Clinical Immunology (2013) 146, 73–76

available at www.sciencedirect.com

Clinical Immunology

www.elsevier.com/locate/yclim

LETTER TO THE EDITOR

Distinct cytokine profile in juvenile systemic lupus erythematosus-associated macrophage activation syndrome

Masaki Shimizu*, Tadafumi Yokoyama, Yuko Tokuhisa, Sayaka Ishikawa, Yasuhisa Sakakibara, Kazuyuki Ueno, Akihiro Yachie

Department of Pediatrics, School of Medicine, Institute of Medical, Pharmaceutical, and Health Sciences, Kanazawa University, Japan

Received 18 September 2012; accepted with revision 7 November 2012
Available online 15 November 2012

KEYWORDS
Macrophage activation syndrome;
Systemic lupus erythematosus

Abstract Macrophage activation syndrome (MAS) has been observed in patients with systemic lupus erythematosus (SLE). Recognition of MAS in patients with SLE may be particularly challenging because it may mimic the clinical features of the underlying disease or be confused with an infectious complication. Massive hypercytokinemia is strongly associated with the pathogenesis of systemic lupus erythematosus-associated macrophage activation syndrome (SLE–MAS) but the pathogenesis and kinetics of cytokine release in SLE–MAS patients is not well studied. We present a case of SLE–MAS. The patient showed the distinct cytokine profile of SLE–MAS compared to systemic juvenile idiopathic arthritis associated MAS and Epstein–Barr virus-induced hemophagocytic lymphohistiocytosis. The observed TNF-α dominant increase appears to be characteristic of SLE–MAS. IgM type antilymphocyte antibody (ALAB) was detected on the surface of lymphocytes during the acute phase and disappeared when the patient was in remission. The patient had a heterozygous P369S-R408Q mutation in the *MEFV* gene. Our results suggest that ALAB and a *MEFV* mutation might play important roles in the pathogenesis of SLE–MAS. Furthermore, the cytokine profile of SLE–MAS differs from that of S-JIA–MAS: the TNF-α dominant increase appears to be characteristic.

Abbreviations: SLE, systemic lupus erythematosus; MAS, macrophage activation syndrome; S-JIA, systemic juvenile idiopathic arthritis; EBV-HLH, Epstein–Barr virus associated hemophagocytic lymphohistiocytosis; FMF, familial Mediterranean fever; ALAB, antilymphocyte antibody.

* Corresponding author at: Department of Pediatrics, School of Medicine, Institute of Medical, Pharmaceutical, and Health Sciences, Kanazawa University, 13-1 Takaramachi, Kanazawa 920-8641, Japan. Fax: +81 76 262 1866.
E-mail address: shimizum@staff.kanazawa-u.ac.jp (M. Shimizu).

1521-6616/$ - see front matter © 2012 Elsevier Inc. All rights reserved.
http://dx.doi.org/10.1016/j.clim.2012.11.004

Macrophage activation syndrome (MAS) has been observed in patients with systemic lupus erythematosus (SLE) [1,2]. Recognition of MAS in patients with SLE may be particularly challenging because it may mimic the clinical features of the underlying disease or be confused with an infectious complication. Massive hypercytokinemia is strongly associated with the pathogenesis of systemic lupus erythematosus-associated macrophage activation syndrome (SLE–MAS) but the pathogenesis and kinetics of cytokine release in SLE–MAS patients are not well studied. In this report, we describe a case of SLE–MAS and show the distinct cytokine profile of SLE–MAS compared to S-JIA–MAS and Epstein–Barr virus-induced hemophagocytic lymphohistiocytosis (EBV-HLH).

A previously healthy 15-year-old girl was referred to our institution with abdominal pain. The patient did not exhibit a butterfly rash, mucous membrane abnormalities, photosensitivity, or arthritis. Laboratory examination revealed lymphopenia (white blood cell count, 7900/mm^3; absolute lymphocyte count, 356/mm^3), increased immunoglobulin (Ig) G levels (3383 mg/dL), positive antinuclear antibody (1:1280; speckle), rheumatic factor (128.7 IU/mL; normal, <20), anti-dsDNA (85.4 IU/mL; normal, <12.0), anti-Sm (index, 15.6; normal, <7.0), anti-SSA (index, 145.5; normal, <10.0), anti-SSB (index, 43.0; normal, <15.0), and anti-RNP (index, 184.8; normal, <15.0). Complement C3 (91 mg/dL) and C4 (16 mg/dL) were normal. Urinary test findings showed proteinuria (0.4 g/day) and hematuria with cellular casts. Renal biopsy showed mesangial proliferative glomerulonephritis with Igs and complement deposition. Treatment with prednisolone (60 mg/day) was initiated. The patient developed high fever and systemic exanthema four days later. Laboratory examination revealed the depression of blood cells (white blood cell count, 8820/mm^3; absolute lymphocyte count, 176/mm^3; hemoglobin, 9.9 g/dL; platelet count, 108,000/mm^3), coagulopathy (fibrinogen, 133 mg/dL), hyperglycemia (203 mg/dL), impaired liver function (alanine aminotransferase,102 U/L; aspartate aminotransferase, 338 U/L), high ferritin levels (20,417 μg/L), and high lactate dehydrogenase (2310 U/L). Epstein–Barr virus (EBV) anti-VCA IgM was negative. EBV anti-VCA-IgG and anti-EB nuclear antigen IgG were positive, as was the direct Coombs test, but the indirect Coombs test was negative. Platelet-associated IgG was positive (109 ng/10^7 cells). Bone marrow aspiration revealed increased histiocytes with evidence of hemophagocytosis. Echocardiography showed pericarditis with pericardial effusion. A diagnosis of SLE–MAS was made based on these findings. The patient was started with the methylprednisolone pulse therapy (1 g/day) administered for 3 consecutive days, followed by intravenous cyclosporine A (1 mg/kg/day). However, her condition worsened and dexamethasone palmitate (10 mg/day) was administered. Thereafter, her condition gradually improved and oral prednisolone and cyclosporine were continued.

We measured the levels of serum cytokines, including IL-1β, IL-18, IL-6, neopterin, and tumor necrosis factor (TNF)-α, and TNF receptor type I (sTNFRI) and type II (sTNFRII) in this patient and compared them with the levels in patients with S-JIA–MAS, EBV-HLH and active SLE without MAS, who are followed up in our hospital. Serum levels of these cytokines were evaluated by commercial enzyme-linked immunosorbent assay. The cytokine profile in the MAS phase in this patient showed TNF-α dominant hypercytokinemia (Fig. 1A). This pattern differs from that of S-JIA–MAS (Fig. 1B), EBV-HLH (Fig. 1C) and active SLE without MAS (Fig. 1D). TNF-α dominant hypercytokinemia persisted until the patient recovered from MAS (Fig. 1E). Serum IL-1β was detected only in MAS phase in this patient (18 pg/ml; normal range<2.0 pg/ml). The patient had a heterozygous P369S-R408Q mutation in the *MEFV* gene. IgM type antilymphocyte antibody (IgM-ALAB) was detected on the surface of lymphocytes during the acute phase (Supplement Fig. 1A) and disappeared when the patient was in remission (Supplement Fig. 1B).

The pathogenesis of MAS remains obscure; however, massive hypercytokinemia is strongly associated with MAS. Although various proinflammatory cytokines had increased in our patient, the pattern of a TNF-α dominant increase is characteristic compared to S-JIA and EBV-HLH patients. TNF-α is one of the cytokines suggested to be connected with the pathogenesis of SLE with both a proinflammatory and an immunoregulatory action with differential effects on B cells, on T cells and on dendritic cells as well as on the process of programmed cell death. Previous reports revealed serum TNF-α level is clearly elevated and is found to correlate with SLE disease activity [3,4]. The data from our patient is consistent with these results. We previously reported that the cytokine release pattern in MAS differs among patients with different etiologies [5]. The cytokine release pattern in SLE–MAS is different among patients with different etiologies. Monitoring the cytokine profile, including TNF-α, may be useful for the evaluation of disease activity in SLE–MAS.

IgM-ALAB is associated with SLE disease flares and lymphopenia forms one line of evidence for a potential role of IgM-ALAB in T cell depletion and cellular immune dysfunction in SLE [6]. Potential mechanisms that have been suggested include elimination of lymphocytes by complement-mediated lysis and/or opsonization, modulation of surface determinants, interaction with soluble products of activated cells, and up- or down-regulation by cross-linking cell surface receptors [6]. IgM-ALAB was detected in our patient and may have played a role in the pathogenesis of SLE–MAS, in particular, cytopenia by hemophagocytosis.

Familial Mediterranean fever (FMF) is caused by mutations in *MEFV*, which encodes pyrin. Pyrin regulates caspase 1 activation and subsequent IL-1β production. It is well known that deregulation of the innate immune system leads to excessive IL-1β secretion and plays a pivotal role in the pathogenesis of FMF and other autoinflammatory diseases. Uncontrolled activation of innate immunity is also considered the main cause of MAS, which has recently been reported in patients with hereditary autoinflammatory diseases [7,8]. P369S/R408Q substitutions in exon 3 of the *MEFV* gene have also recently been associated with a highly variable phenotype and are infrequently associated with typical FMF symptoms [9,10]. Serum Il-1β was detected in the MAS phase in this patient, indicating that excessive IL-1β secretion was associated with the pathogenesis of SLE–MAS. Mutations of *MEFV* might have played a role in amplifying deregulated inflammation in our patient.

