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REVIEW ARTICLE

Review Series: Advances in Consensus, Pathogenesis and Treatment of Urticaria and Angioedema

Wheat-Dependent Exercise-Induced Anaphylaxis Sensitized with Hydrolyzed Wheat Protein in Soap

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ABSTRACT

Wheat-dependent exercise-induced anaphylaxis (WDEIA) is a specific form of wheat allergy typically induced by exercise after ingestion of wheat products. Wheat ω-5 gliadin is a major allergen associated with conventional WDEIA, and detection of serum immunoglobulin E (IgE) specific to recombinant ω-5 gliadin is a reliable method for its diagnosis. Recently, an increased incidence of a new subtype of WDEIA, which is likely to be sensitized via a percutaneous and/or rhinoconjunctival route to hydrolyzed wheat protein (HWP), has been observed. All of the patients with this new subtype had used the same brand of soap, which contained HWP. Approximately half of these patients developed contact allergy several months later and subsequently developed WDEIA. In each of these patients, contact allergy with soap exposure preceded food ingestion-induced reactions. Other patients directly developed generalized symptoms upon ingestion of wheat products. The predominant observed symptom of the new WDEIA subtype was angioedema of the eyelids; a number of patients developed anaphylaxis. This new subtype of WDEIA has little serum ω-5 gliadin-specific serum IgE.

KEY WORDS

angioedema, hydrolyzed wheat protein, percutaneous sensitization, wheat-dependent exercise-induced anaphylaxis, ω-5 gliadin

INTRODUCTION

Wheat protein derivatives are widely used in the composition of products worldwide. Industry uses gluten either with or without modifications. The main modification is hydrolysis, which is used to overcome its insolubility. Hydrolysis is performed either in acid conditions or increasingly with the use of enzymes. The procedure that is chosen and the degree of hydrolysis depend on the desired functionality and the manufacturer. Wheat-dependent exercise-induced anaphylaxis (WDEIA) is a distinct form of wheat allergy induced by the combination of wheat ingestion and physical exercise.[1,2] Aspirin intake is another well-known trigger for allergic symptoms.[3] We identified wheat ω-5 gliadin, a component of water/salt-insoluble protein (gluten), as a major allergen in patients with WDEIA.[1,4-7] When recombinant ω-5 gliadin was used in a fluorescent enzyme immunoassay combined with the CAP system (CAP-FEIA; Phadia, Uppsala, Sweden; detection range, 0.35-100 kUA/L), approximately 80% of patients with WDEIA tested positive.

Recently, increased incidence of a new subtype of WDEIA caused by hydrolyzed wheat protein (HWP) has been observed.[8-10] Patients with this new subtype were likely to be sensitized to HWP primarily through percutaneous and/or rhino-conjunctival routes by using HWP (Glupearl 19S)-supplemented soap (Cha no shizuku). In Japan, this soap was very popular and more than 46 million soaps had been sold from March 2004 to September 2010. More than 1300 individuals who had used the soap developed allergic symptoms after ingesting natural wheat pro-

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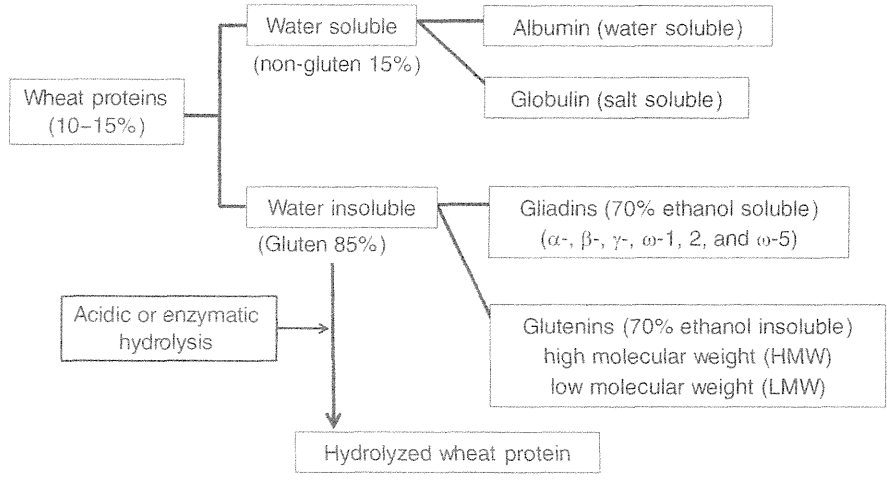

Fig. 1 Components of wheat protein.

teins to date.[11] Patients with this new WDEIA subtype tested positive for the HWP allergy by skin prick test (SPT) and had serum HWP-specific immunoglobulin E (IgE). In addition, the patients had characteristic features of facial angioedema distinct from the patients with conventional WDEIA (CO-WDEIA); they were designated as having WDEIA sensitized by HWP (HWP-WDEIA). We examined the sera of several patients with HWP-WDEIA by CAP-FEIA and found that these patients have no or low levels of ω-5 gliadin-specific IgE.

COMPONENTS OF WHEAT PROTEIN AND HWP[12]

Wheat flour contains 10-15% w/w protein. The wheat proteins are fractionated according to their solubility characteristics: albumins and globulins are soluble in salt solutions, while gluten proteins are precipitated by salt (Fig. 1). Among the gluten proteins, gliadins are soluble in 70% ethanol, while low and high molecular weight glutenin subunits are not. Wheat protein derivatives are used in a variety of products worldwide. HWPs are prepared either from insolubilized total flour proteins or more generally from gluten only. In order to increase the solubility of gluten, acidic or enzymatic hydrolysis is performed. Glupearl 19S, which was added to the soap (Cha no Shizuku), was produced by acid hydrolysis.

CASE REPORT[9]

We present our first patient who was given a diagnosis of HWP-WDEIA. The patient appeared to be sensitized to Glupearl 19S by using Glupearl 19S-supplemented soap.

A 49-year-old woman was referred to our clinic complaining of eyelid edema while working after ingesting bread. She had experienced similar episodes 3 times while working and 4 times while walking during the past 11 months. Her wheat, gluten, and ω-5 gliadin antigen-specific serum IgE levels were 1.35, 1.78, and >0.34 kUA/L (CAP-FEIA, Phadia), respectively, whereas SPTs with wheat and bread were negative (Torii allergen extracts for scratch, Torii Pharmaceutical Co., Ltd., Tokyo, Japan). WDEIA was diagnosed based on a positive challenge test with the combination of wheat (120 g) and aspirin (500 mg). Eyelid edema induced by the challenge test is shown in Figure 2. No symptoms were observed with either the wheat challenge or aspirin intake alone.

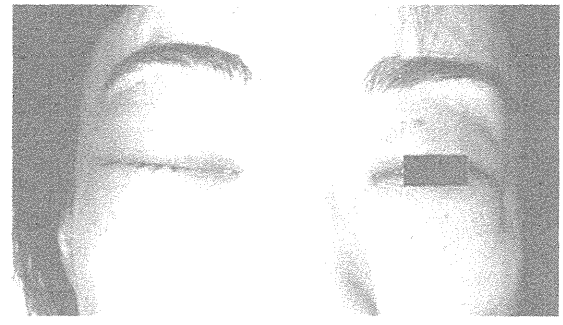

Fig. 2 Eyelid edema induced by challenge test with a combination of wheat and aspirin.

A precise medical history revealed that the patient had used Glupearl 19S-supplemented soap for 1 year, and she had noticed facial wheals and nasal discharge occasionally while washing her face with the soap. She was not atopic. She was confirmed to have no other disease by blood and physical examinations. SPT showed a positive reaction to 0.1% soap solution and 0.01% Glupearl 19S solution. A face wash challenge test with the soap induced facial wheals. Sensitization to HWP was also confirmed by Western blotting. Coomassie blue staining of Glupearl 19S using sodium dodecyl sulfate polyacrylamide gel electro-

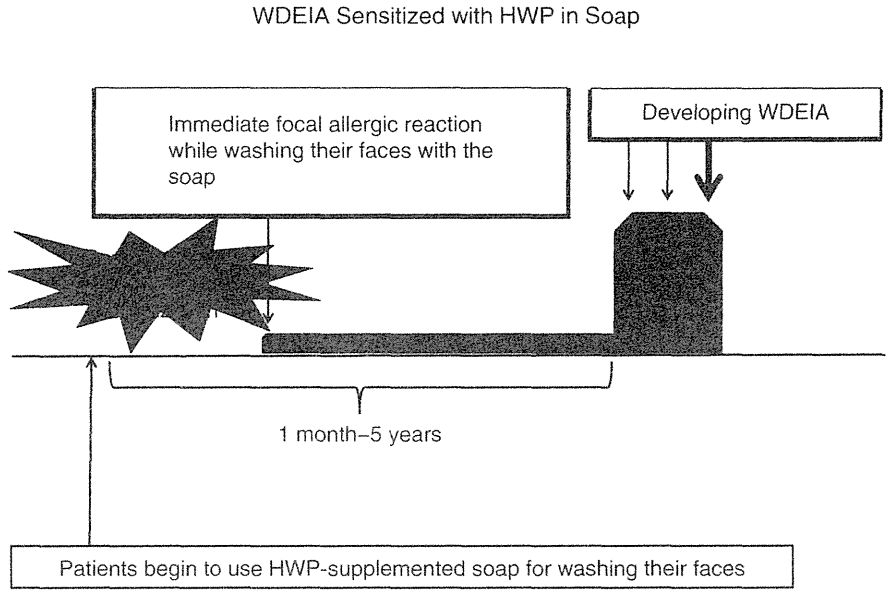

Fig. 3 Presumable time course of wheat-dependent exercise-induced anaphylaxis (WDEIA) patients sensitized to hydrolyzed wheat protein (HWP).

phoresis (SDS-PAGE) showed smears (ranging from 0-250 kDa) characteristic of random degradation and peptide rearrangement. The serum IgE of the patient reacted with the smear proteins ranging from 15-250 kDa, indicating that the IgE was specific to Glupearl 19S. In addition, the IgE of the patient reacted with both water-soluble and water-insoluble wheat proteins but not with ω-5 gliadin.

