TABLE 6. Immunologic Investigation Summary

	IRAK-4-Deficient Patients	MyD88-Deficient Patients
T lymphocytes subset		
Normal pts/tested pts (%)	24/24 (100)	6/6 (100)
B lymphocytes subset		
Normal pts/tested pts (%)	23/23 (100)	7/7 (100)
NK lymphocytes subset		
Normal pts/tested pts (%)	19/19 (100)	6/6 (100)
T cell proliferation		
Normal pts/tested pts (%)	12/12 (100)	3/3 (100)
IgG levels		
Normal pts/tested pts (%)	15/28 (53.6)	3/8 (37.5)
Pts with increased level/tested pts (%)	12/28 (42.9)	4/8 (50)
Pts with decreased level/tested pts (%)	1/28 (3.6)	1/8 (12.5)
IgG1,2,3 levels		
Normal pts/tested pts (%)	13/13 (100)	7/7 (100)
IgG4 levels		
Normal pts/tested pts (%)	8/13 (61.5)	5/7 (71.4)
Pts with increased level/tested pts (%)	5/13 (38.5)	2/7 (28.6)
IgA levels		
Normal pts/tested pts (%)	25/28 (89.3)	7/8 (87.5)
Pts with decreased level/tested pts (%)	3/28 (10.7)	—
Pts with decreased level/tested pts (%)	—	1/8 (12.5)
IgM levels		
Normal pts/tested pts (%)	26/28 (92.9)	6/8 (75)
Pts with increased level/tested pts (%)	2/28 (7.1)	1/8 (12.5)
Pts with decreased level/tested pts (%)	—	1/8 (12.5)
IgE levels		
Normal pts/tested pts (%)	6/20 (30)	3/6 (50)
Pts with increased level/tested pts (%)	14/20 (70)	3/6 (50)
Specific Ab to protein antigens (tetanus, diphtheria, or polio)		
Normal pts/tested pts (%)	17/17 (100)	2/2 (100)
Ab against *H. influenzae*		
Normal pts/tested pts (%)	14/14 (100)	1/1 (100)
Ab against *S. pneumoniae*		
Normal pts/tested pts (%)	6/13 (46.2)	5/5 (100)
Pts with abnormal response/tested pts (%)	7/13 (53.8)	
Ab production after immunization with PNCV23		
Normal pts/tested pts (%)	4/9 (44.4)	
Pts with abnormal response/tested pts (%)	5/9 (55.6)	
Ab production after immunization with PNCV23+PCV7		
Normal pts/tested pts (%)	2/3 (66.7)	
Pts with abnormal response/tested pts (%)	1/3 (33.3)	
Ab production after immunization with PCV7		
Normal pts/tested pts (%)	1/1 (100)	
Allohemagglutinin		
Normal pts/tested pts (%)	7/10 (70)	3/3 (100)
Pts with decreased level/tested pts (%)	3/10 (30)	

Abbreviations: Ab = antibody, PCV7 = 7 valent conjugate vaccine, PNCV23 = 23 valent nonconjugate vaccine, pts = patients.

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 (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,

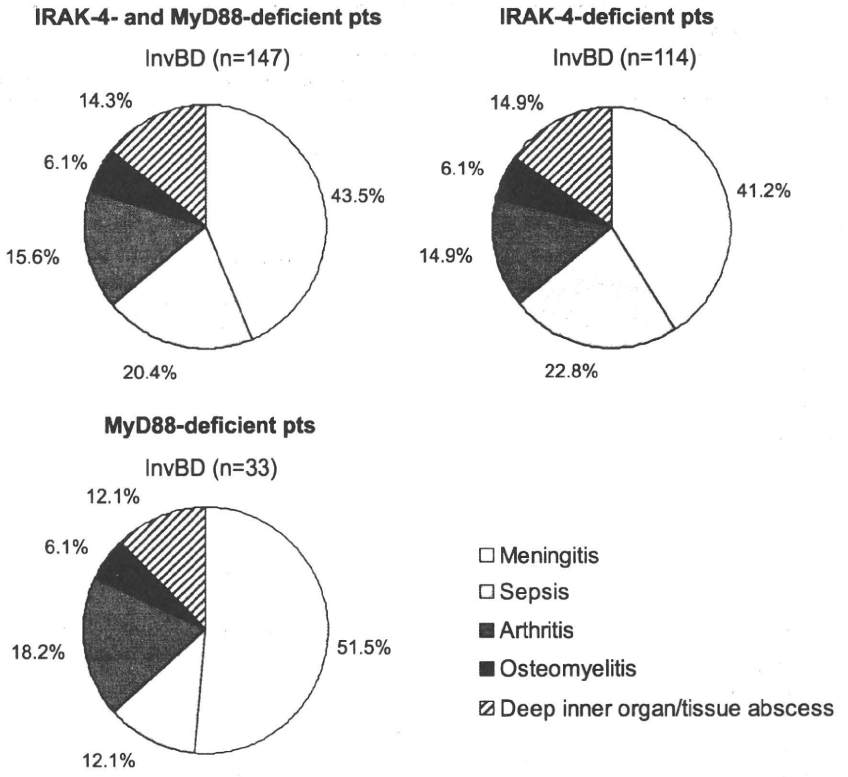

FIGURE 5. Invasive bacterial infections (episodes): in all patients, in IRAK-4-deficient patients, and in MyD88-deficient patients.

30, and 35 years), but the oldest patient, who was aged 35 years, still suffered from occasional skin infections at last follow-up (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. InvBD was recurrent (2–7 episodes) in 5 of the MyD88-deficient patients. There were 114 reported episodes of InvBD in 48 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 (18/48 IRAK-4, 6/12 MyD88), all before the age of 8 years, and most before the age

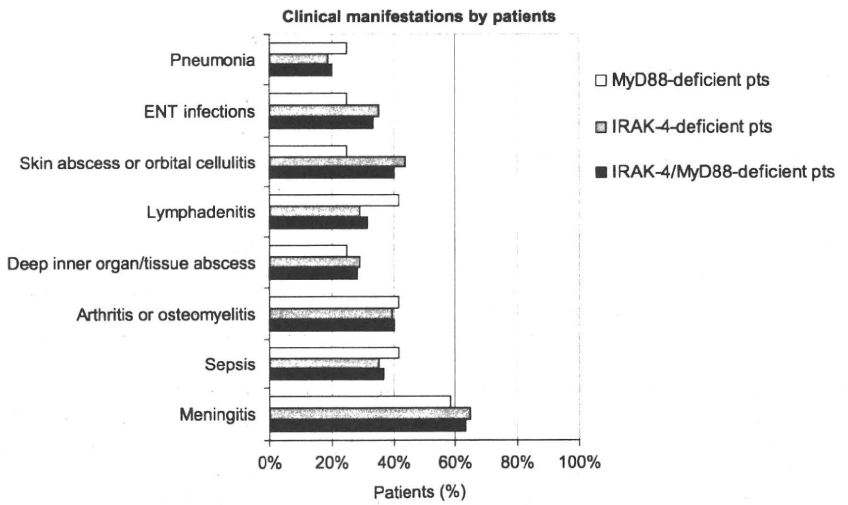

FIGURE 6. Percentage of clinical manifestations found in each patient: in MyD88-deficient patients, in IRAK-4-deficient patients, and in all patients. (ENT = ear, nose, and throat.)