Our results suggest that ALAB and a *MEFV* mutation might play important roles in the pathogenesis of at least some, if not all, SLE–MAS. Furthermore, the cytokine profile of SLE–MAS

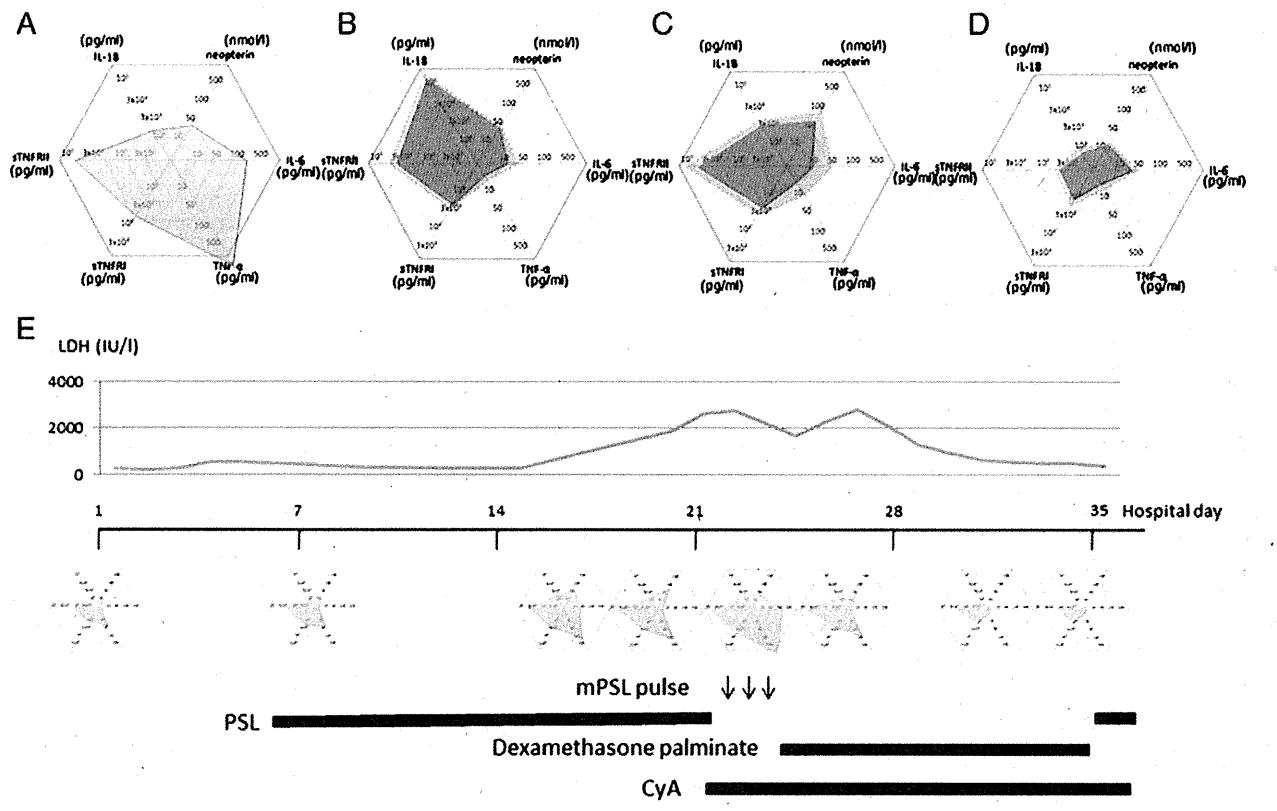


Figure 1 Cytokine profile represented with a radar chart in patients with MAS. A. Our patient with SLE–MAS. B. S-JIA–MAS: the average of 5 patients with S-JIA–MAS. C. EBV-HLH: the average of 10 patients with EBV-HLH. D. Active SLE: the average of 3 patients with active SLE without MAS. The dark red areas show the mean values of the patients. The light red areas show the standard deviation. E. Cytokine profiles at different SLE disease phases in our patient.

differs from that of S-JIA–MAS: the TNF-α dominant increase appears to be characteristic.

The following are the supplementary data related to this article. **Supplement Fig. 1** Detection of IgM type antilymphocyte antibody by flow cytometry. A. Direct immunofluorescent staining of lymphocytes for IgG and IgM type antilymphocyte antibodies. The patient's peripheral blood mononuclear cells (PBMCs) were isolated by Ficoll-Paque and then incubated with fluorescein isothiocyanate conjugated anti-human IgG or IgM. B. Change in mean fluorescence intensity (MFI) of IgM type antilymphocyte antibodies. The control's PBMCs were isolated by Ficoll-Paque and then incubated with the patient's serum. These PBMCs were washed and then incubated with fluorescein isothiocyanate conjugated anti-human IgM.

Conflict of interest statement

We have no conflicts of interest. We have no financial associations to disclose in producing this manuscript.

Acknowledgments

We thank Harumi Matsukawa for technical assistance.

References

[1] A. Parodi, S. Davì, A.B. Pringe, A. Pistorio, N. Ruperto, S. Magni-Manzoni, P. Miettunen, B. Bader-Meunier, G. Espada, G. Sterba, S. Ozen, D. Wright, C.S. Magalhães, R. Khubchandani, H. Michels, P. Woo, A. Iglesias, D. Guseinova, C. Bracaglia, K. Hayward, C. Wouters, A. Grom, M. Vivarelli, A. Fischer, L. Breda, A. Martini, A. Ravelli, Lupus Working Group of the Paediatric Rheumatology European Society, Macrophage activation syndrome in juvenile systemic lupus erythematosus: a multinational multicenter study of thirty-eight patients, Arthritis Rheum. 60 (2009) 3388–3399.

[2] A. Pringe, L. Trail, N. Ruperto, A. Buoncompagni, A. Loy, L. Breda, A. Martini, A. Ravelli, Macrophage activation syndrome in juvenile systemic lupus erythematosus: an under-recognized complication? Lupus 16 (2007) 587–592.

[3] M. Postal, S. Appenzeller, The role of tumor necrosis factor-alpha (TNF-α) in the pathogenesis of systemic lupus erythematosus, Cytokine 56 (2011) 537–543.

[4] A. Sabry, H. Sheashaa, A. El-Husseini, K. Mahmoud, K.F. Eldahshan, S.K. George, E. Abdel-Khalek, E.M. El-Shafey, H. Abo-Zenah, Proinflammatory cytokines (TNF-alpha and IL-6) in Egyptian patients with SLE: its correlation with disease activity, Cytokine 35 (2006) 148–153.

[5] M. Shimizu, T. Yokoyama, K. Yamada, H. Kaneda, H. Wada, T. Wada, T. Toma, K. Ohta, Y. Kasahara, A. Yachie, Distinct cytokine profiles of systemic-onset juvenile idiopathic arthritis-associated macrophage activation syndrome with

particular emphasis on the role of interleukin-18 in its pathogenesis, Rheumatology (Oxford) 49 (2010) 1645–1653.
[6] J.B. Winfield, Are anti-ribosomal P protein antibodies a type of anti-lymphocyte antibody? Clin. Exp. Immunol. 109 (1997) 1–3.
[7] L. Rossi-Semerano, B. Hermeziu, M. Fabre, I. Kone-Paut, Macrophage activation syndrome revealing familial Mediterranean fever, Arthritis Care Res (Hoboken) 63 (2011) 780–783.
[8] D. Rigante, E. Capoluongo, B. Bertoni, V. Ansuini, A. Chiaretti, M. Piastra, S. Pulitanò, O. Genovese, A. Compagnone, A. Stabile, First report of macrophage activation syndrome in hyperimmunoglobulinemia D with periodic fever syndrome, Arthritis Rheum. 56 (2007) 658–661.
[9] J.G. Ryan, S.L. Masters, M.G. Booty, N. Habal, J.D. Alexander, B.K. Barham, E.F. Remmers, K.S. Barron, D.L. Kastner, I. Aksentijevich, Clinical features and functional significance of the P369S/R408Q variant in pyrin, the familial Mediterranean fever protein, Ann. Rheum. Dis. 69 (2010) 1383–1388.
[10] M. Shimizu, Y. Tone, A. Toga, T. Yokoyama, T. Wada, T. Toma, A. Yachie, Colchicine-responsive chronic recurrent multifocal osteomyelitis with MEFV mutations: a variant of familial Mediterranean fever? Rheumatology (Oxford) 49 (2010) 2221–2223.

Cytokine xxx (2012) xxx–xxx

Contents lists available at SciVerse ScienceDirect

Cytokine

journal homepage: www.journals.elsevier.com/cytokine

Short Communication

Distinct subsets of patients with systemic juvenile idiopathic arthritis based on their cytokine profiles

Masaki Shimizu [a,*], Yasuo Nakagishi [b], Akihiro Yachie [a]

[a] Department of Pediatrics, School of Medicine, Institute of Medical, Pharmaceutical and Health Sciences, Kanazawa University, 13-1 Takara-Machi, Kanazawa 920-8641, Japan
[b] Department of Pediatric Rheumatology, Hyogo Prefectural Kobe Children's Hospital, Kobe, Japan

ARTICLE INFO

Article history:
Received 29 July 2012
Received in revised form 8 November 2012
Accepted 27 November 2012
Available online xxxx

Keywords:
Systemic juvenile idiopathic arthritis
Interleukin-6
Interleukin-18
Cytokine profiles

ABSTRACT

To assess the serum interleukin (IL)-6 and IL-18 levels in patients with systemic juvenile idiopathic arthritis (s-JIA) and to identify the clinical features of patient subsets with different cytokine profiles, we analyzed the serum levels of IL-6 and IL-18 in patients with s-JIA and compared them with the clinical features of s-JIA. Eighteen patients were analyzed. IL-6 and IL-18 levels were quantified in serum by enzyme-linked immunosorbent assays. Interestingly, two distinct s-JIA patient subsets based on their serum IL-6 and IL-18 levels were identified: an IL-6 dominant and an IL-18 dominant. The serum IL-6 and IL-18 levels were consistent both at relapse and at the onset of s-JIA in each subset. The IL-6-dominant subset had a significantly greater number of joints with active disease and higher serum levels of matrix metalloproteinase-3, whereas the IL-18-dominant subset was more likely to develop macrophage activation syndrome (MAS). These findings indicate that two subsets of patients with s-JIA, one which is prone for arthritis and another with prone for MAS, can be identified on the basis of their serum IL-6 and IL-18 levels. These two subsets appear to be characterized by certain distinct clinical features.

1. Introduction

Systemic juvenile idiopathic arthritis (s-JIA) is characterized by the clinical features of remitting fever, typical skin rash, and arthritis. Recent investigations into the pathophysiology of s-JIA have focused on mediators of the innate immune system. In particular, interleukin (IL)-1, IL-6, and IL-18 levels are correlated with disease activity and secondary complications [1,2]. Biological agents that inhibit these pivotal inflammatory cytokines (specifically, IL-1 and IL-6) have already changed the approach for the treatment of s-JIA [3,4]. There is accumulating evidence that inhibition of IL-1 or IL-6 is highly efficacious in a significant number of patients with s-JIA, with improvements seen in both systemic symptoms and arthritis [3,4].

A recent study showed that there were two subsets of s-JIA that had some distinct clinical features that could be identified on the basis of patients' responses to IL-1 blockade [5]. We previously reported that some patients with s-JIA had incomplete responses to IL-6 blockade and had macrophage activation syndrome (MAS), the most devastating complication of s-JIA [6]. In these patients, serum IL-18 is a promising marker of s-JIA disease activity [6].

On the basis of these findings, we hypothesized that there are different subsets of s-JIA with distinct cytokine profiles and clinical features. In this study, we examined serum IL-6 and IL-18 levels in patients with s-JIA during the active phase of the disease. We identified two subsets of patients with s-JIA having certain distinct clinical features. These subsets could be identified on the basis of IL-6 and IL-18 levels.

2. Methods

2.1. Patients and samples

Serum samples were obtained from 18 patients with s-JIA. Samples were obtained during the active phase of s-JIA. The diagnosis of s-JIA was made on the basis of the criteria of the International League of Associations for Rheumatology [7]. MAS was diagnosed on the basis of a combination of clinical features, including cytopenia or sudden decrease in white blood cell counts and/or platelet counts, coagulopathy, and liver dysfunction, according to the guidelines proposed by Ravelli et al. [8]. The criteria for the active phase of s-JIA were active arthritis, fever, rash, hepatosplenomegaly, generalized lymphadenopathy, and serositis, as well as increased C-reactive protein (CRP) levels and erythrocyte sedimentation rate (ESR). The clinical characteristics of these patients are shown in Table 1. Some patients had minimal joint disease at the onset of s-JIA and the presence of arthritis was confirmed later.

