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ORIGINAL ARTICLE

Drug eruption caused by the nonionic contrast medium iohexol. "Recall-like phenomenon" appearing on an area previously affected by herpes zoster

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ABSTRACT

We report a case of "recall-like phenomenon" caused by nonionic contrast medium. A 62-year-old woman suffering from postherpetic neuralgia developed erythematous plaques 12 h after an intercostal nerve block under X-ray guidance using iohexol (Omnipaque) as contrast medium. The erythematous plaques were preferentially located in the sites where she had experienced herpes zoster 4 months previously. The lesions cleared spontaneously leaving no pigmentation. Both patch testing and intradermal testing with iohexol and ioversol were positive. We postulate that local immunological changes in the skin, such as an increased number and/or accelerated activity of Langerhans cells and mast cells in the herpes zoster lesions, were responsible for this phenomenon. This "recall-like phenomenon", occurring preferentially in skin previously affected by herpes zoster, could facilitate understanding of the pathology of drug eruptions.

Key words: drug eruption, herpes zoster, nerve block anesthesia, nonionic contrast media, recall-like phenomenon.

INTRODUCTION

"Photo-recall phenomenon" and "photo-recall-like phenomenon" are uncommon types of drug reaction. The former manifests as recurrent radiation or sunlight dermatitis, "recalled" by a chemotherapeutic agent in areas of skin previously affected by radiation dermatitis or sunburn.[1] The latter is erythema multiforme (EM) that is recalled by other kinds of drugs in skin previously affected by severe sunburn or previous allergen injections.[1,2] Most cases of drug eruption caused by nonionic contrast media (NICM) reported to date have been of the EM type.[3] However, some other types of drug eruption such as multiple fixed drug eruption[3] and Stevens–Johnson syndrome[4] have also been reported. We present a case of an eruption caused by the NICM iohexol in which lesions preferentially appeared at sites affected by herpes zoster previously.

CASE REPORTS

A 62-year-old woman was seen at our department on 9 July 2003. A diagnosis of thoracic herpes zoster had been established previously on 15 March 2003 and treated with 1000 mg of valaciclovir hydrochloride three times per day for 5 days. Despite this treatment, postherpetic neuralgia persisted and she had been treated with intercostal nerve block anesthesia at the pain clinic in our hospital on several occasions. On the occasion that she attended our department, she had just received her fifth course of nerve block anesthesia under X-ray guidance, using the NICM iohexol (Omnipaque). She had not received any NICM for her courses of nerve block anesthesia prior to this. Approximately 12 h after she received the iohexol, she noticed itching and an eruption on her chest.

Physical examination at her first visit revealed well-demarcated erythematous plaques on her right

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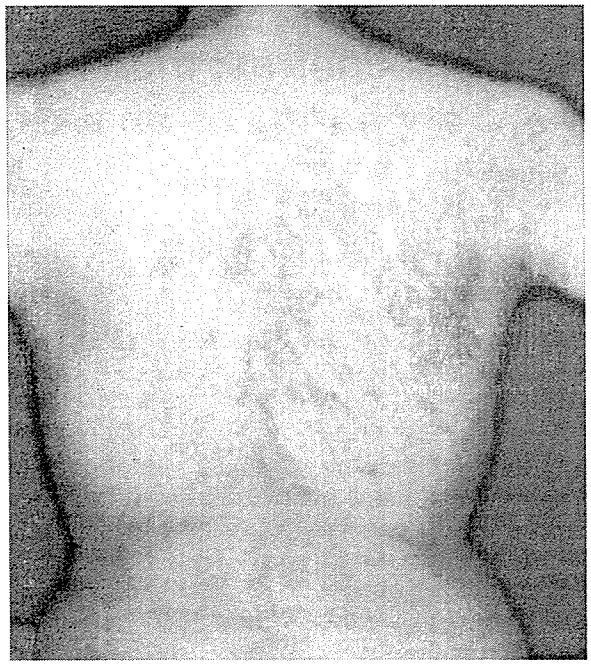

Figure 1. Well-demarcated erythematous plaques on the patient's back. The erythematous plaques were preferentially located in the sites where she had experienced herpes zoster 4 months previously.

anterior chest wall, abdomen, back and upper extremities. The plaques were grouped in a distinctly dermatomal distribution corresponding with the areas of skin previously affected by the eruption of herpes zoster (Fig. 1). The results of laboratory examinations such as full blood count, liver function tests and erythrocyte sedimentation rate were non-contributory. A diagnosis of drug eruption caused by iohexol was made. The lesions cleared within 10 days without any treatment, leaving no pigmentation. We conducted patch testing and intradermal testing with NICM such as iohexol, iotrolan, iopamidol, iomeprol, iopromide and ioversol (as is); ionic contrast media such as amidotrizoic acid, iothalamic acid, ioxaglic acid and iodine (as is); and the 1 and 2% mepivacaine hydrochloride (that had been administered to the patient for her postherpetic neuralgia) and 0.9% NaCl solution. Strong positive reactions (++) to iohexol and ioversol in patch testing were read using the International Contact Dermatitis Research Group scoring system (Fig. 2). Positive reactions were observed on intradermal

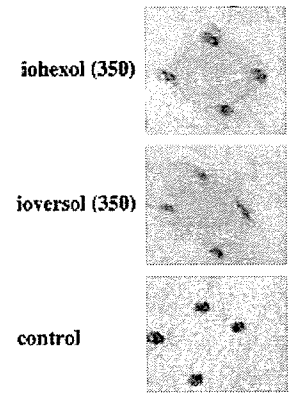

Figure 2. Positive patch testing for iohexol and inversol 48 h after application.

testing with iohexol and ioversol. These results suggest that the mechanism of this eruption must be delayed-type hypersensitivity.

DISCUSSION

The development of the eruption, 12 h after administration of NICM, in anatomical sites corresponding exactly with the sites affected previously by herpes zoster infection, is strongly suggestive of a "recall-like phenomenon". Successful provocation by both patch testing and intradermal testing confirm that this eruption was caused by iohexol.

Most cases of drug eruption attributed to NICM reported to date have been EM-like erythematous plaques and papules, which frequently occur on the trunk and proximal extremities, and a few cases of urticaria.[3] We have diagnosed 78 cases of drug eruption caused by NICM in our department; 75 cases were of the EM-type, two were of the urticaria type, and one multiple fixed drug eruption.[3] However, in the 75 cases of EM-type, there were no cases of asymmetrical distribution as was seen in our present case.

The EM-type drug eruption caused by NICM is a delayed-type hypersensitivity reaction, with the eruption occurring around 6 days after administration in most patients with no previous history of sensitization to contrast agents.[3] In some patients previously sensitized to the contrast agent, when it is administered again for examination or pre-testing, the eruption occurs several hours to 1 day after

administration.[3] Patch testing and intradermal testing are very useful for confirming the causative drug.[3] Cross-sensitization among the NICM examined by patch and intradermal testing was 56% in our series. In the present case, the eruption developed 12 h after administration of iohexol and the positive reactions in both patch testing and intradermal testing confirmed the mechanism of delayed-type hypersensitivity.

We also calculated the patient's cumulative dose of radiation to exclude the possibility that X-ray radiation could have caused the eruption. The dose of radiation received just before the eruption appeared was 0.0393 Gray (Gy) and the cumulative dose was 0.366 Gy. Generally, the threshold at which a single irradiation dose induces acute erythema of skin has been estimated to be in the range of 2–8 Gy.[5,6] Acute radiation dermatitis typically occurs 2–7 days after exposure and tends to manifest as two to three waves of erythema occurring over several weeks. Most acute radiation dermatitis resolves within 3 months, but severe injury can persist indefinitely with postinflammatory pigmentary changes, teleangiectasias and atrophy.[6] The present case developed erythematous plaques 12 h after she received X-ray irradiation and the dose was only 0.0393 Gy. Moreover, the eruption did not have vesiculation, pigmentary changes or atrophy. We can therefore confidently exclude the possibility that our patient's eruption was caused by the radiation she had been exposed to during the intercostal nerve block anesthesia.