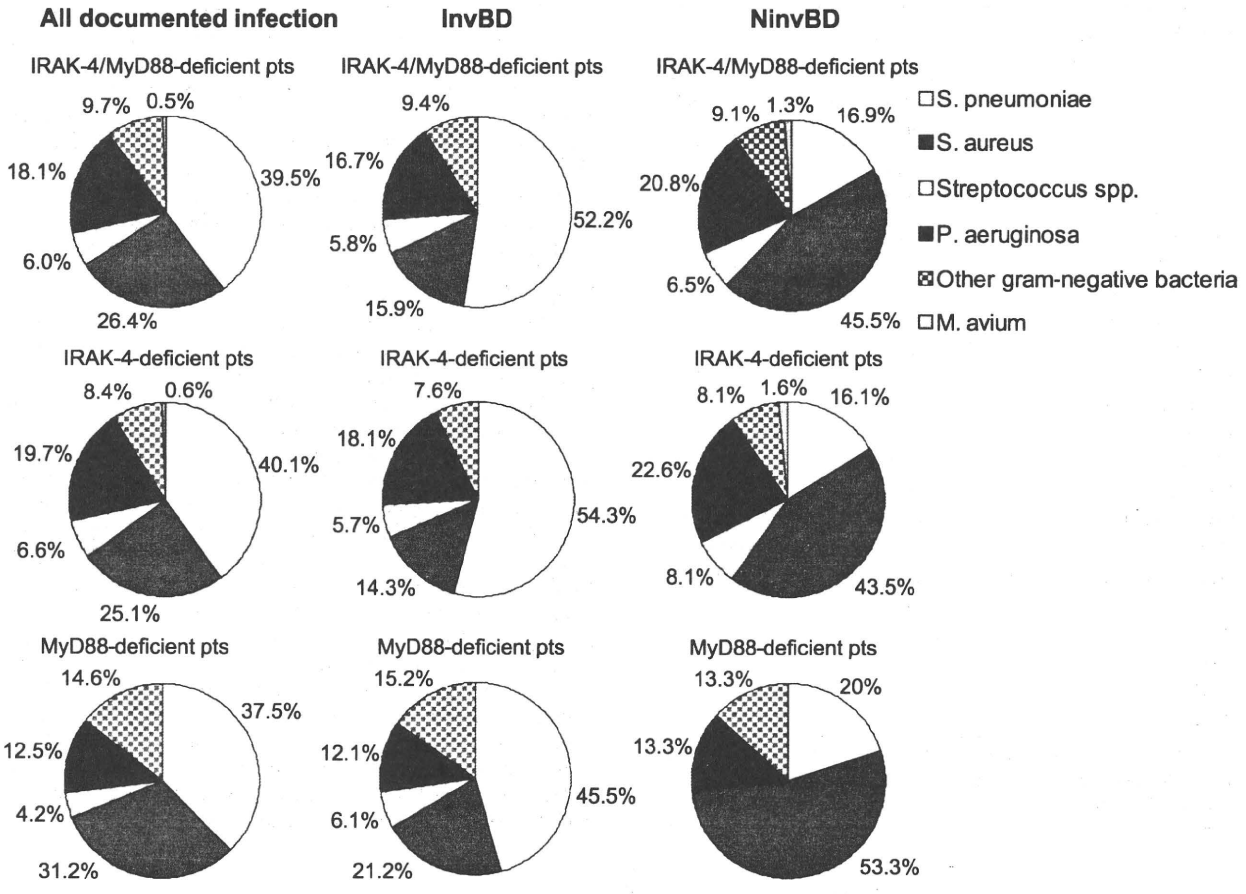

FIGURE 7. Overview of pathogens isolated during bacterial infections of IRAK-4-deficient and MyD88-deficient patients. **Left column,** overview of all pathogens isolated (all documented infection). In IRAK-4-deficient patients: other *Streptococcus* species (*Str. agalactiae, Str. equis, Str. intermedius, Str. milleri, Str. pyogenes,* and *Str. parasanguis*), other gram-negative bacteria (*Shigella sonnei, Neisseria meningitidis, Serratia marcesens, Moraxella catarrhalis, Clostridium septicum, Haemophilus influenzae* type b, *Citrobacter freundii,* and *Escherichia coli*), and *Mycobacterium avium*. In MyD88-deficient patients: other *Streptococcus* species (β-hemolytic *Streptococci*) and other gram-negative bacteria (*Salmonella enteritidis, Haemophilus influnzae* type e, *Moraxella catarrhalis, Klebsiella pneumoniae,* and *E. coli*). **Center column,** pathogens isolated during invasive bacterial infections (InvBD) (meningitis, sepsis, arthritis, osteomyelitis, and deep abscesses). In IRAK-4-deficient patients: other *Streptococcus* species (*Str. agalactiae, Str. milleri, Str. pyogenes,* and *Str. parasanguis*) and other gram-negative bacteria (*Shigella sonnei, N. meningitidis, Serratia marcesens, H. influenzae* type b and *C. septicum*). In MyD88-deficient patients: other *Streptococcus* species (β-hemolytic *Streptococci*) and other gram-negative bacteria (*Salmonella enteritidis, H. influenzae* type e, and *Moraxella catarrhalis*). **Right column,** pathogens isolated during noninvasive bacterial infections (NinvBD). In IRAK-4-deficient patients: other *Streptococcus* species (*Str. equis, Str. intermedius, Str. pyogenes*) and other gram-negative bacteria (*Serratia marcesens, Moraxella catarrhalis, C. septicum, Citrobacter freundii,* and *E. coli*), and *M. avium*. In MyD88-deficient patients: other *Streptococcus* species (β-hemolytic *Streptococci*) and other gram-negative bacteria (*K. pneumoniae* and *E. coli*).

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.

TABLE 7. Humoral Responses to Viruses and *Toxoplasma gondii*

	IRAK-4-Deficient Patients (Positive pts/Tested pts)	MyD88-Deficient Patients (Positive pts/Tested pts)
Herpes simplex virus	0/8	2/4
Varicella zoster virus	5/9	2/3
Cytomegalovirus	2/9	3/4
Epstein-Barr virus	4/8	3/5
HHV6	6/6	Not done
HHV8	0/2	Not done
Parvovirus B 19	2/6	Not done
Rubella	5/6	4/4
Measles	Not done	3/4
Mumps	5/6	2/3
Coxsackie virus B1,2,3,4,6	6/7	Not done
RSV	6/6	Not done
Human metapneumovirus	5/6	Not done
Rotavirus	Not done	1/1
Adenovirus	Not done	1/1
HIV	0/3	Not done
VDRL	0/1	Not done
Toxoplasma	0/3	1/3

Abbreviations: See previous tables. HHV = human herpes virus, HIV = human immunodeficiency virus, VDRL = Venereal Disease Research Laboratory test.

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 (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

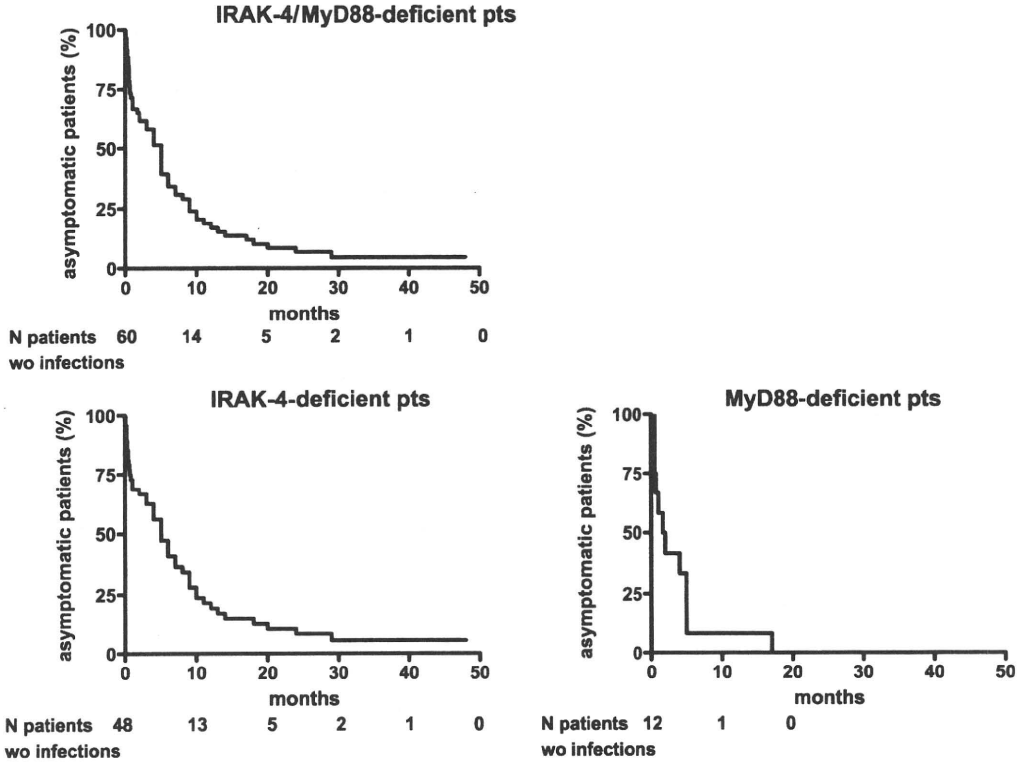

FIGURE 8. Epidemiologic features of IRAK-4 and MyD88 deficiency. Incidence of first bacterial infection in IRAK-4-deficient and MyD88-deficient patients during the first 50 months of life. (wo = without, pts = patients.)

