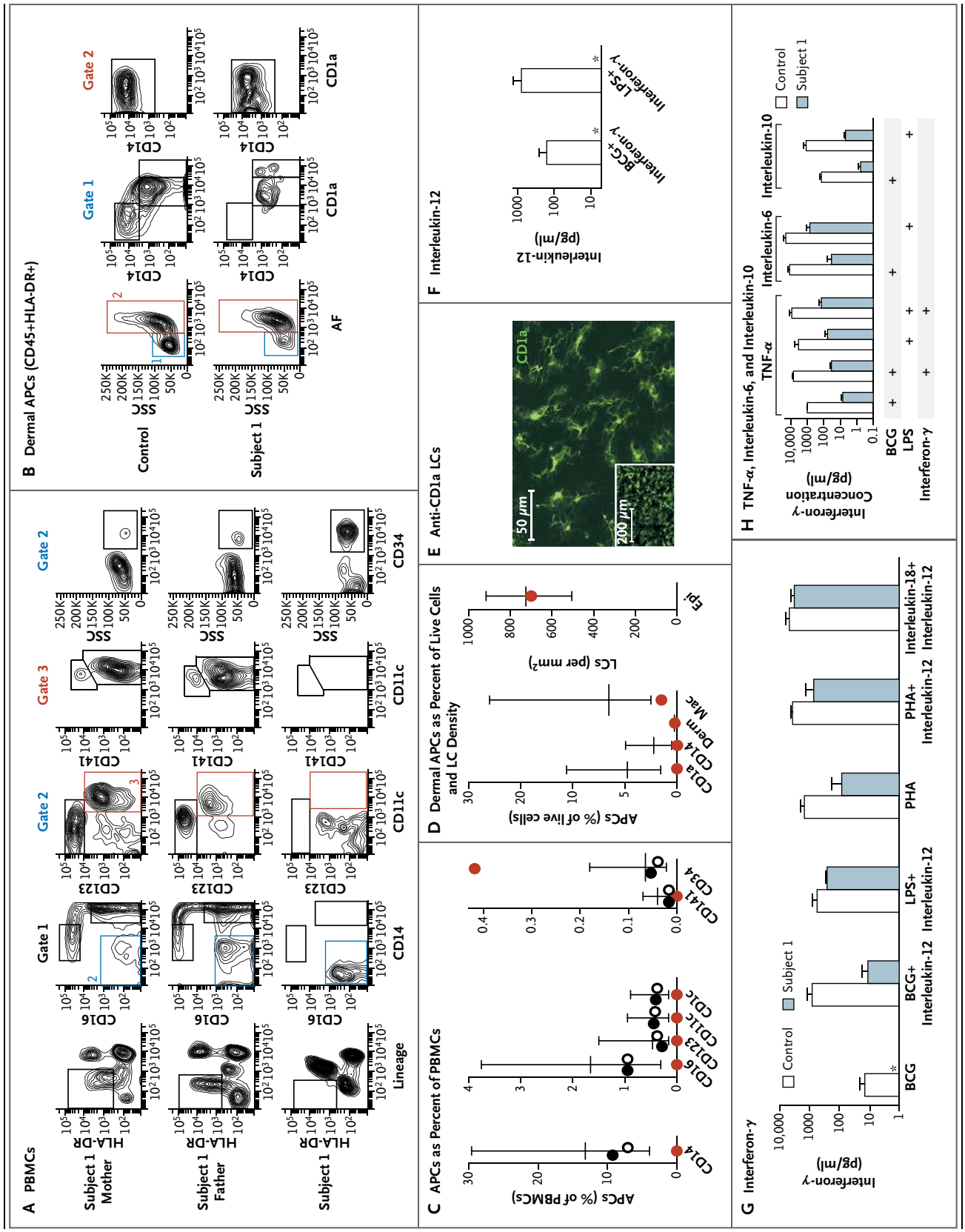


Figure 1 (facing page). Severe Depletion of the Antigen-Presenting-Cell Compartment in an Infant with Autosomal Recessive IRF8 Deficiency.

