Impaired T-cell receptor activation in IL-1 receptor–associated kinase-4–deficient patients

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Background: IL-1 receptor–associated kinase 4 (IRAK-4) is an effector of the Toll-like receptor and IL-1 receptor pathways that plays a critical role in innate immune responses. The role of IRAK-4 in adaptive immune functions in human subjects is incompletely understood.
Objective: We sought to evaluate T-cell function in IRAK-4 deficient patients.
Methods: We compared upregulation of CD25 and CD69 on T cells and production of IL-2, IL-6, and IFN-γ after stimulation of PBMCs from 4 IRAK-4–deficient patients and healthy control subjects with anti-CD3 and anti-CD28.
Results: Upregulation of CD25 and CD69 on T cells and production of IL-6 and IFN-γ, but not IL-2, was significantly reduced in IRAK-4–deficient patients.
Conclusions: IRAK-4–deficient patients have defects in T-cell activation. (J Allergy Clin Immunol 2010;126:332-7.)

Key words: *IL-1 receptor–associated kinase 4, T cell, T-cell receptor, cytokines, TNF-α, IL-2, IL-6, IFN-γ, CD25, CD69*

Toll-like receptors (TLRs) are crucial components of the innate immune system that detect pathogen-associated molecular patterns, such as LPS, single- and double-stranded RNA, and hypomethylated CpG-rich DNA, and initiate inflammatory responses to invading microbes.[1] Activation of TLRs leads to production of proinflammatory cytokines, such as IL-1, IL-6, IL-12, TNF-α, and, in the case of TLR3, TLR7, TLR8, and TLR9, type 1 interferons. Additionally, TLR activation causes upregulation of costimulatory molecules, such as CD40, CD80, and CD86, and enhanced antigen presentation by antigen-presenting cells.[2,3]

Abbreviations used
IKK: IκB kinase
IRAK-4: IL-1 receptor–associated kinase 4
LCMV: Lymphocytic choriomeningitis virus
MAPK: Mitogen-activated protein kinase
NF-κB: Nuclear factor κB
PE: Phycoerythrin
PMA: Phorbol 12-myristate 13-acetate
TCR: T-cell receptor
TLR: Toll-like receptor

The production of IL-12 and type 1 interferons, as well as upregulation of costimulatory molecules, influences subsequent adaptive immune responses by inducing T_H1 differentiation of naive T cells.[4] IL-12 also induces production of IFN-γ by T cells, which further enhances the antimicrobial functions of monocytes and macrophages.

IL-1 receptor–associated kinase-4 (IRAK-4) is an essential effector of the IL-1 receptor and all TLRs, except TLR3.[2] Mice that are deficient in IRAK-4 or express kinase-inactive IRAK-4 have impaired TLR-induced inflammatory responses and impaired host defense against bacterial infection.[5] Investigation of IRAK-4–deficient patients has confirmed that IRAK-4 plays a nonredundant role in immunity against pyogenic bacterial infections.[6] The susceptibility of IRAK-4–deficient patients to invasive bacterial infections diminishes with age, becoming comparable with that of the normal population by roughly age 14 years. The reason for this is not understood. Maturation of the adaptive immune system has been hypothesized to compensate for impaired innate immune function caused by IRAK-4 deficiency.

IRAK-4–deficient murine T cells were shown to be deficient in T-cell receptor (TCR)–induced activation of nuclear factor κB (NF-κB), as well as IL-2 production and proliferation.[7] However, these observations were not replicated in a subsequent report.[8] Recently, IRAK-$4^{-/-}$ mice were found to have reduced splenic and peripheral expansion of $CD8^+$ T cells in response to infection with lymphocytic choriomeningitis virus (LCMV), suggesting that IRAK-4 might be required for optimal antiviral $CD8^+$ T-cell responses *in vivo*.[9] Furthermore, T cells from IRAK-4 kinase–inactive mice and T-cell blasts from IRAK-4–deficient and MyD88-deficient patients were shown to secrete reduced quantities of IL-17, which plays an important role in immunity against bacterial infection.[9,10] Thus, although IRAK-4 plays a crucial role in innate immunity, its role in the development of human adaptive immune responses is incompletely understood.

We describe a new patient with IRAK-4 deficiency who had recurrent invasive infections with *Streptococcus pneumoniae* and

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Pseudomonas aeruginosa. Analysis of T-cell function revealed impaired upregulation of CD25 and CD69 and reduced production of IL-6 and IFN-γ after T-cell activation. Analysis of T-cell function in 3 additional IRAK-4–deficient patients confirmed these findings. These observations provide support for a role of IRAK-4 in human T-cell function.

METHODS

Subjects

Four unrelated patients with IRAK-4 deficiency with distinct molecular defects were studied.[6,11,12] All had clinical features of IRAK-4 deficiency, and the diagnosis was confirmed by means of molecular analysis. Parents of all subjects enrolled in these studies signed informed consent forms that were approved by the University of Iowa Children's Hospital Institutional Review Board and Children's Hospital, Boston, and in accordance with the Declaration of Helsinki. Case reports are included in this article's Online Repository at www.jacionline.org.

