from activated T cells in PBMCs of *STAT3* patients. Fig. S2 shows that MoDC differentiation in vitro and TGF-β1 signaling in MoDCs are intact in *STAT3* patients. Fig. S3 shows that IL-10 treatment does not impair the differentiation of MoDCs, but down-regulation of CD80, CD83, and CD86 is defective in MoDCs from *STAT3* patients. Fig. S4 shows that suppression of proliferation by IL-10 pretreatment is impaired in MoDCs from *STAT3* patients. Fig. S5 shows that up-regulation of FOXP3, CTLA-4, and GITR is impaired in iT_{reg} cells co-cultured with patient IL-10–DCs. Fig. S6 shows that MoDCs from *STAT3* patients produce equivalent amounts of TGF-β1. Fig. S7 shows the characterization of primary DCs and MoDCs from the patient with *TYK2* deficiency. Online supplemental material is available at http://www.jem.org/cgi/content/full/jem.20100799/DC1.

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SUPPLEMENTAL MATERIAL

Saito et al., http://www.jem.org/cgi/content/full/jem.20100799/DC1

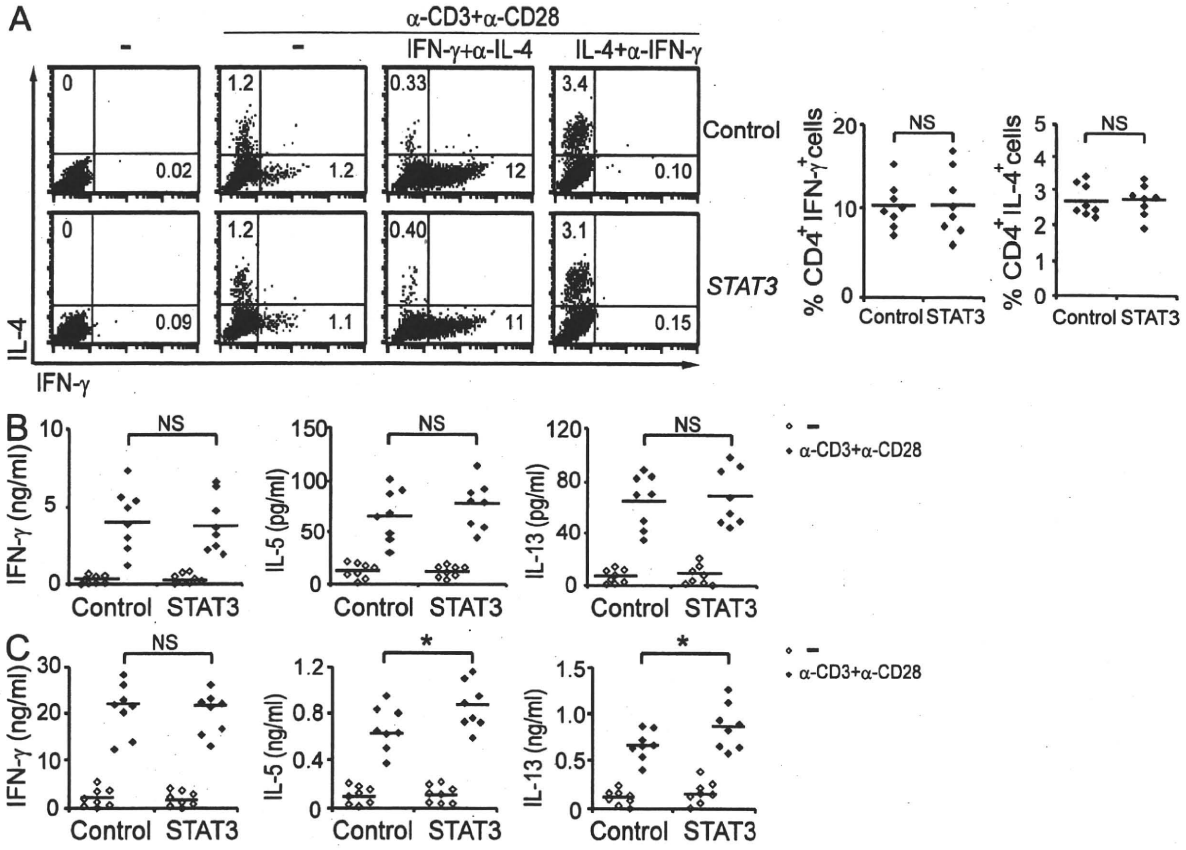

Figure S1. Normal Th1 and Th2 differentiation from naive CD4⁺ T cells but increased Th2 cytokine production from activated T cells in PBMCs of *STAT3* patients. (A) Intracellular staining with mAbs against IFN-γ and IL-4 was performed on naive CD4⁺ T cells from control subjects and *STAT3* patients either left unstimulated or stimulated with mAbs against CD3 and anti-CD28, alone or in combination with IFN-γ and anti–IL-4 mAb or IL-4 and anti–IFN-γ mAb. The percentages of IFN-γ– and IL-4–producing cells are shown. Representative dot plots from a control subject and a *STAT3* patient are shown on the left, and pooled data from eight control subjects and eight *STAT3* patients showing percentages of CD4⁺IFN-γ⁺ cells and CD4⁺IL-4⁺ cells are on the right. (B) Naive CD4⁺ T cells from eight control subjects and eight *STAT3* patients were stimulated with anti-CD3/CD28 mAbs, and the concentration of IFN-γ, IL-5, and IL-13 in the culture supernatants was measured by ELISA. Pooled data from eight control subjects and eight *STAT3* patients are shown. (C) PBMCs from eight control subjects and eight *STAT3* patients were stimulated, and the concentration of IFN-γ, IL-5, and IL-13 was measured as in B. Pooled data from eight control subjects and eight *STAT3* patients are shown. Data are representative of at least two independent experiments performed in triplicate. (A–C) Horizontal bars indicate mean values. *, $P < 0.05$.

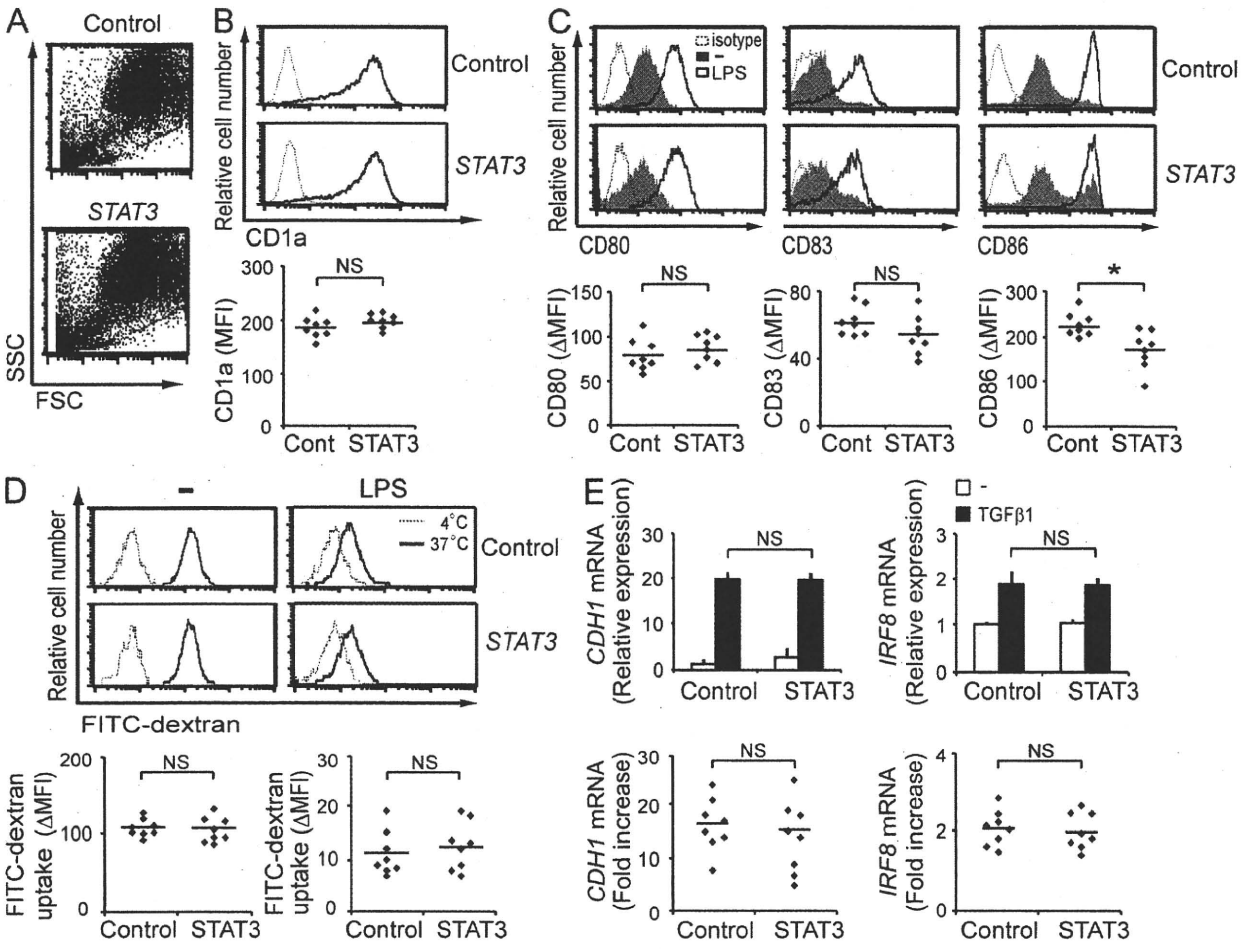

Figure S2. MoDC differentiation in vitro and TGF-β1 signaling in MoDCs are intact in *STAT3* patients. (A) Representative forward and side light scatter (FSC and SSC, respectively) profile of immature DCs from a control subject and a *STAT3* patient. (B) Representative histograms of CD1a expression on immature DCs are at the top, and summary data from eight control subjects and eight *STAT3* patients showing mean fluorescence intensity (MFI) of CD1a expression are at the bottom. Dashed line indicates staining with an isotype-matched control mAb. (C) Representative histograms of CD80, CD83, and CD86 expression on immature MoDCs (−) and LPS-matured MoDCs (LPS) from a control (Cont) subject and a *STAT3* patient are shown at the top, and summary data ($n = 8$ each) showing ΔMFI, LPS stimulated minus immature, are at the bottom. Dashed lines indicate staining with isotype-matched control mAbs. (D) Representative histograms of FITC-dextran uptake by immature (−) and LPS-matured (LPS) MoDCs from a control subject and a *STAT3* patient are shown at the top, and summary data ($n = 8$ each) showing ΔMFI, cultures in 37°C minus cultures in 4°C, are at the bottom. Data are representative of at least two independent experiments. (E) Immature DCs from a control subject and a *STAT3* patient were stimulated with TGF-β1 for 4 h, and the amount of *CDH1* (E-cadherin) and *IRF8*, TGF-β–responsive genes, was evaluated by Q-PCR. Data shown were normalized to *HPRT* levels, and the expression level in unstimulated control cells was defined as 1.0. Representative data are shown at the top, and summary data showing fold increase ($n = 8$ each) are at the bottom. Data are representative of two independent experiments. Graphs show mean ± SD. (B–E) Horizontal bars indicate mean values. *, $P < 0.05$.