Panel A shows the flow cytometric evaluation of peripheral-blood mononuclear cells (PBMCs) obtained from Subject 1, a 10-week-old female infant, and her unaffected mother and father. Lineage refers to the cell population that is positive for the indicated markers. Panel B shows flow cytometric evaluation of CD45+HLA-DR+ cells from collagenase-digested dermis obtained from Subject 1 and a control subject. Panel C shows quantification of peripheral-blood subgroups of antigen-presenting cells (APCs) as a percentage of PBMCs obtained from Subject 1 (red circle), her mother (solid circle), and her father (open circle), as compared with a normal range in 28 adult control subjects. Panel D shows the quantification of dermal APC subgroups as the percentage of live cells obtained from Subject 1 (red circle), as compared with those obtained from 22 adult control subjects, and of Langerhans' cells (LCs) per square millimeter, as compared with those of 12 adult control subjects. Derm denotes dermal Langerhans' cells, Mac macrophages, and Epi epidermal Langerhans' cells. In Panels C and D, the horizontal lines indicate means, and the I bars indicate simple ranges. Panel E shows affected Langerhans' cells obtained from Subject 1 in an epidermal sheet, as revealed by anti-CD1a immunofluorescence (with inset showing reduced magnification). Panels F, G, and H show cytokine responses in whole-blood samples obtained from Subject 1, measured in vitro. Panel F shows interleukin-12 production in response to stimulation with bacille Calmette–Guérin (BCG) or lipopolysaccharide (LPS) plus exogenous interferon-γ. Panel G shows interferon-γ production elicited by the indicated stimuli. Panel H shows the production of tumor necrosis factor α (TNF-α), interleukin-6, and interleukin-10 in response to the indicated combinations of BCG, LPS, and exogenous interferon-γ. The asterisks represent undetectable levels. The bars indicate means, and the T bars indicate standard deviations from three independent experiments. AF denotes autofluorescence, and SSC side scatter.

heterozygous for K108E, and an unaffected sibling lacked the variant allele (Fig. 2C). We sequenced *IRF8* in 454 unrelated persons with clinical susceptibility to mycobacterial infection and did not detect the variant or any other homozygous variant.

Amino acid position 108 is within the DNA-binding domain of *IRF8* (Fig. 2A). The lysine residue is invariant in IRF8 orthologues (Fig. 2B) and is highly conserved among human IRF family members (data not shown). We expressed the IRF8 variant in cultured mouse macrophages. Immunoblotting studies showed similar expression levels of normal (K108) and mutant (K108E) variants and a relatively slower electrophoretic mobility of the mutant variant, suggesting that the mutation affects overall protein structure or folding (Fig. 3E). We tested the ability of normal and mutant isoforms to activate transcription of IRF8 targets — the promoters of *IL12B* (Fig. 3A) and *NOS2* (Fig. 3B) — in mouse macrophages. Although the combination of normal IRF8 and its coactivator, IRF1, induced a dose-dependent stimulation of *IL12B* and *NOS2* promoters, the K108E variant was almost inactive, suggesting that K108E abrogates the IRF1-dependent transcriptional activity of IRF8. Moreover, the mutant IRF8 variant bound the *IL12B* promoter much more weakly than did the normal variant (Fig. 3D).

Molecular modeling with the use of the structure of DNA-bound IRF2[21] places K108 in a short β strand that runs parallel to the major DNA-binding α helix, suggesting that it makes a critical hydrogen bond with the DNA sugar backbone (length, 3.3 Å) that facilitates IRF8 docking onto DNA (Fig. 2E, subpanel a). It is likely that the replacement of a positively charged amino acid (lysine) by one that is negatively charged (glutamic acid) at position 108 would prevent the formation of a hydrogen bond (Fig. 2E, subpanel b) and cause local repulsion of the supporting IRF8 β strand away from DNA, allowing a water molecule to fill the space. This model predicts loss of DNA binding and thus loss of transactivation.

These findings show that Subject 1 carried a loss-of-function mutation in *IRF8*. The heterozygous parents were healthy, despite having received BCG as neonates, and had normal numbers of PBMCs and dendritic cells, findings that are consistent with the autosomal recessive inheritance of the *IRF8* K108E allele.

