**TABLE IV.** (*Continued*)

| Disease | Circulating T cells | Circulating B cells | Serum Ig | Associated features | Inheritance | Gene defects/ presumed pathogenesis |
|---|---|---|---|---|---|---|
| (iv) Caspase 8 defects, ALPS type 2b | Slightly increased CD4- CD8- T cells | Normal | Normal or decreased | Adenopathy, splenomegaly, recurrent bacterial and viral infections, defective lymphocyte apoptosis and activation; | AD | Defects in *CASP8*, intracellular apoptosis and activation pathways |
| (v) Activating N-Ras defect, N-Ras ALPS | Increased CD4- CD8- T cells | Elevation of $CD5^+$ B cells | Normal | Adenopathy, splenomegaly, leukemia, lymphoma, defective lymphocyte apoptosis after IL-2 withdrawal | AD | Defect in *NRAS* encoding a GTP binding protein with diverse signaling functions; activating mutations impair mitochondrial apoptosis |
| (b) APECED (autoimmune polyendocrinopathy with candidiasis and ectodermal dystrophy) | Elevated $CD4^+$ cells | Normal | Normal | Autoimmune disease, particularly of parathyroid, adrenal, and other endocrine organs plus candidiasis, dental enamel hypoplasia, and other abnormalities | AR | Defects in *AIRE*, encoding a transcription regulator needed to establish thymic self-tolerance |
| (c) IPEX (immune dysregulation, polyendocrinopathy, enteropathy [X-linked]) | Lack of $CD4^+$ $CD25^+$ $FOXP3^+$ regulatory T cells | Normal | Elevated IgA, IgE | Autoimmune diarrhea, early-onset diabetes, thyroiditis, hemolytic anemia, thrombocytopenia, eczema | XL | Defects in *FOXP3*, encoding a T-cell transcription factor |

*AD*, Autosomal-dominant inheritance; *AIRE*, autoimmune regulator; *ALPS*, autoimmune lymphoproliferative syndrome; *AP-3*, adaptor-related protein complex 3; *AR*, autosomal-recessive inheritance; *CTL*, cytotoxic T lymphocytes; *GTPase*, guanosine triphosphatase; *IPEX*, immune dysregulation, polyendocrinopathy, enteropathy, X-linked; *NRAS*, neuroblastoma ras viral oncogene homolog; *XL*, X-linked inheritance; *XLP*, X-linked lymphoproliferative syndrome.

**TABLE V.** Congenital defects of phagocyte number, function, or both

| | Disease | Affected cells | Affected function | Associated features | Inheritance | Gene defects/ presumed pathogenesis |
|---|---|---|---|---|---|---|
| 1.-3. | Severe congenital neutropenias | N | Myeloid differentiation | Subgroup with myelodysplasia | AD | *ELA2*: mistrafficking of elastase |
| | | N | Myeloid differentiation | B/T lymphopenia | AD | *GFI1*: repression of elastase |
| | | N | Myeloid differentiation | G-CSF refractory neutropenia | AD | G-CSFR |
| 4. | Kostmann disease | N | Myeloid differentiation | | AR | HAX1: control of apoptosis |
| 5. | Cyclic neutropenia | N | ? | Oscillations of other leukocytes and platelets | AD | *ELA2*: mistrafficking of elastase |
| 6. | X-linked neutropenia/ myelodysplasia | N + M | ? | Monocytopenia | XL | *WASP*: regulator of actin cytoskeleton (loss of autoinhibition) |
| 7. | P14 deficiency | N + L Mel | Endosome biogenesis | Neutropenia<br>Hypogammaglobulinemia<br>↓CD8 cytotoxicity<br>Partial albinism<br>Growth failure | AR | *MAPBPIP*: endosomal adaptor protein 14 |
| 8. | Leukocyte adhesion deficiency (LAD) type 1 | N + M<br>L + NK | Adherence<br>Chemotaxis<br>Endocytosis<br>T/NK cytotoxicity | Delayed cord separation<br>Skin ulcers<br>Periodontitis<br>Leukocytosis | AR | *ITGB2*: adhesion protein |
| 9. | Leukocyte adhesion deficiency type 2 | N + M<br>N + M | Rolling<br>Chemotaxis | LAD type 1 features plus hh-blood group and mental retardation | AR | FUCT1 GDP-fucose transporter |
| 10. | Leukocyte adhesion deficiency type 3 | L + NK | Adherence | LAD type 1 plus bleeding tendency | AR | Cal DAG-GEF1: defective Rap1-mediated activation of β1-3 integrins |
| 11. | Rac 2 deficiency | N | Adherence<br>Chemotaxis<br>$O_2^-$ production | Poor wound healing<br>Leukocytosis | AD | *RAC2*: regulation of actin cytoskeleton |
| 12. | β-Actin deficiency | N + M | Motility | Mental retardation<br>Short stature | AD | *ACTB*: cytoplasmic actin |
| 13. | Localized juvenile periodontitis | N | Formylpeptide-induced chemotaxis | Periodontitis only | AR | *FPR1*: chemokine receptor |
| 14. | Papillon-Lefèvre syndrome | N + M | Chemotaxis | Periodontitis, palmoplantar hyperkeratosis | AR | *CTSC*: cathepsin C activation of serine proteases |
| 15. | Specific granule deficiency | N | Chemotaxis | N with bilobed nuclei | AR | *C/EBPE*: myeloid transcription factor |
| 16. | Shwachman-Diamond syndrome | N | Chemotaxis | Pancytopenia, exocrine pancreatic insufficiency<br>Chondrodysplasia | AR | *SBDS* |
| 17. | X-linked chronic granulomatous disease | N + M | Killing (faulty $O_2^-$ production) | Subgroup: McLeod phenotype | XL | *CYBB*: electron transport protein (gp91phox) |
| 18.-20. | Autosomal chronic granulomatous diseases | N + M | Killing (faulty $O_2^-$ production) | | AR | *CYBA*: Electron transport protein (p22phox)<br>*NCF1*: Adapter protein (p47phox)<br>*NCF2*: Activating protein (p67phox) |
| 21. | Neutrophil G-6PD deficiency | N + M | Killing (faulty $O_2^-$ production) | Hemolytic anemia | XL | *G-6PD*: NADPH generation |

**TABLE V.** *(Continued)*

| | Disease | Affected cells | Affected function | Associated features | Inheritance | Gene defects/ presumed pathogenesis |
|---|---|---|---|---|---|---|
| 22. | IL-12 and IL-23 receptor β1 chain deficiency | L + NK | IFN-γ secretion | Susceptibility to *Mycobacteria* and *Salmonella* | AR | *IL-12Rβ1*: IL-12 and IL-23 receptor β1 chain |
| 23. | IL-12p40 deficiency | M | IFN-γ secretion | Susceptibility to *Mycobacteria* and *Salmonella* | AR | *IL-12p40* subunit of IL12/ IL23: IL12/IL23 production |
| 24. | IFN-γ receptor 1 deficiency | M + L | IFN-γ binding and signaling | Susceptibility to *Mycobacteria* and *Salmonella* | AR, AD | *IFN-γR1*: IFN-γR binding chain |
| 25. | IFN-γ receptor 2 deficiency | M + L | IFN-γ signaling | Susceptibility to *Mycobacteria* and *Salmonella* | AR | *IFN-γR2*: IFN-γR signaling chain |
| 26. | STAT1 deficiency (2 forms) | M + L | IFN α/β/γ signaling | Susceptibility to *Mycobacteria, Salmonella* and *viruses* | AR | *STAT1* |
| | | | IFN-γ signaling | Susceptibility to *Mycobacteria* and *Salmonella* | AD | *STAT1* |

*ACTB*, Actin beta; *AD*, inherited form of IFN-Rγ1 deficiency or of *STAT1* deficiency caused by dominant-negative mutations; *AR*, autosomal recessive inheritance; *Cal DAG-GEF1*, calcium and diacylglycerol-regulated guanine nucleotide exchange factor 1; *ELA*, neutrophil elastase; *FPR*, formyl peptide; *FUCT*, fucosidase regulator; *G-CSF*, granulocyte colony-stimulating factor; *G-CSFR*, G-CSF receptor; *GDP*, guanosine diphosphate; *GFI*, growth factor independent 1; *HAX*, HSLS1-associated protein X1; *ITGB2*, integrin beta-2; *L*, lymphocytes; *M*, monocytes-macrophages; *MAPBPIP*, MAPBP-interacting protein; *Mel*, melanocytes; *N*, neutrophils; *WASP*, Wiskott-Aldrich syndrome protein; *XL*, X-linked inheritance.

**TABLE VI.** Defects in innate immunity

| Disease | Affected cell | Functional defect(s) | Associated features | Inheritance | Gene defects/ presumed pathogenesis |
|---|---|---|---|---|---|
| EDA-ID | Lymphocytes + monocytes | NF-κB signaling pathway | Anhidrotic ectodermal dysplasia + specific antibody deficiency (lack of antibody response to polysaccharides), various infections (mycobacteria and pyogens) | XL | Mutations of *NEMO (IKBKG)*, a modulator of NF-κB activation |
| EDA-ID | Lymphocytes + monocytes | NF-κB signaling pathway | Anhidrotic ectodermal dysplasia + T-cell defect + various infections | AD | Gain-of-function mutation of *IKBA*, resulting in impaired activation of NF-κB |
| IRAK4 deficiency | Lymphocytes + monocytes | Toll and IL-1 receptor–IRAK signaling pathway | Bacterial infections (pyogens) | AR | Mutation of *IRAK4*, a component of TLR-signaling pathway |
| WHIM (warts, hypogammaglobulinemia infections, myelokathexis) syndrome | Granulocytes + lymphocytes | Increased response of the CXCR4 chemokine receptor to its ligand CXCL12 (SDF-1) | Hypogammaglobulinemia, reduced B-cell number, severe reduction of neutrophil count, warts/ human papilloma virus infection | AD | Gain-of-function mutations of *CXCR4*, the receptor for CXCL12 |
| Epidermodysplasia verruciformis | Keratinocytes and leukocytes | ? | Human papilloma virus (group B1) infections and cancer of the skin | AR | Mutations of *EVER1*, *EVER2* |
| Herpes simplex encephalitis | Central nervous system resident cells, epithelial cells, and leukocytes | UNC-93B-dependent IFN-α, IFN-β, and IFN-λ induction | Herpes simplex virus 1 encephalitis and meningitis | AR | Mutations of *UNC93B1* |
| Herpes simplex encephalitis | Central nervous system resident cells, epithelial cells, dendritic cells, cytotoxic lymphocytes | TLR3-dependent IFN-α, IFN-β, and IFN-λ induction | Herpes simplex virus 1 encephalitis and meningitis | AD | Mutations of *TLR3* |

*AD*, Autosomal-dominant inheritance; *AR*, autosomal-recessive inheritance; *EDA-ID*, anhidrotic ectodermal dysplasia with immunodeficiency; *IKBA*, inhibitor of kappa light chain gene enhancer in B cells, alpha; *IRAK*, IL-1 receptor associated kinase; *NEMO*, NF-κB essential modulator; *NF-κB*, nuclear factor-κB; *TLR*, Toll-like receptor.