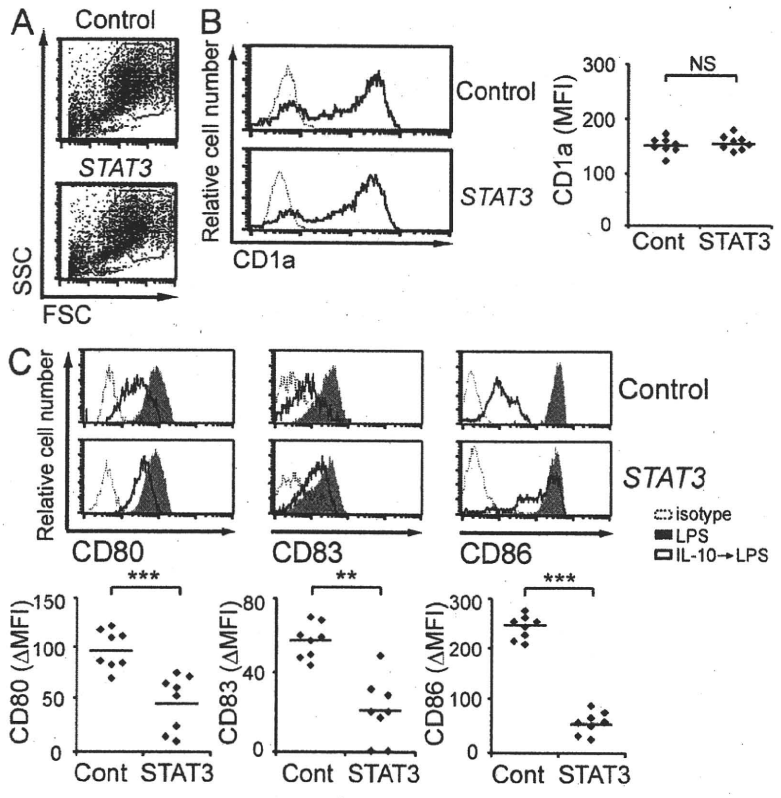

Figure S3. IL-10 treatment does not impair the differentiation of MoDCs, but down-regulation of CD80, CD83, and CD86 is defective in MoDCs from *STAT3* patients. (A) Representative forward and side scatter (FSC and SSC, respectively) profile of IL-10–treated MoDCs. (B) Representative histograms of CD1a expression on IL-10–DCs from a control (Cont) subject and a *STAT3* patient. Dashed lines indicate the staining with isotype-matched control mAb. Representative histograms are on the left, and summary data (n = 8 each) showing mean fluorescence intensity (MFI) of CD1a expression are on the right. Data are representative of at least two independent experiments. (C) Representative histograms of CD80, CD83, and CD86 expression on LPS-matured DCs (LPS) and LPS-matured MoDCs after prior treatment with IL-10 (IL-10 → LPS) from a control subject and a *STAT3* patient. Dashed lines indicate staining with isotype-matched control mAbs. Summary data (n = 8 each) showing ΔMFI, LPS matured minus IL-10 → LPS DCs, of CD80, CD83, and CD86 are on the bottom. Data are representative of at least two independent experiments. (B and C) Horizontal bars indicate mean values. **, $P < 0.01$; ***, $P < 0.001$.

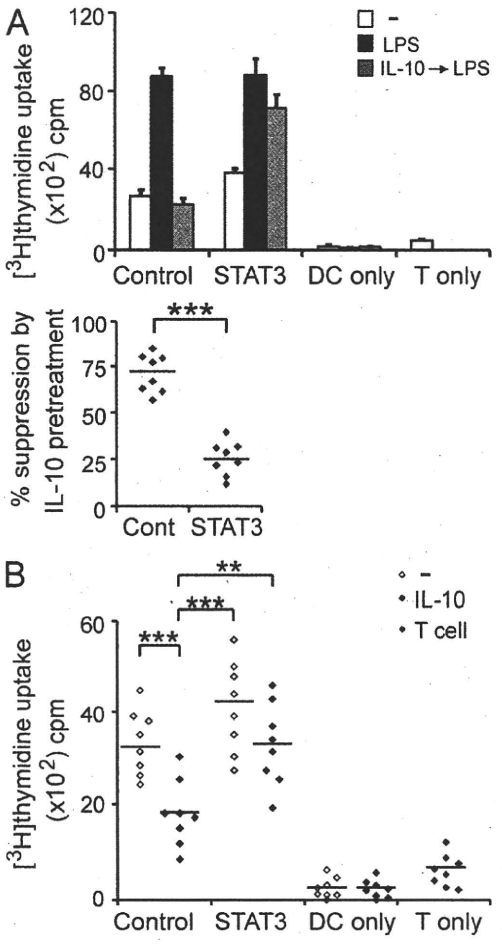

Figure S4. Suppression of proliferation by IL-10 pretreatment is impaired in MoDCs from *STAT3* patients. (A) Third-party allogeneic naive CD4+ T cells from control (Cont) subjects were co-cultured with immature DCs (−), LPS-matured DCs (LPS), or LPS-matured DCs after prior treatment with IL-10 (IL-10 → LPS). After 5 d, proliferation was evaluated by pulsing with 1 µCi (37 kBq) [3H]thymidine for the final 18 h. Representative data from a control subject and a *STAT3* patient are shown at the top, and summary data ($n = 8$ each) showing percent suppression are at the bottom. Graph shows mean ± SD. (B) Allogeneic third-party naive CD4+ T cells from control subjects were co-cultured with immature DCs (−) or IL-10–DCs (IL-10) from control subjects and *STAT3* patients. After 5 d, proliferation was evaluated as in A. Summary data from eight control subjects and eight *STAT3* patients are shown. Data are representative of at least two independent experiments performed in triplicate. (A and B) Horizontal bars indicate mean values. **, $P < 0.01$; ***, $P < 0.001$.

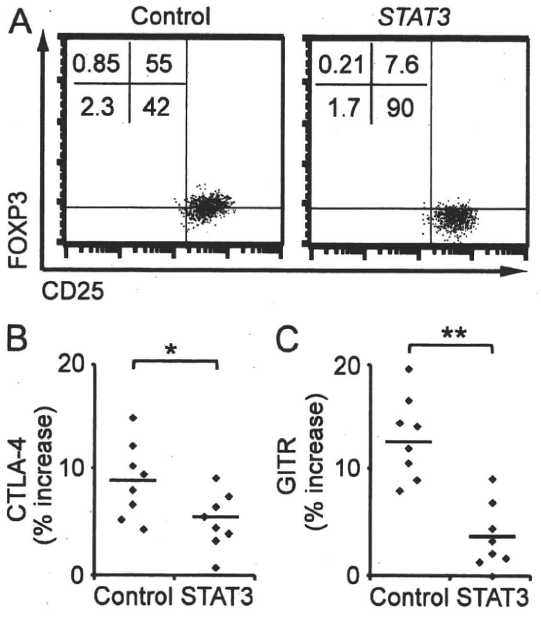

Figure S5. Up-regulation of FOXP3, CTLA-4, and GITR is impaired in naive CD4⁺ T cells co-cultured with patient IL-10–DCs. (A) After the co-culture of naive CD4⁺ T cells with control or patient IL-10 DCs in the presence of plate-bound anti-CD3 mAb for 5 d, CD4⁺ T cells in the small lymphoid gate were evaluated for the expression of CD25 and FOXP3. (B and C) Expression of CTLA-4 (B) and GITR (C) on $CD4^+CD25^+$ T cells cultured as in A from control subjects and *STAT3* patients was evaluated. Summary data ($n = 8$ each) showing percent increase, IL-10–DCs minus untreated DCs, are shown. Data are representative of at least two independent experiments. Horizontal bars indicate mean values. *, $P < 0.05$; **, $P < 0.01$.