Until now, there has only been one reported case of a drug eruption appearing in a dermatome previously affected by herpes zoster.[7] It has already been shown that in regressing skin lesions attributed to viral infection, the number of epidermal Langerhans cells and dermal dendritic cells increases, whereas during developing viral infection they decrease in number.[8,9] This could explain why the erythematous plaques are preferentially distributed in the area previously affected by herpes zoster in the present case. Moreover, recall-like phenomenon in cases of fixed drug eruption has demonstrated that the eruption can appear at the very same site of previous trauma, such as burn scars, insect bites and venepuncture sites.[10] The authors of this report suggested that increased accumulation of mast cells could contribute to this process. Mast cells play a major part in either initiating or perpetuating wound healing.[11,12] They have been shown to release high concentrations of tumor necrosis factor (TNF)-α in the skin through degranulation.[13] TNF-α is required to activate intra-epidermal CD8+ T cells through the expression of intercellular adhesion molecule-1 (ICAM-1) by lesional keratinocytes.[14] We postulate that during the healing of the herpes zoster in our patient, an increased number and/or accelerated activity of Langerhans cells and mast cells in the lesion could be responsible for the recall-like phenomenon which occurred subsequently on administration of iohexol. This is, however, only a theory as we did not perform skin biopsy of the lesional skin to assess the number of Langerhans cells and mast cells present.

In summary, this case demonstrates the appearance of a drug eruption in response to nonionic contrast medium appearing as a recall-like phenomenon in areas of skin previously affected by herpes zoster. Cases such as this may help to develop understanding of the cellular and immunological processes involved in drug eruptions.

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condition the lesions comprise a highly pruritic linear group of excoriated eczematous papules, which are highly refractory to therapy. This condition was also ruled out in this case, as our patient had complained of minimal pruritus; moreover, the histology was not that of ILVEN, i.e. alternate columns of orthokeratosis and parakeratosis.

We believe that this patient has psoriasis confined to specific zones of the left half of the body. The most likely explanation for such a presentation is that of mosaicism for the same genetic predisposition, which is universally present in skins of patients with ordinary psoriasis.

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Adverse reactions to gefitinib (Iressa®): Revealing sycosis- and pyoderma gangrenosum-like lesions

A 55-year-old man, examined in April 2003, with cutaneous lesions of the scalp, face, trunk, both upper extremities, and left thumb, was diagnosed as having nonsmall cell lung cancer (adenocarcinoma, ToN3Mo, Stage IIIB) in March 2002. Since combination chemotherapy (cisplatin + vinorelbine) and radiotherapy were not effective for the lung lesions, gefitinib, 250 mg per day, was administered orally since September 2002. Despite developing nausea and diarrhea, the patient continued to receive gefitinib because it reduced the incidence of respiratory symptoms: cough and sputum. Two weeks after the start of the administration, the patient developed cutaneous lesions on the scalp and face. One month after, erythematous lesions, resistant to topical corticosteroid (betamethasone valerate) therapy, appeared on the trunk and both upper extremities. Additionally, a nodule appeared on the right thumb 6 months after the start of gefitinib.

Physical examination revealed scaly erythematous lesions on the scalp and both cheeks, and follicular papules and pustules on both eyebrows and upper lip. Erythematous lesions studded with follicular papules were seen on the trunk and both upper extremities. On perinail region of the left thumb, erythematous lesions with erosion and a dark reddish vascular node, $9 \times 4 \times 3$ mm in diameter, were visible. Culture of exudates from this lesion revealed *Staphylococcus aureus*. The patient was started on symptomatic therapies, e.g. topical corticosteroids and ketoconazole for erythematous lesions on the scalp and face, acrinol for right thumb, and systemic and topical antibiotics for follicular papules. Two weeks later, papules and pustules on the upper lip grouped and formed sycosis vulgaris-like lesions (Fig. 1). Three months after his initial visit (10 months after starting gefitinib), gefitinib was temporarily discontinued for 1 month because the patient was suspected of having pneumonia. An erythematous lesion

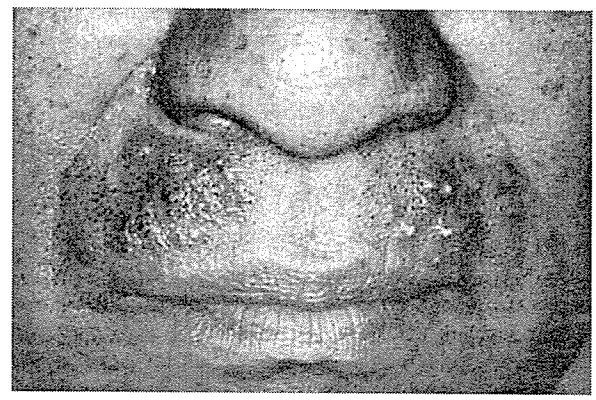

Figure 1 Sycosis vulgaris-like lesions on the upper lip

on the hypogastrium remained the same although other lesions cleared. Three weeks after drug discontinuation, an erythematous plaque on the hypogastrium ulcerated, and subsequently formed a lesion with an elevated and indurated border and a tendency towards central necrosis (Fig. 2). Unfortunately, we have no data of microbiological examinations on this lesion because of technical error, and consent to perform a biopsy could not be gained. Gefitinib was restarted and reduced from daily administration to every other 2 weeks, and the lesion disappeared in the following 1 month.

Discussion

Gefitinib (Iressa®), an anticancer agent that inhibits epidermal growth factor receptors (EGFR) signal transduction,[1,2] has been marketed for nonsmall cell lung cancer patients in Japan since 2002.[3] As for adverse cutaneous reactions to gefitinib, acneiform eruption, seborrheic dermatitis, and paronychia seen at our patient's first visit are common; such reactions are dose-dependent.[3–6] It is assumed that gefitinib inhibits activation of

Figure 2 A pyoderma gangrenosum-like lesion on the hypogastrium

EGFR, which are restricted to the basal and immediately suprabasal layer and the outer root sheath of the hair follicles, and causes an altered terminal keratinocyte maturation.[1,2,7] Sycosis is a pyogenic infection involving the whole depth of the follicle.[8] In our case, sycosis-like lesions should be considered as adverse reactions because the lesions subsided by reduced gefitinib dosing. Although the clinical course of the pyoderma gangrenosum-like lesion was not totally in accord with gefitinib administration, it is presumed, but not proven, that this lesion is also an adverse reaction since it disappeared within 1 month after gefitinib dose reduction.

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Fixed drug reaction may be sexually induced

Dear Sir,

We read with interest the correspondence by George and Ogunbiyi,[1] who described several patients with fixed drug eruption (FDE) or fixed drug-like eruptions (FDLE) but who denied the use of medications. The authors excluded idiopathic eruptive macular pigmentation. They quoted previous reports,[2,3] documenting a significant minority of patients with FDE or FDLE with no significant drug history but persisting to exhibit such eruptions. These situations are frustrating to the patients as well as their physicians.

We concur with George and Ofunbiyi[1] that nonmedicinal products, photoactivation of chemicals, and premenstrual fever are possible underlying mechanisms for some of these reported cases. We wish to point out the possibility of sexually induced FDE. We have previously reported three male patients with typical FDE but who denied drug intake preceding the eruptions. Two had eruptions on the genitalia while one had an eruption on his penis as well as his left axilla. Each attack temporarily followed sexual intercourse with their female partners, who were taking drugs that the male patients had been documented to be allergic to.[4]

We postulated that drug inunction through vaginal fluid could have caused the eruptions. Minor abrasions during intercourse could have led to antigen presentation. The presensitized T cells on the male genitals might release cytokines in the presence of keratinocyte-derived factors, perpetuating the reaction even in extra-genital skin such as the axilla for one of our reported patients.[4]

Interestingly, we also have encountered similar situations in two female patients whose male partners took medications and the females had subsequent eruptions compatible with FDE.

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CASE REPORT

Multiple Chemical Sensitivities Following Intolerance to Azo Dye in Sweets in a 5-year-old Girl

Naoko Inomata[1], Hiroyuki Osuna[1], Hiroyuki Fujita[1], Toru Ogawa[1] and Zenro Ikezawa[1]

ABSTRACT

Background: Cases of multiple chemical sensitivities (MCS) have been reported predominantly in adult patients, but pediatric cases have rarely been reported.

Methods: We present a 5-year-old girl who suffered from recurrent reactions accompanied by urticaria, angioedema, headaches, dyspnea, loss of consciousness, and abdominal pain that were not eradicated, but were instead exacerbated, by various treatments with antihistamines and intravenous corticosteroids. Her diet diary revealed that symptoms occurred after ingestion of colorful sweets such as candies and jellybeans. Open challenge tests with food additives and nonsteroidal anti-inflammatory drugs (NSAIDs) were performed after elimination of these items. Skin prick tests using additives and NSAIDs, which were dissolved in saline, and prick-prick tests using candies and jellybeans, were carried out.