Abbreviations: s-JIA, systemic juvenile idiopathic arthritis; MAS, macrophage activation syndrome; IL, interleukin.

* Corresponding author. Tel.: +81 76 265 2314; fax: +81 76 262 1866.
E-mail address: shimizum@staff.kanazawa-u.ac.jp (M. Shimizu).

1043-4666/$ - see front matter © 2012 Elsevier Ltd. All rights reserved.
http://dx.doi.org/10.1016/j.cyto.2012.11.025

Please cite this article in press as: Shimizu M et al. Distinct subsets of patients with systemic juvenile idiopathic arthritis based on their cytokine profiles. Cytokine (2012), http://dx.doi.org/10.1016/j.cyto.2012.11.025

Table 1
Clinical features of the 2 s-JIA subsets based on serum IL-6 and IL-18 levels.

s-JIA (*n*, % or mean ± SD)	Group A (IL-6 dominant group) *n* = 10	Group B (IL-18 dominant group) *n* = 8	*P* value
Age	7.8 ± 4.2	10.6 ± 8.2	0.9797
Sex (male/female)	3/7	5/5	0.6499
Dosage of prednisolone (mg/kg/day)	0.05 ± 0.13(0–0.4)	0.14 ± 0.30(0–0.84)	0.7604
Clinical symptoms			
Fever	10(100)	8(100)	
Rash	6(60)	6(75)	0.6380
Lymphadenopathy	0(0)	1 (12.5)	1.0000
Hepatosplenomegaly	0(0)	1 (12.5)	1.0000
Arthritis (number of affected joints)	6.3 *t* 8.6	1.1 ± 1.1	0.0104
Macrophage activation syndrome	0(0)	4(50)	0.0229
Laboratory findings			
WBC (/mm^3)	15,189 ± 4436	16,391 ± 8940	0.9052
CRP (mg/dl)	13.2 ± 6.0	7.5 ± 3.4	0.0343
Ferritin (ng/ml)	1151 ± 821	4897 ± 6735	0.3964
MMP-3 (ng/ml)	343.3 ± 500.8	57.4 ± 75.8	0.0321
IL-6 (pg/ml)	133.9 ± 96.3	23.7 ± 42.6	0.0029
IL-18 (pg/ml)	20,420 ± 16,112	160,188 ± 105,344	0.0031

We serially determined the serum IL-6 and IL-18 levels of 4 patients. We measured their levels in active disease at relapses during tapering of the dosage of steroid. Serum was extracted from blood samples, divided into aliquots, frozen, and stored at −80 °C until use. This study was approved by the Institutional Review Board of Kanazawa University and all the patients provided informed consent.

2.2. Serum cytokine level measurements

Serum IL-18 and IL-6 levels were determined using commercial enzyme-linked immunosorbent assays (ELISA) according to the manufacturers' instructions (IL-18: MBL, Nagoya, Japan; IL-6: R&D Systems, Inc, Minneapolis, MN, USA). We also determined serum TNF-α, IFN-β and IL-1β levels using commercial ELISA kit according to the manufacturers' instructions (TNF-α, IFN-β, IL-1β: R&D Systems, Inc.).

2.3. Statistical analysis

Results are given as means ± standard deviations. Comparisons between groups were made by the Mann–Whitney *U*-test or Fisher's exact probability test, as appropriate. $P < 0.05$ was considered statistically significant.

3. Results

3.1. Different subsets of patients with s-JIA based on their serum IL-6 and IL-18 levels

As shown in Fig. 1A, there were two subsets of patients with s-JIA based on their serum IL-6 and IL-18 level: IL-6 dominant ($n = 10$) (Group A) and IL-18 dominant ($n = 8$) (Group B). As shown in Table 1, serum IL-6 levels in Group A patients (mean ± SD, 133.9 ± 96.3 pg/ml) were significantly higher than those in Group B patients (23.7 ± 42.6 pg/ml) ($P < 0.01$). On the other hand, serum IL-18 levels in Group B patients (mean ± SD, 160,188 ± 105,344 pg/ml) were significantly higher than those in Group A patients (20,420 ± 16,112 pg/ml) ($P < 0.01$). We could serially determine the serum IL-6 and IL-18 levels of only two patients in each subset. In both subsets, the serum IL-6 and IL-18 levels at relapse were similar to those at the onset of s-JIA (Fig. 1B).

3.2. Distinct clinical features of the 2 s-JIA subsets based on their serum IL-6 and IL-18 levels

Next, we compared the clinical features of each subset (Table 1). The patients in Group A had a significantly greater number of active joints ($P < 0.05$). Serum matrix metalloproteinase (MMP)-3 levels and C-reactive protein levels were significantly higher in Group A than in Group B. Four of 10 patients in Group B had the complication of MAS, whereas no patients in Group A experienced MAS during their clinical courses. Serum TNF-α, IFN-β and IL-1β levels were not detectable in both groups.

4. Discussion

Inflammatory cytokines are critical for perpetuating the inflammatory processes in s-JIA. Previous studies suggested pivotal roles for the three proinflammatory cytokines IL-6, IL-1, and IL-18 [1,2]. IL-6 has an important role in the systemic manifestations and arthritis observed in s-JIA. IL-6 is markedly elevated in both the peripheral blood and synovial fluid of patients with s-JIA and its expression appears to be correlated with disease activity and certain clinical features, such as fever patterns, growth retardation, and osteoporosis [9].

Recently, the important role of IL-1β in s-JIA has become appreciated. Increased IL-1β can result in fever, anorexia, and pain hypersensitivity, and the dysregulation of its levels can lead to the clinical and laboratory findings of s-JIA. Pascual et al. showed that culturing peripheral blood mononuclear cells from healthy persons with serum from patients with s-JIA increased the IL-1β secretion of these cells; increased production of IL-1β protein by mononuclear cells from patients with active s-JIA was also observed [10].

IL-18 was originally described as an IFN-γ-inducing factor that was primarily produced by activated cells of the macrophage lineage. IL-18 stimulates a variety of inflammatory responses. Serum IL-18 levels are increased in patients with s-JIA, as we previously reported [11]. Another report showed that an imbalance between IL-18 and its natural inhibitor, IL-18-binding protein, resulted in Th-1 lymphocyte and macrophage activation, which escaped the control by natural killer (NK) cell-mediated cytotoxicity and may have allowed for secondary hemophagocytic syndrome [12]. A relationship between high IL-18 levels and impaired NK cell func-

Please cite this article in press as: Shimizu M et al. Distinct subsets of patients with systemic juvenile idiopathic arthritis based on their cytokine profiles. Cytokine (2012), http://dx.doi.org/10.1016/j.cyto.2012.11.025

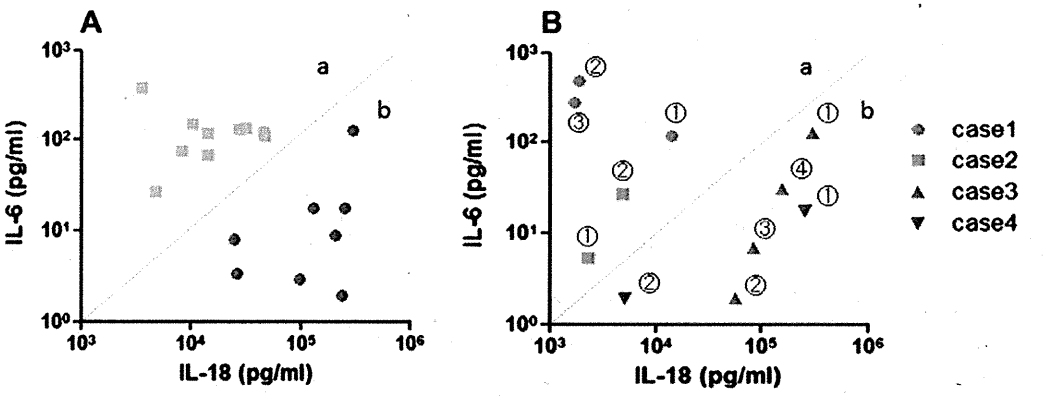


Fig. 1. (A) Two subsets of patients with s-JIA based on their serum IL-6 and IL-18 levels. a: IL-6-dominant subset. b: IL-18-dominant subset. (B) Serum IL-6 and IL-18 levels at time of relapse in 4 s-JIA cases. Cases 1 (blue circles) and 2 (green squares): IL-6-dominant subset; Cases 3 (orange triangle) and 4 (red triangle): IL-18-dominant subset.

tion in s-JIA has been reported [13]. These findings indicate that high serum IL-18 levels may be closely associated with the development of MAS in s-JIA.

Biological agents that inhibit some of these pivotal inflammatory cytokines (e.g., IL-1 and IL-6) have already changed the approach for the treatment of s-JIA. There is accumulating evidence that inhibition of IL-1 or IL-6 is highly efficacious in a significant number of patients with s-JIA, with improvements seen in both systemic symptoms and arthritis. Anakinra, an IL-1-receptor antagonist, has also been reported to be highly effective for treating s-JIA-associated MAS that is refractory to conventional therapy [14].

The disease course in the later stages of s-JIA is highly variable. About half of these patients have a monocyclic course, with remission occurring within 2–4 years [15]. However, other patients have a relapsing course, characterized by flares of systemic features and mild arthritis [15]. The persistence of systemic symptoms without arthritis is unusual and is seldom a cause of permanent disability. Most patients have persistent arthritis, which is usually more prominent after the regression of systemic features and typically lasts no more than 5 years [15]. These findings indicate that s-JIA is a heterogeneous disease both in terms of its severity and disease course, which suggests that there might be disease subsets with certain distinct clinical features.

In this study, we demonstrated that two subsets of patients with s-JIA could be identified on the basis of their serum IL-6 and IL-18 levels. The other cytokines including TNF-α, IFN-β and IL-1β in serum were not detectable in both groups. These two subsets appeared to be characterized by some distinct clinical features. The IL-6-dominant subset appeared to have more severe joint disease, whereas the IL-18-dominant subset appeared to be more likely to develop MAS. We previously reported that MAS developed during therapy with tocilizumab, a humanized anti-human IL-6 receptor monoclonal antibody, which indicated that the inhibition of IL-6 could not prevent this potentially fatal complication [6]. In these patients, serum IL-18 was a promising marker of s-JIA disease activity [6].

Interestingly, Gattorno et al. reported that two subsets of s-JIA with some distinct clinical features could be identified on the basis of patients' responses to IL-1 blockade [5]. In their study, the group with a complete response to anakinra had fewer joints with active disease [5]. On the basis of these findings, it appears that there are two subsets of patients with s-JIA. One is an IL-1/IL-18-dominant subset that has less active joint disease but is more likely to develop of MAS, and the other is an IL-6-dominant subset that has more active joint disease.