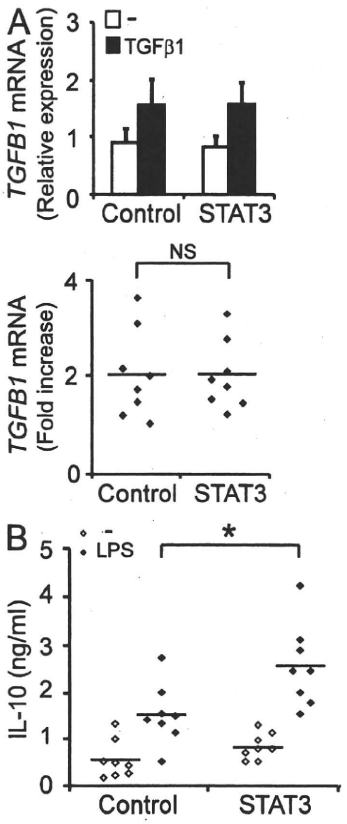

Figure S6. MoDCs from *STAT3* patients produce equivalent amounts of TGF-β1. (A) Immature DCs from a control subject and a *STAT3* patient were stimulated with TGF-β1 for 4 h, and the amount of *TGFB1* mRNAs was analyzed by Q-PCR. Data shown were normalized to *HPRT* levels, and the expression level in unstimulated control cells was defined as 1.0. Representative Q-PCR data are at the top, and summary data ($n = 8$ each) showing fold increase are at the bottom. Graph shows mean ± SD. (B) Summary data of IL-10 levels of culture supernatants of unstimulated immature MoDCs (−) and LPS-stimulated MoDCs (LPS) from eight control subjects and eight *STAT3* patients. Data are representative of at least two independent experiments performed in triplicate. (A and B) Horizontal bars indicate mean values. *, $P < 0.05$.

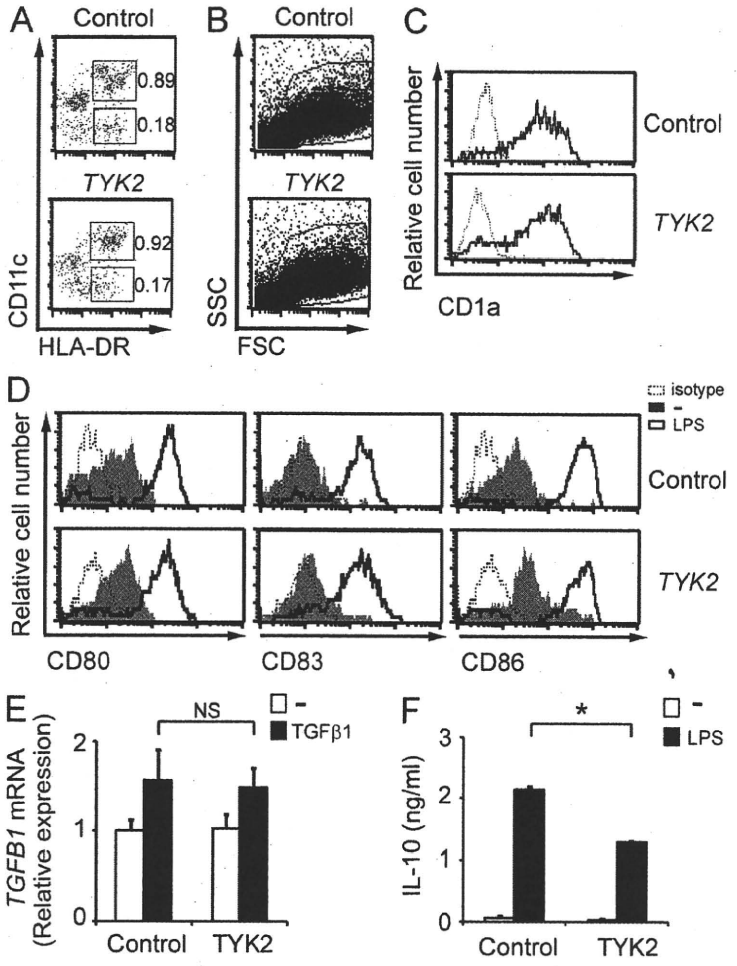

Figure S7. Characterization of primary DCs and MoDCs of the patient with *TYK2* deficiency. (A) Lin-negative cells in PBMCs from a control subject and the *TYK2*-deficient patient were evaluated for the expression of HLA-DR and CD11c. $CD11c^+$ cells are cDCs, and $CD11c^-$ cells are pDCs. (B) Forward and side scatter (FSC and SSC, respectively) profile of immature DCs of a control subject and the *TYK2*-deficient patient. (C) Histogram of CD1a expression on immature DCs of a control subject and the *TYK2*-deficient patient. (D) Expression levels of CD80, CD83, and CD86 on immature MoDCs (−) and LPS-matured MoDCs (LPS). (C and D) Dashed lines indicate staining with isotype-matched control mAbs. (E) Immature DCs from a control subject and the *TYK2*-deficient patient were stimulated with TGF-β1 for 4 h, and the amount of *TGFB1* mRNAs was analyzed by Q-PCR. Data shown are normalized to *HPRT* levels, and the expression level in unstimulated control cells was defined as 1.0. (F) IL-10 levels in the culture supernatants of unstimulated immature MoDCs (−) and LPS-matured MoDCs (LPS) from a control subject and the *TYK2*-deficient patient. Data are representative of at least two independent experiments. (E and F) Graphs show mean ± SD. *, $P < 0.05$.

Clinical Features and Outcome of Patients With IRAK-4 and MyD88 Deficiency

Capucine Picard, MD, PhD, Horst von Bernuth, MD, PhD,* Pegah Ghandil, PhD, Maya Chrabieh, Ofer Levy, MD, PhD, Peter D. Arkwright, MD, Douglas McDonald, MD, PhD, Raif S. Geha, MD, Hidetoshi Takada, MD, Jens C. Krause, MD, C. Buddy Creech, MD, MPH, Cheng-Lung Ku, PhD, Stephan Ehl, MD, PhD, László Maródi, MD, Saleh Al-Muhsen, MD, Sami Al-Hajjar, MD, Abdulaziz Al-Ghonaium, MD, Noorbibi K. Day-Good, PhD, Steven M. Holland, MD, PhD, John I. Gallin, MD, Helen Chapel, MD, David P. Speert, MD, Carlos Rodriguez-Gallego, MD, Elena Colino, MD, Ben-Zion Garty, MD, Chaim Roifman, MD, Toshiro Hara, MD, PhD, Hideto Yoshikawa, MD, Shigeaki Nonoyama, MD, Joseph Domachowske, MD, Andrew C. Issekutz, MD, Mimi Tang, MD, Joanne Smart, MD, Simona Eva Zitnik, MD, Cyrille Hoarau, MD, Dinakantha S. Kumararatne, MD, Adrian J. Thrasher, MD, PhD, E. Graham Davies, MD, Claire Bethune, MD, Nicolas Sirvent, MD, Dominique de Ricaud, MD, Yildiz Camcioglu, MD, Júlia Vasconcelos, MD, Margarida Guedes, MD, Artur Bonito Vitor, MD, Carlos Rodrigo, MD, Francisco Almazán, MD, Maria Méndez, MD, Juan Ignacio Aróstegui, MD, PhD, Laia Alsina, MD, Claudia Fortuny, MD, Janine Reichenbach, MD, PhD, James W. Verbsky, MD, PhD, Xavier Bossuyt, MD, PhD, Rainer Doffinger, PhD, Laurent Abel, MD, PhD, Anne Puel, PhD, and Jean-Laurent Casanova, MD, PhD*

Abstract: Autosomal recessive interleukin-1 receptor-associated kinase (IRAK)-4 and myeloid differentiation factor (MyD)88 deficiencies impair Toll-like receptor (TLR)- and interleukin-1 receptor-mediated immunity. We documented the clinical features and outcome of 48 patients with IRAK-4 deficiency and 12 patients with MyD88 deficiency, from 37 kindreds in 15 countries.

The clinical features of IRAK-4 and MyD88 deficiency were indistinguishable. There were no severe viral, parasitic, and fungal diseases, and the range of bacterial infections was narrow. Noninvasive bacterial infections occurred in 52 patients, with a high incidence of infections of the upper respiratory tract and the skin, mostly caused by *Pseudomonas aeruginosa* and *Staphylococcus aureus*, respectively. The leading threat was invasive pneumococcal disease, documented in 41 patients (68%) and causing 72 documented invasive infections (52.2%). *P. aeruginosa* and *Staph. aureus* documented invasive infections also occurred (16.7% and 16%, respectively, in 13 and 13 patients, respectively). Systemic signs of inflammation were usually weak or delayed. The first invasive infection occurred before the age of 2 years in 53 (88.3%) and in the neonatal period in 19 (32.7%) patients. Multiple or recurrent invasive infections were observed in most survivors (n = 36/50, 72%).

Clinical outcome was poor, with 24 deaths, in 10 cases during the first invasive episode and in 16 cases of invasive pneumococcal disease. However, no death and invasive infectious disease were reported in patients after the age of 8 years and 14 years, respectively. Antibiotic prophylaxis (n = 34), antipneumococcal vaccination (n = 31), and/or IgG infusion (n = 19), when instituted, had a beneficial impact on patients until the teenage years, with no seemingly detectable impact thereafter.

IRAK-4 and MyD88 deficiencies predispose patients to recurrent life-threatening bacterial diseases, such as invasive pneumococcal disease in particular, in infancy and early childhood, with weak signs of inflammation. Patients and families should be informed of the risk of developing life-threatening infections; empiric antibacterial treatment and immediate medical consultation are strongly recommended in cases of suspected infection or moderate fever. Prophylactic measures in childhood are beneficial, until spontaneous improvement occurs in adolescence.

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Abbreviations: CRP = C-reactive protein, ELISA = enzyme-linked immunosorbent assay, IFN = interferon, IKBA = IκBα, IL = interleukin, IL-1R = interleukin-1 receptor, InvBD = invasive bacterial disease, IRAK = interleukin-1 receptor-associated kinase, MyD = myeloid differentiation factor, NEMO = nuclear factor-kappaB essential modulator, NInvBD = noninvasive bacterial disease, TIR = Toll/IL-1R, TLR = Toll-like receptor, TNF = tumor necrosis factor.