**TABLE VII.** Autoinflammatory disorders

| Disease | Affected cells | Functional defect(s) | Associated features | Inheritance | Gene defects |
|---|---|---|---|---|---|
| Familial Mediterranean fever | Mature granulocytes, cytokine-activated monocytes | Decreased production of pyrin permits apoptosis-associated specklike protein with a caspase recruitment domain–induced IL-1 processing and inflammation after subclinical serosal injury; macrophage apoptosis decreased | Recurrent fever, serositis, and inflammation responsive to colchicine; predisposes to vasculitis and inflammatory bowel disease | AR | Mutations of *MEFV* |
| TRAPS | PMNs, monocytes | Mutations of 55-kd TNF receptor leading to intracellular receptor retention or diminished soluble cytokine receptor available to bind TNF | Recurrent fever, serositis, rash, and ocular or joint inflammation | AD | Mutations of *TNFRSF1A* |
| Hyper-IgD syndrome | | Mevalonate kinase deficiency affecting cholesterol synthesis; pathogenesis of disease unclear | Periodic fever and leukocytosis with high IgD levels | AR | Mutations of *MVK* |
| Muckle-Wells syndrome* | PMNs, monocytes | Defect in cryopyrin, involved in leukocyte apoptosis and nuclear factor-κB signaling and IL-1 processing | Urticaria, sensorineural hearing loss, amyloidosis; responsive to IL-1 receptor/antagonist (Anakinra) | AD | Mutations of *CIAS1* (also called PYPAF1 or NALP3) |
| Familial cold autoinflammatory syndrome* | PMNs, monocytes | Same as for Muckle-Wells syndrome | Nonpruritic urticaria, arthritis, chills, fever, and leukocytosis after cold exposure; responsive to IL-1 receptor/antagonist (Anakinra) | AD | Mutations of *CIAS1* |
| Neonatal-onset multisystem inflammatory disease (NOMID) or chronic infantile neurologic cutaneous and articular (CINCA) syndrome* | PMNs, chondrocytes | Same as for Muckle-Wells syndrome | Neonatal-onset rash, chronic meningitis, and arthropathy with fever and inflammation responsive to IL-1 receptor antagonist (Anakinra) | AD | Mutations of *CIAS1* |
| Pyogenic sterile arthritis, pyoderma gangrenosum, acne syndrome | Hematopoietic tissues, upregulated in activated T cells | Disordered actin reorganization leading to compromised physiologic signaling during inflammatory response | Destructive arthritis, inflammatory skin rash, myositis | AD | Mutations of proline/serine/threonine phosphatase-interacting protein 1 (also called CD2BP1) |
| Blau syndrome | Monocytes | Mutations in nucleotide binding site of CARD15, possibly disrupting interactions with lipopolysaccharides and nuclear factor-κB signaling | Uveitis, granulomatous synovitis, camptodactyly, rash and cranial neuropathies, 30% develop Crohn disease | AD | Mutations of *NOD2* (also called CARD15) |

**TABLE VII.** (*Continued*)

| Disease | Affected cells | Functional defect(s) | Associated features | Inheritance | Gene defects |
|---|---|---|---|---|---|
| Chronic recurrent multifocal osteomyelitis and congenital dyserythropoietic anemia (Majeed syndrome) | Neutrophils, bone marrow cells | Undefined | Chronic recurrent multifocal osteomyelitis, transfusion-dependent anemia, cutaneous inflammatory disorders | AR | Mutations of *LPIN2* |

*AD*, Autosomal-dominant inheritance; *AR*, autosomal-recessive inheritance; *CARD*, caspase recruitment domain; *CARD15*, caspase recruitment domain-containing protein 15; *CIAS*, cold-induced autoinflammatory syndrome; *MEFV*, familial Mediterranean fever; *MVK*, mevalonate kinase; *NOD2*, nucleotide-binding oligomerization domain protein 2; *PMN*, polymorphonuclear cells; *TNFRSF1A*, tumor necrosis factor receptor superfamily member 1A; *TRAPS*, tumor necrosis factor receptor–associated periodic syndrome.

*All 3 syndromes associated with similar CIAS1 mutations; disease phenotype in any individual appears to depend on modifying effects of other genes and environmental factors.

**TABLE VIII.** Complement deficiencies

| Disease | Functional defect(s) | Associated features | Inheritance | Gene defects |
|---|---|---|---|---|
| C1q deficiency | Absent C hemolytic activity, defective MAC *Faulty dissolution of immune complexes<br>Faulty clearance of apoptotic cells | SLE-like syndrome, rheumatoid disease, infections | AR | C1q |
| C1r deficiency* | Absent C hemolytic activity, defective MAC Faulty dissolution of immune complexes | SLE-like syndrome, rheumatoid disease, infections | AR | C1r* |
| C1s deficiency | Absent C hemolytic activity | SLE-like syndrome; multiple autoimmune diseases | AR | C1s* |
| C4 deficiency | Absent C hemolytic activity, defective MAC Faulty dissolution of immune complexes<br>Defective humoral immune response | SLE-like syndrome, rheumatoid disease, infections | AR | C4A and C4B† |
| C2 deficiency‡ | Absent C hemolytic activity, defective MAC Faulty dissolution of immune complexes | SLE-like syndrome, vasculitis, polymyositis, pyogenic infections | AR | C2‡ |
| C3 deficiency | Absent C hemolytic activity, defective MAC Defective bactericidal activity<br>Defective humoral immune response | Recurrent pyogenic infections | AR | C3 |
| C5 deficiency | Absent C hemolytic activity, defective MAC Defective bactericidal activity | Neisserial infections, SLE | AR | C5 |
| C6 deficiency | Absent C hemolytic activity, defective MAC Defective bactericidal activity | Neisserial infections, SLE | AR | C6 |
| C7 deficiency | Absent C hemolytic activity, defective MAC Defective bactericidal activity | Neisserial infections, SLE, vasculitis | AR | C7 |
| C8a deficiency§ | Absent C hemolytic activity, defective MAC Defective bactericidal activity | Neisserial infections, SLE | AR | C8α |
| C8b deficiency | Absent C hemolytic activity, defective MAC Defective bactericidal activity | Neisserial infections, SLE | AR | C8β |
| C9 deficiency | Reduced C hemolytic activity, defective MAC Defective bactericidal activity | Neisserial infections‖ | AR | C9 |
| C1 inhibitor deficiency | Spontaneous activation of the complement pathway with consumption of C4/C2<br>Spontaneous activation of the contact system with generation of bradykinin from high-molecular-weight kininogen | Hereditary angioedema | AD | C1 inhibitor |
| Factor I deficiency | Spontaneous activation of the alternative complement pathway with consumption of C3 | Recurrent pyogenic infections, glomerulonephritis, hemolytic-uremic syndrome | AR | Factor I |
| Factor H deficiency | Spontaneous activation of the alternative complement pathway with consumption of C3 | Hemolytic-uremic syndrome, membranoproliferative glomerulonephritis | AR | Factor H |
| Factor D deficiency | Absent hemolytic activity by the alternate pathway | Neisserial infection | AR | Factor D |
| Properdin deficiency | Absent hemolytic activity by the alternate pathway | Neisserial infection | XL | Properdin |
| MBP deficiency¶ | Defective mannose recognition<br>Defective hemolytic activity by the lectin pathway | Pyogenic infections with very low penetrance mostly asymptomatic | AR | MBP¶ |
| MASP2 deficiency# | Absent hemolytic activity by the lectin pathway | SLE syndrome, pyogenic infection | AR | MASP2 |
| Complement receptor 3 deficiency | See LAD1 in Table V | | AR | ITGB2 |
| Membrane cofactor protein (CD46) deficiency | Inhibitor of complement alternate pathway, decreased C3b binding | Glomerulonephritis, atypical hemolytic uremic syndrome | AD | MCP |

**TABLE VIII.** *(Continued)*

| Disease | Functional defect(s) | Associated features | Inheritance | Gene defects |
|---|---|---|---|---|
| MAC inhibitor (CD59) deficiency | Erythrocytes highly susceptible to complement-mediated lysis | Hemolytic anemia, thrombosis | AR | CD59 |
| Paroxysmal nocturnal hemoglobinuria | Complement-mediated hemolysis | Recurrent hemolysis | Acquired X-linked mutation | PIGA |

*AD*, Autosomal-dominant inheritance; *AR*, autosomal-recessive inheritance; *ITGB2*, integrin beta-2; *MAC*, membrane attack complex; *MASP*, mannose-binding protein–associated serine protease; *MBP*, mannose-binding protein; *MCP*, membrane cofactor complex; *PIGA*, phosphatidylinositol glycan class A; *SLE*, systemic lupus erythematosus.

*The C1r and C1s genes are located within 9.5 kb of each other. In many cases of C1r deficiency, C1s is also deficient.

†Gene duplication has resulted in 2 active C4A genes located within 10 kb. C4 deficiency requires abnormalities in both genes, usually the result of deletions.

‡Type 1 C2 deficiency is in linkage disequilibrium with HLA-A25, B18, and DR2 and complotype, SO42 (slow variant of Factor B, absent C2, type 4 C4A, type 2 C4B), and is common in white patients (about 1 per 10,000). It results from a 28-bp deletion resulting in a premature stop codon in the C2 gene; C2 mRNA is not produced. Type 2 C2 deficiency is very rare and involves amino acid substitutions that result in C2 secretory block.

§C8α deficiency is always associated with C8γ deficiency. The gene encoding C8γ maps to chromosome 9 and is normal. C8γ is covalently bound to C8α.

‖Association is weaker than with C5, C6, C7, and C8 deficiencies. C9 deficiency occurs in about 1 per 1000 Japanese.

¶Population studies reveal no detectable increase in infections in MBP-deficient adults.

#A single patient.

# Selective predisposition to bacterial infections in IRAK-4–deficient children: IRAK-4–dependent TLRs are otherwise redundant in protective immunity

Cheng-Lung Ku,[1,2] Horst von Bernuth,[1,2,3] Capucine Picard,[1,2,19] Shen-Ying Zhang,[1,2] Huey-Hsuan Chang,[1,2] Kun Yang,[1,2] Maya Chrabieh,[1,2] Andrew C. Issekutz,[4] Coleen K. Cunningham,[5] John Gallin,[6] Steven M. Holland,[6] Chaim Roifman,[7] Stephan Ehl,[8] Joanne Smart,[9] Mimi Tang,[9] Franck J. Barrat,[10] Ofer Levy,[11,13] Douglas McDonald,[12,13] Noorbibi K. Day-Good,[14] Richard Miller,[15] Hidetoshi Takada,[16] Toshiro Hara,[16] Sami Al-Hajjar,[17] Abdulaziz Al-Ghonaium,[17] David Speert,[18] Damien Sanlaville,[20] Xiaoxia Li,[22] Frédéric Geissmann,[2,23] Eric Vivier,[24] László Maródi,[25] Ben-Zion Garty,[26] Helen Chapel,[27] Carlos Rodriguez-Gallego,[28] Xavier Bossuyt,[29] Laurent Abel,[1,2] Anne Puel,[1,2] and Jean-Laurent Casanova[1,2,21]

CORRESPONDENCE
Jean-Laurent Casanova:
casanova@necker.fr

Abbreviations used: B-EBV, EBV-transformed B lymphocyte cell line; CrP, C-reactive protein; IRAK-4, IL-1R–associated kinase 4; MDC, myeloid DC; MDDC, monocyte-derived DC; MIP-1β, macrophage inflammatory protein 1β; PDC, plasmacytoid DC; SV-40 fibroblast, SV40-transformed fibroblast; TIR, Toll/IL-1 receptor; TLR, Toll-like receptor; TRIF, TIR domain–containing adaptor-inducing IFN-β.

