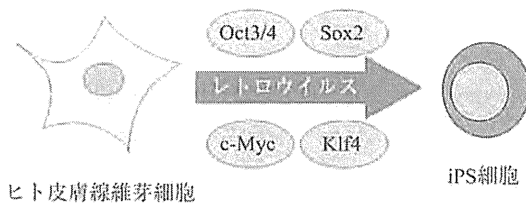

図4　ヒトiPS細胞の作製成功（文献9）より引用）

用いる臨床研究は実施しないこととするとされている．現在，ヒトES細胞を含む新たな指針改正に向けた議論が始まっている．

3．人工多能性幹細胞（iPS細胞）

2006年，京都大学の山中教授らはマウス体細胞にたった四つの転写因子（*OCT3/4*, *Sox2*, *c-Myc*, *Klf4*）遺伝子をレトロウイルスベクターで導入することにより，旺盛な自己複製能と多分化能をもった人工多能性幹細胞（iPS細胞）の樹立に成功し[7]，世界中に大きな衝撃を与えた．

iPS細胞はES細胞特異的なマーカーを発現するようになり，免疫不全マウスに移植すると三胚葉系を含む奇形腫を形成することができ，このiPS細胞は受精卵の胚盤胞に戻すと，生殖細胞を含む全身の細胞に分化し，次の世代では全身がiPS細胞に由来するマウスも正常に誕生したことから，iPS細胞のもつ多能性はES細胞と比べても遜色がないことが示された．iPS細胞を分化に適した条件で培養すると，ES細胞同様，心筋，神経細胞，グリア細胞，各種血液細胞，骨格筋細胞，血管内皮細胞などさまざまな細胞への分化がみられた．筋ジストロフィーへの再生医療の開発を目的に，正常マウスiPS細胞から分化してきた骨格筋を分離し，その中に含まれる骨格筋幹細胞（サテライト細胞）をFACSで分取し，筋ジストロフィーのモデルマウスであるジストロフィンを欠損したmdxマウスに移植すると，多数の正常骨格筋の再生がみられた[8]．

マウスiPS細胞が報告された翌年，山中らはヒト皮膚線維芽細胞にマウスと同様の四つの遺伝子を導入することによりヒトiPS細胞の樹立に成功した（図4）[9]．

ヒトiPS細胞は，受精卵を滅失することなく作成できることから，ES細胞のもつ社会的，倫理的な多くの問題を回避することができる．iPS細胞はES細胞と同様に未分化な状態のままほぼ無限に増やせること，培養条件を変化させると，神経細胞，心筋，骨格筋，血管内皮細胞，軟骨や骨の細胞，膵島細胞，肝細胞，各種血液細胞などさまざまな細胞に*in vitro*で分化可能であることから，幅広い再生医療への応用が期待されている（図3）．現在，臨床応用可能な，より安全性の高いiPS細胞を樹立するためさまざまな検討が行われている．

1）iPS細胞樹立の材料

当初，皮膚線維芽細胞から樹立されていたiPS細胞はその後，骨髄細胞，臍帯血，末梢血，毛根細胞，歯髄細胞，脂肪組織など，さまざまな組織から樹立可能となっている．どの組織を材料にして作製したiPS細胞がもっとも優れているか，さまざまな角度から検討が行われている．

2）最適な誘導法は何か

当初*OCT3/4*, *Sox2*, *Klf4*, *c-Myc*の四つの転写因子遺伝子を導入してiPS細胞の樹立が行われていたが，がん遺伝子でもある*c-Myc*の代りに*L-Myc*が用いられるようになった[10]．遺伝子導入にはレトロウイルスベクターが用いられていたが，染色体に組み込まれないエピゾーマルプラスミドベクターを中心に検討が進んでいる[11]．

3）最適な評価法

どのようなiPS細胞をもっともよい標準的なiPS細胞とするかについても世界的な検討が始まり，目的の細胞にきちんと分化できるか，分化抵抗性をもった細胞が残存しないか，導入遺伝子はしっかりサイレンシングされているか，などの問題が検討されている．

