of 300 μl of RPMI1640 Medium (Invitrogen, Grand Island, NY, USA), including 10 % FBS, for 24 h at 37 °C in a humidified atmosphere with 5 % CO_2. Then, culture medium was replaced with 300 μl of RPMI1640 containing 10 ng/ml LPS. 8 h after medium replacement, the concentrations of IL-1β, IL-6, IL-8, and TNF-α in the culture supernatant were measured by ELISA with specific antibodies (BD Biosciences, San Jose, CA, USA).

Western blotting analyses for p38, ERK, and NF-κB pathways

40 μg of SW982 cell lysates were subjected to SDS-PAGE followed by Western blotting analysis for p38, ERK, and NF-κB, pathways. Signals from the same blotting membrane were detected by Phospho-p38 MAPK (Thr180/Tyr182) (D3F9) XP rabbit monoclonal antibody (Cell Signaling catalog No. #4511) and the p38α MAPK rabbit polyclonal antibody (Cell Signaling catalog No. #9218) for p38 MAPK pathway, or Phospho-p44/42 MAPK (Erk1/2) (Thr202/Tyr204) (D13.14.4E) XP rabbit monoclonal antibody (Cell Signaling catalog No. #4370) and p44/42 MAPK (Erk1/2) (137F5) rabbit monoclonal antibody (Cell Signaling catalog No. #4695) for the ERK pathway, or phospho-NF-κB p65 (Ser536) rabbit polyclonal antibody (Cell Signaling catalog No. #3031) and NF-κB p65 (D14E12) XP rabbit monoclonal antibodies (Cell Signaling catalog No. #8242) for the NF-κB pathway.

Cytokine assays for peripheral blood mononuclear cells

Participation of FMF patients and almost age-matched healthy volunteers regarding the analyses of *MEFV* gene and their blood samples with their written informed consents was approved by the institutional review board at the Shinshu University. We obtained peripheral blood mononuclear cells from five FMF patients with definite diagnosis as FMF according to the 'Tel Hashomer' criteria presented a symptom with typical type of FMF, and exhibited a favorable response to colchicine. All of them had *MEFV* mutations; four patients were E148Q/M694I compound heterozygotes (a 30-years-old woman, an 8-years-old boy, a 25-years-old woman and a 22-years-old woman) and one patient was an E148Q/E148Q homozygote (a 7-years-old girl). 5×10^5/ml of peripheral blood mononuclear cells were incubated in 96-well flat plates (Nunc) with RPMI1640 with 10 % heat-inactivated FBS for 6 h. The supernatants were collected and analyzed for cytokine concentration with the Cytometric Bead Array Flex set (BD Biosciences) according to the manufacturer's instructions. For intracellular cytokine staining, 5×10^5/ml of mononuclear cells including BD GolgiPlug protein transport inhibitor (BD Biosciences) were incubated under the same conditions as described above. After 6 h of incubation, adherent cells were collected by pipetting. The cells were fixed using a BD Cytofix/Cytoperm solution for 20 min at 4 °C, then the fixed cells were permeabilized by washing two times in 1 × BD Perm/Wash buffer. Intracellular IL-8 was stained with FITC-conjugated anti-IL-8 monoclona antibody (BioLegend) and APC-conjugated anti-IL-1β monoclona antibody (BioLegend) at 4 °C for 30 min. After washing with 1 × BD Perm/Wash buffer, resuspension in 1 × PBS was carried out, followed by flow cytometric analysis with a FACSCalibur flow cytometer.

Results

Mutated-pyrin expression plasmids were successfully constructed and expressed in HEK293T cells

Site-specific mutagenesis of plasmids, pFLAG-CMV-4-pyrin-E148Q, pFLAG-CMV-4-pyrin-M694I, pFLAG-CMV-4-pyrin-M694V, pFLAG-CMV-4-pyrin-E148Q+M694I, and pFLAG-CMV-4-pyrin-WT encoding E148Q, M694I, M694V, and E148Q+M694I mutated-pyrin and WT pyrin, generated from pcDNA3-HA-pyrin-WT as a template, was successfully completed and confirmed by sequencing (Fig. 1a). WT pyrin and E148Q, M694I, M694V, and E148Q+M694I mutated pyrin were stably expressed in HEK293T cells transfected with pFLAG-CMV-4-pyrin-WT, pFLAG-CMV-4-pyrin-E148Q, pFLAG-CMV-4-pyrin-M694I, pFLAG-CMV-4-pyrin-M694V, and pFLAG-CMV-4-pyrin-E148Q+M694I, whereas there was an undetectable level of pyrin in HEK293T cells transfected with pFLAG-CMV-4 (Vector) (Fig. 1b).

Wild-type pyrin and E148Q, M694I, M694V, and E148Q+M694I pyrin are detergent-insoluble

HEK293T cells were transfected with the expression plasmids pFLAG-CMV-4-pyrin-WT, pFLAG-CMV-4-pyrin-E148Q, pFLAG-CMV-4-pyrin-M694I, pFLAG-CMV-4-pyrin-M694V, or pFLAG-CMV-4-pyrin-E148Q+M694I, encoding WT, E148Q, M694I, M694V, and E148Q+M694I pyrin, respectively. The cells were suspended in 1.0 % NP-40 buffer and separated into soluble (S: supernatant) and insoluble (P: pellet) fractions by centrifugation at 12,000 rpm for 20 min. Both fractions were subjected to Western blotting. WT pyrin and all mutated pyrins that we tested were fractionated in detergent-insoluble fractions (Fig. 1c; P).

Cytokine secretion from synovial sarcoma SW982 cells

IL-8 and IL-6 were spontaneously secreted from synovial sarcoma SW982 cells (Fig. 2a, b), whereas IL-1β or TNF-α

could not be detected in our ELISA system even when stimulated by LPS (data not shown).

IL-8 secretion from SW982 cells was suppressed by WT pyrin but suppressed much less by mutated pyrins

When SW982 cells were transfected with expression plasmids pFLAG-CMV-4 (Vector), pFLAG-CMV-4-pyrin-E148Q, pFLAG-CMV-4-pyrin-M694I, pFLAG-CMV-4-pyrin-M694V, pFLAG-CMV-4-pyrin-E148Q+M694I, and pFLAG-CMV-4-pyrin-WT, IL-8 but not IL-6 secretion from SW982 seemed to be suppressed (Fig. 2a, b). After standardation to the β-galactosidase activity, related % of IL-8 secretion versus WT pyrin suppression, IL-8 secretion was significantly suppressed by WT pyrin but suppressed much less by E148Q, M694I, M694V, and E148Q+M694I pyrin in that order (Fig. 2c). In terms of IL-6 secretion from SW982 cells, there was no significant difference among all the mutations (Fig. 2d).

Neither ASC nor caspase-1 was expressed in 982 synovial sarcoma cells

The expressions of inflammasome components ASC and caspase-1 were analyzed by Western blotting. Although both ASC and caspase-1 were expressed in THP-1 monocytic leukemia cells, they were not expressed in SW982 cells as well as HEK293T cells (Fig. 3).

IL-1β, IL-8, TNF-α but not IL-6 secretion from THP-1 cells was suppressed by stably expressed WT pyrin but suppressed much less by stably expressed mutated pyrin proteins

We generated stable THP-1 cells transfected with expression plasmids pFLAG-CMV-4 (Vector), pFLAG-CMV-4-pyrin-E148Q, pFLAG-CMV-4-pyrin-M694 V, and pFLAG-CMV-4-pyrin-WT, which express no pyrin (vector control), or stably express mutant pyrin proteins such as M694V, E148Q, or WT pyrin (Fig. 4a inset). These cells secreted IL-