The limitation of this study is the small number of patients with s-JIA. It is necessary to perform larger scale study to confirm our preliminary data and draw firm conclusion. In spite of this limitation, our results indicate that two subsets of patients with s-JIA can be identified on the basis of their serum IL-6 and IL-18 levels. These two subsets appear to be characterized by certain distinct clinical features. Further mechanistic studies to determine the interplay among IL-1, IL-6, and IL-18 may help to identify predictors of a response to IL-1 or IL-6 inhibition, allowing a personalized approach to treatment.

Acknowledgment

We thank Harumi Matsukawa for technical assistance.

References

[1] Frosch M, Roth J. New insights in systemic juvenile idiopathic arthritis—from pathophysiology to treatment. Rheumatology 2008;47:121–5.
[2] de Jager W, Hoppenreijs EP, Wulffraat NM, Wedderburn LR, Kuis W, Prakken BJ. Blood and synovial fluid cytokine signatures in patients with juvenile idiopathic arthritis: a cross-sectional study. Ann Rheum Dis 2007;66:588–9.
[3] Verbsky JW, White AJ. Effective use of the recombinant interleukin 1 receptor antagonist anakinra in therapy resistant systemic onset juvenile rheumatoid arthritis. J Rheumatol 2004;31:2071–5.
[4] Yokota S, Imagawa T, Mori M, Miyamae T, Aihara Y, Takei S, et al. Efficacy and safety of tocilizumab in patients with systemic-onset juvenile idiopathic arthritis: a randomised, double-blind, placebo-controlled, withdrawal phase III trial. Lancet 2008;371:998–1006.
[5] Gattorno M, Piccini A, Lasiglié D, Tassi S, Brisca G, Carta S, et al. The pattern of response to anti-interleukin-1 treatment distinguishes two subsets of patients with systemic-onset juvenile idiopathic arthritis. Arthritis Rheum 2008;58:1505–15.
[6] Shimizu M, Nakagishi Y, Kasai K, Yamasaki Y, Miyoshi M, Takei S, et al. Tocilizumab masks the clinical symptoms of systemic juvenile idiopathic arthritis-associated macrophage activation syndrome: the diagnostic significance of interleukin-18 and interleukin-6. Cytokine 2012;58:287–94.
[7] Petty RE, Southwood TR, Manners P, Baum J, Glass DN, Goldenberg J, et al. International league of associations for rheumatology classification of juvenile idiopathic arthritis: second revision, Edmonton. J Rheumatol 2001;31(2004):390–2.
[8] Ravelli A, Magni-Manzoni S, Pistorio A, Besana C, Foti T, Ruperto N, et al. Preliminary diagnostic guidelines for macrophage activation syndrome complicating systemic juvenile idiopathic arthritis. J Pediatr 2005;146:598–604.
[9] de Benedetti F, Massa M, Robbioni P, Ravelli A, Burgio GR, Martini A. Correlation of serum interleukin-6 levels with joint involvement and thrombocytosis in systemic juvenile rheumatoid arthritis. Arthritis Rheum 1991;34:1158–63.
[10] Pascual V, Allantaz F, Patel P, Palucka AK, Chaussabel D, Banchereau J. How the study of children with rheumatic diseases identified interferon-α and interleukin-1 as novel therapeutic targets. Immunol Rev 2008;223:39–59.
[11] Shimizu M, Yokoyama T, Yamada K, Kaneda H, Wada H, Wada T, et al. Distinct cytokine profiles of systemic-onset juvenile idiopathic arthritis-associated

Please cite this article in press as: Shimizu M et al. Distinct subsets of patients with systemic juvenile idiopathic arthritis based on their cytokine profiles. Cytokine (2012), http://dx.doi.org/10.1016/j.cyto.2012.11.025

macrophage activation syndrome with particular emphasis on the role of interleukin-18 in its pathogenesis. Rheumatology (Oxford) 2010;49:1645–53.
[12] Mazodier K, Marin V, Novick D, Farnarier C, Robitail S, Schleinitz N, et al. Severe imbalance of IL-18/IL-18BP in patients with secondary hemophagocytic syndrome. Blood 2005;106:3483–9.
[13] de Jager W, Vastert SJ, Beekman JM, Wulffraat NM, Kuis W, Coffer PJ, et al. Defective phosphorylation of interleukin-18 receptor beta causes impaired natural killer cell function in systemic-onset juvenile idiopathic arthritis. Arthritis Rheum 2009;60:2782–93.
[14] Miettunen PM, Narendran A, Jayanthan A, Behrens EM, Cron RQ. Successful treatment of severe paediatric rheumatic disease-associated macrophage activation syndrome with interleukin-1 inhibition following conventional immunosuppressive therapy: case series with 12 patients. Rheumatology (Oxford) 2011;50:417–9.
[15] Woo P. Systemic juvenile idiopathic arthritis: diagnosis, management, and outcome. Nat Clin Pract Rheumatol 2006;2:28–34.

Please cite this article in press as: Shimizu M et al. Distinct subsets of patients with systemic juvenile idiopathic arthritis based on their cytokine profiles. Cytokine (2012), http://dx.doi.org/10.1016/j.cyto.2012.11.025

Rheumatology Advance Access published July 23, 2012

Letter to the Editor (case report)

doi:10.1093/rheumatology/kes181

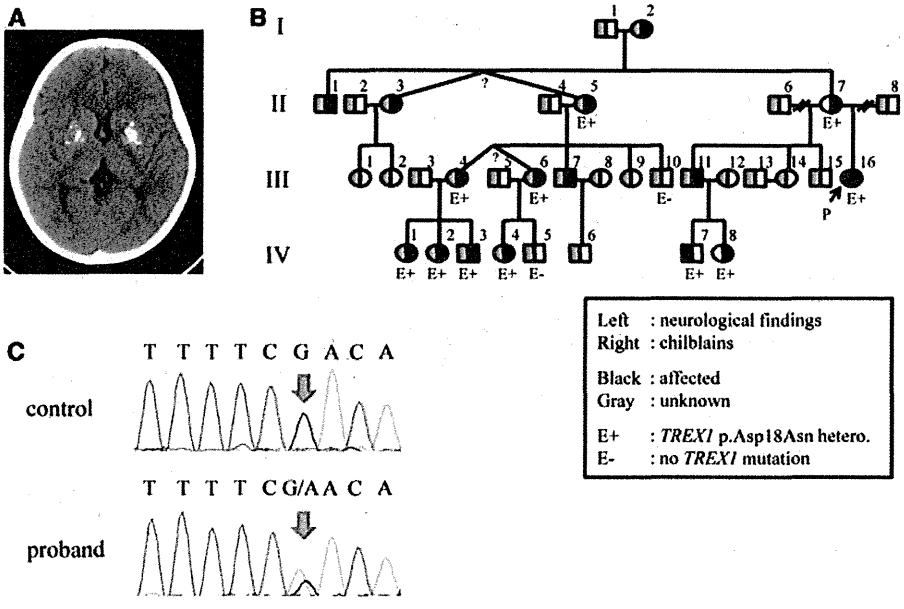
Heterozygous *TREX1* p.Asp18Asn mutation can cause variable neurological symptoms in a family with Aicardi-Goutières syndrome/familial chilblain lupus

SIR, we present the first case of Aicardi-Goutières syndrome (AGS) and familial chilblain lupus (FCL) in the same family as a result of the evaluation of a three-generation family with chilblains caused by a heterozygous *TREX1* p.Asp18Asn mutation. AGS is a genetically determined early-onset encephalopathy that features acquired microcephaly, cerebral calcifications, leukodystrophy and cerebral atrophy, as well as chilblains (~40%) [1, 2]. As previous reports have highlighted the phenotypic overlap, AGS is one of the important disorders for differential diagnosis of SLE [3]. To date, five genes responsible for AGS have been identified: *TREX1*, *RNASEH2B*, *RNASEH2C*, *RNASEH2A* and *SAMHD1* [4, 5]. *TREX1* and *SAMHD1* are also responsible for FCL, a Mendelian-inherited chilblain lupus that is a rare cutaneous form of SLE and lacks neurological abnormalities [3, 6–8]. In spite of the similarities between AGS and FCL, the variation and continuity of the two conditions are unknown because of the extreme rarity.

The proband, a 12-year-old girl, was born at 38 weeks after an uncomplicated pregnancy to unrelated parents. Her birth weight was 2840 g and no congenital infections were documented. From infancy, she had poor weight gain. Beginning at 1 year of age, she regularly developed severe chilblains on her fingers and toes during the winter. She had mental retardation (full-scale Intelligence Quotient 49) and short stature (−3.1 S.D.). Laboratory tests showed mild hypothyroidism with normal levels of serum immunoglobulins and complement; ANAs, aCLs, LA, cryoglobulin and cold agglutinin were all negative. A cranial CT scan revealed bilateral calcifications in the basal ganglia (Fig. 1A).

The proband has a family history of early-onset chilblains that led to finger amputations in patients I-2, II-1 and II-3 (Fig. 1B). The mother of the proband had suffered from chilblains in winter since childhood but her

FIG. 1 Examinations and pedigree.



(**A**) CT scan of the proband's head revealed bilateral calcifications in the basal ganglia. (**B**) Pedigree of the family. The left sides of the symbols indicate the presence of neurological findings and the right sides indicate chilblains. Affected individuals are indicated by black symbols, and individuals in whom symptoms could not be determined are indicated by grey symbols. Molecular investigation of the *TREX1* gene was used to identify family members heterozygous for the *TREX1* p.Asp18Asn mutation (E+), and those with no *TREX1* mutation (E−). (**C**) Molecular investigation of the *TREX1* gene of the proband.

symptoms had ameliorated with age. She never showed neurological symptoms. At the age of 47 years, a cranial CT scan revealed very mild bilateral calcifications in the basal ganglia, comparable to those of age-matched controls. The niece of the proband, a 6-year-old girl, has also suffered from chilblains in winter since infancy but lacks any evidence of neurological symptoms (FIQ 90), and her cranial CT scan was normal.

This study was approved by the ethical committee of Kyoto University, and after obtaining written informed consent according to the Declaration of Helsinki, genes related to cold-induced inflammation, namely *NLRP3*, *NLRP12* and the genes responsible for AGS were examined by direct sequencing of their exons and exon–intron boundaries. The heterozygous c.52G>A of *TREX1* leading to p.Asp18Asn (rs121908117) was identified in the proband as well as in her mother and niece (Fig. 1C). The proband was diagnosed with AGS according to the diagnostic criteria [1], and her mother and niece were diagnosed with FCL. Genetic analysis of the family members showed complete penetrance of the *TREX1* p.Asp18Asn mutation, except in patient IV-7 in terms of chilblains, whereas thorough neurological examinations were not performed because of lack of consent.