Results: Open challenge tests with Tartrazine, aspirin and acetaminophen were positive, whereas skin prick tests using additives and NSAIDs and prick-prick tests using candies and jellybeans were all negative. Consequently, intolerance to azo dyes and NSAIDs such as aspirin was diagnosed. However, she appeared to react to multiple chemical odors such as those of cigarette smoke, disinfectant, detergent, cleaning compounds, perfume, and hairdressing, all while avoiding additives and NSAIDs. On the basis of her history and the neuro-ophthalmological abnormalities, a diagnosis of severe MCS was made and she was prescribed multiple vitamins and glutathione.

Conclusions: The present results suggest that in pediatric MCS, food and drug additives containing azo dyes might play important roles as elicitors.

KEY WORDS

azo dye, food additives, inheritance, multiple chemical sensitivities, nonsteroidal anti-inflammatory drugs

INTRODUCTION

Multiple chemical sensitivities (MCS) syndrome, also known as idiopathic environmental intolerance, is a controversial diagnosis that encompasses a wide range of waxing and waning, symptoms related to more than one body system and provoked by exposure to low levels of chemicals, foods, or other agents in the environment. Although MCS has been studied extensively, a unifying mechanism explaining the illness remains obscure. Cases of adult MCS have been predominantly reported, but pediatric cases are apparently rare. In this report, we present a pediatric case of MCS that appeared to have been triggered by repeated exposure to food additives such as azo dyes. Notably, the patient's mother had also experienced symptoms of MCS.

CLINICAL SUMMARY

A 5-year-old girl was referred for evaluation after 10 days of recurrent episodes, which were accompanied by generalized urticaria, angioedema, dyspnea, nausea, headache, a slight fever, abdominal cramps, and loss of consciousness, and were not eradicated, but were instead exacerbated, by various treatments with antihistamines and intravenous corticosteroids. She was admitted to our hospital for urgent care and hydration. Her symptoms gradually improved, although

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hives and slight fever did not completely resolve. After discharge, her mother was instructed to keep a diet diary for her. Consequently, the diet diary revealed that hives and angioedema developed immediately after ingestion of sweets containing vivid coloring agents, such as jellybeans and candies, indicating an association between azo dyes and the several events. The association between past recurrent episodes and colorful sweets led her mother to recall that she had eaten a purple-colored candy (found to contain Tartrazine and Brilliant blue1) immediately prior to the first, severe event. Thereafter, once she began to avoid azo dyes, hives and slight fever rarely recurred.

The patient's mother had suffered from angina, bronchial asthma, allergic rhinitis, allergic conjunctivitis, atopic dermatitis, food allergy, and intolerance to aspirin, theophilline, and lidocaine. She often developed headaches and nausea after being exposed to chemical odors, resulting in a diagnosis of MCS. The patient's father had suffered from frequent urticaria in his childhood.

The past medical history of the patient was bronchial asthma from the age of 2 years. The patient had an incomplete, restricted diet up to the age of 4 years, because her mother was worried that she might be as sensitive as her mother to several drugs, although she had no prior history of reactions to foods or medications. When she was 5 years old, her mother lifted the restriction on medications and foods, hoping to expose her to some drugs and foods so that she would be better able to tolerate the less restricted environment she would experience in kindergarten. The exam for nose, ears, and throat showed no nasal polyp. Blood tests, including a peripheral eosinophil count and serum IgE level, were normal.

To test for hypersensitivity to azo dye and drugs such as nonsteroidal anti-inflammatory drugs (NSAIDs), skin testing and open oral challenge tests with Tartrazine (FD&C Yellow No.5, Wako Pure Chemical, Osaka, Japan), Brilliant blue No.1 FCF (FD&C Blue No.1, Kyoritsu Foods, Tokyo, Japan), *p*-Hydroxybenzoic acid (Wako Pure Chemical, Osaka, Japan), aspirin, and acetaminophen were performed under closely supervised conditions, after obtaining the informed consent of her parents. Her parents preferred open challenge tests to blind challenge tests for evaluation. The substances tested were given in the lowest dose. If no objective reaction could be noted, additional and increased doses were given at approximately 3-hour intervals. As a result, the patient tested positive not only to additives such as Tartrazine 300 μg and Brilliant blue No.1 FCF 210 μg, but also to 50 mg aspirin and 10 mg acetaminophen (Table 1). Furthermore, additional challenge tests proved that she is sensitive to other drugs, such as theophilline and lidocaine. According to the standard methods proposed by Dreborg, skin prick tests using these drugs and chemicals dissolved in saline were all negative, whereas prick-prick tests using candies, jellybeans, and glutinous starch syrup, which were melted for 1 minute by an electronic oven, were all negative.

Table 1 Results of challenge tests using additives, NSAIDs, and other drugs

Substance	Symptoms	Dose
Tartrazine (FD&C Yellow No.5)	U, Dy, Ab	300 μg
Brilliant blue No.1 FCF (FD&C Blue No.1)	Dy, He, Ab	210 μg
P-Hydroxybenzoate	–	10 mg
Aspirin	U, He, Ab	50 mg
Acetaminophen	U, Dy, He, Ab	10 mg
Lidocaine (s.c.)	Dy, Ab, Nau	1.25 μg
Theophiline (i.v.)	Dy, He, Nau	6 mg

U, urticaria; *Dy*, dyspnea; *He*, headache; *Ab*, abdominal pain; *Nau*, nausea.

Thereafter, she frequently complained of urticaria, dizziness, headache, fatigue, chest tightness, and nausea, although the suspected foods and drugs were avoided. She appeared to react to multiple chemical odors such as those of cigarette smoke, disinfectant, ethanol, detergent, volatile organic chemicals, cleaning compounds, perfume, and hairdressing. On the basis of her history and her neuro-ophthalmological abnormalities, she was given a diagnosis of severe MCS, and prescribed multiple vitamins and glutathione. Her activities had begun to be severely limited due to her symptoms of MCS in public areas. Because she had olfactory symptoms in some areas of our hospital, we set up an air conditioner in a consulting room before her visits. In addition, her mother feared that her imminent transition into public school would likely be made difficult by the school's routine use of stationery, felt-tipped pens and crayons, and cleaning products. School officials permitted an air conditioner to be stationed on beside her in the classroom. Nevertheless, her symptoms appeared to be worsened by the school's use of cleaning products and chalk. Finally, her parents moved from the town to the country, where she could attend elementary school in a wooden frame schoolhouse and experienced no symptoms.

PATHOLOGICAL FINDINGS

Pathological findings were not obtained because her parents did not consent for her to undergo skin biopsy.

DISCUSSION

Despite the numerous agents that are added to foods, there are relatively few documented hypersensitivity

reactions to food additives, especially in children. We report a pediatric case of severe reactions immediately after ingestion of sweets containing azo dyes. Further, our patient developed hypersensitivity not only to food additives but also to multiple other chemicals, including aspirin. The recurrent reactions due to azo dyes might have been a kindling phenomenon of the subsequent symptoms in response to various chemically-unrelated compounds because the first reactions to azo dyes, which were most severe, were consecutively followed by these symptoms. However, the pathogenic link between these two events has not yet been elucidated in the present case.

The prevalence of food-additive intolerance in children age 5–16 is 1–2%,[1] whereas in children aged less than 10 years, including children with asthma, the prevalence of aspirin intolerance is less than 10%.[2] Carefully blinded studies have shown the incidence of a cross-reaction between aspirin and Tartrazine as a representative of azo dyes to be less than 2.4% in asthmatic patients.[3] Unlike in aspirin, the most likely mechanism for reactions to Tartrazine has been reported to be dose-related histamine release from mast cells.[4]

In our patient, not only food additives but also the drugs administered for treatment, which contained several additives such as Food Yellow No. 5 (Sunset Yellow FCF) and sodium benzoate aggravated her symptoms before she was diagnosed. Children might be more frequently exposed than adults to multiple chemicals such as vibrantly colored foods and medications. The original statement concerning adverse reactions associated with pharmaceutical excipients, which was issued by the American Academy of Pediatrics in 1997, warned that although many excipients have been implicated in causing adverse reactions, and these are the most significant in the pediatric population.[5] An outline of the diagnostic criteria of MCS in children based on those in adult cases was presented by Woolf.[6] Because there is no single objective test finding to confirm the diagnosis of MCS, its diagnosis in children will often depend largely on historical information obtained from the parents. Olfactory warning of inciting odors appears to be a hallmark of MCS. A mechanism of neurotoxicity from chemical toxicants carried to the central nervous system by way of the olfactory bulb has been offered as one mechanism of causation.[7] A significantly higher prevalence of the panic disorder-associated cholecystokinin B receptor allele 7 has been reported in subjects with idiopathic environmental intolerance, as a synonym of MCS (9/22 [40.9%]), compared with control subjects (2/22 [9.1%]).[8] It should be noted that her mother had also suffered from MCS, indicating that MCS might be a hereditary disorder in a portion of the MCS population.