2010年11月1日に改正された「ヒト幹細胞を用いる臨床研究に関する指針」では，ヒトiPS細胞を用いた再生医療も指針の中に含まれている．

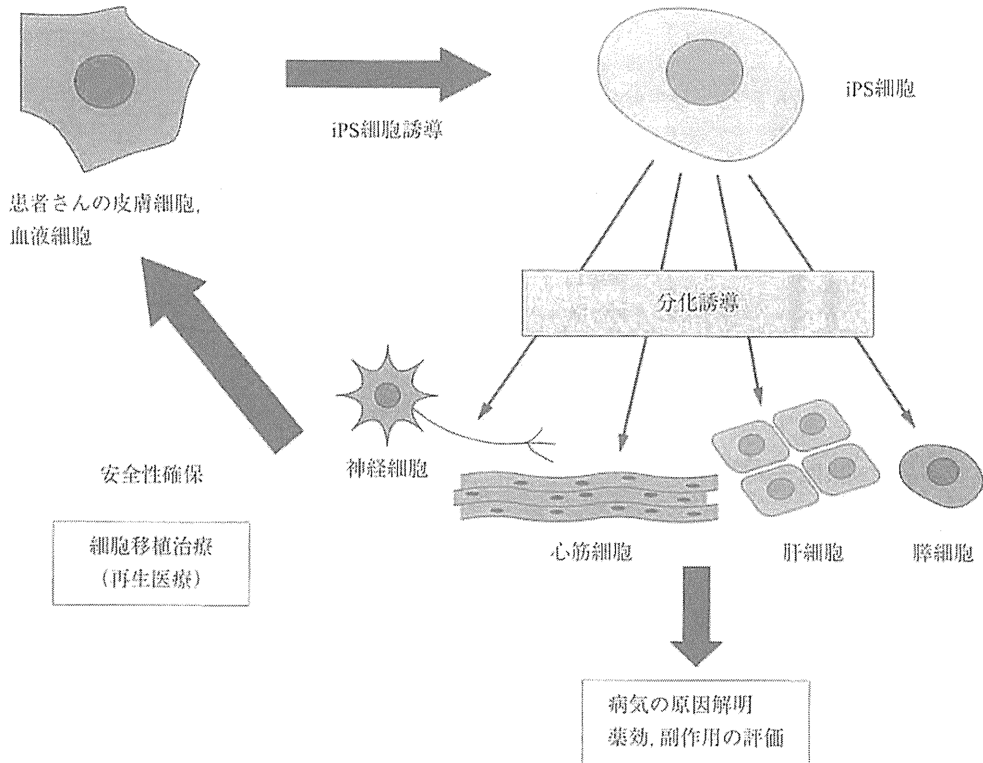

図5　疾患特異的iPS細胞の医療応用への可能性

表　再生医療の対象となる疾患

血液，腫瘍疾患	白血病，再生不良性貧血，小児がんなどに対する造血幹細胞移植，間葉系幹細胞（MSC）の移植への応用，人工血液
神経疾患	Perkinson病，Alzheimer，脳性小児麻痺，脳室周囲白質軟化症（PVL），神経変性疾患，脊髄損傷，末梢神経障害，など
循環器疾患	心筋梗塞（川崎病），拡張型心筋症，慢性閉塞性動脈硬化症（ASO），Buerger病，など
筋疾患	各種筋ジストロフィー
肝疾患	劇症肝炎，肝硬変，など
内分泌疾患	1型糖尿病，など
骨疾患	骨系統疾患，骨欠損，など
皮膚疾患	重症熱傷，など
その他	先天性難聴，網膜色素変性症，未熟児網膜症，など

MSC：mesenchymal stem cell，PVL：periventricular leukomalacia，ASO：arteriosclerosis obliterans

iPS細胞を用いた再生医療はそう遠くない将来わが国でも始まり，さまざまな疾患が対象となるであろう（表）．

おわりに

造血幹細胞や間葉系幹細胞などの体性幹細胞を用いた再生医療で始まったわが国においても，ヒトES細胞やiPS細胞を用いた再生医療が始まろうとしている．とくにiPS細胞は患者自身の皮膚や血液から樹立可能である．この疾患特異的iPS細胞は，疾患の病因，病態の解明や新規薬剤の開発に有用であるとともに，遺伝子治療と組み合せた再生医療への発展が期待されている（図5）．この分野の研究の発展を期待したい．

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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].