AUTOSOMAL DOMINANT IRF8 DEFICIENCY

In parallel with our analysis for Subject 1 with recessive disease, we sequenced *IRF8* in 454 persons with MSMD in whom known MSMD-associated mutations had already been excluded (for details, see the Supplementary Appendix). Two unrelated persons from Brazil (Subject 2) and Chile (Subject 3) who had recurrent episodes of disseminated BCG disease were each found to carry the same de novo heterozygous mutation that was predicted to cause a threonine-to-alanine substitution at position 80 (T80A) of IRF8 (Fig. 2A and 2D). We genetically confirmed the reported biologic paternity of these two subjects. The finding that each mutation was de novo sug-

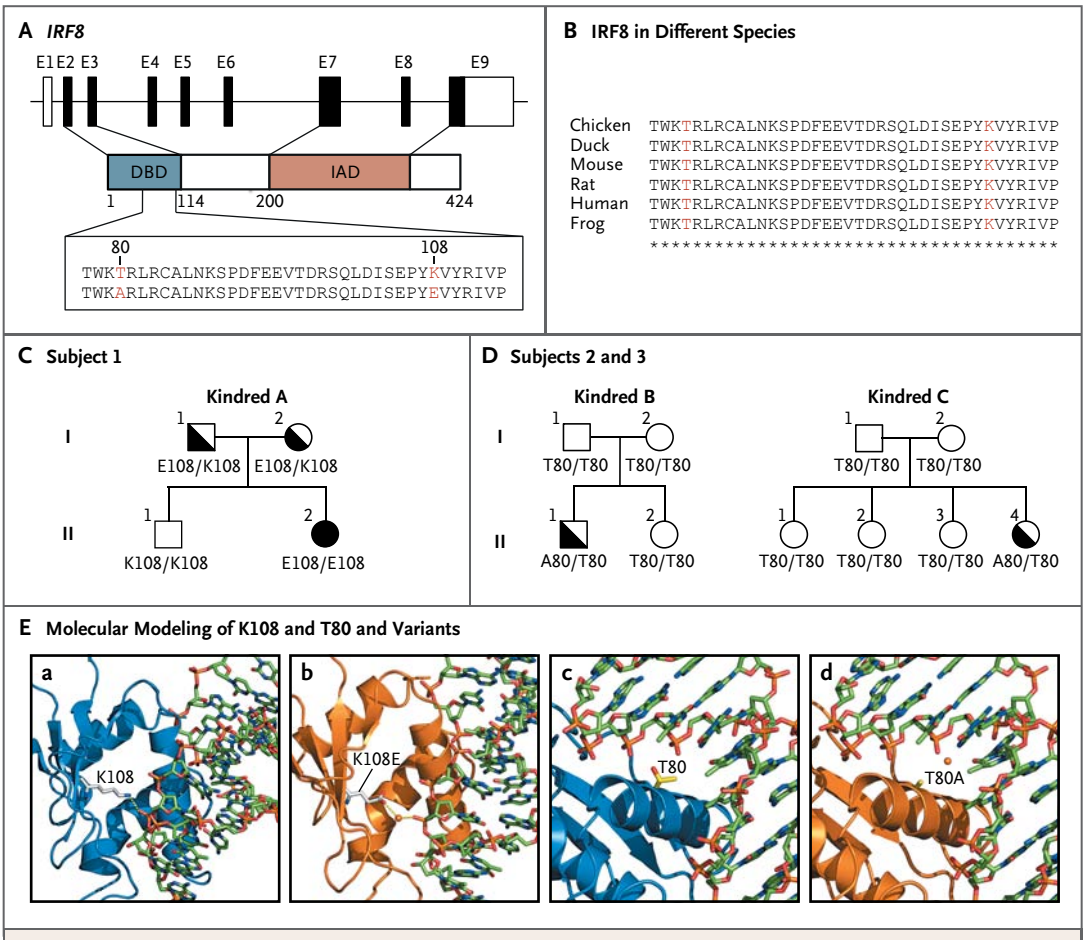


Figure 2. Genetic Analysis of Autosomal Recessive and Autosomal Dominant IRF8 Deficiency.

