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Key words: CCR4, cutaneous lymphocyte-associated antigen, IL-17, IL-22, psoriasiform and pustular eruption, Th17, tumour necrosis factor- α inhibitor

Conflicts of interest: none declared.

Interleukin-17 expression in the urticarial rash of familial cold autoinflammatory syndrome: a case report

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MADAM, Familial cold autoinflammatory syndrome (FCAS) is an autosomal dominant disease characterized by recurrent episodes of fever, urticarial rash, arthralgia and conjunctivitis associated with exposure to cold temperatures.¹ FCAS is an autoinflammatory disorder related to the innate immune system. This syndrome is caused by mutations of NLRP3 (NOD-like receptor family, pyrin domain containing 3).^{2,3} The urticarial rash in FCAS is considered to be different from the typical urticaria induced by histamine, but the pathophysiology underlying the urticarial rash in FCAS remains poorly understood. We hypothesized that certain cytokines induced by interleukin (IL)-1 β may attract neutrophils and mononuclear cells in this disease. Therefore, we performed immunohistochemical detection of IL-17A in the urticarial lesions of FCAS.

A 34-year-old Japanese man presented with a recurrent, urticarial rash, which developed following physical exhaustion or cold exposure and resolved after rest and warming. The patient had experienced recurrent episodes of the rash since infancy, but the condition had not been previously diagnosed. There was no history of aseptic meningitis, renal amyloidosis or perceptive deafness. The patient's 3-month-old daughter had the same symptoms (H. Ohnishi, T. Teramoto, H. Iwata, Z. Kato, T. Kimura, K. Kubota, R. Nishikomori, H. Kaneko, M. Seishima, N. Kondo, unpublished data).

On examination, a mildly itchy, urticarial rash was distributed over his whole body (Fig. 1). In addition, bilateral



Fig 1. Clinical appearance of the urticarial rash on the back of the patient.

conjunctivitis was observed. Histopathological examination of a biopsy specimen from the lower back revealed mild dermal perivascular inflammation with prominent neutrophils and mononuclear cells (Fig. 2a). Blood tests showed an elevated total white blood cell count of $13\,120\,\mu\text{L}^{-1}$ with 74.5% neutrophils and an elevated C-reactive protein of $3.05\,\text{mg dL}^{-1}$. A heterozygous missense mutation (Y563N) at exon 3 of NLRP3 was detected in the DNA from the father and daughter by direct sequencing. These findings confirmed the diagnosis of FCAS.

An immunohistochemical analysis of IL-17A expression was performed with an antihuman IL-17A monoclonal antibody (eBioscience, San Diego, CA, U.S.A.), using the Dako EnVision method (Dako, Glostrup, Denmark). Sections ($5\,\mu\text{m}$) were cut and deparaffinized using two xylene exchanges followed by rehydration through an ethanol gradient. Antigen retrieval was performed by boiling the slides in buffer containing $6.5\,\text{mmol L}^{-1}$ sodium citrate (pH 6.0). The slides were immersed in absolute methanol containing 0.75% hydrogen peroxide for 30 min to neutralize endogenous peroxidase activity, followed by a 20-min incubation in SuperBlock Blocking Buffer (Thermo Scientific, Rockford, IL, U.S.A.) to block nonspecific binding. Slides were incubated with the IL-17A antibody at 1 : 100 dilution for 45 min at room temperature. The slides were washed three times for 10 min with phosphate buffered saline, and incubated with Dako EnVision for 30 min. Staining was visualized using 3,3'-diaminobenzidine tetrahydrochloride substrate and counterstained with haematoxylin. IL-17A predominantly stains T helper (Th) 17 cells. Immunohistochemistry of the paraffin sections of the biopsy showed positivity for IL-17A in some of the dermal perivascular infiltrate (Fig. 2b); stained cells had multilobulated nuclei suggestive of neutrophils (Fig. 2c). Replacement of the primary antibody with mouse cocktail isotype immunoglobulin (Dako) and omission of the primary antibody resulted in negative staining.