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

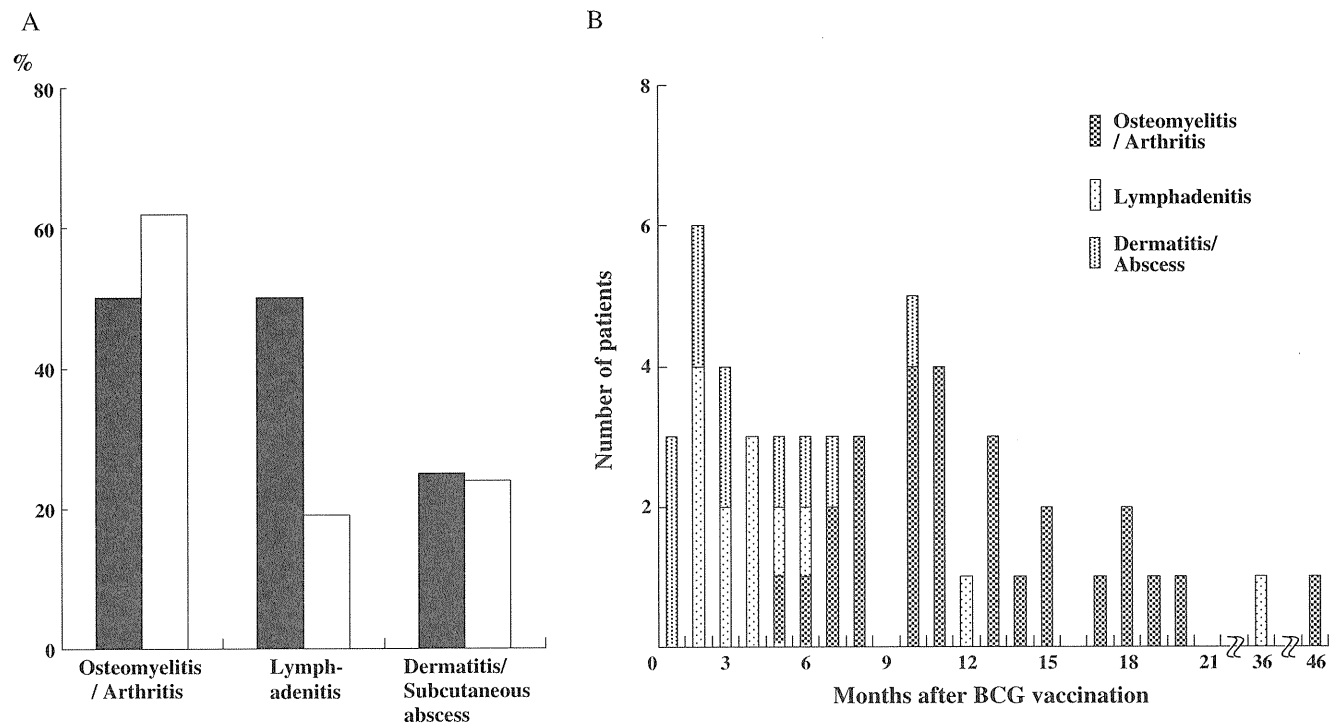

Fig. 1 The clinical features of the patients with BCG infection. The distribution of the sites of infections (**a**) and the intervals between BCG vaccination and the first onset of BCG infection (**b**) are shown. The *black bar* and the *white bar* represent the proportion of the patients with and 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