Panel A shows a schematic representation of the gene encoding interferon regulatory factor 8 (*IRF8*), including a total of nine noncoding (white) and coding (black) exons, with the major structural features of the protein shown. DBD denotes DNA-binding domain, and IAD IRF association domain. The positions of the T80A and K108E mutations (bold) in the DBD are shown. Panel B shows multiple sequence alignment of IRF8 from different species (T80 and K108 are shown in red; stars identify invariant residues). Panel C shows the segregation of K108E in the family of Subject 1. Panel D shows that T80A is a de novo mutation that has arisen independently in two subjects with mendelian susceptibility to mycobacterial disease from unrelated families in Brazil (Subject 2) and Chile (Subject 3). Panel E shows molecular modeling of wild-type K108 (subpanel a) and T80 (subpanel c), as compared with mutant variants K108E (subpanel b) and T80A (subpanel d) on the three-dimensional structure of DNA-bound IRF8. The hydrogen bond that is formed between the side-chain amino group of K108 and the sugar backbone of DNA is interrupted by K108E. The threonine-to-alanine substitution at position 80 in the key DNA-binding helix (inserted into the major groove) alters the hydrophobic interface between the protein and the DNA.

gests that the same T80A allele arose independently on two occasions. We did not detect the T80A variant in 1064 healthy control subjects of diverse relevant ancestry (for details, see the Supplementary Appendix). Amino acid position 80 of IRF8 is within the DNA-binding domain (Fig. 2A). The threonine residue is strictly conserved across IRF8 orthologues (Fig. 2B) and human paralogous genes (data not shown).

The T80A mutation had no effect on the level or stability of IRF8 in immortalized B-cell lines derived from the subjects (Fig. 3E) or after expression in transfected macrophages (data not shown). When the T80A mutant was tested for its ability to transactivate the promoters of *IL12B* (Fig. 3A) and *NOS2* (Fig. 3B) in mouse macrophages, it showed relatively low activity, similar to that of the hypomorphic R294C Irf8 variant found