In the present case, a mother-child relationship seems to have been one of the key factors in the development of clinical symptoms. In this study, challenge tests were openly conducted because informed consent was not obtained from the parents for double-blind tests. Double-blind tests should, however, have been carried out to rule out various factors, including emotional factors. Additional medical examinations as well as socio-medical and psychological approaches to both the patient and her mother need to be carried out in the future.

The present results suggest that in pediatric MCS, food and drug additives containing azo dyes might play important roles as elicitors. Clinicians should therefore consider this possibility before patients with additive intolerance definitively develop MCS.

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SHORT COMMUNICATION

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Increased production of vascular endothelial growth factor in the lesions of atopic dermatitis

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Abstract Atopic dermatitis (AD) is a chronic inflammatory skin disease characterized by severe itching, erythema and edema resistant to anti-histamine therapy. Vascular endothelial growth factor (VEGF) is a potent agent that causes hyperpermeability of blood vessels and endothelial cell proliferation, and might be involved in the persisting erythema and edema in AD. In this study, we used extraction of stratum corneum with physiological saline to detect VEGF produced in the lesions of AD. Biological activity of VEGF was assayed by proliferation of cultured human umbilical vein endothelial cells in vitro. As a result, we found that the amount of VEGF produced in lesional scales was approximately 25 times higher than that in normal stratum corneum. Moreover, VEGF 121 isoform that exclusively induces hyperpermeability of blood vessels was a predominant component in the lesional scales suggesting that this factor plays an important role in the persisting erythema and edema in the AD lesions.

Keywords AD · Scale · VEGF

Atopic dermatitis (AD) is a chronic inflammatory skin disease characterized by severe itching and prolonged erythematous eruptions. Histological examination shows dilated and tortuous vessels within the papillae, accompanied by perivascular edema and prominent collections of mononuclear cells with proportionately rare neutrophilic or eosinophilic polymorphonuclear leukocytes [14, 19]. In acute lesions of AD, mast cells are quantitatively normal but in an active state, as substantiated by various stages of degranulation. In chronic lesions, the number of mast cells increased [9]. Mast cell-derived histamine is believed to be a major vasoactive mediator in these lesions. In fact, elevated histamine concentration has been detected in the plasma and skin of AD [8, 11], which may initiate skin lesions by increasing vascular permeability, eliciting erythema and causing an itchy sensation. However, anti-histamine therapy is less effective in the case of such functional and morphological disorders in AD [20]. This observation prompts speculation that there may be other contributing factor(s).

Vascular endothelial growth factor (VEGF) is a multifunctional cytokine that not only promotes angiogenesis but also enhances vascular permeability [5]. The vascular permeabilizing activity is 50,000-fold more potent than histamine [6]. Human VEGF is a homodimeric 36–46 kDa protein consisting of six differentially spliced variants, giving rise to mature isoforms containing 121, 145, 165, 183, 189 and 206 amino acids [10, 13, 18, 24]. Among them, the VEGF 121 and VEGF 165 isoforms are synthesized and secreted predominantly in normal keratinocytes [1, 10]. Changes of the capillary system in the dermis are closely associated with epidermal proliferation under the influence of epidermis-derived angiogenic molecules, which are considered to originate in the epidermis [2, 21]. It has been reported that the expression of VEGF is upregulated in the lesional epidermis of inflammatory diseases, such as psoriasis and contact eczema [3, 4]. Both of the diseases are characterized by prolonged dermal vascular dilatation, whilst vascular proliferation is also prominent in psoriasis [4]. Since prolonged dermal capillary dilatation is seen in the typical lesions of AD, it is likely that VEGF may also participate in the onset and development of AD [19]. Our purpose is to assess the involvement of VEGF by analyzing the content of VEGF in the lesional stratum corneum of patients with AD.

Twenty-three patients with AD were enrolled in the study (8 very severely affected, 13 severely affected and 2 moderately affected). Stratum corneum from skin lesions

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of the patients was collected from the patients' bedsheets, as scales peeled off naturally or by scratching. Control scales were collected from normal sole stratum corneum from patients with AD and from 11 healthy donors by scraping. The patients received neither systemic corticosteroids nor topical treatment with corticosteroids for at least 3 weeks prior to the study.

Scale extracts were prepared by a method as described previously [23]. The contents of VEGF protein in the scales were measured by an enzyme-linked immunosorbent assay (ELISA) kit (R&D Systems, Minneapolis, USA). This kit detects both VEGF 165 and 121 isoforms. Statistical analyses were conducted using the Wilcoxon nonparametric tests.

Vascular endothelial growth factor was detected in all samples collected from lesional scales of patients ($n=23$). The mean content of VEGF was 202.03 ± 120.44 pg/mg scale in the lesional scales, and this was significantly higher than that of patients' heel stratum corneum (10.31 ± 5.64 pg/mg scale) ($P<0.01$) (Fig. 1). VEGF was also detected in heel stratum corneum obtained from normal healthy subjects (8.55 ± 6.62 pg/mg scale). There was no significant difference in VEGF content of heel stratum corneum between the patients with AD and normal healthy subjects (data not shown).

To identify the isoforms of VEGF in the scales, the extracts were analyzed with Sodium Dodecyl Sulfate-Polyacrylamide Gel Electrophoresis (SDS-PAGE) followed by immunoblotting. The protein concentration of each sample was determined using protein assay Dye Reagent Concentrate (Bio-Rad Protein Assay, Bio-Rad, München, Germany). Four milligram of protein scale lysates per sample were incubated overnight at 4°C with 5 μg of agarose-conjugated anti-VEGF rabbit polyclonal antibodies (VEGF (A-20): sc-152, Santa Cruz Biotechnology, Inc., USA). After centrifugation at 15,000×g for 10 min at 4°C, the precipitates were washed 4 times with 0.1% Tween-Tris-buffered saline (TTBS). The precipitates were boiled for 5 min in a sample buffer

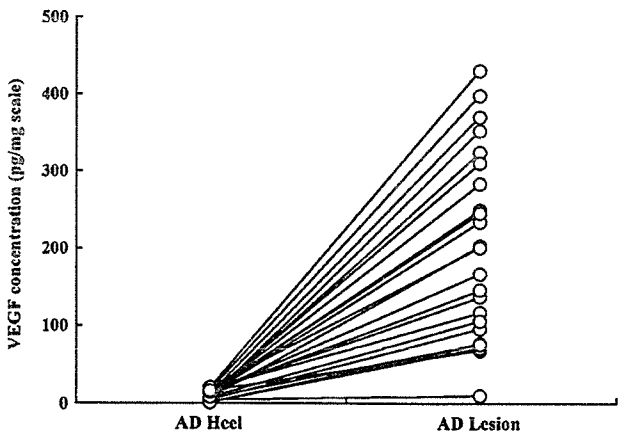

Fig. 1 The content of VEGF in stratum corneum. VEGF was extracted from stratum corneum obtained from AD lesions (*AD Lesion*) and normal heel (*AD Heel*), and then quantified using a specific ELISA kit

(25 mM Tris, 1% SDS, 1% β-mercaptoethanol, 0.005% bromphenol blue, 5% glycerol) and subjected to electrophoresis on 12.5% polyacrylamide gels. They were then electroblotted onto a polyvinylidene difluoride microporous membrane (Immobilon PVDF, Millipore Corporation, MA, USA). After being blocked with 5% skim milk in TTBS for 4 h at room temperature, the membrane was incubated overnight at 4°C with goat anti-human VEGF polyclonal antibodies (R&D Systems) at a concentration of 0.2 μg/ml. Afterward, the membrane was extensively washed with TTBS and incubated with horseradish peroxidase-conjugated donkey anti-goat IgG antibodies (Chemicon International, Inc., CA, USA) at a concentration of 0.2 μg/ml for 1 h. Bound antibodies were reacted with ECL plus Western Blotting Detection Reagents™ (Amersham Biosciences, Uppsala, Sweden) and exposed on Hyperfilm ECL™ (Amersham Pharmacia, Biotech AB, Sweden). Recombinant VEGF 165 isoform and recombinant VEGF 121 isoform (R&D Systems) were used as controls.

