

ORIGINAL ARTICLE

# Levels of immunoglobulin E specific to the major food allergen and chemokine (C-C motif) ligand (CCL)17/thymus and activation regulated chemokine and CCL22/macrophage-derived chemokine in infantile atopic dermatitis on Ishigaki Island

Hitokazu ESAKI,<sup>1</sup> Satoshi TAKEUCHI,<sup>1,2</sup> Norihiro FURUSYO,<sup>3,4</sup> Kazuhiko YAMAMURA,<sup>1</sup> Sayaka HAYASHIDA,<sup>1</sup> Gaku TSUJI,<sup>1</sup> Masaki TAKEMURA,<sup>1</sup> Jun HAYASHI,<sup>3,4</sup> Masutaka FURUE<sup>1</sup>

<sup>1</sup>Department of Dermatology, Graduate School of Medical Sciences, Kyushu University, <sup>2</sup>Department of Dermatology, Federation of National Public Service Personnel Mutual Aid Associations, Hamanomachi Hospital, <sup>3</sup>Department of General Internal Medicine, Kyushu University Hospital, <sup>4</sup>Department of Environmental Medicine and Infectious Disease, Graduate School of Medical Sciences, Kyushu University, Fukuoka, Japan

## ABSTRACT

Atopic dermatitis (AD) is a multifactorial T-helper (Th)2-mediated skin disease frequently associated with elevated serum immunoglobulin (Ig)E and food allergy is also a Th2- and IgE-mediated adverse immunological reaction. Our previous study indicated the relation of egg allergy history and disease severity of AD. Thus, the purpose of the study was to investigate the levels of IgE specific to major food allergens (egg, milk, wheat) and Th2 chemokines (chemokine [C-C motif] ligand [CCL]17/thymus and activation regulated chemokine [TARC] and CCL22/macrophage-derived chemokine [MDC]) and the relationship between them. A total of 743 nursery school children were enrolled. Dermatologist-based physical examination and a questionnaire survey were also conducted. Significantly increased levels of disease severity markers (CCL17/TARC and CCL22/MDC) were confirmed in children with AD. The levels of CCL22/MDC in all of the children were markedly high compared with those reported in adults. IgE specific to egg white, ovomucoid, wheat and mite antigen were significantly higher in the AD group than in the non-AD group. Among them, IgE specific to egg allergens were well associated with disease severity markers, and IgE specific to ovomucoid seemed particularly well correlated with the presence of egg allergy history. In conclusion, the markedly high level of CCL22/MDC in children as compared with those reported in adults may partly explain the AD-prone nature of children and their spontaneous remission afterwards. Mild but significant correlation of IgE specific to egg allergens and Th2 chemokines may explain correlation of disease severity and comorbidity of egg allergy in our previous study.

**Key words:** atopic dermatitis, chemokine (C-C motif) ligand 17/thymus and activation regulated chemokine, chemokine (C-C motif) ligand 22/macrophage-derived chemokine, cohort, food allergy.

## INTRODUCTION

Atopic dermatitis (AD) is a chronic, relapsing, pruritic and multifactorial inflammatory skin disease, frequently associated with peripheral blood eosinophilia and raised serum immunoglobulin (Ig)E levels, with the IgE being specific to environmental allergens such as mites and food.<sup>1</sup> Food allergy is an adverse immunological reaction to food. The central pathophysiology is the generation of allergen-specific IgE, and most of its manifestations are T-helper (Th)2-mediated,<sup>2–4</sup> which are similar to

those of AD. In Japan, three major types of food (eggs, milk and wheat) have been identified as the most common sources of allergens in children,<sup>5</sup> and egg allergy is the most common in young children.<sup>6</sup> Egg sensitization is closely associated with AD, the level of which is apparently correlated with the severity of this disease.<sup>7,8</sup>

In 2001, we initiated a population-based cohort study on nursery school children aged 6 years and under, on Ishigaki Island, Okinawa, Japan, which was named the Kyushu University Ishigaki Atopic Dermatitis Study (KIDS). Through this

Correspondence: Satoshi Takeuchi, M.D., Ph.D., Department of Dermatology, Federation of National Public Service Personnel Mutual Aid Associations, Hamanomachi Hospital, 3-3-1 Nagahama, Chuo-ku, Fukuoka 810-8539, Japan. Email: takeuchi-s@hamanomachi.jp  
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cohort study, we have reported AD prevalence, serum total IgE and chemokine (C-C motif) ligand (CCL)17/thymus and activation regulated chemokine (TARC) levels, spontaneous regression ratio, risk factors for AD, and the relationship between skin infections and childhood AD.<sup>9–12</sup> Recently, we found that a history of egg allergy was associated with higher disease severity as assessed by CCL17/TARC in the KIDS cohort.<sup>13</sup> To extend this work, we investigated the levels of IgE specific to the major food allergens (egg, milk and wheat); we also evaluated the serum levels of CCL17/TARC and CCL22/macrophage-derived chemokines (MDC), which share the same chemokine receptor, C-C chemokine receptor type 4, and have been shown to reflect the disease activity well in both adult and infantile AD.<sup>14,15</sup>

