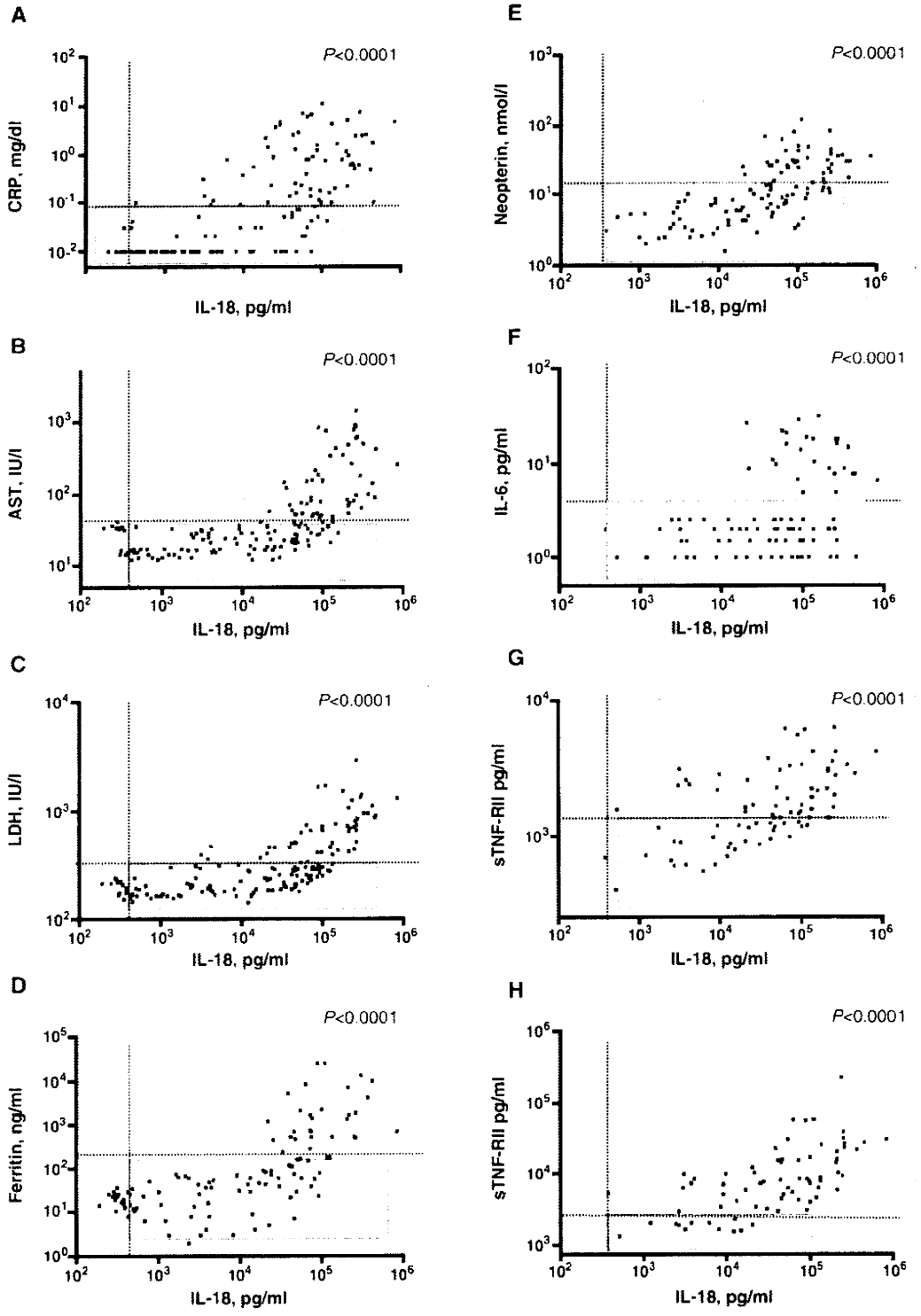
FIG. 5 Positive correlations between IL-18 and other measures of the disease activity. Serum IL-18 concentrations were compared with other serum markers and cytokines, in five cases of s-JIA. (**A**) CRP, (**B**) AST, (**C**) LDH, (**D**) ferritin, (**E**) neopterin, (**F**) IL-6, (**G**) sTNF-RI and (**H**) sTNF-RII. Red boxes indicate the areas where IL-18 concentrations are increased whereas other measures remain within normal limits.



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Clinical and Host Genetic Characteristics of Mendelian Susceptibility to Mycobacterial Diseases in Japan

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Abstract
Purpose The aim of this study is to investigate clinical characteristics and genetic backgrounds of Mendelian susceptibility to mycobacterial diseases (MSMD) in Japan.
Methods Forty-six patients diagnosed as having MSMD were enrolled in this study. All patients were analyzed for the *IFNGR1*, *IFNGR2*, *IL12B*, *IL12RB1*, *STAT1*, and *NEMO* gene mutations known to be associated with MSMD.
Results Six patients and one patient were diagnosed as having partial interferon-γ receptor 1 deficiency and nuclear factor-κB-essential modulator deficiency, respectively. Six of the seven patients had recurrent disseminated mycobacterial infections, while 93% of the patients without these mutations had only one episode of infection.
Conclusions The patients with a genetic mutation were more susceptible to developing recurrent disseminated mycobacterial infections. Recurrent disseminated mycobacterial infections occurred in a small number of patients even without these mutations, suggesting the presence of as yet undetermined genetic factors underlying the development and progression of this disease.

Keywords Disseminated mycobacterial infection · IFN-γR1 deficiency · NEMO deficiency · flow cytometric analysis

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Introduction

Although the outcome of mycobacterial infection is influenced by many factors, including the virulence of the pathogen and the environment of the host, it has been demonstrated that host genetic factors play important roles in the defense against mycobacteria [1]. Mendelian susceptibility to mycobacterial diseases (MSMD, MIM 209950) is a rare primary immunodeficiency syndrome characterized by a predisposition to develop infections caused by weakly virulent mycobacteria, such as *Mycobacterium bovis* bacille Calmette-Guerin (BCG) and environmental non-tuberculous mycobacteria (NTM) [2–4]. These patients are vulnerable to systemic salmonellosis and infections with *Mycobacterium tuberculosis*, the virulent mycobacterial species, to a lesser extent [5, 6]. Diseases caused by other intracellular pathogens, such as *Nocardia*, *Listeria*, *Paracoccidioides*, *Histoplasma*, and *Leishmania*, and some viruses, such as human herpes virus-8, have only rarely been reported, mostly in single patients [7–12].