CLINICAL FEATURES OF THE PATIENTS WITH HWP-WDEIA

Almost all of the patients with HWP-WDEIA had used the same brand of soap (Cha no Shizuku), which included HWP (Glupearl 19S). Several patients developed contact urticaria on their faces several months later, and subsequently developed WDEIA. We summarized a presumable time course of patients with HWP-WDEIA (Fig. 3). After starting to use HWP-supplemented soap, patients were likely to be sensitized to HWP through the percutaneous and/or rhino-conjunctival route within 1 month to 5 years. Several patients had an immediate focal allergic reaction while washing their faces with the soap. Patients had systemic allergic symptoms when they were exposed to wheat products and subsequently exercised. In each of these patients, contact urticaria with soap preceded the food ingestion-induced reactions. The other patients directly developed generalized symptoms upon ingestion of wheat products. In some cases, allergies were triggered by mild exercise such as walking and bathing. The predominant observed symptom in all of the patients was angioedema of the eyelids; most of the patients developed anaphylaxis, and some of them developed anaphylactic shock. Several patients had pollinosis, and a few patients had atopic dermatitis.

IMMUNOLOGICAL STUDIES OF HWP-WDEIA PATIENTS

In our hospital, approximately half of the patients with HWP-WDEIA tested positive in the SPT to wheat and bread allergens. Almost all of the patients tested positive by SPT to 0.1% solution of soap supplemented with HWP in saline and 0.01% HWP solution diluted with saline. None of the patients with CO-WDEIA had positive reactions to diluted soap and HWP solution. None of the healthy subjects reacted to SPT with any of these allergens. Wheat protein-specific IgE was detected by CAP-FEIA. Wheat- and gluten-specific IgE were detected in almost all of the patients with HWP-WDEIA. A few patients with HWP-WDEIA had ω-5 gliadin-specific IgE. However, the level of IgE to ω-5 gliadin was significantly lower in patients with HWP-WDEIA than in those with CO-WDEIA. Challenge tests including exercise, wheat ingestion, aspirin intake, and/or a combination of these tests were performed for some patients.[13] In almost all of the patients, a combination of aspirin/exercise and wheat challenge induced allergic symptoms such as angioedema, urticaria, and dyspnea.

SDS-PAGE AND WESTERN BLOTTING[9,10]

To detect HWP-specific IgE in HWP-WDEIA patients, we performed SDS-PAGE and Western blotting. Coomassie blue staining of Glupearl 19S electrophoresed using SDS-PAGE showed smears characteristic of random degradation and rearrangement of peptides (Fig. 4). These smears spanned most of the gel, ranging from 0-250 kDa. In contrast, SDS-PAGE of wheat proteins showed characteristic bands mainly existing in the area of 25-100 kDa.

Representative blots are shown in Figure 4. The

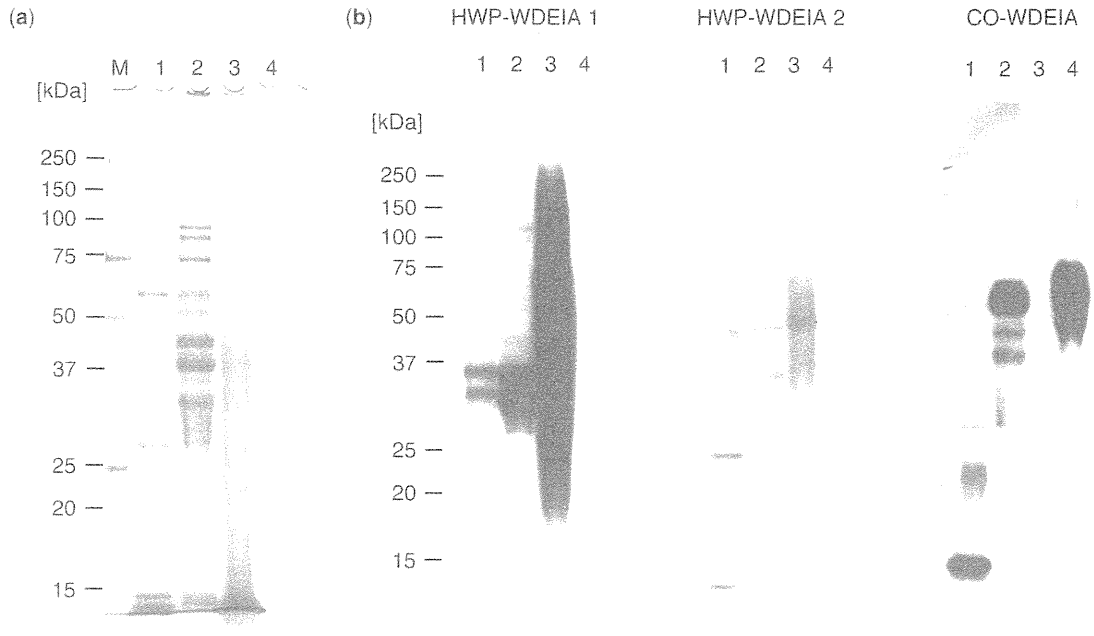

Fig. 4 Sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS-PAGE) and Western blotting analyses of wheat protein fractions and hydrolyzed wheat protein (HWP [Glupearl 19S]). (**a**) Gel stained with Coomassie Brilliant Blue; (**b**) Immunoglobulin E (IgE) immunoblotting with the patients' sera (2 HWP-wheat-dependent exercise-induced anaphylaxis [WDEIA] patients and 1 conventional [CO]-WDEIA-patient). M, marker proteins; Lane 1, salt-soluble wheat proteins; Lane 2, salt-insoluble wheat proteins; Lane 3, Glupearl 19S; Lane 4, purified ω-5 gliadin.

IgE of all of the HWP-WDEIA patients reacted to Glupearl 19S with a smear pattern. The intensity of the reaction varied among the patients. The IgE of all of the patients also reacted with both water-soluble and water-insoluble wheat proteins, whereas they did not react with ω-5 gliadin. The IgE of the CO-WDEIA patients did not react with HWP, but reacted with water-soluble and water-insoluble wheat proteins, and ω-5 gliadin.

To perform immunoblotting inhibition assays for determining cross-reactivity of wheat proteins and Glupearl 19S, 100 μL of serum was previously incubated with Glupearl 19S serially diluted from 100 to 5 μg/mL. The reaction of the IgE to water-soluble and water-insoluble wheat proteins was inhibited by Glupearl 19S in a dose-dependent manner when the sera of HWP-WDEIA patients were preincubated with a series of Glupearl 19S concentrations (Fig. 5).

INDUCTION OF BASOPHILIC CD203c EXPRESSION BY GLUPEARL 19S AND ω-5 GLIADIN

Flow cytometry-based tests of basophil activation status have been used to diagnose or confirm sensitization in allergic patients.[14-25] CD203c is an ectoenzyme that belongs to a family of ectonucleotide pyrophosphatases and phosphodiesterases. It is expressed on the cell membrane of human peripheral basophils and mast cells, and cross-linking of the high-affinity IgE receptor upregulates membrane CD203c expression.

Glupearl 19S enhanced CD203c expression of basophils in a concentration-dependent manner in all of the HWP-WDEIA patients.[10] In these patients, no significant enhancement of CD203c was observed with purified ω-5 gliadin. In contrast, purified ω-5 gliadin induces enhancement of CD203c expression in a concentration-dependent manner in CO-WDEIA patients, whereas no significant enhancement of CD203c is observed in the presence of Glupearl 19S. A representative reaction is shown in Figure 6.

INFORMATION FROM THE JAPANESE SOCIETY OF ALLERGOLOGY[11]

Information concerning the wheat allergy associated with the use of soap bars containing Glupearl 19S provided in the Rheumatism & Allergy Information Center website includes "FAQs (for general consumers)," the "Definition of the disorder and diagnostic guidelines (for healthcare professionals)," and a "List of institutions offering treatment for wheat allergy associated with the use of 'Cha no Shizuku' soap." The "Diagnostic criteria for immediate wheat allergy to the hydrolyzed wheat (Glupearl 19S) contained in

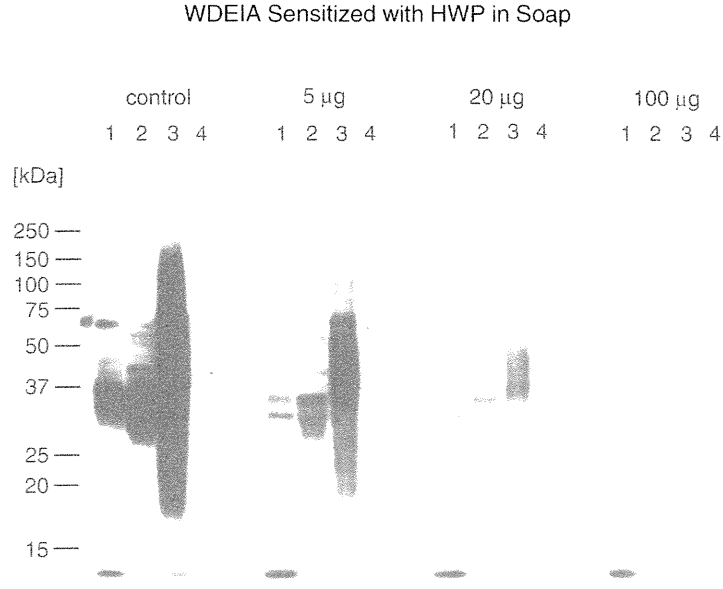

Fig. 5 Immunoblot inhibition assay for determining cross-reactivity of wheat proteins and Glupearl 19S. Lane 1, salt-soluble wheat proteins; Lane 2, salt-insoluble wheat proteins; Lane 3, Glupearl 19S; Lane 4, purified ω-5 gliadin. Electrophoresed membranes were blotted against patient sera (hydrolyzed wheat protein wheat-dependent exercise-induced anaphylaxis [HWP-WDEIA]) without Glupearl 19S (control) or with increasing amounts of Glupearl 19S (5 μg, 20 μg, and 100 μg).