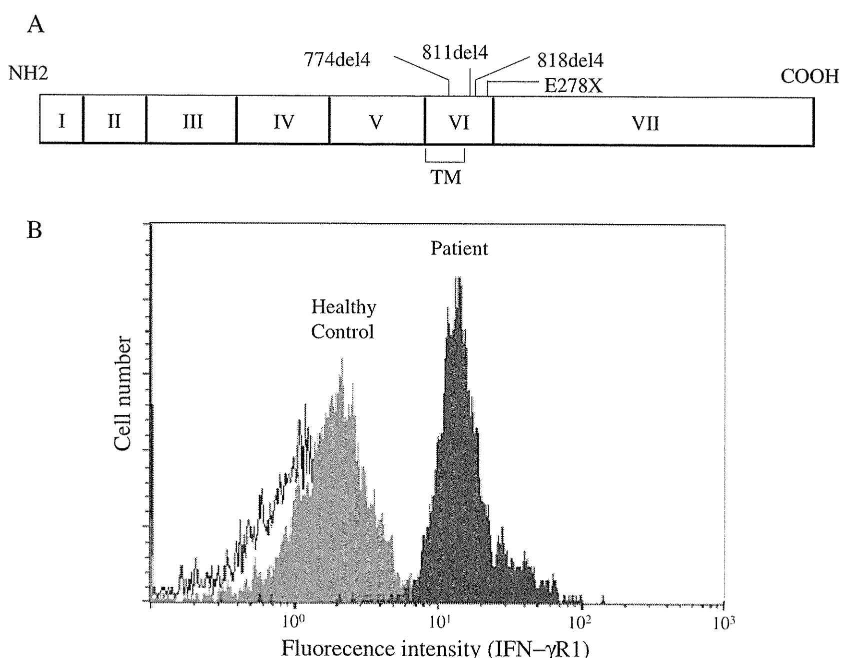

Fig. 2 *IFNGR1* gene mutations and the analysis of IFN-γR1 expression on monocytes. The sites of *IFNGR1* gene mutations in the six IFN-γR1-deficient patients (**a**) and the increased IFN-γR1 expression level on monocytes in patient 2 are shown (**b**)

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 25% and 25% of patients). 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 = 32), and/or IgG infusion

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(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.

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 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.).

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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 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 13 newly diagnosed patients (10 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 that 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 (see 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

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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]

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 (E52del and L93P), whereas the R196C mutant allele was associated with the quantitatively normal production of a nonfunctional protein.[49]

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 (see 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 (see Tables 2 and 3). There thus seemed to 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 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 (see 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 (see Figure 5; Table 1). Neurologic complications secondary to meningitis and brain abscesses occurred in 5 IRAK-4-deficient patients (K-P17, Q-P27, R-P28, W-P35, W-P36). Three MyD88-deficient patients (c-P3, c-P4, e-P9) developed secondary deafness, and 2 other IRAK-4-deficient patients developed hemiplegia (B-P2) or developmental delay (M-P22). 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

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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* (see 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%) (see 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 *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%) (see 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 de Calmette Guerin (BCG) vaccination without adverse effect. One IRAK-4-deficient patient (R-P28)

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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 zona 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 suffered 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).

Similarly, bacterial infections occurred early in most MyD88-deficient patients, before the age of 2 years in 91.7% (n = 11) of these patients. The first InvBD occurred before the age of 2 years in 50% (n = 6), and the first NInvBD in 66.7% (n = 8) of these patients. The first bacterial infection occurred before the age of 6 months in 91.7% (n = 11) of MyD88-deficient patients. The first InvBD occurred before the age of 6 months in 50% (n = 6), and the first NInvBD in 66.7% (n = 8) of the cases. The first bacterial infection occurred in the neonatal period in 33.3% (n = 4) of MyD88-deficient patients. The first InvBD occurred during the neonatal period in 16.7% (n = 2), and NInvBD in 16.7% (n = 2) of these patients (see Figures 8 and 9).