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To date, interferon (IFN)-γ receptor 1 (*IFNGR1*) [13–15], IFN-γ receptor 2 (*IFNGR2*) [16], interleukin (IL)-12 p40 subunit (*IL12B*) [17], IL-12 receptor β subunit (*IL12RB1*) [18–20], signal transducer and activator of transcription-1 (*STAT1*) [21], and nuclear factor-κB-essential modulator (*NEMO*) [22] mutations were identified as the causes of this primary immunodeficiency. On the other hand, no genetic etiology has yet been reported to be identified for about half of all patients with MSMD [3]. In addition, there have been no precise reports on the clinical characteristics and genetic backgrounds of MSMD in Asian countries, including Japan, which has a high prevalence of tuberculosis.

In this study, we analyzed patients who had a recurrent or disseminated infection with intracellular pathogens to clarify the clinical manifestations and host genetic backgrounds of MSMD in Japan.

Materials and Methods

Subjects

We studied 46 patients (30 males and 16 females) diagnosed as having MSMD because of recurrent infections, or blood-borne infections such as osteomyelitis/arthritis, and multiple infections at different anatomic sites by intracellular bacteria including BCG, NTM, *Salmonella* species, *Listeria monocytogenes*, or *M. tuberculosis* in 34 hospitals in Japan from 1999 to 2009. There was no consanguinity in these families. The clinical information on each patient was collected using a standardized case report form. Informed consent was obtained from the parents of the subjects before the study. This study was approved by the Ethics Committee of Kyushu University.

Flow Cytometric Analysis

Two-color flow cytometric analysis was performed to investigate IFN-γ receptor 1 (IFN-γR1) expression levels on the patients' monocytes by using an EPICS XL instrument (Beckman Coulter, Miami, FL, USA). Peripheral blood mononuclear cells (PBMCs) were stained with mouse anti-IFN-γR1 monoclonal antibody (MAb) (Genzyme, Cambridge, MA, USA), followed by rat phycoerythrin anti-mouse immunoglobulin antibody (BD Bioscience Pharmingen, San Diego, CA, USA). Cells were washed twice and stained with a phycoerythrin 5.1 (PC5)-anti-CD14 MAb (Beckman Coulter). IFN-γR1 expression was analyzed on monocytes determined by their side scatter and CD14 positivity.

Genomic DNA and cDNA Sequence Analysis

The *IFNGR1*, *IFNGR2*, *IL12B*, *IL12RB1*, *STAT1*, and *NEMO* genes were analyzed for coding exons and flanking intronic sequences. These genes were amplified by polymerase chain reaction (PCR) after whole genome amplification with a GenomiPhi V2 DNA Amplification Kit (GE Healthcare, Little Chalfont, UK). The PCR products were treated with an Exo-SAP-IT kit (GE Healthcare, Amersham, UK) and then were analyzed by direct sequencing with an ABI 3130 DNA sequencer (Perkin-Elmer, Foster City, CA, USA). Detected mutations were confirmed by sequencing the PCR product using cDNA as a template.

Statistical Analysis

Comparisons of the proportions were analyzed by the χ^2 test. The Mann–Whitney *U* test was used to compare differences between quantitative variables. A *P* value less than 0.05 was considered to be statistically significant.

Results

The median age of the patients was 8 years (range, 6 months–41 years), and the median age at the onset of infection was 1 year and 4 months (range, 4 months–6 years). The male to female ratio was 1.9:1. Only one patient had not received a BCG vaccination. There were 59 episodes of disseminated mycobacterial infections in the 46 patients. Nine (19%) of 46 patients had two or more episodes of these infections. Two of the patients had three episodes, and one had four episodes of these infections. In all episodes, BCG was the most common pathogen (82.6%, Table I). The *Mycobacterium avium* complex (MAC) was isolated during eight episodes of these infections. *M. tuberculosis* was also confirmed in two episodes of infection. No severe *Salmonella* species, *L. monocytogenes*, or viral infections were observed.

The common clinical manifestations were osteomyelitis/arthritis, lymphadenitis, and subcutaneous abscess/dermatitis (Table I and Fig. 1a). Only one patient was diagnosed as having arthritis, and the lesion spread to the adjacent bone. Two patients showed hepatosplenomegaly during the BCG infection, and two patients with the MAC infection developed pulmonary abscess. Among the BCG infections, the median intervals of time between BCG vaccination and the development of primary BCG infection were 3 (1–10 months), 4 (2–36 months), and 11 months (5–46 months) for the subcutaneous abscess/dermatitis, lymphadenitis, and osteomyelitis/arthritis, respectively (Fig. 1b).

We performed the genetic analysis on these patients for the *IFNGR1*, *IFNGR2*, *IL12B*, *IL12RB1*, *STAT1*, and *NEMO* genes. Six patients (five families) and one patient had mutations in the *IFNGR1* and *NEMO* genes, respectively (Table II). Five of the seven patients who had a mutation in the *IFNGR1* gene were the patients that we

Table I The clinical manifestations of the patients with MSMD

	Patients with genetic mutation, *n* (%)	Patients without a genetic mutation, *n* (%)	Total *n* (%)
Causative pathogen[a]			
BCG	3 (42.9)	35 (89.7)	38 (82.6)
M. avium complex	1 (14.3)	3 (10.2)	4 (8.7)
BCG+*M. avium* complex	2 (28.5)	0 (0)	2 (4.3)
M. avium complex+*M. tuberculosis*	1 (14.3)	1 (2.6)	2 (4.3)
Sites of infection[b]			
Osteomyelitis/arthritis	7 (43.8)	24 (55.8)	31 (52.5)
Lymphadenitis	8 (50.0)	8 (18.6)	16 (27.1)
Dermatitis/subcutaneous	3 (18.8)	11 (25.6)	14 (23.7)
Pulmonary abscess	0 (0)	2 (4.7)	2 (3.4)

The total number exceeds 59 because some patients had multiple lesions at the same time

[a] $n=7$ for patients with a genetic mutation and $n=39$ for patients without a genetic mutation

[b] $n=16$ for patients with a genetic mutation and $n=43$ for patients without a genetic mutation

reported previously [14, 15], and the other two patients were newly identified. All of the IFN-γR1-deficient patients were heterozygotes, and the mutation was in the transmembrane domain in one patient (774del4: patient 5) and in the intracellular domain in five patients (811del4: patient 1, 818del4: patients 2–4, and 832 G>T, E278X: patient 6), which led to the expression of a truncated protein with a dominant negative effect on the IFN-γR1 signaling (Table II and Fig. 2a). The IFN-γR1 expression levels were significantly increased in all six patients with IFN-γR1 deficiency (Fig. 2b). Patient 7 had a missense mutation in *NEMO* (943 G>C, E315Q). The CD14-positive cells from this patient produced a lower level of TNF in response to LPS stimulation (data not shown), which was consistent with the defect in NF-κB signaling.