FCAS belongs to the group of cryopyrin-associated periodic syndromes (CAPS), which has been mapped to the gene NLRP3, encoding the NLRP3 (cryopyrin) protein.² CAPS is a

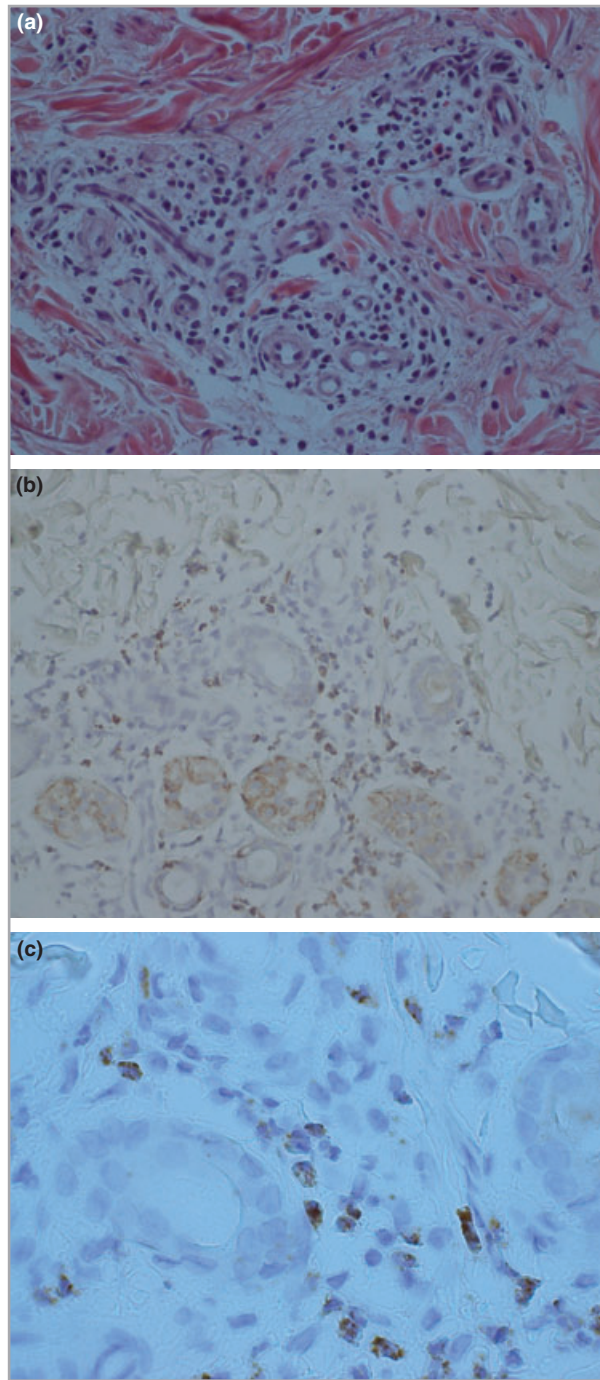


Fig 2. (a) A mild dermal perivascular inflammation with prominent neutrophils and mononuclear cells (haematoxylin and eosin stain; original magnification $\times 400$). (b) Immunohistochemical analysis of interleukin-17A expression in the dermal infiltrate. The brown coloration indicates positive staining (original magnification $\times 400$). (c) Positive-stained cells have multilobulated nuclei suggestive of neutrophils (original magnification $\times 1000$).

spectrum of rare, autoinflammatory disorders which also include Muckle–Wells syndrome and chronic infantile neurological, cutaneous, articular (CINCA) syndrome.⁴ CINCA syndrome is the most severe syndrome, while FCAS is the least

severe. In some patients with CAPS, the severity of the phenotype correlates with the genotype.⁵ However, additional genetic and environmental factors might be influential in some cases.⁵

NLRP3 plays a critical role in innate immunity, as it responds to intracellular pathogens and some danger signals. NLRP3 is composed of three domains: a pyrin domain, a nucleotide oligomerization domain (NOD) and a leucine-rich repeat domain.² All mutations have been identified in NLRP3 and most of them are found in exon 3 encoding NOD. In the present case of the father and his daughter, a heterozygous mutation (Y563N) of NLRP3 was detected. This mutation has previously been reported in two cases of FCAS.⁵

NLRP3 participates in the formation of a large protein complex called the inflammasome.⁶ NLRP3 is maintained in an inactive state in the cell cytoplasm. The NLRP3 inflammasome is activated by bacterial toxins, bacterial or viral RNA and uric acid crystals.⁷ Activated NLRP3 leads to activation of caspase-1, which subsequently activates pro-IL-1 β and pro-IL-18 to become active IL-1 β and IL-18, respectively. These cytokines participate in systemic and local responses to infection or injury. A mutation in NLRP3 might constitutively activate NLRP3, resulting in increased IL-1 β secretion.⁸ Until recently, nonsteroidal anti-inflammatory drugs and oral corticosteroids have been the only treatment options for CAPS. Their efficacy was unremarkable. With identification of the underlying molecular pathophysiology, treatments targeting the IL-1 pathway are being developed. Anakinra, an IL-1 receptor antagonist, has shown promising results in the treatment of patients with CAPS.⁴