As a result, 20.5, 18.0 and 15.7 kDa bands were specifically detected as immunoreactive VEGF protein in lesional AD scales (Fig. 2a). The 15.7 kDa band was found to be predominant. Image analysis revealed that the amount ratio was 1:1.6:2.8 for the 20.5, 18.0 and 15.7 kDa bands, respectively. Relative molecular mass of recombinant VEGF 165 isoform was 19.5 kDa, and that of recombinant VEGF 121 isoform was 17.2 and 15.7 kDa in this condition.

A similar result was reported in SDS-PAGE analysis of the culture supernatant from human keratinocytes under reducing conditions. This yielded three bands of approximately 24, 20 and 15 kDa [1]. Because VEGF 189 isoform is not secreted [22], these three subunits are most likely differentially glycosylated VEGF 121 isoform and VEGF 165 isoform, where VEGF 121 isoform is a predominant component [1]. *N*-deglycosylation experiments of those samples supported this assumption because a predominant band with a molecular weight of about 15 kDa appeared in the keratinocyte-derived VEGF, corresponding to the expected size of the VEGF 121 protein [1]. The profile of scale-derived VEGF was compatible with the data of keratinocyte-derived VEGF, suggesting that main source of VEGF in the scales was keratinocytes. The 15.7 kDa band, found to be predominant, possibly corresponds to the protein described for deglycosylated VEGF 121 isoform, and the 18.0 kDa band may correspond to glycosylated VEGF 121 isoform. The 20.5 kDa band is likely to correspond to VEGF 165 isoform in these conditions. Like VEGF 121 isoform, VEGF 165 isoform may also exist as both glycosylated and deglycosylated species, resulting in two types of monomers, having a molecular mass of 23 and 18.0 kDa, respectively [7]. It is thus conceivable that the 18.0 kDa band found in the scales derives from both glycosylated VEGF 121 isoform and deglycosylated VEGF 165 isoform.

To further analyze the molecular mass, the VEGF preparation was separated by means of a TSKgel

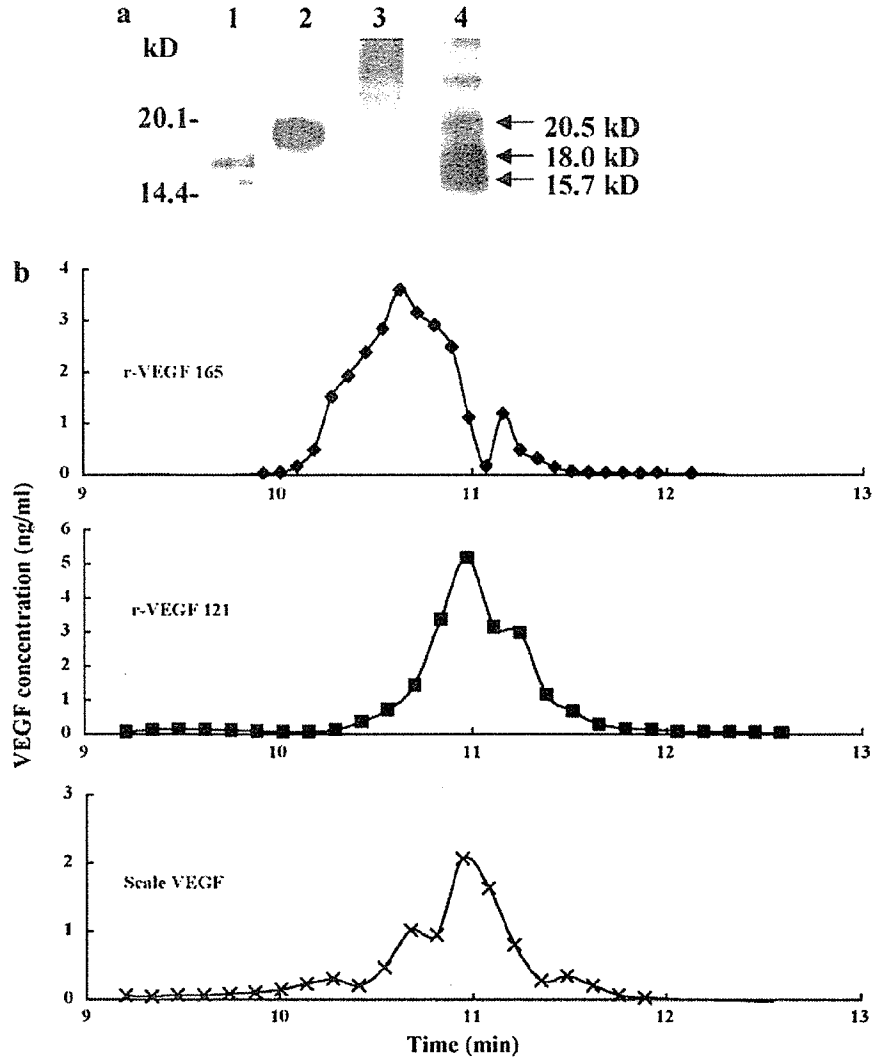

Fig. 2 a Determination of VEGF isoforms in lesional AD scale by western blot. Scale extracts were analyzed by SDS-PAGE and immunoblotted using goat anti-human VEGF polyclonal antibodies. *Lane 1* recombinant VEGF 121 isoform (5 ng); *Lane 2* recombinant VEGF 165 isoform (5 ng); *Lane 3* normal stratum corneum extracts; *Lane 4* AD scale extracts. **b** Determination of VEGF isoforms in lesional AD scale by gel filtration chromatography. Chromatography was performed with TSKgel G2000SW$_X$L column for 20 ng of recombinant VEGF 165 isoform (*r-VEGF 165*), 20 ng of recombinant VEGF 121 isoform (*r-VEGF 121*), and 30 ng of partially purified VEGF preparation from AD scales (*Scale VEGF*). Relative molecular weights of scale VEGF were calculated as 34 and 30 kDa using ribonuclease A, chymotrypsinogen A, ovalbumin and albumin as molecular markers

G2000SW$_X$L column (Tosoh, Tokyo, Japan). The column was equilibrated with 0.1 M PB. Then a 20 µl sample was applied and chromatographed at a flow rate of 0.8 ml/min at room temperature. Fractions of three drops were collected, and VEGF contents in the fractions were determined by VEGF ELISA kit. Recombinant VEGF 165 isoform eluted at 10.63 min and recombinant VEGF 121 isoform eluted at 10.98 min in this condition (Fig. 2b). The gel-chromatography of the scale extracts showed a peak at 10.95 min with a shoulder at 10.68 min for VEGF (Fig. 2b). The molecular size of the VEGF was calculated to be 30 and 34 kDa, respectively. The main peak of 30 kDa upon gel-filtration profile confirmed the finding that in AD lesions VEGF 121 isoform is predominantly produced though VEGF 165 isoform may be another participant.

To determine whether the VEGF recovered from lesional AD scales was biologically active, activity of the samples was determined by proliferation assay with the cultured human umbilical vein endothelial cells. For the assay, the VEGF in the scale extracts was partially purified by ammonium sulfate precipitation followed by Phenyl Sepharose chromatography. Ammonium sulfate was added to scale extracts (390 mg/ml) to make a 60% saturated solution. The solution was mixed for 1 h at 4°C, and then the precipitated protein was recovered by centrifugation at 15,000*g* for 40 min at 4°C. The pellets were resuspended with 5 ml of 1.0 M ammonium sulfate in 0.1 M phosphate buffer (PB, pH 7.4). After centrifugation at 15,000*g* for 5 min at 4°C, supernatant from the suspension was put onto a Phenyl Sepharose 6 Fast Flow (high sub) column (1 ml, Amersham Biosciences). The column was washed with 5 ml of 1.0 M ammonium sulfate in 0.1 M PB, then stepwise-eluted with 5 ml of 0.5 M ammonium sulfate in 0.1 M PB, 10 ml of 0.1 M PB and 10 ml of sterilized water at a flow rate of approximately 1 ml/min. One-ml fractions were collected, and the concentration of VEGF in the fractions was measured with a VEGF ELISA kit. Fractions containing VEGF were collected, lyophilized and dissolved in PBS. Human umbilical vein endothelial cells were purchased from Cambrex Bio Science Walkersville, Inc.,