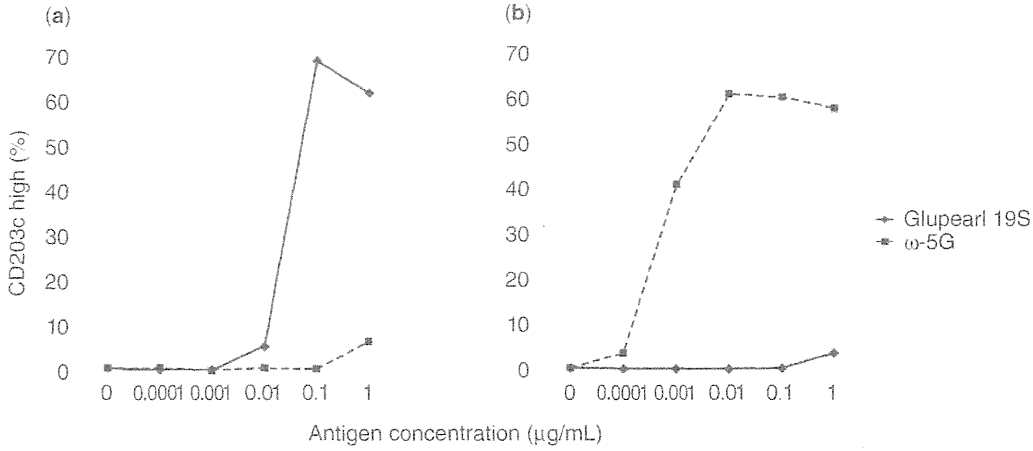

Fig. 6 Expression of CD203c on basophils induced by Glupearl 19S and ω-5 gliadin (ω-5G). (**a**) Results from a 44-year-old woman with hydrolyzed wheat protein wheat-dependent exercise-induced anaphylaxis (HWP-WDEIA). Serum allergen-specific IgE to wheat, gluten, and ω-5G was 9.26 kUA/L, 15.8 kUA/L, and 1.16 kUA/L, respectively. (**b**) Results from a 39-year-old woman with conventional wheat-dependent exercise-induced anaphylaxis (CO-WDEIA). Serum allergen-specific IgE to wheat, gluten, and ω-5G was <0.34 kUA/L, 1.90 kUA/L, and 3.82 kUA/L, respectively.

'Cha no Shizuku' soap and some other products" have been published by the Special Committee for the Safety of Protein Hydrolysate in Cosmetics of the Japanese Society of Allergology (see below).

Diagnostic criteria for immediate wheat allergy to the hydrolyzed wheat (Glupearl 19S) contained in "Cha no Shizuku" soap and some other products (Prepared by the Special Committee for the Safety of Protein Hydrolysate in Cosmetics on October 11, 2011)

[Definitive diagnosis]

Table 1 Comparison between CO-WDEIA and HWP-WDEIA

	CO-WDEIA	HWP-WDEIA
Age	School child age-old age	Adults in their 20s-60s
Gender	Both genders	Predominantly female
History of HWP-supplemented soap use	None	Essential
Symptoms occur while using the soap before developing WDEIA	None	Often
Predominant symptom of WDEIA	Urticaria (wheal)	Angioedema (especially on the eyelids)
Anaphylactic shock	Sometimes	Occasionally

Abbreviations: CO-WDEIA, conventional wheat-dependent exercise-induced anaphylaxis; HWP-WDEIA, hydrolyzed wheat protein wheat-dependent exercise-induced anaphylaxis.

Satisfying all of the following criteria:

1. Have used "Cha no Shizuku" soap or other products containing hydrolyzed wheat (Glupearl 19S)
2. Have had at least 1 of the following symptoms:
   - 2-1. Itching, eyelid edema, nasal discharge, and or wheals within several to 30 minutes after using "Cha no Shizuku" soap or other products containing hydrolyzed wheat (Glupearl 19S)
   - 2-2. General symptoms such as itching, wheals, eyelid edema, nasal discharge, dyspnea, nausea, vomiting, abdominal pain, diarrhea, and decreased blood pressure within 4 hours after eating wheat products
3. Have tested positive in at least 1 of the following tests:
   - 3-1. Prick test using ≤0.1% Glupearl 19S solution
   - 3-2. Immunoassay such as dot blot, enzyme-linked immunosorbent assay (ELISA), and Western blot to identify specific IgE antibody to Glupearl 19S in the blood
   - 3-3. Basophil activation test using Glupearl 19S as the antigen

[Exclusion criterion]

4. Tested negative in a prick test using 0.1% Glupearl 19S solution

[Suspected cases]

Satisfying Criteria 1 and 2 but not 3

*Wheat allergy is strongly suspected if sensitization to wheat or gluten is shown in a specific IgE antibody test or a prick test but there is no hypersensitivity to ω-5 gliadin or milder hypersensitivity to ω-5 gliadin compared with that to wheat and gluten.

COMPARISON BETWEEN CO-WDEIA AND HWP-WDEIA

Clinical features of CO-WDEIA and HWP-WDEIA observed in our hospital are summarized in Table 1. CO-WDEIA occurred more often in expanded age-group. HWP-WDEIA developed more commonly in women who use soap as cosmetics. In HWP-WDEIA, history of HWP-supplemented soap use is essential, and symptoms that occur while using the soap before the development of WDEIA often appear. A predominant symptom of CO-WDEIA is the development wheals on the entire body, whereas that of HWP-WDEIA is angioedema on the eyelids.

Anaphylactic shock sometimes developed in CO-WDEIA patients and occasionally developed in HWP-WDEIA patients.

Measurement of gluten-specific IgE as well as wheat-specific IgE is possible in the diagnosis of WDEIA using the CAP-FEIA; however, more than 60% of patients with definite CO-WDEIA are considered negative by these tests. Recently, recombinant food allergens, which are consistent in quality, have been produced and applied for the diagnosis of many food allergies. Measurement of IgE that is specific to ω-5 gliadin and high molecular weight-glutenin is highly useful in diagnosing CO-WDEIA when compared with the routine diagnostic CAP-FEIA for wheat and gluten.[26] As shown in Table 2, in our hospital, 79.6% of the patients with CO-WDEIA have IgE that reacted to recombinant ω-5 gliadin. Additionally, 94.4% of the CO-WDEIA patients were positive according to the combined recombinant ω-5 gliadin-specific IgE test and the recombinant high molecular weight-glutenin-specific IgE test, whereas gluten- and

Table 2 Positivity rate of ω-5 gliadin-specific IgE and high molecular weight glutenin-specific IgE measurement in CO-WDEIA and HWP-WDEIA patients

CAP-FEIA	CO-WDEIA (%, *n* = 54)	HWP-WDEIA (%, *n* = 30)
Wheat	31.4	70.0
Gluten	37.0	76.6
ω-5 gliadin	79.6	6.6
High molecular weight-glutenin	18.5	16.6
ω-5 gliadin and/or high molecular weight-glutenin	94.4	16.6

Abbreviations: CAP-FEIA, fluorescent enzyme immunoassay combined with the CAP system; CO-WDEIA, conventional wheat-dependent exercise-induced anaphylaxis; HWP-WDEIA, hydrolyzed wheat protein wheat-dependent exercise-induced anaphylaxis.

wheat-specific IgE tests positively recognized only 31.4% and 37.0% of these patients, respectively. In contrast, the positive rate of ω-5 gliadin-specific IgE was only 6.6% in HWP-WDEIA patients, whereas gluten- and wheat-specific IgE tests positively recognized 70.0% and 76.6% of these patients, respectively.

PERCUTANEOUS SENSITIZATION TO FOOD ALLERGENS

The importance of percutaneous sensitization in the development of food allergies has been well recognized in the case of latex allergies in which fruits and vegetables are the causative agents.[27] Direct percutaneous sensitization by peanuts has recently been suggested by Fox *et al.*[28] who identified a dose-response relationship between environmental (non-oral) peanut exposure and the development of peanut allergies. HWP should now be considered a possible allergen for percutaneous sensitization, because they are currently used globally as ingredients of cosmetic products, and HWP cross-reacts to the wheat allergen in foods and may cause life-threatening anaphylaxis once sensitized. Several cases of contact urticaria due to HWP in cosmetics have been described.[12,29-32] These findings indicate percutaneous and/or rhinoconjunctival penetration of HWP in patients. Some of these patients have developed generalized allergic symptoms upon ingestion of wheat products. Some of our HWP-WDEIA patients also experienced contact urticaria by using Glupearl 19S-supplemented soap and later developed generalized symptoms upon ingestion of wheat products. Another group of patients directly developed generalized symptoms upon ingestion of wheat products. Hydrophilic moieties of allergens might be necessary for percutaneous and/or rhinoconjunctival sensitization, as Glupearl 19S was hydrolyzed under acidic conditions and was highly allergenic. This process yields new terminal amino- and carboxyl-charged groups. Matsuo *et al.* reported that serum IgE of patients with wheat protein contact dermatitis reacted to water-soluble proteins rather than water-insoluble proteins.[33] They identified 3 water-soluble proteins, peroxidase, purple acid phosphatase, and wheat 27-kDa allergen, as candidate allergens for wheat protein contact dermatitis. These results suggest that glycan moieties in these proteins are involved in IgE binding.

When considering sensitization by the percutaneous and/or rhinoconjunctival route, a penetration of the molecule through the epithelia must occur. Large molecules such as proteins are generally not expected to cross the skin barrier, unless the skin has been damaged.[34] However, a few patients with HWP-WDEIA had atopic dermatitis, a condition arising from impaired function of the skin barrier. In addition to creation of new terminal amino- and carboxyl-charged groups due to the hydrolysis of peptide bonds, soap containing HWP might facilitate the penetration of HWP into the epidermis, because soap contains surfactants that may cause destruction of skin barriers. It has been proposed by Lack that antigen exposure through inflamed skin might be involved in the establishment of allergy and tolerance.[35] He reported in his review that allergic sensitization to food could occur through low-dose cutaneous sensitization and that early consumption of food protein induced oral tolerance. He argued that low-dose exposure to environmental foods (on tabletops, hands, and dust) penetrates the skin barrier and is taken up by Langerhan's cells. This leads to T helper type 2 (T_H2) responses and IgE production by B cells. In contrast, early high-dose oral consumption induces tolerance, and T helper type 1 (T_H1) and regulatory T-cell responses occur in the gut-associated lymphoid tissue. The timing and balance of cutaneous and oral exposure determines whether a child has allergy or tolerance (dual-allergen-exposure hypothesis). Leduc *et al.* have suggested that acidic hydrolysis induces a conformational change in HWP and produces a conversion of a glutamine residue to glutamic acid and a conversion of an asparagine residue to aspartic acid.[36] As a result, new epitopes that differ from the epitopes of natural wheat proteins might be produced. It is conceivable that humans do not have sufficient tolerance to HWP, which are not natural proteins. Thus, humans appear to be easily sensitized to HWP once HWP penetrates into the skin or mucosa. As wheat proteins contain repetitive amino acid structures highly rich in glutamine and proline, it is likely that IgE produced against HWP cross-reacts to natural wheat proteins. In fact, preincubation of sera with HWP clearly revealed a decrease in the binding of IgE to natural wheat proteins (Fig. 5).