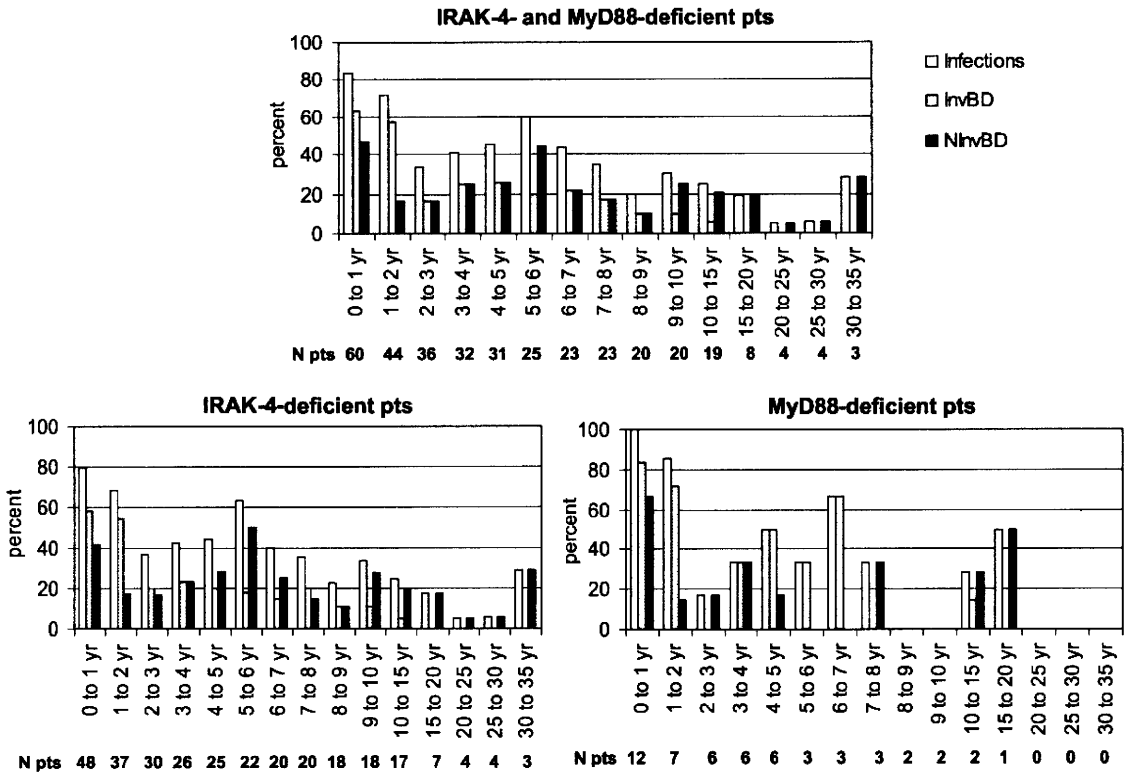

FIGURE 9. Annual rate of bacterial infections per patient, as a percentage. P = patients presenting at least 1 infection over the course of a year. Percent = P over the total number of patients.

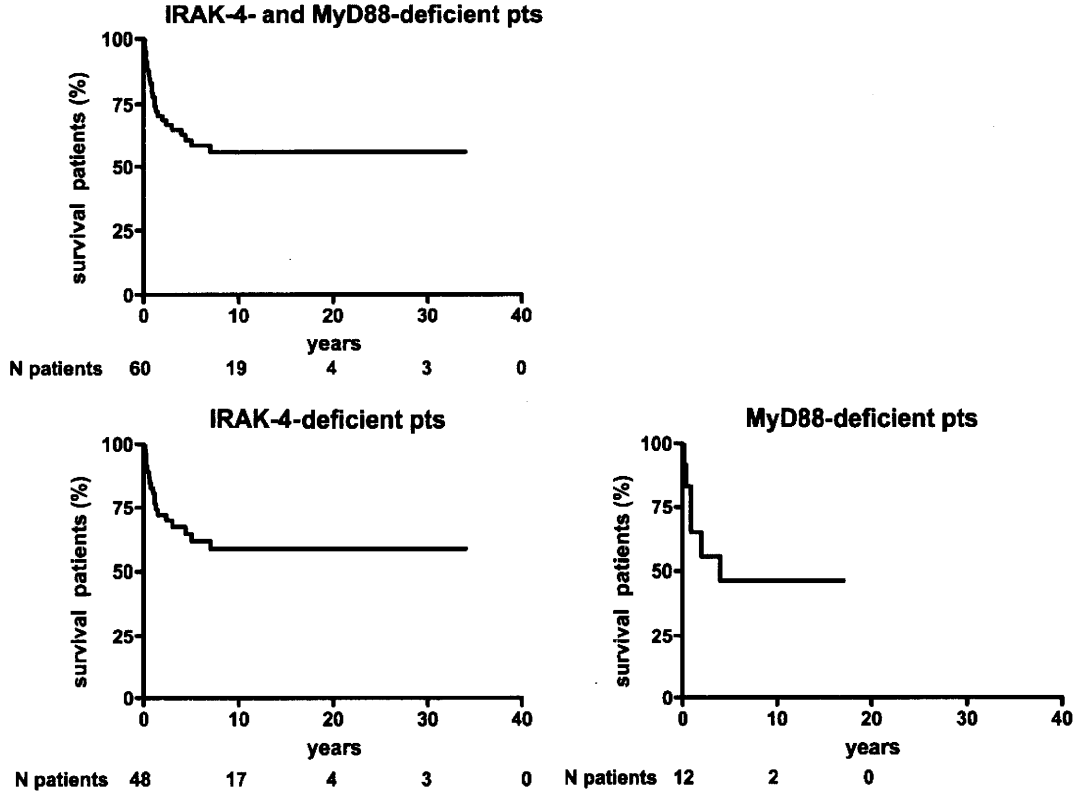

FIGURE 10. Survival curve of IRAK-4-deficient and MyD88-deficient patients.

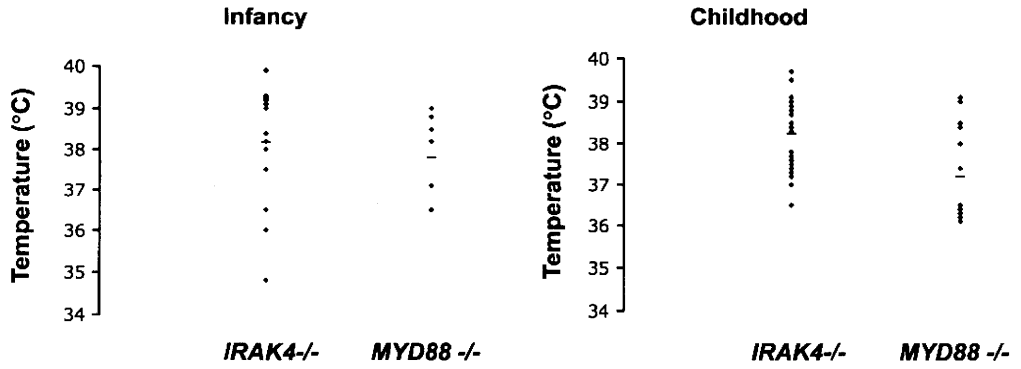

FIGURE 11. The inflammatory phenotype of IRAK-4/MyD88-deficiency. Temperature during bacterial infection in infancy and childhood.

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) (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.

In conclusion, both IRAK-4 deficiency and MyD88 deficiency confer a predisposition to InvBD, mostly caused by *Str. pneumoniae*, *Staph. aureus*, and *P. aeruginosa*. In addition, both conditions confer a predisposition to NInvBD, often severe skin infections, mostly caused by *Staph. aureus*, and severe forms of ear, nose, and throat infections caused by *P. aeruginosa*. Clinical status and outcome both improve with age. There seems to be a beneficial role of prophylaxis combining intensive vaccinations, oral antibiotics, and IgG injections.

The most important advice for the families and physicians of IRAK-4-deficient and MyD88-deficient patients is to initiate empiric parenteral antibiotic treatment as soon as infection is suspected or the patient develops a moderate fever, without taking inflammatory parameters into account, because patients may die from rapid invasive bacterial infection even if prophylactic measures are taken.

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

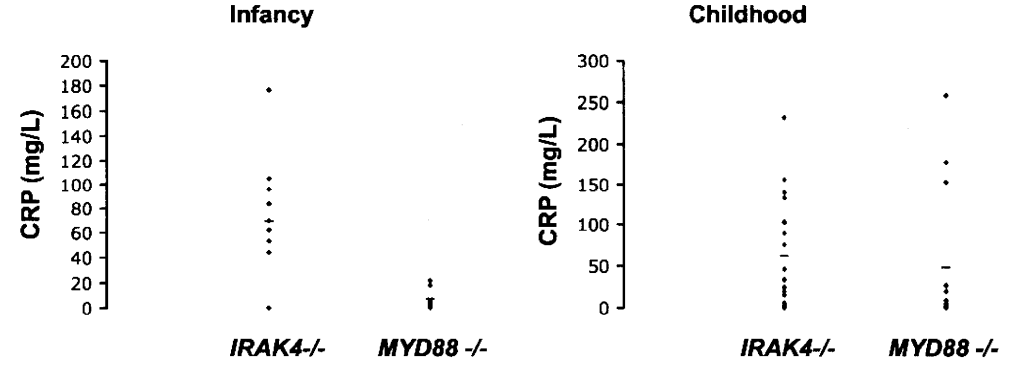

FIGURE 12. CRP concentration during bacterial infection in infancy and childhood.