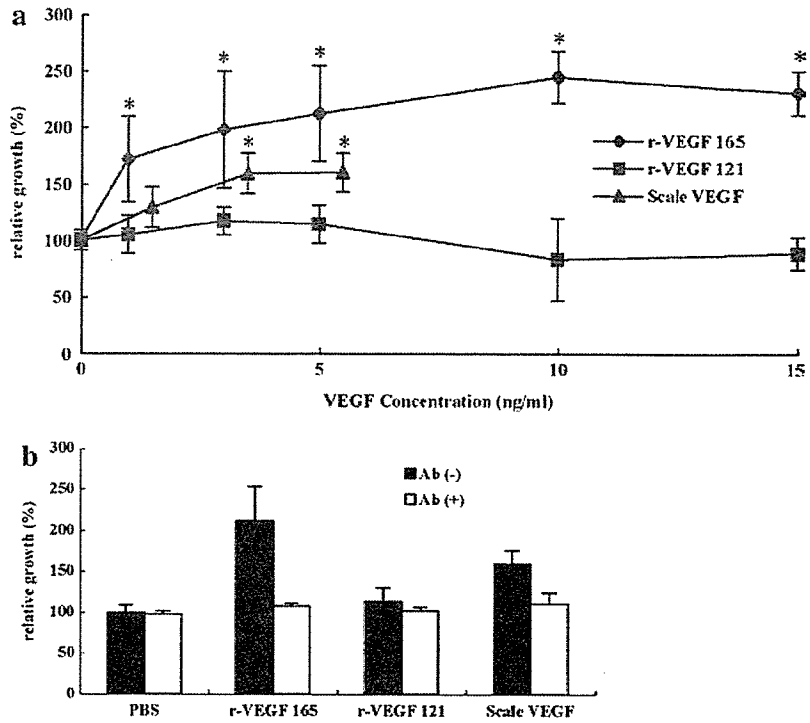

Fig. 3 Effect of partially purified VEGF preparation on the proliferation of human umbilical vein endothelial cells. **a** Biological activity of partially purified VEGF in the AD scale extracts (*Scale VEGF*), recombinant VEGF 165 isoform (*r-VEGF 165*) and recombinant VEGF 121 isoform (*r-VEGF 121*) were determined using cultured human umbilical vein endothelial cells. Each value is expressed as a percentage change in growth from cells cultured without VEGF (mean ± SD), and significant growth is shown with *asterisks*. **b** Neutralization was performed using anti-VEGF polyclonal goat antibodies. Cell growth is displayed as a percentage change from cells cultured with medium alone (mean ± SD)

Minneapolis, USA, and were maintained at 37°C in a humidified atmosphere of 5% CO_2 in EGM-2 complete medium (EGM-2 BulletKit, Cambrex Bio Science Walkersville, Inc.). Third passaged cells were used in all experiments. The cells were plated in 96-well plates at 2,800 cells/well in 150 µl EGM-2 complete medium. Sixteen hours later, the medium was replaced with EBM-2 medium supplemented with 0.2% fetal calf serum, 0.1% ascorbic acid, 0.4 µg/ml hydrocortisone, 0.1% heparin, 0.05 mg/ml gentamicin and 0.05 µg/ml amphotericin-B (Cambrex Bio Science Walkersville, Inc.). Recombinant VEGF isoforms or partially purified VEGF sample from the scale extracts were added and further incubated. After 30 h, 15 µl Cell Proliferation Reagent WST-1 (Roche, Mannheim, Germany) was added to each well, reacted for 2 h, and then the cells were counted on a Ceres 900 Hdi plate reader (Biotek Instruments, Inc., Winooski, VT, USA) at a measuring wavelength of 450 nm and a reference wavelength of 690 nm. Assays were performed in five wells per sample, and the experiments were performed three times. Statistical analyses were performed using a Tukey test for comparing cell growth between and within groups. Values of $P<0.05$ were considered statistically significant. When comparing percentage change in cell growth, recombinant VEGF 165 was found to have an ability to elicit cell proliferation ($P<0.01$), whereas recombinant VEGF 121 failed to significantly enhance the proliferation of vascular endothelial cells (Fig. 3a). Maximum proliferation was observed at a concentration of 10 ng/ml of recombinant VEGF 165. The VEGF recovered from the lesional scales was still biologically active to induce the proliferation of vascular endothelial cells at a concentration of 3 ng/ml and above ($P<0.01$) (Fig. 3a). However, compared to recombinant VEGF isoforms, the activity was significantly lower than that of recombinant VEGF 165 isoform ($P<0.01$), but higher than that of recombinant VEGF 121 isoform ($P<0.01$) at a concentration of 5 ng/ml. This may reflect the fact that scale VEGF consists of mostly VEGF 121 isoform, but is possibly contaminated with VEGF 165 isoform.

To further confirm the biological activity of samples, neutralization was performed using anti-VEGF polyclonal goat antibodies (R&D Systems). The neutralization antibodies were added to the samples (VEGF 5 ng/ml) at a concentration of 0.5 µg/ml and incubated for 2 h at 37°C. The remaining biological activity was determined by proliferation assay. By pretreating with anti-VEGF goat polyclonal antibodies, the proliferation activity of partially purified VEGF was completely abrogated, similar to the recombinant VEGF isoforms (Fig. 3b).

This study shows that significant amounts of VEGF (mainly VEGF 121 isoform) are stored in the stratum corneum of the AD lesions and the VEGF is extractable and still biologically active, indicating an involvement of VEGF in the pathogenesis of AD, especially in initiating prolonged capillary dilatation and dermal edema. The source of VEGF is likely to be keratinocyte itself, because biochemical features of the VEGF are very similar to those of cultured keratinocytes. In addition, immunohistochemical examination using anti-VEGF mouse monoclonal antibodies revealed strong cytoplasmic staining for VEGF in the keratinocytes from the basal to granular layer as well as horny layer in the clinically involved AD skin (data not shown). In contract, little

VEGF expression was detected in the epidermis of normal subjects except intensive immunoreactivity to VEGF in the granular layer. The amount of VEGF extracted from lesional scales was at least 25 times higher than that extracted from uninvolved sole scales, suggesting an overproduction of VEGF in the lesions of AD. Our investigation provided the evidence that VEGF 121 isoform is a prominent component though approximately 20% of VEGF could possibly be VEGF 165 isoform.

Recent investigations have identified that in contrast to VEGF 165 isoform, VEGF 121 isoform is not able to induce angiogenic sprouting, but causes extensive dilatation and hyperpermeability of pre-existing blood vessels [12]. Accordingly, VEGF 121 isoform produced from the keratinocytes in the AD lesions accounted for the altered features of microvessels in the AD lesions. The overproduced VEGF may also account for the mechanisms of anti-histamine resistant erythema.

The present study failed to resolve mechanisms of VEGF overproduction in AD lesional keratinocytes. It is possible that VEGF production is upregulated by other cytokines such as tumor necrosis factor-α, fibroblast growth factor, and transforming growth factor, because with these factors the production of VEGF in cultured keratinocytes was known to be upregulated [17].

Because keratinocytes degenerate into the horny layer for approximately 2-week turnover periods, stratum corneum might contain cytokines produced by the keratinocytes during inflammatory events. Thus, analysis of cytokine contents in the lesional scales might be a useful tool for investigating the pathogenesis of inflammatory skin diseases. In fact, we also revealed that some members of the chemokine family, such as thymus and activation-regulated chemokine and regulated on activation, normal T expressed and secreted, were detectable in the lesional scales obtained from patients with AD [15, 16].

In conclusion, we revealed that VEGF is overproduced in AD lesions, and that VEGF 121 isoform mainly participates in a process leading to vessel dilatation and hyperpermeability in the AD lesions. In this regard, down-regulation of VEGF production or blockade of VEGF action is likely to become an exciting new therapeutic strategy for AD.

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CASE REPORT

Cross-reactivity among shrimp, crab and scallops in a patient with a seafood allergy

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ABSTRACT

Shrimp is known to be the most common causative agent in seafood allergy. Patients with shrimp allergy often exhibit allergic symptoms to a variety of seafoods such as crabs and clams. We experienced a 14-year-old girl with shrimp allergy who developed oral swelling and pain accompanied with an uncomfortable feeling after ingestion of scallops followed by intensive exercise. Laboratory investigation showed that she had serum immunoglobulin (Ig)E molecules reacting with several kinds of crustaceans and mollusks, including shrimp, crab and scallops. Immunoblotting revealed that her serum IgE reacted with the 38 kDa bands for shrimp, crab and scallops, suggesting that tropomyosin was the major allergen. Dot-blot inhibition analysis showed a cross-reactivity among shrimp, crab and scallops. We conclude that the cross-reactivity of IgE in this patient resulted from the high homology of tropomyosins.