The proportions of the patients with recurrent mycobacterial infection or multiple osteomyelitis/arthritis were

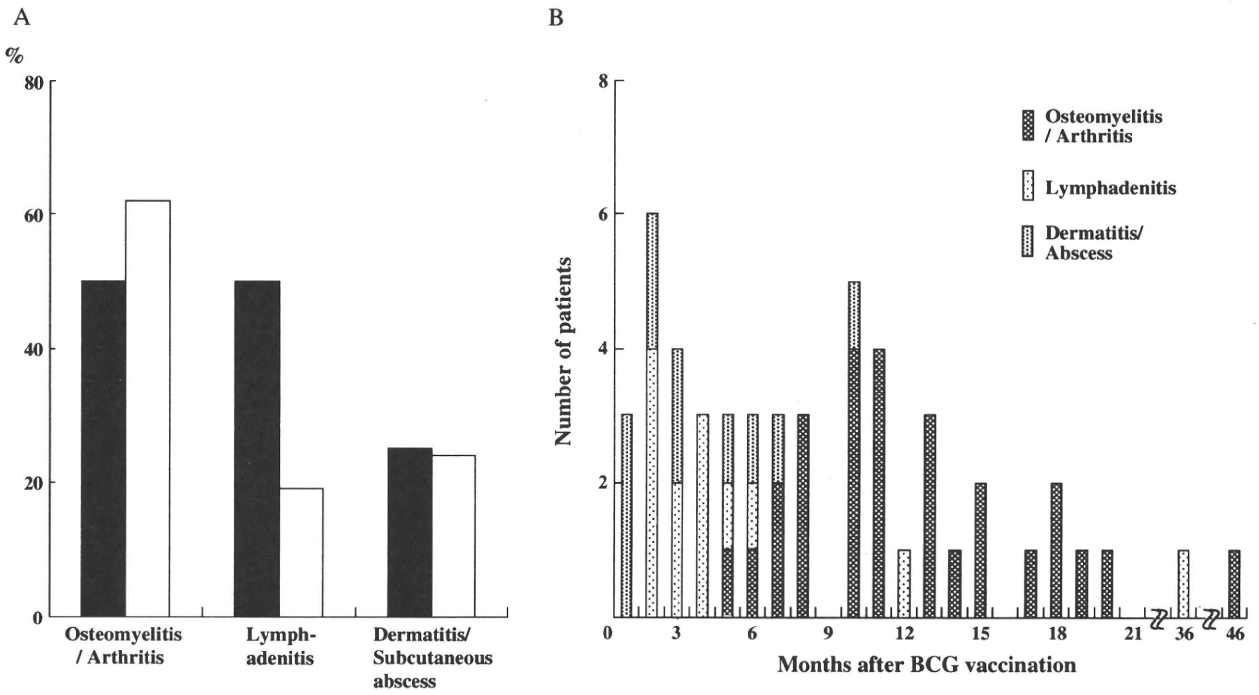

Fig. 1 The clinical features of the patients with BCG infection. The distribution of the sites of infections (**a**) and the intervals between BCG vaccination and the first onset of BCG infection (**b**) are shown. The *black bar* and the *white bar* represent the proportion of the patients with and without genetic mutations, respectively

Table II Characteristics of the patients with a genetic mutation

Patient no.	Sex	Age	Age of onset	Episodes of infections prior to detection of the genetic mutation	Genetic mutation
1[a] [14]	F	1 year 7 months	10 months	BCG lymphadenitis and dermatitis Multiple BCG osteomyelitis	*IFNGR1* 811del4
2[a] [14]	M	1 year 9 months	8 months	BCG lymphadenitis, hepatomegaly Multiple BCG osteomyelitis	*IFNGR1* 818del4
3[a] [14]	M	2 years	2 years	Multiple BCG osteomyelitis	*IFNGR1* 818del4
4[a] [14]	M	41 years	3 years	*M. tuberculosis* lymphadenitis (twice) Multiple MAC octeomyelitis	*IFNGR1* 818del4
5[a] [15]	F	12 years	6 months	BCG lymphadenitis Multiple MAN osteomyelitis	*IFNGR1* 774del4
6	M	19 years	4 months	BCG lymphadenitis and dermatitis Multiple BCG osteomyelitis MAC subcutaneous abscess Multiple MAC osteomyelitis	*IFNGR1* E278X
7	M	10 years	10 months	*M. tuberculosis* lymphadenitis Multiple MAC lymphadenitis Sepsis, bacterial pneumonia (four times)	*NEMO* E315Q

Patient 4 is the father of patient 2

MAC Mycobacterium avium complex

[a] These patients were reported previously

significantly higher in those with the genetic mutations (Table III). There were no significant differences in the age at the onset of mycobacterial infection, or in the interval of time between BCG vaccination and the first onset of BCG infection between the patients with and without genetic mutations. One patient diagnosed with BCG dermatitis died of persistent diarrhea of unknown etiology, while the others are still alive.

Discussion

In the present study, we investigated the clinical characteristics and the genetic backgrounds of the patients diagnosed as having MSMD in Japan. We observed that the patients with the genetic mutation were susceptible to developing recurrent mycobacterial infections and multiple osteomyelitis/arthritis, and IFN-γR1 deficiency was the most