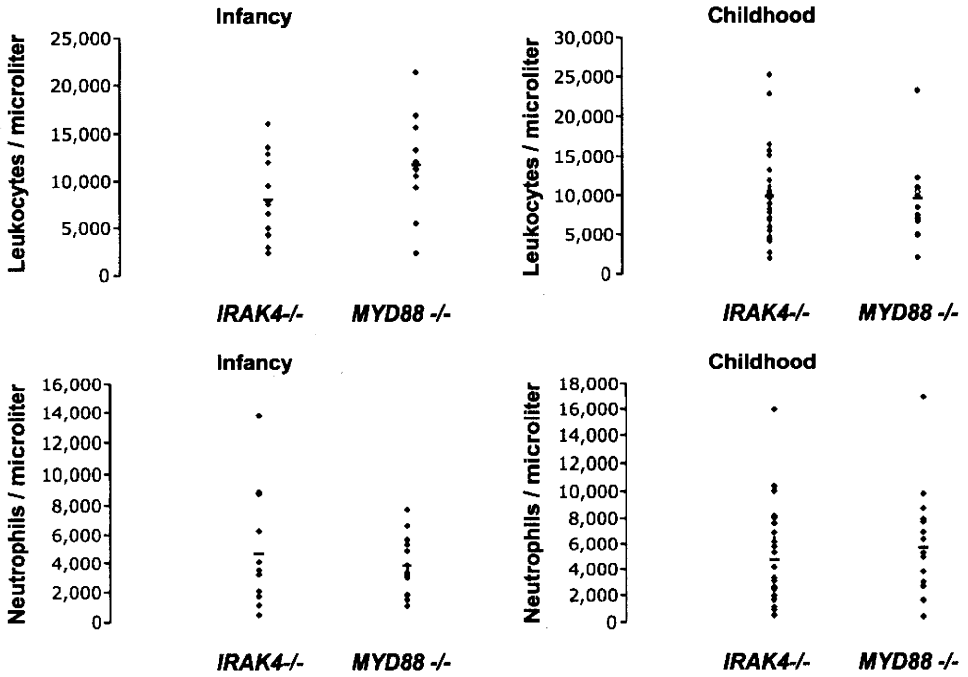

FIGURE 13. Polymorphonuclear neutrophil counts during bacterial infection in infancy and childhood.

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 most patients present multiple infections, although some display a specific predisposition to pneumococcal or mycobacterial diseases.[7] Almost all patients present infections caused by pyogenic bacteria, and only a few patients suffer from mycobacterial, fungal, and/or viral diseases. The most frequent pathogens observed include gram-positive (*Str. pneumoniae* and *Staph. aureus*) and gram-negative pyogenic bacteria (*P. aeruginosa* and *H. influenzae*). Patients bearing mutations in NEMO almost invariably have an impaired antibody response to glycans, including pneumococcal capsules in particular, as in half the IRAK-4- and MyD88-deficient patients explored for antibody responses to a subset of glycan antigens.[40] Thus, the bacterial diseases seen in NEMO-deficient patients are probably due in part to the impact of NEMO mutations on the TIR-signaling pathway. Conversely, the other infections seen in NEMO-deficient patients but not in IRAK-4-deficient and MyD88-deficient patients probably reflect the impairment of other signaling pathways.

The association of clinical disease caused by *Str. pneumoniae*, *Staph. aureus*, and *P. aeruginosa* is unique among primary immunodeficiencies other than IRAK-4, MyD88, NEMO, and IκBα deficiencies.[37] Primary immunodeficiencies affecting bacterial opsonization and splenic phagocytosis are associated with invasive pneumococcal disease. These conditions include most B- and T-cell defects, congenital asplenia, deficiencies of C3, the early component of the classical and alternative complement pathway.[39] These patients develop recurrent invasive pneumococcal disease due to *Str. pneumoniae*, but are less susceptible to *Staph. aureus* and *P. aeruginosa* infections.

Other primary immunodeficiencies, such as STAT3 and TYK2 deficiencies in HyperIgE syndromes, are associated with staphylococcal infections,[7] but patients with these primary immunodeficiencies do not suffer from invasive pneumococcal disease and *Pseudomonas* infection. Notably, two-thirds of the explored IRAK-4- and MyD88-deficient patients were found to have high levels of IgE, but these levels were modest with respect to the very high IgE levels described in STAT-3-deficient patients.

Finally, most primary immunodeficiencies involving phagocyte defects, including congenital neutropenia, leukocyte adhesion deficiency, and chronic granulomatous disease, are associated with severe infections caused by *P. aeruginosa* and *Staph. aureus*, but patients with these disorders are not particularly prone to invasive pneumococcal disease.[39] A diagnosis of IRAK-4 or MyD88 deficiency or of NEMO/IκBα-related defects should be considered even with only 1 or 2 of these 3 infections. Neonates, infants, and children with invasive pneumococcal disease, severe staphylococcal disease, or *Pseudomonas* lesions of the upper respiratory tract or peritoneum, particularly in cases of recurrence, should be tested for the NF-κB pathways, including the TIR pathway in particular.[2,9] This list is not exclusive, as systemic shigellosis was

TABLE 8. Inflammatory Signs at Admission in Patients With IRAK-4 Deficiency Who Had InvBD

Age Group	Age at Onset	No. of Episodes*	Temperature (°C)			CRP (mg/L)			Whole Leukocyte Count (WLC/μL)			Neutrophil Count (NC/μL)		
			Mean	Max	SD	Mean	Max	SD	Mean	Max	SD	Mean	Max	SD
Neonatal period	7 d to 17 d	5 (T) 5 (CRP) 5 (WLC) 3 (NC)	37.2	38.0	0.6	43.6	150	61.2	9550 (N: 2700–13,000)	18,000	6807	3525	5308	11,500
Infancy	5 wk to 11 mo	16 (T) 12 (CRP) 13 (WLC) 12 (NC)	38.2	39.9	1.4	69	176.4	51.1	8034 (N: 4300–9700)	16,000	4422	4643	13,760	3999
Childhood	1 yr to 14 yr	27 (T) 22 (CRP) 36 (WLC) 31 (NC)	38.3	41.0	1.0	61.5	156	65.3	9875 (N: 4300–9700)	25,200	4894	4731	15,940	3593

*No. of patients for whom the following data were available: T = temperature, CRP = C-reactive protein concentration, WLC = whole leukocyte count, NC = neutrophil count.

TABLE 9. Inflammatory Signs at Admission in Patients With Myd88 Deficiency Who Had InvBD

Age Group	Age at Onset	No. of Episodes*	Temperature (°C)			CRP (mg/L)			Whole Leukocyte Count (WLC/μL)			Neutrophil Count (NC/μL)		
			Mean	Max	SD	Mean	Max	SD	Mean	Max	SD	Mean	Max	SD
Infancy	5 wk to 11 mo	7 (T) 12 (CRP) 14 (WLC) 12 (NC)	38.1	39.0	1.3	7.2	6.5	21.8	11,691 (N: 4300–9700)	21,300	4964	3783	7680	1998
Childhood	1 yr to 10 yr	17 (T) 14 (CRP) 16 (WLC) 15 (NC)	37.2	39.1	1.1	47.7	153	83.7	9515 (N: 4300–9700)	23,200	4694	5693	16,900	4070

*No. of patients for whom the following data were available: T = temperature, CRP = C-reactive protein concentration, WLC = whole leukocyte count, NC = neutrophil count.

TABLE 10. Prophylaxis

	IRAK-4-Deficient Patients	MyD88-Deficient Patients
Antibiotic prophylaxis	28/48	6/12
Penicillins	6	1
Cotrimoxazole	10	1
Penicillins plus cotrimoxazole	8	4
Cephalosporin	1	—
Azythromycin	1	—
Quinolone	2	—
IgG treatment	15/48	4/12
Antibiotic prophylaxis plus IgG treatment	13/48	4/12
No prophylaxis	18/48	6/12

documented in 2 patients, and other infectious diseases associated with these primary immunodeficiencies may be revealed by the investigation of other patients in the future.