Key words: crab, cross-reactivity, scallop, shrimp, tropomyosin.

INTRODUCTION

Seafood allergy is common in coastal countries such as Japan and Scandinavia. Among the seafood allergens, shrimp is the most frequent culprit.[1] Moreover, patients with shrimp allergies often exhibit allergic symptoms in response to a variety of other seafoods such as crabs and clams. Patrick *et al.* reported both clinical and experimental evidence of significant cross-reactivity among crustaceans (shrimp, lobster, crab and crawfish).[2] In such patients, immunoglobulin (Ig)E molecules against shrimp allergens have been demonstrated to cross-react with homologous allergens from multiple seafoods. Herein, we report a case of shrimp allergy whose serum IgE reacted with both crustaceans and mollusks.

CASE REPORT

A 14-year-old girl was referred to our hospital because of oral swelling and pain accompanied with an uncomfortable feeling after ingestion of scallops followed by intensive exercise. No systemic symptoms were noted. The symptoms faded within 2 hours without any medications. No sign of infection or history of medication intake was present before onset of the symptoms.

The patient had a 3-year history of atopic dermatitis and one of conjunctivitis since birth. From the age of 5 years, she had often had oral pain and mucosal swelling after eating shrimp. Similar episodes occurred after eating other crustaceans and mollusks such as crab, squid and octopus. There had been no symptoms with foodstuffs devoid of crustaceans and mollusks. Sometimes oral swelling appeared when she ate foods supplemented with shrimp, either raw or cooked. There was no history of fish allergy.

Laboratory investigation showed an elevated concentration of serum IgE (1072.7 IU/ml). Serum-specific IgE antibodies are summarized in Table 1. The allergen-specific IgE test revealed positive reactions to tropomyosin (shrimp), shrimp, crab, squid, octopus,

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Table 1. Food-specific IgE Levels (Ua/ml)

Seafood	Value*	Other food	Value*
Tropomyosin (shrimp)	19.2	Egg white	< 0.35
Shrimp	18.9	Milk	< 0.35
Crab	18.9	Wheat	0.56
Squid	10.5	Gluten	< 0.35
Octopus	8.99	Buckwheat	< 0.35
Scallop	4.95	Soybean	< 0.35
Clam	4.64	Cod roe	< 0.35
Mackerel	2.89		
Anisakis	2.65		
Tuna	2.65		
Oyster	1.98		
Horse mackerel	1.34		

*Food-specific IgE antibodies were measured by CAP System fluorescent-enzyme immunoassay (Pharmacia Diagnostics, Uppsala, Sweden).

scallop, clam, mackerel, anisakis, tuna, oyster and horse mackerel. Strongly positive reactions were observed to extracts of shrimp and crab by skin testing using allergen extracts (Torii Pharmaceutical, Tokyo, Japan) (Fig. 1).

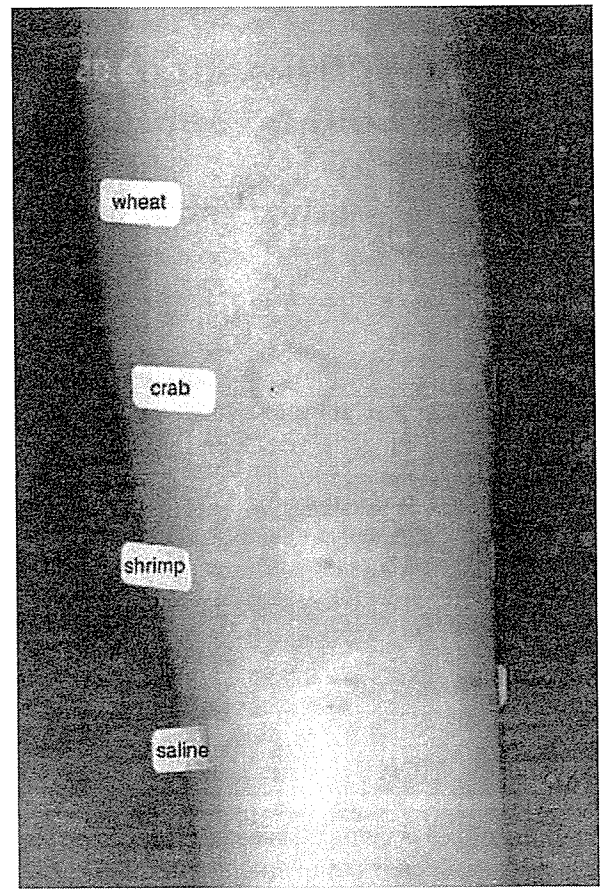

Figure 1. Skin testing showed strongly positive reactions to extracts of shrimp and crab.

Immunoblotting

A piece of raw shrimp, crab or scallop was ground separately in a Petri dish with distilled water at a concentration of approximately 3 mg/ml. Aliquots of the extracts were boiled for 15 min. All the extracts were filtered, dialyzed, lyophilized, and quantitated for protein content. To identify the allergenic protein, the extracts were analyzed with sodium dodecyl sulfate-polyacrylamide gel electrophoresis followed by immunoblotting. Ten μg of protein lysate per sample were subjected to electrophoresis on 12.5% polyacrylamide gel. Then they were electroblotted onto a polyvinylidene difluoride (PVDF) microporous membrane (Millipore Corporation, MA, USA). After being blocked with 5% skim milk in 0.1% Tween-Tris-buffered saline (TTBS), the membrane was incubated overnight at 4°C with the patient's serum diluted 1:10 with 5% skim milk in TTBS. Afterward, the membrane was extensively washed and incubated with goat antihuman IgE antibodies conjugated with horseradish peroxidase (BioSource, Camarillo, CA, USA) at a concentration of 0.04 μg/ml for 1 h at room temperature. Bound antibodies were reacted with enhanced chemiluminescence plus Western Blotting Detection Reagents (Amersham Biosciences, Uppsala, Sweden). The results revealed that the serum IgE of the patient reacted with similar bands of 38 kDa for shrimp, crab and scallop (Fig. 2). Boiling had no effect on the intensities of the bands.

Inhibition assays

Specific-IgE inhibition assays were performed using a dot-blotting test. A PVDF microporous membrane was spotted with 2 μg of extracted proteins (boiled shrimp, crab and scallop), blocked with 5% skim milk in TTBS, and incubated with the patient's serum. Before incubation with the membrane, 40 μl of the serum were pre-incubated at 37°C for 2 h with the extracted proteins serially diluted from 333 to 0.03 μg/ml with 5% skim milk in TTBS. After washing with TTBS, the membrane was incubated with goat antihuman IgE antibodies conjugated with horseradish peroxidase for 1 h at room temperature.

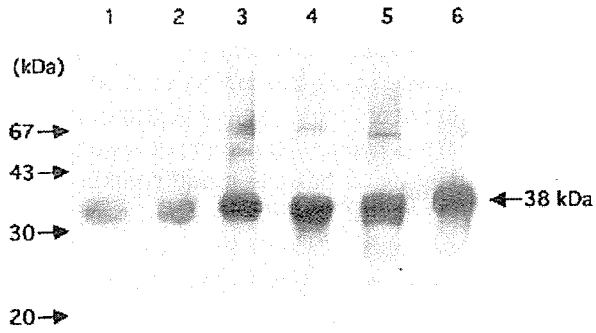

Figure 2. Immunoblot analyses using extracts from scallop, shrimp and crab. Lane 1, raw scallop; lane 2, boiled scallop; lane 3, raw shrimp; lane 4, boiled shrimp; lane 5, raw crab; lane 6, boiled crab.

The inhibition of the specific IgE binding to extracted proteins was visualized using ECL plus Western Blotting Detection Reagents. The resulting light was detected with autoradiography film (Hyperfilm ECL, Amersham Biosciences). After scanning the film, the spot intensities were measured using the Gel-Pro Analyzer software (Media Cybernetics, Silver Spring, MD, USA).

The inhibition was expressed as a percentage change from the patient's serum treated with Tris-buffered saline. The incubation of patient's serum and shrimp extracts caused dose-dependent inhibitions in IgE binding to extracted shrimp and crab proteins. Both of the IgE bindings were completely inhibited by extracted shrimp proteins at the concentration of 333 μg/ml. A similar result was obtained with extracted crab proteins (Fig. 3a,b). However, IgE binding to extracted scallop proteins was only partially inhibited even at the highest concentration: 60% by shrimp and 35% by crab, although almost 100% inhibition was obtained by scallop itself at 333 μg/ml extracted proteins (Fig. 3c). Scallop extracts inhibited IgE binding to extracted proteins from shrimp or crab by only about 20% even at the highest concentration (Fig. 3a,b).