The majority of AGS patients show autosomal recessive traits. In the dominant type of AGS because of a *TREX1* mutation, the previously reported neurological findings associated with the p.Asp18Asn mutation ranged from normal to milder than the p.Asp200Asn and p.Asp200His mutations [3, 5–7, 9]. In the presented family, one of the three patients with the p.Asp18Asn mutation showed mild mental retardation and cerebral calcifications. All the reported patients with heterozygous *TREX1* mutations suffered from chilblains or vasculitis compatible with AGS [3, 5–7, 9]. In our study, 10 of the 11 patients with the p.Asp18Asn mutation suffered from chilblains. Thus, the majority of patients with heterozygous *TREX1* mutations have skin lesions, although the neurological complications vary among genotypes.

The TREX1 protein is a major component of 3′ to 5′ exonucleases [4]. The main pathophysiological mechanism of AGS is the overproduction of type I IFN, caused by unprocessed DNA because of reduced TREX1 enzymatic activity [4]. The TREX1 p.Asp18Asn, p.Asp200Asn and p.Asp200His heterodimers were shown to lack enzymatic activity, and the more aggressive neurological phenotypes can be explained by the greater inhibitory effects on wild-type TREX1 by mutations at Asp200 than at Asp18 [10]. It is intriguing that AGS patients in general have a lower rate of chilblains than those carrying the p.Asp18Asn mutation. To our knowledge, a correlation has yet to be established between the severity of neurological and skin lesions in AGS patients. A further accumulation of AGS and FCL cases and a delineation of the pathophysiology underlying the respective disorders are needed to understand how *TREX1* mutations result in the various clinical phenotypes of AGS and FCL.

In conclusion, we present a three-generation Japanese family with AGS and FCL caused by a heterozygous *TREX1* p.Asp18Asn mutation. The occurrence of AGS and FCL in the same family is noteworthy and points out the need for clinicians to carefully evaluate neurological complications in FCL patients.

Rheumatology key message

- Careful evaluation of neurological complications in FCL is important.

Funding: This work was supported by grants from the Japanese Ministry of Health, Labor, and Welfare [H23-ippan-nanchi-003, H22-ippan-nanchi-123].

Disclosure statement: The authors have declared no conflicts of interest.

Junya Abe[1], Kazushi Izawa[1], Ryuta Nishikomori[1], Tomonari Awaya[1], Tomoki Kawai[1], Takahiro Yasumi[1], Naoko Hiragi[2], Toru Hiragi[2], Yusei Ohshima[3] and Toshio Heike[1]

[1]*Department of Pediatrics, Kyoto University Graduate School of Medicine, Kyoto,* [2]*Department of Pediatrics, Tsuruga Municipal Hospital, Tsuruga and* [3]*Department of Pediatrics, Faculty of Medical Sciences, University of Fukui, Fukui, Japan.*
Accepted 28 May 2012
Correspondence to: Ryuta Nishikomon, Department of Pediatrics, Kyoto University Graduate School of Medicine, 54 Kawahara-cho, Shogoin, Sakyo-ku, 606-8507 Kyoto, Japan.
E-mail: rnishiko@kuhp.kyoto-u.ac.jp

References

1 Orcesi S, La Piana R, Fazzi E. Aicardi-Goutieres syndrome. Br Med Bull 2009;89:183–201.

2 Rice G, Patrick T, Parmar R *et al.* Clinical and molecular phenotype of Aicardi-Goutieres syndrome. Am J Hum Genet 2007;81:713–25.

3 Rice G, Newman WG, Dean J *et al.* Heterozygous mutations in TREX1 cause familial chilblain lupus and dominant Aicardi-Goutieres syndrome. Am J Hum Genet 2007;80:811–15.

4 Crow YJ, Rehwinkel J. Aicardi-Goutieres syndrome and related phenotypes: linking nucleic acid metabolism with autoimmunity. Hum Mol Genet 2009;18:R130–6.

5 Ramantani G, Kohlhase J, Hertzberg C *et al.* Expanding the phenotypic spectrum of lupus erythematosus in Aicardi-Goutières syndrome. Arthritis Rheum 2010;62:1469–77.

6 Lee-Kirsch MA, Gong M, Schulz H *et al.* Familial chilblain lupus, a monogenic form of cutaneous lupus erythematosus, maps to chromosome 3p. Am J Hum Genet 2006;79:731–7.

7 Lee-Kirsch MA, Chowdhury D, Harvey S *et al.* A mutation in TREX1 that impairs susceptibility to granzyme A-mediated cell death underlies familial chilblain lupus. J Mol Med 2007;85:531–7.

8 Ravenscroft JC, Suri M, Rice GI, Szynkiewicz M, Crow YJ. Autosomal dominant inheritance of a heterozygous mutation in SAMHD1 causing familial chilblain lupus. Am J Med Genet A 2011;155A:235–7.

9 Haaxma CA, Crow YJ, van Steensel MA *et al*. A de novo p.Asp18Asn mutation in TREX1 in a patient with Aicardi-Goutières syndrome. Am J Med Genet A 2010; 152A:2612–17.

10 Fye JM, Orebaugh CD, Coffin SR, Hollis T, Perrino FW. Dominant mutation of the TREX1 exonuclease gene in lupus and Aicardi-Goutieres syndrome. J Biol Chem 2011; 286:32373–82.

臨床免疫・アレルギー科，57(5)：582-586, 2012

Lecture

解　説

新生児期の自然免疫能*

高　田　英　俊**

Key Words : innate immunity, NK cells, neonate, IFN-γ

はじめに

新生児は，種々の感染症に罹患しやすく，健康成人と比較して免疫能が低下していると考えられているが，具体的に，生体防御機構のどの部分が低下しているのかを明確に理解する必要がある．今回は自然免疫能，特にNK細胞の機能に焦点をあて，新生児と健康成人との比較という観点から，われわれの知見を含めて述べることとする．

新生児の免疫能の特徴[1)2)]

新生児は好中球貯蔵プールが少ない．好中球や組織マクロファージの貪食能や殺菌能は成人と比較して大きく低下しているわけではないが，細菌に対する易感染性に関与している可能性はある．

NK細胞活性は低下しているが，IL-2を添加すると新生児NK細胞は成人NK細胞と同等のNK細胞活性を呈するようになる．対照的にIFN-γを加えた際のNK細胞活性の増強は低下している．

新生児の末梢血樹状細胞(DC)はpreDC2が75%を占めており，残りの25%は成人のDC1に類似するがCD83の発現がない．これらのDCの未熟性が，抗原刺激に対するT細胞の低反応性やTh2優位性，細胞内寄生菌に対する低反応性などに関与していると考えられる．

新生児の補体成分は全体的に成人の約40～60%である．特に，C8とC9はそれぞれ成人の20%，およびそれ以下と低下している．補体成分が成人値に達するのは生後6～18か月である．

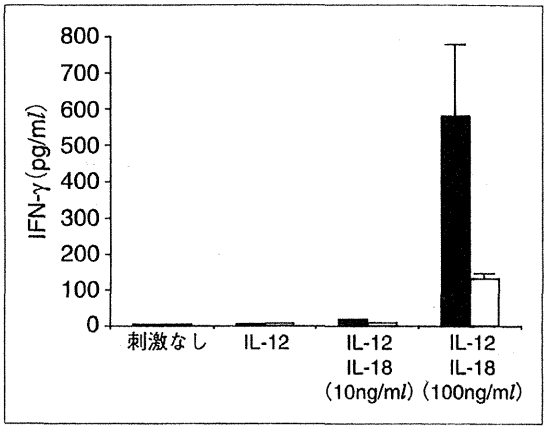


図1
臍帯血NK細胞は成人末梢血NK細胞よりもIL-18刺激に対するIFN-γ産生能が高い．
臍帯血NK細胞(■)と，成人末梢血NK細胞(□)をマイクロビーズにより純化し，IL-12(40ng/ml)の存在下にIL-18で刺激し，培養上清中のIFN-γ濃度を測定した．
(文献[4]より改変)

新生児のT細胞はナイーブT細胞がほとんどを占めており生態圏の病原体に対する免疫学的メモリー機構が成立していない．また，Th1細胞や細胞傷害性T細胞の分化能が低下していることは，新生児の細胞性免疫能の低下の重要な要因である．満期産新生児の場合，母体からの移行抗体により出生直後の血清IgG値は成人レベルであるが，生後4～6か月頃に最も低下し成人の30%程度となる．早産時やIUGR児では出生時の血清IgG値は定値であり，長期間低γグロブリン

* Characteristic of neonatal innate immunity.

** Hidetoshi TAKADA, M.D., Ph.D.: 九州大学大学院医学研究院成長発達医学分野〔〒812-8582 福岡県福岡市東区馬出3-1-1〕; Department of Pediatrics, Graduate School of Medical Sciences, Kyushu University, Fukuoka 812-8582, JAPAN

