cells, *SOX9* promoter activity was 2.3-fold higher in forskolin-treated cells than in vehicle-treated cells, whereas *SOX9* promoter activity was 2-fold lower in SQ22536-treated cells than in vehicle-treated cells (Figure 6B). Similar effects were observed in wild-type chondroprogenitor cells, although they were less pronounced. These data correlated well with the effects of forskolin and SQ22536 on *SOX9* mRNA expression. We also examined the effects of forskolin and SQ22536 on 3-D chondrocyte pellet formation (Figure 6C). Compared to pellets of vehicle-treated mutant cells, pellets of mutant cells treated with forskolin and SQ22536 were 2.0-fold larger and 2.1-fold smaller, respectively. Similar effects were observed in wild-type cells, although they were less pronounced. These data clearly indicate that up-regulation of *SOX9* following activation of adenylate cyclase is involved in the enhanced chondrogenesis of mutant iPSCs.

We next measured the cAMP concentration to demonstrate that the activity of adenylate cyclase is increased in mutant chondroprogenitor cells. The concentration of cAMP was 4-fold higher in mutant chondroprogenitor cells than in wild-type chondroprogenitor cells on days 15 and 36 (Figure 6D). By contrast, the concentration of cAMP was similar in mutant and wild-type iPSCs, in which NLRP3 expression was low.

Finally, we examined the level of phosphorylated CREB in chondroprogenitor cells. CREB is phosphorylated by cAMP-activated PKA. According to Western blot analysis, the level of phosphorylated CREB was higher in mutant chondroprogenitor cells than in wild-type chondroprogenitor cells on days 15 and 36 (Figure 6D). Taken together, these data indicate that the cAMP/PKA/CREB pathway plays an important role in the up-regulation of *SOX9*, and therefore enhanced chondrogenesis, in chondroprogenitor cells with mutant NLRP3 (Figure 6E).

DISCUSSION

Disease-specific iPSCs have been used extensively to investigate the pathogenesis of diseases and to discover novel drugs. This approach is particularly useful to study rare diseases because tissues are often difficult to obtain from patients with such diseases. In this study, we used disease-specific iPSCs to study NOMID. Using this approach, we produced chondrocyte tissues with mutant and wild-type NLRP3, and revealed a previously unidentified connection between the inflammasome-associated molecule NLRP3 and the master regulator of chondrocyte differentiation SOX9.

SOX9 was up-regulated during the differentiation of iPSCs into chondrocytes, and this was particularly pronounced in mutant iPSCs. During cartilage development, SOX9 is highly expressed in immature chondrocytes and is required for the condensation and differentiation of mesenchymal cells. During the early stages of chondrogenesis, SOX9 activates the transcription of many cartilage-specific ECM genes, including *COL2A1*, *ACAN*, and *COMP*, by directly interacting with SOX5 and SOX6 (28,29). Overexpression of *SOX9* in chondrocytes using a recombinant adeno-associated virus significantly increases the synthesis of major ECM components in chondrocytes, without affecting their proliferation, in vivo and in vitro (30,31). In addition, retroviral transduction of SOX9 increases ECM production in human chondrocytes in vitro (32). These data correlate well with our observation that SOX9 overexpression driven by mutated NLRP3 caused overproduction of ECM, but did not increase chondrocyte proliferation.

It remains to be determined how enhanced expression of SOX9 in chondrocytes leads to epiphyseal overgrowth in NOMID patients. Conditional transgenic mice have been used to show that overexpression of *SOX9* in *COL2A1*-positive cells inhibits terminal differentiation of hypertrophic chondrocytes and endochondral bone formation (29). Although we have not directly confirmed the expression level of SOX9 in samples derived from NOMID patients, this previous study might help to link the findings of the present study with the clinical phenotype of NOMID patients.

We identified the cAMP/PKA/CREB pathway as being critical for the up-regulation of *SOX9* mRNA in a mutant NLRP3–dependent manner. cAMP is an intracellular second messenger that is involved in a variety of cellular processes (33). cAMP/PKA/CREB signaling is crucial in chondrogenesis, and synergism between cAMP and SOX9 is particularly important (34–36). Cotransfection of CREB binding protein (CBP) and p300 increases SOX9 activity (35). PKA phosphorylates SOX9 and thereby increases SOX9 activity, which results in the up-regulation of the *COL2A1* promoter through the interaction between CBP and SOX9 (34). In addition, the PKA inhibitor H89 blocks chondrogenesis in the chick limb bud (36). These data support the idea that cAMP/PKA/CREB signaling up-regulates SOX9 to enhance chondrogenesis.

Using stromal cells established from a tumor-like lesion in a NOMID patient, Almeida et al (37) demonstrated that activation of the cAMP/PKA/CREB pathway leads to caspase 1 activation, release of IL-1β, and

consequently the proliferation of bone stromal cells. This suggests that bone lesions in NOMID are caused in an NLRP3 inflammasome–dependent manner. One explanation for the discrepancy between their data and ours is that no disease-causing NLRP3 mutation was identified in the patient in that previous study; therefore, an unknown genetic alteration may have caused the NOMID phenotype. Another explanation is that different cell types were analyzed in the two studies. The previous study analyzed bone stromal cells established from a tumor-like lesion that might have been a heterogeneous population, while we focused on a single cell type, namely, chondrocytes.

The lack of environmental factors and interactions with other cell populations in our model might have eliminated some contributions of the NLRP3 inflammasome and IL-1β pathway that occur in NOMID patients. Furthermore, our observations relied on an artificial differentiation system in which iPSCs were first differentiated into cells of neural crest character and then into chondrocytes by culture in the presence of various exogenous factors. Abnormal epiphyseal growth is specifically observed around the knee joints of NOMID patients; therefore, additional events might be required to trigger abnormal chondrocyte proliferation in vivo. It is also possible that specific factors produced by surrounding cells in unaffected joints prevent mutant chondrocytes from manifesting their phenotype. Further analyses of patients or patient-derived samples would provide a better understanding of the pathophysiology of arthropathy in NOMID.

The interaction between cAMP and NLRP3 has been studied in monocyte/macrophages, in which the NLRP3 inflammasome is activated following binding of extracellular Ca^{2+} to Ca^{2+}-sensing receptors (CaSRs) (38,39). One study reported that an increase in extracellular Ca^{2+} is detected by CaSRs, which leads to phospholipase C activation and subsequently the release of Ca^{2+} from the endoplasmic reticulum and down-regulation of cAMP. cAMP binds directly to NLRP3 and inhibits assembly of the NLRP3 inflammasome. Therefore, this decrease in the level of intracellular cAMP relieves this inhibition and thereby induces activation of the NLRP3 inflammasome (38). On the other hand, another study reported that an increase in the extracellular Ca^{2+} concentration induces an increase in the intracellular Ca^{2+} concentration, thereby leading to activation of the NLRP3 inflammasome, and this mechanism requires the CaSRs GPRC6A and CaSR, but not the down-regulation of cAMP (39). Thus, the effects of cAMP on the NLRP3 inflammasome in monocyte/macrophages remain a subject of controversy.

In the chondrocyte differentiation system used in the present study, mutated NLRP3 caused SOX9 overexpression via the cAMP/PKA/CREB pathway, which is at odds with the relationship between cAMP and activation of the NLRP3 inflammasome in monocyte/macrophages. This discrepancy might be explained by the absence of other NLRP3 inflammasome components, such as ASC and procaspase 1, in the chondrocytes generated in the present study. Further analysis is needed to determine why cAMP/PKA/CREB signaling elicits different effects on mutated NLRP3 in chondrocytes and monocyte/macrophages, as well as how intracellular cAMP is up-regulated in chondrocytes derived from mutant iPSCs.