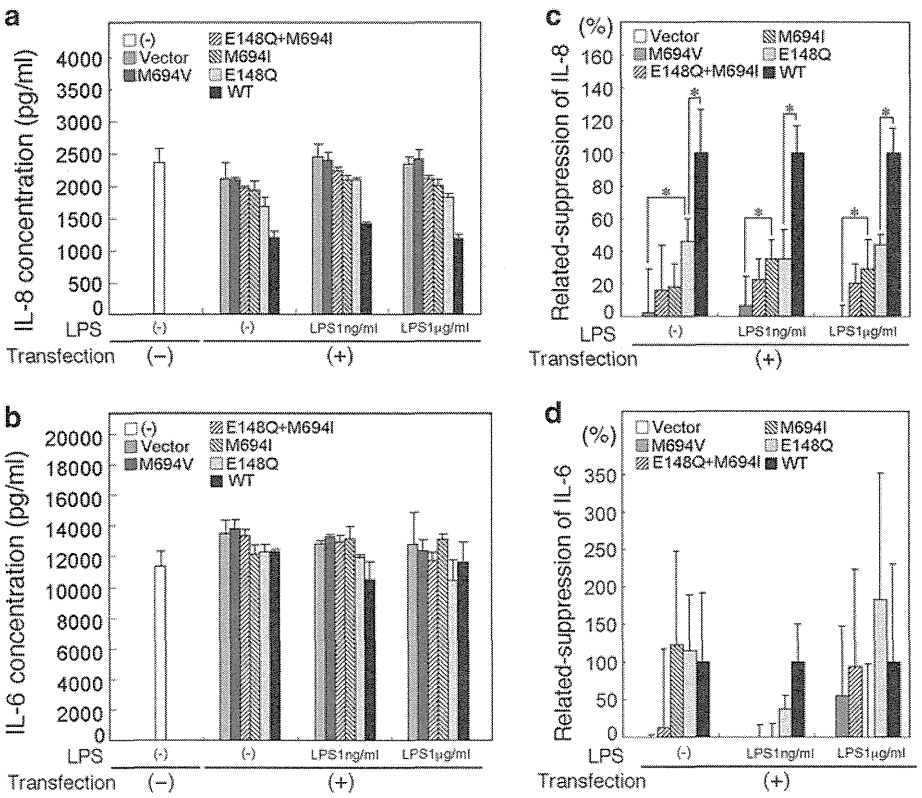

Fig. 2 Interleukin-8 and interleukin-6 secretion from SW982 synovial sarcoma cells transfected with expression plasmids. **a**, **b** 1×10^6 SW982 cells were transfected with 1.67 μg of pFLAG-CMV-4 (Vector), pFLAG-CMV-4-pyrin-M694V (M694V), pFLAG-CMV-4-pyrin-E148Q+M694I (E148Q+M694I), pFLAG-CMV-4-pyrin-M694I (M694I), pFLAG-CMV-4-pyrin-E148Q (E148Q), pFLAG-CMV-4-pyrin-WT (WT), or left untransfected (−) in the presence of 0.67 μg of pEF1-BOS-β-gal. 8 h after transfection, culture medium was replaced with 1 ml of DMEM alone [LPS(−)], or DMEM containing 1.0 ng/ml or 1.0 μg/ml LPS. 8 h after medium replacement, concentrations of interleukin-8 (IL-8) (**a**) and interleukin-6 (IL-6) (**b**) in the culture supernatant were measured by ELISA. Values are from triplicate cultures. **c**, **d** Percentiles are relative suppression of mutated pyrin versus WT pyrin. Percentiles of relative suppression of IL-8 (**c**) or IL-6 (**d**) secretion from SW982 cells transfected with mutated pyrin versus WT pyrin were normalized to the transfection efficiency by β-galactosidase activity from triplicate cultures. *A p value <0.05 was considered statistically significant

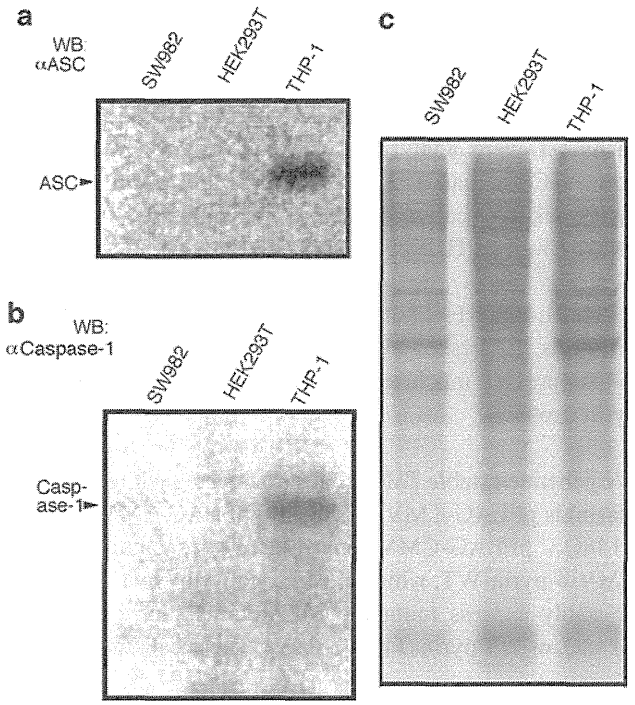

Fig. 3 Expression of ASC and caspase-1 in THP-1 cells, SW982 cells, and HEK293T cells by Western blotting analysis. Thirty μg of whole cell lysates of THP-1 cells, SW982 cells, and HEK293 cells was subjected to Western blotting. **a** Blotting membranes were detected using mouse anti-human ASC monoclonal antibody [26]. **b** Blotting membranes were detected using rabbit anti-human caspase-1 polyclonal antibody (Cell Signaling Technology, Danvers, MA, USA). **c** Gel was stained with Coomassie Brilliant Blue

1β, IL-6, IL-8, or TNF-α with 10 ng/ml LPS stimulation (Fig. 4a); for IL-1β, IL-8, and TNF-α, each cytokine secretion was significantly suppressed by WT pyrin but suppressed much less by M694V pyrin (Fig. 4a, b). In terms of IL-6 secretion from THP-1 cells, there was no significant difference among all the mutations (Fig. 4a, b).

Pyrin affects ERK1/2 phosphorylation of SW982 cells

We found that p38 and ERK 1/2 were spontaneously phosphorylated even when mutated M694V and E148Q pyrin proteins were ectopically expressed in SW982 cells (Fig. 5a, b). ERK1/2 was found to be less phosphorylated when WT pyrin was ectopically expressed in SW982 cells (Fig. 5b). On the other hand, there was no significant phosphorylation in NF-κB p65 for NF-κB activation (Fig. 5c).

Peripheral blood mononuclear cells from FMF patients secreted IL-8 when incubated on a culture plate

We obtained peripheral blood mononuclear cells from five FMF patients with *MEFV* mutations; four patients were

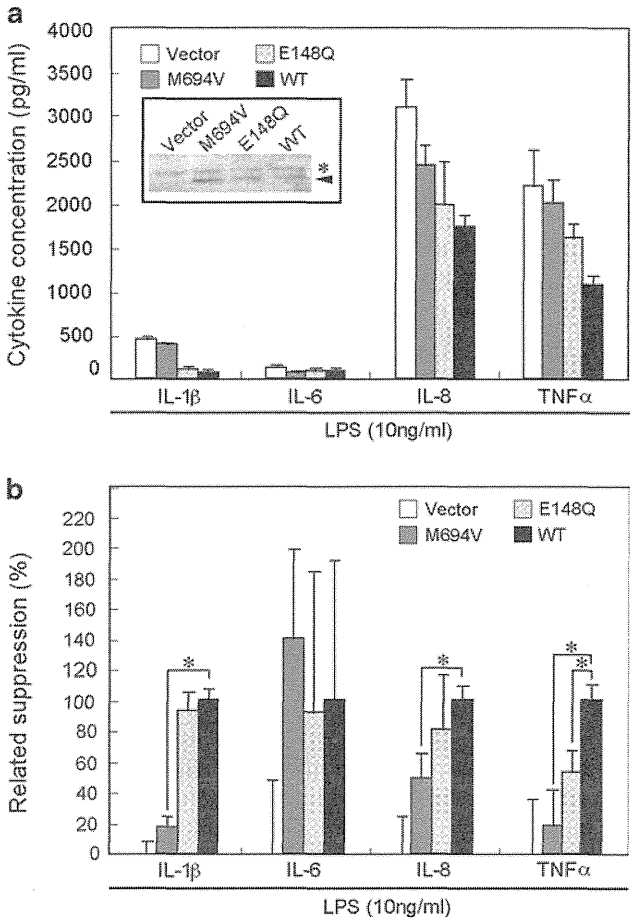

Fig. 4 Interleukin 1β, interleukin-6, interleukin-8, and TNF-α secretion from stable THP-1 cells. **a** 1×10^7 monocytic leukemia THP-1 cells were transfected with 5 μg of pFLAG-CMV-4 (Vector), pFLAG-CMV-4-pyrin-WT, pFLAG-CMV-4-pyrin-E148Q, or pFLAG-CMV-4-pyrin-M694V. After incubation with 500 μg/ml G418 (Sigma) in RPMI 1640 medium including 10 % FBS for 4 weeks to generate stable THP-1 cells. Wild-type (WT) and mutated pyrin expressions were confirmed by Western blotting (*inset*, *arrowhead*; *asterisk* is non-specific band). THP-1-derived stable cells expressing WT and mutated pyrin proteins were pre-cultured in 24-well flat-bottomed plates to a final cell density of 2×10^7/ml in a volume of 300 μl of RPMI1640 Medium including 10 % FBS for 8 h at 37 °C in a humidified atmosphere with 5 % CO_2. Then, the culture medium was supplemented with 300 μl of RPMI1640 containing 20 ng/ml LPS. 8 h after medium replacement, the concentrations of IL-1β, IL-6, IL-8, and TNF-α in the culture supernatant were measured by enzyme-linked immunosorbent assay with specific antibodies (BD Biosciences, San Jose, CA, USA). **b** Percentiles of relative suppression of IL-1β, IL-6, IL-8, or TNF-α secretion from THP-1 cells expressing mutated pyrin versus WT pyrin were calculated from triplicate cultures. *A p value <0.05 was considered statistically significant