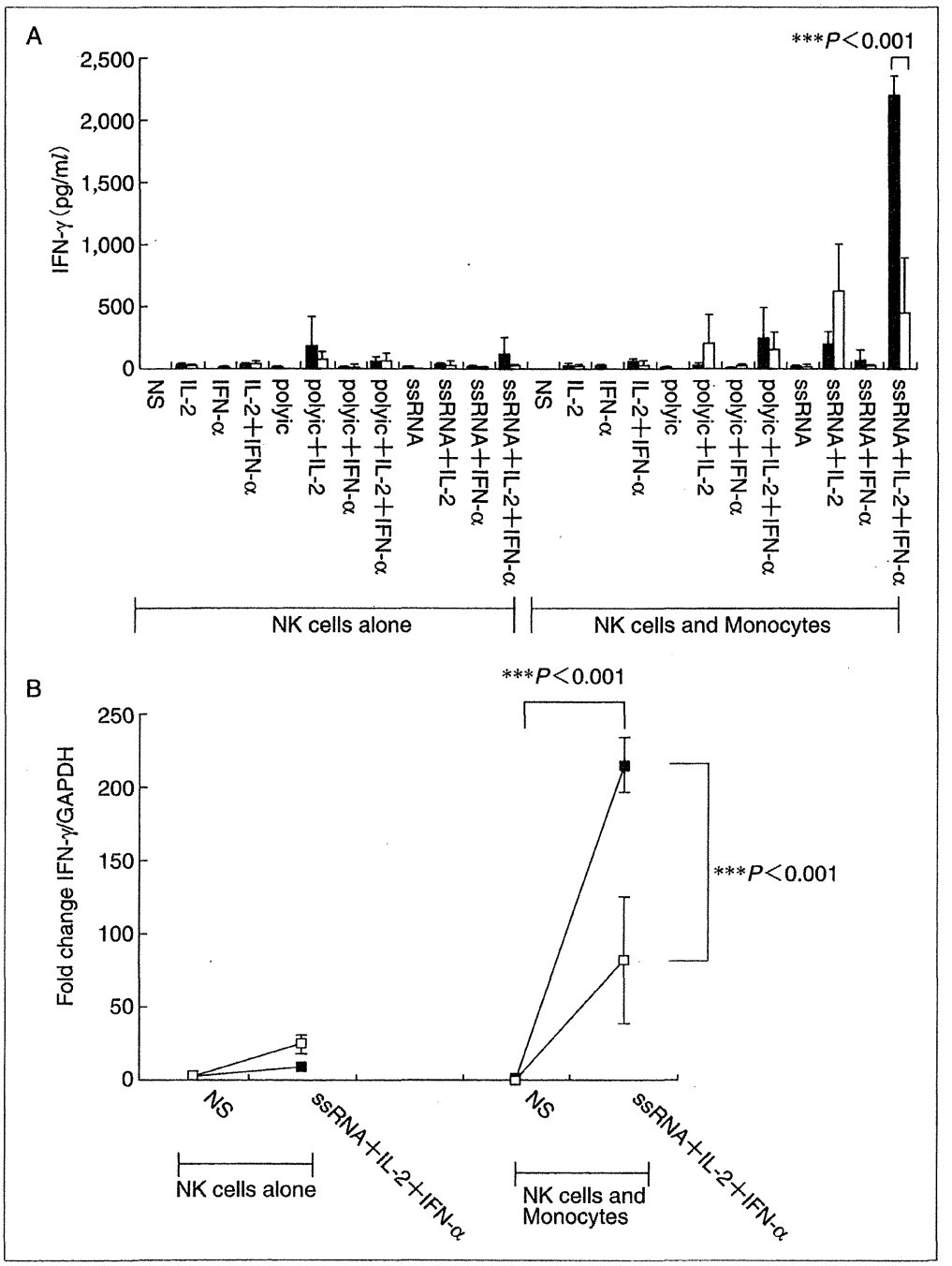


図2

NK細胞はssRNA(TLR8 ligand)に反応してIFN-γを産生し，臍帯血NK細胞がIFN-γ産生能が高い．

A：純化したNK細胞を種々のTLRで刺激し，培養上清中のIFN-γ濃度を測定した．ssRNA：2 μg/ml，IL-2：1,000U/ml，IFN-α：1,000U/ml，■：臍帯血NK細胞，□：成人末梢血NK細胞

B：培養後に細胞を回収し，IFN-γ mRNAの発現量を定量的PCR法で測定した．■：臍帯血NK細胞，□：成人末梢血NK細胞

血症が持続する．Th2細胞の分化能やCD40リガンド発現低下などによって，T細胞依存性抗体産生能が低下しており，特に多糖体抗原に対する抗体産生能が低下している．

新生児NK細胞の特徴[3]

新生児NK細胞は，inhibitory receptor complexであるCD94/NKG2A, CD158b/jの発現が新生児

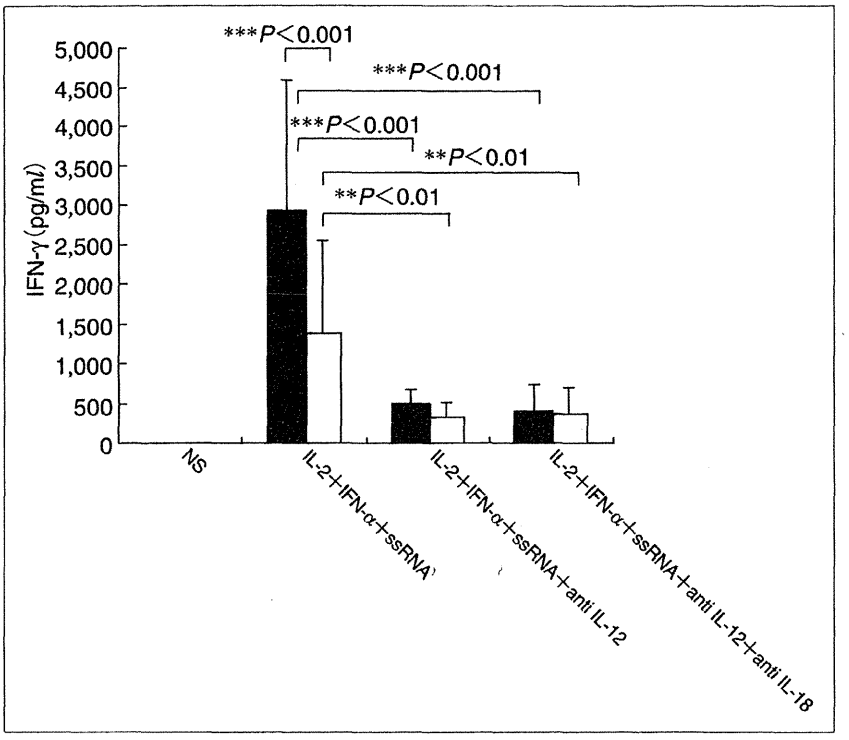


図4
ssRNA刺激によるNK細胞からのIFN-γ産生はIL-12 dependentである．
純化したNK細胞を，抗IL-12抗体あるいは抗IL-18抗体の存在下あるいは非存在下に，ssRNA, IL-2, IFN-α，単球とともに48時間培養した．■：臍帯血NK細胞，□：成人末梢血NK細胞

新生児NK細胞は成人末梢血NK細胞よりも細胞傷害性が低下している．しかし，IL-2やIL-12, IL-15で刺激した場合，細胞傷害性は成人のものと同等である．CD56^{bright} NK細胞とCD56^{dim} NK細胞の割合は，新生児NK細胞と成人末梢血NK細胞とで差を認めない．CD56^{bright} NK細胞は細胞傷害性が低い集団であるが，新生児NK細胞と成人NK細胞で細胞傷害性に差はみられない．新生児CD56^{dim} NK細胞の細胞傷害性は，成人のものよりも明らかに低下している．この理由の一つとして，新生児NK細胞はtarget細胞K562への結合能が低いことがあげられ，CD2, CD11a, CD18, CD54などのadhesion moleculeの発現が低いことと関連していると考えられている．新生児NK細胞では，IL-12やIL-15刺激によってこれらのadhesion moleculeの発現が急速に起こる．機能抑制性のCD94/NKG2やKIRなどのinhibitory receptor complexの発現が高いことも新生児NK活性が低いことと関連していると考えられている．Glucocorticoid-induced tumor necrosis factor receptor(GITR)の発現も新生児NK細胞が高い．また，制御性T細胞が新生児に高い割合でみられることも関連している可能性がある．

新生児NK細胞のサイトカイン産生能[3)]

Mitogen刺激後のNK細胞からのGM-CSF, TNF-α, IFN-γ産生能は新生児で低下しているとの報告があり，これはNFAT1の発現が新生児NK細胞で低いことと関連していると考えられている．一方，IFN-γ産生能は成人とかわらないとする報告もある．

CD56^{bright} NK細胞はINF-γ産生の主体となる細胞である．IFN-γはウイルス感染防御に重要な役割を果たしている．新生児がヘルペスウイルスなどのウイルスに対して易感染性を呈することの原因としてT細胞による細胞性免疫能が未熟であることがあげられる．しかし，新生児NK細胞のIFN-γ産生能が新生児の易感染性にどの程度関与しているかは明らかになっていない．以前われわれは，NK細胞のIL-18に対するIFN-γ産生

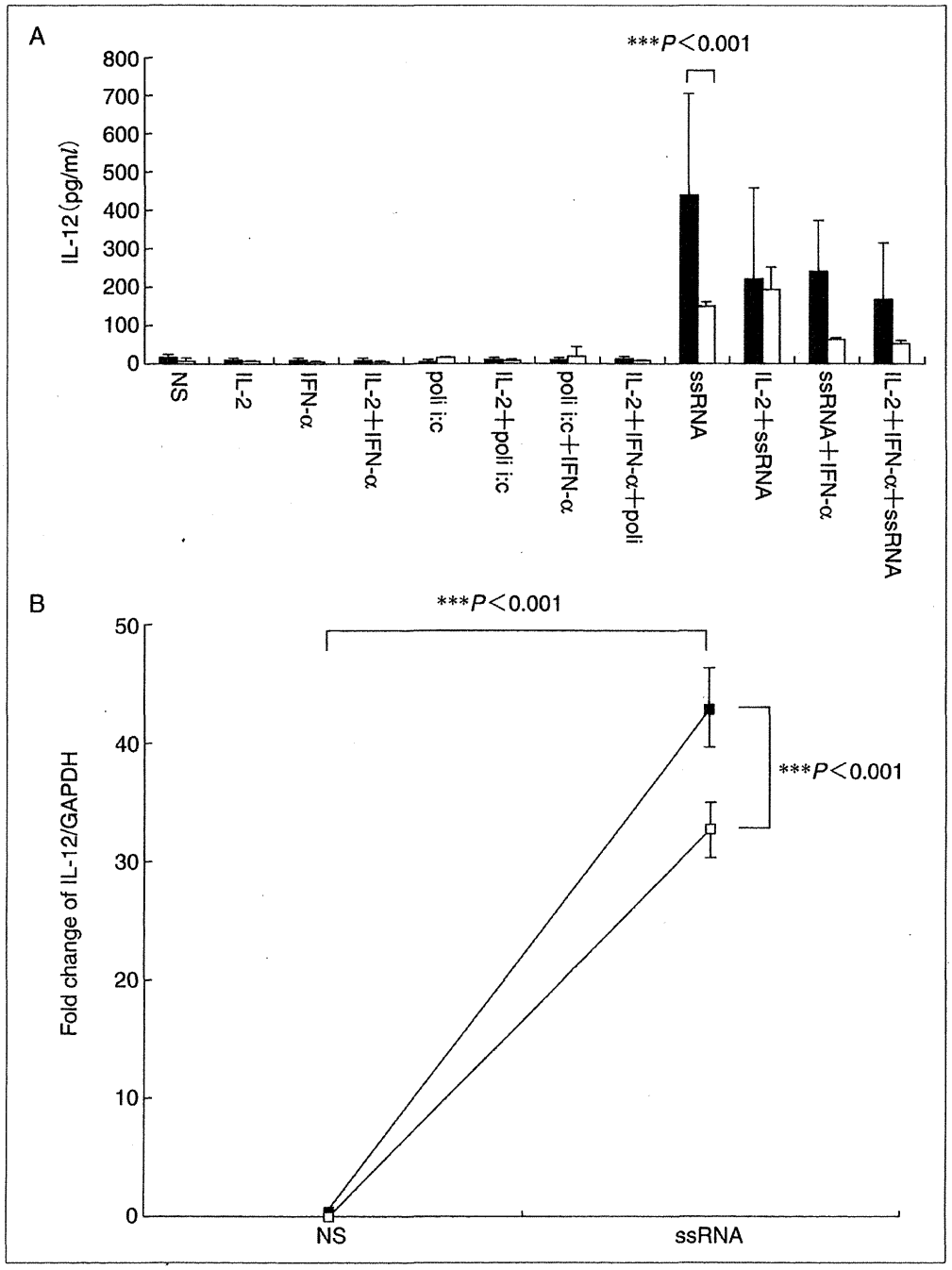


図3

単球はssRNA刺激によりIL-12を産生し，臍帯血単球が成人末梢血単球よりIL-12産生能が高い．

A：マイクロビーズで純化した単球をssRNAで刺激し，48時間後に培養上清中のIL-12濃度を測定した．■：臍帯血NK細胞，□：成人末梢血NK細胞

B：培養後に細胞を回収し，IL-12 mRNAの発現量を定量的PCR法で測定した．■：臍帯血NK細胞，□：成人末梢血NK細胞

で高いという報告がある．新生児NK細胞はL-selectinの発現が低くリンパ節へのhoming能が低いと考えられている．また，CD2, CD11a, CD18, CD54などのadhesion moleculeの発現も低い．CD8やCD57などのNK細胞の最終的な成熟を示唆するマーカーの発現は弱い．他方，新生児NK細胞はTLR4の発現が高く，またeffector moleculeであるperforinやgranzyme Bの発現も高い．