Reagents

TLR ligands used include PAM3CSK4 (TLR1/2), poly I:C (TLR3), ultrapure LPS from *Salmonella minnesota* (TLR4), flagellin (TLR5), and CpG DNA (ODN2216; TLR9), all of which were obtained from Invivogen (San Diego, Calif). The ligands for TLR7 (3M-2) and TLR8 (3M-13) were kind gifts of Dr Richard Miller, 3M Pharmaceuticals.

Antibodies used include anti-CD3 (HIT3a), which was from BioLegend (San Diego, Calif), and anti-CD28 and conjugated murine anti-human mAbs, including CD4 fluorescein isothiocyanate, CD69 phycoerythrin (PE), CD8 peridinin-chlorphyll-protein–Cy5.5, and CD25 PE, which were from BD Biosciences (San Jose, Calif). Human IL-1β, TNF-α, and ELISA kits for human TNF-α were obtained from Invitrogen (Carlsbad, Calif). Phorbol 12-myristate 13-acetate (PMA) and ionomycin were obtained from Calbiochem (La Jolla, Calif).

TLR stimulation of TNF-α production

PBMCs were isolated and stimulated with TLR ligands or PMA plus ionomycin, as previously described.[12] Cell-culture supernatants were collected after 24 hours, and TNF-α levels were measured by means of ELISA.

For Western blotting, patients' and control subjects' primary fibroblasts were stimulated with IL-1β (10 ng/mL) or TNF-α (20 ng/mL) for the indicated times in RPMI plus L-glutamine and penicillin/streptomycin with 10% FCS. Cells were lysed in Sample Buffer (62.5 mmol/L Tris [pH 6.8], 2% wt/vol SDS, 10% glycerol, 2% β-mercaptoethanol, and 0.01% bromophenol blue). Proteins were resolved with 10% SDS-PAGE (Bio-Rad Laboratories, Hercules, Calif) and transferred to polyvinylidene difluoride membranes (Millipore, Billerica, Mass). Western blotting with anti–phospho p38 mitogen-activated protein kinase (MAPK) and anti–IRAK-4 (Cell Signaling, Danvers, Mass) and anti-IκBα and anti–IκB kinase (IKK) γ (sc-8330; Santa Cruz Biotechnology, Santa Cruz, Calif) was performed according to the manufacturer's recommendations.

Mutational analysis of IRAK-4

RNA from PBMCs was prepared with Trizol reagent (Invitrogen), and cDNA was generated with Superscript II reverse transcriptase (Invitrogen). IRAK-4–specific primers were used to amplify the full-length message with the following primer sets: forward, 5′-TTCTTCTGTCGCCGGCTTCAG-3′; reverse, 5′-TGTCAACCATTGCTGCAAGC-3′; and forward, 5′-ATGGGAGAGGGAG GATTTGG-3′; reverse, 5′-ACGCTATGCCTTGTTAAAGG-3′. Genomic DNA from patients' fibroblasts was prepared by using phenol/chloroform extraction to confirm the mutations observed in exon 7. The patient's mutation was identified in genomic DNA in exon 7 of IRAK-4 by using the following primers, as previously described[12]: forward, 5′-GCTATAACATCATCTT CAGTTGTTG-3′; reverse, 5′-GGATGAGTACTGGAAGTAGGTC-3′. The individual exon 7 alleles were isolated by means of TA cloning (pCR2.1-TOPO vector, Invitrogen). TA clones were sequenced with T3 primers. All sequencing was performed by the Molecular Genetics Core facility at Children's Hospital, Boston.

Upregulation of activation markers on T cells

PBMCs were plated at a density of 1 to 2×10^6/mL and incubated in tissue-culture flasks at 37°C in 5% CO_2 for 2 hours to remove adherent cells. Nonadherent lymphocytes were suspended in RPMI 1640 supplemented with 10% FCS, penicillin (1,000 U/mL), streptomycin (1,000 U/mL), and glutamine (20 mmol/L) and then plated onto 24-well Costar plates. Cells were incubated for 18 hours under one of the following conditions: PBS, plate-bound biotinylated anti-CD3 (0.5 mg/mL, diluted 1:100 in 1× PBS) with or without soluble anti-CD28 (0.5 mg/mL), or PMA (75 ng/mL) plus ionomycin (1 mmol/L). Lymphocytes were removed from the media, washed, and resuspended in staining buffer (0.1% BSA, PBS, and 0.01% sodium azide). Cells were incubated for 30 minutes on ice with conjugated murine anti-human mAbs that included CD4 fluorescein isothiocyanate, CD69 PE, CD8 peridinin-chlorphyll-protein–Cy5.5, and CD25 PE (BD Biosciences) and washed twice. Fluorescence was determined with a FACScan flow cytometer (Becton-Dickinson, San Jose, Calif), and data analysis was performed with Cell Quest software (Becton-Dickinson), as previously described.[13]

Cytokine production in T cells

PBMCs (2.5×10^6 cells/mL) were plated in tissue-culture flasks, as above, and nonadherent lymphocytes were incubated for 18 hours in media, as above, with PBS, PMA plus ionomycin, or immobilized anti-CD3 plus soluble anti-CD28, as above. Cell-culture supernatants were harvested, diluted 1:3 with RPMI, and analyzed in triplicate by using Bioplex assays (Bio-Rad Laboratories), according to the manufacturer's protocol with a Luminex 200 multiplexing system.