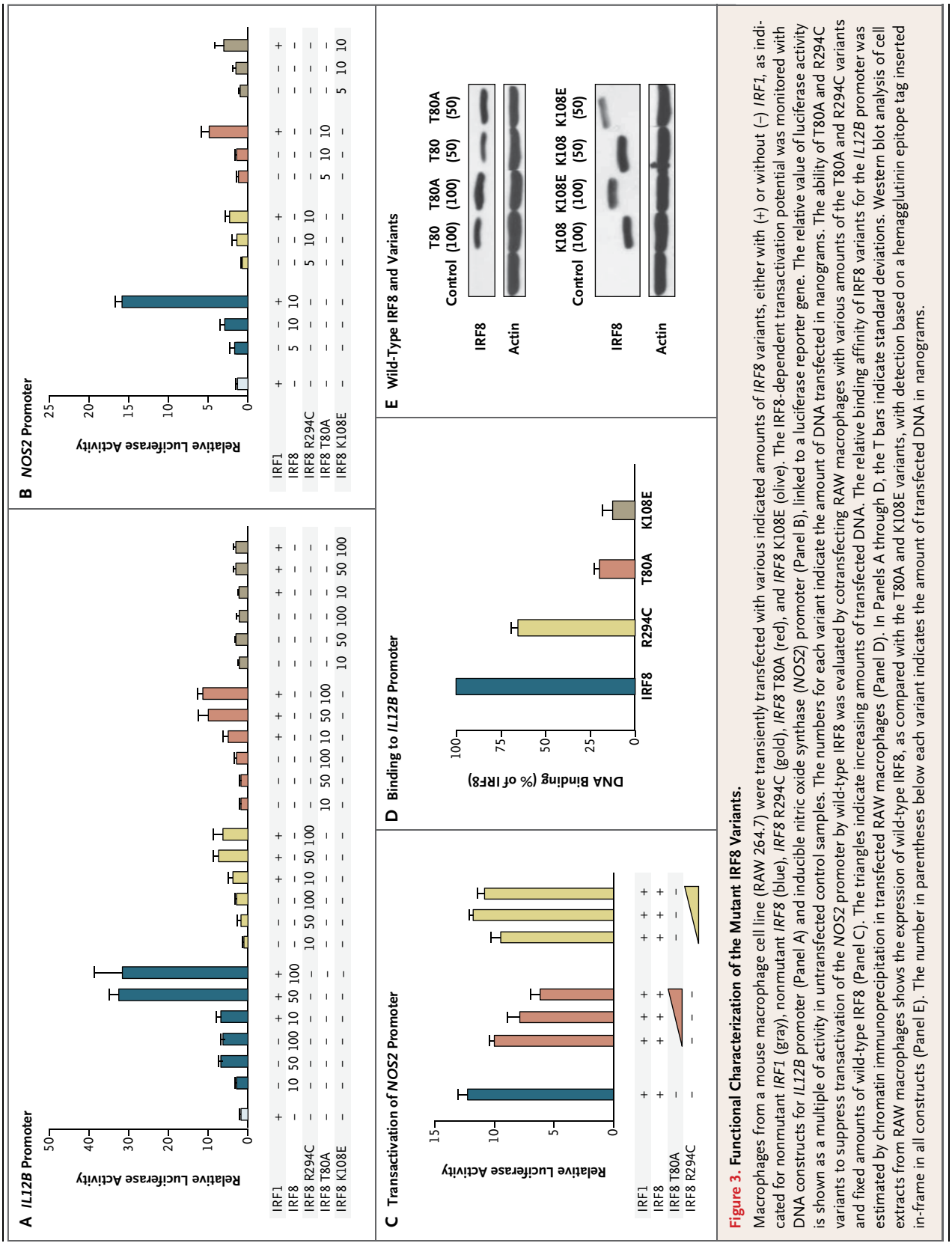


Figure 3. Functional Characterization of the Mutant IRF8 Variants.

Macrophages from a mouse macrophage cell line (RAW 264.7) were transiently transfected with various indicated amounts of *IRF8* variants, either with (+) or without (–) *IRF1*, as indicated for nonmutant *IRF1* (gray), nonmutant *IRF8* (blue), *IRF8* R294C (gold), *IRF8* T80A (red), and *IRF8* K108E (olive). The IRF8-dependent transactivation potential was monitored with DNA constructs for *IL12B* promoter (Panel A) and inducible nitric oxide synthase (*NOS2*) promoter (Panel B), linked to a luciferase reporter gene. The relative value of luciferase activity is shown as a multiple of activity in untransfected control samples. The numbers for each variant indicate the amount of DNA transfected in nanograms. The ability of T80A and R294C variants to suppress transactivation of the *NOS2* promoter by wild-type IRF8 was evaluated by cotransfecting RAW macrophages with various amounts of the T80A and R294C variants and fixed amounts of wild-type IRF8 (Panel C). The triangles indicate increasing amounts of transfected DNA. The relative binding affinity of IRF8 variants for the *IL12B* promoter was estimated by chromatin immunoprecipitation in transfected RAW macrophages (Panel D). In Panels A through D, the T bars indicate standard deviations. Western blot analysis of cell extracts from RAW macrophages shows the expression of wild-type IRF8, as compared with the T80A and K108E variants, with detection based on a hemagglutinin epitope tag inserted in-frame in all constructs (Panel E). The number in parentheses below each variant indicates the amount of transfected DNA in nanograms.

in Irf8-deficient BXH2 mutant mice. The T80A mutant showed weak recruitment to the *IL12B* promoter (20% of the amount of the nonmutant IRF8 protein), suggesting that the mutation interferes with DNA binding (Fig. 3D).