From Study Center of Primary Immunodeficiencies, Assistance Publique Hôpitaux de Paris (CP); Necker Hospital, Paris, France. Laboratory of Human Genetics of Infectious Diseases (CP, HvB, PG, MC, CLK, L. Abel, AP, JLC), Necker Branch, INSERM U980, Paris, France. Paris Descartes University (CP, HvB, PG, MC, CLK, L. Abel, AP, JLC), Paris, France. Department of Pediatric Pneumology and Immunology (HvB), Charité Hospital-Humboldt University, Berlin, Germany. Prince Naif Center for Immunology Research (PG, SAM, SAH, AAG, JLC), College of Medicine, King Saud University, Riyadh, Saudi Arabia. Division of Infectious Diseases (OL); and Division of Immunology (DM, RSG), Children's Hospital Boston (OL), Boston, Massachusetts. Harvard Medical School (OL, DM, RSG), Boston, Massachusetts. University of Manchester (PDA), Royal Manchester Children's Hospital, Manchester, United Kingdom. Department of Pediatrics (HT, TH), Graduate School of Medical Sciences, Kyushu University, Fukuoka, Japan.

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Department of Pediatrics (JCK, CBC), Division of Pediatric Infectious Diseases, Vanderbilt University, Nashville, Tennessee. Centre of Chronic Immunodeficiency (SE), University of Freiburg, Freiburg, Germany. Department of Infectious and Pediatric Immunology (LM), Medical and Health Science Center, University of Debrecen, Debrecen, Hungary. Department of Pediatrics (SAM, SAH, AAG), King Faisal Specialist Hospital and Research Centre, Riyadh, Saudi Arabia. Department of Pediatrics (NKDG), Division of Allergy and Immunology, University of South Florida and All Children's Hospital, St. Petersburg, Florida. Laboratory of Clinical Infectious Diseases (SMH, JIG), National Institute of Allergy and Infectious Diseases, National Institutes of Health, Bethesda, Maryland. University of Oxford and Oxford Radcliffe Hospital (HC), Oxford, United Kingdom. Division of Infectious and Immunological Diseases (DPS), Department of Pediatrics, University of British Columbia, Vancouver, British Columbia, Canada. Department of Immunology (CRG), Dr Negrin University Hospital of Gran Canaria, Las Palmas de Gran Canaria, Spain. Department of Pediatrics (EC), Unit of Infectious Diseases, Insular-Materno-Infantil University Hospital, Las Palmas

INTRODUCTION

Autosomal recessive interleukin-1 receptor-associated kinase (IRAK)-4 and myeloid differentiation factor (MyD)88 deficiencies are recently described primary immunodeficiencies.[38,49] MyD88 is a key cytosolic adapter molecule, providing a bridge from Toll-like receptors (TLRs) and interleukin-1 receptors (IL-1Rs) to the IRAK complex, which consists of 2 active kinases (IRAK-1 and IRAK-4) and 2 noncatalytic subunits (IRAK-2 and IRAK-3/M). MyD88 interacts with TLRs and IL-1Rs via a shared Toll and IL-1R (TIR) domain. The MyD88- and IRAK-4-dependent TIR pathway leads to the synthesis of inflammatory cytokines, such as IL-1β, IL-6, IL-8, tumor necrosis factor (TNF)-α, interferon (IFN)-α/β, and IFN-λ, at least after TLR7, TLR8, and TLR9 stimulation (Figure 1).[1] MyD88 and IRAK-4 deficiencies can thus be considered phenocopies with respect to their immunologic phenotype.[49] Blood leukocytes derived from MyD88- and IRAK-4-deficient patients display impaired responses to most of the TLR and IL-1R agonists tested.[38,49] All human TLRs other than TLR3 use both MyD88 and IRAK-4.[42,43] This pathway is also used by a number of IL-1Rs, including IL-1R, IL-18R, and IL-33Ra (ST2).[3,17] (unpublished data) It is unknown whether other TIR-containing IL-1Rs, such as IL-1Rrp-2, SIGIRR/TIR8, TIGIRR-1, and TIGIRR-2/IL-1RAPL, use MyD88 and IRAK-4.[17,41] IL-1α and IL-33 may also exert alternative, intracellular effects leading to transcriptional regulation.[17] To our knowledge, no mutation affecting the MyD88-independent IL-1R pathway has yet been identified. An alternative, MyD88-independent but TRIF-dependent pathway can be triggered by TLR-3 and TLR-4. The alternative TLR-3 pathway is impaired in patients with UNC-93B and TLR-3 deficiencies, whose alternative TLR-4 pathway is not affected.[11,54] By contrast, mutations in NEMO and IKBA genes are associated with a much broader signaling defect, including both the classical and alternative pathways.[7]

Given such a broad and profound immunologic phenotype, we would expect the clinical infectious phenotype of IRAK-4 and MyD88 deficiencies to be extremely severe. However, available clinical data for 45 patients with MyD88 and IRAK-4 deficiencies suggest instead a narrow susceptibility to invasive bacterial infections, mostly caused by gram-positive bacteria, such as *Streptococcus pneumoniae* and *Staphylococcus aureus* in particular, with rare infections caused by gram-negative bacteria, such as *Pseudomonas aeruginosa* and *Shigella sonnei*.[6,19,25,26,38,49] Both MyD88- and IRAK-4-deficient patients seem to have normal resistance to common fungi, parasites, viruses, and to a large fraction of bacteria. Moreover, although 16 of the 45 reported patients died in childhood, the clinical features of the survivors seemed to improve with age.[6,8,12,14–16,18–20,23–27,30,32,38,44,52] The clinical history of these patients seems otherwise unremarkable, with the exception of a late detachment of the umbilical cord, reported in 2 patients.[44]

This clinical information, however, is based principally on the description of individual case reports and small series of patients, with a single large series of 28 individuals.[25] Moreover, most publications, including that dealing with the large series,[25] have focused on the genotype and cellular phenotype of patients, providing little clinical information—infectious and immunologic information in particular. To our knowledge, the actual clinical presentation of patients with MyD88 and IRAK-4 deficiency and their overall immunologic evaluation have yet to be described. The nature and severity of the infectious diseases to which these patients are susceptible and the impact of prophylaxis and age on clinical outcome have not been described. The impact of these defects on the development and function of the myeloid and lymphoid cell subsets also remains to be characterized. We therefore undertook a detailed and thorough description of the clinical features and outcome of an international series of patients with MyD88 or IRAK-4 deficiency.

PATIENTS AND METHODS

Subjects and Kindreds

The current study was conducted in accordance with the Helsinki Declaration, with informed consent obtained from each patient or the patient's family. The study was approved by the local ethics committee of Necker-Enfants Malades Hospital, Paris, France. A detailed questionnaire was completed by the physicians caring for the patients with MyD88 and IRAK-4 deficiencies and sent to 2 of the authors (CP and HvB) for thorough review. During follow-up, communications were sent

de Gran Canaria, Spain. Schneider Children's Medical Center (BZG), Petah Tiqva, Israel. Division of Immunology and Allergy (C. Roifman), Department of Pediatrics, Hospital for Sick Children, University of Toronto, Toronto, Ontario, Canada. Department of Neurology (HY), Miyagi Children's Hospital, Sendai, Japan. Department of Pediatrics (SN), National Defense Medical College, Saitama, Japan. Pediatrics, Microbiology and Immunology (JD), SUNY Upstate Medical University, Syracuse, New York. Dalhousie University (ACI), Halifax, Nova Scotia, Canada. Royal Children's Hospital (MT, JS), Parkville, Victoria, Australia. University Children Hospital Ljubljana (SEZ), Ljubljana, Slovenia. Unité transversale d'Allergologie, Néphrologie et Immunologie Clinique (CH), Centre hospitalier universitaire de Tours, Tours, France. Department of Clinical Biochemistry and Immunology (DSK, RD), Addenbrookes Hospital, Cambridge, United Kingdom. Paediatric Immunology and Molecular Immunology Unit (AJT), Institute of Child Health, London, United Kingdom. Department of Clinical Immunology (EGD), Great Ormond St Hospital, London, United Kingdom. Immunology Department (CB), Derriford Hospital, Plymouth, United Kingdom. University Hospital Archet 2 (NS), Nice, France. Lenval Foundation (DDR), Children's Hospital, Nice, France. Cerrahpasa Medical School (YC), Istanbul University, Istanbul, Turkey. Clinical Pathology and Pediatric Department (JV, MG), General Hospital of Santo António, Porto, Portugal. Pediatric Department (ABV), Hospital S. João, Porto, Portugal. Pediatric Department (C. Rodrigo, FA, MM), Germans Trias i Pujol Hospital, Barcelona Autonomous University, Barcelona, Spain. Immunology Department-CDB (JIA), Hospital Clínic-IDIBAPS, Barcelona University, Barcelona, Spain. Pediatric Department (L. Alsina, CF), Hospital Sant Joan de Deu, Barcelona University, Barcelona, Spain. Immunology Department (JR), Universitäts-Kinderspital Zürich, Zürich, Switzerland. Pediatrics and Microbiology and Molecular Genetics (JMV), Medical College of Wisconsin, Milwaukee, Wisconsin. Experimental Laboratory Medicine (XB), Department of Medical Diagnostic Sciences, Biomedical Science Group, Catholic University Leuven, Leuven, Belgium. St. Giles Laboratory of Human Genetics of Infectious Diseases (JLC), Rockefeller Branch, The Rockefeller University, New York, New York. Pediatric Hematology-Immunology Unit (JLC), Necker Hospital, Assistance Publique Hôpitaux de Paris, Paris, France.

*Both authors contributed equally to this work.

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Reprints: Jean-Laurent Casanova, MD, PhD, St. Giles Laboratory of Human Genetics of Infectious Diseases, The Rockefeller University, 1230 York Avenue, New York, NY 10021, USA (e-mail: jean-laurent.casanova@rockefeller.edu); or Capucine Picard, MD, PhD, Laboratory of Human Genetics of Infectious Diseases, INSERM U980- Necker Faculty, 156 rue de Vaugirard, 75015, Paris, France (e-mail: capucine.picard@inserm.fr).