There have been many reports on the differentiation of chondrocytes from embryonic stem cells (ESCs) or iPSCs (40–42). However, previously, it was difficult to differentiate a sufficient number of chondrocytes with a relatively mature phenotype from ESCs or iPSCs, especially human ESCs or iPSCs. We have recently established a cartilage differentiation system in which iPSCs first differentiate into cells of neural crest character and then into chondrocytes, which enabled us to obtain a large number of chondrocytes with the phenotype of growth plate cartilage chondrocytes. An important aspect of the present study is that this differentiation system can generate a large number of chondrocytes that could share functional properties causing the arthropathy observed in NOMID. This system could thereby be used to screen for novel therapeutic agents.

In conclusion, we showed that SOX9 is overexpressed via the cAMP/PKA/CREB signaling pathway in chondrocytes with disease-causing mutations in NLRP3, and this causes overproduction of ECM independently of the NLRP3 inflammasome. We used iPSC technology to elucidate the role of chondrocytes in the pathophysiology of the human disease NOMID.

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AUTHOR CONTRIBUTIONS

All authors were involved in drafting the article or revising it critically for important intellectual content, and all authors approved the final version to be published. Drs. Nishikomori and Toguchida had

full access to all of the data in the study and take responsibility for the integrity of the data and the accuracy of the data analysis.
Study conception and design. Yokoyama, Ikeya, Tanaka, Nishikomori, Nakayama, Nakahata, Heike, Toguchida.
Acquisition of data. Yokoyama, Umeda, Nodomi, Horigome, Kusaka, Ohara.
Analysis and interpretation of data. Yokoyama, Umeda, Oda, Nodomi, Nasu, Matsumoto, Izawa, Kusaka, Saito, Yasumi, Nishikomori, Ohara.

ADDITIONAL DISCLOSURES

Author Horigome is an employee of Dainippon Sumitomo Pharma.

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Concise report

Early progression of atherosclerosis in children with chronic infantile neurological cutaneous and articular syndrome

Kenichiro Yamamura[1], Hidetoshi Takada[1], Kiyoshi Uike[1], Yasutaka Nakashima[1], Yuichiro Hirata[1], Hazumu Nagata[1], Tomohito Takimoto[1], Masataka Ishimura[1], Eiji Morihana[1], Shouichi Ohga[2] and Toshiro Hara[1]

Abstract

Objective. Chronic inflammation plays a key role in the development of atherosclerosis. Early progression of atherosclerosis has been reported in patients with RA. Cryopyrin-associated periodic syndromes (CAPS) are autosomal dominant autoinflammatory disorders caused by heterozygous *NLRP3* gene mutations. Chronic infantile neurological cutaneous and articular (CINCA) syndrome is the most severe form of CAPS and patients display early onset of rash, fever, uveitis and joint manifestations. However, there has been no previous report on atherosclerosis in patients with CAPS. The objective of this study is to assess the development of atherosclerosis in patients with CINCA syndrome.

Methods. Intima–media thickness (IMT) of the carotid arteries, stiffness parameter β, ankle brachial index (ABI) and pressure wave velocity (PWV) were evaluated by ultrasonography in 3 patients with CINCA syndrome [mean age 9.0 years (S.D. 5.3)] and 19 age-matched healthy controls [9.3 years (S.D. 4.3)].

Results. The levels of carotid IMT, stiffness parameter β and PWV in CINCA syndrome patients were significantly higher than those in healthy controls [0.51 mm (S.D. 0.05) *vs* 0.44 (0.04), $P = 0.0021$; 6.1 (S.D. 1.7) *vs* 3.9 (1.0), $P = 0.0018$; 1203 cm/s (S.D. 328) *vs* 855 (114), $P = 0.017$, respectively].

Conclusion. Patients with CINCA syndrome showed signs of atherosclerosis from their early childhood. The results of this study emphasize the importance of chronic inflammation in the development of atherosclerosis. Further analysis on atherosclerosis in young patients with CINCA syndrome may provide more insights into the pathogenesis of cardiovascular disease.

Key words: ankle–brachial index, atherosclerosis, chronic infantile neurologic cutaneous and articular syndrome, cryopyrin-associated periodic syndromes, intima-media thickness, pulse wave velocity.

Introduction

It is well known that chronic inflammation is a predisposing factor for atherosclerosis. There has been considerable interest regarding the possible causal role of inflammation in the development of atherosclerosis in adult patients with RA, SLE and familial Mediterranean fever (FMF). Patients with SLE, APS or RA have increased mortality rates related to early atherosclerosis. Relative risk of 5 for myocardial infarction, 6–10 for stroke in SLE patients and 3.6 for cardiovascular deaths in RA patients has been reported [1]. Furthermore, the American Heart Association has reported that chronic inflammatory disease is one of the eight high-risk factors for atherosclerosis, even in children [2].

Cryopyrin-associated periodic syndromes (CAPS), including chronic infantile neurological cutaneous and articular (CINCA) syndrome, Muckle–Wells syndrome and familial cold autoinflammatory syndrome, are autosomal dominant autoinflammatory syndromes caused by heterozygous mutations of the *NLR family pyrin domain*

[1]Department of Pediatrics and [2]Department of Perinatal and Pediatric Medicine, Graduate School of Medical Sciences, Kyushu University, Fukuoka, Japan.

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Correspondence to: Kenichiro Yamamura, Department of Pediatrics, Graduate School of Medical Sciences, Kyushu University, 3-1-1, Maidashi, Higashi-ku, Fukuoka 812-8582 Japan.
E-mail: yamamura@pediatr.med.kyushu-u.ac.jp

containing 3 (*NLRP3*) gene. It has been reported that disease associated *NLRP3* mutation causes IL-1β oversecretion by caspase-1 activation. CINCA syndrome, the most severe form among them, is characterized by chronic systemic inflammation manifested as early onset of rash, fever, uveitis, chronic meningitis and joint symptoms [3]. However, there has been no previous report evaluating atherosclerosis in patients with CAPS.

Several physiological examinations are applied to assess atherosclerosis. Carotid intima–media thickness (cIMT) is known to be an indicator of atherosclerosis for adults and children [4]. In fact, increased cIMT has been shown in children with obesity, hyperlipidaemia and diabetes mellitus [5]. It has been reported that stiffness parameter β is more useful in detecting atherosclerotic changes in earlier stages than cIMT [6]. Also, pulse wave velocity (PWV) and ankle–brachial index (ABI) are simplified parameters of the severity of atherosclerosis and predictors of prognosis in adult patients with cardiovascular disease [7, 8]. The objective of this study is to assess the development and progression of atherosclerosis in young patients with CINCA syndrome by measuring cIMT, stiffness parameter β, PWV and ABI.

Patients and methods

Study population

Three patients (a 5-year-old boy [9], a 7-year-old girl [10] and a 15-year-old boy [11]) with CINCA syndrome and 19 age-matched healthy controls were enrolled in this study. *NLRP3* mutations were observed in all three patients. The parameters of atherosclerosis were investigated in these three patients who were in remission for 1 year after the initiation of canakinumab treatment. The Institutional Review Board of Kyushu University Hospital approved the study and informed consent was obtained from each subject.