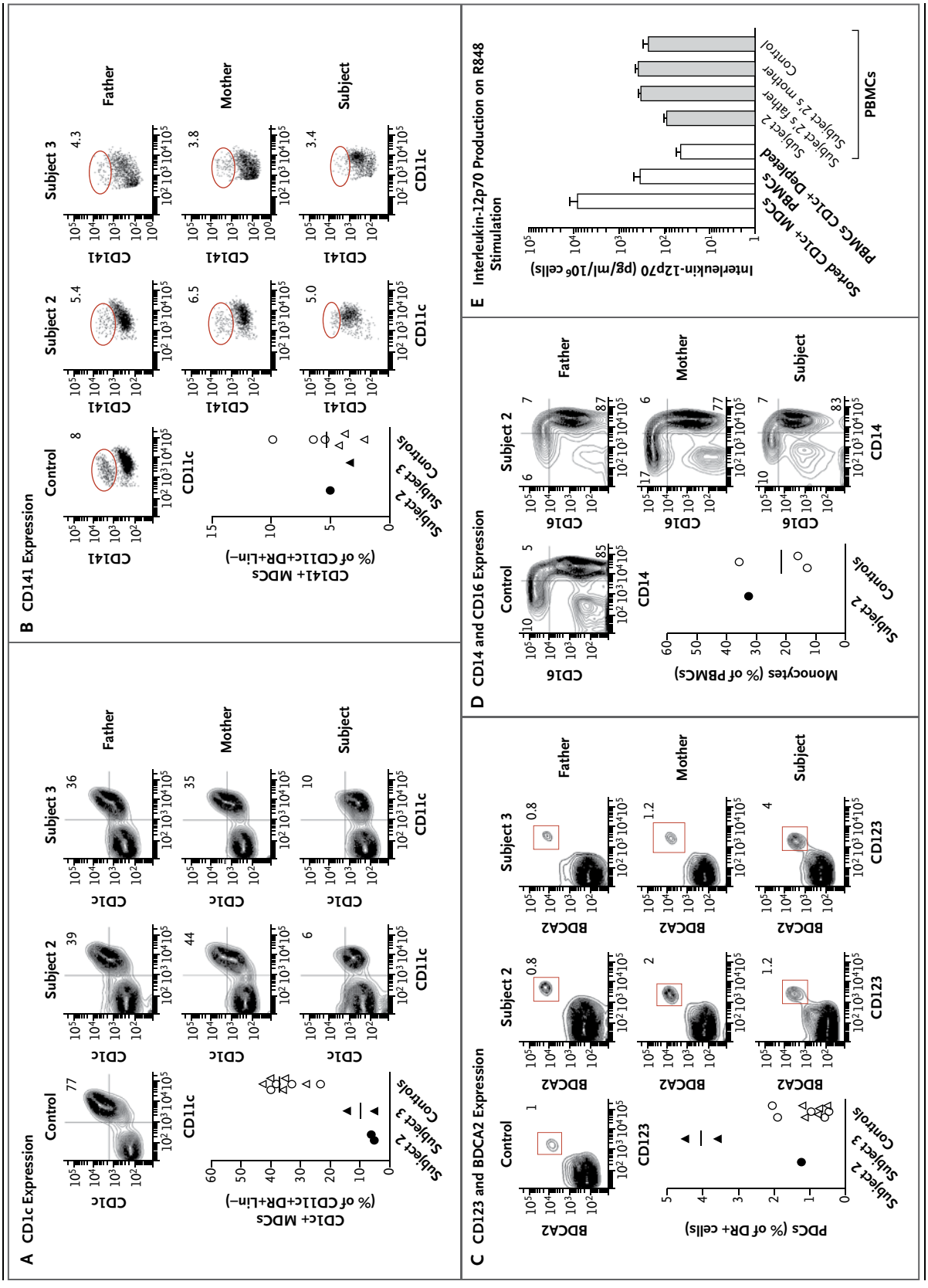
Homology modeling shows that T80 maps to the DNA-binding α helix of IRF8 that fits into the major groove, with its side chain directed toward the DNA bases (Fig. 2E, subpanel c). Molecular-dynamics simulations indicate that T80A alters the hydrophobic interface between the protein and DNA and possibly modulates the DNA-binding specificity of IRF8 (Fig. 2E, subpanel d). Alanine has a smaller side chain than does threonine, which may allow the entry of a water molecule at the DNA–IRF8 interface. Although functional data suggest that T80A is severely hypomorphic, we investigated whether the T80A variant is negatively dominant (i.e., whether it interferes with the function of the nonmutant IRF8 protein). We coexpressed the mutant and nonmutant alleles in macrophages (Fig. 3C) and observed decreasing activation of the *IL12B* promoter (not shown) and the *NOS2* promoter with increasing levels of mutant T80A IRF8 added to fixed levels of nonvariant IRF8. The effect was T80A-specific; we did not observe it to be associated with K108E (data not shown) or R294C mutant proteins. We conclude that the T80A mutation has a dual effect on IRF8 function and causes autosomal dominant MSMD.

We observed subtle deficits in the PBMCs of Subjects 2 and 3. There were no deficiencies of circulating lymphocytes and granulocytes (data not shown), monocyte subgroups, or BDCA2+CD123+ plasmacytoid dendritic cells (Fig. 4C and 4D). However, within CD11c+ myeloid dendritic cells, which are normally divided into minor CD141+ and major CD1c+ subgroups, there was marked loss of CD1c+ dendritic cells (Fig. 4A), whereas the CD141+ subgroup and the total number of CD11c+ dendritic cells remained intact (Fig. 4B). CD1c+ dendritic cells from unaffected persons can produce large amounts of interleukin-12 when stimulated with the toll-like receptor 7/8 ligand R848, as compared with PBMCs, whereas their depletion from PMBCs was associated with relatively low levels of interleukin-12 production by the remaining PBMCs ($P<0.02$ for all comparisons) (Fig. 4E). PBMCs with the *IRF8* T80A allele produced one third the amount of interleukin-12 produced by control cells in response to R848 stimulation ($P<0.004$ for all comparisons) (Fig. 4E).