In IRAK-4- and MyD88-deficient patients, clinical and laboratory signs of inflammation develop slowly even in cases of severe infection. The current study confirms and expands previous work indicating that CRP concentration, total leukocyte counts, and neutrophil numbers are typically low, but may also rise to appropriately high levels during prolonged infections, whereas temperature frequently remains inappropriately low even in such infections.[18] Thus, weak signs of inflammation despite severe infection provide a further clue to possible defects in TIR signaling, although appropriately high levels of inflammatory signs do not rule out the diagnosis of TIR deficiency.[18] Impairment of the production of IL-6-inducible molecules, such as CRP, may be observed. IRAK-4- and MyD88-deficient cells produce small amounts of IL-6 and IL-8 in vitro upon activation with IL-1 and TLR agonists.[25,38,49] As CRP contributes to the clearance of pyogenic bacteria including pneumococcus,[35,47] susceptibility to *Str. pneumoniae*, *Staph. aureus*, or *P. aeruginosa* may be increased by the slow rise in CRP levels. Similar delays in the development of signs of inflammation are observed in patients with NEMO and IκBα deficiencies, whose broader susceptibility to infections includes these pyogenic bacteria.[7]

Some IRAK-4-deficient patients (n = 10) had a delay in umbilical cord detachment and/or omphalitis. Other primary immunodeficiencies, such as leukocyte adhesion deficiency type I and Rac2 deficiency, have been associated with late loss of the umbilical cord and/or omphalitis, but extremely high levels of circulating neutrophils and a lack of pus formation in peripheral tissues are classically found in these disorders.[36] By contrast, in IRAK-4- and MyD88-deficient patients, impaired polymorphonuclear neutrophil mobilization and/or frank neutropenia occurs from the onset of infection, perhaps secondary to the lack of IL-8 production. Despite this neutropenia, pus formation is normal in IRAK-4- and MyD88-deficient patients. The precise mechanism of cord separation is unknown, but it does require MyD88- and IRAK-4-dependent signals, as well as CD18-expressing leukocytes. Conversely, unlike patients with various phagocyte defects, such as chronic granulomatous disease, none of the IRAK-4- and MyD88-deficient patients had inflammatory bowel disease.[36]

Despite conferring selective susceptibility to only a few bacteria, IRAK-4 and MyD88 deficiencies are nonetheless life-threatening in infancy and childhood, with a mortality rate of 38% in our series. Strikingly, however, although IRAK-4 and MyD88 appear to be vital in childhood, infections in patients lacking these proteins become rarer with age, with no death recorded in patients after the age of 8 years and no invasive infection after the age of 14 years, even in the absence of antibiotics or/and IgG prophylaxis in 7 patients over the age of 14 years. In total, this represents a cumulative time of 49 years without any InvBD for these patients. This dramatic improvement with age may be accounted for by adaptive antigen-specific T- and B-lymphocyte responses. Indeed, our patients displayed no detectable defect of protein antigen-specific T- and B-cell responses, although some patients were found to have weak antibody responses to a subset of glycan antigens.

Recent studies of neonatal bacterial sepsis in newborn mice suggest a reliance on innate immunity early in life, which progressively diminishes with age.[51] An alternative complementary hypothesis is that innate immune responses may also mature with age.[4,25] Other sensors, such as RIG-I-like helicases and NOD-like receptors, may progressively play a compensatory role. In any event, clinical improvement did not result solely from prophylaxis following diagnosis of the first infection or of the underlying deficit. The TIR pathway, including TLR responses in particular, remains dependent on IRAK-4/MyD88 with age, but the maturation of other pathways may gradually compensate for the lack of TIR signaling.

In this study, we show that the prognosis of IRAK-4 and MyD88 deficiencies is severe in infancy and early childhood, but improves substantially in adolescence. This finding is probably unique so far in the field of primary immunodeficiencies, which classically do not improve with age. This improvement with age is a hallmark of these conditions, not observed in other primary immunodeficiencies. A similar but less striking spontaneous improvement has been reported only in children with IL-12p40 and IL-12Rβ1 deficiencies.[10]

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Short Report

NEMO mutation as a cause of familial occurrence of Behçet's disease in female patients

Takada H, Nomura A, Ishimura M, Ichiyama M, Ohga S, Hara T. *NEMO* mutation as a cause of familial occurrence of Behçet's disease in female patients.
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Behçet's disease is a chronic, relapsing, multisystem inflammatory disease of unknown etiology. Nuclear factor κB (NF-κB) essential modulator (NEMO) that is required for the activation of NF-κB plays an important role in inflammation. To investigate the role of NEMO in the pathogenesis of Behçet's disease, we analyzed *NEMO* gene and its expression pattern in tissues in a family with Behçet's disease. We found a heterozygous mutation (1217A>T, D406V) in a 6-year-old girl and her mother. Skewed X-chromosome inactivation was not observed in the peripheral blood mononuclear cells as well as in oral and intestinal mucosa of the patients. Accordingly, there was a significant proportion of peripheral blood monocytes that did not produce sufficient intracellular tumor necrosis factor-α with the stimulation of lipopolysaccharide. Heterozygous *NEMO* mutation is a cause of familial occurrence of Behçet's disease in female patients.

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Key words: Behçet's disease – nuclear factor κB essential modulator – Incontinentia pigmenti – X-linked anhidrotic ectodermal dysplasia with immunodeficiency

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Nuclear factor κB (NF-κB) essential modulator (NEMO) is required for the activation of the transcription factor NF-κB (1). The *NEMO* gene has been mapped to the chromosome location Xq28 (1). Large genomic rearrangements or amorphic mutations of *NEMO* cause incontinentia pigmenti, a disorder that is usually prenatally lethal in males, and contribute to abnormalities of skin, hair, nails, teeth and central nervous system in female heterozygotes (2). On the other hand, hypomorphic *NEMO* mutations cause X-linked anhidrotic ectodermal dysplasia with immunodeficiency (XL-EDA-ID) in male, characterized by immunodeficiency associated with an impaired development of skin adnexa (hair, sweat glands, and teeth) (3).

Behçet's disease is a chronic, relapsing, multisystem inflammatory disease of unknown etiology characterized by mucocutaneous, ocular, articular, vascular, urogenital, neurological, and gastrointestinal involvements, such as ulcerative colitis and congestive gastritis (4, 5). We found heterozygous *NEMO* mutation in two female patients with Behçet's disease.

Materials and methods

Patient 1 was a 6-year-old girl who suffered from ulcers in oral cavity and perianal area for 7 months. Her elder brother was diagnosed as XL-EDA-ID and died of gastrointestinal bleeding when he was 9 years old. On admission, her skin showed hypopigmented lesions without atrophy in the abdominal area and extremities (Fig. 1a), which had been observed since early infancy. A small ulcer was observed in oral

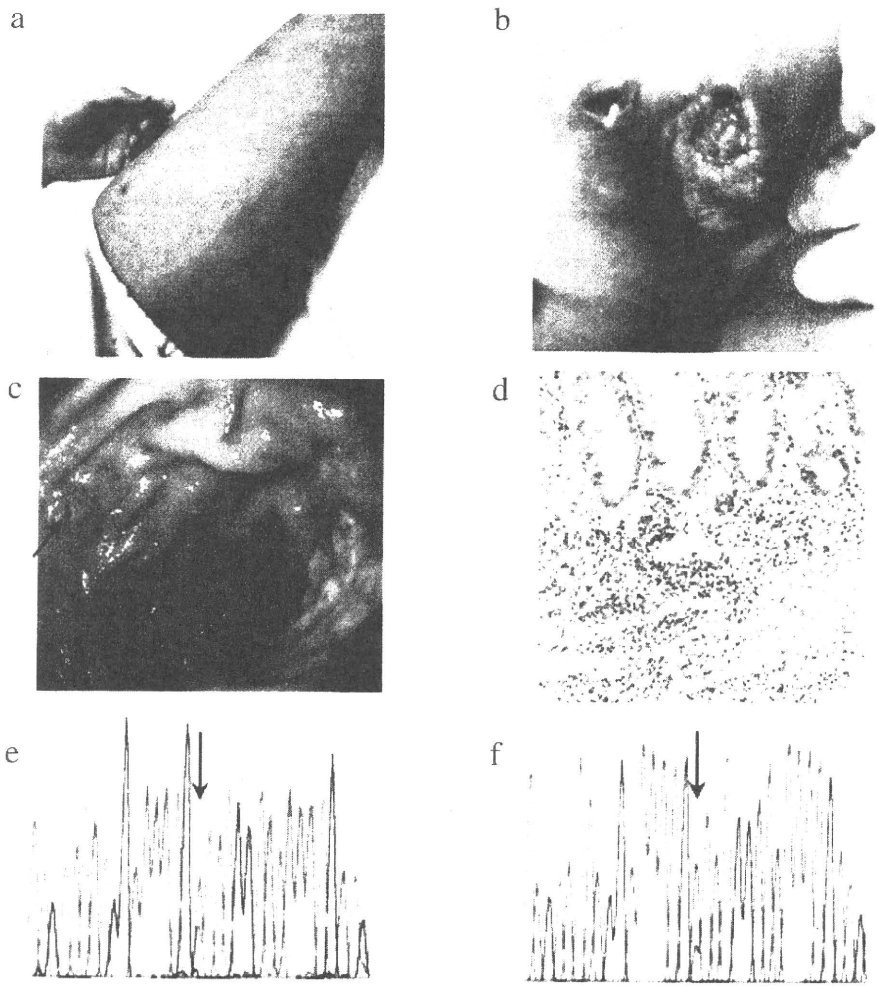

Fig. 1. Clinical manifestations and *NEMO* mutation in the patients. The hypopigmented skin lesions on the lower extremities observed along the curvilinear lines of Blaschko (**a**) and the ulcerative lesions in perianal area (**b**) are shown. The endoscopic finding with ulcerative lesions (arrows) in ascending colon and the histology (hematoxylin–eosin staining, ×100) of the ulcerative lesion are shown in (**c**) and (**d**), respectively. Sequencing results of the peripheral blood cells from patients 1 and 2 on *NEMO* are shown in (**e**; patient 1) and (**f**; patient 2).