RESULTS

Impaired TLR and IL-1 receptor signaling in a compound heterozygous IRAK-4–deficient patient

The clinical history of the index patient is detailed in this article's Online Repository. Severe and recurrent pyogenic infections and a diminished febrile response to invasive bacterial infections suggested a defect in innate immunity. Stimulation of the patient's blood cells with TLR ligands failed to elicit production of TNF-α (Fig 1, *A*). TLRs and IL-1 receptor use the adaptor MyD88 and IRAK-4 to activate IKK, which phosphorylates the NF-κBα inhibitor IκBα. Stimulation of the patient's fibroblasts with IL-1β for 5 and 15 minutes failed to induce phosphorylation and degradation of IκBα, as demonstrated by the absence of a mobility shift and disappearance of IκBα on Western blotting, respectively. IL-1β also failed to cause phosphorylation of p38 MAPK (Fig 1, *B*, compare lanes 2 and 3 with lanes 6 and 7). In contrast, stimulation of the patient's fibroblasts with TNF-α induced complete degradation of IκBα and normal p38 phosphorylation (Fig 1, *B*, lane 8), ruling out a defect in the IKK subunits IKKα, IKKβ, and IKKγ or in IκBα.

The absence of TLR-induced cytokine production coupled with absent IL-1β–induced IκBα phosphorylation and degradation and p38 MAPK activation suggested a proximal defect in IL-1/TLR pathways. Western blotting of the patient's fibroblast lysates revealed absence of IRAK-4 protein, which is consistent with IRAK-4 deficiency (Fig 1, *B*, lanes 5-8). *IRAK4* was sequenced from cDNA derived from the patient's PBMCs, revealing compound heterozygous mutations within exon 7. The mutant alleles were confirmed by amplifying exon 7 from genomic DNA

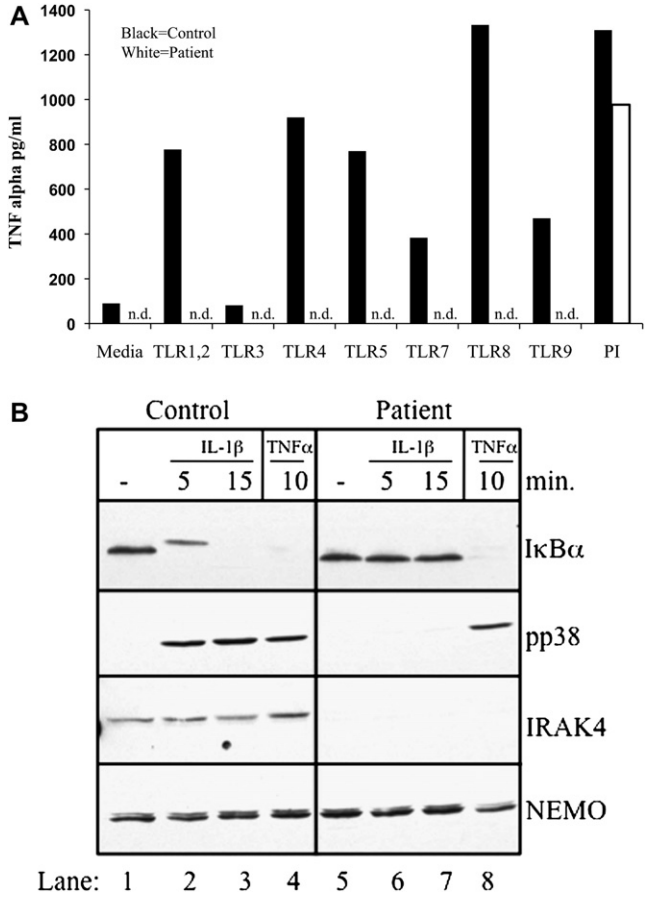

FIG 1. Defective TLR-induced cytokine production and absent IL-1–induced signaling associated with IRAK-4 deficiency. **A**, TNF-α production in PBMCs in response to TLR ligands or PMA plus ionomycin *(PI). n.d.*, Not detected. **B**, Control subjects' and patients' fibroblasts were stimulated for the times shown with IL-1β (10 ng/mL) or TNF-α (20 ng/mL). Lysates were analyzed by means of Western blotting with anti-IκBα, anti–phospho p38 MAPK, anti–IRAK-4, and anti–NF-κB essential modifier (*NEMO*; loading control). Data are representative of 2 independent experiments.

generated from the patient's fibroblasts, followed by TA cloning to isolate clones derived from both alleles. One mutation consisted of a novel 17-bp deletion (870-887del) that results in a premature stop codon and was found to be inherited from the father. The other mutation consisted of the previously described C to T transition at codon 877 (C877T), which also results in a premature stop codon (Q293X) and was found to be inherited from the mother (Table I).[6,11,12] The patient's healthy sister is heterozygous for the C877T mutation.