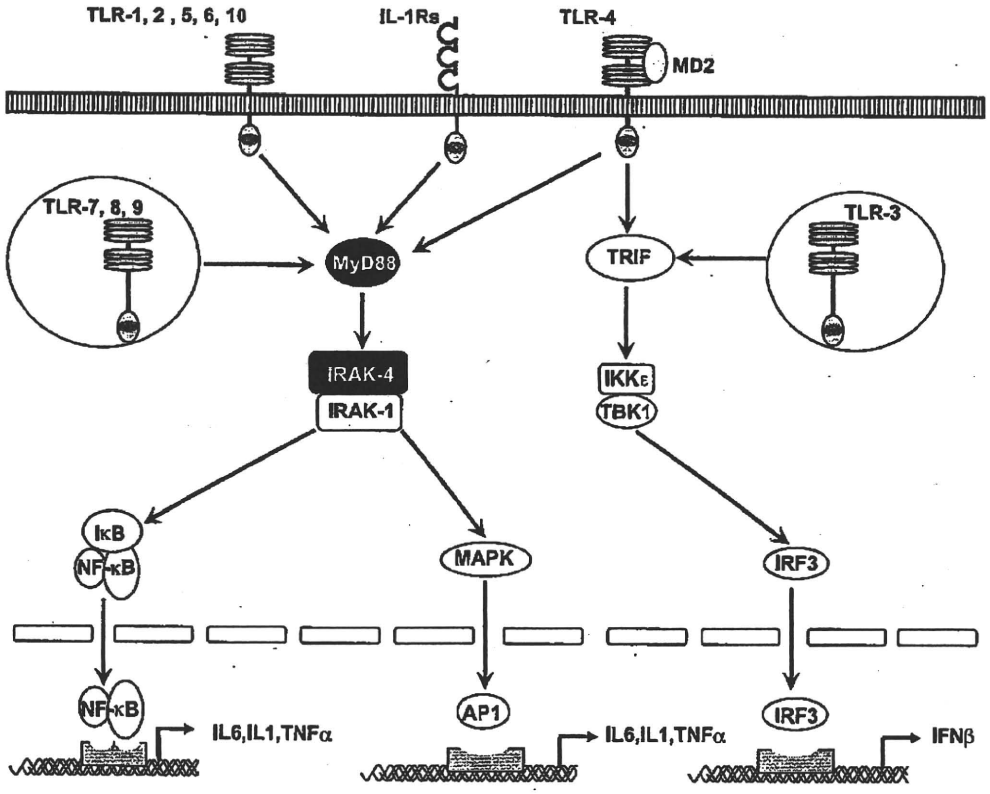

FIGURE 1. Schematic representation of TIRs signaling pathway. MyD88 interacts with TLRs and IL-1Rs through a shared TIR domain. MyD88 is a key cytosolic adapter molecule, providing a bridge from TLRs and IL-1Rs to the 2 active kinases IRAK-4 and IRAK-1. IRAK-4 and IRAK-1 then activate at least the 2 signaling NF-kB and MAPK pathways. The MyD88- and IRAK-4-dependent TIR pathway leads among others to the synthesis of inflammatory cytokines, such as IL-1β, IL-6, IL-8, TNF-α, and to IFN-α/β and IFN-λ, at least for TLR7, TLR8 and TLR9. The MyD88- and IRAK-4-independent TIR pathway uses TRIF pathway after stimulation of TLR3 and TLR4. This pathway is important for IFN-α and IFN-β production.

to confirm clinical information, including the prevalence, clinical presentation, and histologic features of noninvasive infections, such as otitis media, dermatitis, lymphadenitis, and necrotizing pharyngitis. Clinical and laboratory data were collected for the patients from their birth until December 2009, or until their death if they died before this date.

Activation by TLR Agonists and Cytokine Determinations

The activation of cells in whole-blood samples and the levels of TNF-α and IL-6 secretion were determined by enzyme-linked immunosorbent assay (ELISA), as previously described.[25] Granulocytes were isolated by Ficoll density gradient centrifugation, activated with TLR agonists, stained with anti-CD62L-FITC (BD) antibody, and analyzed by flow cytometry, as previously described.[48] Twenty kindreds with IRAK-4 deficiency and the 6 kindreds with MyD88 deficiency were explored in our laboratory, by 1 or by both exploratory methods. The remaining 11 kindreds with IRAK-4 deficiency were identified by other teams.

Sequencing Analysis

Genomic DNA was isolated by phenol/chloroform extraction. RNA was isolated with Trizol (GibcoBRL Life Technologies, Invitrogen SARL). Genomic DNA and cDNAs for IRAK4 and MYD88 were amplified, sequenced, and analyzed on an ABI Prism 3700 apparatus (BigDye Terminator sequencing kit, Applied Biosystems), as previously described.[25] Twenty kindreds with IRAK-4 deficiency and the 6 kindreds with MyD88 deficiency were identified in our laboratory by sequencing analysis. The remaining 11 kindreds with IRAK-4 deficiency were identified by other teams.

Western Blotting

Proteins for Western blotting were extracted from peripheral blood mononuclear cells, Epstein-Barr virus-transformed B cells, and SV40-transformed fibroblasts. Western blots were probed with rabbit antibodies against IRAK-4 (Tularik and Cell Signaling Technology), MyD88 (CSA-510, Stressgen), and glyceraldehyde-3-phosphate dehydrogenase (GAPDH) (Santa Cruz Biotechnology, Inc.).

Immunologic Investigations

Immunologic investigations were based on those described in previous studies and/or the questionnaires sent to physicians. Lymphocyte subsets were determined by routine flow cytometry. Serum levels of the IgM, IgA, IgG, and IgG subclasses were assessed by standard nephelometry techniques. Total IgG antibody levels against multiple pneumococcal serotypes (23 serotypes),[5,22] levels of IgG against *Haemophilus influenzae* PRP antigens, tetanus toxoid, and diphtheria were assessed by standard ELISA techniques. We carried out a prospective study in 9 IRAK-4-deficient patients, for whom antibody titers against serotype-specific pneumococcal capsular polysaccharides were determined, as previously described, before and after immunization with nonconjugate antipneumococcal vaccine.[22,50] The United States Pneumococcal Reference Serum Lot 89-SF was

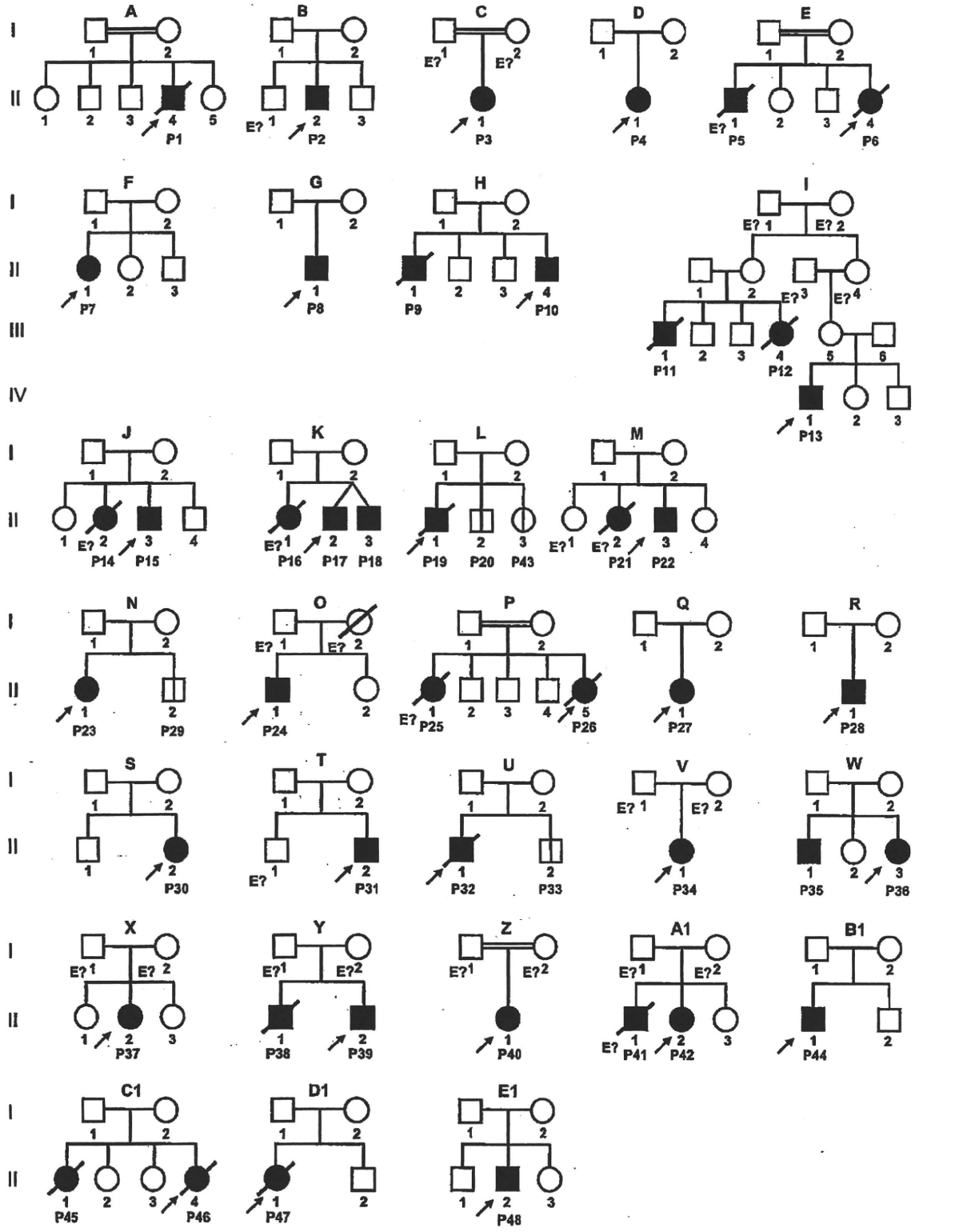

FIGURE 2. Pedigrees of the 31 kindreds identified with IRAK-4 deficiency. Each kindred with IRAK-4 deficiency is designated by a capital letter (A–E1) each generation is designated by a Roman numeral (I–IV), and each individual is designated by an Arabic numeral (from left to right). Patients with a clinical phenotype are indicated by closed symbols. Patients with confirmed IRAK-4 deficiency but no clinical phenotype as yet are indicated by an open square or circle divided by a black line. In each family, the proband is indicated by an arrow. Individuals whose genetic status could not be evaluated are indicated by "E?".

used as a reference. We determined IgG concentrations against serotype 3 (a strong immunogen), serotypes 4, 14, and 19F (intermediate immunogens), and serotypes 6B, 9N, and 18C (weak immunogens). A normal response is defined as an increase in antibody titers by a factor of at least 3. All antibody determinations were performed before or several months after the end of immunoglobulin treatment.