The histological features of the urticarial rash in patients with CAPS show dermal perivascular infiltration with neutrophils.⁹ The mode of neutrophil recruitment in the urticarial rash of CAPS is still unclear. In 2009, Nakamura *et al.*⁹ reported that mast cells constitutively produce IL-1 β in the skin of patients with CAPS. NLRP3 mutants promoted the production of IL-1 β and neutrophil recruitment in mice.⁹ Patients with FCAS respond markedly to therapy with IL-1 β receptor antagonists,¹⁰ suggesting that the urticarial rash in FCAS is mediated predominantly by IL-1 β and not histamine. IL-1 β is essential for the differentiation of Th17 cells,¹¹ whose major interleukin, IL-17, induces a variety of neutrophil-activating cytokines such as IL-1, IL-6, tumour necrosis factor- α , CXCL8 and granulocyte colony-stimulating factor.¹² In contrast, Li *et al.*¹³ reported that neutrophils are the predominant source of IL-17A, and IL-17A from neutrophils regulates neutrophil infiltration in the kidney. Meng *et al.*¹⁴ reported that a mutation in NLRP3 potentiates Th17 cell-dominant immune responses. NLRP3 mutant mice showed spontaneous skin inflammation in which the inflammatory cell infiltrate was mainly neutrophils. In addition, expression of IL-17A was increased in the inflamed skin as demonstrated by real-time polymerase chain reaction analysis. Blockage of IL-17A decreased skin inflammation and IL-17A expression in NLRP3 mutant mice. On the basis of these results, IL-17 might induce neutrophils into skin and cause the urticarial rash of CAPS. In the present case, we

showed that the dermis in an urticarial lesion of a patient with FCAS was infiltrated with IL-17-positive cells, which appeared to be neutrophils. This is the first report of IL-17 expression in an urticarial lesion of a patient with FCAS.

In conclusion, we hypothesize that the urticarial rash in FCAS is induced by IL-1 β as a result of NLRP3 activation; IL-1 β activates Th17 cells leading to IL-17-associated neutrophil recruitment into the dermis. In addition, the neutrophil infiltrate might secrete more IL-17, and cause further inflammation. The blockage of IL-1 β by anakinra provides therapeutic benefit for patients with CAPS; however, long-term benefit and safety data are needed. We speculate that IL-17 might be a potential therapeutic target.

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Departments of Dermatology and

*Pediatrics, Graduate School of Medicine,

Gifu University, 1-1 Yanagido,

Gifu City 501-1194, Japan

Correspondence: Hiroaki Iwata.

E-mail: hiriwata0927@yahoo.co.jp

A. YAMAUCHI

H. IWATA

H. OHNISHI*

T. TERAMOTO*

N. KONDO*

M. SEISHIMA

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Hair casts are a dermoscopic clue for the diagnosis of traction alopecia

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MADAM, Prolonged hair traction may cause cicatricial alopecia, which is more commonly localized at hair margins behind the frontal hairline or along the temporoparietal margin (marginal alopecia) but may involve any scalp area depending on the cause of traction. Traction alopecia is clinically characterized by loss and thinning of hair in the affected area. Traction alopecia due to traumatic hair care practice is a very common problem in African-American women and children. Hairstyling associated with considerable traction includes braids, twists, dreadlocks, braiding and weaving with extensions, and the use of overnight rollers. According to a large epidemiological study, traction alopecia is more common when traction is applied to relaxed hair and in subjects using combined hairstyles.^{1,2}

However, marginal traction alopecia due to hairstyle can also occur in straight-haired individuals, particularly in girls wearing a tight ponytail. Occipital loss has been reported in women wearing a chignon or a bun, in nurses who used to fix their cap with a pin and recently in African-American girls wearing sculptured hairstyles requiring multiple pins.³

Hair casts are small freely movable cylindrical structures that envelop the proximal hair shaft and may clinically be misdiagnosed as nits.^{4,5}

The formation of hair casts is a well-known sign of persistent hair traction. Traction-induced hair casts encircle a single hair shaft, consist of the inner and/or outer root sheath and are not characterized by prominent parakeratosis.⁶ The presence of hair casts can give the clinician a clue in the differential diagnosis of alopecias affecting the scalp margin.

In the last 6 months we detected hair casts in five patients with traction alopecia including one Caucasian woman with patchy alopecia that had been previously misdiagnosed as alo-