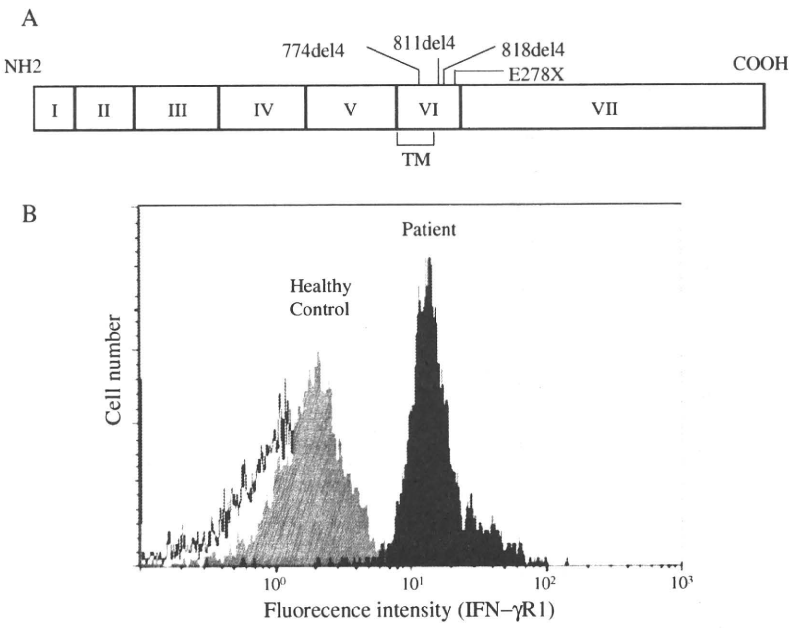

Fig. 2 *IFNGR1* gene mutations and the analysis of IFN-γR1 expression on monocytes. The sites of *IFNGR1* gene mutations in the six IFN-γR1-deficient patients (**a**) and the increased IFN-γR1 expression level on monocytes in patient 2 are shown (**b**)

Table III Comparison of the patients with and without a genetic mutation

	Patients with a genetic mutation (n=7)	Patients without a genetic mutation (n=39)
Age of onset (months)	10 (4–36)	14 (4–75)
Male to female ratio	2.5:1	1.8:1
Familial history (n)	2	0
Median interval between BCG vaccination and the first onset of BCG infection (months)	9.5 (7–15, n=4)	10 (1–46, n=35)
Recurrent cases (%)	85.7*	7.7
Patients with multiple osteomyelitis/arthritis (%)	100* (n=6)	4.2 (n=24)

*p<0.0001

frequent genetic defect identified in these patients. The prevalence of MSMD is estimated to be at least 0.59 cases per million births, and the disease does not seem to be confined to any ethnic group or geographic region, according to a national retrospective study of idiopathic disseminated BCG infection in France [23, 24]. This is the first epidemiological study associated with MSMD in Japan which showed the difference in the clinical manifestation and the genetic background between Japan and Western countries.

The *IFNGR1* mutations identified in this study were in exon IV, within the transmembrane domain, or the intracellular domain of the *IFNGR1* gene (Fig. 2a), which led to a truncated protein lacking signaling motifs [25]. The truncated protein also lacks the recycling motif, which leads to the overexpression of the mutant protein (Fig. 2b) [25]. These mutations are located in important hot spots in the patients diagnosed with dominant partial IFN-γR1 deficiency [13], and the flow cytometric analysis of IFN-γR1 expression levels may be a useful method for the screening for this disease [15]. The *NEMO* mutation found in patient 7 was in exon VIII within the leucine zipper domain of the *NEMO* gene. A previous study reported that a mutation in this region disrupted a common salt bridge in the leucine zipper domain and impaired T-cell-dependent IL-12 production [22].

The patients with the genetic mutations were susceptible to recurrent mycobacterial infections and multiple osteomyelitis/arthritis as described previously [3], but no fatal mycobacterial infection was observed in this study. Unlike complete IFN-γR1 and IFN-γR2 deficiencies, which often cause fatal mycobacterial infections [13, 16], the patients with dominant partial IFN-γR1 and NEMO deficiencies have been reported to have a relatively mild disease and a better prognosis [13, 22]. These factors might have contributed to the good outcome of the patients in this study. In addition, the low virulence of BCG might contribute to the characteristics of BCG infection in Japan, because the BCG Tokyo 172 strain that is used in Japan for vaccination is the least virulent BCG substrain.

The *IL12RB1* mutation has been reported to be the most common cause of MSMD [4]. However, none of the patients in this study was diagnosed as having an IL-12 receptor β1 deficiency. In Japan, this disease was reported in only one patient with disseminated lymphadenitis caused by *M. avium* complex [18]. It has been suggested that most complete IL-12 receptor β1-deficient individuals may be asymptomatic, and only those that also have a second mutation in another gene may be more prone to infections [26, 27]. These symptomatic IL-12 receptor β1-deficient patients are mainly found in families with consanguineous parents [19, 27]. Consanguineous marriages are uncommon in Japan, and there were no consanguineous families in this study. This might be the reason why no IL-12 receptor β1-deficient patients were observed. Alternatively, it is possible that the causative gene mutations associated with MSMD are different among races, because the number of patients with IL-12 receptor β1 deficiency was also lower than those with IFN-γR1 deficiency in Taiwan [28].

Although another patient had multiple osteomyelitis, and three patients had recurrent disseminated mycobacterial infections in these studies, they did not have mutations in any of the six genes. It was previously reported that no genetic etiology has yet been identified in about half of patients with disseminated and recurrent mycobacterial infections [3, 4]. This suggests the presence of as yet undetermined genetic factors in the development of this disease.

In the present study, the number of patients with genetic mutations might be too small to conclusively indicate the differences in the clinical manifestations and the host genetic backgrounds of MSMD between Japan and Western countries. However, in terms of the genetic etiology and the prognosis, it remains possible that the features of the patients diagnosed as having MSMD in the present study are different from those in previous reports [3]. Further investigations of a large number of patients are therefore warranted to more precisely evaluate the clinical manifestations and the host genetic background of MSMD in Japan.

Conclusions

We found that the patients diagnosed as having MSMD in Japan seem to have different genetic features, as well as

different clinical manifestations, compared with those in Western countries. A few patients with recurrent mycobacterial infections without mutations in the six known genes might suggest a contribution of other genetic, as well as environmental, factors in the susceptibility to recurrent infections.