Statistical Analysis

Infection-free status and survival curves as a function of age were estimated by the Kaplan-Meier method, and, when necessary, curves were compared by log-rank tests.

RESULTS

Description of Patients and Kindreds

We studied 48 patients (26 male and 22 female patients) from 31 kindreds with IRAK-4 deficiency (kindred A to E1)[6,8,12,14–16,18,20,23–25,27,30,32,38,44] (present report) and 12 patients (7 male and 5 female patients) from 6 kindreds with MyD88 deficiency (kindred a to f)[49] (present report) (Figures 2 and 3; Table 1). This series includes all 45 patients (36 IRAK-4 and 9 MyD88) described in previous reports (24 and 5 kindreds, respectively) and 15 newly diagnosed patients (12 IRAK-4 and 3 MyD88 patients, corresponding to 7 kindreds and 1 kindred, respectively). In all probands, diagnosis was based on the detection of homozygous or compound heterozygous mutations in IRAK4 or MYD88 accompanied by a lack of production of IL-6 by whole blood or of CD62L shedding from granulocytes following activation with TLR/IL-1Rs agonists.[38,48,49] In addition, 16 relatives were found to be homozygous or compound heterozygous for mutations in IRAK4 or MYD88. Finally, 7 sibs who had died of bacterial infection were considered to have IRAK-4 or MyD88 deficiency retrospectively, by inference from the personal and familial history.

The parents were consanguineous in 7 of the 37 kindreds. Up to 18 cases were sporadic, whereas 42 cases were familial (19 kindreds). The 37 families originated from 15 countries on 4 continents, including North America (Canada, El Salvador, United States), Asia (Israel, Japan, Saudi Arabia, Turkey), Australia, and Europe (France, Hungary, Portugal, Serbia, Slovenia, Spain, United Kingdom). Most patients and their families were living in their countries of origin, with the exception of a Portuguese family living in France, a Serbian family living in Switzerland, a Turkish family living in Germany, and a family from El Salvador living in the United States (Figure 4; Table 1).

IRAK4 and MYD88 Mutations

Patients with IRAK-4 deficiency were homozygous in 17 kindreds, whereas those from 14 other kindreds were compound heterozygous for IRAK4 mutations (Table 1). One seemingly homozygous patient (B-P2) was actually compound heterozygous for the Q293X mutation, inherited from his mother, and for a large de novo deletion (designated BAC210N13del) encompassing the IRAK4 gene.[25] Two other patients from the same family (I-P11 and I-P12) had 1 parent who did not carry the mutant allele. Not enough material was available to explore the IRAK4 locus further in deceased patients P11 and P12 from kindred I.[8] Two of the newly identified mutations were nonsense mutations (R183X and Y430X), 1 was a splice mutation (1126-1 G>T), 2 were frameshift insertions and deletions (43insA and 897_900delCAT), and 2 were missense mutations (M1V and G298D). All the mutations other than the missense mutations were predicted to be loss-of-expression and loss-of-function, as they create a premature termination codon or delete a large segment of the gene. The M1V mutation affecting the initiation codon was also likely to be severely deleterious. No IRAK-4 protein was detected in the patient bearing the M1V/1188+520A>G mutant alleles, whereas the patient bearing the G298D mutation at compound heterozygous state (G298D/Q293X) did produce IRAK-4 protein in peripheral blood mononuclear cells and in B cell lines. All the previously reported mutations are loss-of-expression,[25] with the exception of the R12C and 831+5G>T mutant alleles in patient T-P31, which are associated with residual IRAK-4 protein production.[20]

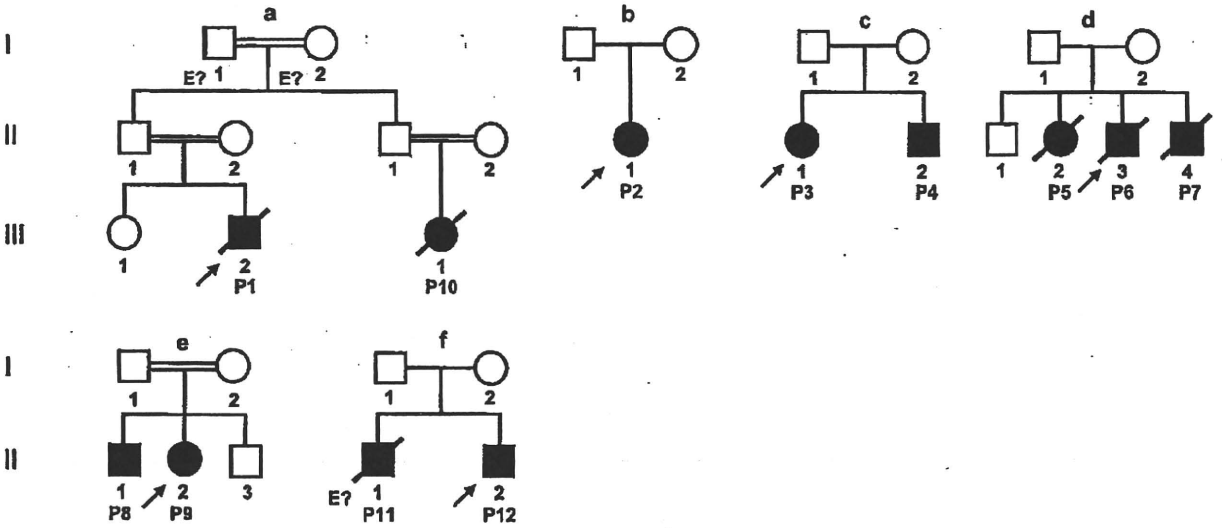

FIGURE 3. Pedigrees of the 6 kindreds with MyD88 deficiency identified. Each kindred with MyD88 deficiency is designated by a lower case letter (a–f); each generation is designated by a Roman numeral (I–IV), and each individual is designated by an Arabic numeral (from left to right). Patients with a clinical phenotype are indicated by closed symbols. In each family, the proband is indicated by an arrow. Individuals whose genetic status could not be evaluated are indicated by "E?".

TABLE 1. Country of Origin, Genotype, Infectious Phenotype, and Outcome in the Cohort of IRAK-4- and MyD88-Deficient Patients

Kindred	P	Age/Sex (yr)	Mutation of IRAK4	Country of Origin	Outcome Status	Invasive Infections With Gram-Positive Bacteria	Invasive Infections With Gram-Negative Bacteria	Other	Ref.
A	1	7/M	821delT/821delT	Saudi Arabia	†	*S. pneumoniae, S. aureus*			38
B	2	17/M	Q293X/BAC210N13del	Portugal	Alive	*S. pneumoniae, S. aureus*			25,38
C	3	14/F	Q293X/Q293X	USA	Alive	*S. pneumoniae, S. aureus*		*Enterovirus, Curvularia* species	15,19,25,38
D	4	27/F	Q293X/620-621delAC	USA	Alive	*S. pneumoniae, C. septicum*	*N. meningitidis*		25,26,32
E	5	16 mo/M	ND	Turkey	†	*S. pneumoniae, S. parasanguis*			18
E	6	2 mo/F	523delA/523delA	Turkey	†	*S. pneumoniae,*			18
F	7	35/F	Q293X/Q293X	UK	Alive	*S. pneumoniae,*	*S. sonnei*		12,25
G	8	11/M	1189-1G>T/ 1188+520A>G	Hungary	Alive	*S. pneumoniae*			24,25
H	9	5.5/M	Q293X/Q293X	Canada	†	*S. pneumoniae*	*P. aeruginosa*		14,25
H	10	10/M	Q293X/Q293X	Canada	Alive	*S. pneumoniae*			14,25
I	11	2.5/M	E402X/del (?)	Spain	†	*S. aureus*	*P. aeruginosa*		8,25
I	12	8 mo/F	E402X/del (?)	Spain	†	*S. pneumoniae*	*P. aeruginosa*		8,25
I	13	12/M	E402X/ E402X	Spain	Alive	*S. pneumoniae*			8,25
J	14	3 mo/F	ND	Israel	†	*S. milleri*			25
J	15	12/M	1-1096_40+23del/ 1-1096_40+23del	Israel	Alive	*S. pneumoniae*			25
K	16	5 mo/F	ND	Canada	†	*S. aureus*			25,27
K	17	30/M	Q293X/Q293X	Canada	Alive	*S. pneumoniae*			25,27
K	18	30/M	Q293X/Q293X	Canada	Alive	*S. pneumoniae*		*M. avium*	25,27
L	19	2.5/M	118insA/118insA	Japan	†	*S. pneumoniae*			25,44
L	20	4/M	118insA/118insA	Japan	Alive				25,44
M	21	4 mo/F	ND	USA	†			Undocumented meningitis	25
M	22	3/M	Q293X/620-621delAC	USA	Alive	*S. pneumoniae*			25
N	23	5/F	Y48X/631delG	Canada	Alive				25
O	24	19/M	1240insA/ 942-1481_1125+547del	Canada	Alive	*S. pneumoniae, S. aureus*			25
P	25	4 mo/F	ND	Australia	†	*S. pneumoniae*			25
P	26	6 mo/F	Q293X/Q293X	Australia	†	*S. pneumoniae, S. aureus*			25
Q	27	14/F	Q293X/Q293X	USA	Alive	*S. pneumoniae*			25
R	28	10/M	Q293X/Q293X	USA	Alive	*S. pneumoniae, S. aureus*	*P. aeruginosa*	*Enterovirus*	25,30