E148Q/M694I compound heterozygotes and one patient was an E148Q/E148Q homozygote. We found a significant difference between five FMF patients and five healthy volunteers in terms of IL-8 secretion from mononuclear cells, even when incubated on a culture plate for 6 h (Fig. 6a, b). Peripheral blood mononuclear cells from FMF

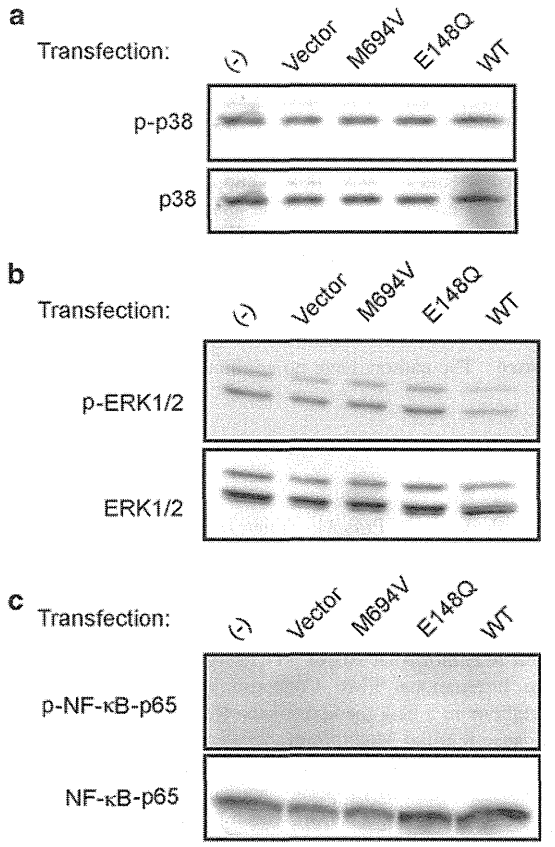

Fig. 5 Western blotting analyses for p38, ERK, and NF-κB pathways. 40 μg of SW982 cell lysates were subjected to SDS-PAGE followed by Western blotting analysis for p38, ERK, and NF-κB pathways. Signals from the same blotting membrane were detected by phospho-p38 MAPK (Thr180/Tyr182) rabbit monoclonal antibody and p38α MAPK rabbit polyclonal antibody for the p38 pathway (**a**), or phospho-p44/42 MAPK (Erk1/2) (Thr202/Tyr204) rabbit monoclonal antibody and p44/42 MAPK (Erk1/2) rabbit monoclonal antibody for the ERK pathway (**b**), or phospho-NF-κB p65 (Ser536) rabbit polyclonal antibody and NF-κB p65 rabbit monoclonal antibodies for the NF-κB pathway (**c**)

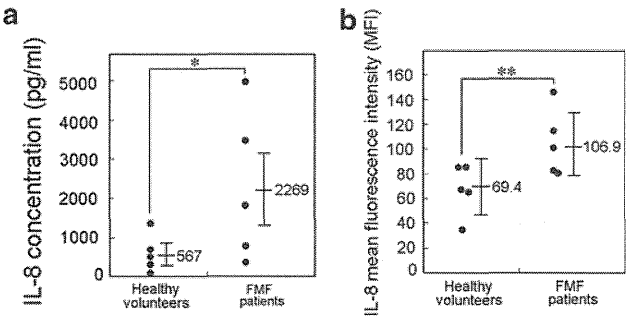

Fig. 6 IL-8 secretion from mononuclear cells from FMF patient with pyrin mutations compared with healthy volunteers. **a** 5×10^5/ml peripheral blood mononuclear cells were incubated in 96-well flat plates with RPMI1640 with 10 % heat-inactivated FBS for 6 h. The supernatants were collected and analyzed for IL-8 concentration (pg/ml) with the Cytometric Bead Array Flex set. **b** 5×10^5/ml mononuclear cells including BD GolgiPlug protein transport inhibitor (BD Biosciences) were incubated under the same conditions as described above. After 6 h of incubation, adherent cells were collected by pipetting. The cells were fixed using a BD Cytofix/Cytoperm solution for 20 min at 4 °C, then fixed cells were permeabilized by washing two times in 1 × BD Perm/Wash buffer. Intracellular IL-8 was stained with FITC-conjugated anti-IL-8 monoclonal antibody and flow cytometric analysis was performed with a FACSCalibur flow cytometer and mean fluorescence intensity (MFI) was calculated. *, ** p values <0.05 and <0.01 were considered statistically significant, respectively

patients were found to exhibit higher IL-8 secretion than those from healthy volunteers (Fig. 6a, b). IL-1β concentrations were at an undetectable level under the same conditions (data not shown).

Discussion

We have investigated the relationship between the main pyrin mutations of FMF patients and the suppression of IL-8 secretion from synovial sarcoma SW982 cells. Pyrin was discovered as a causative gene product of FMF, and E148Q, M694I, and E148Q/M694I mutations of pyrin have been found to be the major mutations of Japanese FMF patients [7, 9]. We constructed mutated-pyrin expression plasmids corresponding to the above mutations (Fig. 1a), and found no apparent difference among WT pyrin and mutated pyrin proteins in terms of expression stability and detergent solubility (Fig. 1b, c). We also found that WT pyrin suppressed IL-8 secretion from SW982 cells, but this was less suppressed by E148Q, M694I, M694V, and E148Q+M694I pyrin in that order, and WT pyrin and mutated pyrin proteins did not affect IL-6 secretion from SW982 cells (Fig. 2). Although it is unusual, compared with normal synovia, for SW982 cells spontaneously to secrete IL-8 without any stimulation, it is likely that a similar model is involved in sterile arthritis, which has been found in FMF patients.

Arthritis is one of the major symptoms of FMF patients [8, 19]. The attacks of FMF arthritis are usually acute inflammatory responses, of which the hallmark in the tissue is self-limiting neutrophil infiltration in synovial stroma [20]. Neutrophils are usually recruited by chemotactic factors such as IL-8, which was shown to be induced by epithelial cells or leukocytes in microbial infection or rheumatoid arthritis [21, 22]. However, sterile inflammation in pleura, peritonea, and synovia, which is common in FMF patients, is thought to occur without any microbial infection or rheumatoid factors [23]. FMF-related sterile inflammation is reported to be triggered by dysregulation of inflammasome, an IL-1β processing platform composed of Nod-like receptor (NLR), ASC, and caspase-1 [24]. It was also reported that NLRC4-inflammasome-related NF-κB activation leads to IL-8 secretion from MEIL-8 cells [12]. The NLRC4-inflammasome-related NF-κB activation

is reported to be inhibited by pyrin [17]. Consistent with this, our results indicated that WT pyrin suppresses IL-8 secretion from SW982 cells (Fig. 2). However, unexpectedly, both ASC and caspase-1 were at undetectable levels in SW982 cells (Fig. 3), suggesting that inflammasome may have been dispensable in the mechanism of suppression of IL-8 secretion from SW982 cells in our experiment (Fig. 3).

To investigate whether pyrin can suppress IL-8 secretion from another cell line, THP-1, monocytic leukemia cells, we generated stable THP-1 cells stably expressing WT pyrin or mutant pyrin proteins such as E148Q, M694V, or vector control. We found that pyrin can suppress IL-8 secretion from THP-1 cells as well as IL-1β and TNF-α (Fig. 4). Because pyrin was reported to inhibit ASC-related inflammasome signaling [15, 16], suppression of IL-1β and TNF-α secretion from THP-1 cells may be inflammasome-dependent. Considering the results from SW982 and THP-1, we speculate that pyrin may contribute to the suppression of IL-8 secretion by an inflammasome-independent pathway.