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

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

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

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

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

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

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INTRODUCTION

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

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

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

PATIENTS AND METHODS

Subjects and Kindreds

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

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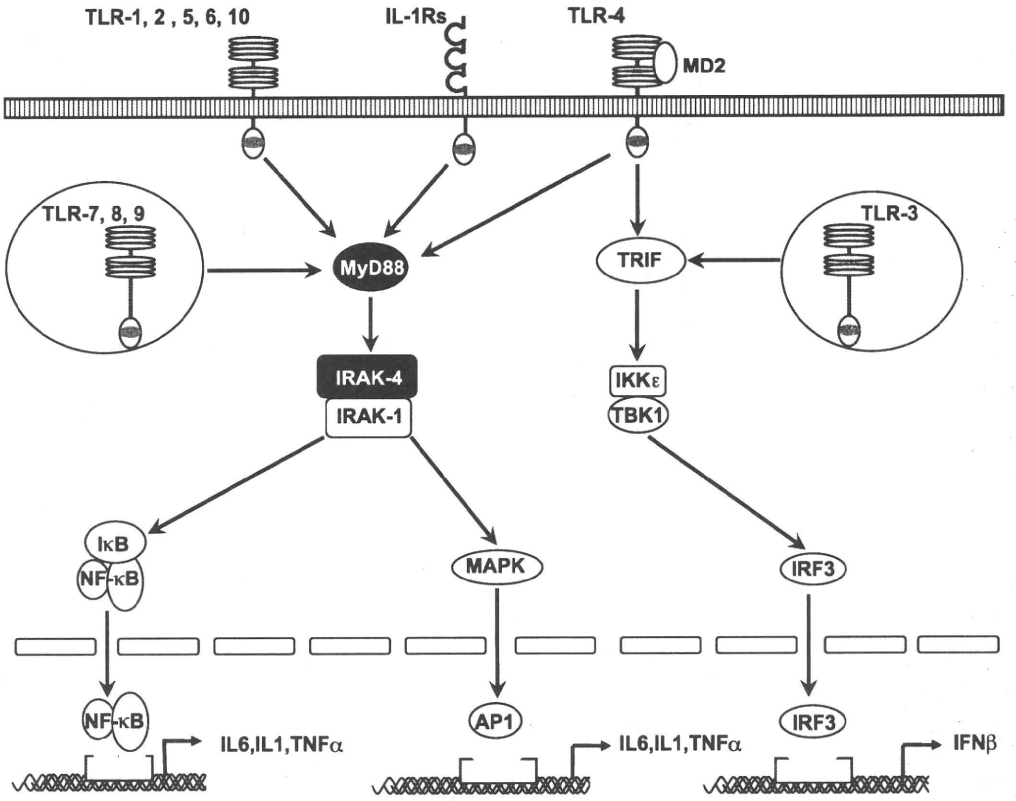

FIGURE 1. Schematic representation of TIRs signaling pathway. MyD88 interacts with TLRs and IL-1Rs through a shared TIR domain. MyD88 is a key cytosolic adapter molecule, providing a bridge from TLRs and IL-1Rs to the 2 active kinases IRAK-4 and IRAK-1. IRAK-4 and IRAK-1 then activate at least the 2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

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

Activation by TLR Agonists and Cytokine Determinations

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

Sequencing Analysis

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

Western Blotting

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

Immunologic Investigations

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

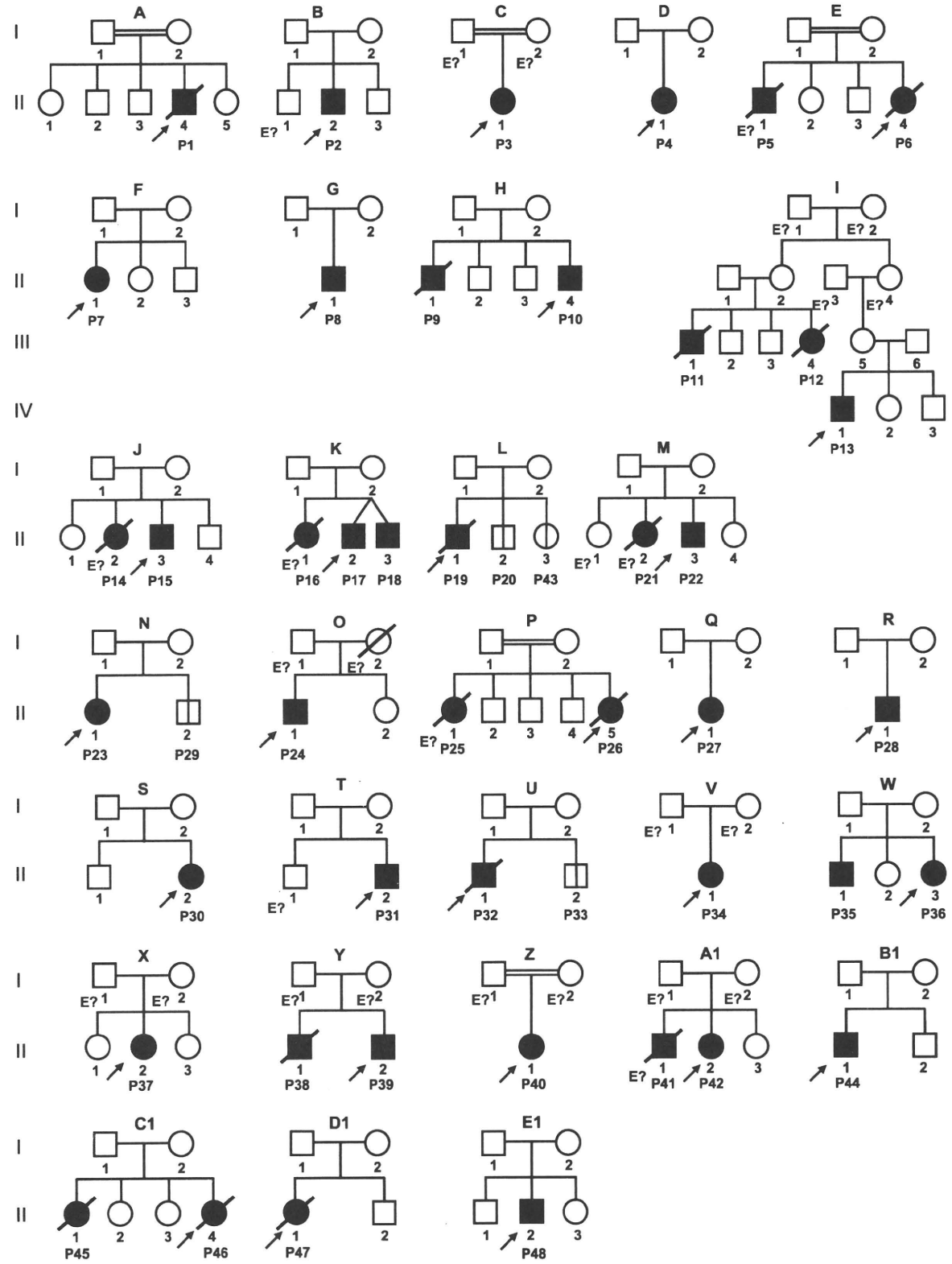

FIGURE 2. Pedigrees of the 31 kindreds identified with IRAK-4 deficiency. Each kindred with IRAK-4 deficiency is designated by a capital letter (A–E1) each generation is designated by a Roman numeral (I–IV), and each individual is designated by an Arabic numeral (from left to right). Patients with a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