Sonographic study

Carotid artery US was performed with an iE33 ultrasound machine (Philips, Amsterdam, The Netherlands) using an 11 MHz probe. Measurements were obtained with subjects in the supine position by experienced sonographers blinded to the subjects' clinical status. Ultrasonographic images of the right and left common carotid arteries (CCAs) of each subject at the lower third cervical region proximally and 1 cm above the carotid bulb distally in the longitudinal plane were obtained. CCA IMT measurements of the distal CCA posterior wall were done manually by the distance measurement system of the sonography device after magnification of the images. Three measurements were made in a non-neighbouring fashion within an ~1 cm segment from both the left and right CCA proximal and distal portions. The IMT was measured during end diastole. Mean IMT was calculated as the average of three consecutive measurements of maximum far wall thickness obtained from the CCA. Measurement of the internal diameter of the CCA was performed for three consecutive heartbeats. Intraobserver variability was 1.7% for IMT and 3.1% for arterial wall diameter measurements. The stiffness parameter β was calculated from this formula [12]: $\beta = [\ln(SBP/DBP)]/(\Delta D/D)$, where SBP is the systolic blood pressure, DBP is the diastolic blood pressure, D is carotid artery diastolic diameter and ΔD is the change in artery diameter during systole.

PWV and ABI

PWV and ABI were measured using a BP-203RPEIII (Omron Colin, Tokyo, Japan). PWV, ABI, the blood pressure of the extremities, ECG and heart sounds were synchronously measured and then automatically recorded. Electrodes were contacted on both wrists and a microphone was attached to the left margin of the sternum. The extremities were then wrapped by cuffs that were connected to a pulse monitor. The volume wave and time difference emitted from the pulse monitor were recorded. The pulse wave was defined as the value obtained by dividing the distance between the two points by the time spent in transferring the pulse. In the current study, the pulse wave was measured in the brachial artery and ankle (baPWV). The ABI was defined as the ratio between the systolic pressure measured in the ankle and that measured in the brachial artery.

Laboratory evaluation

In the morning, after an overnight fast, venous blood was sampled for the measurement of serum concentrations of glucose, total cholesterol, triglycerides and standard CRP.

Statistical analysis

Data are expressed as mean (S.D.). Differences between data were studied using the Student's *t* test. Analytical statistics of data between group comparisons of categorical data parameters were performed by using the chi-square test. Statistical significance was taken as $P < 0.05$. All statistical analyses were performed using JMP8 (SAS Institute, Tokyo, Japan).

Results

Clinical characteristics of the study group are presented in Table 1. Age, sex and triglyceride levels were similar between patients with CINCA syndrome and control subjects ($P = 0.65$, 0.53 and 0.17, respectively). Total cholesterol levels in CINCA syndrome patients were significantly lower than those in healthy controls, although they were within normal ranges in both groups. CRP concentrations in the patient group were significantly higher than in healthy controls [5.76 mm (S.D. 2.05) *vs* 0.08 (0.16), $P < 0.0001$].

All subjects tolerated the sonographic examination well. Sonographic study results and normal values of the parameters for the age of the patients [13, 14] are summarized in Table 2. Carotid artery analysis revealed that the IMT and stiffness parameter β of patients with CINCA syndrome were significantly higher than those of healthy controls [0.51 mm (S.D. 0.05) *vs* 0.44 (0.04), $P = 0.0021$, and 6.1 (S.D. 1.7) *vs* 3.9 (1.0), $P = 0.018$, respectively).

TABLE 1 Clinical and laboratory characteristics of the subjects

	Patient 1	Patient 2	Patient 3	CINCA syndrome ($n=3$), mean (S.D.)	Controls ($n=19$), mean (S.D.)	P-value
Gender, male/female	Male	Female	Male	2/1	9/10	0.53
Age, years	5	7	15	9.0 (5.3)	9.3 (4.3)	0.65
BMI, kg/m^2	16.0	15.5	16.8	16.1 (0.6)	17.3 (2.9)	0.51
Systolic blood pressure, mmHg	91	96	128	105 (20)	99 (8)	0.38
Diastolic blood pressure, mmHg	45	50	68	54 (12)	53 (4)	0.73
Total cholesterol, mg/dl	123	122	131	125 (5)	159 (17)	0.0046
Triglycerides, mg/dl	61	79	157	99 (51)	70 (28)	0.17
Glucose, mg/dl	93	85	102	94 (3)	94 (6)	0.95
CRP, mg/dl	0.26	1.62	5.55	2.48 (2.75)	0.08 (0.16)	<0.0001

CINCA syndrome: chronic infantile neurological cutaneous and articular syndrome.

TABLE 2 Ultrasonographic examination, baPWV and ABI in CINCA syndrome patients and control subjects

	Patient 1	Patient 2	Patient 3	CINCA syndrome ($n=3$), mean (S.D.)	Controls ($n=19$), mean (S.D.)	P-value
Intima–media thickness, mm (normal value for each age) [13]	0.47 (0.40)	0.5 (0.40)	0.57 (0.50)	0.51 (0.05)	0.44 (0.04)	0.0021
Systolic diameter, mm	5.5	5.8	5.8	5.7 (0.2)	6.2 (0.8)	0.30
Diastolic diameter, mm	4.8	5.2	5.4	5.1 (0.3)	5.3 (1.7)	0.63
Stiffness parameter β (normal value for each age) [14]	4.8 (3.4)	5.7 (3.7)	7.6 (4.5)	6.1 (1.7)	3.9 (1.0)	0.018
Right baPWV, cm/s	1068	920	1566	1185 (338)	850 (114)	0.0025
Left baPWV, cm/s	1053	1022	1587	1221 (318)	859 (114)	0.0014
Averaged baPWV, cm/s (normal value for each age) [15]	1061 (<941)	971 (<919)	1577 (1041)	1203 (328)	855 (114)	0.0017
Right ABI	1.15	0.91	0.98	1.00 (0.10)	1.04 (0.10)	0.67
Left ABI	1.16	0.95	0.92	0.99 (0.10)	1.06 (0.10)	0.48
Averaged ABI (normal value for each age) [15]	1.16 (>1.00)	0.93 (>1.00)	0.95 (>1.00)	0.99 (0.10)	1.05 (0.10)	0.54

CINCA syndrome: chronic infantile neurological cutaneous and articular syndrome; baPWV: brachial artery pulse wave velocity; ABI: ankle–brachial index.

The averaged baPWV of the patients was significantly higher than that of controls [1203 cm/s (S.D. 328) *vs* 855 (114), $P=0.017$) (Table 2). There was no significant difference in ABI between the two groups, although the values of two patients were lower than the normal range [15].

Discussion

In the present study we found that patients with CINCA syndrome develop atherosclerosis from early childhood. There have been many previous studies describing atherosclerosis associated with inflammatory diseases such as RA, SLE and FMF [1]. However, this is the first report showing the youngest group of patients who developed atherosclerosis associated with inflammatory disorders.

It has been shown that inflammation plays an important role in the development of atherosclerosis. The presence of macrophages and activated lymphocytes within the plaques supports the nature of an immune system-mediated inflammatory disorder of atherosclerosis. It has been shown that higher disease activity representing higher inflammatory burden is associated with increased cardiovascular events in patients with RA and SLE [16]. It may be induced by elevated inflammatory cytokines, which can cause the development of endothelial dysfunction in atherosclerotic processes. In addition, changes in lipid metabolism and a wide variety of immune and inflammatory alterations that directly affect the endothelium, vascular smooth muscle cells and inflammatory cellular components of the atherosclerotic plaque may also play important roles in the development and progression of atherosclerosis in patients. CINCA syndrome is the most severe form of CAPS, and patients display severe systemic inflammation from the neonatal period [3].