What kind of signaling pathway does pyrin affect? We performed Western blotting analyses for the p38, ERK, and NF-κB pathways of SW982 cells. Interestingly, we found that p38 and ERK were spontaneously phosphorylated (Fig. 5a, b) and just ERK was less phosphorylated when WT pyrin was ectopically expressed in SW982 cells (Fig. 5b). We also found that NF-κB p65 was not phosphorylated (Fig. 5c). Thus, we speculate that pyrin affects at least the ERK pathway independently of inflammasome.

Notably, peripheral blood mononuclear cells from FMF patients exhibit higher IL-8 secretion than those from healthy volunteers, even when plated on a culture dish (Fig. 6a, b), suggesting that only mechanical stress may affect clinical manifestations of FMF patients.

The most frequent mutation of FMF patients in Middle Eastern countries is reported to be M694V, which is associated with arthritis and severe clinical manifestations [25], whereas no M694V mutation was found among Japanese FMF patients [6–9]. Japanese FMF patients exhibit atypical clinical manifestations, and approximately half of FMF patients exhibit E148Q/M694I compound heterozygosity, E148Q heterozygosity, or M694I homozygosity [9]. As for the clinical significance of our results in correlation with the above description, pyrin-M694V hardly suppressed IL-8 secretion from SW982 and THP-1 cells (Figs. 2, 4, respectively), whereas E148Q, M694I, and E148Q+M694I still had the ability to suppress IL-8 secretion from SW982 cells (Fig. 2).

In conclusion, our data demonstrate that FMF-related mutated pyrin proteins have a low ability to suppress IL-8 secretion from SW982 cells independently of inflammasome. Common mutations in Japanese FMF patients of E148Q, M694I, and E148Q/M694I result in retention of the power to suppress IL-8 secretion from SW982 cells, rather than the M694V mutation, which may explain why atypical clinical manifestations are common in Japanese FMF populations.

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REVIEW ARTICLE

Tumor necrosis factor receptor-associated periodic syndrome (TRAPS) in Japan: a review of the literature

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Abstract Tumor necrosis factor receptor-associated periodic syndrome (TRAPS) is a dominantly inherited autoinflammatory syndrome that is characterized by recurrent episodes of fever attacks associated with rashes, abdominal pain, myalgia, conjunctivitis, chest pain, and arthralgia. Some patients have severe abdominal pain leading to abdominal surgery. Most reported cases of TRAPS involve patients of European ancestry, but there have been nine reports of patients with TRAPS in Japan. Here, we review these nine case reports. Reported *TNFRSF1A* gene mutations in these nine index patients were C70S, T61I, C70G, C30Y, C30R, N101K, and N25D. Fever (100 %) was seen in all 23 cases. Most patients developed rash (erythema) (84.6 %) and arthralgia (73.3 %), and half suffered from myalgia (54.5 %) and abdominal pain (50.0 %). Although one-half of the patients suffered from abdominal pain, none underwent surgery. In contrast, only a small percentage of patients suffered from chest pain (20.0 %), conjunctivitis (20.0 %), and headache (10.0 %). Almost all cases (95.7 %) concerned patients whose relatives suffered from periodic fever. These findings suggest that the clinical features of Japanese TRAPS patients may be milder than those of patients in Western countries.

Keywords TRAPS · Autoinflammatory disease · *TNFRSF1A* gene mutation · Japan

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Introduction

Tumor necrosis factor (TNF) receptor-associated periodic syndrome (TRAPS), formerly known as Familial Hibernian Fever [1], is an inherited autoinflammatory syndrome that is caused by mutations in the TNF receptor type 1 (*TNFRSF1A*), the gene encoding for the 55-kDa receptor for TNF [2–6]. The *TNFRSF1A* gene mutations were first thought to be associated with a deregulation of the shedding of TNFRSF1A [2], and in 2006 Lobito and colleagues [7] reported that the mutations may spontaneously induce alternative signaling, independent of binding TNF-α. Recently, the concerted pro-inflammatory action of cell-surface wild-type and accumulated intracellular mutant TNF receptors has been reported [8]. Since TRAPS was first proposed as a genetic diagnosis, only those patients with demonstrable TNF receptor mutations should be included as having TRAPS [2–6].

More than 100 different mutations have been reported for patients with TRAPS in INFEVERS, a mutational database accessible on the World Wide Web at http://fmf.igh.cnrs.fr/infevers. However, a few *TNFRSF1A* gene mutations are also found in about 1 % of the general population [9]. The *TNFRSF1A* gene mutations that are present in unaffected individuals are considered to be low-penetrance gene mutations [10].

TRAPS is characterized by recurrent episodes of fever attacks associated with rash, abdominal pain, myalgia, conjunctivitis, chest pain, and arthralgia [2–6]. Hull and colleagues [3] reported the clinical characteristics of 50 genetically confirmed American TRAPS patients (i.e., 47 of European ancestry, 2 Puerto Ricans, and 1 African American). Common symptoms associated with periodic fever were myalgia (98.0 %), conjunctivitis/periorbital edema (90.0 %), abdominal pain (88.0 %), rash (86.0 %), arthralgia (84.0 %), and pleuritis (54.0 %). Among these 50 patients, 26 (52.0 %) had abdominal pain which led to abdominal surgery. Pelagatti and colleagues [11] reported the clinical profiles of 11 Italian patients with TRAPS, noting that the recurrent fever attacks were associated with abdominal pain (81.8 %), arthralgia (63.6 %), myalgia (63.6 %), rash (54.4 %), headache (54.4 %), chest pain/pleuritis (27.2 %), and conjunctivitis (9.0 %). Although these authors reported that arthralgia (63.6 %) was frequently associated with periodic fever, arthritis (27.2 %) was less common [11].

TRAPS was originally described as occurring among Irish and Scottish descendants [1], and most reported patients with TRAPS are of Northern European ancestry [2–6], although any ethnic group may be afflicted in this disease [2–6].

We have reported Japanese TRAPS families with the C70G mutation [12] and C30R mutation [13] of the *TNFRSF1A* gene. However, including our reports [12, 13], there have been only nine reported cases of patients with genetically confirmed TRAPS in Japan [12–20]. Moreover, to our knowledge, no TRAPS patients have been reported in East Asian countries, with the exception of Japan. In order to clarify the characteristics of TRAPS in the East Asian population, as well as to explore the modified diagnostic criteria for Japanese TRAPS patients, we reviewed the published case reports on Japanese patients with genetically confirmed TRAPS.

Japanese patients with TRAPS

Table 1 presents a summary of the reported cases of Japanese patients with TRAPS and information on their families [12–20]. Seven different mutations of the *TNFRSF1A* gene were reported in these nine Japanese patients with TRAPS: C70S (T295A) [14], T61I (C269T) [15, 20], C70G (T295G) [12], C30Y (G176A) [16, 19], C30R (T175C) [13], N101K (C390G) [17], and N25D (A160G) [18].

All mutations reported among these Japanese patients are located in the exon regions. Among the nine reports of gene mutations of *TNFRSF1A*, six reports demonstrated that family members also had gene mutations as well as clinical symptoms [12–16, 20]. One report showed that family members had episodes of periodic fever [18, 19], but no genetic analysis was performed [18, 19]. Another paper reported that a patient with TRAPS had no family history of the disease [17].

Three of the nine index cases (30.0 %) and six of 23 patients (26.1 %) had been misdiagnosed as having juvenile idiopathic arthritis (JIA) or adult onset Still's disease prior to the diagnosis of TRAPS being established [13, 16, 19] (see remarks in Table 1). All of these patients had a family history of periodic fever [13, 16, 19].

As shown in Table 2, fever was observed in all Japanese index cases [12–20]. Almost 90 % of all index patients developed rash (erythema) [12–18, 20], and nearly 80 % suffered from arthralgia [12, 14–19]; only half suffered from myalgia (55.6 %) [15, 16, 18–20]. In contrast, only a small percentage of Japanese index cases suffered from abdominal pain (33.3 %) [16, 19, 20], chest pain (22.2 %) [16, 19], conjunctivitis (22.2 %) [14, 15], and headache (11.1 %) [18].