Impaired upregulation of activation markers on activated T cells from IRAK-4–deficient patients

T cells were activated by cross-linking with anti-CD3 and anti-CD28 antibodies and evaluated 18 hours later for upregulation of the activation markers CD25 and CD69 by means of flow cytometry. Upregulation of CD25 and CD69 on both $CD4^+$ and $CD8^+$ T cells was impaired in the patient (Fig 2, *A*). In 3 experiments the mean percentage of $CD4^+/CD25^+$ cells in the patient was 42% ± 5% compared with 72% ± 18% in the control subject ($P = .049$), and the mean percentage of $CD8^+/CD25^+$ cells in the patient was 21% ± 5% compared with 59% ± 22% in the control subject ($P = .043$). In the same experiments the mean percentage of $CD4^+/CD69^+$ cells in the patient was 38% ± 12% compared with 74% ± 17% in the control subject ($P = .04$), and the mean percentage of $CD8^+/CD69^+$ cells in the patient was 23% ± 2% compared with 56% ± 4% in the control subject ($P = .0002$). Upregulation of CD25 and CD69 expression on $CD8^+$ T cells after stimulation with PMA and ionomycin, which bypass TCR signaling by activating protein kinase C and increasing the intracellular calcium concentration, respectively, was comparable in the patient and control subject (Fig 2, *B*). The effect of PMA plus ionomycin on CD25 and CD69 expression on $CD4^+$ T cells was not examined because PMA strongly downregulates CD4 expression on T cells.[14]

To confirm these findings, we examined the expression of T-cell activation markers in 3 other previously characterized, unrelated IRAK-4–deficient patients (Table I). The combined data from these 3 patients and our patient were pooled. Upregulation of CD25 expression on $CD4^+$ and $CD8^+$ T cells after cross-linking with anti-CD3 alone or anti-CD3 plus anti-CD28 was significantly impaired in IRAK-4–deficient patients relative to that seen in 4 healthy control subjects. This was evident by a decrease in the percentage of $CD25^+$ T cells (Fig 3, *A*) and by decreased mean fluorescence intensity of CD25 expression by these T cells (Fig 3, *B*). Additionally, upregulation of CD69 after cross-linking with anti-CD3 alone or anti-CD3 plus anti-CD28 was significantly impaired in $CD8^+$ T cells in IRAK-4–deficient patients. Upregulation of CD69 in $CD4^+$ T cells in IRAK-4–deficient patients was reduced, although not significantly (Fig 3). Upregulation of CD25 and CD69 expression on $CD8^+$ T cells after stimulation with PMA and ionomycin, which bypass TCR signaling, was comparable in patients and control subjects.

TABLE I. Genotypes of IRAK-4 deficient patients

Patient no.	Patient mutation	Protein	Reference
1	C877T/870-887del	ND	This report
2	631del_G/C144G	ND	6
3	C877T/G893A	ND	11
4	C877T/C877T	ND	12

ND, None detected.

Impaired secretion of IL-6 and IFN-γ by activated T cells from IRAK-4–deficient patients

Production of IL-6 and IFN-γ by T cells after cross-linking of CD3 plus CD28 was significantly impaired in IRAK-4–deficient patients (Fig 4). For IL-6, the mean was 409 pg/mL for the patients compared with 45,490 pg/mL for the control subjects ($P = .006$). For IFN-γ, the mean was 506 pg/mL for the patients compared with 7,829 pg/mL for the control subjects ($P = .02$). Decreased production of IL-6 and IFN-γ in the patients was not due to decreased percentages of T cells because all 4 patients had normal populations of circulating $CD4^+$ and $CD8^+$ cells (see Table E1 in this article's Online Repository at www.jacionline.org). Furthermore, IL-6 and IFN-γ production after stimulation with PMA and ionomycin, which bypass TCR signaling, was comparable in patients and control subjects. IL-2 production by T cells after cross-linking of CD3 plus CD28 was not significantly different in patients and control subjects (4,141 pg/mL in patients vs 9,646 pg/mL in control subjects, $P = .14$).