[1]Laboratory of Human Genetics of Infectious Diseases, U550, Institut National de la Santé et de la Recherche Médicale, 75015 Paris, France
[2]Necker Medical School, University Paris Rene Descartes, 75015 Paris, France
[3]Department of Pediatrics, Technical University Dresden, 01309 Dresden, Germany
[4]Department of Pediatrics, Dalhousie University, Halifax B3K 6R8, Nova Scotia, Canada
[5]Department of Pediatrics, Duke University Medical Center, Durham, NC 27710
[6]Laboratory of Clinical Infectious Diseases, National Institute of Allergy and Infectious Diseases, National Institutes of Health, Bethesda, MD 20892
[7]Divison of Immunology and Allergy, Department of Pediatrics, Hospital for Sick Children, University of Toronto, Toronto M5G 1X8, Ontario, Canada
[8]Center for Pediatrics and Adolescent Medicine, University of Freiburg, 79106 Freiburg, Germany
[9]Royal Children's Hospital, Parkville, Victoria 3052, Australia
[10]Dynavax Technologies, Berkeley, CA 94710
[11]Division of Infectious Diseases and [12]Division of Immunology, Children's Hospital Boston, Boston, MA 02115
[13]Harvard Medical School, Boston, MA 02115
[14]Department of Pediatrics, Division of Allergy and Immunology, University of South Florida and All Children's Hospital, St. Petersburg, FL 33701
[15]3M Center, St. Paul, MN 55144
[16]Department of Pediatrics, Graduate School of Medical Sciences, Kyushu University, Fukuoka 812-8582, Japan
[17]Department of Pediatrics, King Faisal Specialist Hospital and Research Center, Riyadh 11211, Kingdom of Saudi Arabia
[18]Centre for Infectious and Inflammatory Diseases, Child and Family Research Institute, Vancouver V5Z 4H4, British Columbia, Canada
[19]Immunodeficiency Investigation Center, [20]Laboratory of Histology, Embryology and Cytogenetics, Department of Molecular Cytogenetics and [21]Pediatric Hematology-Immunology Unit, Necker Hospital, 75015 Paris, France
[22]Department of Immunology, Cleveland Clinic Foundation, Cleveland, OH 44195
[23]U838, Institut National de la Santé et de la Recherche Médicale, 75015 Paris, France
[24]Centre d'Immunologie de Marseille-Luminy, Institut National de la Santé et de la Recherche Médicale–Centre National de la Recherche Scientifique, Université Mediterranee, Campus de Luminy, 13288 Marseille, France
[25]Department of Infectious and Pediatric Immunology, Medical and Health Science Center, University of Debrecen, 4032 Debrecen, Hungary
[26]Department of Pediatrics, Schneider Children's Medical Center of Israel, Petah Tiqva 49202, Israel
[27]Department of Immunology, Nuffield Department of Medicine, John Radcliffe Hospital, Headington, Oxford OX3 9DU, England UK
[28]Department of Immunology, Hospital Universitario de Gran Canaria Dr. Negrín, 35020 Las Palmas de Gran Canaria, Spain
[29]Experimental Laboratory Medicine, University Hospital Leuven, 3000 Leuven, Belgium

C.-L. Ku and H. von Bernuth contributed equally to this work.
The online version of this article contains supplemental material.

JEM © The Rockefeller University Press $30.00
Vol. 204, No. 10, October 1, 2007 2407-2422 www.jem.org/cgi/doi/10.1084/jem.20070628



Supplemental Material can be found at:
http://www.jem.org/cgi/content/full/jem.20070628/DC1

JEM

**Human interleukin (IL) 1 receptor–associated kinase 4 (IRAK-4) deficiency is a recently discovered primary immunodeficiency that impairs Toll/IL-1R immunity, except for the Toll-like receptor (TLR) 3– and TLR4–interferon (IFN)-α/β pathways. The clinical and immunological phenotype remains largely unknown. We diagnosed up to 28 patients with IRAK-4 deficiency, tested blood TLR responses for individual leukocyte subsets, and TLR responses for multiple cytokines. The patients' peripheral blood mononuclear cells (PBMCs) did not induce the 11 non-IFN cytokines tested upon activation with TLR agonists other than the nonspecific TLR3 agonist poly(I:C). The patients' individual cell subsets from both myeloid (granulocytes, monocytes, monocyte-derived dendritic cells [MDDCs], myeloid DCs [MDCs], and plasmacytoid DCs) and lymphoid (B, T, and NK cells) lineages did not respond to the TLR agonists that stimulated control cells, with the exception of residual responses to poly(I:C) and lipopolysaccharide in MDCs and MDDCs. Most patients (22 out of 28; 79%) suffered from invasive pneumococcal disease, which was often recurrent (13 out of 22; 59%). Other infections were rare, with the exception of severe staphylococcal disease (9 out of 28; 32%). Almost half of the patients died (12 out of 28; 43%). No death and no invasive infection occurred in patients older than 8 and 14 yr, respectively. The IRAK-4–dependent TLRs and IL-1Rs are therefore vital for childhood immunity to pyogenic bacteria, particularly *Streptococcus pneumoniae*. Conversely, IRAK-4–dependent human TLRs appear to play a redundant role in protective immunity to most infections, at most limited to childhood immunity to some pyogenic bacteria.**

Inherited IL-1R–associated kinase 4 (IRAK-4) deficiency is an autosomal recessive disorder that was first described in three unrelated children (1). IRAK-4–deficient patients' fibroblasts and/or leukocytes show an impaired response to most Toll-like receptor (TLR) and IL-1R agonists tested (1–12). Specifically, the patients' whole blood cells or PBMCs do not respond to IL-1β, in terms of IL-6 secretion (1), or to IL-18, in terms of IFN-γ production (1, 4). Moreover, agonists of TLR1/2 ($Pam_3CSK_4$), TLR2/6 ($Pam_2CSK_4$), TLR3 (poly(I:C)), TLR4 (LPS), TLR5 (flagellin), and TLR9 (CpG DNA), do not induce the production of major inflammatory cytokines (TNF-α, IL-6, and IL-12) and growth factors (G-CSF and GM-CSF) in whole blood cells and PBMCs (1–9, 11, 12). However, the patients' PBMCs do respond to the nonspecific TLR3 agonist poly(I:C) and the TLR4-specific agonist LPS by producing IFN-β mRNA (for poly(I:C) and LPS) or IFN-α protein (for poly(I:C) only) (13). Moreover, the patients' fibroblasts have been shown to respond to poly(I:C) by inducing IFN-β, IFN-λ, and IL-6 (13). The human IRAK-4–independent TLR3/4 pathway is reminiscent of the mouse MyD88-independent, Toll/IL-1 receptor (TIR) domain–containing adaptor-inducing IFN-β (TRIF)–dependent TLR3/4 pathway (14, 15), which also controls the induction of cytokines other than IFNs, at least for TLR3 (16, 17). Despite the lack of IL-6 and TNF-α induction in response to poly(I:C) in human IRAK-4–deficient whole blood cells (1), the normal induction of IFN-α, -β, and -λ in response to poly(I:C) and LPS (13) raises the possibility that IRAK-4 deficiency may not prevent the induction of other cytokines in response to these two and possibly other TLR agonists.

The lack of response of IRAK-4–deficient whole blood cells and PBMCs to TLR and IL-1R agonists also does not exclude the possibility that individual leukocyte subsets may respond to at least some agonists. Several human leukocyte subsets produce TLR mRNAs and/or proteins. In the myeloid lineage, neutrophilic granulocytes express TLR1, 2, 4, 5, 6, 7, 8, and 10, as well as TLR9 upon induction with GM-CSF (18); monocytes express TLR1, 2, 4, 5, 6, 7, 8, and 9 (19–21); myeloid DCs (MDCs) express TLR1, 2, 3, 4, 5, 6, 7, 8, and 10 (22); and plasmacytoid DCs (PDCs) express TLR1, 6, 7, 9, and 10 (19, 21–23). Monocyte-derived DCs (MDDCs) express TLR1, 2, 3, 4, 5, 6, 8, 9, and 10, but hardly any TLR7 (24, 25). In basophilic and eosinophilic granulocytes, substantial expression has been confirmed only for TLR7 in eosinophils (26). In the lymphoid lineage, blood B cells express TLR1, 6, 7, 9, and 10 (20, 23, 27); NK cells express TLR1, 2, 3, 5, 6, 7, and 8 (20); CD4 α/β T cells express at least TLR1, 2, and 5 (28); and effector α/β CD8 T cells and γ/δ T cells express TLR3 (29, 30). In healthy controls, most subsets could be activated by the corresponding TLR agonists tested. In contrast, the range of blood cells in which TLR responses are affected by IRAK-4 deficiency remains unclear.

IRAK-4 deficiency may have an even broader impact, given the well-established role of IRAK-4 downstream from multiple IL-1Rs (1, 31) and the recently proposed role of IRAK-4 in TCR signaling (32). It is thus surprising that the first three patients identified were alive and well and had experienced only a few infectious diseases (1). To date, 21 IRAK-4–deficient patients have been reported in individual case reports or small series (1, 4–13, 33–36). Most presented with peripheral (e.g., pharyngotonsillitis, sinusitis, cellulitis, and endophthalmitis) and/or invasive bacterial diseases (e.g., meningitis, arthritis, septicemia, and visceral abscess) caused mostly by *Streptococcus pneumoniae* and *Staphylococcus aureus* (1, 4–13, 33–36). Only seven patients also presented infectious disease caused by Gram-negative bacteria (*Pseudomonas aeruginosa* in most cases) (1, 4–6, 8, 13, 33, 36). Although IRAK-4 deficiency appears to be more severe than initially thought (1), with seven reported deaths (5, 7–9, 13, 34, 36), the condition seems to improve with age, even without prophylaxis (4, 6, 36). The apparent broad resistance of IRAK-4–deficient patients challenges the prevailing view that TLRs are the principal sentinels of innate immunity (37–39). However, it has been difficult to draw firm conclusions in the absence of a large series of patients. Moreover, the rarity of infections may reflect the TLR-dependent, yet IRAK-4–independent, induction of certain cytokines in specific leukocyte subsets. We thus investigated the contribution of human TLRs to host defense by documenting

the clinical course of a large number of IRAK-4–deficient patients and testing the TLR responses of their PBMCs for multiple cytokines, as well as the TLR responses of their individual leukocyte subsets.