IRAK-4-deficient patients presented no InvBD from the age of 14 years on (a total of 10 patients, aged 14, 15, 17, 18, 19, 27, 30, and 35 years), but the oldest patient, who was aged 35 years, still suffered from occasional skin infections at last follow-up (see Figures 8 and 9). MyD88-deficient patients presented no InvBD from the age of 11 years on (2 patients aged 11 and 17 years), but the oldest patient, aged 17 years, still suffered from NInvBD at last follow-up. InvBD was recurrent (2–10 episodes) in 33 of the IRAK-4-deficient patients. In 3 IRAK-4-deficient patients, 2–3 recurrences of invasive pneumococcal disease due to the same serotype (6A, 14, or 19F) were identified at intervals of 1–24 months. One MyD88-

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deficient patient had 4 recurrences of InvBD (range for MyD88-deficient patients, 2–7). There were 114 reported episodes of InvBD in 46 IRAK-4-deficient patients (n = 2.38 episodes per patient; range, 0–10), and 33 reported episodes of InvBD in 12 MyD88-deficient patients (n = 2.75 episodes per patient; range, 1–7). Finally, 24 patients died of InvBD (18/46 IRAK-4, 6/12 MyD88), all before the age of 8 years, and most before the age of 2 years (n = 17) (Figure 10; Table 1). Sixteen of these patients died of invasive pneumococcal disease (11 IRAK-4-deficient and 5 MyD88-deficient patients).

Inflammatory Response

Impaired ability to mount inflammation during invasive infections has been previously described in isolated case reports and smaller series.[12,18,25,46] In the current study we evaluated temperature, C-reactive protein (CRP) levels, total leukocyte counts, and neutrophil counts in invasive infections during 3 periods of life that are known to have different levels of inflammatory responses: the neonatal period (day 1 to day 28), infancy (day 29 to 1 year), and childhood (children aged >1 year). In analyses carried out on admission to the hospital, we often observed inflammatory signs within the normal range, despite infection (Figures 11–13; Tables 8 and 9). Little (n = 3) or no (n = 2) increase in body temperature above 37°C was observed in neonates with IRAK4-deficiency. By contrast, a significant increase in CRP concentration (>10 mg/L) was observed in all neonates with IRAK-4 deficiency and InvBD. Counts of total leukocytes and of neutrophils remained low despite InvBD; none of the neonates showed neutrophil counts above the 95th percentile adjusted for age.[29] Initial temperature on admission was below 38°C in 10 of the 23 cases of InvBD in infants and in 22 of the 44 cases of InvBD in children admitted. Similarly, initial CRP concentration was below 10 mg/L in 12 of 23 cases of InvBD in infancy and in 16 of 36 cases of InvBD in childhood. Despite the presence of InvBD, total leukocyte counts remained below 14,000/μL in 21 of 35 episodes in infancy and in 46 of 52 episodes in childhood. One frequently documented abnormality was a neutrophil count below 6000/μL, observed in 20 of 26 episodes in infancy and 30 of 47 InvBD episodes in childhood.

Thus, both MyD88 and IRAK-4 deficiencies confer a predisposition to severe InvBD impairment of the ability to increase plasma CRP concentrations and mount fever. However, patients with IRAK-4 and MyD88 deficiency and InvBD may also present with high temperature and high levels of CRP, total leukocytes, and neutrophils (see Figures 11–13). Pus formation was observed in the liver, joints, lymph nodes, saliva glands, and in the meninges, as well as in skin infections. Finally, separation of the umbilical cord later than 28 days after birth was observed in 10 IRAK-4-deficient patients.

Prophylaxis of Infections

Thirty-six patients with IRAK-4 deficiency or MyD88 deficiency received prophylaxis following diagnosis of the corresponding primary immunodeficiency, a diagnosis that occurred after 1 episode of InvBD in 30 patients (24 IRAK-4-deficient and 6 MyD88-deficient) and before any InvBD episode in 6 IRAK-4-deficient patients. Prophylactic treatment was discontinued in 7 (6 IRAK-4-deficient and 1 MyD88-deficient) of the 11 patients who reached the age of 14 years, and was continued in all others.