In almost 70 % of the Japanese index cases, fever and rash improved after glucocorticoid therapy (66.7 %) [13–17, 19]. With the exception of two index cases who had no chance to use glucocorticoid [12, 18], fever and rash improved after glucocorticoid therapy in 6 out of 9 Japanese index cases with TRAPS (77.8 %) [13–17, 19]. All Japanese index cases but one (88.9 %) had patients with periodic fever occurring among their relatives [12–16, 18–20].

Table 2 also presents the summary of the clinical symptoms of Japanese index cases and TRAPS patients in their families [12–20]. Fever was seen in all cases [12–20], and most patients presented with rash (erythema) (84.6 %) [12–18, 20] and arthralgia (73.3 %) [12–19], and half suffered from myalgia (54.5 %) [15, 16, 18–20] and abdominal pain (50.0 %) [15, 16, 19, 20]. Although half of the Japanese TRAPS patients suffered from abdominal pain, none underwent surgery [15, 16, 19, 20]. In contrast, only a small percentage of patients suffered from chest pain (20.0 %) [16, 19], conjunctivitis (20.0 %) [14, 15], and headache (10.0 %) [17]. Among the ten patients who received glucocorticoid therapy, 80 % were responsive [13–17, 19]. All cases but one (95.7 %) had relatives who suffered from periodic fever attacks [12–16, 18–20].

Discussion

Hereditary periodic fevers are a group of inherited systemic disorders characterized by episodes of fever with localized inflammation that often affects serosal membranes, joints, and skin [4]. The clinical features of periodic fevers are episodes of fever and localized inflammation, which can include abdominal pain, pleuritic chest pain, arthritis or

Table 1 Reported cases of Japanese patients with TRAPS and their families

References	Age (years) and sex	Kinship	Age at onset (years)	*TNFRSF1A* gene mutation	Fever	Abdominal pain	Myalgia	Rash (erythema)	Conjunctivitis (periorbital edema)	Chest pain (pleuritis)	Arthralgia	Other symptoms	Response to glucocorticoids	Remarks
Kusuhara et al. [14]	14F	Proband	0 (2 months)	C70S (T295A)	Positive	Negative	Negative	Positive	Positive	Negative	Positive	Negative	Responder	Her 17-year-old sister was a mutation carrier with C70S (T295A)
	48F	Mother	Childhood	C70S (T295A)	Positive	NA	NA	NA	NA	NA	NA	NA	NA	Similar symptoms were observed as the proband
	45M	Maternal uncle	NA	Not examined	NA	NA	NA	NA	NA	NA	NA	NA	NA	Similar symptoms were observed as the proband
	Deceased (85F)	Maternal great grandmother	NA	Not examined	NA	NA	NA	NA	NA	NA	NA	NA	NA	Similar symptoms were observed as the proband
	Deceased (57M)	Maternal grandfather	NA	Not examined	NA	NA	NA	NA	NA	NA	NA	NA	NA	Similar symptoms were observed as the proband
Ida et al. [15]	27F	Proband	6 or 7	T61I (C269T)	Positive	Negative	Positive	Positive	Positive	Negative	Positive	Negative	Responder	She was diagnosed with SLE at the age of 21 years. Mother, 3 sisters, 2 brothers, and 1 nephew were mutation carriers with T61I (C269T)
	18F	Niece	NA	T61I (C269T)	Positive	Positive	NA	Positive	NA	NA	Negative	NA	NA	Established the diagnosis of TRAPS but did not fulfil the criteria for SLE
	6M	Nephew	NA	T61I (C269T)	Positive	Positive	NA	Negative	NA	NA	Negative	NA	NA	Established the diagnosis of TRAPS but did not fulfil the criteria for SLE
	4F	Niece	NA	T61I (C269T)	Positive	Positive	NA	Positive	NA	NA	Positive	NA	NA	Established the diagnosis of TRAPS but did not fulfil the criteria for SLE
Horiuchi et al. [12]	32M	Proband	Childhood	C70G (T295G)	Positive	Negative	Negative	Positive	Negative	Negative	Positive	Negative	No chance to use	
	87M	Grandfather	NA	C70G (T295G)	NA	NA	NA	NA	NA	NA	NA	NA	NA	Similar symptoms were observed as the proband
	55M	Father	NA	C70G (T295G)	NA	NA	NA	NA	NA	NA	NA	NA	NA	Similar symptoms were observed as the proband
	33M	Brother	NA	Not examined	NA	NA	NA	NA	NA	NA	NA	NA	NA	Similar symptoms were observed as the proband

Table 1 continued

References	Age (years) and sex	Kinship	Age at onset (years)	*TNFRSF1A* gene mutation	Fever	Abdominal pain	Myalgia	Rash (erythema)	Conjunctivitis (periorbital edema)	Chest pain (pleuritis)	Arthralgia	Other symptoms	Response to glucocorticoids	Remarks
Manki et al. [16]	10M	Proband	0 (6 months)	C30Y (G176A)	Positive	Positive	Positive	Positive	Negative	Positive	Positive	Negative	Responder	He was misdiagnosed as systemic JIA based on the ILAR criteria (prolonged spike-fever, skin rash, arthritis, and pericarditis) at 3 years of age
	7F	Sister	3	C30Y (G176A)	Positive	Positive	NA	NA	NA	NA	Positive	NA	Responder	She was suspected of having systemic JIA at 3 years of age although the clinical symptoms did not fulfill the ILAR criteria
	38M	Mother	28	Not examined	Positive	NA	Positive	NA	NA	NA	Positive	NA	NA	She had recurrent fever, arthralgia, and myalgia after the delivery of a daughter
Takagi et al. [13]	36F	Proband	22	C30R (T175C)	Positive	Negative	Negative	Positive	Negative	Negative	Negative	Negative	Responder	She had experienced periodic high-grade fever after the birth of her elder son. Fever was often accompanied with skin rash and lymphadenopathy. She was diagnosed as having adult onset Still's disease before the diagnosis of TRAPS
	11M	Son	0 (7 months)	C30R (T175C)	Positive	Negative	Negative	Positive	Negative	Negative	Positive	Negative	Responder	Tentatively diagnosed as having JIA before the diagnosis of TRAPS
	9M	Son	3	C30R (T175C)	Positive	NA	NA	NA	NA	NA	NA	NA	Non-responder	Tentatively diagnosed as having JIA before the diagnosis of TRAPS. Fever did not respond to either corticosteroid or non-steroidal anti-inflammatory drugs, but disappeared for several months independent of treatment
Nakamura et al. [17]	17F	Proband	17	N101K (C390G)	Positive	Negative	Negative	Positive	Negative	Negative	Positive	Negative	Responder	Sporadic case. No family history of TRAPS was described

Table 1 continued

References	Age (years) and sex	Kinship	Age at onset (years)	*TNFRSF1A* gene mutation	Fever	Abdominal pain	Myalgia	Rash (erythema)	Conjunctivitis (periorbital edema)	Chest pain (pleuritis)	Arthralgia	Other symptoms	Response to glucocorticoids	Remarks
Nakamura and Tokura [18]	29F	Proband	29	N25D (A160G[a])	Positive	Negative	Positive	Positive	Negative	Negative	Positive	Positive[b]	No chance to use	Her son had fever one to three times a month since he was 2 months old. Her grandfather also had episodes of periodic fever for a long time
Kai et al. [19]	21F	Proband	Childhood	C30Y (G176A)	Positive	Positive	Positive	Negative	Negative	Positive	Positive	Negative	Responder	She was misdiagnosed as systemic JIA at 11 years of age. Her newborn baby had unexplained fever and the plan was to examine gene mutations related to TRAPS. Her father had died with amyloidosis
Ohmori et al. [20]	16F	Proband	15	T61I (C269T)	Positive	Positive	Positive	Positive	Negative	Negative	Negative	Positive[c]	Non-responder	Oral prednisolone (20 mg/day) could not reduce inflammation but anti-TNF antibody and infliximab did so. Her mother and grandfather were diagnosed as TRAPS patients with similar episodes of inflammation and T61I mutation

TRAPS tumor necrosis factor receptor-associated periodic syndrome, *F* female, *M* male, *NA* not available, *TNFRSF1A* TNF receptor superfamily 1A, *SLE* systemic lupus erythematosus, *JIA* juvenile idiopathic arthritis, *ILAR* International League Against Rheumatism

[a] Changed from A73G [14] to A160G according to a mutational database accessible on the World Wide Web at http://fmf.igh.cnrs.fr/infevers