DISCUSSION

Herein, we report a case of shrimp allergy exhibiting a cross-reactivity with crab and scallop. Immunoblot analysis with the patient's serum detected a major band of 38 kDa in every extract, indicating that tropomyosin is the common and major antigen.[2,3] This is supported by the highest score of serum-specific

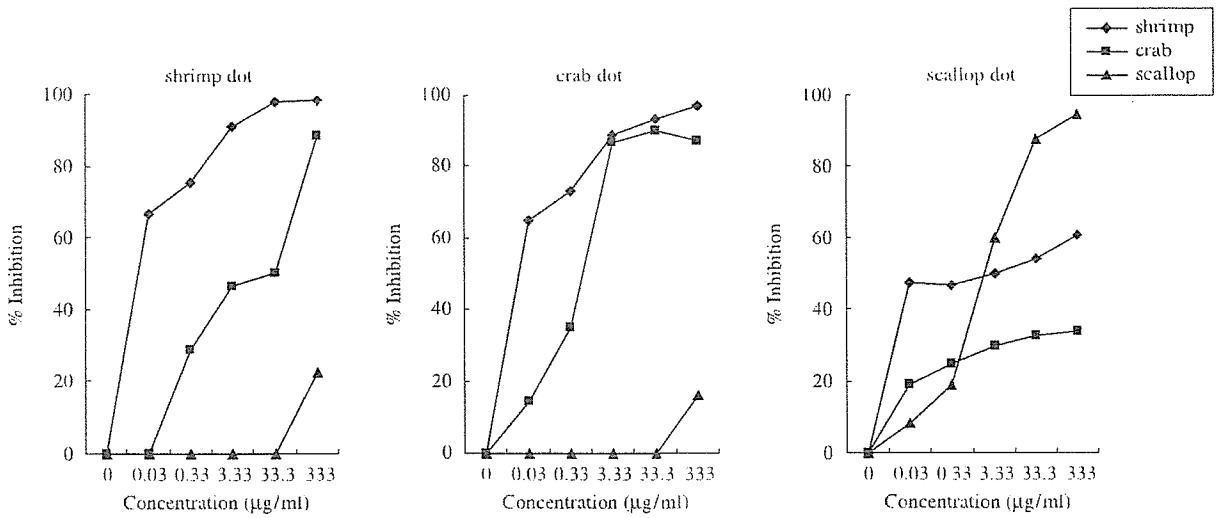

Figure 3. Inhibitions of immunoglobulin (Ig)E binding to extracted proteins from shrimp (◆), crab (■) and scallop (▲). (a) Two μg of shrimp extract was spotted and incubated with the patient's serum treated with serially diluted shrimp extract, crab extract and scallop extract; (b) 2 μg of crab extract was spotted and incubated with the patient's serum treated with serially diluted shrimp extract, crab extract and scallop extract; (c) 2 μg of scallop extract was spotted and incubated with the patient's serum treated with serially diluted shrimp extract, crab extract and scallop extract.

IgE antibodies to shrimp tropomyosin. The specific IgE inhibition analyses with the patient's serum demonstrated a marked cross-reactivity between shrimp and crab, paralleling the great homology in the amino acid sequences of shrimp and crab tropomyosins (91.7%).[4] The IgE binding to extracted scallop proteins was remarkably inhibited by shrimp or crab extracts, indicating a cross-reactivity among shrimp, crab and scallops. However, the inhibition percentage with scallop extracts was found to be lower in the IgE binding to the shrimp extracts and the crab extracts. This may be attributed to the lesser homology in the amino acid sequence of scallop tropomyosin with the shrimp and crab tropomyosins. The homologies are 61.6% between shrimp and scallop and 61.7% between crab and scallop.[4,5] In this patient, scallop-induced allergic symptoms were exhibited only when the patient did intensive exercise after ingestion of scallops. The scallop-specific IgE level is lower than those for shrimp and crab; thus, the symptoms may have been induced by a mechanism of food-dependent exercise-induced anaphylaxis. Goetz *et al.* reported a similar cross-reactivity between shrimp and scallop in a patient with occupational asthma using a specific IgE inhibition test and an immunoblotting test.[6] Therefore, it appears that individuals allergic to shrimp or crab are at risk for reacting to mollusks, because these seafoods share common antigenic epitopes in tropomyosin, even though their homologies are not so high.

Only the IgE binding to scallop protein was inhibited up to 60% by the shrimp extracts and up to 35% by the crab extracts. This partial inhibition may be due to the presence of IgE antibodies reacting only to scallop-specific epitopes of the tropomyosin in the patient.

The patient also exhibited an oral allergy syndrome after eating squid and octopus, but no symptoms after ingestion of fish. Her specific IgE scores to squid and octopus were 10.5 and 8.99, respectively, much higher than those of fishes such as mackerel and tuna (2.89 and 2.65, respectively). These scores are well correlated with the amino acid sequence homology between those seafood tropomyosins and shrimp tropomyosin. The amino acid sequence homology of squid tropomyosin and shrimp tropomyosin is 70.3%, but that of tuna tropomyosin and shrimp tropomyosin is only 57.2%.[4]

In conclusion, we demonstrated marked cross-reactivities of IgE among shrimp, crab and scallop extracts in the patient with seafood allergy, which may be a result of the high homology of their tropomyosins. Further examination of IgE-binding epitopes is necessary in order to substantiate this speculation.

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Phenotypic Investigation of Human Eyes with Transplanted Autologous Cultivated Oral Mucosal Epithelial Sheets for Severe Ocular Surface Diseases

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Purpose: To determine the epithelial lineage of origin of surgically removed grafts after autologous cultivated oral mucosal epithelial transplantation (COMET).

Design: Retrospective comparative case series.

Participants: We studied 6 eyes from 5 patients with total corneal stem cell destruction; 3 eyes were from patients with Stevens–Johnson syndrome and 3 eyes had sustained chemical injury.

Methods: Autologous cultivated oral mucosal epithelial sheets on human amniotic membrane (AM) were transplanted onto the ocular surface. Regrafting (2 eyes) or penetrating keratoplasty (4 eyes) was performed after the initial transplantation procedure for further visual rehabilitation.

Main Outcome Measures: The excised grafts were subjected to clinical evaluation and to light, scanning, and transmission electron microscopic (EM) study and to immunohistochemical analysis.

Results: In clinically failed grafts, EM and immunohistochemical analysis disclosed only small areas where the original cultivated oral epithelial cells persisted. Neighboring conjunctival epithelial cells had apparently invaded a large portion of the corneal surface (keratin 3[−], Muc5ac[+]); there were many blood vessels and inflammatory cells. In clinically successful grafts, transplanted cultivated oral epithelial cells survived and had adapted well to the host corneal tissues (keratin 3[+], Muc5ac[−]); there was no infiltration by inflammatory cells, nor was there dissolution of the AM substrate.

Conclusions: We posit that the process of graft opacification after COMET is responsible for the loss of transplanted cultivated oral epithelial cells and that this is followed by conjunctival cell invasion onto the corneal surface. We confirmed that in clinically successfully grafted eyes, autologous cultivated oral epithelial cells survived on the corneal surface and maintained ocular surface integrity. *Ophthalmology 2007;xx:xxx © 2007 by the American Academy of Ophthalmology.*

Severe ocular surface disease (OSD) that arises from Stevens–Johnson syndrome (SJS) and thermal and chemical burns can be devastating and result in significant visual complications. It is a disease characterized by the destruction of corneal epithelial stem cells in the limbus that results in invasion of the corneal surface by surrounding conjunctiva, neovascularization, chronic inflammation, ingrowth of fibrous tissue, and stromal scarring.[1–3] Conventional corneal transplantation has proven to be a less than satisfactory curative measure in patients with severe OSD. Reconstructive methods such as keratoepithelioplasty and limbal transplantation have improved the clinical outcomes in these severely damaged eyes.[4,5] As the transplantation of cultivated corneal epithelial stem cells has yielded promising results, it has gained acceptance as an effective treatment modality.[6–9] Our experimental[10] and clinical[11,12] studies demonstrated the efficacy of autologous cultivated oral epithelial transplantation for the treatment of severe OSD. This method offers the distinct advantage of reducing the risk of allograft rejection and immuno-

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