## RESULTS

### *IRAK4* mutations

We report 28 patients with IRAK-4 deficiency. The patients originate from 18 unrelated kindreds and 11 countries (Table I and Fig. 1). All *IRAK4* exons, flanking intron regions, and, when appropriate, entire introns, were sequenced in 24 patients (P1–4, 6–13, 15, 17–20, and 22–28). IRAK-4 deficiency was diagnosed on clinical grounds in four deceased relatives (P5, 14, 16, and 21) for whom no biological material was available. The patients of 13 kindreds were apparently homozygous (kindreds A–C, E, F, H–L, and P–R), and those from 5 kindreds were compound heterozygous (D, G, and M–O) for *IRAK4* mutations. However, four seemingly homozygous patients from three unrelated families (P2 from kindred B, P7 from kindred F, and P11 and 12 from kindred I) had one parent who did not carry the mutant allele. Fluorescence in situ hybridization with BAC210N13, which covers the entire *IRAK4* locus, and the genotyping of polymorphic markers showed that P2 was heterozygous for a large de novo deletion (designated BAC210N13del) encompassing *IRAK4* (Fig. S1, top, available at http://www.jem.org/cgi/content/full/jem.20070628/DC1; and not depicted). For P7, using the same BAC as for P2, fluorescence in situ hybridization revealed two signals, consistent with homozygosity owing to segmental uniparental disomy or compound heterozygosity with an undetected deletion encompassing a fraction of *IRAK4* (Fig. S1, bottom; and not depicted). Not enough material was available to explore the *IRAK4* locus in the deceased patients P11 and 12 from kindred I (8). 3 out of the 14 mutant alleles identified carried nonsense mutations (Y48X, Q293X, and E402X) (1, 3, 4, 6, 8, 9, 11, 36), 3 carried large deletions (*1-1096_40+23del*, *BAC210N13del*, and *942-1481_1125+547del*), 2 carried splice mutations (*1188+520A>G* and *1189-1G>T*) (12), and 6 carried frameshift insertions and deletions (*167_172insA*, *573delA*, *620_621delAC*, *631delG*, *821delT*, and *1240insA*) (1, 4, 7, 34) (Table I and Fig. 2 A). All mutations are predicted to be null, as they create a premature termination codon or delete a large segment of the gene. No missense mutation was found. The 14 mutations were not found in 100 healthy controls sequenced.

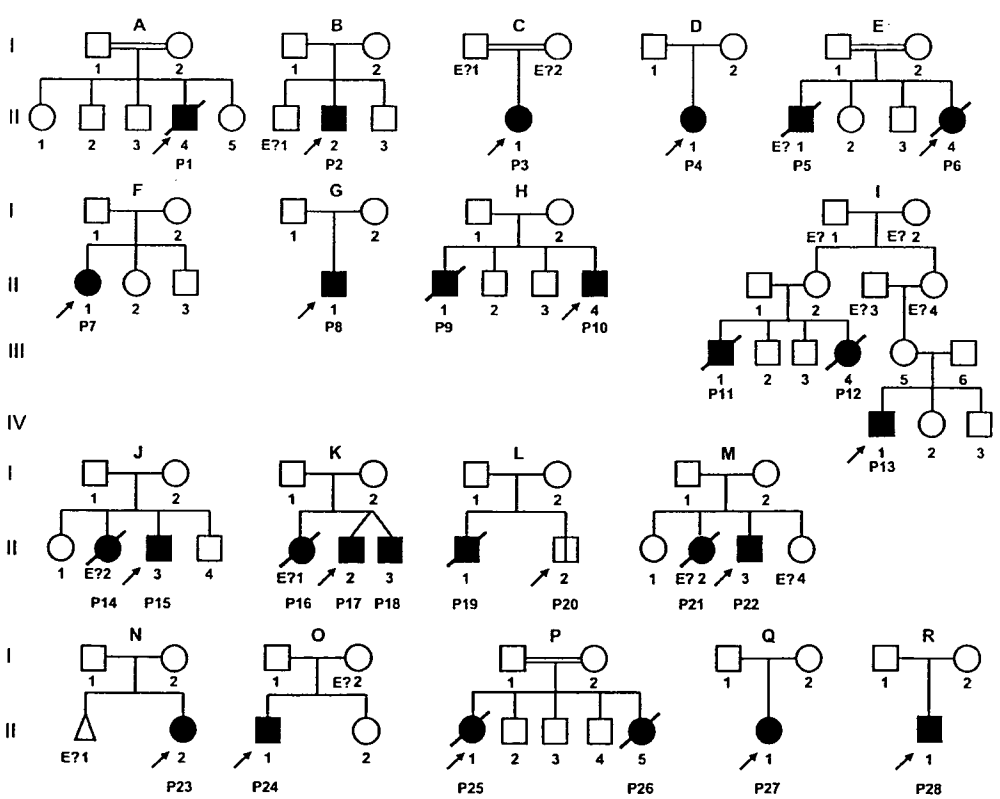



**Figure 1. Pedigree of the 18 kindreds identified with IRAK-4 deficiency.** Each kindred is designated by a capital letter (A–R), each generation is designated by a Roman numeral (I–IV), and each individual is designated by an Arabic numeral (from left to right). IRAK-4–deficient patients with a clinical phenotype are represented as closed symbols. P20, the only patient with confirmed IRAK-4 deficiency but no known clinical phenotype, is represented with an open square 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?"; they include four individuals (P5, 14, 16, and 21) thought to be IRAK-4 deficient based on their clinical phenotypes.

**Table I.** Genotypes, origin, and clinical phenotypes of IRAK-4–deficient patients

| Kindred | Patient | Mutation | Origin | Follow-up | Age | Pathogens causing severe Gram-positive infections | Pathogens causing severe Gram-negative infections | References |
|---|---|---|---|---|---|---|---|---|
| A | P1 (II-4) | *821delT* | KSA | deceased | 7 yr | Sp, Sa | – | (1, 13) |
| B | P2 (II-2) | Q293X/ *BAC210N13del* | Portugal | alive | 14 yr | Sp, Sa | – | (1, 10, 13) |
| C | P3 (II-1) | Q293X | USA | alive | 11 yr | Sp, Sa | Ec | (1, 3, 33) |
| D | P4 (II-1) | Q293X/ *620-621delAC* | USA | alive | 24 yr | Sp, Cs | Nm | (2, 4, 35) |
| E | P5 (II-1) | ND | Turkey | deceased | 16 mo | Sp, Spa | – | (34) |
| E | P6 (II-4) | *573delA* | Turkey | deceased | 2 mo | Sp | – | (34) |
| F | P7 (II-2) | Q293X | UK | alive | 32 yr | Sp | Ss | (6, 10, 13) |
| G | P8 (II-1) | *1188+520A>G/ 1189-1G>T* | Hungary | alive | 9 yr | Sp | – | (10, 12, 13) |
| H | P9 (II-1) | Q293X | Canada | deceased | 6 yr | Sp | Pa | (5, 9, 13) |
| H | P10 (II-4) | Q293X | Canada | alive | 7 yr | Sp | – | (5, 9, 13) |
| I | P11 (III-1) | E402X | Spain | deceased | 2 yr | Sa | Pa | (8, 13) |
| I | P12 (III-4) | E402X | Spain | deceased | 8 mo | Sp | Pa | (8, 13) |
| I | P13 (IV-1) | E402X | Spain | alive | 9 yr | Sp | – | (8, 10, 13) |
| J | P14 (II-2) | ND | Israel | deceased | 3 mo | Sm | – | (13) |
| J | P15 (II-3) | *1-1096_40+23del* | Israel | alive | 9 yr | Sp | – | (10, 13) |
| K | P16 (II-1) | ND | Canada | deceased | 5 mo | Sa | – | (13, 36) |
| K | P17 (II-2) | Q293X | Canada | alive | 27 yr | Sp | Pa | (13, 36) |
| K | P18 (II-3) | Q293X | Canada | alive | 27 yr | Sp | – | (13, 36) |
| L | P19 (II-1) | *167_172insA* | Japan | deceased | 2 yr | Sp | – | (7) |
| L | P20 (II-2) | *167_172insA* | Japan | alive | 24 mo | – | – | (7) |
| M | P21 (II-2) | ND | USA | deceased | 4 mo | bacterial meningitis | – | this study |
| M | P22 (II-3) | Q293X/ *620-621delAC* | USA | alive | 10 yr | Sp, Sa | – | this study |
| N | P23 (II-1) | Y48X/ *631delG* | Canada | alive | 2 yr | Sa | – | this study |
| O | P24 (II-1) | *1240insA/ 942-1481_1125+547del* | Canada | alive | 16 yr | Sp, Sa | – | this study |
| P | P25 (II-1) | Q293X | Australia | deceased | 4 mo | Sp | – | this study |
| P | P25 (II-5) | Q293X | Australia | deceased | 6 mo | Sp, Sa | – | this study |
| Q | P27 (II-2) | Q293X | USA | alive | 11 yr | Sp | – | this study |
| R | P28 (II-1) | Q293X | USA | alive | 6 yr | Sp | – | (11) |

Cs, *Clostridium septicum*; Ec, *E. coli*; KSA, Kingdom of Saudi Arabia; Nm, *N. meningitidis*; Pa, *P. aeruginosa*; Sa, *S. aureus*; Sm, *S. milleri*; Sp, *S. pneumoniae*; Spa, *S. parasanguis*; Ss, *S. sonnei*.

The Q293X mutant allele was found in homozygotes from six kindreds (C, H, K, P, Q, and R) and compound heterozygotes from four kindreds (B, D, M, and possibly F). The recurrence of this mutation may reflect a mutational hotspot, a founder effect, or both (unpublished data).