IgE produced against HWP in HWP-WDEIA patients do not react to ω-5 gliadin but instead to other undetermined protein components with specific epitopes. The patients with HWP-WDEIA had no or decreased levels of ω-5 gliadin-specific IgE.

HWPs are prepared either from insolubilized total flour proteins or more generally from gluten alone. The main modification of gluten is hydrolysis performed to overcome its insolubility. HWP, which includes large polypeptide aggregates, has a greater ability to induce sensitization than the HWP that is digested to lower molecular weight polypeptides.[37] This finding is supported by the previous observation by Palosuo *et al.*[38] that artificial polymerization of ω-5 gliadin increases its direct reactivity with IgE in an immunosorbent assay and in patients using SPT. They also hypothesized that large polymers have better IgE-bridging capacities.

CONCLUSIONS

We experienced an outbreak of wheat allergy with systemic symptoms, which may be due to percutaneous and/or a rhinoconjunctival sensitization following

the use of cosmetics. Such an event was almost completely unknown by healthcare professionals until recently. Thus, there is limited information regarding the clinical course. The serum IgE levels of many HWP-WDEIA patients against wheat and gluten decreased after the cessation of HWP-containing soap usage. In some patients, serum IgE against these antigens had disappeared. A remission case of HWP-WDEIA has been reported[39] and we also experienced some patients who had remission of WDEIA-symptoms (unpublished observation). However, many patients with HWP-WDEIA have not recovered from WDEIA-related symptoms. Large-scale studies are needed to clarify the prognosis of HWP-WDEIA patients.

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Tinea caused by *T. verrucosum* is very rare in young children.[9] In suspected cases, special attention is required to avoid misdiagnosis. DNA sequence analysis has become the standard method for identifying the causative fungus, but morphological diagnosis remains important.

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Drug eruption with eosinophilia and systemic syndrome associated with reactivation of human herpesvirus 7, not human herpesvirus 6

Dear Editor,

Drug eruption with eosinophilia and systemic syndrome (DRESS), also known as drug-induced hypersensitivity syndrome (DIHS), is a severe type of drug eruption.[1] It is characterized by cutaneous eruption, lymphadenopathy, liver or renal dysfunction, leukocytosis with mainly eosinophilia and sometimes atypical lymphocytes. The causative drugs of DRESS/DIHS are limited to a relatively narrow range of drugs including anticonvulsants, sulfasalazine, diaphenylsulfone, allopurinol, minocycline and several other drugs.[2] DRESS/DIHS develops 2–6 weeks after the initiation of these drugs. Recently, it has been shown that reactivation of human herpesvirus (HHV)-6 may be implicated in the pathogenesis of this disease.[3] We report a rare case of DRESS with HHV-7 reactivation but without HHV-6 reactivation.

A 62-year-old Japanese male began to receive oral carbamazepine therapy at the Department of Urology of our hospital because of pain after surgery for prostate cancer on 30 June 2004. Fever (38.5°C) and systemic diffuse erythema developed on 11 July (day 0). He consulted a local clinic, and was diagnosed as having hepatic dysfunction. Three days later, he was urgently admitted to the Department of Gastroenterology of our hospital and referred to our department. Physical examination revealed mild facial edema and systemic diffuse erythema (Fig. 1a,b). Laboratory findings showed eosinophilia (white blood cell count, $5.6 \times 10^3/\mu L$; eosinophils, 30%) and liver dysfunction (aspartate aminotransferase, 648 IU/L; alanine aminotransferase, 879 IU/L; lactate dehydrogenase, 1055 IU/L). A skin biopsy of erythema showed perivascular lymphocytic infiltration in the upper dermis with small amounts of eosinophils and nuclear dust (Fig. 1c,d).

The patient was suspected of having DRESS/DIHS because of high fever, systemic diffuse erythema, eosinophilia and hepatic dysfunction on admission. Thus, his carbamazepine therapy was discontinued, and oral prednisolone (30 mg/day) was initiated on day 3, which gradually improved his eruption and high grade fever. Figure 2 shows the clinical course of the patient. On day 4, HHV-7 DNA was detected in the peripheral blood (1.2×10^4 copies/mL) by polymerase chain reaction. High titers of immunoglobulin (Ig)G antibodies to HHV-7 were also recorded (1:160) on day 5. After the remission of the eruption, HHV-7 DNA became negative on day 15, followed by reduction of antibody titers to HHV-7 (1:40) on day 25. During the course of management, HHV-6, cytomegalovirus and Epstein–Barr virus DNA remained undetectable in the peripheral

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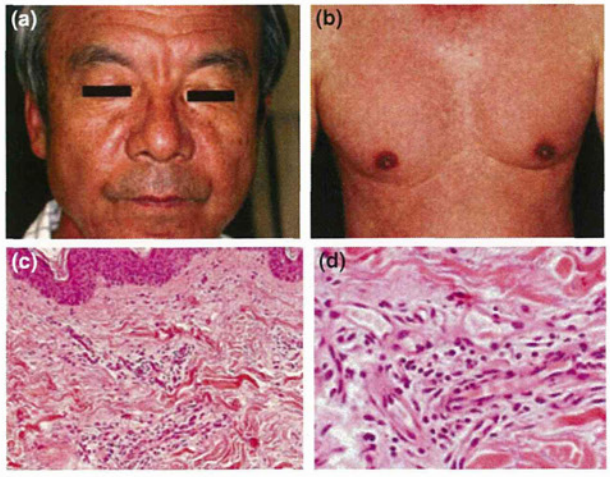

Figure 1. (a) Mild facial edema was observed. (b) Diffuse erythematous skin eruption in the patient. (c,d) The skin biopsy showed perivascular lymphocytic infiltration in the upper dermis. (hematoxylin–eosin, original magnifications: [c] ×100; [d] ×400).

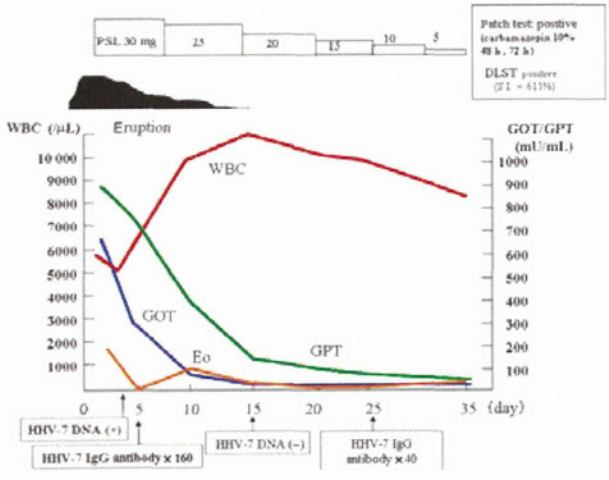

Figure 2. Clinical course in relation to serological data. DLST, drug lymphocyte stimulation test; Eo, eosinophils; GOT, aspartate aminotransferase; GPT, alanine aminotransferase; HHV, human herpesvirus; IgG, immunoglobulin G; PSL, prednisolone; WBC, white blood cell.

blood, and HHV-6 IgG antibody titers showed no elevation on days 9 (1:20) and 25 (1:10). A patch test was positive for carbamazepine after both 48 and 72 h. A drug-induced lymphocyte stimulation test for carbamazepine was also positive (stimulation index, 611%).

The clinical features and laboratory findings of DRESS/DIHS have a lot in common, although there are differences in the diagnostic criteria between DRESS and DIHS.

Our case took carbamazepine p.o. for 11 days before the onset of the disease. Because the diagnostic criteria for DIHS[4] include developing more than 3 weeks after starting with a limited number of drugs, we consider that this case cannot be diagnosed as DIHS strictly. Instead, diagnostic criteria for DRESS are fulfilled and our patient showed solely HHV-7 reactivation as far as we examined.

Human herpesvirus-7 was first isolated from $CD4^+$ T lymphocytes by Frenkel *et al.*[5] Tanaka *et al.*[6] reported that HHV-7 was another causative agent of exanthem subitum in addition to HHV-6. DRESS/DIHS patients with co-reactivation of HHV-6 and HHV-7 have been reported previously.[7] There have also been some reports of DRESS/DIHS patients with reactivation of herpesviruses other than HHV-6, such as cytomegalovirus and Epstein–Barr virus.[8,9] Interestingly, our case showed no reactivation of any herpesvirus examined other than HHV-7. Although the clinical finding of our case was almost identical to typical DRESS/DIHS with HHV-6 reactivation, there was a difference: the lag phase between the onset of skin eruption and HHV-7 reactivation was only 4 days, and relatively short compared to that of typical DRESS/DIHS cases.[10] Accumulation of cases will be necessary to characterize the clinical features of DRESS/DIHS with HHV-7 reactivation.

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JAPANESE DERMATOLOGICAL ASSOCIATION
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Case of drug-induced hypersensitivity syndrome involving multiple-drug hypersensitivity

Dear Editor,

Drug-induced hypersensitivity syndrome (DIHS) is a life-threatening adverse reaction characterized by skin rashes, fever, leukocytosis with eosinophilia and/or atypical lymphocytosis, lymph node enlargement, and liver and/or renal dysfunction. DIHS usually occurs 3 weeks to 3 months after the start of therapy with certain drugs.[1] Shiohara *et al.* and Hashimoto *et al.* reported that there is a close relationship between human herpes virus-6 (HHV-6) reactivation and the development of this syndrome.[2,3]

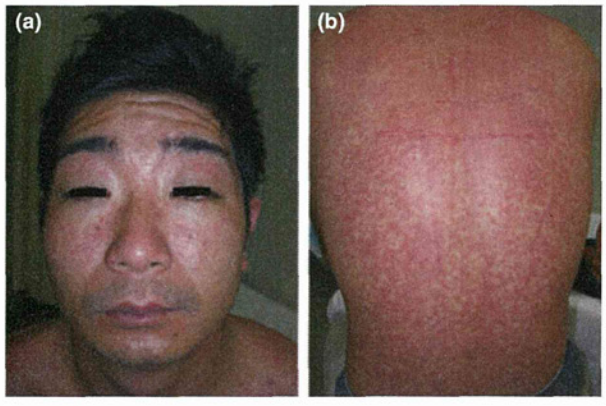

Figure 1. (a) Facial edema and diffuse maculopapular rash over the subject's face, which spared the periocular skin, and (b) diffuse maculopapular rash over his trunk.