Therefore it is reasonable to assume that the progression of atherosclerosis from childhood in three patients with CINCA syndrome is closely related to chronic systemic inflammation. It was reported that the incidence of atherosclerosis could be reduced by aggressive disease-modifying therapies in patients with RA and SLE [16]. In patients with CINCA syndrome, we can investigate the association between inflammation and atherosclerosis without any effect of classical risk factors such as obesity, smoking, hyperlipidaemia or diabetes. This may provide a novel clue to clarify the role of inflammation in the development of atherosclerosis.

In patients with FMF and SLE, age and disease duration were reported to be associated with the severity of atherosclerosis [17]. In the present study we found that the oldest patient (patient 3) with the longest disease duration had the most advanced atherosclerosis, which is in line with this report. Early diagnosis and effective treatment for chronic inflammation in these patients have been emphasized in preventing cardiovascular disease because a negative correlation between the duration of anti-inflammatory treatment and IMT has been observed in SLE patients [18].

Interestingly, improvements in PWV and cIMT [19] were reported in patients with RA after sufficient infliximab treatment. In patients with CINCA syndrome, canakinumab was reported to induce rapid and sustained remission of symptoms [20]. It is possible that a significant improvement in atherosclerosis will be observed in our patients with CINCA syndrome after canakinumab treatment in the near future.

However, there are some limitations in the present study. First, our study contains only a small number of patients because of the extremely rare incidence of this disease. Second, the parameters investigated in this study are considerably variable with the age of the subjects. It is also possible that the values of the parameters change because of the measurement equipment. Multicentre and long-term follow-up analysis with standardized procedures and tools on a larger number of the patients are necessary to provide more precise information on the pathogenesis of atherosclerosis.

Conclusion

Patients with CINCA syndrome developed atherosclerosis from early childhood. Atherosclerosis in CINCA syndrome patients may be a prototype of cardiovascular disease predominantly induced by chronic inflammation.

Rheumatology key messages

- Patients with CINCA syndrome develop atherosclerosis from early childhood.
- This report shows the youngest group of patients who developed atherosclerosis associated with inflammatory disorders.
- Early treatment with anti-IL-1β antibody might be beneficial in preventing atherosclerosis in CINCA syndrome.

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特集 自己炎症症候群の診断と治療

中條-西村症候群

金澤伸雄
KANAZAWA Nobuo
和歌山県立医科大学皮膚科

Key Words ■中條-西村症候群 ■凍瘡様皮疹 ■脂肪筋肉萎縮 ■免疫プロテアソーム ■*PSMB8*

中條-西村症候群は，1939年の中條，1950年の西村らの報告した「凍瘡を合併せる続発性肥大性骨骨膜症」に由来する，わが国発の古くて新しい遺伝性自己炎症疾患であり，慢性反復性の炎症と進行性のやせ・消耗を特徴とする．2010年に欧米，中東から報告されたJMP症候群，CANDLE症候群とともに，*PSMB8*遺伝子変異にもとづくプロテアソーム機能不全症である．不要蛋白質の細胞内貯留に対するストレス応答として炎症，組織変性をきたすと考えられ，新たな慢性炎症カスケードとして注目される．今後の有効な特異的治療の開発，さらに他の慢性炎症性疾患への応用が期待される．

はじめに

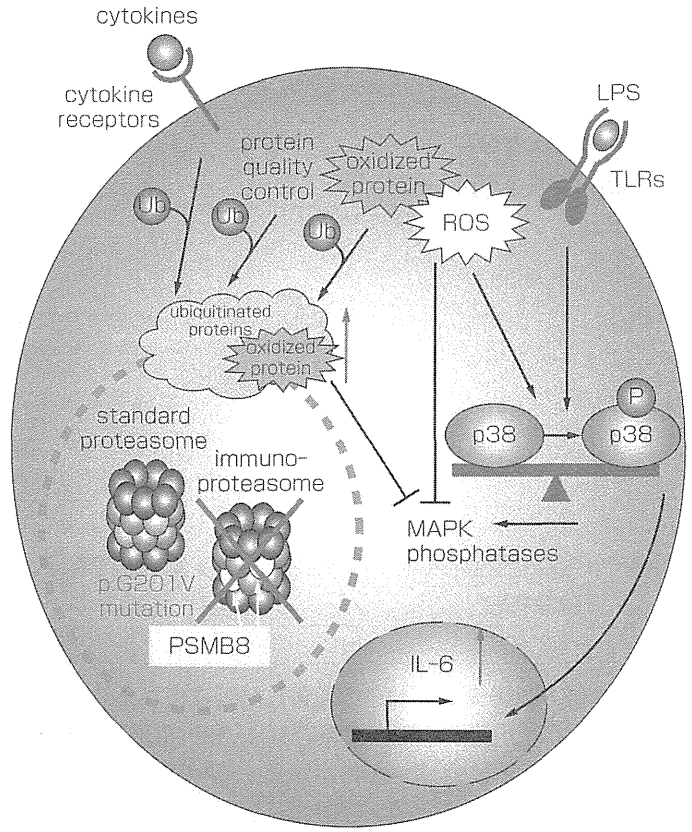
中條-西村症候群は，慢性反復性の炎症と進行性のやせ・消耗を特徴とする，特異な遺伝性自己炎症疾患である[1]．1939年の中條，1950年の西村らの「凍瘡を合併せる続発性肥大性骨骨膜症」の報告以来，症例は和歌山・泉南を中心とした関西と関東・東北地方に集中する[2)3)]．わが国特有とされたが，2010年に欧米，中東から臨床的に酷似する症例が報告され，JMP（joint contractures, muscular atrophy, microcytic anemia and panniculitis-associated lipodystrophy）症候群，CANDLE（chronic atypical neutrophilic dermatosis with lipodystrophy and elevated temperature）症候群と命名された[4)5)]．その後，原因となる*PSMB8*遺伝子変異がつぎつぎに同定され，3疾患ともプロテアソーム機能不全症であることが明らかとなった[6)~9)]．

1．疫学

中條の報告以来，東北・関東に5家系7症例（宮城，秋田，新潟，東京），関西に20家系23症例（大阪，奈良，和歌山）あり，そのうち現在も継続してフォローされている症例は，和歌山と奈良の小児例を含む関西の12症例のみである[1)10)11)]．一方，JMP症候群はポルトガルとメキシコの2家系4症例，CANDLE症候群はスペイン，米国，イスラエル，バングラデシュの9家系12症例が報告されている[4)~6)9)12)]．