能はIL-12依存性に起こり，新生児はIL-18に対する反応性が高いことを明らかにした(図1)[4]．

今回，NK細胞のウイルス感染に対するIFN-γ産生能を明らかにするため，臍帯血NK細胞を用いて，ウイルス感染に関連するToll-like receptor (TLR)からの刺激によるIFN-γ産生能を調べた[5]．

NK細胞をマイクロビーズで純化し，ウイルスの認識に関連すると考えられているTLR ligandで刺激し48時間後に培養上清中のIFN-γ濃度を測定したところ，図2-Aに示すようにTLR-8 ligandであるssRNA刺激の場合のみ高いIFN-γ産生能を示した．その場合，これまでの報告にあるように，IL-2, IFN-α，単球の存在が必要であった．このssRNA刺激に対するIFN-γ産生能は臍帯血NK細胞が成人末梢血NK細胞よりも高かった(図2-A)．NK細胞を回収し，IFN-γのmRNA発現を定量した場合でも同様の結果が得られた(図2-B)．

単球を加えないとNK細胞からのIFN-γ産生が起こらないことから，単球がssRNAを認識し，単球から産生されるIL-12やIL-18がNK細胞からのIFN-γ産生に重要な役割を果たしている可能性が考えらえる．そこで，単球を純化し，ssRNAで48時間刺激し，培養上清中のIL-12およびIL-18濃度を測定した．その結果，図3-Aに示すようにssRNA刺激によって単球はIL-12を産生した．有意なIL-18の産生は認められなかった．培養後に単球を回収し，IL-12 mRNA発現を定量しても同様の結果が得られた(図3-B)．これらのことから，単球由来のIL-12がNK細胞からのIFN-γ産生に重要であることが推測された．

このことを確認するために，抗IL-12抗体，抗IL-18抗体の存在/非存在下に，ssRNA, IL-2, IFN-α，単球とともに純化したNK細胞を培養し，培養上清中のIFN-γ濃度を測定した．その結果，図4に示すように，抗IL-12抗体の存在によりNK細胞からのIFN-γの産生はほぼ完全に抑制された．抗IL-18抗体の効果はみられなかった．これらの結果から，ssRNA刺激による単球からのIL-12産生が，NK細胞からのIFN-γ産生に重要であることがわかった．

考　察

新生児は単純ヘルペスウイルス，サイトメガロウイルス，水痘帯状疱疹ウイルス，RSウイルスなど種々のウイルスに対して易感染性を呈する．新生児のT細胞機能の未熟性がこれらの原因としてあげられているが，NK細胞がどの程度関与しているのかは不明であった．今回，新生児NK細胞は，TLR8 ligand刺激によって成人末梢血NK細胞よりも高いIFN-γ産生能を有していることがわかり，新生児NK細胞は，IL-2やIFN-α, IL-12などinnate immunityが誘導されている環境であれば十分機能していることを意味していると考える．実際にウイルス感染の場では，IFN-α, IL-2などのサイトカインがfibroblastやT細胞から産生されていることが確認されており，ウイルスの排除にはたらくNK細胞を活性化し，IFN-γ産生を誘導しているものと考えられる．新生児単球は，成人単球と同程度のTLRを発現していると報告されている．臍帯血単球がssRNAに反応して成人末梢血単球よりも多くのIL-12を産生したことは，臍帯血NK細胞が成人末梢血NK細胞よりも高いIFN-γ産生能を示した要因の一つであると考えられる．

以上のことを考慮すれば，新生児の未熟な獲得免疫系を，NK細胞や単球の機能が代償している可能性も考えられる．

文　献

1) 牛島廣治．免疫系の個体発生とその成熟．小林登，ほか・編．新小児医学体系19A, 小児感染免疫学I．東京：中山書店；1979．p. 207.
2) 原　寿郎．免疫能低下を合併する疾患：発育に伴う免疫能の変化．小児内科 2005；37：739.
3) Verneris MR, Miller JS. The phenotypic and functional characteristics of umbilical cord blood and peripheral blood natural killer cells. Br J Haematol 2009；147：185.
4) Nomura A, Takada H, Jin CH, et al. Functional analyses of cord blood natural killer cells and T cells : a distinctive interleukin-18 response. Exp Hematol 2001；29：1169.
5) Eljaafari FM, Takada H, Tanaka T et al. Potent induction of IFN-γ production from cord blood NK cells by the stimulation with single-stranded RNA. J Clin Immunol 2011；31：728.

IRAK4 欠損・MyD88 欠損をはじめとする Toll 様受容体（TLR）

—シグナル伝達の異常症に関する最近の知見—

大西秀典* 山本崇裕

はじめに

病原体に対するほ乳類の生体防御システムは，自然免疫と獲得免疫の大きく2つに区分される。2011年のノーベル医学・生理学賞は，自然免疫に関する受賞であったが，Hoffmanらはショウジョウバエの Toll 遺伝子の同定，Beutlerらはグラム陰性菌の細胞膜構成成分であるリポポリサッカライド（LPS）を認識する受容体である Toll 様受容体（TLR）4の同定がそれぞれ評価されたものである。彼らの発見以後，わが国でも Akiraらによって TLR ファミリーをはじめとする，種々のパターン認識受容体（pattern recognition receptor：PRR）や，それらのシグナル伝達機構が詳細に明らかにされてきている。

一方，これら自然免疫の構成分子が明らかになるに伴い，その分子の異常，ヒトにおける遺伝子変異が相ついで報告されている。インターロイキン1受容体関連キナーゼ（interleukin-1 receptor associated kinase：IRAK）4や骨髄分化因子88（myeloid differential factor 88：MyD88）は，TLR シグナルを細胞内で仲介する主要な分子である。2002年に Suzuki らによって，IRAK4 欠損マウスが作成され，そのマウスが TLR シグナル全般に不応答を示すことが報告されているが，次いで2003年に Picard らによってヒト IRAK4 欠損症が報告された。その後，TLR シグナル経路の遺伝子異常により発症する免疫不全症が相ついで報告されており，これらは当初高 IgM 症候群の1型として分類されていた NEMO 異常症を含める形で，2003年度に改訂された原発性免疫不全症の国際分類から innate immune defect として新たにカテゴリー分けされている（表）[1]。その中でとくに TLR シグナルに関連する疾患を，さらに大きく3グループに分けて以下に概説する。

Ohnishi Hidenori Yamamoto Takahiro
* 岐阜大学大学院医学系研究科小児病態学
〔〒501-1194 岐阜市柳戸1-1〕
TEL 058-230-6000 FAX 058-230-6387
E-mail：ohnishih@gifu-u.ac.jp

Ⅰ．Myddosome 異常症（IRAK4 欠損症，MyD88 欠損症）

1．IRAK4 欠損症

ヒトの IRAK は IRAK1，IRAK2，IRAK4，IRAK-M の4種類のファミリーメンバーで構成される分子群であるが，その中で IRAK4 は，上流の MyD88 と下流の IRAK2（あるいは IRAK1）をシーケンシャルにつなぐ必須分子であることが，タンパク立体構造解析の結果明らかにされている[2]。

IRAK4 欠損症は，前述のとおり2003年に報告された，乳幼児期にとくに侵襲性肺炎球菌感染症や黄色ブドウ球菌，緑膿菌などに対する易感染性を示す常染色体劣性遺伝形式の疾患である。IRAK4 は N 末端の Death ドメインと C 末端のキナーゼドメインの2つの機能性ドメインから構成される分子で，すでにこの分子上に20以上

表　自然免疫不全症

疾患名	影響を受ける細胞	機能障害	関連症状	遺伝型式	責任遺伝子
1．無汗性外胚葉形成不全免疫不全症（EDA-ID）					
a）X 連鎖（XL-）EDA-ID（NEMO 異常症）	リンパ球＋単球	NF-κB シグナル系	無汗性外胚葉形成不全＋特異抗体欠損＋種々の感染症（抗酸菌と化膿性細菌）	XL	*NEMO*
b）常染色体優性遺伝（AD-）EDA-ID	リンパ球＋単球	NF-κB シグナル系	無汗性外胚葉形成不全＋T 細胞欠損＋種々の感染症	AD	*IKBA*
2．IRAK4 欠損症	リンパ球＋単球	TIR-IRAK シグナル系	細菌感染症（化膿性細菌）	AR	*IRAK4*
3．MyD88 欠損症	リンパ球＋単球	TIR-IRAK シグナル系	細菌感染症（化膿性細菌）	AR	*MyD88*
4．WHIM 症候群	顆粒球＋リンパ球	CXCR4 のケモカインに対する反応過剰	低γグロブリン血症，B 細胞数減少 重度の好中球減少，疣贅/HPV 感染	AD	*CXCR4*
5．疣贅状表皮異形成症	ケラチノサイト＋リンパ球	不明	ヒトパピローマウイルス（B1）感染，皮膚癌	AR	*EVER1, EVER2*
6．家族性単純ヘルペス脳炎					
a）TLR3 欠損症	中枢神経系細胞＋線維芽細胞	TLR3 依存性 IFN-α，β，λ誘導	単純ヘルペスⅠ型脳炎	AD	*TLR3*
b）UNC93B1 欠損症	中枢神経系細胞＋線維芽細胞	UNC93B 依存性 IFN-α，β，λ誘導	単純ヘルペスⅠ型脳炎	AR	*UNC93B1*
c）TRAF3 欠損症	中枢神経系細胞＋線維芽細胞	TRAF3 依存性 IFN-α，β，λ誘導	単純ヘルペスⅠ型脳炎	AD	*TRAF3*
7．易真菌感染症	単核球	CARD9 シグナル系	侵襲性カンジダ感染，末梢性皮膚糸状菌症	AR	*CARD9*
8．慢性粘膜皮膚カンジダ症（CMC）					
a）IL-17RA 欠損症	上皮細胞，線維芽細胞，単核球	IL-17RA シグナル系	CMC	AR	*IL-17RA*
b）IL-17F 欠損症	T 細胞	IL-17F 2 量体	CMC	AD	*IL-17F*
c）STAT1 異常症（機能獲得型変異）	T 細胞	STAT1 機能獲得変異による Th17 細胞分化障害	CMC	AD	*STAT1*
9．トリパノゾーマ病	不明	APOL-I	トリパノゾーマ感染	AD	*APOL-I*