cavity. She had large and deep painful ulcerative lesions in perianal area (Fig. 1b). The laboratory examinations showed a white blood cell count of 13.2×10^9/l with 80.9% neutrophils, hemoglobin of 12.3 g/dl, and erythrocyte sedimentation rate of 69 mm/h. Human leukocyte antigen (HLA) typing showed A2/A24, B61/B54, Cw1, Cw15, DR4, and DR12. Endoscopic examination showed multiple ulcerative lesions in colon, lacking reactive change in their marginal area (Fig. 1c). Histologically, chronic active inflammation was observed (Fig. 1d). These findings met the diagnostic criteria for Behçet's disease (entero-Behçet type) (6).

Patient 2 was a 42-year-old mother of the patient 1, who also suffered from ulcers in oral cavity and perianal area since she was 8 years old. She was diagnosed as having Behçet's disease when she was 12 years old. She also had hypopigmented skin lesions in the abdominal area and extremities, which had been observed since early infancy.

Genomic DNA and cDNA were amplified by polymerase chain reaction (PCR) as reported previously (2). The direct sequencing was performed using ABI PRISM 3100 Genetic Analyzer (Perkin-Elmer, Foster City, CA, USA).

Intracellular tumor necrosis factor (TNF)-α staining was performed using the Fastimmune Intracellular Staining System (BD Bioscience Pharmingen, San Diego, CA, USA) (7). Flow cytometric analysis was performed using EPICS XL (Beckman Coulter, Miami, FL, USA).

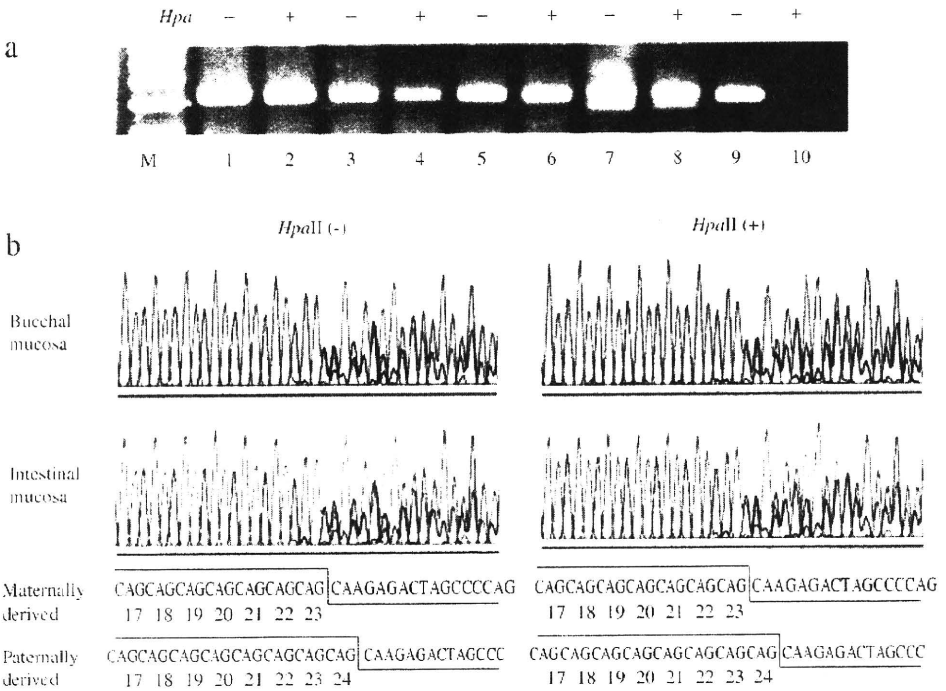

Fig. 2. X-chromosome inactivation of the patients. (**a**) Exon 1 of the HUMARA locus that contains CAG repeats was amplified by PCR after the digestion by methylation-sensitive *Hpa*II. Lanes 1 and 2: PBMNC, lanes 3 and 4; buccal mucosa, lanes 5 and 6; intestinal mucosa of patient 1, lanes 7 and 8; PBMNC of patient 2, lanes 9 and 10; PBMNC of the father of patient 1. (**b**) Sequencing results of the PCR products of the HUMARA locus from PBMNC, and buccal and intestinal mucosa of the patient 1 with and without *Hpa*II treatment are shown. The CAG repeats in the HUMARA locus of the maternally derived and paternally derived X-chromosome were 23 and 24 in number, respectively.

X-chromosome inactivation was analyzed as previously described (8). In brief, DNA was digested with the methylation-sensitive *Hpa*II (New England BioLabs, Beverly, MA, USA), amplified by the PCR at the exon 1 of human androgen receptor (HUMARA) gene locus that contains a highly polymorphic trinucleopeptide repeat (CAG), and sequenced.

Results

The cDNA and genomic DNA were obtained from peripheral blood mononuclear cells (PBMNC), and *NEMO* gene was amplified by PCR and sequenced. Heterozygous mutation (1217A→T, D406V) was observed in patients 1 and 2 (Fig. 1e,f), and elder brother of patient 1 had the same mutation (data not shown). We then investigated X-chromosome inactivation pattern of PBMNC, buccal mucosa, and intestinal mucosa by analyzing the effect of methylation-sensitive *Hpa*II on the HUMARA locus. Although we could not detect the difference of CAG repeat number in HUMARA locus between maternally and paternally derived X-chromosomes by electrophoresis of PCR products due to the minimal (1 repeat) difference in repeat number between them in patient 1 (Fig. 2a), the lack of extreme skewing was confirmed in PBMNC of patient 2 (Fig. 2a). The sequencing of these PCR products showed the lack of extreme skewing in all these tissues in patient 1 (Fig. 2b).

We analyzed lipopolysaccharide (LPS)-induced monocytic TNF-α production using flow cytometer to investigate individual cell function caused by the *NEMO* mutation and X-chromosome inactivation. As shown in Fig. 3, there was a significant proportion of monocytes that did not produce sufficient intracellular TNF-α with the stimulation of LPS, which functionally supported the lack of extensive X-inactivation skewing in the patient.

Discussion

A familial aggregation of Behçet's disease has been reported previously (9–14). Although *Familial Mediterranean fever* (*MEFV*) gene mutation is reported to be one of the genetic backgrounds of Behçet's disease (15), most of the patients as well as our patients did not have *MEFV* mutation (data not shown). There are several

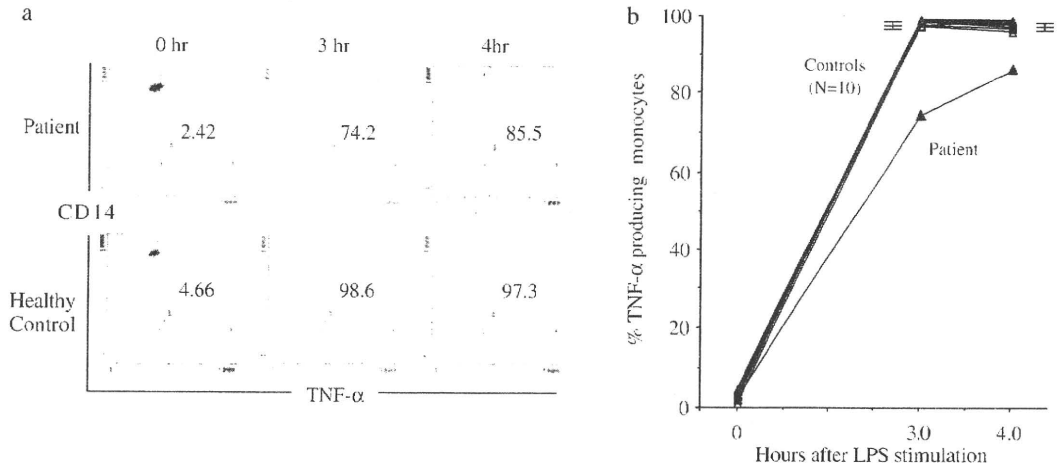

Fig. 3. A population of peripheral blood monocytes with insufficient production of intracellular TNF-α by the stimulation of LPS. A representative data of intracellular TNF-α staining (**a**) and the percentage of TNF-α producing cells in monocytes (**b**) without and with LPS stimulation are shown. Horizontal bars indicate the mean value and standard deviation in healthy controls.

reports of the association of Behçet's disease and incontinentia pigmenti (16–18). All the patients were females and developed Behçet's disease in childhood (16–18), which further supports our results.