2．病因・病態

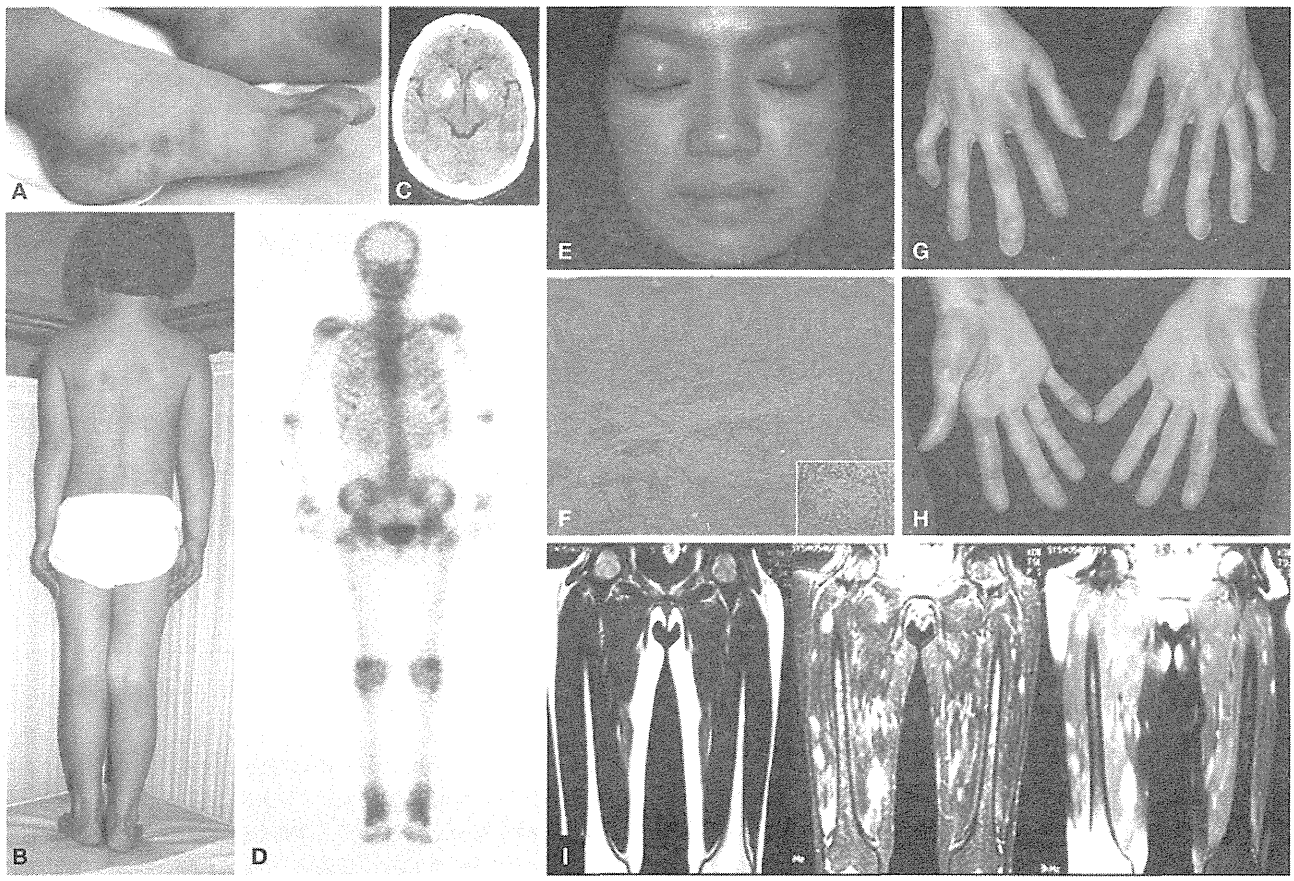
近親婚や家族内発症が多くみられることから常染色体劣性遺伝と予想され，ホモ接合部マッピング法により，プロテアソームの誘導型サブユニットであるβ5iをコードする*PSMB8*遺伝子のG201Vホモ変異が同定され



図❶ 中條-西村症候群において想定される炎症惹起メカニズム
PSMB8 変異のために免疫プロテアソームの機能が低下することによって免疫担当細胞をはじめ各種細胞にユビキチン化・酸化蛋白質が蓄積し，これがストレスとなって p38 MAP キナーゼが活性化し，IL-6 に代表されるサイトカイン産生が亢進する．
（Arima K *et al*, 2011[7] より改変引用）

た[7][8]．検索したすべての症例に同じ変異を認め，強い創始者効果を伴った．一方，JMP 症候群のすべてと CANDLE 症候群の多くの症例に *PSMB8* 遺伝子の T75M ホモ変異が同定された一方，CANDLE 症候群の 2 症例に T75M ヘテロ変異，ユダヤ人の 1 症例に C135X ホモ変異，バングラデシュ人の 1 症例に M117V ホモ変異が認められ，変異のない症例も 1 例報告されている[6][9][12]．

プロテアソームはポリユビキチン鎖によってラベルされた分子を選択的に分解する巨大分子複合体であり，不要な蛋白質を除去するだけでなく細胞周期やシグナル伝達など多彩な細胞機能に関わる[13]．酵素活性をもつ β1，β2，β5 サブユニットが誘導型のより活性の高い β1i，β2i，β5i サブユニットに置き換わったものを免疫プロテアソームとよび，免疫担当細胞で恒常的に発現し，また体細胞においても炎症時に誘導される．*PSMB8* の G201V 変異によって，β5i がもつキモトリプシン様活性が著しく低下するだけでなく，隣接サブユニットとの接合面の変化のために複合体の形成不全が起こり，成熟した免疫プロテアソームの量が減少するとともに，β1i と β2i がもつトリプシン様，カスパーゼ様活性も大きく低下する．その結果，炎症局所に浸潤する組織球など各種細胞内にユビキチン化・酸化蛋白質が蓄積する．シグナル伝達系の検討でリン酸化 p38 の核内貯留を認め，血清中と患者由来培養線維芽細胞が分泌するサイトカインの検討で IL-6 の過剰産生を認めたことから，不要蛋白質の貯留に対するストレス応答として炎症，組織変性をきたすと考えられる（図❶）[7]．プロテアソーム阻害薬が各種免疫疾



図❷　代表的な中條-西村症候群症例の臨床像
A：右足外側縁の凍瘡様紫紅色斑（5歳時）．B：下腿筋炎による尖足位．C：CTでの大脳基底核石灰化（24歳時）．D：骨シンチにおける関節部異常集積像．E：ヘリオロープ様眼瞼紅斑を伴うやせて骨ばった顔貌（27歳時）．F：手掌の結節性紅斑様皮疹の病理組織像．G：長く節くれだった指．H：右手首，左手掌に結節性紅斑様皮疹を認める．I：大腿筋のMRI像（左よりT1，T2，ガドリニウム強調T1像．24歳時）
（金澤伸雄ほか，2011[1]より引用）

患の治療に応用されつつある一方で，プロテアソーム機能不全によって惹起される新たな慢性炎症カスケードとして注目される[14]．

3．臨床症状・診断

代表的な1症例における臨床像を図❷に示す．幼小児期，とくに冬季に手足や顔面の凍瘡様皮疹にて発症し，その後四肢・体幹の結節性紅斑様皮疹や弛張熱を不定期にくり返すようになる．組織学的には，真皮から筋層に至るまで巣状に，リンパ球・組織球を主体とした稠密だが多彩な炎症細胞の浸潤を認め，内皮の増殖を伴う血管障害を伴う．筋炎を伴うこともあるが，筋力は比較的保たれる．早期より大脳基底核の石灰化を認めるが，精神発達遅滞ははっきりしない．次第に特徴的な長く節くれだった指と顔面・上肢を主体とする脂肪筋肉萎縮・やせが進行し，手指や肘関節の屈曲拘縮をきたす．LDH，CPK，CRP，AAアミロイドのほか，γ-グロブリン，とくにIgGが高値となる．さらに進行すると抗核抗体や各種自己抗体が陽性になることがある．早期より肝脾腫を認めるが，脂質代謝異常ははっきりしない．胸郭の萎縮を伴う拘束性呼吸障害や，心筋変性や心電図異常を伴う心機能低下のために早世する症例がある一方，著明な炎症所見を認めず病院を受診しない症例もある．