Figure 4 (facing page). Selective Depletion of Dendritic Cells in Autosomal Dominant IRF8 Deficiency.

In Panel A, blood CD1c+ myeloid dendritic cells (MDCs) are gated on HLA-DR+ lineage (Lin)-negative cells (CD14–CD16–CD19–), and the expression of CD11c+ and CD1c+ is shown. The percentage of DR+CD11c+ lineage-negative cells that are positive for CD1c+ is shown for Subjects 2 and 3 (who carry the T80A allele), their relatives, and control subjects. Horizontal lines indicate mean values, and the numbers on contour plots represent the mean of two independent samples. In Panel B, blood CD141+ MDCs are gated on HLA-DR+ lineage-negative cells (CD14–CD16–CD19–), and the expression of CD11c and CD141 is shown. The percentage of DR+CD11c+ lineage-negative cells with a high level of CD141 expression on the cell surface is shown. In Panel C, plasmacytoid dendritic cells (PDCs) are gated on HLA-DR+ lineage-negative cells (CD14–CD16–), and the expression of CD123 and BDCA2 is shown. The percentage of DR+ cells that are positive for CD123 and BDCA2 is shown. In Panel D, monocytes are gated on HLA-DR+ lineage-negative cells (CD2–CD15–CD19–Nkp46–), and the expression of CD14 and CD16 is shown. The numbers on the contour plots represent the percentage of each subgroup (low and high expression of CD14 and CD14+CD16+) among total monocytes. In Panel D, the percentage of peripheral-blood mononuclear cells (PBMCs) that are positive for CD14 or CD16 is shown for Subject 2, his relatives, and a control subject. In Panel E, the mean (±SD) production of IL-12p70 protein in response to R848 is shown in four samples of blood CD1c+ MDCs, in three samples of PBMCs depleted in CD1c+ MDCs (PBMCs CD1c+ depleted), and in three samples of PBMCs not depleted (PBMCs), obtained by fluorescence-activated cell sorting from healthy control subjects (white bars) and in PBMCs from Subject 2, his parents, and a control subject collected at the same time (gray bars).

We suggest that depletion of interleukin-12–producing CD1c+ dendritic cells contributes to the susceptibility to mycobacterial disease in these subjects. In vitro assays of whole blood from the affected subjects showed no detectable effect on the production of interferon-γ (Fig. S6 in the Supplementary Appendix) or in the subjects' PBMCs in response to stimulation by purified protein derivative (PPD), BCG, or PHA (Fig. S7 in the Supplementary Appendix) or by PHA-driven T-cell blasts stimulated with interleukin-12 (data not shown). The presence of T80A does not seem to cause a generalized defect in interleukin-12 production, as shown by normal levels of the cytokine in a whole-blood assay in response to BCG, in monocyte-derived dendritic cells stimulated with CD40L, and by Epstein–Barr virus–trans-



formed B cells stimulated with phorbol dibutyrate (Fig. S6, S8A, and S8B in the Supplementary Appendix). Because the two healthy heterozygous parents of the infant with autosomal recessive IRF8 deficiency had normal levels of functional dendritic cells, the marked deficit of CD1c+CD11c+ dendritic cells was probably caused by the dominant-negative effect of the T80A mutant protein.

DISCUSSION

We have described three subjects, an infant with autosomal recessive IRF8 deficiency and two unrelated persons with an autosomal dominant form of the disease, whose conditions were characterized by either a complete loss of mononuclear phagocyte subgroups (Subject 1) or a selective loss of such subgroups (Subjects 2 and 3).

The *IRF8* mutation underlying the autosomal recessive disorder (K108E) results in an impairment of DNA binding and transactivation potential of IRF8. This defect caused a life-threatening pediatric syndrome, characterized by the absence of blood monocytes and dendritic cells, myeloproliferation of granulocyte precursors, and severe opportunistic infections, which together required stem-cell transplantation in infancy. This finding suggests that any deficiency of dendritic cells and monocytes will result in susceptibility to various infectious pathogens.