Several clinically unique features were observed in our patients. The first was the occurrence of incontinentia pigmenti and XL-EDA-ID in a family. The second was the hypopigmented skin lesions since early infancy, because they are usually observed in early teens to adulthood (19). The third was the lack of extremely skewed X-chromosome inactivation (Fig. 2). Most of the patients with incontinentia pigmenti showed skewed X-chromosome inactivation in PBMNC and hepatocytes, which spared any apparent phenotype of these cells (20). This *NEMO* mutation was reported previously only in one patient with XL-EDA-ID (21), not in females. The D406V mutation locates in zinc finger domain, which is important in phosphorylation of NEMO, binding with ubiquitin, and full NF-κB activation (22–24). The development of Behçet's disease may be restricted only in a small proportion of the patients with incontinentia pigmenti caused by some particular type of *NEMO* mutation. Alternatively, it is possible that there are unrecognized patients with Behçet's disease and atypical or mild skin lesions caused by *NEMO* mutations.

NEMO-deficient mice developed intestinal inflammation by the impaired intestinal integrity caused by increased sensitivity to TNF-induced cell death, diminished expression of antimicrobial peptides such as defensins, and recruitment of inflammatory cells into damaged tissues (25). It is possible that this occurs in female patients with heterozygous *NEMO* mutation if they do not have skewed X-chromosome inactivation in the intestine. Immunocompetent inflammatory cell fraction in these patients can be recruited and it accelerates the inflammatory reaction in the intestine. The latter seems to be more important for the development of the Behçet's disease, because low-dose corticosteroid treatment was effective in both patients. This mechanism would also be applied to the lesions in oral mucosa and perianal tissues where continuous bacterial stimulation and infection occur.

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Conflict of interest

Nothing to declare.

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TABLE 2 Details of response to sequential treatments where applicable ($n = 10$)

No.	Severity of disease	First treatment		Second treatment		Third treatment	
1	Severe	Amlodopine	×	Nifedipine	√	–	–
2	Moderate	Amlodopine	×	GTN	×	–	–
3	Moderate	Amlodopine	×	GTN	×	–	–
4	Severe	Nifedipine	×	Amlodopine	×	–	–
5	Severe	Nifedipine	×	Amlodopine	×	GTN	√
6	Moderate	Nifedipine	×	GTN	×	–	
7	Severe	GTN	×	Amlodopine	×	Nifedipine	√
8	Moderate	Nifedipine	×	GTN	√	–	–
9	Severe	Amlodopine	×	Nifedipine	×	GTN	×
10	Moderate	Amlodopine	√	GTN	√	–	–

×: no response/inadequate response; √: response.

Overall, GTN patches were effective in 55% of the treated patients. Efficacy was better than that of nifedipine and amlodipine (33 *vs* 25% response rate, respectively), but small numbers and retrospective analysis does not allow statistical comparison. Response was similar in primary and secondary RP. Children with severe RP had a better response to nifedipine and amlodopine than children with moderate disease. The sub-group with severe disease was more likely to be using a disease-modifying drug, which may have had an impact. However, numbers are too small for any conclusion to be drawn from this.

Application of GTN patches allows removal if adverse events occur. Together with absence of tablets, this may make treatment with GTN attractive in paediatric practice. All patients received Deponit GTN patches. Alternative brands may not have adequate skin adhesion when cut into quarters for this off-license use.

GTN patches, nifedipine and amlodipine offer symptomatic relief for patients with moderate primary/secondary RP. Further studies, including head-to-head trials, are needed to determine if one agent is superior. Meanwhile, GTN patches offer an alternative to oral calcium channel blockers for symptomatic relief of paediatric RP.

Rheumatology key message

- GTN patches are an efficacious treatment option in paediatric RP.

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A case of early-onset sarcoidosis with a six-base deletion in the *NOD2* gene

SIR, We present the first case of early-onset sarcoidosis (EOS, MIM no. 609464) with a six-base deletion in the *NOD2* gene, resulting in the replacement of one amino acid and the deletion of two additional amino acids. All previous mutations reported for EOS and Blau syndrome (BS, MIM no. 186580) were single-base substitutions that resulted in the replacement of a single amino acid [1–3].

The patient was a Japanese male born after an uncomplicated pregnancy and delivery. His family had no symptoms of skin lesions, arthritis or uveitis. At 5 years of age, he was diagnosed with bilateral severe uveitis. He became blind in both eyes during adolescence. He had swollen ankles without pain during childhood,

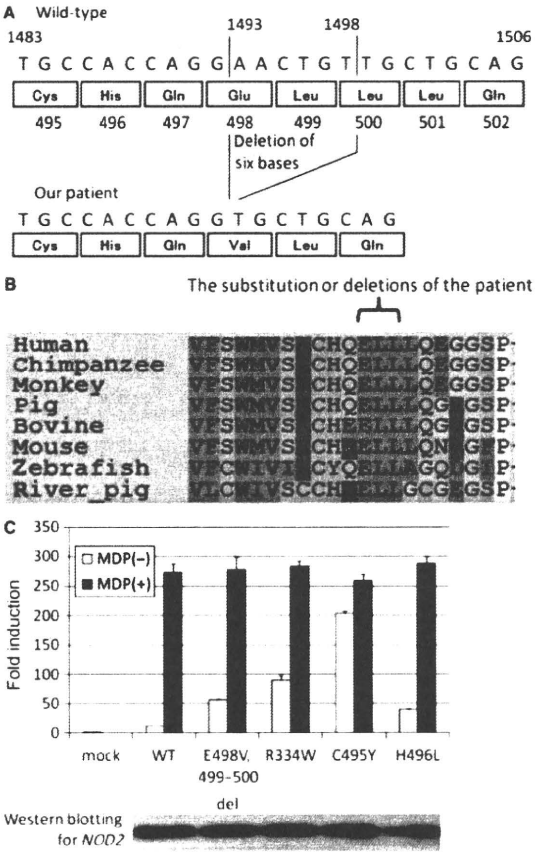
and developed arthritis in his both knees and ankles at 15 years of age. At 30 years, a skin rash had developed on his extremities after his first BCG vaccination. The skin lesions were scaly erythematous plaques with multiple lichenoid papules and some pigmentation. At the same age, camptodactyly without obvious synovial cysts of the hands was observed, and the deformity in all fingers developed by 35 years. At 41 years, he had low-grade fever for 1 year. He had no pulmonary lesions. His laboratory investigations showed normal white blood cell count, mildly elevated CRP (1.0 mg/dl) and ESR (20 mm/h). A skin biopsy from his left forearm revealed non-caseating granulomas without lymphocyte infiltration. There were no indications of infection by *Mycobacterium*.

The clinical symptoms and pathological findings on the biopsied skin indicated that the patient suffered from EOS. It has been reported that EOS and BS have a common genetic aetiology due to mutations in the *NOD2* gene that cause constitutive Nuclear Factor (NF)-κB activation [4, 5]. Thus we analysed the *NOD2* gene from the patient to look for mutations that might correlate with the pathology of EOS. A written informed consent was obtained from the patient and his families, according to the protocol of the institutional review board of Kyoto University Hospital and in accordance with the Declaration of Helsinki. Genomic sequencing analysis of the patient's *NOD2* gene showed the presence of a heterozygous deletion of six bases in exon 4, which resulted in c.1493_1498delAACTGT, p.E498V, 499–500del (Fig. 1A). The mutation was novel and was not identified in 100 normal controls. A genome alignment of *NOD2* among several species showed that E498, L499 and L500 are conserved from zebrafish to human (Fig. 1B). These data strongly suggested that the identified deletion of six bases in the *NOD2* gene is not a single nucleotide polymorphism (SNP), but is probably responsible for EOS in the patient.

Previous studies report that *NOD2* mutations causing EOS/BS show constitutive activation of NF-κB [6–8]. Therefore, we investigated the level of NF-κB activity associated with the new mutation identified here. First, we confirmed the level of mRNA expression of the mutated allele by subcloning analysis of *NOD2*-cDNA, which showed that the mutated allele was expressed as well as the wild type allele (data not shown). We then evaluated the ability of the *NOD2* mutant to constitutively activate NF-κB by using an *in vitro* reporter system in HEK293T cells transfected with both *NOD2* mutants and NF-κB reporter plasmids (Fig. 1C). The deletion mutant demonstrated almost five times more NF-κB activity than wild type without muramyl dipeptide (MDP) stimulation. Western blot analysis confirmed that NOD2 mutant protein expression was similar to that of wild type (Fig. 1C). Thus, like other mutations of *NOD2* identified previously, the deletion mutant identified here also showed constitutive activation of NF-κB.