### IRAK-4 expression and function

We assessed *IRAK4* mRNA levels in EBV-transformed B lymphocyte cell lines (B-EBVs; Fig. 2 B) derived from most patients and a healthy control by RT-PCR. The two patients carrying the *573delA* mutation died before cell lines could be established (34). Most other patients lacked detectable full-length *IRAK4* mRNAs species, presumably because of nonsense-mediated mRNA degradation. However, P7 (mutation Q293X), P8 (mutations *1188+520A>G* and *1189-1G>T*), P13 (mutation E402X), P19 (mutation *167_172insA*), and P22 (mutation Q293X/*620-621del*) had low levels of detectable full-length *IRAK4* mRNA. We then assessed IRAK-4 protein levels in B-EBVs (Fig. 2 C). No IRAK-4 protein was detected in any of the patients tested, even in P7, 8, 13, 19, and 22, all of whom had detectable full-length mRNAs, excluding a potential role of IRAK-4 as a scaffold protein in our patients (40, 41). Finally, we assessed the functional impact of *IRAK4* mutations. B-EBVs bearing mutations *821delT* (P1), Q293X (P2, 3, and 7), *1188+520A>G/1189-1G>T* (P8), E402X (P13), and *1-1096_40+23del* (P15) did not respond to TLR7 and 8 agonists, as measured by TNF-α production (Fig. 3 A). SV40-transformed fibroblasts (SV40-fibroblasts)

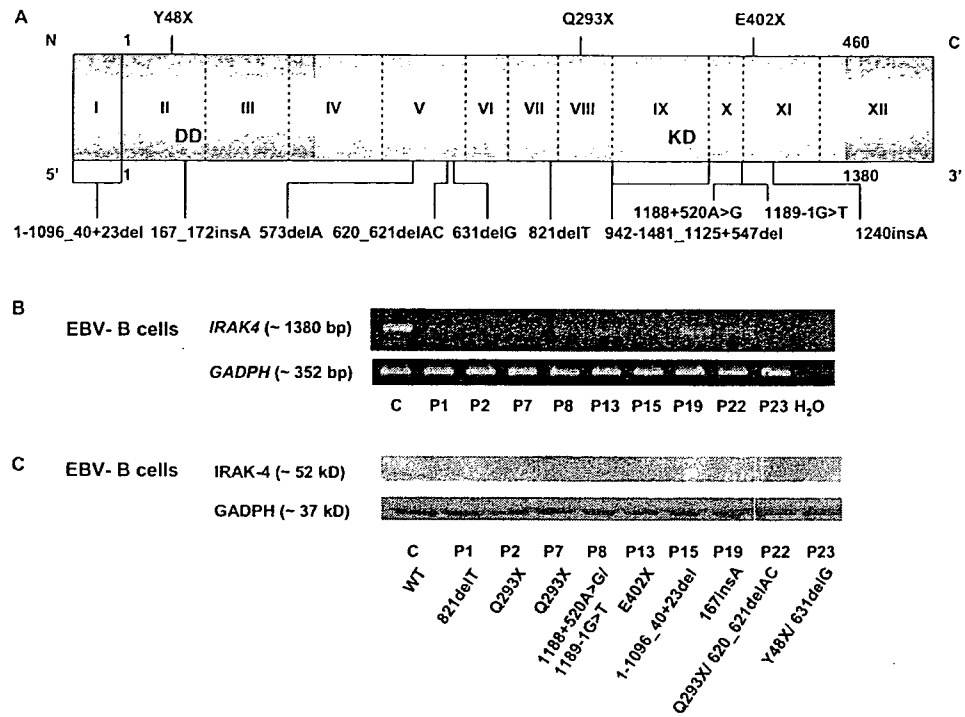



**Figure 2. IRAK-4 deficiency.** (A) Schematic representation of *IRAK4* with all identified mutations. The gene is composed of 12 exons, with exon 1 and a part of exon 12 noncoding. The N-terminal death domain (DD) and C-terminal kinase domain (KD) are shown in light gray. (B) RT-PCR of the full-length *IRAK4* and *GAPDH* genes in B-EBVs from a healthy control (C) and nine IRAK-4–deficient patients. (C) IRAK-4 and GAPDH protein levels in B-EBVs from a healthy control and nine IRAK-4–deficient patients, as shown by Western blotting. White lines indicate that intervening lanes have been spliced out.

bearing mutations *821delT* (P1), Q293X (P2 and 3), *1188+520A>G/1189-1G>T* (P8), E402X (PI3), *1-1096_40+23del* (P15), Y48X/*631delG* (P23), and *1240insA/942-1481_1125+547del* (P24) did not respond to IL-1β, as assessed by measuring IL-6 production. However, IRAK-4–deficient SV40-fibroblasts did produce IL-6 upon activation by poly(I:C) (Fig. 3 B) (13). Thus, all patients had complete IRAK-4 deficiency and a complete absence of IRAK-4–dependent TIR signaling, owing to the inheritance of two loss-of-expression, loss-of-function *IRAK4* alleles.

### Development and function of blood leukocyte subsets

We analyzed blood leukocyte subsets in 12 IRAK-4–deficient patients. We previously showed that granulocytes, $CD14^+$, $CD16^+$, and $CD14^+/CD16^+$ monocyte subsets, and MDCs and PDCs, were present in normal numbers in three patients (13). We now report that T cell subsets, including $CD4^+$ and $CD8^+$, and $CD45RA^+$ and $CD45RO^+$ T cells, are also present in normal numbers (Table S1, available at http://www.jem.org/cgi/content/full/jem.20070628/DC1), with the possible exception of normal to low levels of T cells in P17 and 18 (36). T cells proliferated normally in response to the mitogen PHA, CD3, and recall antigens in vitro (Table S2). B cells and memory B cells ($CD27^+$) were also present in normal numbers (Table S1). Serum Ig levels for IgA were normal in five, high in two (P8 and 11), and low in four (P1, 2, 17, and 18) patients (36). IgG levels were normal in seven and high in four (P7, 8, 11, and 17) patients, and IgM levels were normal in seven, high in three (P7, 11, and 19), and low in one (P2) patients. IgE levels were high in 8 (P1, 7, 8, 11, 13, 15, 17, and 23) out of the 11 patients evaluated (Table S2). Antibody responses to protein antigens were normal in all but two patients, who had slightly low titers (P7 and 15); however, the date of recall vaccination before serological testing was unknown. The antibody response to glycans was impaired in some (P2, 8, 17, 18, and 29) but not all patients, and in response to some but not all pneumococcal and erythrocyte AB antigens (Table S2 and unpublished data) (11, 12, 33). Finally, the surface expression of CD16 and CD56 on NK cells was normal (Table S1). IFN-γ secretion and surface expression of CD107 (degranulation) by the patients' NK cells were normal (unpublished data). Overall, there seemed to be no overt defect of leukocyte development in IRAK-4–deficient patients. Thus, antigen-specific T and B cell responses seemed to be normal, except for an impaired glycan-specific antibody response in at least some patients and against some glycans, and except for an overproduction of IgE in most of the patients tested.

### Impaired production of multiple cytokines by blood mononuclear leukocytes

We previously reported that IRAK-4–deficient whole blood cells and PBMCs produce only very small amounts of TNF-α,

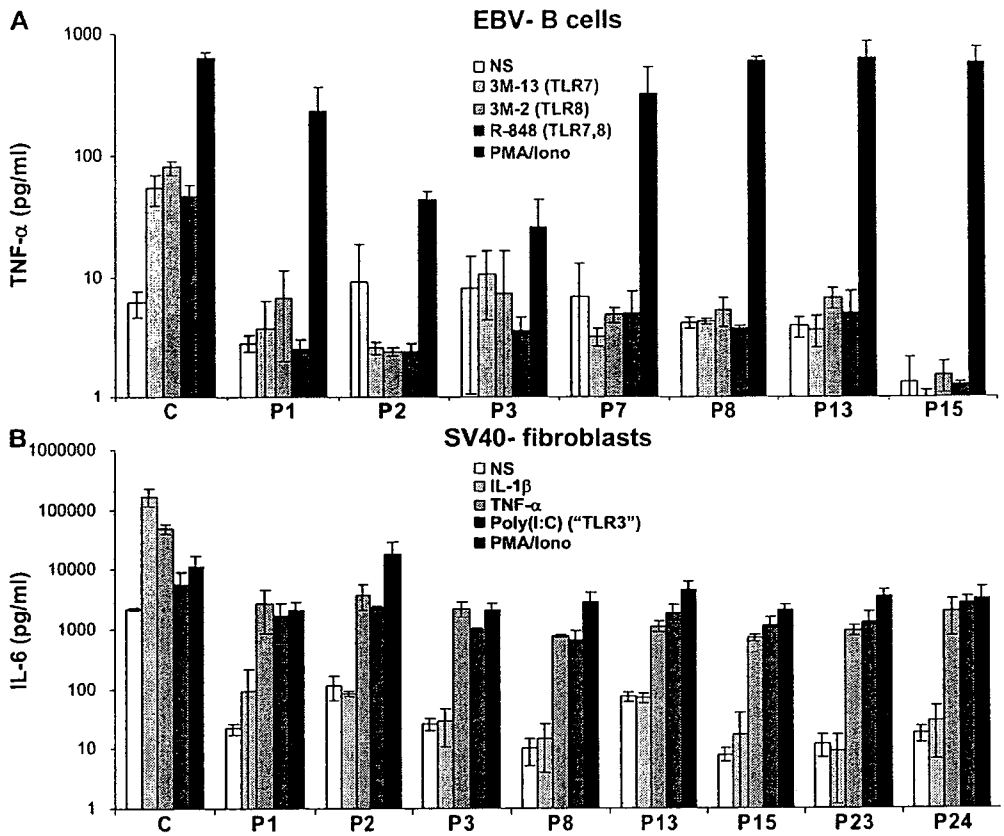



**Figure 3. Impaired cellular responses to TIR agonists in IRAK-4–deficient cell lines.** (A) TNF-α production by B-EBVs from a healthy control (C) and seven IRAK-4-deficient patients 24 h after stimulation with various TLR agonists and PMA/ionomycin. (B) IL-6 production by SV40-fibroblasts from a healthy control and eight IRAK-4-deficient patients after 24 h of stimulation with IL-1β, TNF-α, poly(I:C), and PMA/ionomycin. Mean values and SDs are shown for triplicates of a single experiment.

IL-6, IL-12, G-CSF, GM-CSF, and IFN-γ in vitro in response to all IL-1R and TLR agonists tested (1–9, 11, 12). We wondered whether the induction of other cytokines, chemokines, IFNs, and growth factors was also dependent on IRAK-4 after TLR stimulation. We therefore activated PBMCs from IRAK-4–deficient patients with $Pam_3CSK_4$ (TLR1/2), $Pam_2CSK_4$ (TLR2/6), poly(I:C) (a nonspecific TLR3 agonist), LPS (TLR4), flagellin (TLR5), 3M-13 (TLR7), 3M-2 (TLR8), R-848 (TLR7 and 8), and CpG (TLR9) for 24 h. We did not assess TLR10 responses, as there is no known agonist for this receptor (23). Cytokine secretion into the supernatant was assessed using a multiplex cytometry-based system. 11 out of the 25 cytokines assayed were induced and detectable after TLR stimulation in healthy controls. IRAK-4–deficient cells did not respond to seven out of nine agonists for all cytokines tested (Fig. 4). Upon activation with poly(I:C), the patients' PBMCs displayed induction of IL-12, monocyte chemoattractant protein 1, and macrophage inflammatory protein 1β (MIP-1β) to levels similar to those in healthy controls, as well as some induction of IFN-inducible protein 10 (Fig. 4). However, the induction of IL-12 and MIP-1β was weak in both patients and healthy controls (Fig. S2, available at http://www.jem.org/cgi/content/full/jem.20070628/DC1). IL-7 induction was abolished in the patients, whereas other cytokines were not induced in controls. The patients' PBMCs showed detectable IL-8 and MIP-1β (an IFN-inducible cytokine) responses to LPS, but these responses were weaker than those of healthy controls (Fig. 4). The other cytokines were not induced in the patients. These data are reminiscent of our previous observation that IRAK-4–deficient PBMCs respond to poly(I:C) by producing IFN-α protein, and to poly(I:C) and LPS by producing IFN-β mRNA (13). However, whereas LPS responses can be specifically ascribed to TLR4, we recently showed, in TLR3-deficient patients, that the poly(I:C) responses of PBMCs are TLR3-independent (42). These data indicate a broad immunological impact of IRAK-4 deficiency, as the production of 11 key cytokines was completely impaired in response to all TLR agonists, with the exception of a couple of cytokines in response to poly(I:C) and LPS.