N	29	2/M	Y48X/631delG	Canada	Alive				PR
S	30	4/F	M1V/1188+520A>G	Slovenia	Alive		*P. aeruginosa*		16
T	31	18/M	R12C/831+5G>T	France	Alive	*S. aureus*	*P. aeruginosa*		20
U	32	15 mo/M	Q293X/Q293X	UK	†	*S. pneumoniae, S. aureus*			PR
U	33	6 mo/M	Q293X/Q293X	UK	Alive				PR
V	34	6/F	Q293X/G298D	UK	Alive	*S. pneumoniae*			6
W	35	13/M	Y430X/1126-1 G>T	El Salvador	Alive			Bacterial infec.	23
W	36	4/F	Y430X/1126-1 G>T	El Salvador	Alive	*S. agalactiae, S. pneumoniae*	*S. sonnei*		23
X	37	14/F	Q293X/Q293X	UK	Alive	*S. pneumoniae, S. pyogenes*	*P. aeruginosa, H. influenzae* type b		6
Y	38	18 mo/M	Q293X/593delG	UK	†	*S. pneumoniae*	*P. aeruginosa*		6
Y	39	2/M	Q293X/593delG	UK	Alive				6
Z	40	6 mo/F	Q293X/Q293X	France	Alive		*P. aeruginosa*		PR
A1	41	14 mo/M	ND	UK	†		*P. aeruginosa*		PR
A1	42	4/F	Q293X/897_900delCAAT	UK	Alive	*S. pneumoniae*	*N. meningitidis*		PR
L	43	3/F	118insA/118insA	Japan	Alive				PR
B1	44	2/M	118insA /R183X	Japan	Alive	*S. pneumoniae*			PR
C1	45	3/F	118insA/118insA	Japan	†	*S. pneumoniae*	*P. aeruginosa*		PR
C1	46	9 mo/F	118insA/118insA	Japan	†	*S. pneumoniae*			PR
D1	47	12 mo/F	Q293X/831+5G>T	USA	†	*S. pneumoniae*			PR
E1	48	12/M	Q293X/Q293X	France	Alive	*S. pneumoniae*			PR

Kindred	P	Age/Sex (yr)	Mutation of MyD88*	Country of Origin	Outcome Status	Invasive Infections With Gram-Positive Bacteria	Invasive Infections With Gram-Negative Bacteria	Other	Ref.
a	1	11 mo/M	E65del/E65del	France	†	*S. pneumoniae*		Adenovirus Rotavirus	49
b	2	5/F	R209C/L106P	Turkey	Alive	*S. pneumoniae*			49
c	3	17/F	R209C/R209C	Portugal	Alive	*S. pneumoniae*	*Salmonella* spp.		49
c	4	11/M	R209C/R209C	Portugal	Alive	*S. pneumoniae, S. aureus,* β-hemolytic *Streptococci*	*S. enteriditis*		49
d	5	11 mo/F	E65del/E65del	Spain	†	*S. pneumoniae*		RSV	49
d	6	4/M	E65del/E65del	Spain	†	*S. pneumoniae, S. aureus*			49
d	7	2 mo/M	E65del/E65del	Spain	†	*S. pneumoniae*			49
e	8	8/M	E65del/E65del	Spain	Alive	β-hemolytic *Streptococci*			49
e	9	5/F	E65del/E65del	Spain	Alive	*S. pneumoniae, S. aureus*		*C. albicans*	49
a	10	2/F	E65del/E65del	France	†	*S. aureus, S. pneumoniae*	*H. influenzae* type e	*M. catarrhalis*	PR
f	11	5 mo/M	ND	Serbia	†		*P. aeruginosa*		PR
f	12	1/M	E65del/E65del	Serbia	Alive		*P. aeruginosa*		PR

Abbreviations: † = dead, ND = no data, P = patient, PR = present report, RSV = respiratory syncytial virus, UK = United Kingdom, USA = United States of America.

*The mutation numbering has been adjusted from the original report (reference 49) to correspond to the dominant ATG initiation in the new reference sequence of GenBank (NM 001172567.1, GI 289546502).

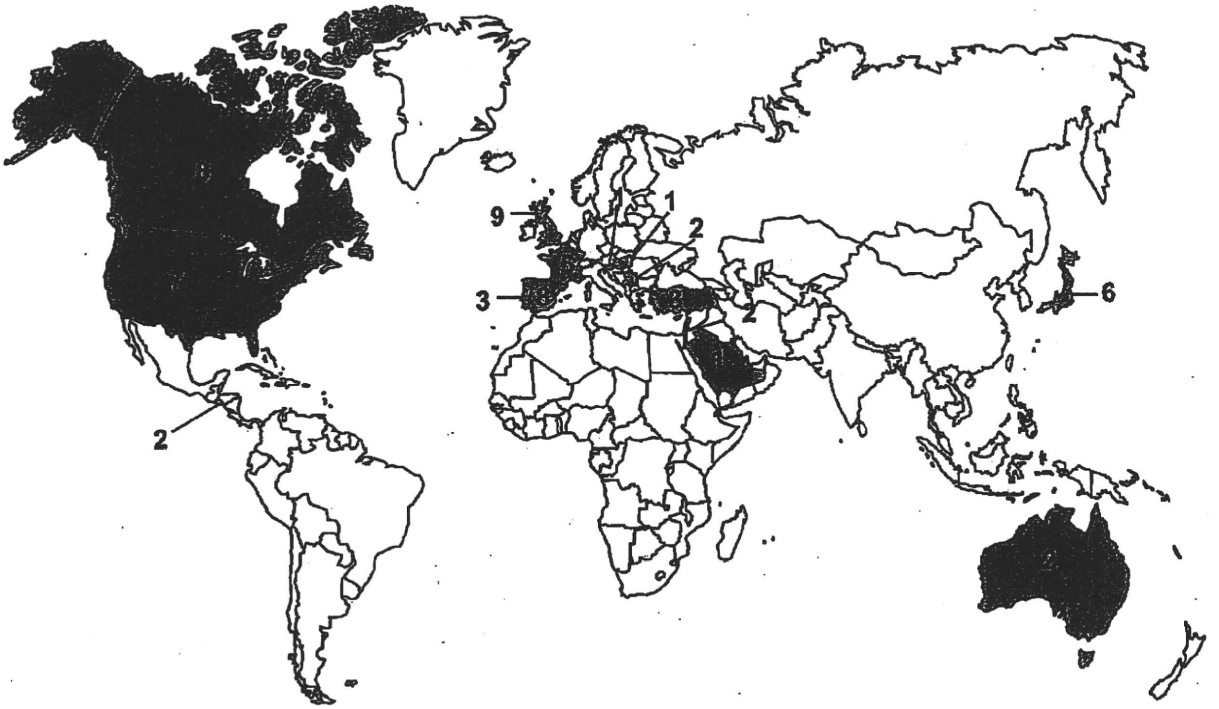

FIGURE 4. Countries of origin of the 31 kindreds with IRAK-4 deficiency and the 6 kindreds with MyD88 deficiency identified. The number of patients identified in each country is indicated.

Patients with MyD88 deficiency from 5 kindreds were homozygous, and 1 patient (b-P2) was compound heterozygous.[49] Two MyD88 mutant alleles were found to be associated with the production of very small amounts of a nonfunctional protein (E65del and L106P), whereas the R209C mutant allele was associated with the quantitatively normal production of a nonfunctional protein.[49] (The mutation numbering has been adjusted from the original report [reference 49] to correspond to the dominant ATG initiation in the new reference sequence of GenBank [NM 001172567.1, GI 289546502].)

Immunologic Investigations

We analyzed blood leukocyte subsets in 29 patients with IRAK-4 deficiency and 10 patients with MyD88 deficiency. We previously showed that monocyte and dendritic cell subsets were present in normal numbers in 3 patients with IRAK-4 deficiency.[25] T-cell subsets, including CD4 and CD8 T cells, were also present in normal numbers (24 patients with IRAK-4 deficiency and 6 with MyD88 deficiency tested) (Tables 2 and 3). T cells proliferated normally in response to the mitogen phytohemagglutinin, CD3-specific antibodies, and recall antigens in vitro (12 patients with IRAK-4 deficiency and 3 with MyD88 deficiency tested).

IgM, IgG, and IgA levels were normal for age in 15 IRAK-4-deficient and in 3 MyD88-deficient patients, and high in 12 IRAK-4-deficient and 4 MyD88-deficient patients. Among these patients, 5 patients with IRAK-4 deficiency and 2 with MyD88 deficiency had very high IgG4 levels. IgG level was low in 1 IRAK-4-deficient patient (U-P32) and 1 MyD88-deficient patient (d-P5). Two IRAK-4-deficient patients had high levels of IgM (I-P13, V-P34). In particular, IgE levels were high in 14 IRAK-4-deficient patients and in 3 MyD88-deficient patients, with a total of 26 patients evaluated (Tables 4–6). The highest IgE-levels in IRAK4- and MyD88-deficient patients were, however, not as high as those in patients with STAT3 or DOCK8 deficiency, with the exception of 1 patient with IRAK-4 deficiency (A-P1).[34,53] Antibody responses to protein antigens (tetanus toxoid, poliovirus and/or diphtheria) were normal in the 17 IRAK-4-deficient and 2 MyD88-deficient patients tested. Six of the 13 IRAK-4-deficient (A-P1, F-P7, J-P15, O-P24, S-P30, V-P34) and all 5 MyD88-deficient (b-P2, c-P3, e-P4, e-P9, a-P10) patients tested had detectable IgG antibodies against pneumococcus after infection and/or immunization with conjugate or nonconjugate vaccines. The antibody response (serotypes 3, 4, 6B, 9N, 14, 18C, 19F) to glycans following nonconjugated pneumococcal vaccine was impaired in 5 (B-P2, G-P8, K-P17, K-P18, R-P28) of the 9 IRAK-4-deficient patients explored. Three IRAK-4-deficient patients (H-P10, O-P24, W-P36) received conjugated and nonconjugated pneumococcal vaccine, and the response to vaccination was in the normal range in 2 of these patients (O-P24, W-P36), at least at the time points 1 and 5 months after the last booster vaccination. One IRAK-4-deficient patient (S-P30) received only conjugated pneumococcal vaccine, and the response to immunization was normal. Unfortunately, we found no correlation between the presence or absence of antipneumococcal antibodies and the occurrence of invasive pneumococcal disease. The antibody response to conjugated *H. influenzae* type b vaccine was normal in the 13 IRAK-4-deficient patients and 1 MyD88-deficient patient explored. One IRAK-4-deficient patient (X-P37) who developed meningitis caused by *H. influenzae* type b had antibodies against *H. influenzae* type b after infection. The production of IgM allo-hemagglutinins directed against erythrocyte AB antigens was impaired in 3 of the 10 IRAK-4-deficient and in 1 of the 3 MyD88-deficient patients explored (Tables 4–6).