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

Statistical Analysis

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

RESULTS

Description of Patients and Kindreds

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

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

IRAK4 and MYD88 Mutations

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

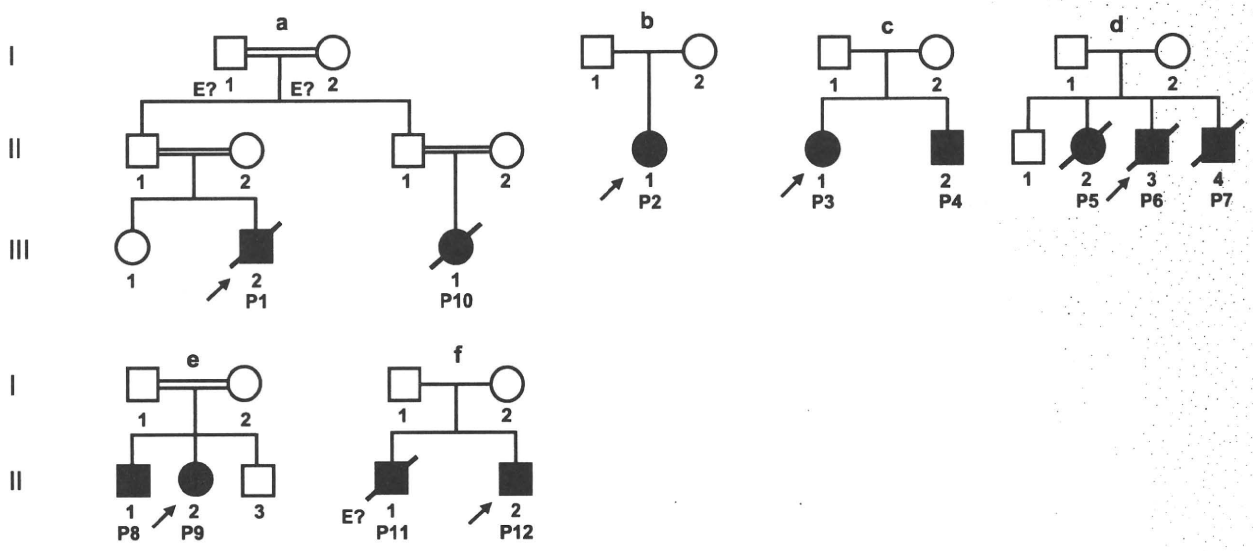

FIGURE 3. Pedigrees of the 6 kindreds with MyD88 deficiency identified. Each kindred with MyD88 deficiency is designated by a lower case letter (a–f); each generation is designated by a Roman numeral (I–IV), and each individual is designated by an Arabic numeral (from left to right). Patients with a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

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

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

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

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

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

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

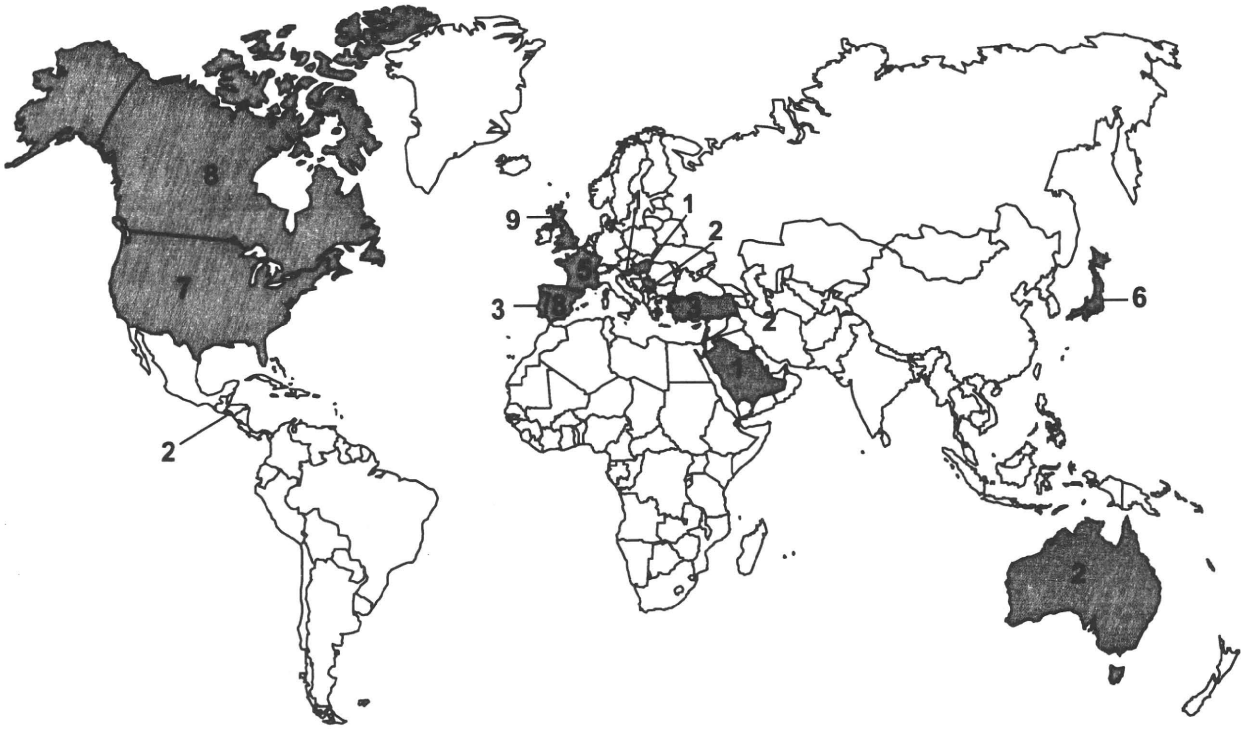

FIGURE 4. Countries of origin of the 31 kindreds with IRAK-4 deficiency and the 6 kindreds with MyD88 deficiency identified. The number of patients identified in each country is indicated.

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

Immunologic Investigations

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

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

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

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

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

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

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

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

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

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

Invasive Bacterial Infections

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

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

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

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

abscesses affected the brain (3 episodes), peritoneum (8 episodes), liver (4 episodes), and muscles (2 episodes: subfascial calf and psoas abscesses).

There were 33 reported episodes of InvBD in 12 MyD88-deficient patients (n = 2.75 episodes per patient; range, 1–7), including meningitis (17 episodes, 51.5% of all invasive episodes), sepsis (4 episodes, 12.1%), arthritis (6 episodes, 18.2%), osteomyelitis (2 episodes, 6.1%), and deep inner organ/tissue abscesses (4 episodes, 12.1%).