（Al-Herz ら[1] 2011 より一部改変）

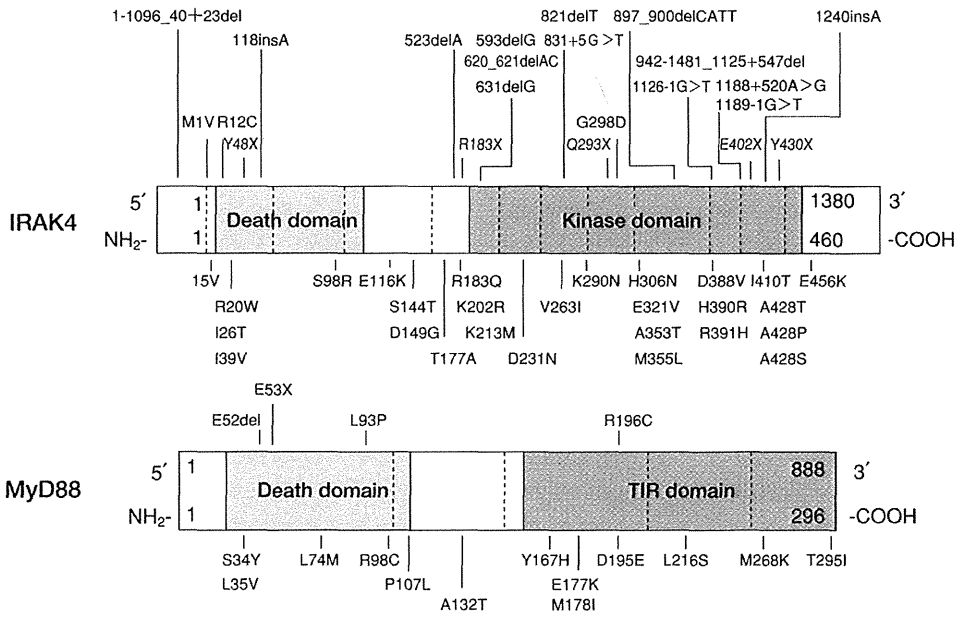


図 1　IRAK4 および MyD88 の遺伝子構造

それぞれの遺伝子の既報の遺伝子変異（Gene graph の上側），および dbSNP 上に掲載されているアミノ酸変異を伴う SNP（Gene graph の下側）を表記した。

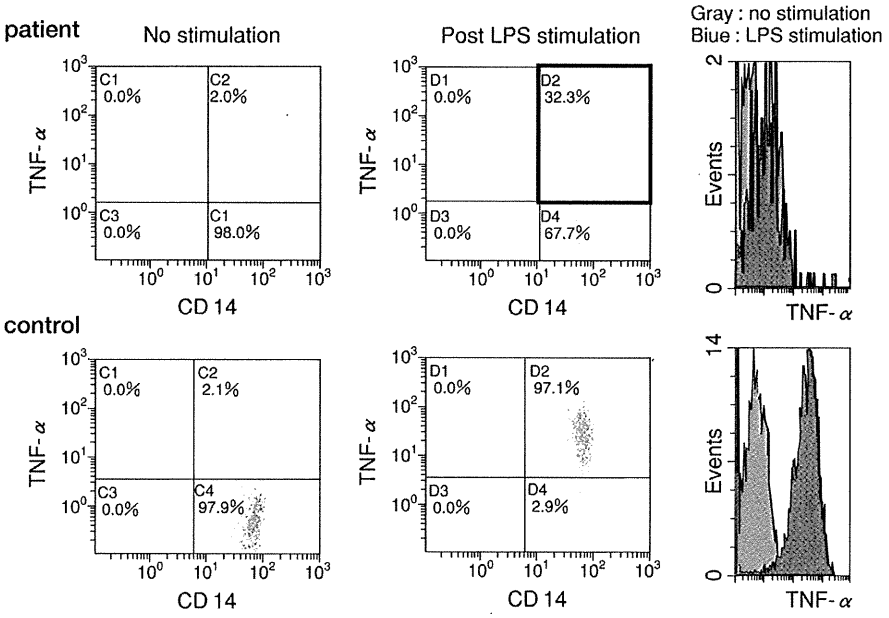


図 2　LPS 刺激後の末梢血単球内 TNF-α 産生率の検討
IRAK4 欠損症患者の LPS 刺激後の末梢血単球内 TNF-α 産生率は，健常者（90%以上）と比較して著しく低下している。

の遺伝子変異型が報告されている（図 1）。欧米では Q293X 変異の報告が最も多いのに対し，わが国では c.118insA の報告が多い。これら遺伝子変異の結果，IRAK4 タンパクの欠失あるいは，IRAK4 と MyD88 の相互作用の低下をきたし，IRAK4 欠損症が発症するものと考えられている。

IRAK4 欠損症では，上記の易感染性を示すにもかかわらず，血清免疫グロブリン値や末梢血中の免疫細胞数に明らかに異常を認めないため，診断を見逃す恐れがある。そこで，IRAK4 欠損症，ならびに後述の MyD88 欠損症では，Takada らが提唱している LPS 刺激後の末梢血単球内 TNF-α 産生率の検討が，患者の迅速診断に非常に有用である[3]。わが国で報告された IRAK4 欠損症症例の末梢血を，同法で検査した結果を提示する（図 2）。

厚生労働省難治性疾患克服研究事業の一環として，IRAK4 欠損症研究班が 2010～2011 年度に施行した全国症例調査によると，国内での IRAK4 欠損症の発生患者数は 7 名であった。IRAK4 欠損症患者 7 名中 4 名がすでに死亡しており，3 名が生存していた。IRAK4 の遺伝子型は 6 例が c.118insA のホモ接合性変異で，1 例は c.118insA と R183X のコンパウンドヘテロ接合性変異であった。主要な感染症罹患歴として，肺炎球菌化膿性髄膜炎，緑膿菌肝膿瘍，ブドウ球菌皮下膿瘍，緑膿菌壊死性筋膜炎があげられ，死亡例はそのいずれかによって致死的となっていた。臨床的な特徴として臍帯脱落遅延が指摘されており，わが国の症例でも 7 名中 6 名で生後 2 週間以上の遅延を認めている。従来から白血球接着不全症においても臍帯脱落遅延が特徴的な症状としてあげられているが，本症例でも特徴的な所見であるといえ，早期診断の指標となる可能性がある。検査データでは，致死的細菌感染症への罹患であることに比して，CRP の上昇が緩やかな症例がみられ，また肺炎球菌化膿性髄膜炎罹患症例では，致死的な程重篤であるにもかかわらず，髄液細胞数の上昇が少ないことがあることが判明した。IRAK4 欠損症症例 7 名全例で，血球分画や免疫グロブリン値（IgG，IgA，IgM）に明らかな異常を認めていない。

IRAK4 欠損症患者は，興味深いことに Picard らの全世界症例調査の報告によると 8 歳以降の重症感染症罹患の頻度が著しく低下しているため，それまでの乳幼児期の感染予防が本疾患の治

療の主体となる[4]。わが国の症例では，生存例3例のうち2例は，兄弟がIRAK4欠損症に合併した肺炎球菌化膿性髄膜炎で死亡したため，早期に診断されており，生後1歳まではγグロブリン製剤の補充療法を施行し，7価肺炎球菌ワクチンを抗体価の推移を追跡しながら3回接種，および23価肺炎球菌ワクチンを1回接種していた。残る1例でも同様に7価肺炎球菌ワクチンを3回，23価肺炎球菌ワクチンを1回接種していた。なお，3例とも肺炎球菌特異的IgG2抗体価の上昇が確認されている。加えて，ST合剤およびペニシリン製剤，あるいはST合剤単独の予防投与が行われており，感染罹患時に早めの抗菌薬投与（静注）を行うことで重篤な感染症への罹患を免れている。

2．MyD88欠損症

MyD88は，Toll-interleukin-1-receptor（TIR）ドメインとDeathドメインの2つの機能性ドメインから構成されるアダプター分子であり，TLRのTIRドメインとIRAK4のDeathドメインをつなぐ役割を有し，TLR3以外のTLRファミリーによる病原体認識後のシグナル伝達に必須の分子である。2008年にヒトでのMyD88遺伝子変異が化膿性細菌感染症罹患と関連することが報告された。Picardら[4]の全世界症例調査によると，MyD88欠損症は，IRAK4欠損症とほぼ同じ臨床的特徴をもち，肺炎球菌，黄色ブドウ球菌，緑膿菌に対する易感染性があること，年長者では重症感染罹患頻度が低下することが明らかにされた。遺伝形式は常染色体劣性遺伝であり，片側アレルのみの遺伝子変異では発症しない。MyD88欠損症では，Deathドメイン上のミスセンス変異2種（E52del，L93P），TIRドメイン上のミスセンス変異1種（R196C）が報告されている。デスドメイン構造内でE52，L93アミノ酸残基は，ドメイン構造の内部に位置し，この残基が置換されることで，ドメイン構造の安定性が失われることが推測される。また，同じくデスドメイン上に機能減損型亜型（S34Y，R98C）が報告されているが，これらもMyD88欠損症の発症遺伝子型になりうると推測している。一方，TIRドメイン上でR196残基は，われわれの解析したタンパク立体構造によると，タンパク表面に位置していることがわかる[5]。このアルギニンがシステインに置換されても，タンパク立体構造には影響しない。しかし，この残基はTIRドメイン同士の相互作用に重要なBBループとよばれるTIRドメイン間で保存性の高い配列領域に位置している。BBループ部分の機能変化の結果として，TLR4とMyD88を仲介するアダプター分子であるMalとMyD88側のタンパク間相互作用が減弱することが，われわれの検討で明らかにされている。また，ナンセンス変異も1種（E53X）報告されている[6]。興味深いことに，このナンセンス変異を有する家系の解析で，TLRシグナルの応答性が明らかに低下していながらも，過去に明らかな易感染性を示していない症例が存在しており，本疾患では特定の病原体（とくに肺炎球菌）の偶発的な感染が起きない限り，臨床症状が顕在化しない可能性が指摘される。すなわち，わが国ではMyD88欠損症の報告例は存在しないが，いまだ診断されていない症例が存在している可能性が考えられる。

3．Myddosome異常症の概念

IRAK4欠損症，MyD88欠損症の種々の遺伝子型の考察から，どちらの疾患もTLR-Mal-MyD88-IRAK4の連鎖的に会合する多量体構造，Myddosomeの形成不全により発症することが明らかになっている。臨床症状もほぼ共通であるため，われわれはこの2つの疾患を総称して，Myddosome異常症とよぶことを提案している。Myddosome異常症は，乳幼児期に肺炎球菌髄膜炎の反復罹患や緑膿菌による侵襲性感染をきたし，致死的経過をたどる例が多いため，早期診断による予防治療が重要となる。前述のとおり，8歳以降は重症感染症罹患頻度が低下することから，乳幼児期の重点的な感染対策が本疾患の対応に最も重要である。臍帯脱落遅延を見逃さないことや，本疾患が疑われた際に迅速診断スクリーニングを行うことで患者の早期診断が可能となり，適切な対応をすることが可能である。