臨床診断基準案を表❶に示すが，症状が出揃う前の，発症後間もない乳幼児期に診断することは容易ではない[10][11]．皮疹は凍瘡様あるいは結節性紅斑様であり，寒冷刺激で増悪する点はクリオピリン関連周期熱症候群や

表❶ 中條-西村症候群臨床診断基準案

1．常染色体劣性遺伝（血族婚や家族内発症あり）
2．手足の凍瘡様紫紅色斑（乳幼児期から冬期に出現）
3．強い浸潤・硬結を伴う結節性紅斑が出没（環状のこともある）
4．くり返す弛張熱（周期熱：必発ではない）
5．手足の長く節くれだった指・関節拘縮
6．進行性の限局性脂肪筋肉萎縮・やせ（顔面・上肢に著明）
7．肝脾腫
8．大脳基底核石灰化

8項目中5項目以上陽性で他疾患を除外できれば確定

（金澤伸雄ほか，2011[1]より引用）

Aicardi-Goutières症候群と共通するため，萎縮や精神症状の有無で鑑別する．

4．治療・予後

標準的治療法はない．ステロイド全身投与は発熱，皮疹などの炎症の軽減には有効だが，減量により容易に再燃し，また脂肪筋肉萎縮には無効である．むしろ幼小児期からのステロイド全身投与は，長期内服による成長障害，代償性肥満，緑内障，骨粗鬆症など弊害も多く，慎重な投与が必要である．他の自己炎症疾患と同様，抗TNFα製剤や抗IL-1β製剤などの生物学的製剤，とくに病態から抗IL-6受容体抗体製剤が有効である可能性があり，小児例において全身型若年性特発性関節炎に準じた投与が奏効しquality of life（QOL）の回復に寄与したと報告されている[11]．一方，JMP症候群やCANDLE症候群では，関節リウマチに準じてメトトレキサートや各種生物学的製剤が投与されているが，いずれもステロイドと同様，炎症には有効だが脂肪筋肉萎縮には無効と報告されている[6][9]．最近では，IFNγシグナルの過剰活性化を抑制するjanus kinase（JAK）阻害薬の有効性も報告されている[15]．

早期から拘束性呼吸障害や心機能低下をきたして30代で突然死する症例もあるが，心肺のほかにも肝臓などの障害が緩やかに進み，60代で亡くなる症例が多いようである．手の拘縮のほか，咬合不全や重度の鶏眼などによりQOLが低下する．

おわりに

古くより存在が知られていたものの，地域性が強く症例も散発的で広く顧みられることがなかった疾患であるが，変異遺伝子の同定によって疾患単位として確立し，同時に世界各地から報告が集積しつつある．プロテアソーム機能不全から症状発現に至る病態の解明にもとづき，早期に診断し介入することで，反復性炎症のみならず萎縮・拘縮の進行が抑制できる，有効な特異的治療の開発が望まれる．さらに各種慢性炎症性疾患におけるプロテアソーム機能不全の関与が明らかとなり，広く応用される治療となることが期待される．

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Autoimmunity versus Autoinflammation - Friend or Foe?

Nobuo Kanazawa · Georgi Tchernev · Uwe Wollina

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Abstract "Autoimmunity" is a designation dependent on the conventional immunological issue of self/non-self discrimination. Identification of novel target autoantigens is still an important issue ongoing in classical tissue-specific autoimmune bullous diseases and autoimmune connective tissue diseases. In contrast, synchronized with the paradigm shift of the fundamental aspect of immunity to danger sensing/signaling, distinct collagen-like diseases have been defined by the genetic mutations causing dysregulated innate immunity/inflammation and have been designated as "autoinflammatory" diseases. Due to the clinical and etiological similarities, the concept of autoinflammatory diseases has expanded to include non-hereditary collagen-like diseases, tissue-specific chronic idiopathic inflammatory diseases and metabolic diseases. On the other hand, various genetic causes of autoimmune diseases have been identified and the border of these two pathophysiologies is becoming obscure. Instead, a variable mixture of both autoimmunity and autoinflammation can cause each inflammatory phenotype with a variable level of antigen specificity.

N. Kanazawa, MD, PhD (✉)
Department of Dermatology, Wakayama Medical University,
811-1 Kimiidera,
641-0012 Wakayama, Japan
e-mail: nkanazaw@wakayama-med.ac.jp

G. Tchernev
Polyclinic for Dermatology and Venereology, Medical Faculty,
University Hospital Lozenetz, Sofia University,
Sofia, Bulgaria
e-mail: georgi_tchernev@yahoo.de

U. Wollina
Department of Dermatology and Allergology, Hospital Dresden-Friedrichstadt, Academic Teaching Hospital of the Technical University of Dresden,
Dresden, Germany
e-mail: wollina-uw@khdf.de

Keywords Autoimmunity · Autoinflammation

Autoimmunität versus Autoinflammation - Freund oder Feind?

Zusammenfassung Unter „Autoimmunität" versteht man konventionell das immunologische Problem der Diskriminierung von Selbst und Nicht-Selbst. Die Identifizierung weiterer Target-Autoantigene bei den klassischen gewebe-spezifischen autoimmunen bullösen Dermatosen und den autoimmunen Bindegewebserkrankungen bleibt auch weiterhin ein wichtiges Anliegen. Dabei hat sich das Verständnis der fundamentalen Aspekte der Immunologie hin entwickelt zum Themenkomplex der Gefahrenerkennung (danger sensing) und Signalübertragung. Bei den Kollagenosen wurden teils genetische Mutationen entdeckt, die verantwortlich zeichnen für Störungen der Immunität und Entzündungskaskade. Die autoinflammatorischen Erkrankungen wurden definiert. Aufgrund klinischer und ätiologischer Ähnlichkeiten wurde das Konzept der autoinflammatorischen Erkrankungen auf nicht hereditäre Bindegewebserkrankungen, gewebe-spezifische chronisch idiopathisch-entzündliche Erkrankungen und metabolische Erkrankung ausgedehnt. Andererseits wurden verschiedene genetische Ursachen der Autoimmunerkrankungen entdeckt, so dass das die Grenzen der klassischen Pathologien verschwimmen. In der Tat kann eine Mischung von Autoimmunität und Autoinflammation nahezu jeden Entzündungs-Phänotyp mit variablem Level der Antigenspezifität auslösen.

Schlüsselwörter Autoimmunität · Autoinflammation

The immune system is working for protection of the living things from harmful things, such as invasive patho-

gen and internal malignancy. At first, discriminating self and nonself had been considered the fundamental aspect of immunity, and "autoimmunity" was designated for immune reaction to the self ("auto") antigens. Identification of major histocompatibility complex and concept of central and peripheral tolerance (clonal deletion and anergy as the mechanism, respectively) explained the basis of self-recognition. However, recent research on regulatory T cells (T_{reg}) and tolerogenic dendritic cells has added further implications in keeping unresponsiveness to the self [1, 2].