### TLR responses of individual myeloid subsets

We then assessed the role of IRAK-4 in TLR signaling pathways in discrete leukocyte cell populations. Cell subsets other

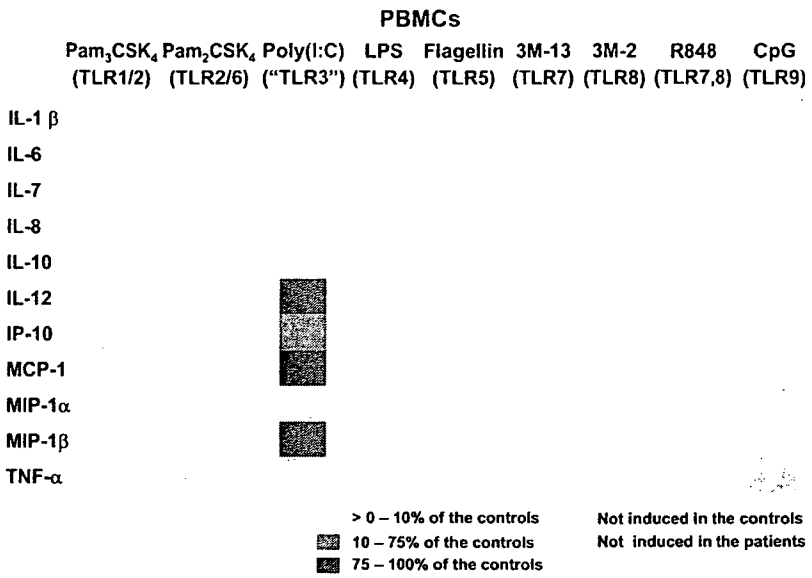
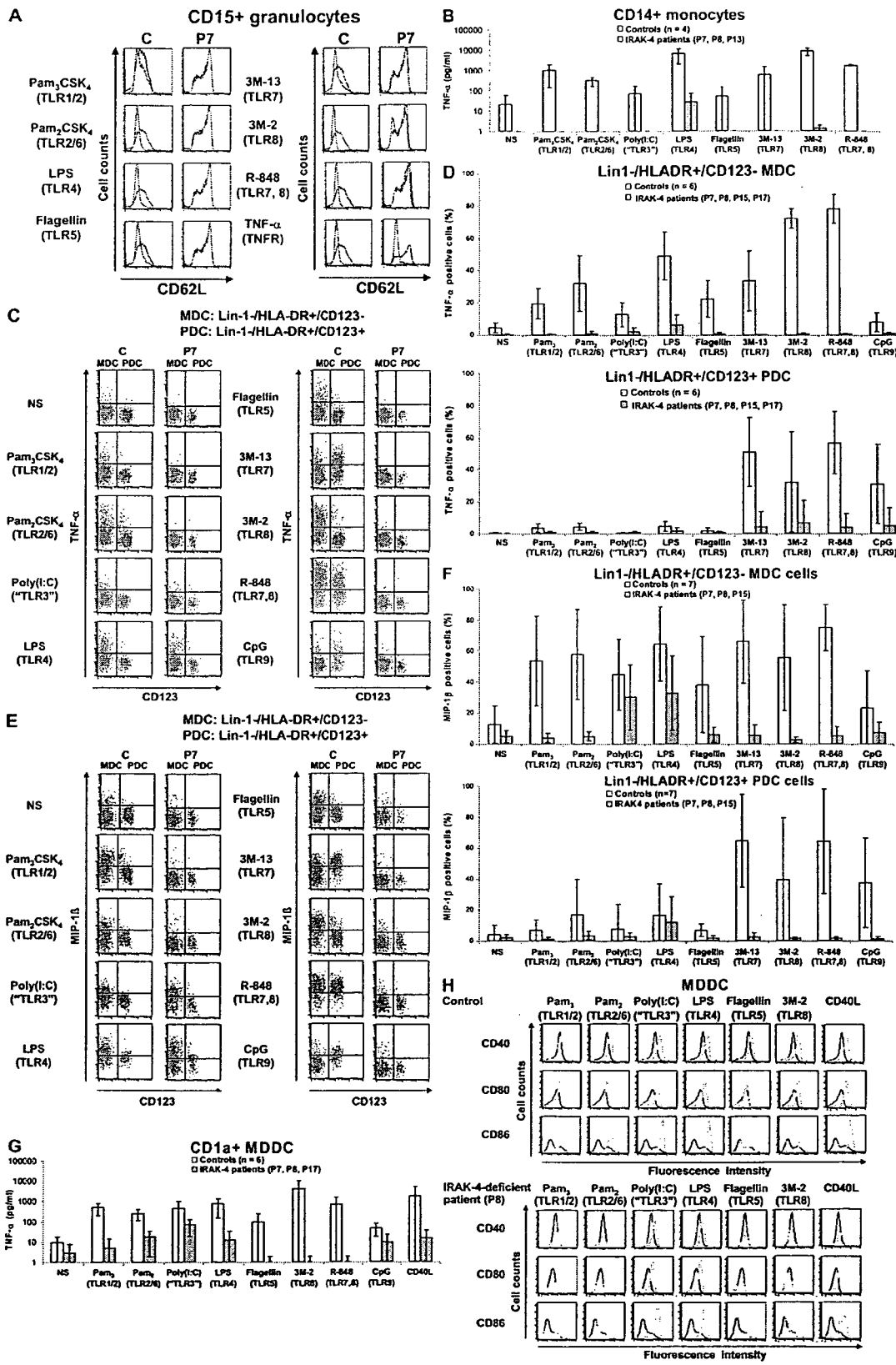



**Figure 4. Multiple cytokine secretion in IRAK-4–deficient PBMCs.** PBMCs from three healthy controls and three IRAK-4-deficient patients (P17, 18, and 22) were activated with various TLR agonists for 24 h. Cytokine levels are represented as ratios of the mean secretion observed in the three IRAK-4–deficient patients to that in three healthy controls. Cytokines represented in gray are not induced upon the stimulation of control PBMCs.

than granulocytes and DCs were purified by cell sorting (purity >99.5%). More than 95% of the granulocytes purified on Ficoll were $CD15^+$. The response of DCs (MDCs and PDCs) was tested in PBMCs. We assessed the CD62L shedding of granulocytes from four healthy controls and four IRAK-4–deficient patients after activation with $Pam_3CSK_4$, $Pam_2CSK_4$, LPS, flagellin, 3M-13, 3M-2, R-848, and TNF-α (10). The response to all TLR agonists was impaired in the granulocytes of all four patients tested (Fig. 5 A). $CD14^+$ monocytes from healthy controls responded to TLR1–8 agonists but not to TLR9 agonists. The monocytes of IRAK-4–deficient patients did not respond to these agonists, with the possible exception of very weak TNF-α production upon LPS stimulation (Fig. 5 B). Finally, we tested MDCs and PDCs by stimulating PBMCs from seven healthy donors and three IRAK-4–deficient patients with the TLR agonists $Pam_3CSK_4$, $Pam_2CSK_4$, poly(I:C), LPS, flagellin, 3M-13, 3M-2, R-848, and CpG for 3 h. We assessed TNF-α and MIP-1β production for MDCs ($Lin\text{-}1^-$, $HLA\text{-}DR^+$, and $CD123^{low}$) and PDCs ($Lin\text{-}1^-$, $HLA\text{-}DR^+$, and $CD123^{high}$) by intracellular staining. In healthy individuals, MDCs responded to all of the TLR agonists tested, except the TLR9 agonist, with the induction of TNF-α and MIP1-β. In contrast, only upon activation with poly(I:C) (nonspecific TLR3 agonist) and LPS (TLR4), did MDCs from the patients display normal levels of MIP1-β induction and some induction of TNF-α. PDCs from healthy individuals responded only to agonists of TLR7 and 9, whereas IRAK-4–deficient PDCs did not respond to any of the agonists tested (Fig. 5, C–F). As poly(I:C) activation in MDCs appears to be TLR3 independent (42), we further evaluated the production of TNF-α and the up-regulation of IFN-inducible surface-expressed CD40, CD80, and CD86 by in vitro MDDCs, which respond to poly(I:C) in a TLR3-dependent manner (42). MDDCs from healthy controls responded normally to the TLR agonists $Pam_3CSK_4$, $Pam_2CSK_4$, poly(I:C), LPS, flagellin, and 3M-2. In contrast, the patients' MDDCs did not respond to $Pam_3CSK_4$, $Pam_2CSK_4$, flagellin, and 3M-2. However, IRAK-4–deficient MDDCs showed a weak but not abolished TNF-α response and normal induction of CD40, CD80, and CD86 upon activation with poly(I:C) (TLR3). Normal induction of CD40, CD80, and CD86 was also observed upon activation with LPS (TLR4) (Fig. 5, G and H). These data indicate that the IRAK-4–deficient individual myeloid cell subsets tested displayed no response to most TLR agonists, with the exception of normal responses to poly(I:C) and LPS detected in MDCs for MIP-1β, an IFN type I–inducible cytokine, and in MDDCs for CD40, CD80, and CD86, which are induced by type I IFNs and TNF-α.

### TLR responses of individual lymphoid subsets

We then tested the TLR responses of the B, T, and NK lymphoid cell subsets. The subsets were purified by cell sorting (purity >99.5%). $CD19^+$ B cells were activated by incubation with the TLR agonists $Pam_3CSK_4$, $Pam_2CSK_4$, poly(I:C), LPS, flagellin, 3M-13, 3M-2, R-848, and CpG for 24 h, and their response was measured by assessing IL-10 production. Highly purified control B cells showed a unique pattern of activation, with no response to agonists of TLR1/2, TLR2/6, TLR3, TLR4, TLR5, and TLR8, and only weak IL-10 production in response to TLR7, TLR7 and TLR8, and TLR9 agonists (Fig. 6 A and not depicted). In contrast, no response to these TLR agonists



was observed in the three IRAK-4–deficient patients tested (Fig. 6 A). Moreover, the response to TLR7 and 9, as measured by cell-surface expression of CD40, CD80, and CD86 after 3 d of incubation with IL-4 and various TLR agonists, was also impaired in the patients' B cells (Fig. 6 B) (13). $CD3^+$ T cells from healthy individuals were activated by $Pam_3CSK_4$, $Pam_2CSK_4$, poly(I:C), LPS, flagellin, 3M-13, 3M-2, R-848, and CpG. Control T cells displayed a weak but detectable response to $Pam_3CSK_4$ and flagellin in terms of IFN-γ production, whereas T cells from IRAK-4–deficient patients were not activated by any of the TLR agonists (Fig. 6 C). Finally, control NK cells were shown to respond to TLR3, 7, and TLR7 and 8 agonists in terms of IFN-γ production, but no response was observed in NK cells from IRAK-4–deficient patients (Fig. 6 D). NK cells respond to poly(I:C) through TLR3 (42), suggesting that at least some TLR3 pathways are IRAK-4 dependent. These data indicate that the three major blood lymphoid subsets require IRAK-4 for TLR responses, including TLR3 responses in NK cells.