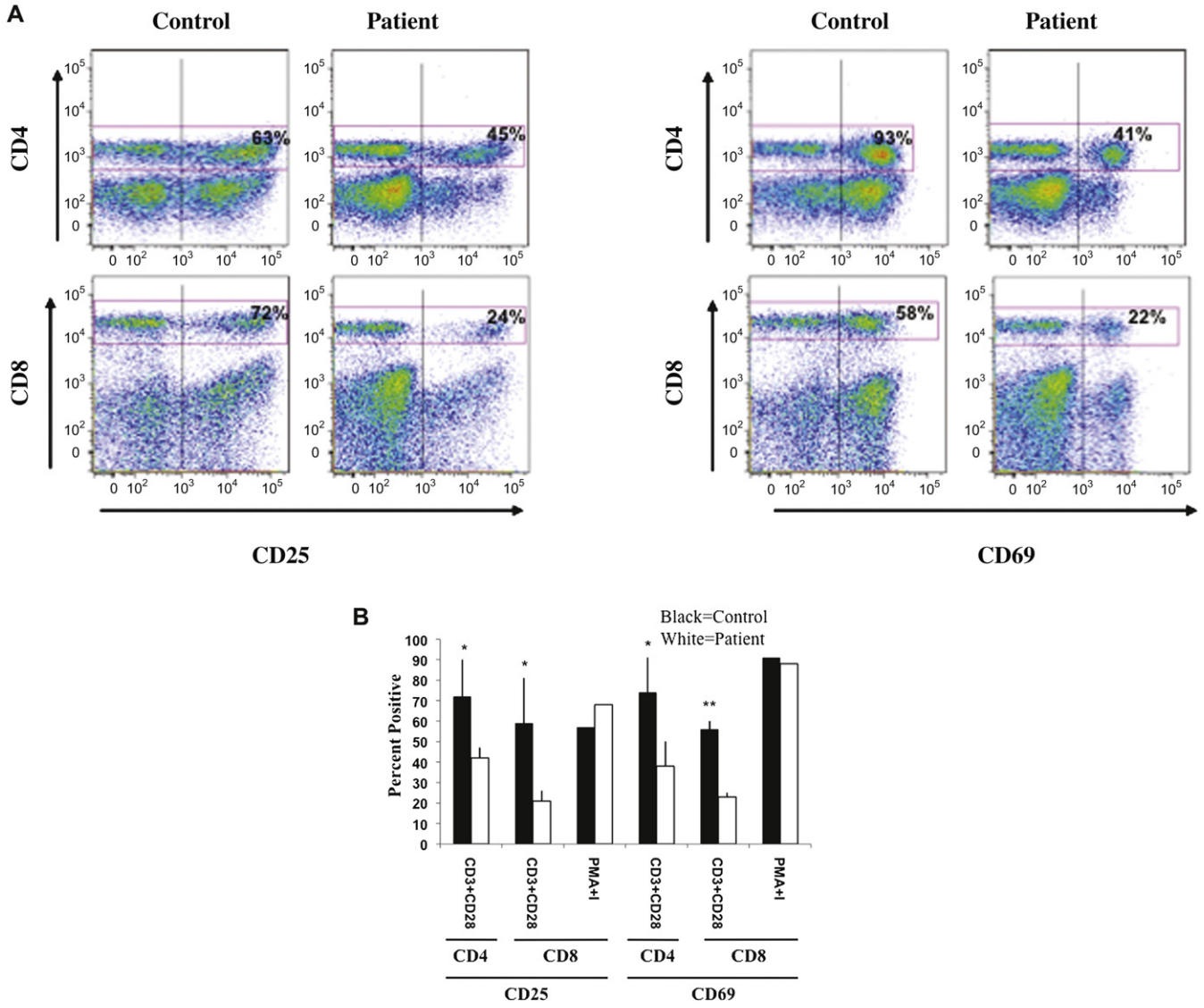

FIG 2. Impaired expression of activation markers by T cells in an IRAK-4–deficient patient. CD25 and CD69 expression on $CD4^+$ and $CD8^+$ cells in nonadherent lymphocytes from the index patient and a control subject after 18 hours' stimulation with plate-bound anti-CD3 plus soluble anti-CD28 is shown. Representative data from 3 experiments **(A)** and the mean ± SD of 3 experiments and plotted as percent positive **(B)** are shown. *PMA+I*, PMA plus ionomycin. $*P < .05$ and $**P < .001$, Student *t* test.

DISCUSSION

The results of this study support a role for IRAK-4 in T-cell activation. Stimulation of T cells through the TCR leads to upregulation of CD69 and CD25, which are early markers of T-cell activation.[15,16] Upregulation of the activation markers CD25 and CD69 after TCR ligation and ligation of both the TCR and CD28 was impaired in all 4 IRAK-4–deficient patients analyzed. CD69 is the earliest marker of lymphocyte activation and might be involved in lymphocyte proliferation. CD25 is a component of the high-affinity IL-2 receptor required for T-cell responsiveness to IL-2. Reduced IL-2 responsiveness resulting from reduced TCR-induced upregulation of CD25 might underlie the observed reduction in splenic and peripheral expansion of $CD8^+$ T cells in LCMV-infected IRAK-$4^{-/-}$ mice.[9]