A 31-year-old man with lung tuberculosis and hyperuricemia developed a fever of 38.8°C and a maculopapular rash over his face, trunk and extremities (Fig. 1) at 2, 4 and 6 weeks, respectively, after the initiation of therapy involving ethambutol (EB), allopurinol, isoniazid (INH), rifampicin (RFP) and pyrazinamide (PZA) (Fig. 2). Laboratory studies showed leukocytosis (15 400/μL) with eosinophilia (19.4%) and liver dysfunction (aspartate aminotransferase, 218 U/L; alanine aminotransferase, 287 U/L). On the 16th day of onset, real-time polymerase chain reaction detected HHV-6 DNA in his whole blood. We diagnosed him with DIHS and oral prednisolone (60 mg/day) was effective. However, the skin eruption relapsed on day 32, 1 day after the restart of the anti-tuberculosis drug therapy (INH, RFP, EB and PZA). The skin eruption subsequently disappeared 1 week after treatment with discontinuation of these anti-tuberculosis drugs.

The patch test and lymphocyte transformation test (LTT) demonstrated positive reactions to INH. We changed the patient's anti-tuberculosis treatment to RFP, EB and levofloxacin (LVX) on day 47, but itching appeared 1 month later. We again changed anti-

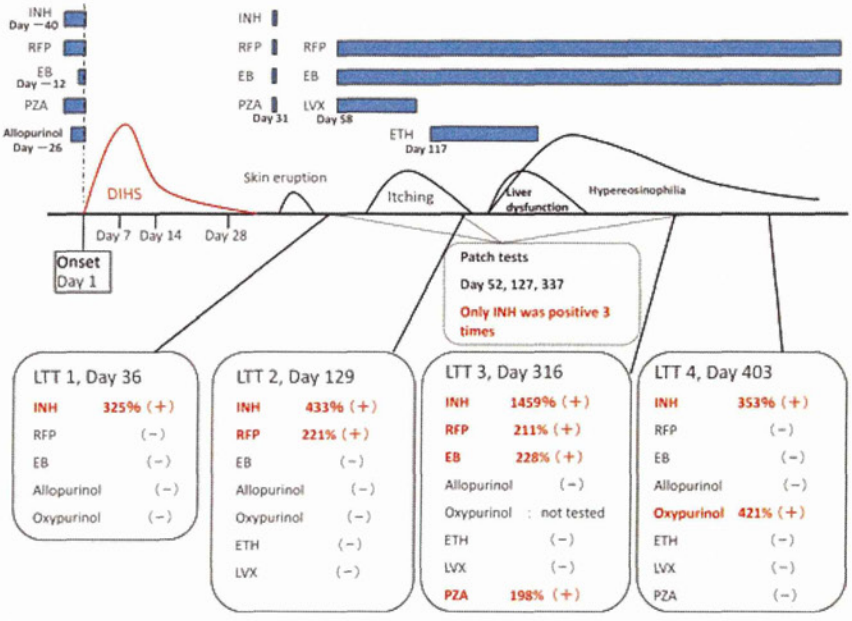

Figure 2. Clinical course and results of patch tests and LTT. DIHS, drug-induced hypersensitivity syndrome; EB, ethambutol; ETH, ethionamide; INH, isoniazid; LTT, lymphocyte transformation test; LVX, levofloxacin; PZA, pyrazinamide; RFP, rifampicin.

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tuberculosis treatment to RFP, EB and ethionamide(ETH) on day 115. Although his itching disappeared, liver dysfunction and eosinophilia newly developed on day 164. We had to stop the ETH treatment but continued to administrate RFP and EB (Fig. 2). LTT to INH consistently demonstrated positive reactions for over a year, and transient mildly positive reactions to RFP, EB and PZA were also detected. Patch tests for INH also demonstrated three positive reactions within a year. LTT to oxypurinol produced a positive result 1 year later (Fig. 2). We also examined his human leukocyte antigen (HLA) genotypes and found that he possessed the HLA-B*1301 and HLA-B*5201 alleles.

We initially thought that this case was INH-induced DIHS, because repeated patch tests and LTT for INH were both consistently positive after the onset of the patient's DIHS. We used LTT for oxypurinol to support the diagnosis of allopurinol hypersensitivity. Finally, on day 403, the LTT for oxypurinol produced a positive result, more than 1 year after the onset of the condition. Allopurinol is one of the few causative drugs of DIHS and INH is a very rare cause of that.[4] Furthermore, it is difficult to believe that the patient was newly sensitized with allopurinol after remission of DIHS without further administration of this drug. Though we could not determine the true culprit drug, taken together, we considered that this case was allopurinol-induced DIHS involving multiple-drug hypersensitivity. Results of LTT for DIHS depend on the timing performed and the timing of positive transformation has not been confirmed. In this case, it took more than a year for oxypurinol to produce a positive result.

A unique feature of DIHS is its unexplained cross-reactivity to multiple drugs.[1] We think multiple-drug allergy to RFP, EB, PZA and INH occurred based on the results of LTT. In addition, though the results of LTT and patch tests were negative, we think that he developed hypersensitivity to LVX and ETH because itching and liver dysfunction occurred and improved after the administration and discontinuation of LVX and ETH, respectively.

In summary, we report a case of allopurinol-induced DIHS complicated by multiple hypersensitivity to drugs that were used during the course of the disease.

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Inflammatory disseminated superficial porokeratosis with an unusual clinical feature of the pruritic, erythematous papules preceding annular brownish pigmentation

Dear Editor,

Porokeratosis is a heritable disorder of keratinization that is histologically characterized by the presence of a cornoid lamella. Clinically, the basic lesion is sharply demarcated and hyperkeratotic and it may be annular with central atrophy. Five clinical variants are recognized: (i) classic porokeratosis of Mibelli; (ii) disseminated superficial porokeratosis (DSP) and disseminated superficial actinic porokeratosis (DSAP); (iii) porokeratosis palmaris et plantaris disseminata (PPPD); (iv) linear porokeratosis; and (v) punctate porokeratosis.[1]

Porokeratosis may be associated with inflammatory changes and is known as inflammatory porokeratosis. Inflammatory porokeratosis has been reported as various diagnostic terms; eruptive pruritic papular porokeratosis (EPPP), inflammatory stage of porokeratosis or porokeratosis accompanied by eosinophilic spongiosis.[2–5] Generally, most of the patients with inflammatory porokeratosis demonstrated that the pre-existing annular pigmented lesions caused erythematous changes and inflammation.

We herein describe a unique case of inflammatory DSP in which multiple, pruritic, erythematous papules transformed to the typical annular, pigmented porokeratosis lesions during the clinical course.

A 68-year-old Japanese man visited us with pruritic eruption on his trunk and extremities of 4 months' duration. The patient had been treated with topical corticosteroid agents and oral antihista-

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Identification of thymus and activation-regulated chemokine (TARC/CCL17) as a potential marker for early indication of disease and prediction of disease activity in drug-induced hypersensitivity syndrome (DIHS)/drug rash with eosinophilia and systemic symptoms (DRESS)

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ABSTRACT

Background: Drug-induced hypersensitivity syndrome (DIHS)/drug rash with eosinophilia and systemic symptoms (DRESS) is a serious acute drug reaction with fever, cutaneous eruption, lymphadenopathy, and several visceral dysfunctions. Eosinophilia is a common hematological abnormality in DIHS/DRESS suggesting that the Th2-type immune response is involved. Thymus and activation-regulated chemokine (TARC/CCL17) is a family of CC chemokines known to play an important role in Th2-mediated immune-inflammatory processes.
Objective: We investigated the pathogenic role of TARC in patients with DIHS.
Methods: Sera were obtained from 8 patients with DIHS, 7 patients with Stevens–Johnson syndrome/Toxic epidermal necrolysis (SJS/TEN), and 14 patients with drug-induced maculopapular exanthema (MPE). Serum TARC levels were measured by ELISA. TARC levels were then compared with clinical symptoms and various hematological parameters. In addition, a biopsy was taken from the lesional skin of patients with DIHS and stained with anti-TARC Ab and anti-CD11c Ab.
Results: Serum TARC levels in patients with DIHS were significantly higher than those in patients with SJS/TEN and MPE during the acute phase. Serum TARC levels in DIHS patients correlated with skin eruptions, serum sIL-2R levels, eosinophil counts, and serum IL-5 levels. Immunohistochemical staining revealed that TARC was mainly expressed on CD11c+ dermal dendritic cells in patients with DIHS.
Conclusion: Serum TARC levels may be associated with the initial presentation of DIHS as well as disease activity during the course. Thus, they could be useful as an indicator for early diagnosis and assessment of disease activity in DIHS. CD11c+ dendritic cells may be the main source of TARC in patients with DIHS.