Clinically, several kinds of chronic inflammatory disorders have been applied for autoimmune diseases, which target self-antigens. They are divided into two categories: tissue-specific autoimmune diseases caused by type II allergy and immune complex-mediated systemic autoimmune diseases caused by type III allergy. In the skin-specific autoimmune bullous diseases, such as pemphigus and pemphigoid, molecular identification of pathogenic antibodies and the corresponding antigens, desmoglein, and type XVII collagen, respectively, provided insights into the molecular basis of skin structure [3]. Interestingly, another severe bullous disease, staphylococcal scalded skin syndrome is caused by a proteolytic exotoxin, which injures homophilic desmoglein junction [4]. Type VII collagen is a target antigen in autoimmune epidermolysis bullosa aquisita, and is genetically deficient in congenital dystrophic epidermolysis bullosa, suggesting that an autoantibody causes functional defect of the corresponding antigen. By analysis of the cases showing lesional immunoglobulin deposit without detection of known autoantibodies, novel target antigens are still continuously discovered [5]. However, it is mostly unclear how the autoantibodies are generated [6].

Systemic autoimmune diseases include the classical connective tissue diseases (rheumatoid arthritis, systemic lupus erythematosus, systemic sclerosis, and polymyositis/dermatomyositis) except for rheumatoid fever, in which self-antigen mimicking a part of *Group A Streptococcus* becomes the target after streptococcal infection. Serum antinuclear antibody level is elevated in most of these diseases, and the disease-specific tissue-nonspecific autoantibodies against DNA, DNA-binding proteins, and other nuclear/cytoplasmic proteins, are used as markers for diagnosis and for appreciation of the disease activity. However, their pathogenic role is mostly unclear as compared with the case of skin-specific autoimmune bullous diseases. Nevertheless, recent identification of autoantibodies against novel antigens has defined some specific subtypes of dermatomyositis [7]. Interestingly, antibody against MDA5, which acts as an intracellular receptor for viral RNA and belongs to RIG-I-like receptors, is specific for clinically amyopathic dermatomyositis, which is frequently accompanied with rapidly progressing interstitial pneumonitis [8, 9].

Besides the classical autoimmune connective tissue diseases, some related disorders are still accompanied with specific autoantibodies, such as Sjögren's syndrome, antiphospolipid antibody syndrome, and antineutrophil cytoplasmic antibody-related vasculitis. In contrast, the remaining systemic disorders with chronic inflammation, such as adult Still's disease, Behcet's disease, Sweet syndrome, Weber-Christian disease, and sarcoidosis, are negative for autoantibodies and are driven by activated neutrophils and/or macrophages. Therefore, these diseases are considered to be autoimmune-like, but rather related with nonspecific hyper-reactivity or latent infection. These characteristic features are shared with other tissue-specific chronic idiopathic inflammatory diseases, such as urticaria, psoriasis, and inflammatory bowel diseases including Crohn's disease and ulcerative colitis.

Recently, several distinct diseases, whose phenotypes are similar to these chronic idiopathic inflammatory diseases, have been defined by the causative genetic mutations. As these mutations cause dysregulation of innate immunity/inflammation, the defined diseases have been designated as "autoinflammatory" diseases [10]. These processes have been synchronized with the paradigm shift of the fundamental aspect of immunity, from self/nonself discrimination to danger sensing/signaling [11]. Familial Mediterranean fever (FMF) and related hereditary periodic fever syndromes are the prototypic autoinflammatory disorders and most of them are caused by dysregulated activation of NOD-like receptors (NLR) P3 inflammasome, which senses various dangerous stimuli to induce interleukin (IL)-1β secretion, such as bacterial RNA, imidazoquinolin, and contact allergen [12]. As referred to the membranous toll-like receptors (TLR), NLR have been shown to act as intracellular sensors for various pathogen- or danger-associated molecular patterns [13]. Therefore, it is conceivable that these hereditary diseases resemble infectious or allergic diseases. Although heterozygous *NLRP3* mutations-oriented cryopyrin-induced periodic fever syndromes (CAPS) include formerly called familial cold urticaria, febrile attacks seem to occur periodically or even "automatically", without any apparent triggers. The category of hereditary autoinflammatory diseases has rapidly expanded to include more numbers of diseases, such as pyogenic pustular diseases and systemic granulomatosis [14]. Differentiation of pyogenic arthritis, pyoderma gangrenosum and acne syndrome with *PSTPIP1* mutations among pyoderma gangrenosum and/or cystic acne patients, identification of deficiency for IL-36 receptor antagonist with *IL36RN* mutations among generalized pustular psoriasis patients, and distinction of early onset sarcoidosis with *NOD2* mutations from sarcoidosis have indicated the fact that such monogenic diseases constitute at least a part of sporadic common diseases [15–17].

Then, the previously described autoantibody negative chronic idiopathic inflammatory disorders are considered as acquired autoinflammatory diseases, which share clinical features and the putatively dysregulated inflammatory pathways with hereditary autoinflammatory diseases. Adult Still's disease, Behcet's disease, and Weber-Christian disease show periodic febrile

attacks with skin rash and are similar to CAPS, FMF, and Nakajo-Nishimura syndrome with *PSMB8* mutations, respectively. Sweet syndrome is associated with Majeed syndrome with *LPIN2* mutations [18, 19]. Moreover, Schnitzler syndrome with various clinical similarities to CAPS shows dramatic improvement by anti-IL-1β therapy, suggesting the underlying activation of NLRP3 inflammasome [20]. Thus, autoimmune and autoinflammatory diseases seem apparently distinguished by their clinical and/or genetic features.

Furthermore, some metabolic diseases such as gout, pseudogout, and type 2 diabetes mellitus, which are caused by chronic inflammation due to monosodium urate crystals, calcium pyrophosphate crystals, and hyperglycemia, respectively, are categorized as another class of autoinflammatory diseases [21]. In these diseases, self molecule-induced activation of NLRP3 inflammasome is considered responsible, and anti-IL-1β therapy is effective. Similarly, it has been shown that obesity-induced metabolic syndrome is mediated by TLR4 activation in adipocytes through saturated fatty acid, which can be called as homeostatic inflammation rather than autoinflammation [22].

On the other hand, it is well known that genetic background is important for development of systemic autoimmune diseases. Various mouse strains have been reported to be autoimmune-prone, such as *lpr* (deficient for *fas*), *gld* (deficient for *fas ligand*), *Dnase1-/-*, *Ctla4-/-*, and *Pd1-/-* mice [23, 24]. These mutations are related with dysregulation of apoptosis, clearance of apoptosis, and negative costimulatory (coinhibitory) signaling, which lead to breakage of tolerance. In humans, Aicardi-Goutieres syndrome, which shows the characteristic features of overlapping with cerebellar viral infection and systemic lupus erythematosus, is caused by deficiency for various exonucleases, such as TREX1/DNaseIII [25]. Identification of such various genetic causes for autoimmune diseases is making the border obscure between these diseases and hereditary autoinflammatory diseases.