Production of IL-6 and IFN-γ after T-cell activation by anti-CD3 and anti-CD28 was significantly lower in IRAK-4–deficient patients compared with that seen in healthy control subjects, which is consistent with reduced LCMV-induced proinflammatory cytokine production observed in T cells from IRAK-$4^{-/-}$ mice.[9] IL-6 is a proinflammatory cytokine that has been shown to play a critical role in resistance against *S pneumoniae*.[17] TLR-induced IL-6 production in IRAK-4–deficient monocytes, macrophages, and dendritic cells is virtually absent.[18] Our observation that TCR/CD28-induced IL-6 production is impaired in T cells from IRAK-4–deficient patients is novel and might contribute to the susceptibility of these patients to *S pneumoniae*. Specific antibody responses to polysaccharide antigens, such as those contained in the 23-valent pneumococcal vaccine (Pneumovax; Merck Pharmaceuticals, Whitehouse Station, NJ), are variably impaired in IRAK-4–deficient patients, and this was observed in 2 of 4 of the IRAK-4–deficient patients analyzed in this study. Although IL-6 augments production of immunoglobulins by

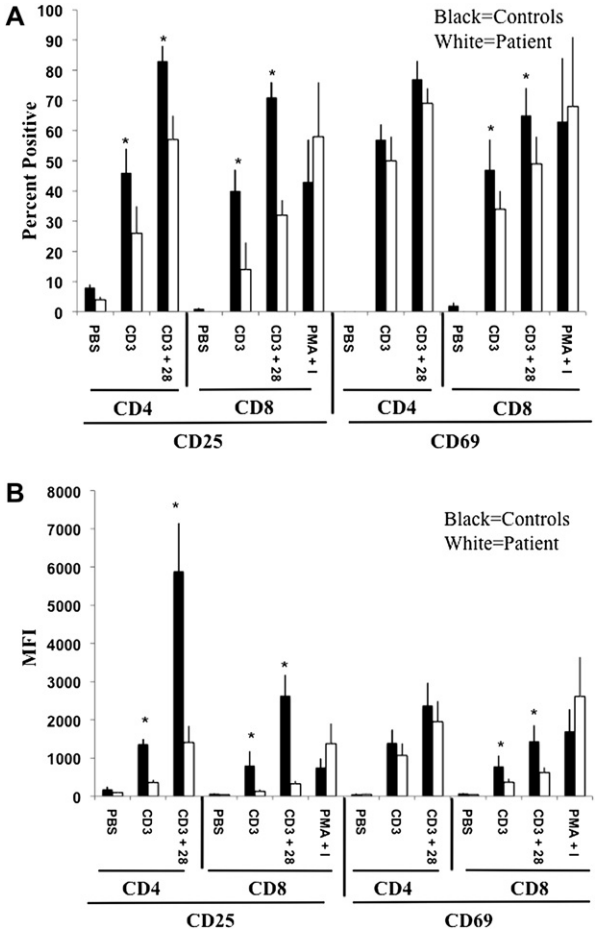

FIG 3. Reduced expression of activation markers by T cells from IRAK-4–deficient patients. Control subjects' and patients' nonadherent lymphocytes were cultured with PBS, immobilized anti-CD3 *(CD3)*, immobilized anti-CD3 plus soluble anti-CD28 *(CD3/28)*, or PMA and ionomycin *(PMA+I)* and stained as in Fig 2. Data from 4 patients and control subjects were averaged and plotted as percent positive **(A)** and mean fluorescence intensity (*MFI*; **B**). **P* < .05, Student *t* test.

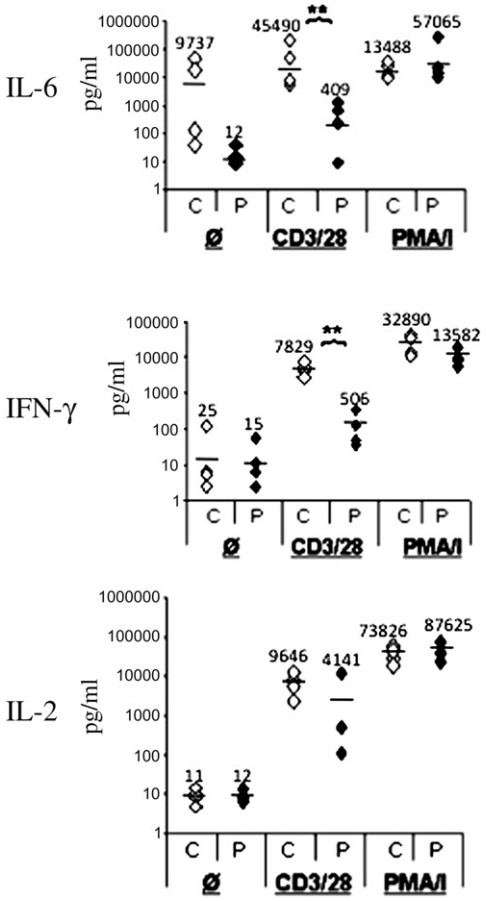

FIG 4. Impaired production of IL-6 and IFN-γ by activated T cells of IRAK-4–deficient patients. IL-2, IL-6, and IFN-γ production in 4 control subjects' *(C)* and patients' *(P)* nonadherent lymphocytes stimulated with PBS *(Ø)*, immobilized anti-CD3 plus soluble anti-CD28 *(CD3/28)*, or PMA plus ionomycin *(PMA/I)* is shown. Measurements were performed in triplicate. Average values are indicated by a *horizontal line* and listed above each dataset. ***P* < .05, Student *t* test.