## METHODS

### Study design

The study design has been described previously.<sup>12</sup> A total of 743 children aged 6 years and under were enrolled from 13 nursery schools on Ishigaki Island, Okinawa, Japan, in 2008. The study was approved by the ethics committee of Kyushu University and by the directors and classroom teachers of the nursery schools. Written informed consent to allow participation of the children was obtained from their parents or guardians. Possible selection bias could occur by not recruiting infants who did not attend nursery schools.

### Physical examination and questionnaire

A medical skin examination for the children was conducted by dermatologists from the Department of Dermatology, Kyushu University Hospital. AD was diagnosed according to the criteria for AD by the Japanese Dermatological Association.<sup>16</sup> A structured questionnaire that included questions on personal history of AD-related allergic diseases and food allergy was filled out by the children's parents or guardians. Of the 743 children, blood samples were successfully obtained from 600, among which we received valid questionnaires from 520.

### Measurement of serum CCL17/TARC, CCL22/MDC, total IgE and IgE specific to food- and mite-antigens

Serum CCL17/TARC and CCL22/MDC levels were measured by enzyme-linked immunoassay (Shionogi [Osaka, Japan] and R&D Systems [Minneapolis, MN, USA], respectively), in accordance with the manufacturers' protocols. Total serum IgE was determined by radioimmunoassay (Shionogi). The serum levels of the IgE specific to hen's egg white (EW), ovomucoid (OVM), wheat, milk and the mite *Dermatophagoides pteronyssinus* (Dp) were measured in a laboratory at Siemens Healthcare Diagnostics (Tokyo, Japan) using IMMULITE<sup>®</sup> 3gAllergy™ technology.

### Statistical analysis

The Mann–Whitney *U*-test was used to compare variables between two groups. Spearman's rank correlation coefficient test was used for correlation analyses. Data were presented as mean ± standard error of the mean. *P* < 0.05 was considered statistically significant.

## RESULTS

### Study population

Of the 743 children, 45 were diagnosed as having current AD by dermatologists (AD prevalence, 6.1%) in 2008. Blood samples were obtained from 600 children (including 36 children with AD), and CCL17/TARC, CCL22/MDC, total serum IgE, and IgE specific to EW, OVM, wheat, milk and mite Dp antigens were successfully measured in 600, 519, 600, 599, 588, 559, 592 and 577 children, respectively, due to the limited volume of the obtained blood samples.

### Levels of Th2 chemokines and total IgE in AD and controls

The levels of serum CCL17/TARC, CCL22/MDC and total IgE were significantly higher in children with current AD than in children without this condition (Fig. 1a–c). There were slight decreases in the levels of CCL22/MDC (Fig. 1d) and CCL17/TARC (data not shown) with age in children without AD. However, there was no such decrease for CCL22/MDC in the current AD group (Fig. 1e), the same as for CCL17/TARC as shown in our very recent study,<sup>13</sup> in children with AD. There was a strong positive correlation between CCL17/TARC and CCL22/MDC (Fig. 1f).

### Levels of IgE specific to major food allergens in children from Ishigaki

We then examined levels of IgE specific to the major food allergens in children from Ishigaki. IgE specific to EW, OVM, wheat and mite Dp antigen were significantly higher in AD children than in non-AD children (Fig. 2a–d). A tendency of increased IgE specific to milk observed in AD children, but the difference was not statistically significant (Fig. 2e).