The mechanism underlying EOS/BS has not been totally understood, although two pathways downstream from *NOD2* have been identified: NF-κB activation through receptor-interacting protein (RIP) like interacting caspase-like apoptosis regulatory protein kinase (RICK) and MAP kinase activation through the caspase recruitment domain 9 (CARD9) [9]. We previously tested 10 *NOD2* missense mutations that have been identified in our cohort of EOS/BS patients in Japan, and all of them demonstrated constitutive activation of NF-κB [3]. By analysing this newly identified deletion mutant, we have further confirmed the importance of constitutive activation of NF-κB by mutated *NOD2* for the pathogenesis of EOS/BS. We would like to emphasize the

FIG. 1 (**A**) Summary of the mutations identified in our patient. (**B**) NOD2 protein alignment among different species on the mutated amino acids. (**C**) NF-κB reporter assay using the *NOD2* deletion mutant. *In vitro* NF-κB reporter assays were performed as previously described [1, 3, 6, 7]. Mock vector, wild type *NOD2* (WT) and three *NOD2* variants (R334W, C495Y, H496L) derived from EOS/BS patients, were used as controls. Values represent the mean of normalized data (mock without MDP = 1) of triplicate cultures, and error bars indicate S.D. Shown is one representative result of three independent experiments. Protein expression levels of *NOD2* mutants analysed by western blotting are shown in the bottom panel.



usefulness of the NF-κB reporter assay with mutant *NOD2* for observing its role in EOS/BS, although the MAP kinase activation pathway and other possible pathways need to be evaluated to more completely understand the pathogenesis of the *NOD2* mutation in EOS/BS.

We have identified the first deletion mutation in the *NOD2* gene responsible for EOS/BS, and the mutant showed constitutive activation of NF-κB, which is one of the key features that lead to the pathogenesis of EOS/BS.

Rheumatology key message

- A six-base deletion in *NOD2* gene causes EOS.

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Comment on: Hepatotoxicity rates do not differ in patients with rheumatoid arthritis and psoriasis treated with methotrexate

Sir, We read with interest the recent article by Amital *et al.* [1] that compared hepatotoxicity rates in PsA and RA patients treated with MTX based on the evaluation of standard liver function tests. The authors conclude that the incidence of hepatotoxicity does not differ between the two disease groups after adjusting for the cumulative dose of MTX.

Several studies in MTX-treated psoriasis patients have reported that isolated abnormalities of liver enzymes (i.e. alkaline phosphatase, aspartate aminotransferase and alanine aminotransferase) were poor predictors of the severity of liver histopathology. The authors state that the combined sensitivity of aspartate aminotransferase, alanine aminotransferase and bilirubin for detecting an abnormal liver biopsy has been rated at 0.86 based on a previous study [2]. This figure implies that 14% of those with normal liver function tests will have undetected hepatic disease. Larger studies have suggested that 30–50% of the psoriasis patients on MTX have normal standard liver function test results despite histology showing fibrosis and cirrhosis [3]. The lack of correlation between liver enzymes and hepatic fibrosis and cirrhosis has been the major factor leading to the recommendation that liver biopsies be done to monitor potential hepatotoxicity. In this study, the liver function tests were performed with varying frequency which could allow abnormal liver function tests to be missed. The authors acknowledge that the rates of other hepatotoxic agents such as alcohol use and the occurrence of other hepatic comorbidities were not known. We believe that these are significant confounding variables, which make the interpretation of the results of this study difficult. The British Association of Dermatologists recommends serial monitoring

Promoting tolerance to proteolipid protein-induced experimental autoimmune encephalomyelitis through targeting dendritic cells

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Contributed by Jack L. Strominger, August 9, 2010 (sent for review May 20, 2010)

In T cell-mediated autoimmune diseases, self-reactive T cells with known antigen specificity appear to be particularly promising targets for antigen-specific induction of tolerance without compromising desired protective host immune responses. Several lines of evidence suggest that delivery of antigens to antigen-presenting dendritic cells (DCs) in the steady state (i.e., to immature DCs) may represent a suitable approach to induce antigen-specific T-cell tolerance peripherally. Here, we report that anti-DEC205–mediated delivery of the self-peptide proteolipid protein (PLP)139–151 to DCs ameliorated clinical symptoms in the PLP-induced SJL model of experimental autoimmune encephalomyelitis. Splenocytes from treated mice were anergized to PLP139–151, and IL-17 secretion was markedly reduced. Moreover, we show directly, using transgenic CD4$^+$ Vβ6$^+$ TCR T cells specific for PLP139–151, that, under the conditions of the present experiments, these cells also became anergic. In addition, evidence for a CD4$^+$ T cell-mediated suppressor mechanism was obtained.

DEC205 | multiple sclerosis | anergy | monophosphoryl lipid A | T cells

Multiple sclerosis is a T cell-mediated autoimmune disease characterized by immune cell infiltration, inflammatory demyelination of neuronal axons, and axonal loss in the human central nervous system (1, 2). Studies of multiple sclerosis are facilitated by the animal model experimental autoimmune encephalomyelitis (EAE) that recapitulates many aspects of the human disease (3). Active induction of EAE is accomplished by stimulation of T cell-mediated immunity to myelin, the insulating phospholipid layer surrounding the neuronal axons, through immunization with myelin proteins or synthetic peptide antigens derived from myelin and then emulsified in adjuvant (4). This treatment leads to activation of autoreactive myelin-specific CD4$^+$ T cells that circulate in the periphery of naïve animals. Activated autoreactive T cells will cross the blood–brain barrier (5). Within the central nervous system, local and infiltrating antigen-presenting cells, such as dendritic cells (DCs) derived from microglia, present MHC class II molecule-associated myelin peptides to infiltrating T cells in the context of costimulation. Myelin-specific CD4$^+$ T cells are reactivated, initiating a cascade of neuroinflammatory responses that ultimately leads to demyelination in the central nervous system and neurodegeneration. EAE can also be passively induced by adoptive transfer of preactivated myelin-specific T cells (6).

Although T helper 1 (Th1) cells secreting IFN-γ were considered to be the primary mediators of EAE, T helper 17 (Th17) cells recently were shown to exhibit greater pathogenicity, suggesting that they play a more decisive role in mediating severe tissue damage (7, 8). However, both Th1 and Th17 cells, generated with kinetic differences and/or involved at different stages, may be involved in development of EAE (9). In fact, the relative contribution of both Th subsets was recently suggested to affect the anatomical location of lesion distribution between brain and spinal cord parenchyma (10).

Self-reactive T cells with known antigen specificity, which can be found in T cell-mediated autoimmune diseases such as multiple sclerosis, appear particularly promising targets for antigen-specific tolerance induction without compromising host immunity to infectious insults. Various protocols have been used to interfere with unwanted immunity using peptide-induced tolerance (11), including the administration of antigens over extended periods of time via osmotic minipumps (12, 13). In addition, peptide antigens can also be directly delivered to antigen-presenting cells via targeting approaches. In particular, antigens delivered to different subsets of DCs after fusion with antibodies to the endocytic receptors DEC205 (αDEC205) or 33D1 are efficiently processed and presented by MHC class I and class II molecules (14). This route of antigen delivery to murine (15) or human (16) DCs is several orders of magnitude more efficient than free peptides and in conjunction with maturation stimuli represents an effective method for inducing strong T-cell responses, i.e., vaccination. By contrast, targeting antigen to immature DCs in the steady state has been described as promoting immunological tolerance but through different mechanisms in different studies (15, 17–20). It may lead to deletion of antigen-specific T cells with residual cells becoming immunologically unresponsive, a mechanism that in one study increased CD5 expression on activated T cells (17). In addition, delivering minute amounts of peptides via αDEC205 fusion proteins to steady-state immature DCs can lead to the de novo generation of antigen-specific Foxp3$^+$ Treg in vivo (18, 21).

Previous studies indicated that αDEC205-mediated targeting of an encephalogenic peptide of the myelin oligodendrocyte glycoprotein (MOG), a minor myelin component, to DCs in vivo prevents EAE induction by subsequent injection of the same peptide in complete Freund's adjuvant (CFA) in C57BL/6 mice (17). In this model, pretreatment with large doses of the free peptide in the absence of adjuvants also leads to protection from subsequent challenge. Here, we report experiments with αDEC205-mediated targeting of the autoantigen of the proteolipid protein peptide (PLP139–151) (derived from a major myelin constituent) in the EAE model in SJL mice, which is much more prone to disease and

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