B cells,[19] it is unknown whether decreased IL-6 production by IRAK-4–deficient patients contributes to impaired specific antibody responses to pneumococcus. A defect in specific antibody production in response to Pneumovax that has been observed in TLR2-deficient mice, TLR4-deficient mice, or both has been hypothesized to result from a deficient response to small amounts of TLR ligands found in the 23-valent pneumococcal vaccine.[20] A similar mechanism could operate in IRAK-4–deficient patients.

Additionally, IL-6 is critical for the development of IL-17– and IL-22–producing T cells.[9,21-23] IL-17 contributes to host defense against bacterial infections. In murine models IL-17 plays a critical role in the clearance of nasopharyngeal colonization with *S pneumoniae*.[24] IL-17 promotes the recruitment of monocytes and macrophages to the nasopharyngeal mucosa that are responsible for phagocytosis and clearance of pneumococcus. Additionally, IL-17 contributes to host defense against invasive infections with salmonella and *P aeruginosa*, which have been observed in many IRAK-4–deficient patients.[25] Antigen-stimulated T cells from mice with an inactivating mutation in IRAK-4 kinase secrete reduced amounts of IL-17.[26] T-cell blasts from patients with IRAK-4 and MyD88 deficiency have been shown to secrete reduced quantities of IL-17 when cultured in the presence of IL-1β, presumably because of a lack of responsiveness to IL-1.[10] Reduced TCR/CD28-driven IL-6 production, in addition to reduced TLR- and IL-1–driven IL-6 production, might contribute to impaired development of T_H17 T cells in IRAK-4–deficient patients. Reduced IL-17 production might contribute to the infectious susceptibility of these patients.

IFN-γ produced by T cells plays a critical role in enhancing antimicrobial activity of macrophages and monocytes, recruiting inflammatory cells to sites of infection, and increasing antigen presentation by antigen-presenting cells.[27] Thus the crosstalk between T cells and monocytes and macrophages that augments antimicrobial activity might be diminished in IRAK-4–deficient patients as a result of reduced IFN-γ production by T cells.

At present, it is unclear how IRAK-4 promotes T-cell activation. It is not known whether IRAK-4 kinase activity plays an essential role or whether IRAK-4 functions as a scaffolding protein in the TCR signaling pathway of human subjects. The observation that antigen-induced activation of $CD8^+$ T cells in mice with an inactivating mutation in IRAK-4 kinase is impaired suggests that IRAK-4 kinase activity might be required.[9]

Longitudinal studies in IRAK-4 deficiency are needed to determine whether the defective T-cell functions that we have characterized in these patients will improve as they reach adolescence and become less susceptible to infections.

Clinical implications: Defects in T-cell activation might contribute to the susceptibility of IRAK-4–deficient patients to infections.

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CASE REPORTS

Patient 1

The patient, a 3-year-old girl, was born at term without complications to unrelated parents. Family history is negative for immune deficiency. Infectious history is remarkable for recurrent skin abscesses and impetigo, 2 urinary tract infections, recurrent otitis media, *S pneumoniae*–induced sepsis, *P aeruginosa*–induced brain abscess, and *S pneumoniae*–induced cervical abscess. The maximum recorded temperature with her brain abscess was 37.3°C. Physical examination was unremarkable. Initial immunologic evaluations revealed white blood cell counts from 3,300 to 17,000/μL, neutrophil counts from 560 to 10,000/μL, and lymphocyte counts from 1,463 to 12,075/μL. Immunoglobulin levels were normal, except for low IgA levels (<5-13 mg/dL). Antibody titers to diphtheria and tetanus antigens, *Haemophilus influenzae*, and rubella were protective. Postimmunization antibody titers after immunization with Pneumovax (Merck Pharmaceuticals, Whitehouse Station, NJ), which contains 23 pneumococcal polysaccharide antigens, were protective for 9 of 14 serotypes measured. T- and B-cell subsets were normal, and T-cell mitogen and antigen proliferation was normal. Natural killer cell function was normal. CH50 (total complement activity) results were normal. HIV test results were negative. The patient has compound heterozygous mutations in *IRAK4* consisting of a nonsense mutation, C877T (Q293X), on 1 allele and a 17-bp deletion (870-887del) that results in a premature stop codon (I291X).