1. Introduction

Drug-induced hypersensitivity syndrome (DIHS) or drug rash with eosinophilia and systemic symptoms (DRESS) is a severe acute adverse drug-induced reaction characterized by cutaneous eruption often develop to erythroderma, fever, leukocytosis with eosinophilia, and/or atypical lymphocytosis, lymph node enlargement, and several visceral dysfunctions. DIHS has a delayed onset and it usually occurs 3 weeks to 3 months after the initiation of medication with a limited number of drugs including carbamazepine, phenytoin, phenobarbital, dapsone, mexiletine, salazosulfapyridine, allopurinol, and minocycline [1]. Recently, it has been suggested that the severe systemic symptoms of DIHS are associated with reactivation of human herpesvirus-6 (HHV-6). HHV-6 reactivation, evidenced by the rise in HHV-6 IgG titers and HHV-6 DNA levels, usually occurs 2 to 3 weeks after the onset of a rash. It has been observed despite

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the high variability of clinical manifestations in the vast majority of patients with DIHS [2]. Additionally, it is also known that other herpes viruses such as Epstein–Barr virus (EBV), HHV-7, or cytomegalovirus (CMV) are reactivated sequentially in the course of the disease [1]. Thus, this syndrome is regarded as a reaction induced by a complicated interplay between drug-specific immune responses and several herpes viruses. Recently, dramatic expansions of functional regulatory T (Treg) cells in the acute stage and functional deficiency in the resolution stage of DIHS have been reported. Treg cells were suggested to be involved in the pathological condition of DIHS, such as delayed onset and the risk of subsequently developing autoimmune disease [3].

Thymus and activation-regulated chemokine (TARC/CCL17) is a member of the CC chemokines [4]. It is a ligand for CC chemokine receptor (CCR) 4 that is expressed on type 2 helper T (Th2) lymphocytes [5–7]. TARC plays important roles in Th2-type immune responses by selectively recruiting CCR4+ Th2-polarized memory/effector T cells into inflamed tissues. It has been reported that atopic dermatitis (AD) is characterized by an expansion of the population of Th2 cells in the acute phase [8] and serum TARC levels are associated with disease activity [9]. It has been also reported that TARC levels were significantly increased in inflammatory erythroderma and Sézary syndrome [10]. Furthermore, Treg cells are known as another subset of CD4(+) T cells expressing CCR4 and responding to TARC [11].

In this study, to examine the role of TARC in the pathophysiology of DIHS, that is erythroderma, the Th2-shift, and increased Treg cells, we measured serum TARC levels of DIHS, Stevens–Johnson syndrome/Toxic epidermal necrolysis (SJS/TEN), and drug-induced maculopapular exanthema (MPE). In addition, we examined the association between serum TARC levels and the severity of skin eruption and other laboratory data such as soluble interleukin-2 receptor (sIL-2R), IL-5, eosinophil numbers in peripheral blood, and ALT. To the best of our knowledge, the association between TARC and DIHS has not yet been described.

2. Materials and methods

2.1. Patients

In the period between December, 2009 and December, 2011, 8 patients with DIHS (6 men and 2 women with a median age of 46.0 years old, ranging from 16 to 79 years old), 7 patients with SJS/TEN (3 men and 4 women with a median age of 49.1 years old, ranging from 33 to 72 years old), and 14 patients with MPE (8 men and 6 women with a median age of 59.5 years old, ranging from 32 to 75 years old) were enrolled in this study. Criteria for diagnosis of DIHS were maculopapular rash and/or erythroderma, high fever, leucocytosis with hypereosinophilia, and/or atypical lymphocytosis, lymphadenopathy, and liver dysfunction or other organ involvement [12]. Characteristics of these patients are summarized in Table 1. Causative drugs were identified by the lymphocyte transformation test (LTT) and/or history of drug administration and clinical course; allopurinol and salazosulfapyridine were involved in one patient each and carbamazepine and lamotrigine in three patients each. HHV reactivation was detected in all 8 cases. Of these, HHV-6 was detected in 6 cases, and HHV-7 and CMV in one each. We defined "day 0" as the onset day of characteristic clinical symptoms of DIHS, that is development of skin eruption. In case 4, the serum anti HHV-6 IgG titer was elevated from ×10 on day 4 to ×80 on day 25. In case 8, the serum anti HHV-6 IgG titer was elevated from ×40 on day 21 to ×640 on day 44. In case 6, unfortunately, detailed clinical symptoms and laboratory data were unspecified before consultation on day 15. Case 6 did not fulfill the diagnostic criteria of DIHS exactly, but fulfilled the criteria of DRESS (probable case) based on a skin rash, prolonged clinical course, liver dysfunction, and lymphadenopathy. Four cases (cases 1, 2, 5, and 7) developed erythroderma and the other 4 cases remained as maculopapular erythema. Liver dysfunction developed in 7 cases (cases 1–3, and 5–8) and renal disorder occurred in 3 cases (cases 2, 3, and 4) during the course (Table 1).

2.2. Detection of human herpes virus DNA

Peripheral blood was obtained twice or three times a week until the remission period. DNA was extracted from whole blood using a QIAamp DNA Blood mini-kit (Qiagen, Tokyo, Japan), according to the manufacturer's instructions, and subjected to a real-time polymerase chain reaction (PCR) for the detection of HHV-6, HHV-7 [13], and CMV [14].

2.3. Assay for serum TARC and other cytokines

Serum samples from patients were obtained several times from the acute stage to the remission stage and were stored at −80 °C until use. Serum levels of TARC, sIL-2R, and IL-5 were retrospectively measured by enzyme-linked immunosorbent assays (ELISA) (R&D systems, Minneapolis, USA).

2.4. Immunohistochemical staining

Lesional skin biopsies were performed in 7 patients with DIHS in acute stage (day 4–21) and 4 patients with MPE (day 2–13). Biopsy samples were placed in 10% buffered formalin for processing to paraffin blocks and sections were stained with goat polyclonal anti-human TARC antibodies (R&D systems,

Table 1
Summary of DIHS patients' number, age, sex, causative drugs, types of human herpes virus, day of viral reactivation, skin rash, body temperature, existence or non-existence of lymphadenopathy, and laboratory data.

Case	Age/Sex	Causative drug	Viral reactivation	Day of viral detection after onset	Skin rash	BT (°C)	Leukocytosis (μl)	Eosinophil (μl)	Aty-lym (%)	ALT (U/l)	Lymph adenopathy
1	32/M	Allopurinol	HHV-6	day 16	E	39.3	18,000	3400	12	449	(+)
2	36/F	Lamotrigine	HHV-6	day 19	E	39.7	32,700	1200	5	107	(+)
3	16/M	Lamotrigine	CMV	day 18	MP	37.4	17,400	3100	0	60	(+)
4	79/M	Carbamazepine	HHV-6		MP	38.5	18,100	3500	1	32	(−)
5	44/M	Carbamazepine	HHV-6	day 16	E	39.8	22,700	2400	17	108	(+)
6	60/M	Lamotrigine	HHV-7	day 22	MP	36.5	10,700	700	0	104	(+)
7	57/F	Salazosulufa-pyridine	HHV-6	day 19	E	38.6	22,300	5800	33	383	(+)
8	44/M	Carbamazepine	HHV-6		MP	40.0	16,200	3200	15	80	(+)

BT: body temperature; Aty-lym: atypical lymphocyte; ALT: alanine aminotransferase; HHV: human herpes virus; CMV: cytomegalovirus; MP: maculopapular rash; E: erythroderma.

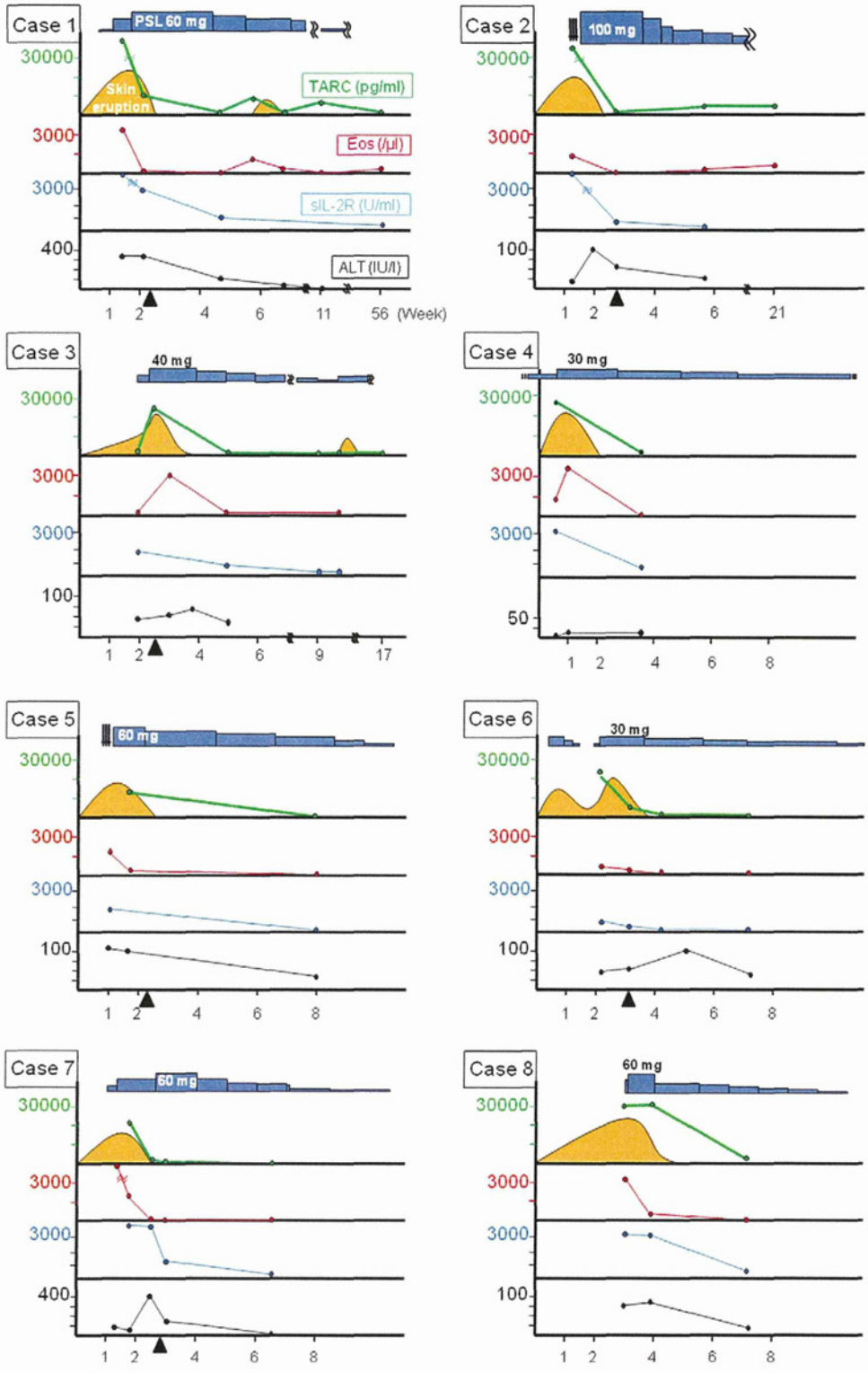

Fig. 1. Clinical course and laboratory data. Clinical course, treatment, and time-dependent changes in serum TARC levels, blood eosinophil counts, serum sIL-2R levels, and ALT in 8 DIHS patients are shown. Human herpes viral reactivation is shown as a black triangle (▲) on the abscissa axis. Three arrow lines in case 2 and case 5 represent steroid pulse therapy.