On the other hand, in the two subjects with autosomal dominant IRF8 deficiency, the disease was associated with a heterozygous mutation resulting in a dominant-negative *IRF8* allele (T80A) that suppresses the transactivation potential of nonmutant IRF8 in vitro. The associated syndrome was less severe than that in Subject 1 and was characterized by an abnormal peripheral-blood myeloid phenotype with a marked loss of CD11c+ CD1c+ dendritic cells. Autosomal dominant IRF8 deficiency causes selective susceptibility to mycobacterial infections and represents a novel, albeit rare, cause of MSMD. Together, these results establish a critical role of IRF8 in the ontogeny of the human mononuclear phagocyte lineage and of circulating monocytes and dendritic cells in particular.

After the cultivation of circulating stem cells from Subject 1 with growth factors that support the formation of granulocyte and monocyte or macrophage colonies, we found that the newly formed myeloid colonies were almost exclusively granulocytic (>98%), thus establishing that IRF8 is critical in the differentiation of myeloid progenitors into monocytes. Irf8 deficiency in mice is also associated with myeloproliferation of granulocyte precursors[10,11,20] and very low levels of circulating monocytes (Ginhoux F, Merad M: personal communication). The finding that tissue macrophages and Langerhans' cells are well represented in autosomal recessive IRF8 deficiency suggests heterogeneity within the mononuclear phagocyte compartment with respect to IRF8 independence or a potential for local self-renewal.[22,23] Further work will be required to establish the relative capacity of IRF8-deficient human CD34+ progenitors to produce fully functional macrophages and dendritic cells in vitro.[24] Studies in Irf8-deficient mice also show normal numbers of F4/80+ tissue macrophages (unpublished data), although these cells are abnormally susceptible to infection with intracellular pathogens in vitro.[25,26] Finally, we observed that although CD4+ T cells from Subject 1 had normal proliferation in response to stimulation with CD3 and CD28 (Fig. S5A and S5C in the Supplementary Appendix), they had poor secretion of effector cytokines interferon-γ and interleukin-17 and, to a lesser extent, interleukin-10 (Fig. S5B in the Supplementary Appendix), with a similar pattern detected on ex vivo stimulation with phorbol myristate acetate and ionomycin (data not shown). These results strongly suggest a defect in the function of helper T cells that may be caused by abnormal T-cell differentiation in an environment deficient in antigen-presenting cells.

The finding that autosomal dominant IRF8 deficiency is associated with a loss of interleukin-12–producing CD1c+CD11c+ myeloid dendritic cells suggests that these cells are essential for protective immunity to mycobacteria in humans. The marked reduction in CD1c+CD11c+ myeloid dendritic cells may be caused by altered ontogeny and maturation of this subgroup of CD11c+cells, which is linked to target-specific or global transcriptional effects of the IRF8 T80A variant.[27] Despite the fact that mutant whole-blood cells produced normal amounts of interleukin-12 on BCG stimulation in vitro, specific impairment of interleukin-12 secretion by CD1c+ dendritic cells could contribute to mycobacterial susceptibility. Alternatively, the lack of CD1c+CD11c+ myeloid dendritic cells may contribute to MSMD by other mechanisms, such as impairment of cell migra-

tion between draining lymph nodes and infected tissues or of priming of CD1c-restricted T cells for subsequent activation of infected macrophages. Indeed, CD1c-restricted T cells that are specific for a mycobacterial phospholipid antigen have been reported.[28,29]

In Subjects 2 and 3, we observed normal levels of CD141+ (BDCA3+) dendritic cells, a subgroup that investigators have recently suggested may be the functional equivalent of mouse CD8α+ dendritic cells that are absent in Irf8-deficient mice.[30-33] Cells other than CD1c+ dendritic cells may also be involved in the pathogenesis of MSMD, and it is possible that IRF8 deficiency impairs the effector function of tissue macrophages.

These findings also suggest that mutations in heterodimerization partners of IRF8 (e.g., IRF1 and PU.1) or specific transcriptional targets of such complexes[27] may impair antimycobacterial immunity. Finally, our study illustrates the value of performing genetic studies in mouse models of infection to identity candidate genes for human primary immunodeficiencies.[34]

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APPENDIX

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