Finally, the counts of CD16-positive and CD56-positive NK cells were normal in the 19 IRAK-4-deficient and 6 MyD88-deficient patients tested (Tables 2 and 3). There thus seemed to

TABLE 2. Immunologic Investigation: Blood Lymphocyte Subsets and T-Cell Proliferation in IRAK-4–Deficient Patients*

Patients	P40	P26	P46	P10	P23	P30	P38	P43	P11	P19	Normal Values	P36	P13	P34	P37	P1	P15	P28	P8	Normal Values	P3	P31	P24	P2	Normal Values	P17	P18	Normal Values
(age)	3 mo	6 mo	9 mo	1 yr	1 yr	1 yr	1 yr	1 yr	2 yr	2 yr	3 mo–2 yr	3 yr	4 yr	4 yr	4 yr	5 yr	5 yr	5 yr	6 yr	2–6 yr	7 yr	11 yr	13 yr	14 yr	7–14 yr	27 yr	27 yr	Adult
Lympl., 10^9/μL	7.5	4.2		5.97	6.2	5.2	5.25	6.38	5.0		(3.4–9)	2.95	2.8	3.6	3.82	3.3	4.8	3.2	1.9	(2.3–5.4)	5.0	2.4	2.1	2.6	(1.9–3.7)	1.3	1.3	(1.4–3.3)
T cells, %																												
CD3	68	62		59	77	66	45	77	71	73	(53–84)	79	58	63	60	75	86	57	73	(56–75)	68	85	76	75	(56–84)	72	76	(63–84)
CD4	42	35	36	45	48	50	33	57	48		(31–64)	49	35	39	33	55	63	35	46	(28–47)	52	48	48	54	(31–52)	56	46	(34–62)
CD8	24	21	29	11	26	14	10	18	29		(12–30)	29	19	19	27	32	19	24	22	(16–30)	14	28	24	22	(18–35)	13	20	(14–41)
CD45RA/CD4											(64–95)									(53–86)				58	(46–77)			
CD45RO/CD4																								45				
NK cells, %	12	15			5	12	7	8			(4–18)	6	20	9	8		3	3	5	(4–17)	2	13	2	12	(3–22)	11	8	(5–20)
B cells, %	18	22		34	10	20	45	14	7	17	(6–41)	13	19	27	26	23	6	38	18	(14–33)	28	10	10	16	(6–23)	15	12	(6–17)
Prolif. × 10 3cpm																												
CD3											(>30)									(>30)				80	(>30)	26	48	(>30)
PHA		64		44	59			40.7	169		(>50)				50		242	387		(>50)			235	238	(>50)	112	135	(>50)
PPD											(>10)						7			(>10)				94	(>10)	1.7	10	(>10)
Candidin											(>10)				10		7			(>10)				16	(>10)	7	19	(>10)
Tetanus					35						(>10)									(>10)				123	(>10)	0.5	1	(>10)
S. aureus				237							(>10)									(>10)					(>10)			(>10)

*Data given as total lymphocyte counts and percentages of T cells, NK cells, and B cells. Age-specific normal values are shown in parentheses. Proliferative responses to OKT3 (50 ng/mL) ("CD3"), the mitogen PHA, and various antigens (PPD, candidin, tetanus) are listed.

TABLE 3. Immunologic Investigation: Blood Lymphocyte Subsets and T-Cell Proliferation in MyD88-Deficient Patients*

Patients	P1	P10	P2	Normal Values	P6	P9	Normal Values	P8	P4	Normal Values
(age)	10 mo	15 mo	2 yr	3 mo–2 yr	2.5 yr	3 yr	2–6 yr	7 yr	8 yr	6–12 yr
Lymph., $10^9/\mu L$		5.5	2.4	(3.4–9)		3.32	(2.3–5.4)	2.43		(1.9–3.7)
T cells, %										
CD3		69	64	(53–84)	68	64	(56–76)	47	77	(60–76)
CD4		39	39	(31–64)	35	39	(28–47)	25	43	(31–47)
CD8		22	27	(12–30)	33	25	(16–30)	22	27	(18–35)
CD45RA/CD4		79		(64–95)			(53–86)		46	(46–77)
CD45RO/CD4		25							59	
NK cells, %		5	20	(4–18)	7	11.5	(4–17)	26	4	(4–17)
B cells, %	27	24	18	(6–41)	23	17.5	(14–33)	17	18	(13–27)
Prolif. × 10 3cpm										
CD3			46	(>30)					56	(>30)
PHA			175.5	(>50)		50			113	(>50)
PPD			4.2	(>10)					10.3	(>10)
Candidin			1.0	(>10)					15.2	(>10)
Tetanus			26.0	(>10)					21	(>10)

*Data given as total lymphocyte counts and percentages of T cells, NK cells, and B cells. Age-specific normal values are shown in parentheses. Proliferative responses to OKT3 (50 ng/mL) ("CD3"), the mitogen PHA, and various antigens (PPD, candidin, tetanus) are listed.

be no overt defect of leukocyte development in IRAK-4- and MyD88-deficient patients. Antigen-specific T- and B-cell responses seemed to be normal, as detected with these routine immunologic evaluations, with 2 notable exceptions. First, the glycan-specific IgG and IgM antibody response against at least pneumococcal and AB glycans was impaired in half of the patients tested. Second, serum IgG4 and IgE levels were high in up to 35% (n = 7/20) and 65% (n = 17/26), respectively, of the patients tested (both were high in 4 patients). Nevertheless, none of the MyD88- and IRAK-4-deficient patients in this cohort suffered from allergic asthma, and a chronic eczematous skin disease was reported only in patient F-7. A survey is underway to assess laboratory and clinical manifestation of allergy in patients with MyD88 and IRAK-4 deficiency (Gallego and Picard, unpublished data).

Invasive Bacterial Infections

Invasive bacterial disease (InvBD) is defined here as clinical disease due to the presence of a disease-causing bacterium in a normally sterile fluid or tissue. There were 114 reported episodes of InvBD in 48 IRAK-4-deficient patients (n = 2.38 episodes per patient; range, 0–10), including meningitis (47 episodes, 41.2% of all invasive episodes), sepsis (including bacteremia, septicemia, and shock; 26 episodes, 22.8%), arthritis (17 episodes, 14.9%), osteomyelitis (7 episodes, 6.1%), and deep inner organ/tissue abscesses (17 episodes, 14.9%) (Figure 5). Deep-seated

TABLE 4. Immunologic Investigation: Ig Levels and Humoral Responses to Recall Antigens and to Glycans in IRAK-4-Deficient Patients*

Patients	P6	P22	P40	P26	P46	Normal Values	P5	P10	P23	P30	P32	P38	P43	P11	P39	Normal Values
(age)	2 mo	3 mo	3 mo	6 mo	9 mo	(3–9 mo)	1 yr	1 yr	1 yr	1 yr	1 yr	1 yr	1 yr	2 yr	2 yr	(1–3 yr)
Serum Ig (g/L)																
IgG	5.7	2.45	7.38	5.68	3.84	(2.35–5.49)	5.4	11.4	8.9	16.9	2.21	4.55	13.8	17	6.31	(3.35–8.96)
IgG1					1.37	NA		7.2		13.05			8.17			(>3)
IgG2					0.30	NA		1.26		2.09			2.01			(>0.30)
IgG3					0.07	NA		0.20		0.68			0.9			(>0.12)
IgG4					0.03	NA		0.28		0.41			<0.3			(<1)
IgA	0.6	0.16	0.25	0.14	0.3	(0.12–0.62)	0.2	0.34	0.47	1.09	0.26	0.34	0.86	0.9	0.25	(0.27–1.22)
IgM	0.6	0.51	0.63	0.40	0.33	(0.34–0.95)	1.1	1.02	1.11	0.98	0.43	0.33	2.27	2.0	0.52	(0.58–1.53)
IgE (kU/L)				19	203	(<15)		129	801	257		13.8	38.2	198		(<40)
Specific antibodies																
Antitetanus				0.21		(>0.1 IU/mL)			0.12	1.15		0.32		>0.1		(>0.1 IU/mL)
Poliovirus						(>40)										(>40)
Diphtheria				0.12		(>0.1 IU/mL)			0.26	0.7						(>0.1 IU/mL)
S. pneumoniae						(>0.3 μg/mL)			<0.3	2.04						(>0.3 μg/mL)
H. influenzae b				0.31		(>0.15 μg/mL)				>9	0.56	0.16				(>0.15 μg/mL)
Allohemagglutinin						NA								1/16		(>1/8)

*Serum immunoglobulin levels and titers for specific antibodies. Age-specific normal values are shown in parentheses.