Minneapolis, US) followed by biotin-conjugated rabbit anti-goat IgG. Sections were then incubated with avidin–peroxidase conjugate followed by diaminobenzidine. In some experiments, double staining was performed using goat polyclonal anti-human TARC antibodies and rabbit monoclonal anti-human CD11c antibodies (Abcam, Cambridge, UK). For negative control staining, normal goat serum was used as a primary antibody.

3. Results

3.1. In the acute stage, serum TARC levels in patients with DIHS were significantly higher than those in patients with SJS/TEN and MPE

We evaluated serum TARC levels in 8 patients with DIHS, 7 patients with SJS/TEN, and 14 patients with MPE during the course.

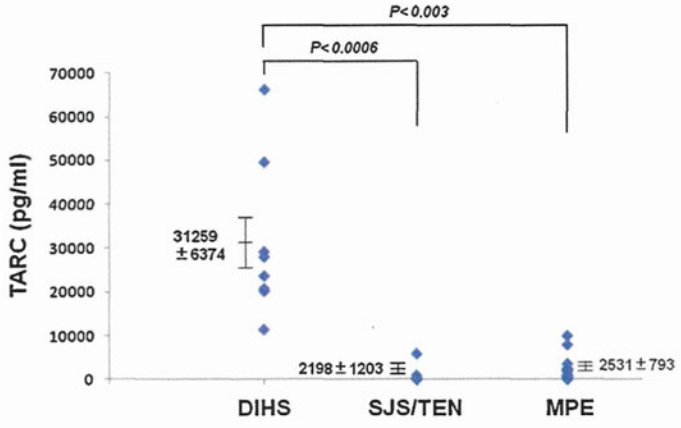

Fig. 2. Serum TARC levels in patients with DIHS, SJS/TEN, and MPE. Serum TARC levels in patients with DIHS were significantly higher than those in patients with SJS/TEN and MPE. Average TARC levels in patients with DIHS were 31,259 ± 6374 pg/ml (average ± SEM), while they were 2198 ± 1203 pg/ml in patients with SJS/TEN (including five cases with normal values; <450 pg/mL) and 2531 ± 793 pg/ml in patients with MPE (including two cases with normal values).

Clinical course, treatment, and time-dependent changes in serum TARC levels, blood eosinophil counts, serum sIL-2R levels, and ALT in 8 DIHS patients were shown in Fig. 1.

We observed an increase in serum TARC levels in DIHS, SJS/TEN, and MPE patients, but the former had significantly higher TARC levels than the others ($P < 0.0006$ and $P < 0.003$, respectively; Fig. 2). Average TARC levels in patients with DIHS were 31,259 ± 6374 pg/ml (average ± SEM), while it was 2198 ± 1203 pg/ml in patients with SJS/TEN (including five cases with normal values; <450 pg/mL) and 2531 ± 793 pg/ml in patients with MPE (including two cases with normal values). Time course evaluations showed that serum TARC levels in patients with DIHS were extremely high during active skin eruptions, but decreased in accordance with improvements in rashes (Figs. 1 and 3). Average TARC levels in patients with DIHS in the remission stage were 514.0 ± 215.1 pg/ml, which were measured in sera on day 22–68 (Fig. 3).

3.2. Serum TARC levels strongly correlated with sIL-2R and eosinophil counts in patients with DIHS

We compared serum TARC levels in DIHS patients with the number of eosinophils in peripheral blood, serum sIL2-R levels, and

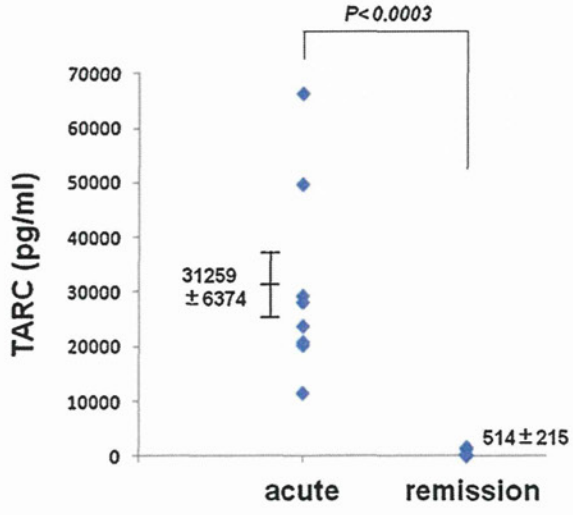

Fig. 3. Serum TARC levels in patients with DIHS. In patients with DIHS, serum TARC levels were extremely high (31,259 ± 6374 pg/ml, average ± SEM) during active skin eruptions, but decreased in accordance with improvements in rashes.

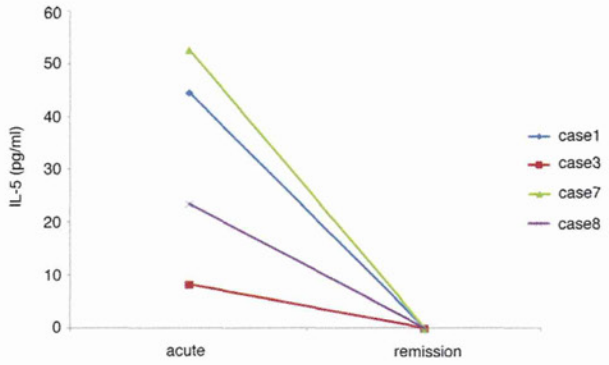

Fig. 4. Serum IL-5 levels in patients with DIHS. We measured serum IL-5 levels in 4 cases (cases 1, 3, 7, and 8). Serum IL-5 levels were elevated in the acute stage and decreased in the remission stage. The average serum IL-5 level in the acute stage was 32.2 pg/ml.

ALT. Serum TARC levels correlated significantly with serum sIL-2R levels ($r = 0.91$, $P < 3 \times 10^{-19}$) and the number of eosinophils in peripheral blood ($r = 0.73$, $P < 5 \times 10^{-10}$). However, serum TARC levels correlated weakly with ALT ($r = 0.34$, $P < 0.03$).

3.3. Serum IL-5 levels in patients with DIHS

Given that eosinophilia is usually observed in DIHS/DRESS and IL-5 is an important cytokine for activation of eosinophils, we measured serum IL-5 levels in 4 cases of DIHS. We found that there was an increase in IL-5 levels in all 4 cases in the acute stage (day 10–22). In the remission stage (day 44–63), serum IL-5 levels were within normal ranges in all cases. Having observed that TARC, Th2-attracting chemokine, correlated well with disease activity in DIHS, we then compared serum TARC levels with IL-5 levels. Both serum TARC and IL-5 levels were elevated in the acute stage and decreased in the remission stage as shown in Fig. 4.

3.4. TARC increased in the lesional skin of DIHS and CD11c+ dermal DCs may be the main source

Immunohistochemical staining was next conducted to determine whether TARC was increased in skin lesions of DIHS. As shown in Fig. 5A–D, we found that dermal infiltrating cells had strong immunoreactivity for TARC. On the other hand, skin from MPE patients showed scatter staining for TARC (Fig. 5E and F). Enlarged image of Fig. 5A showed morphologically dendritic cells stained well (Fig. 5G). To further determine which dermal infiltrating cells expressed TARC, we performed double staining for TARC and CD11c using skin biopsy samples from DIHS patients (Fig. 5H). Results showed that TARC was mainly expressed on CD11c+ dermal dendritic cells in DIHS.

4. Discussion

In this study, serum TARC levels were markedly elevated in all patients with DIHS. Although systemic corticosteroids had been administered before testing for TARC, serum TARC levels stayed significantly high in 3 cases (cases 1, 6, and 7). Furthermore, they decreased in accordance with improvements in skin eruption with sufficient treatment in all cases but they were not parallel with liver dysfunction (Fig. 1). From these findings, we concluded that serum TARC levels may reflect mainly the activity of skin rash of DIHS. Additionally, serum TARC levels in patients with DIHS were significantly higher than those in patients with SJS/TEN and MPE. Therefore serum TARC levels may be a useful diagnostic marker and an indicator of a certain type of clinical activity.