[b] Headache

[c] Dyspnea, localized edema

Table 2 Summary of clinical symptoms of Japanese TRAPS patients

Patients with TRAPS	Female sex (%)	Age at onset (years)	*TNFRSF1A* gene mutation (%)	Fever	Abdominal pain	Myalgia	Rash (erythema)	Conjunctivitis (periorbital edema)	Chest pain (pleuritis)	Arthralgia	Headache	Response to glucocorticoids	Family history of periodic fever
Index cases [12–20]	$n = 9$	$n = 9$	$n = 9$	$n = 9$	$n = 9$	$n = 9$	$n = 9$	$n = 9$	$n = 9$	$n = 9$	$n = 9$	$n = 9$	$n = 9$
	7 (77.8 %)	0 (2 months) to 29	9 (100 %)	9 (100 %)	3 (33.3 %)	5 (55.6 %)	8 (88.9 %)	2 (22.2 %)	2 (22.2 %)	7 (77.8 %)	1 (11.1 %)	6 (66.7 %)[b]	8 (88.9 %)
Index cases and patients in their family [12–20]	$n = 23$	$n = 14$	$n = 23$	$n = 17$	$n = 14$	$n = 11$	$n = 13$	$n = 10$	$n = 10$	$n = 15$	$n = 10$	$n = 12$	$n = 23$
	12 (52.2 %)	0 (2 months) to 29	18 (78.3 %)[a]	17 (100 %)	7 (50.0 %)	6 (54.5 %)	11 (84.6 %)	2 (20.0 %)	2 (20.0 %)	11 (73.3 %)	1 (10.0 %)	8 (66.7 %)[c]	22 (95.7 %)

[a] 7 of 9 patients (77.8 %) who received glucocorticoids were responders

[b] 5 family members were diagnosed as having TRAPS with clinical symptoms and family histories

[c] 8 of 10 patients (80.0 %) who received glucocorticoids were responders

arthralgia, myalgia, rashes (erythematous macular rash), and conjunctivitis [3, 4]. Periodic fevers can be characterized according to their mode of inheritance—recessively or dominantly inherited [3, 4], with familial Mediterranean fever (FMF) and hyperimmunoglobulinemia D with periodic fever syndrome (HIDS) composing the group of recessively inherited periodic fevers and TRAPS, familial cold autoinflammatory syndrome (FCAS), Muckle–Wells syndrome (MWS), and neonatal-onset multisystem inflammatory disease [NOMID; which is also known as chronic infantile neurological cutaneous and articular syndrome (CINCA)] composing the group of dominantly inherited periodic fevers [3, 4].

TRAPS, a dominantly inherited periodic fever [2–6], can be clinically distinguished from other hereditary periodic fevers by a number of characteristics: (1) recurrent attacks that often last >5 days and sometimes several weeks; (2) localized myalgia that is often associated with an overlying macular rash, which together display a centrifugal migratory pattern; (3) conjunctivitis and/or periorbital edema; (4) attenuation of symptoms with glucocorticoid but not with colchicine; (5) an autosomal-dominant mode of inheritance [21].

More than 100 different mutations have been reported for patients with TRAPS in INFEVERS, a mutational database accessible on the World Wide Web at http://fmf.igh.cnrs.fr/infevers. Of the seven mutations identified in the reported cases of Japanese TRAPS, five (C70S [14], T61I [15, 20], C70G [12], N101K [17], N25D [18]) have been reported only in Japan, while the remaining two (C30Y [16, 19], C30R [13]) have also been reported in Western countries. Since there are only 18 genetically confirmed TRAPS patients in Japan [12–20], further studies should be carried out to search for additional mutations among Japanese patients with TRAPS.

In our review of the nine case reports on Japanese TRAPS patients, eight index cases have family histories with periodic fever [12–16, 18–20], while there is only one sporadic case of TRAPS [17]. Ida and colleagues [15], who reported the T61I variant in TRAPS patients associated with systemic lupus erythematosus (SLE), reported that this variant was detected in five of 60 SLE patients (8.3 %) and five of the 120 healthy Japanese individuals in their study (4.2 %). They could not detect any significant difference in the proportion of this variant between the SLE patients and the healthy controls [15]. In addition, Horiuchi and colleagues [12], who reported on TRAPS patients with the C70G *TNFRSF1A* gene mutation, also reported that they identified the T61I variant in one of the 100 healthy Japanese volunteers in their study (1.0 %) [12]. In contrast, Aksentijevich and colleagues [9] also reported the presence of P46L and R92Q *TNFRSF1A* gene variants in TRAPS patients, which were also found in about 1 % of the U.S.

population. In particular, the R92Q mutation is supposed to be a low-penetration mutation that is associated with a milder disease course [11]. Assuming that these three substitutions confer susceptibility to autoinflammatory disease, the penetrance (i.e., the probability of having a disease if a person has a mutation) must be low because the frequency of TRAPS does not reach 1 % of the general population. The T61I, P46L, and R92Q mutations are generally considered to be low-penetrance *TNFRSF1A* gene variants because they are present in symptomatic patients as well as unaffected individuals [10].

The functional significance of T61I on *TNFRSF1A*, which augments TNF signaling, has been reported in Japan. The T61I variant has been associated with a defect in *TNFRSF1A* shedding in peripheral blood mononuclear cells [12]. However, other Japanese investigators did not find any effect on *TNFRSF1A* shedding in the monocytes collected from their patients carrying T61I [15]. In a specific cell population, such as lymphocytes, T61I may be related to the pathogenesis of TRAPS. As the T61I variation has not been reported in Caucasian patients with TRAPS, the clinical and functional importance of T61I variation needs to be clarified within the Japanese population.

There have been many sporadic cases of TRAPS in the absence of the *TNFRSF1A* gene mutation in both Japan [5] and Western countries [9]. However, we cannot eliminate the possibility sporadic cases without any gene mutation, which have been reported in the literature, had a novel gene mutation, as Nakamura and colleagues reported [18]. Since TRAPS was first proposed as a genetic diagnosis, only those patients with demonstrable TNF receptor mutations should have been included as being cases of TRAPS [2–6]. Further studies are recommended to argue this issue.

A review of the literature compiling 153 TRAPS patients from all over the world demonstrated that the most frequent symptom associated with fever is abdominal pain (77 %), which can occasionally lead to surgery in 33 % of TRAPS patients [10]. Other common clinical symptoms reported in this review are myalgia (63.5 %), rash (55.2 %), arthralgia (51 %), ocular involvement (48.8 %), and pleuritis (32 %) [10]. Arthralgia is more frequent than arthritis in TRAPS patients [4]. Chronic arthritis of the type seen in FMF has not been observed in TRAPS [4], and characteristic migratory myalgia and rashes, which distinguish TRAPS from FMF, typically occur as a localized area of cramping muscle pain with warmth and tenderness to palpation and an overlying erythematous, blanchable rash [4].

In our review of nine reports on Japanese TRAPS patients, the common clinical symptoms associated with fever were rash (erythema) (84.6 %), arthralgia (73.3 %), myalgia (54.5 %), and abdominal pain (50.0 %). Although half of the Japanese TRAPS patients suffered from abdominal pain, they had no history of abdominal surgery [15, 16, 19, 20]. In contrast, only small percentage of Japanese TRAPS patients suffered from chest pain (20.0 %), conjunctivitis (20.0 %), and headache (10.0 %). These findings suggest that the clinical symptoms of TRAPS may be milder in Japanese patients than in Caucasian ones, which is similar to the presentation of other inherited autoinflammatory syndromes, such as FMF [22] and HIDS [23]. The difference of the disease-causing mutations, the genetics or environmental background may be responsible for the discrepancies in the results. Further research is required before definitive conclusions can be drawn.

Since colchicine is ineffective in preventing the fever attacks, glucocorticoid can be used to treat the attacks of TRAPS, but patients will require escalating dosages over time [4–6]. Etanercept, an anti-TNF agent, is recommended for chronic therapy to prevent attacks [4–6]. Interleukin 1β blockade may be effective in cases resistant to anti-TNF therapy [24]. In our review of the Japanese literature, fever and rash improved after the administration of glucocorticoid in all patients [13–17, 19, 20], with the exception of the two patients who did not receive corticosteroid therapy [12, 18].