Patient 2

The patient, a 22-month-old boy, was born at term to unrelated parents. Family history is significant for an older sister with IRAK-4 deficiency. The patient was given a diagnosis in the first month of life based on absence of neutrophils to shed CD62 ligand (L-selectin) in response to LPS, poly I:C, R848, and FSL lipopeptide. IL-6, TNF-α, and IL-8 production by whole blood cells in response to IL-1, lipopeptide, LPS, poly I:C, and R848 were absent. Immunoglobulin levels were normal at 11 months of age (IgG, 386 mg/dL; IgA, 29 mg/dL; and IgM, 59 mg/dL) and at 22 months of age (IgG, 651 mg/dL; IgA, 31 mg/dL; and IgM, 64 mg/dL), although the IgE level was increased at 330 U/mL (<110 U/mL). Response to specific immunizations showed protective titers to hepatitis B vaccine but failure to develop protective titers to varicella after 2 vaccinations with Varivax (Merck). He did respond to measles, mumps, rubella vaccine with mumps IgG, measles IgG, and rubella IgG with protective titers. The patient did not have protective titers to pneumococcus after vaccination with the polysaccharide antigens contained in Prevnar (diphtheria-pneumococcal conjugate vaccine; Pfizer, Kirkland, Quebec, Canada). The patient had a normal complete blood count, including normal lymphocyte populations. The patient has remained healthy since he was started on antibiotic prophylaxis shortly after birth. He has not had any invasive bacterial or fungal infections and has only had 1 episode of mild bronchiolitis with parainfluenza 3. The patient has compound heterozygous mutations in *IRAK4* consisting of a deletion (631del_G) on 1 allele resulting in a premature stop codon at amino acid 212 (V212X). The other allele contains the point mutation C144G, resulting in a tyrosine to stop codon (Y48X) substitution at position 48.

Patient 3

The patient, a 4-year-old girl, was born at term to unrelated parents. Family history was negative for immune deficiency. Infectious history was remarkable for pneumococcal meningitis. The patient was discharged to home after therapy but was subsequently readmitted with proptosis and complete opthalmoplegia of the left eye. A computed tomographic scan/magnetic resonance image showed a retro-orbital mass. Biopsy showed skeletal muscle infiltrated with chronic inflammatory cells. Complete resolution of the retro-orbital mass occurred after treatment with antibiotics and steroids, although she was left with residual left optic atrophy. She later experienced an episode of pneumococcal arthritis of the left ankle. She subsequently had fever and meningismus, but no organisms were isolated from the cerebrospinal fluid. She has had recurrent impetigo and cellulitis of the foot, and no organisms were isolated. Immunologic evaluations revealed normal complete blood counts, normal T- and B-cell subsets, normal immunoglobulin levels, protective immune responses to the polysaccharide antigens contained in Pneumovax, normal classical and alternative complement function, and a heterozygous mutation in mannose binding lectin. The patient has compound heterozygous mutations in *IRAK4* consisting of C877T (Q293X) and G893A (G298D).

Patient 4

The patient is an 11-year-old boy born at term to unrelated parents. Family history was negative for immune deficiency. Infectious history was significant for 2 episodes of pneumococcal meningitis 1 year apart. Sequelae of his episodes of meningitis included stroke, seizures, mild hearing loss, and, possibly, mild learning impairment. The patient had multiple episodes of otitis media, requiring placement of myringotomy tubes at age 2 years. At 11 months of age, he had intestinal intussusception complicated by perforation of the intestine and abscess formation in the peritoneum. He had several episodes of furuncles of the scalp and several skin infections, all of which responded well to treatment with antibiotics. He was also noted to have a weak febrile response, usually having only low-grade fevers late in the course of an illness. Physical examination was unremarkable. Immunologic evaluation revealed a normal complete blood count and normal T- and B-cell subsets. Immunoglobulin levels were all within the normal range. Specific antibody titers demonstrated protective immune responses to protein antigens, such as tetanus toxoid, HiB conjugate vaccine, and the pneumococcal polysaccharide conjugate vaccine Prevnar. However, immunization with pure polysaccharide antigens, contained in Pneumovax, did not result in increased titers. Complement function was normal. The patient is homozygous for a nonsense mutation in *IRAK4* consisting of C877T (Q293X).

TABLE E1. T- and B-lymphocyte populations in IRAK-4–deficient patients

Patient no.	1	2	3	4
CD3	2.7 (2.3-6.5)	4.6 (2.3-6.5)	3.0 (2.4-6.9)	1.8 (1.6-6.7)
CD3/4	1.6 (1.5-5)	3 (1.5-5)	1.9 (1.4-5.1)	1.02 (1-4.6)
CD3/8	0.9 (0.5-1.6)	1.4 (0.5-1.6)	1.04 (0.6-2.2)	0.7 (0.4-2.1)
CD19	1.3 (0.6-3)	0.6 (0.6-3)	1.7 (0.7-2.5)	1.2 (0.6-2.7)
CD56	0.05 (0.1-1.3)	0.3 (0.1-1.3)	0.36 (0.1-1.3)	0.95 (0.2-1.2)

Values are $\times$1,000. Normal ranges are shown in parentheses.