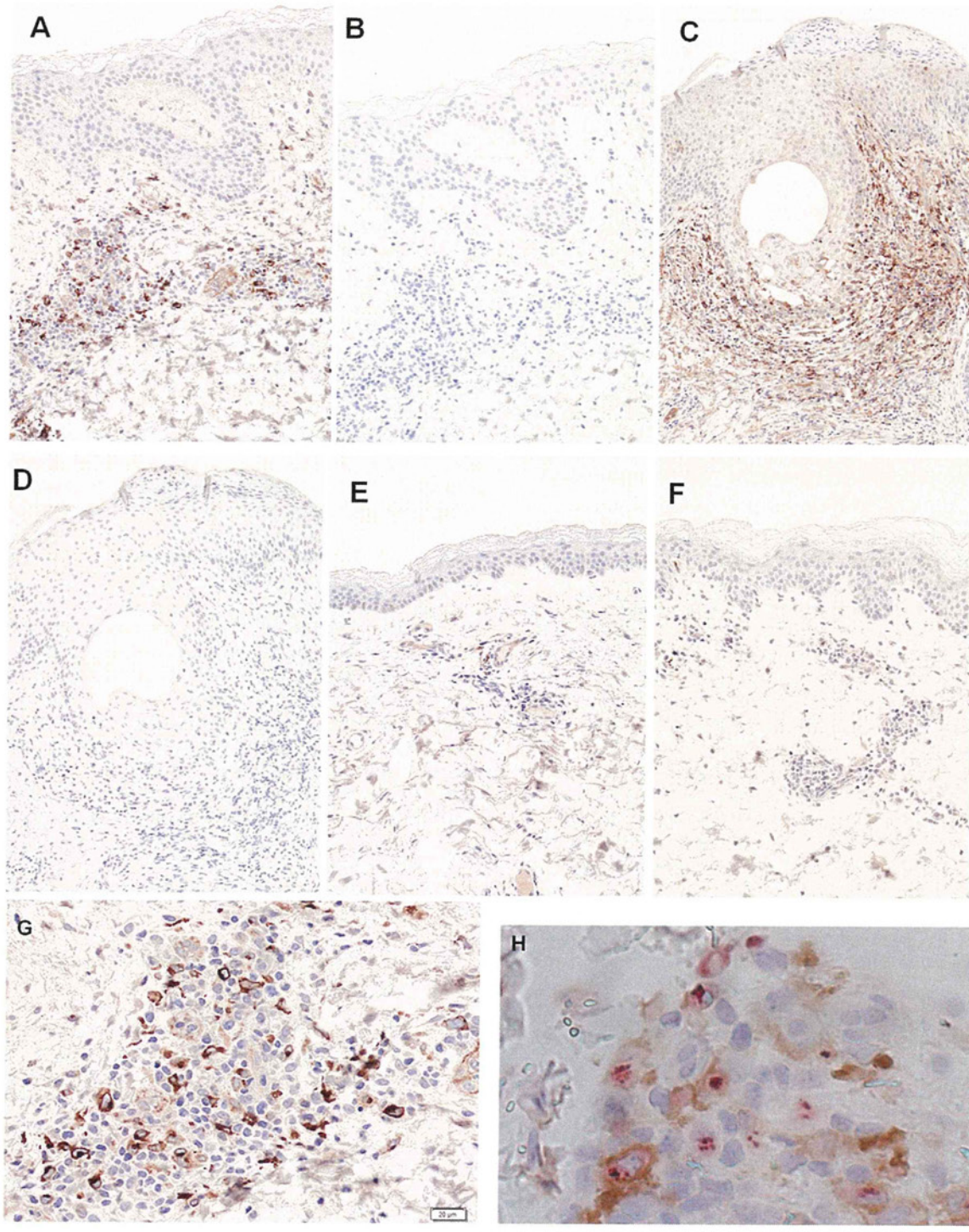

Fig. 5. Immunohistochemical staining for TARC in the lesional skin of DIHS and MPE. Dermal infiltrating cells had strong immunoreactivity for TARC in the lesional skin of DIHS: TARC staining (A) and negative control (B) of case 1 (skin biopsy was performed on day 8), and TARC staining (C) and negative control (D) of case 6 (skin biopsy was performed on day 15). On the other hand, skin from MPE patients showed scatter staining for TARC (E and F). Panel (G) is enlarged image of panel (A). Panel (H) is double staining for TARC and CD11c of case 1. TARC (red) is expressed on CD11c+ (brown) dendritic cells. (For interpretation of the references to color in Figure legend, the reader is referred to the web version of the article.)

TARC is a key chemokine for migration of CCR4 positive T cells and may play an important role in Th2 immunoreactions. Th2 cells produce IL-4, IL-5, IL-10, and IL-13, which are responsible for antibody production, eosinophil activation, and inhibition of several macrophage functions. Hypereosinophilia (>1500/μl) was observed in 6 of 8 cases. As our results show, serum TARC levels were strongly correlated with the number of eosinophils in peripheral blood ($r = 0.73$). Furthermore, serum IL-5 levels in the acute stage were increased in 4 cases with DIHS (Fig. 4). From these findings, the Th2-type immune reaction is induced by significantly elevated TARC in DIHS, and it is consistent with the role of TARC previously reported [5–7]. In other words, an accelerated Th-2 immunoreaction may develop hypereosinophilia through increased IL-5 levels in patients with DIHS. Additionally, there is a possibility that TARC also functions as an inducer of Treg cells expressing CCR4 [11]. Indeed, a previous report demonstrated that

CCR4-positive Treg cells in PBMCs expanded at the acute stage of DIHS [3]. Elevations of TARC during an early period may play an important role in DIHS, involving Treg cells expansion and migration to lesional skin, and Th2-type immune reactions.

Serum TARC levels are also strongly correlated with serum sIL-2R levels in DIHS. Soluble IL2-R is the extracellular domain of the 55-kd α-chain of the IL-2 receptor and is released from activated T cells. Therefore sIL-2R is considered to be a marker of T-cell activation. There is the possibility that high levels of serum TARC induce Th2 cell activation, which involves soluble IL-2 receptor production.

Previous report showed that TARC levels were increased in inflammatory erythroderma [10]. In this study, 4 of 8 DIHS cases were developed erythroderma and the other 4 cases showed widespread maculopapular exanthema. Although serum TARC levels were elevated in all 8 cases with DIHS, the levels were especially elevated in case 1 and 2, respectively 66,520 pg/ml and 49,740 pg/ml, and these two cases developed erythroderma. Thus we guessed that elevated TARC levels had some association with erythroderma in DIHS.

It has been previously reported that TARC is produced by monocyte-derived dendritic cells (DCs) [4,15–17] and endothelial cells [18]. Previous articles also indicated that TARC is expressed in lesional keratinocytes in atopic dermatitis (AD) [9] and mycosis fungoides (MF) [19], and bronchial epithelial cells in bronchial asthma [20].

In our study, the double staining result showed that TARC was expressed on CD11c+ dendritic cells in the lesional dermis in patients with DIHS. In the case of DIHS, CD11c+ dendritic cells may be the main source of TARC that is different from AD, MF, and asthma. This result suggests that the cells that produce TARC may be important in determining disease pathogenesis and final clinical presentation.

A previous report showed that CD11c+ DCs are potently activated by thymic stromal lymphopoietin (TSLP) and induced production of TARC and CCL-22/macrophage-derived chemokine [21]. We conducted immunohistochemical staining for TSLP in lesional skin of DIHS, MPE, and normal skin. TSLP was expressed on the epidermis in both DIHS and MPE, stronger than that on a normal epidermis. However, we could not find a difference in TSLP expression between DIHS and MPE (data not shown). TSLP expression in skin was not associated with overexpression of TARC in sera and dermal DCs in patients. Further research is necessary to identify the TARC inducer in patients with DIHS.

In conclusion, serum TARC levels may be associated with initial presentation of DIHS as well as disease activity during the course. Thus, they could be useful as an indicator for early diagnosis and assessment of disease activity in DIHS.

Acknowledgments

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Eur J Dermatol 2012; 22(6): 1-3

CORRESPONDENCE

Toxic epidermal necrolysis complicated by sepsis, haemophagocytic syndrome, and severe liver dysfunction associated with elevated interleukin-10 production

Toxic epidermal necrolysis (TEN) is a condition of blistering and widespread purpuric macules involving more than 30% [1] of the total skin surface area. It is a life-threatening condition associated with a mortality rate of 20-70%, which is attributed to sepsis (33%) or other causes [1]. In more than 90% of cases, TEN is characterised as an adverse reaction to an administered drug [1]. Severe liver dysfunction is not typical during TEN as it affects 10% of cases.

Here, we report a case of TEN complicated by haemophagocytic syndrome (HPS) and severe liver dysfunction, in which increased interleukin (IL)-10 levels were detected in the patient's serum.

A 76-year-old Japanese woman, who was treated for 2 weeks with etodolac for cervical spondylosis, developed a cough and sore throat similar to a common cold 4 days after treatment began. On examination, severe liver dysfunction was found (serum levels of 1,166 IU/l aspartate aminotransferase and 1,302 IU/L alanine aminotransferase) accompanied for 3 days by extensive skin eruption. After these findings, etodolac administration was discontinued, and the patient was transferred to our hospital.

In addition to skin symptoms, extensive bulla formation and erosions were found in the oral and vulva mucous membrane. An abdominal lesional skin biopsy showed full-thickness epidermal necrosis and eosinophil infiltration. A drug lymphocyte stimulation test was etodolac-negative. Results of paired serological tests for mycoplasma, Epstein-Barr virus, herpes simplex virus, and cytomegalovirus were all negative. Moreover, no serum anti-hepatitis A, -hepatitis B surface antigen, or -hepatitis C virus antibody was found. Antinuclear antibody was negative. Despite steroid pulse therapy (1 g/day for 3 days), plasma exchange (total of five times), intravenous immune globulin administration (0.4 g/kg/day for 5 days), and antibiotic therapy during hospitalisation, the severe liver dysfunction and progression of epidermal detachment (approximately 60% of the body surface area) was prolonged, and the patient died due to sepsis.

Serum levels of IL-10 and other indicator molecules were monitored over the course of hospitalisation of the patient (summarized in *figure 1A*). In this case of TEN with HPS complication, increased IL-10 production appears to be a marker of poor prognosis. The elevated IL-10 is proposed to be a mediator of ongoing advanced liver degeneration during immunoenhancement, in an hypothesis we propose to explain our observations (*figure 1B*).

In cases of TEN, attention to sepsis followed by erosions is critical. The complicating HPS observed is a rare but frequently fatal disorder of immune regulation, characterized by pancytopenia, hepatosplenomegaly, and increased proliferation and activation of macrophages [2]. Clinical symptoms of HPS are not specific, and therefore diagnosis is frequently delayed or found at autopsy [3]. From our observations, we hypothesise that HPS may have begun at a period during sepsis when additional elevation of IL-10 was observed.

IL-10 is a central and critical anti-inflammatory cytokine produced by various cell populations including $Foxp3^+$ regulatory T cells, Th1, Th2, Th17, B-cells, and dendritic cells [4]. Serum concentration of IL-10 is significantly higher in fulminant versus severe acute hepatitis [5]. It has been observed that serum IL-10 is elevated in patients with both sepsis and HPS and proposed to be a prognostic factor [3, 6]. In this case report, serum IL-10 levels were apparently elevated after plasma exchange during progression of epidermal detachment. We propose that while modest increases in IL-10 levels may have a positive effect, a massive anti-inflammatory response may in fact lead to a poor prognosis.

In conclusion, this report is the first to show that a case of TEN complicated by sepsis, HPS, and severe liver dysfunction was accompanied by enhanced and prolonged

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