In our review of the Japanese literature, three of the nine index cases (30.0 %) and six of the 23 patients (26.1 %) had been misdiagnosed as having JIA or adult onset Still's disease prior to the diagnosis of TRAPS being established [13, 16, 19]. All of these patients had a family history of periodic fever [13, 16, 19]. Since glucocorticoid attenuates the clinical symptoms of TRAPS patients [21], patients misdiagnosed as having JIA or adult onset Still's disease may experience an improvement in their clinical symptoms with glucocorticoid therapy. There may be more TRAPS patients misdiagnosed as having JIA or adult Still's disease in Japan. Thus, an important issue should be whether Japanese patients diagnosed with JIA or adult onset Still's disease are actually TRAPS patients, especially if there is a family history of periodic fever.

There are a number of limitations to our review. First, the number of reported Japanese TRAPS patients is very small. In addition, insufficient clinical information was available for some of the family member patients of the index cases. Second, although all index cases are genetically confirmed TRAPS patients, some family members of index cases have been diagnosed as having TRAPS with clinical symptoms and this has been identified in their family history (i.e., kinship of index cases). Third, we cannot provide the answer to whether the differences between Japanese and Western TRAPS is dependent on the difference in the disease-causing mutations, genetics, or environmental background. Further studies are recommended to provide this answer.

The major strength of this review is that it demonstrates that clinical symptoms are milder in Japanese TRAPS patients than in TRAPS patients in Western countries. Compared with their counterparts, Japanese patients are less likely to suffer from severe abdominal pain, and none of the patients in this review had a medical history of abdominal surgery.

In conclusion, our review of the Japanese literature possibly suggests that the clinical features of Japanese patients with TRAPS are milder than those of TRAPS patients in Western countries. We have launched a national survey of TRAPS patients in Japan [6] and are investigating the modified diagnostic criteria for Japanese patients with TRAPS.

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Conflict of interest None.

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REVIEW ARTICLE

Guidance on the use of canakinumab in patients with cryopyrin-associated periodic syndrome in Japan

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Abstract Cryopyrin-associated periodic syndrome (CAPS) is an orphan disease with incidence of about one in 1,000,000 persons. This autoinflammatory disease develops in the neonatal period or early childhood, with various inflammatory symptoms occurring repeatedly throughout the patient's lifetime. It is caused by abnormality of the NLRP3 protein which mediates the intracellular signal transduction mechanism of inflammatory processes, resulting in continuous overproduction of interleukin (IL)-1β, which induces chronic inflammation and progressive tissue damage. Definitive diagnosis of CAPS is difficult, and treatment has also been difficult because of a lack of effective medications in Japan. Clinical studies of human anti-human IL-1β monoclonal antibody (canakinumab) treatment were conducted in Japan, and approval was granted for therapeutic use of canakinumab for CAPS in September 2011. Similar to other biological drugs, canakinumab is clinically highly effective. However, sufficient attention to the method of use and adverse drug reactions is necessary. This guidance describes the use of canakinumab in Japan for CAPS in relation to exclusion criteria, method of use, evaluation criteria, and adverse drug reactions.

Keywords Canakinumab · Cryopyrin-associated periodic syndrome · Human anti-human IL-1β monoclonal antibody · Interleukin-1β

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Introduction

Cryopyrin-associated periodic syndrome (CAPS) is an autoinflammatory disease that develops in the neonatal period or early childhood, with various inflammatory symptoms. Patients experience recurrent rash, articular symptoms, fever, and headache associated with chronic meningitis, as well as progressive visual and auditory impairment. Many patients have poor prognosis, and a large proportion develop amyloidosis.

CAPS is classified into the following three types according to its symptoms: familial cold autoinflammatory syndrome (FCAS), Muckle–Wells syndrome (MWS), and neonatal-onset multisystem inflammatory disease (NOMID). All types are associated with overproduction of interleukin (IL)-1β, which induces inflammation [1, 2] and chronic tissue damage. The overproduction is caused by a mutation of the *NLRP3* gene [3–6], which mediates responses to infectious agents, tissue damage, and intracellular proteins derived from apoptosis.

The incidence of CAPS is about one in 1,000,000 persons. Definitive diagnosis of CAPS is difficult, and treatment has also been difficult because of a lack of effective medications in Japan. Therefore, clinical studies of human anti-human IL-1β monoclonal antibody (canakinumab) treatment were conducted in Japan, and approval was granted for therapeutic use of canakinumab for CAPS in

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September 2011. However, canakinumab can also suppress physiological inflammation by neutralizing the activity of IL-1β. Although canakinumab is clinically highly effective, adverse drug reactions will be carefully monitored.

This guidance describes the use of canakinumab for treatment of CAPS in Japan in relation to exclusion criteria, method of use, evaluation criteria, and adverse drug reactions.

Overview of canakinumab

Canakinumab (Ilaris®; Novartis Pharma K.K.) is a recombinant human immunoglobulin G1 monoclonal antibody against human IL-1β expressed in mouse hybridoma SP2/0-Ag14 cells. It neutralizes the activity of IL-1β by binding to IL-1β and inhibiting binding of IL-1β to its receptor [7]. Clinical studies conducted in Japan and other countries have demonstrated that canakinumab promptly improves various inflammation-related symptoms and abnormal laboratory values in CAPS patients by inhibiting the action of IL-1β [8, 9], and that these effects persist for long time [10].

Canakinumab was approved for treatment of CAPS in the USA and Europe in 2009, and had been approved in at least 50 countries as of August 2011. In Japan, the phase I clinical study in healthy volunteers began in December 2006, and the phase III clinical study in CAPS patients began in October 2009. Canakinumab was designated as an orphan drug in August 2010. Based on the results of the Japanese phase I and phase III clinical studies and overseas clinical studies, canakinumab was approved for treatment of CAPS in September 2011.

Guideline for canakinumab treatment of CAPS patients

Indications and use

Canakinumab is indicated for treatment of CAPS. CAPS is classified according to its severity into three types: FCAS [11], MWS [12], and NOMID [13]. Patients with FCAS, which is considered to be a mild form, experience urticarial rash, fever, conjunctivitis, and other symptoms due to cold stimuli. MWS and NOMID are classified according to differences in the timing of onset and severity of symptoms, but there are no other specific differences between these types. Symptoms include fever, urticarial rash, headache, central nervous system inflammation, arthritis, and amyloidosis, depending on the severity. Inflammatory indices such as C-reactive protein (CRP) and serum amyloid A (SAA) are elevated. If these common findings and the following characteristic findings are observed, CAPS should be suspected, specialists who have experience in the prescription of canakinumab should be consulted, and *NLRP3* genetic testing should be performed.

The age of onset of FCAS [11] is just after birth or in early infancy in about 95 % of cases. Inflammatory episodes including rash, fever, and arthralgia occur repeatedly following cold exposure. Inflammatory reactions may last less than 24 h. Conjunctivitis occurs during inflammatory episodes, but hearing loss, periorbital edema, lymphadenopathy, and serositis are not observed. Concomitant amyloidosis is rare. The *NLRP3* gene is mutated in most patients.

The age of onset of MWS [12] is usually during infancy, but some patients develop the disease in childhood or adolescence. Abnormality of the *NLRP3* gene is detected in 65–75 % of cases. Inflammatory symptoms occur repeatedly due to stress, and persist for almost 3 days. Patients experience fever, rash, arthritis, myalgia, headache, conjunctivitis, and uveitis. Sensorineural hearing loss or hearing impairment occurs in 50–70 % of patients, and renal failure due to amyloidosis occurs in about 25 %.

Inflammatory symptoms occur continuously and repeatedly from soon after birth in NOMID [13]. About half of patients have low birth weight. Patients experience fever, urticarial rash, arthritis, headache, conjunctivitis, and episcleritis almost every day. Headache associated with chronic aseptic meningitis, vomiting, and irritability can also occur. Hydrocephalus, sensorineural hearing loss, psychomotor retardation, growth disorders, joint disorders, and amyloidosis develop in the long term. Joint disorders during the developmental stage lead to gait disturbance. About 20 % of patients have poor prognosis before the age of 20 years, and many patients experience progression to amyloidosis.

Contraindications and careful administration of canakinumab are presented in Table 1. There have been no cases of discontinuation of canakinumab to date.

Predose testing

Patients should be carefully screened for common infections, especially otitis media, sinusitis, and respiratory tract infections (including bronchiectasis). Patients must also be screened for tuberculosis with an interview and the following tests: chest X-ray, tuberculin reaction, chest computed tomography (CT), and QuantiFERON® (QFT). As chest X-ray and tuberculin reaction do not always give a definitive diagnosis, chest CT and/or QFT should be performed as necessary. Chest CT is necessary for all pediatric patients. Patients with history of tuberculosis or a suspected tuberculosis infection should be evaluated by physicians with experience in the treatment of tuberculosis, including pulmonologists and radiologists.