### Clinical features of IRAK-4 deficiency

In total, 28 IRAK-4–deficient patients from 18 families were studied, including the 7 patients (P21–27) from 5 families described in this study for the first time (Table I and Fig. 1). Most IRAK-4–deficient patients had had at least one Gram-positive bacterial infection: 22 out of the 28 (79%) had had invasive disease caused by *S. pneumoniae* (meningitis, septicemia, or arthritis), and 9 out of the 28 (32%) had suffered severe disease caused by *S. aureus* (meningitis, septicemia, or liver abscess; Table I). If we also take into account peripheral staphylococcal disease (cellulitis and subcutaneous abscess), 14 patients could be considered particularly susceptible to *S. aureus*. One patient (P20) had had no major infectious disease. This patient is 25 mo old and was diagnosed with IRAK-4 deficiency as a neonate. He was placed on IgG substitution and antibiotic prophylaxis shortly after birth. Seven patients also suffered from severe Gram-negative bacterial infections, which were invasive in four cases (*Shigella sonnei* and *P. aeruginosa*) and peripheral in four cases (*Escherichia coli*, *Serratia marcescens*, *Neisseria meningitidis*, and *P. aeruginosa*). As previously reported in a smaller series (13), no severe viral, fungal, or parasitic infections were observed in the patients. Most patients developed their first invasive infection before the age of 2 yr (20 out of 28; 71%), often before the age of 6 mo (9 out of 28; 32%) and in the neonatal period (4 out of 28; 14%), when maternal antibodies are still present. Remarkably, no invasive infection was documented in the six patients over the age of 14 yr (P2, 14 yr; P4, 24 yr; P7, 32 yr; P17 and 18, 27 yr; and P24, 16 yr), even in the absence of prophylaxis (P2, 4, 7, 17, and 18; $n = 5$; Fig. 7 A) (4, 6, 36). 12 patients died of invasive Gram-positive infections, all before the age of 8 yr and most before the age of 2 yr (Fig. 7 B). IRAK-4 deficiency is thus associated with a selective predisposition to pyogenic bacterial infections, mostly caused by Gram-positive bacteria (*S. pneumoniae* in particular and *S. aureus* to a lesser extent), and clinical status and outcome both improve with age. The detailed clinical features of IRAK-4 deficiency will be reported elsewhere (unpublished data).

## DISCUSSION

The 28 patients reported in this study suffered from complete IRAK-4 deficiency. The patients had been exposed to an extremely diverse range of microorganisms, including many potential viral, bacterial, and fungal pathogens, as well as parasites (Tables S2 and S3, available at http://www.jem.org/cgi/content/full/jem.20070628/DC1). However, IRAK-4–deficient patients presented a strikingly narrow infectious phenotype (Table I), similar to the three patients initially reported (1). 27 patients suffered from invasive infectious disease, typically caused by Gram-positive *S. pneumoniae* ($n = 22$; 79%) and/or *S. aureus* ($n = 9$; 32%). Seven patients (25%) also presented severe infections with Gram-negative bacteria (*P. aeruginosa*, *N. meningitidis*, *S. sonnei*, and *S. marcescens*). 15 patients had peripheral infectious disease. When identified, the causal pathogens were *S. aureus*, *P. aeruginosa*, and *Streptococcus* species. The susceptibility of IRAK-4–deficient patients to *S. aureus* is consistent with that observed in IRAK-4– and MyD88-deficient mice (31, 43). MyD88-deficient mice are susceptible to *P. aeruginosa* (44) and, in some models, to *S. pneumoniae* (45, 46). Intriguingly, the 28 IRAK-4–deficient patients were not particularly susceptible to most other microorganisms, including common viruses (e.g., herpes viruses, enteroviruses, adenoviruses, and papillomaviruses), and widespread bacteria (e.g., *Listeria*, *Mycobacterium*, and Enterobacteriaceae), parasites (e.g., *Toxoplasma*), and fungi (e.g., *Cryptococcus*, *Pneumocystis*, *Candida*, and *Aspergillus*). As five of these patients have had

**Figure 5. Impaired responses to TLR agonists in IRAK-4–deficient individual myeloid subsets.** (A) Cleavage of CD62 ligand (CD62L) at the surface of granulocytes from a healthy control and an IRAK-4–deficient patient (P7) after activation for 1 h with various TLR agonists and TNF-α. The black line shows CD62L expression on nonactivated granulocytes, and the red line shows CD62L expression after 1 h of activation with various agonists (induced CD62L shedding). One experiment representative of four (P7, 8, 13, and 15) is shown. (B) TNF-α secretion by $CD14^+$ monocytes after 24 h of activation with various TLR agonists. Mean values and SDs were calculated from four healthy controls and three IRAK-4–deficient patients. (C–F) Ex vivo MDC and PDC responses. PBMCs from healthy controls and IRAK-4–deficient patients were stimulated with various TLR agonists. In both subsets, responses were measured by staining for intracellular TNF-α (C) and MIP-1β (E). Mean values and SDs were calculated from six different controls and four IRAK-4–deficient patients for TNF-α (D), and from seven different controls and three IRAK-4–deficient patients for MIP-1β (F). (G) TNF-α secretion in vitro by MDDCs after 24 h of activation. Means and SDs were calculated from six different controls and three different IRAK-4–deficient patients. (H) Induction of CD40, CD80, and CD86 surface expression on MDDCs from a control (top) and an IRAK-4–deficient patient (bottom) after 24 h of stimulation with various TLR agonists. Black and green lines indicate the expression of CD40, CD80, and CD86 without and after stimulation, respectively. The experiment shown is representative of three independent experiments (also performed on patients P15 and 18). C, control.

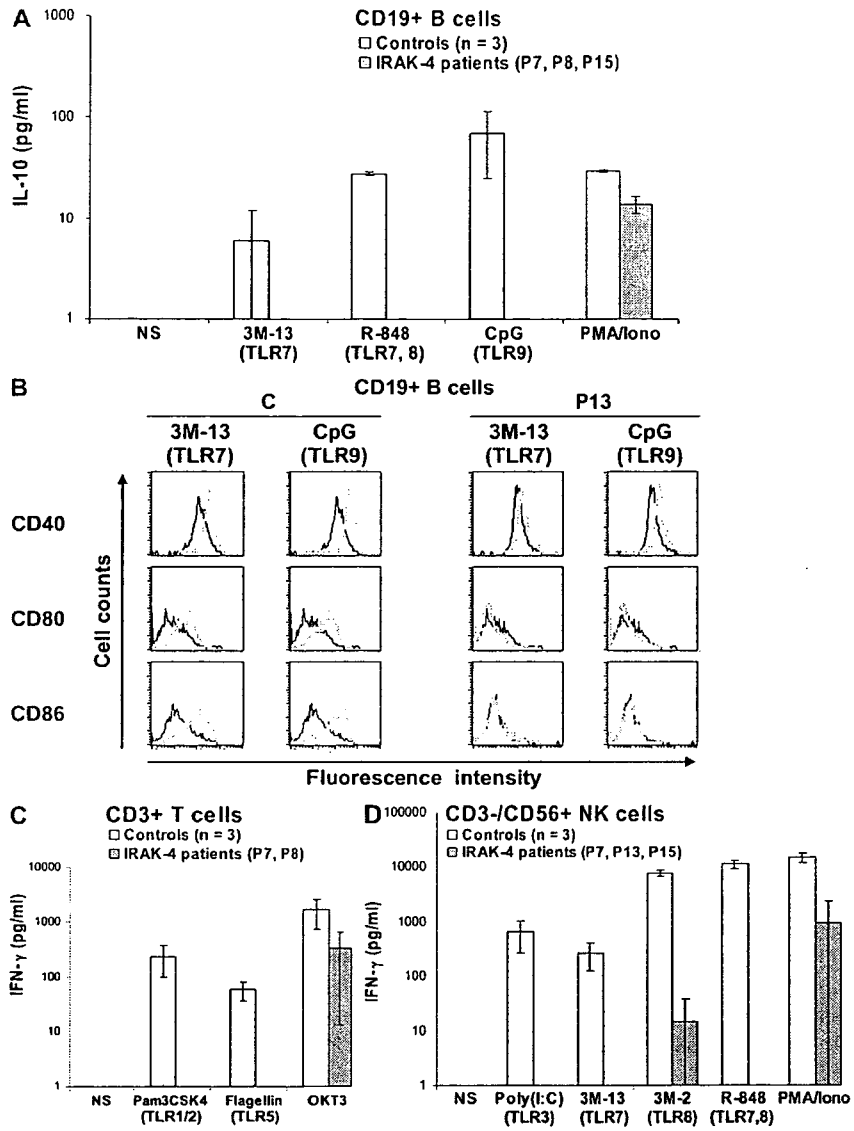



**Figure 6. Lack of response to TLR agonists of individual IRAK-4–deficient lymphoid subsets.** (A) IL-10 secretion by CD19$^+$ B cells after 24 h of activation with various TLR agonists and PMA/ionomycin. Mean values ± SD were calculated from the data obtained for three different controls and three IRAK-4–deficient patients. (B) Induction of CD40, CD80, and CD86 surface expression on CD19$^+$ B cells after activation for 72 h with 3M-13 and CpG. Black and green lines indicate the expression of CD40, CD80, and CD86 without and after stimulation, respectively. Data are representative of two independent experiments. (C) IFN-γ secretion by CD3$^+$ T cells after stimulation for 24 h with various TLR agonists and anti-CD3 (50 ng/ml OKT3) antibody in the presence of 100 U/ml IL-2 for 2 d. Mean values ± SD were calculated for three different controls and two IRAK-4–deficient patients. (D) IFN-γ secretion by CD3$^-$/CD56$^+$ NK cells after activation for 24 h with various TLR agonists and PMA/ionomycin. Mean values and SDs were calculated for three different controls and three IRAK-4–deficient patients.

no prophylaxis for 60 patient years (Fig. 7 B) (4, 6, 36), the resistance to most microbes observed is unlikely to be caused by the early death of some patients or to the prophylactic treatment of the survivors. Ascertainment bias cannot be excluded, but remains unlikely, as 10 affected relatives with causal mutations shared the case-definition clinical phenotype of index cases. In contrast, MyD88-deficient mice were found to be susceptible to mouse CMV (47), HSV-1(48), *Listeria monocytogenes* (49, 50), *Mycobacterium avium* (51), *Toxoplasma gondii* (52), *Cryptococcus neoformans* (53), *Candida albicans*, and *Aspergillus fumigatus* (54), among other relevant infections (37–39).