Five IRAK-4-deficient patients never developed InvBD, 4 of whom were diagnosed at birth and remained asymptomatic on prophylactic treatment (Figure 2; Table 1). The remaining patient without InvBD was diagnosed at the age of 2 years following an episode of *Staph. aureus* adenitis (N-P23) and received prophylactic treatment from that time to the end of follow-up. All the MyD88-deficient patients reported have presented InvBD (Figure 5; Table 1). Neurologic complications secondary to meningitis and brain abscesses occurred in 7 IRAK-4-deficient patients; 5 of them developed secondary deafness (K-P17, Q-P27, R-P28, W-P35, X-P37), and 2 other patients developed hemiplegia (B-P2) or developmental delay (M-P22). Three MyD88-deficient patients (c-P3, c-P4, e-P9) developed secondary deafness. The overall frequency and the sites of InvBD were found to be indistinguishable in IRAK-4-deficient and MyD88-deficient patients.

Noninvasive Bacterial Infections

Noninvasive bacterial disease (NInvBD) most frequently presented as skin infections, such as recurrent localized cellulitis, furunculosis, and folliculitis, often prompting intravenous and prolonged antibiotic treatment (in 21 of 48 IRAK-4-deficient and 3 MyD88-deficient patients) (Figure 6). IRAK-4-deficient patients also presented with adenitis (14 patients), omphalitis (6 patients), maxillary sinusitis (6 patients), tonsillar abscesses (4 patients), necrotizing epiglotitis (1 patient), necrotizing pharyngitis (1 patient), necrotizing palate infection (1 patient), recurrent otitis media (12 patients), and orbital cellulitis or endophthalmitis (6 patients). MyD88-deficient patients developed adenitis (5 patients), sinusitis (2 patients, a-P1 and c-P3), recurrent otitis media (2 patients), gingivitis and periodontal disease (1 patient, c-P3). Intriguingly, only 21 episodes of pneumonia were reported, in only 9 IRAK4-deficient patients and 2 MyD88-deficient patients. There were no episodes of acute bronchitis and no chronic bronchopulmonary disease. Acute upper urinary tract infections were found in only 2 IRAK-4-deficient patients and 1 MyD88-deficient patient. Most NInvBD in MyD88-deficient and IRAK-4-deficient patients affected the skin and the upper respiratory tract—sites at which necrotizing infections are particularly common.

Documented Bacterial Infections

In both IRAK-4 and MyD88 deficiency, *Str. pneumoniae, Staph. aureus,* and *P. aeruginosa* were, by far, the most commonly isolated pathogens causing InvBD and NInvBD (Figure 7; Table 1). In IRAK-4-deficient patients, *Str. pneumoniae* accounted for 40.1% (67/167), *Staph. aureus* for 25.1% (42/167), and *P. aeruginosa* for 19.7% (33/167) of all documented bacterial infections (a total of 84.9%). *Str. pneumoniae* was involved in 54.3% (57/105) of InvBD episodes, whereas *Staph. aureus* and *P. aeruginosa* were found in 14.3% (15/105) and 18% (19/105) of such episodes, respectively, accounting together for 87% of all cases of InvBD. The other bacteria causing invasive disease were *Streptococcus* species, *Shigella sonnei, Neisseria meningitidis, H. influenzae* type b, and *Clostridium septicum* (Table 1). In cases of NInvBD, the principal bacterium isolated was *Staph. aureus*, which was implicated in 43.5% (27/62) of documented episodes of NInvBD, whereas *P. aeruginosa* and *Str. pneumoniae* were found in 22.6% (14/62) and 16.1% (10/62), respectively. These 3 bacteria altogether accounting for 82% of all episodes of NInvBD.

In patients with MyD88 deficiency, *Str. pneumoniae* accounted for 37.5% (18/48), *Staph. aureus* for 31.2% (15/48), and *P. aeruginosa* for 12.5% (6/48) of all bacterial infections (81%) (Figure 7). *Str. pneumoniae* caused InvBD in 45.5% of cases (15/33), whereas *Staph. aureus* and *P. aeruginosa* were involved in 21.2% (7/33) and 12.1% (4/33) of the episodes, respectively (78.8% of all cases of InvBD). The other pathogens identified during invasive infections were β-hemolytic *Streptococci, Salmonella enteritidis, H. influenzae* type e, and

P36	P13	P37	P1	P15	P28	P34	P8	P3	Normal Values	P31	P24	P2	P17	P18	P7	Normal Values
3 yr	4 yr	4 yr	5 yr	5 yr	5 yr	5 yr	6 yr	7 yr	(3–7 yr)	11 yr	13 yr	14 yr	27 yr	27 yr	32 yr	(11 yr-adult)
11.2	**13.6**	9.29	11.7	10.3	**18.99**	11.1	**13.6**		(5.49–11.54)	12.8	7.6	**14.4**	**15**	**13**	**16.7**	(6.55–12.78)
8.82	10.2		6.85	6.88	8.44		8.41		(>4)	10.6	7.16	8.62				(>4)
0.65	2.63		0.4	1.18	5.37		3.12		(>0.40)	2.5	0.95	0.71				(>0.60)
0.36	0.13		0.19	0.45	0.29		0.34		(>0.16)	0.72	0.38	0.47				(>0.17)
0.29	**1.85**		**1.41**	0.5	**4.89**		**3.54**		(<1)	0.65	0.06	**1.46**				(<1)
1.19	0.64	0.57	0.63	1.31	1.51	0.6	2.42		(0.41–1.57)	1.02	**0.49**	0.88	**0.3**	**0.6**	1.1	(0.70–3.44)
1.59	**1.65**	0.85	0.72	0.50	0.96	**3.5**	0.52		(0.54–1.55)	1.38	1.91	0.64	1.3	1.5	1.9	(0.50–2.09)
6	**977**	**106**	**17,400**	**356**			**187**		(<60)	**180**	36.6	11	**255**	96.5	**400**	(<150)
0.58	>10			0.06	0.26	0.47	0.59	1,81	(>0.1 IU/mL)		0.47	0.47	0.34	0.46	**0.06**	(>0.1 IU/mL)
				>40					(>40)			40				(>40)
0.55			2.04	**0.002**			0.10	0.88	(>0.1 IU/mL)		0.72				0,18	(>0.1 IU/mL)
	0.2		1.9	>0.6	**<0.3**	>0.3	**<0.3**		(>0.3 μg/mL)		3.59	**<0.3**	**<0.3**	**<0.3**	>0.3	(>0.3 μg/mL)
>1	5.1	0.84	>1		1.98	12.3	>1	>1	(>0.15 μg/mL)			>1			1.36	(>0.15 μg/mL)
	1/8	**1/1**	1/128	1/16			**1/2**		(>1/16)		1/32		1/16	1/16	**1/2**	(>1/16)