Table 1 Contraindications and careful administration of canakinumab

Contraindications	Careful administration
Patients with serious infection (Infection may worsen)	Patients with infection or suspected infection (Infection may worsen)
Patients with active tuberculosis (Symptoms may worsen)	Patients with history of tuberculosis or suspected tuberculosis infection (Tuberculosis may be activated)
Patients with history of hypersensitivity to any of the ingredients of canakinumab	Patients with history of recurring infection (Infection may recur)
	Immunocompromised patients (Infection may be induced)

Canakinumab should only be given after the administration of an antituberculous drug in patients who meet any of the followings criteria:

- Patients with shadows consistent with or indicative of old tuberculosis on chest imaging
- Patients with history of treatment for tuberculosis (including extrapulmonary tuberculosis)
- Patients strongly suspected of having tuberculosis infection in a tuberculin test or interferon gamma response test (QFT)
- Patients with history of close contact with a tuberculosis patient

Dosage and administration (Fig. 1)

Canakinumab is usually administered at 2 mg/kg for CAPS patients with body weight $\leq$40 kg or 150 mg for body weight >40 kg, every 8 weeks as a single dose via subcutaneous injection.

If satisfactory clinical response (resolution of rash and other generalized inflammatory symptoms) has not been achieved, the dose should be gradually increased as appropriate. Maximum dose is 8 mg/kg for body weight $\leq$40 kg or 600 mg for body weight >40 kg [10].

If the patient experiences relapse within 8 weeks after an administration with the maximum dose, an increase of dosing frequency of up to every 4 weeks can be considered.

The dose may be adjusted according to the condition.

Treatment evaluation

Remission criteria (clinical and serological remission) and relapse criteria (clinical and serological relapse) were used in the Japanese clinical studies to evaluate the therapeutic effects of canakinumab in patients with CAPS (Table 2). Clinical remission and relapse were evaluated using the following five levels of symptoms: absent, minimal, mild, moderate, and severe. These five levels of evaluations should be evaluated based on physician's assessment because there are no criteria for each level.

Evaluations of adverse reactions

In clinical studies in Japan, adverse drug reactions occurred in 12 of 19 subjects (63.2 %). Common adverse reactions were nasopharyngitis in three subjects (15.8 %) and stomatitis in two subjects (10.5 %). In clinical studies in other countries, adverse drug reactions occurred in 68 of 169 subjects (40.2 %). Common adverse reactions were headache in seven subjects (4.1 %), weight gain in seven subjects (4.1 %), vertigo in six subjects (3.6 %), and bronchitis in five subjects (3.0 %).

Canakinumab may affect the inflammatory and immunological reactions to viruses, bacteria, and *Mycobacterium tuberculosis* by inhibiting the action of IL-1β, which may lead to worsening of infection. In clinical studies conducted in Japan and other countries, infections including upper respiratory tract infection were reported frequently, and some of these infections were serious. Patients should therefore be carefully monitored for the occurrence, recurrence, and exacerbation of infection during canakinumab therapy.

Immunization during canakinumab therapy

Inactivated vaccines may be administered during canakinumab therapy. Live vaccines should not be given, because a risk of developing infection cannot be ruled out. It is desirable to administer necessary vaccines prior to canakinumab therapy.

Caution

Canakinumab must be used with strict adherence to the indications and contraindications. It is recommended that canakinumab be used by physicians with appropriate

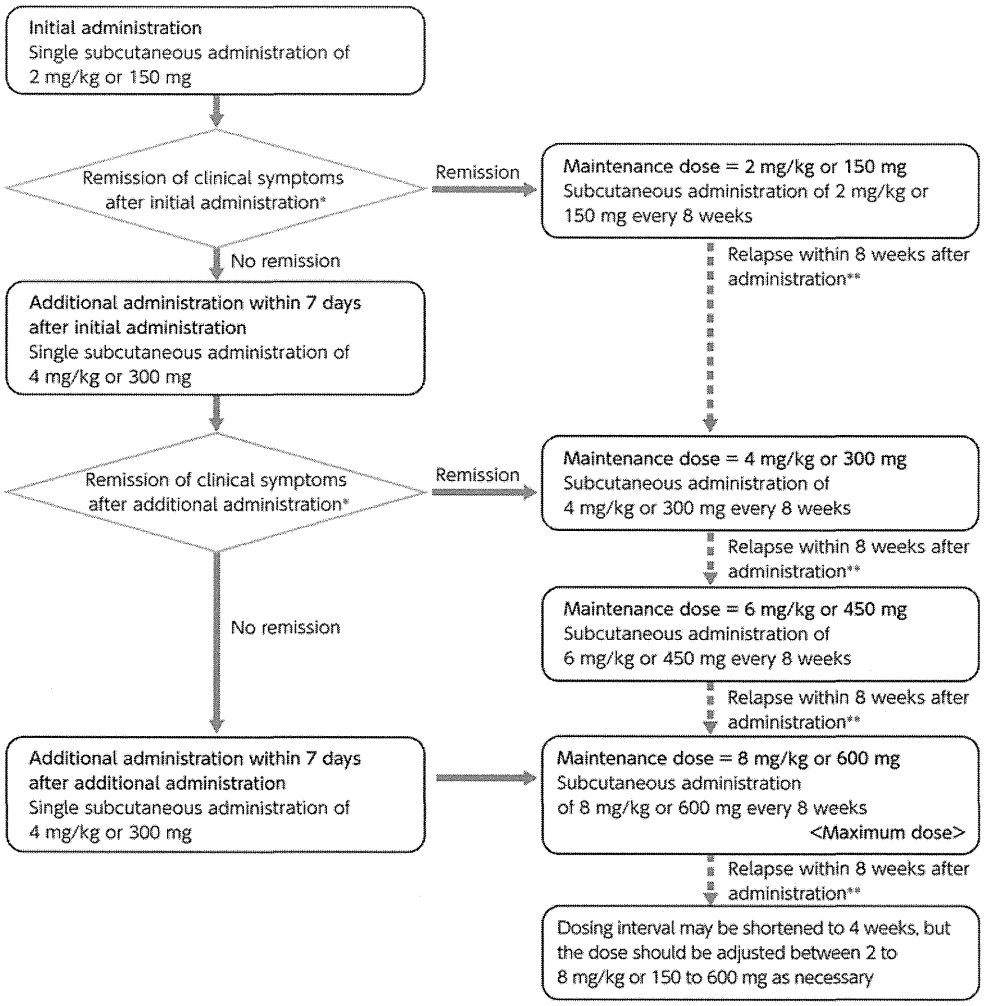

Fig. 1 Dosing regimen of canakinumab (Ilaris®) for CAPS patients [10]. Dosing regimen for CAPS patients who do not experience sufficient symptomatic relief. If sufficient clinical effects are not observed with the initial dose, the dose should be increased as shown until clinical effects are observed. The dose at which effects are observed should be the maintenance dose. * Criteria for remission in Japanese clinical studies. ** Criteria for relapse in Japanese clinical studies (modified from Ilaris® Product Information, Novartis Pharma K.K.)

Table 2 Criteria for remission and relapse used in clinical studies in Japan to evaluate the therapeutic effects of canakinumab in patients with CAPS

Remission: If the following criteria are all met, the disease is considered to have remitted	Relapse: If the following criteria are both met, the disease is considered to have relapsed
Clinical remission	Clinical relapse
Overall evaluation of autoinflammatory disease activity by physicians is minimal or lower	Overall evaluation of autoinflammatory disease activity by physicians is mild or higher, or overall evaluation of autoinflammatory disease activity by physicians is minimal and evaluation of skin disease is mild or higher
Evaluation of skin disease is minimal or lower	
Serological remission	Serological relapse
CRP is less than 10 mg/L (=1 mg/dL) or SAA is less than 10 mg/L (=10 μg/mL)	CRP is higher than 30 mg/L (=3 mg/dL) or SAA is higher than 30 mg/L (=30 μg/mL)

Evaluation grades are in 5 levels: absent, minimal, mild, moderate, and severe. These five levels of evaluations should be evaluated based on physician's assessment because there are no criteria for each level

education, in cooperation with physicians who have experience in the treatment of CAPS. It is the responsibility of pediatricians to optimize the effects of pharmaceutical products and minimize adverse drug reactions.

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Conflict of interest None.

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Induced pluripotent stem cells from CINCA syndrome patients as a model for dissecting somatic mosaicism and drug discovery

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