So why are the infectious phenotypes of MyD88/IRAK-4–deficient mice and IRAK-4–deficient humans so different? An overrepresentation of MyD88 deficiency with respect to IRAK-4 deficiency in mouse studies may be involved,

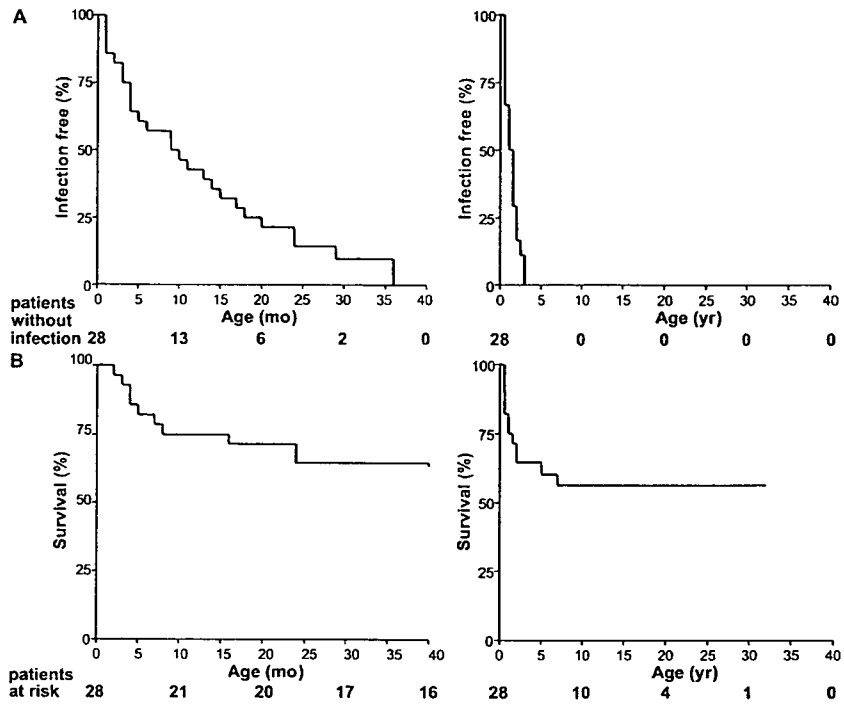



**Figure 7. Epidemiological features of IRAK-4 deficiency.** (A) Incidence of invasive infections in IRAK-4–deficient patients during the first 40 mo of life (left) and the first 40 yr of life (right). Invasive infections included meningitis, septicemia, and arthritis. (B) Survival curve of 28 IRAK-4–deficient patients during the first 40 mo of life (left) and the first 40 yr of life (right).

although IRAK-4– and MyD88-deficient mice, when infected by the same pathogens, are indistinguishable (31, 43). We provide an experimental demonstration in this paper that the occurrence of human-specific IRAK-4–independent TLR pathways is not involved. We show that IRAK-4–deficient PBMCs do not secrete any of 11 cytokines tested when stimulated with agonists of TLR1, 2, 5, 6, 7, 8, and 9. The TLR4 response was abolished for all but two cytokines, which were weakly induced. One of these two cytokines was the IFN-inducible MIP-1β, consistent with the IFN-β mRNA response to LPS in IRAK-4–deficient PBMCs (13). IRAK-4–deficient PBMCs also responded to poly(I:C), producing IFN-inducible monocyte chemoattractant protein 1 and IFN-inducible protein 10, as expected from the previously reported induction of IFN-α, -β, and -λ in IRAK-4–deficient PBMCs and fibroblasts (13). However, poly(I:C) activates PBMCs normally in patients with TLR3 deficiency (42), making it difficult to infer conclusions about TLR3 responses from the data for poly(I:C) stimulation. In any event, the MyD88- and IRAK-4–independent TLR3 and TLR4 pathways, present in mice, cannot account for humans being more resistant (13, 15). The "conventional" MyD88-dependent pathway downstream from TLRs appears to be strictly IRAK-4–dependent in humans; no detectable leakiness can apparently account for the narrow infectious phenotype. We cannot, however, exclude the possibility that other TLR-inducible genes may be IRAK-4 independent.

We further excluded the possibility that human IRAK-4 deficiency may be milder than mouse MyD88/IRAK-4 deficiency owing to the occurrence of human-specific IRAK-4–independent TLR pathways in discrete leukocyte subsets, as suggested by the normal induction of both IL-6 and IFN-β/λ in IRAK-4–deficient fibroblasts (13). We showed that IRAK-4 deficiency impaired the TLR responses of all lymphoid and myeloid leukocyte subsets tested ex vivo, including granulocytes, monocytes, PDCs, MDCs, NK, T, and B cells. With the exception of the induction of IFN-inducible MIP-1β production in MDCs in response to poly(I:C) and LPS (Fig. 5, E and F), there was no detectable TLR response in individual subsets. The LPS response is TLR4 dependent, whereas the poly(I:C) response in MDCs appears to be TLR3 independent (42). Even IRAK-4–deficient NK cells did not respond to poly(I:C), suggesting that responses to poly(I:C) in NK cells are largely TLR3- (42) and IRAK-4–dependent. Moreover, MDDCs generated in vitro did not respond to TLR agonists, with the exception of poly(I:C) and LPS. The poly(I:C)-triggered induction of TNF-α, CD40, CD80, and CD86 in MDDCs was IRAK-4 independent (Fig. 5, G and H) and seemed to be TLR3 dependent (42). These data extend previous findings (1, 13) and show that human IRAK-4 plays a nonredundant role in the conventional TLR signaling pathway in at least seven major leukocyte subsets. In contrast, IRAK-4 may be dispensable for the "alternative," TRIF-dependent pathways downstream from TLR3 (for IFNs and other cytokines) and

TLR4 (for IFNs). Obviously, we cannot formally exclude the possibility that specific leukocyte subsets in certain tissues (55) and nonleukocyte cell types (56–59) display IRAK-4–independent TLR responses involved in host defense.

There are, therefore, no overt immunological differences between MyD88/IRAK-4–deficient mice and IRAK-4–deficient patients. Nonetheless, MyD88 and IRAK-4 are critical for protective immunity to numerous pathogens in the mouse, whereas IRAK-4 is largely redundant for protective immunity in humans. Intrinsic differences between mice and humans, affecting receptors other than TLRs, may account for the observed discrepancies. There may be non-TLRs governing the innate immune recognition of pathogens in humans but not in mice. An alternative, complementary hypothesis is that immunity to infection in animals is studied in experimental conditions, whereas immunity to infection in humans operates in natural conditions, accounting for considerable differences in the hosts, microbes, and routes of infection (60, 61). The human model can be used to define the function of host genes in a natural ecosystem in which species live and undergo selection. The ecologically relevant and evolutionarily selected function of human *IRAK4* appears to be narrower than predicted from experimental studies in the mouse. This is reminiscent of the narrow infectious phenotype of patients with mycobacterial disease and mutations in the IL-12–IFN-γ circuit (62), or patients with herpes simplex encephalitis and mutations in the TLR3–UNC-93B pathway (42, 63). In any event, whether owing to species differences or to the conditions of infection, our findings for this series of IRAK-4–deficient patients strongly suggest that human IRAK-4–dependent TLRs are redundant for protective immunity to most microbes.

IRAK-4 seems to be crucial for protective immunity to Gram-positive *S. pneumoniae* and *S. aureus* and a few Gram-negative bacteria. It remains unknown whether invasive bacterial disease in patients with IRAK-4 deficiency results from an upstream impairment of IL-1R and TLR signaling or a combination of both pathways, from the defective induction of one or a combination of specific target genes downstream, or a combination of upstream and downstream defects. Impaired IL-1R and TLR2 signaling may play a role in the observed infections. Indeed, studies of experimental infection models in knockout mice have indicated that defense against *S. pneumoniae* and *S. aureus* may depend on IL-1R (64, 65), TLR2 (43, 66), and, for *S. pneumoniae*, perhaps also TLR4 (67, 68). Interestingly, the role of TLR2 in mouse defense against *S. pneumoniae* has been called into question in some experimental conditions (69, 70). Impaired stimulation of TLR7, 8, and 9 is probably not involved in predisposition to pneumococcal disease, as UNC-93B–deficient patients with impaired TLR3, 7, 8, and 9 signaling do not suffer from invasive pneumococcal disease (63). The impaired production of IL-6–inducible molecules, such as C-reactive protein (CrP), may also be involved. IRAK-4–deficient cells produce small amounts of IL-6 in vitro upon activation with IL-1β and TLR agonists. Moreover, most patients have weak or delayed acute inflammatory responses in vivo (low serum CrP levels in particular) (34, 71). As CrP contributes to the clearance of *S. pneumoniae* (72, 73), susceptibility to *S. pneumoniae* may be enhanced by the delayed increase in CrP levels. The contribution of individual molecules upstream or downstream from IRAK-4 to infectious phenotypes should be clarified by the identification of new patients with mutations in the corresponding genes (74).

Despite conferring selective susceptibility to only a few bacteria, IRAK-4 deficiency is life-threatening in infancy and childhood, with a mortality rate of 43% in our series. Most, if not all, patients would have probably died in the absence of antibiotic treatment. Strikingly, although IRAK-4 is absolutely vital in childhood, infections become rarer with age, with no deaths recorded after the age of 8 yr and no invasive infection after the age of 14 yr, even in the absence of antibiotic or IgG prophylaxis for more than 60 patient years (4, 6, 36). This dramatic improvement with age may be accounted for by the modest impact, if any, of IRAK-4 deficiency on antigen-specific T and B lymphocyte responses. Human T cells do not need IRAK-4 for activation by OKT3 in vitro (Table S2), in contrast to the results obtained for mice in a previous report (32) and in accordance with a more recent study (75). Moreover, our patients displayed no detectable global defect of protein antigen–specific T and B cell responses. However, most of the patients displayed IgE overproduction, and some patients have been shown to have weak antibody responses to a subset of glycan antigens (11, 12, 33). A more thorough investigation of B cells and antibody responses in IRAK-4–deficient patients is therefore currently underway (unpublished data). Our data are consistent with the apparently intact primary and secondary antigen-specific responses in mice with MyD88 deficiency, TRIF deficiency, or both (76, 77). Adaptive immunity may therefore progressively compensate for the poor innate immunity in our patients. An alternative and complementary hypothesis, accounting for the clinical improvement of IRAK-4–deficient patients with age, is that innate immune responses may also mature with age (78, 79). As shown in this study, the TIR pathway, including TLR responses in particular, remains dependent on IRAK-4 with age, but the maturation of other innate pathways may gradually compensate for the lack of TIR–IRAK-4 signaling.

## MATERIALS AND METHODS

**Subjects and kindreds.** Our study was conducted according to the principles expressed in the Helsinki Declaration, with informed consent obtained from each patient or the patient's family. The study was approved by the Comité d'Éthique, CCPPRB, Hôpital Necker–Enfants Malades.

**Molecular genetics.** Genomic DNA was isolated from whole blood cells or from B-EBVs. The cells were lysed by incubation overnight at 37°C in extraction buffer (10 mM Tris, 0.1 M EDTA, 0.5% SDS, 1 mg/ml proteinase K) and subjected to phenol/chloroform extraction. DNA was precipitated in ethanol. Amplified PCR products were analyzed by electrophoresis in a 1% agarose gel purified by centrifugation through superfine resin (Sephadex G-50; GE Healthcare), sequenced by dideoxynucleotide termination with the BigDye terminator kit (Applied Biosystems), and analyzed on an ABI Prism 3730 apparatus (Applied Biosystems).