Preventive treatment included antibiotic prophylaxis (oral penicillin and/or cotrimoxazole in most cases (Table 10) in 28 IRAK-4-deficient and 6 MyD88-deficient patients, and empirical intravenous or subcutaneous IgG injections (400 mg/kg every 3 wk) in 15 IRAK-4-deficient and 4 MyD88-deficient patients. Patients were also immunized with *Str. pneumoniae* conjugated vaccine only (7/48 IRAK-4-deficient patients, 3/12 MyD88-deficient patients), nonconjugated vaccine only (8/48 IRAK-4-deficient patients, 1/12

MyD88-deficient patients), or both (9/48 IRAK-4-deficient patients, 3/12 MyD88-deficient patients); *H. influenzae* conjugated vaccine (21/48 IRAK-4-deficient patients, 8/12 MyD88-deficient patients); and *N. meningitidis* conjugated or nonconjugated vaccine (12/48 IRAK-4-deficient patients, 7/12 MyD88-deficient patients).

We evaluated the impact of prophylaxis on the incidence of InvBD and their prognosis in all patients. Of all patients with documented bacterial infections, there was a total of 227 years and 152 years of follow-up without or with prophylaxis, respectively. At least 1 InvBD was observed in 35% of years without prophylaxis and in 16.4% of years on prophylactic treatment, and this difference was highly significant ($p = 10^{-5}$). We noted that no InvBD was documented in the 11 patients over the age of 14 years (10 IRAK-4-deficient patients and 1 MyD88-deficient patient), although only 4 of these patients continued to receive prophylactic treatment (antibiotics in 3 cases and antibiotics plus IgG infusions in the fourth case) (see Figure 8; Table 10). For the 7 patients aged >14 years without prophylactic treatment, there was a total cumulative follow-up time of 49 years without any InvBD.

DISCUSSION

We provide here the first detailed description, to our knowledge, of the clinical features and outcome of a large series of patients with IRAK-4 and MyD88 deficiencies, a novel group of primary immunodeficiencies characterized by a selective and profound defect of TLR and IL-1R signaling. Patients with these 2 deficiencies are highly susceptible to InvBD caused by *Str. pneumoniae* and *Staph. aureus*, and to NInvBD caused by *Staph. aureus* and *P. aeruginosa*. NInvBD is largely restricted to the skin (*Staph. aureus*) and the upper respiratory tract (*P. aeruginosa*). By contrast, several sites are affected during InvBD, with abscesses of inner organs, lymph nodes and saliva glands, meningitis, and septicemia frequently observed. Recurrent invasive pneumococcal disease is a hallmark of these 2 primary immunodeficiencies. Infections typically run an acute, as opposed to chronic course. However, they may be difficult to diagnose, due to weak inflammatory signs that appear late. No chronic pulmonary disease is observed in these patients, and both acute bronchitis and pneumonitis are rare. Gastrointestinal and urogenital infections are also rare.

Finally, the lack of viral, parasitic, and fungal disease in these patients is striking and cannot merely result from medical prophylaxis, as proposed elsewhere,[33] because the prophylaxis used targets mostly pyogenic bacteria, and patients with no prophylaxis do not present such infections. The nature and sites of infections in patients with IRAK-4 and MyD88 deficiencies seem to be well delineated: mostly invasive pneumococcal disease, cutaneous and invasive staphylococcal disease, and *Pseudomonas* infection of the upper respiratory tract or peritoneum. It is striking that the range of infectious agents is much narrower than predicted from the mouse model of experimental infection: MyD88-deficient and IRAK-4-deficient mice are susceptible to more than 40 infectious agents.[25,45] The sites of infection also provide us with unique information about the anatomical role of the TIR pathway in host defense.

The infectious phenotype of MyD88- or IRAK-4-deficient patients is related to but different from that observed in most patients with NEMO or IκBα deficiency, who generally display impairment of both TIR-signaling and other NF-κB-dependent immunologic pathways.[7] Indeed, up to 85 patients with hypomorphic mutations of NEMO and 5 patients with hypermorphic mutations of IKBA have been reported.[7,13,21,28,31] Some of these patients had developmental signs ranging from ectodermal dysplasia with osteopetrosis and lymphoedema to a complete absence of a developmental phenotype, whereas IRAK-4-deficient and MyD88-deficient patients have no signs of developmental impairment.[7] The spectrum of infectious diseases is broad in NEMO-deficient and IκBα-deficient patients, as

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