TABLE 5. Immunologic Investigation: Ig Levels and Humoral Responses to Recall Antigens and to Glycans in MyD88–Deficient Patients*

Patients	P5	P10	Normal Values	P2	P6	P9	Normal Values	P8	P4	Normal Values	P3	Normal Values
(age)	8 mo	15 mo	(6–15 mo)	2 yr	2.5 yr	3 yr	(2–4 yr)	7 yr	8 yr	(5–8 yr)	15 yr	(14 yr–adult)
Serum Ig (g/L)												
IgG	**2.23**	6.2	(2.35–6.23)	**12.4**	**13.6**	**29.2**	(4.82–8.96)	**12.5**	8.31	(5.49–11.54)	12.5	(6.55–12.78)
IgG1		5.0	NA	10.3	10.36	20.4	(>4)	8.0	6.61	(>4)	9.8	(>4)
IgG2		0.88	NA	0.148	2.52	5.19	(>0.3)	3.2	0.96	(>0.40)	0.85	(>0.6)
IgG3		0.78	NA	0.59	0.3	1.7	(>0.15)	0.47	0.24	(>0.16)	0.56	(>0.17)
IgG4		0.07	NA	0.44	0.4	**3.51**	(<1)	**3.7**	0.1	(<1)	0.84	(<1)
IgA	0.36	0.52	(0.12–0.62)	0.5	0.32	**1.6**	(0.33–1.22)	1.32	1.21	(0.41–1.57)	1.36	(0.70–3.44)
IgM	0.41	0.93	(0.34–1.1)	**1.59**	0.61	1.24	(0.50–1.53)	**0.29**	0.9	(0.54–1.55)	0.69	(0.50–2.09)
IgE (U/mL)	**53**		(<30)	24.5	**115**	34	(<40)		**199**	(<60)	10.4	(<150)
Specific antibodies												
Antitetanus			(>0.1 IU/mL)			0.9	(>0.1 IU/mL)		1.3	(>0.1 IU/mL)		(>0.1 IU/mL)
S. pneumoniae		0.9	(>0.3 µg/mL)	17.96		11.5	(>0.3 µg/mL)		2.74	(>0.3 µg/mL)	3.85	(>0.3 µg/mL)
H. influenzae b			(>0.15 µg/mL)			3.4	(>0.15 µg/mL)			(>0.15 µg/mL)		(>0.15 µg/mL)
Allohemagglutinin			NA	1/4			(>1/8)		1/16		1/16	(>1/16)

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

Moraxella catarrhalis. In cases of NInvBD, the principal bacterium isolated was *Staph. aureus*, which was implicated in 53.3% (8/15) of NInvBD episodes, whereas *Str. pneumoniae* was found in 20% (3/15) and *P. aeruginosa* in 13.3% (2/15) of NInvBD episodes. These 3 bacteria accounting altogether for 86% of all cases of NInvBD.

In summary, in both IRAK-4 and MyD88 deficiencies, *Str. pneumoniae*, *Staph. aureus*, and *P. aeruginosa* were by far the most commonly isolated pathogens causing InvBD (52.2%, 15.9%, and 16.7% of cases, respectively), and *Staph. aureus* was by far the most commonly isolated pathogen causing NInvBD (45.5%) (Figure 7).

Other Infections

Among infections caused by agents other than pyogenic bacteria, there were no severe mycobacterial, viral, parasitic, and fungal diseases. One IRAK-4-deficient patient (K-P18) had a *Mycobacterium avium* lung infection and otitis at the age of 15 years. Nine patients (8 IRAK-4-deficient patients and 1 MyD88-deficient patient) received Bacille Calmette-Guérin (BCG) vaccination without adverse effect. One IRAK-4-deficient patient (R-P28) had *Staph. aureus* meningitis at the age of 6 years, and *Enterovirus* was isolated from the cerebral spinal fluid by polymerase chain reaction. Another IRAK-4-deficient patient (C-P3) had an episode of diarrhea caused by *Enterovirus* at the age of 7 years. One MyD88-deficient patient (a-P1) experienced 2 hospital-acquired episodes of diarrhea caused by adenovirus and rotavirus, with both infections following a normal course during the first year of life. One MyD88-deficient patient (d-P5) had 3 episodes of respiratory syncytial virus bronchilitis at 2, 3, and 4 months of age, with a spontaneous favorable outcome. One IRAK-4-deficient patient (T-P31) developed localized warts at the age of 16 years. One MyD88-deficient patient (d-P6) developed chickenpox 10 days after varicella zoster virus vaccination. Several IRAK-4- and MyD88-deficient patients had humoral responses to viruses and *Toxoplasma gondii* without abnormal clinical manifestations (Table 7). Two IRAK-4-deficient patients (C-P3, W-P36) and 2 MyD88-deficient patients (c-P4, e-P9) had oral thrush, even in the absence of antibiotic treatment. Finally, *Curvularia* species were isolated from the maxillary sinus of 1 IRAK-4-deficient patient (C-P3) living in the southern United States.

In conclusion, it is noteworthy that IRAK-4-deficient and MyD88-deficient patients were not particularly susceptible to most other microorganisms, including common viruses (for example, herpes viruses, enteroviruses, adenoviruses, and papillomaviruses), and widespread bacteria (for example, *Listeria* and *Mycobacterium*), parasites (for example, *Toxoplasma*), and fungi (for example, *Cryptococcus, Pneumocystis, Candida,* and *Aspergillus*).

Patient Outcome

Most IRAK-4-deficient patients had their first bacterial infection early in life, before the age of 2 years in 87.5% (n = 42) of cases. The first InvBD occurred before the age of 2 years in 79.2% (n = 38), and the first NInvBD in 48% (n = 23) of these patients. The first bacterial infection occurred before the age of 6 months in 54% (n = 26) of IRAK-4-deficient patients. The first InvBD occurred before the age of 6 months in 35.4% (n = 17), and the first NInvBD in 37.5% (n = 18) of these patients. The first bacterial infection even occurred during the neonatal period in 31.2% (n = 15) of IRAK-4-deficient patients. The first InvBD occurred during the neonatal period in 14.5% (n = 7) and the first NInvBD in 27% (n = 13) of these patients (5 patients had both InvBD and NInvBD in the neonatal period) (Figures 8 and 9).