Interestingly, *Il1rn-/-* mice show rheumatoid arthritis-like autoimmune and psoriasis-like T cell-independent autoinflammatory phenotypes, while *IL1RN* deficiency causes an autoinflammatory syndrome termed deficiency for IL-1 receptor antagonist in humans [26–28]. It may sound strange that both systems can be dysregulated on a monogenic background. However, considering that innate and adaptive immunity not only regulate but also activate each other, it is not surprising that both phenotypes of autoimmunity and autoinflammation are observed in the same body. Actually, elevation of nonspecific autoantibodies can be observed during development of Nakajo-Nishimura syndrome [17]. To develop the autoimmune phenotype (T/B cell-mediated autoantigen-specific hyper-reactivity), autoinflammatory responses (dysregulated signaling from the autoantigen through some pattern recognition receptor such as TLRs) should be involved [29]. Very recently, it has been reported that the repetitive percutaneous stimulation with imidazoquinolin generated a new model of systemic lupus erythematosus with high serum level of anti-double-stranded DNA antibodies [30]. Collectively, a variable mixture of both autoinflammation and autoimmunity can cause the inflammatory phenotype with a variable level of antigen specificity, depending on the lesional tissue, triggering factors, and other genetic backgrounds.

Conflict of interest
None declared.

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SHORT COMMUNICATION

Serum IL-18 as a potential specific marker for differentiating systemic juvenile idiopathic arthritis from incomplete Kawasaki disease

Tadamori Takahara · Masaki Shimizu · Yasuo Nakagishi · Noriko Kinjo · Akihiro Yachie

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Abstract Clinical features and laboratory parameters in patients with incomplete Kawasaki disease (KD) and systemic juvenile idiopathic arthritis (s-JIA) tend to overlap. Furthermore, there have been no definite biomarkers for these diseases. This situation makes the clinical diagnosis of these patients difficult. In this study, we aimed to measure serum interleukin (IL)-18 and IL-6 levels in patients with s-JIA who were initially diagnosed with incomplete KD and compare these data with those in patients with complete KD and arthritis. Serum IL-18 levels in patients with s-JIA were significantly elevated compared with those in patients with KD and arthritis. Pediatricians should be aware that the presentation of s-JIA can mimic incomplete KD. Because the clinical features overlap, a high index of suspicion is warranted. The measurement of serum IL-18 may be useful for differentiating s-JIA from KD.

Keywords Interleukin 18 · Systemic juvenile idiopathic arthritis · Incomplete Kawasaki disease

T. Takahara
Department of Pediatrics, Hyogo Prefectural Tsukaguchi Hospital, Amagasaki, Japan

M. Shimizu (✉) · A. Yachie
Department of Pediatrics, School of Medicine, Institute of Medical Pharmaceutical, and Health Sciences, Kanazawa University, 13-1 Takaramachi, Kanazawa 920-8641, Japan
e-mail: shimizum@staff.kanazawa-u.ac.jp

Y. Nakagishi
Department of Pediatric Rheumatology, Hyogo Prefectural Kobe Children's Hospital, Kobe, Japan

N. Kinjo
Department of Pediatrics, School of Medicine, University of the Ryukyus, Nakagami-gun, Japan

Introduction

Kawasaki disease (KD) is one of the most common childhood vasculitis disorders. However, there are some patients who do not fulfill the classic diagnostic criteria for KD and are termed as incomplete KD. It is important to recognize and treat this entity because it is not a milder form of KD, and it carries a risk of coronary artery aberrations similar to that of complete KD [1]. For this reason, physicians possibly diagnose patients with KD prematurely, even though they do not meet the complete diagnostic criteria, in the fear of losing the opportunity to administer high-dose intravenous immunoglobulin (IVIG) and for preventing the potentially life-threatening complication of coronary aneurysm.

Furthermore, it has been reported that some patients with KD have arthritis [2]. Systemic juvenile idiopathic arthritis (s-JIA) is characterized by remitting fever, a typical skin rash, and arthritis. Diagnosis of s-JIA is often challenging, particularly before children have had symptoms for 6 weeks as required by the International League of Associations for Rheumatology and American College of Rheumatology criteria [3]. Pericardial effusion is a well-recognized feature of s-JIA along with myocarditis, which is a rare feature [4]. Coronary artery dilation has been recently identified as one of the cardiac manifestations in patients with s-JIA [5].

Differentiation of KD and s-JIA is important to avoid multiple courses of IVIG because of suspected refractory KD; however, clinical features and laboratory parameters in patients with s-JIA and incomplete KD tend to overlap. Furthermore, there are no definite biomarkers for these diseases, which makes the clinical diagnosis of these patients difficult [6–9].

We recently reported that serum levels of IL-18 are highly elevated in patients with s-JIA, and the abnormal

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Table 1 Clinical characteristics of the patients in this study

Case	1	2	3	4	5	7	8	9
Age	3	4	3	2	1	4	2	5
Sex	Male	Male	Male	Male	Male	Female	Male	Female
Final diagnosis	s-JIA	s-JIA	s-JIA	s-JIA	s-JIA	KD	KD	KD
The day of drawing blood after disease onset	22	9	33	10	20	10	8	5
Clinical manifestations								
Fever persisting at least 5 days	+	+	+	+	+	+	+	+
Changes in extremities	+	−	+	−	+	+	+	+
Exanthema	+	+	+	+	+	−	+	+
Bulbar conjunctival injection	−	−	−	−	+	+	+	+
Changes in lips and oral cavity	+	−	−	−	+	+	+	+
Cervical lymphadenopathy	−	−	−	−	+	+	+	+
Dilatation of coronary artery	+	−	−	−	−	−	−	−
Hepatosplenomegaly	−	−	−	−	+	−	−	−
Serositis	−	−	−	−	−	−	−	−
Redness at the BCG inoculation site	−	−	−	−	−	−	+	−
Macrophage activation syndrome	−	−	−	−	+	−	−	−
Laboratory findings								
WBC	24,250	23,880	29,920	18,000	7,700	28,380	17,700	9,400
CRP	6.9	20.7	9.5	14.2	11.65	14.8	22	10
AST	84	47	71	25	1,096	23	23	642
LDH	955	597	414	286	4,830	212	285	634
Ferritin	15,000	3,686	nd	1,129	68,310	nd	nd	nd
IL-6	580	64	58	7	22	250	1,200	106
IL-18	181,000	123,000	10,800	36,500	330,000	298	660	260
Treatments								
IVIG	No response	No response	No response	No response	No response	No response	No response	Responded
Steroid	+	+	+	+	+	−	−	+
Tocilizumab	−	−	+	−	−	−	−	−

WBC white blood cells, *CRP* C-reactive protein, *AST* aspartate aminotransferase, *LDH* lactate dehydrogenase

production of IL-18 appears to be specific to s-JIA [10]. In this study, we aimed to demonstrate that the serum level of IL-18 may be useful for differentiating incomplete KD from s-JIA, for which we measured serum interleukin (IL)-18 and IL-6 levels in patients with s-JIA who were initially diagnosed with incomplete KD and compared that data with those in patients with KD and arthritis.

Methods

Patients and samples

Serum samples were obtained from five patients with s-JIA who were initially diagnosed with incomplete KD (cases 1–5) and three patients diagnosed with complete KD and arthritis (cases 6–8). Serum samples were drawn at 5–33 days after disease onset (Table 1). Serum samples were also obtained from 15 patients with s-JIA, 10 patients with typical KD without arthritis, and 20 patients with age- and sex-matched healthy controls. Serum was extracted from the blood samples, divided into aliquots, frozen, and stored at −80 °C until analysis. The protocol of this study was approved by the Institutional Review Board of Kanazawa University, and all the patients provided informed consent.

The patients were diagnosed with KD on the basis of a classic clinical criteria [11]. Complete KD was diagnosed if all diagnostic criteria were fulfilled [11]. Incomplete KD was diagnosed if fewer than three diagnostic criteria were