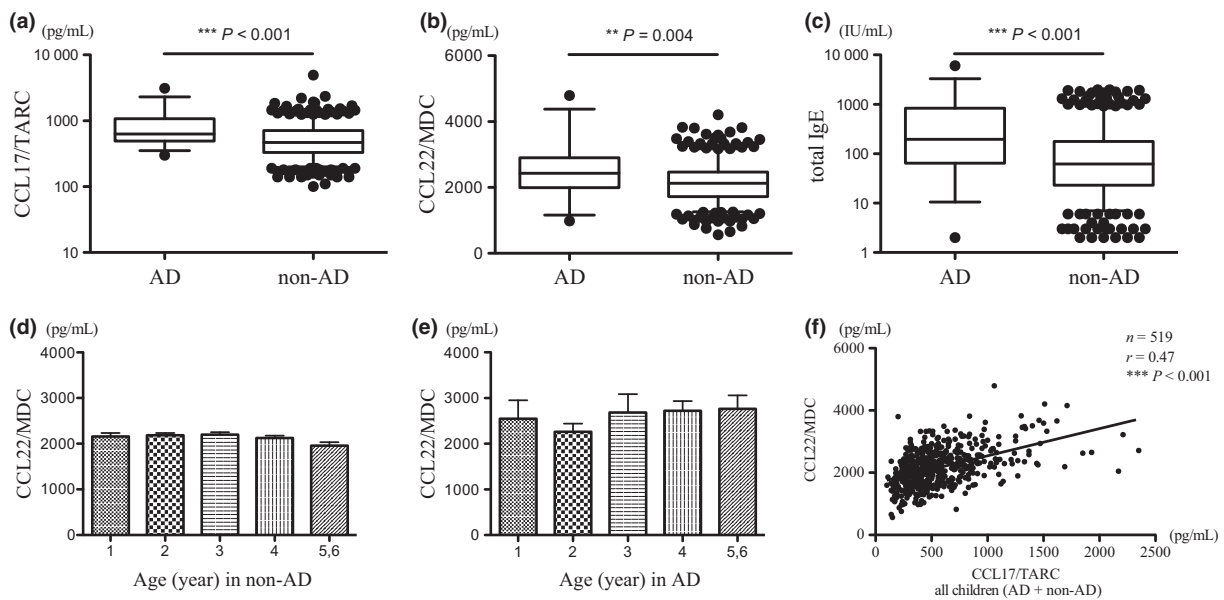
### Association between Th2 chemokines and degree of food sensitization

Among the food allergens examined, OVM showed the strongest correlation with CCL17/TARC, followed by wheat and EW (Fig. 3). Although the correlations were weaker, similar relationships were also observed between the levels of CCL22/MDC and the degree of food allergen sensitization (Fig. 3).

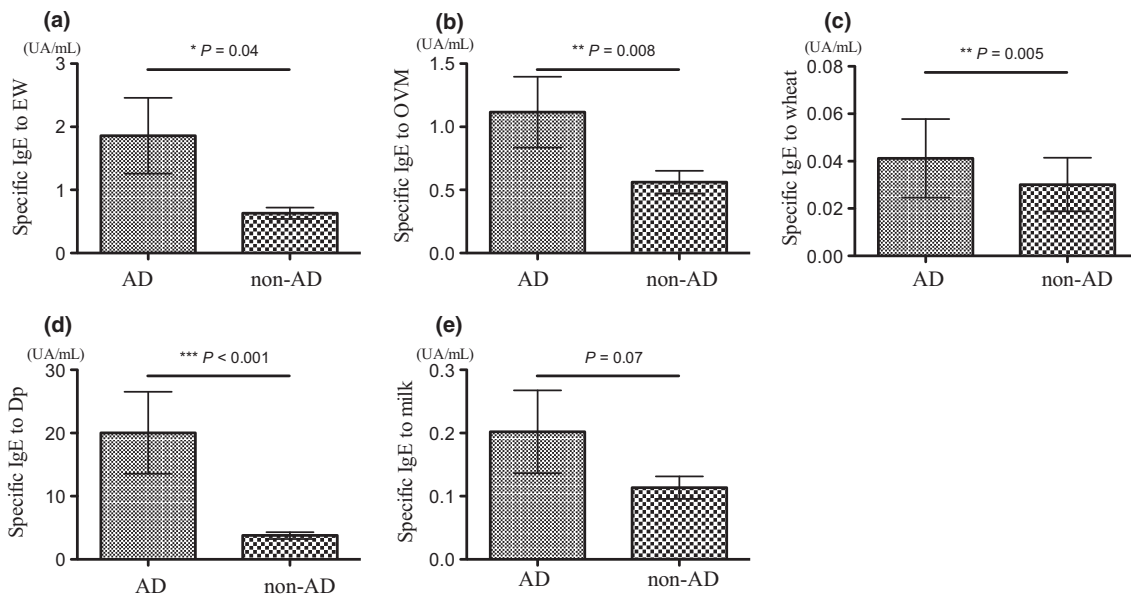
### Levels of IgE specific to major food allergens in children with AD and/or food allergy from Ishigaki

From the results of the questionnaire survey, the incidences of egg, milk and wheat allergies were 22.6%, 6.5% and 0% in children with AD, and 4.7%, 0.61% and 0.41% in children without AD, respectively (Table 1).

We examined the levels of IgE specific to EW and OVM in conjunction with the presence of egg allergy in children with or without AD. IgE specific to EW and OVM were significantly increased in children with egg allergy than those without it in both the AD and non-AD group (Fig. 4). IgE specific to OVM, when compared with that to EW, did not seem confounded by the presence of AD in assessing egg allergy in children. We could not conduct the same analysis for wheat and milk allergy because of insufficient number of patients.



**Figure 1.** Levels of serum T-helper (Th)2 chemokines and total immunoglobulin (Ig)E in children with and without atopic dermatitis (AD). The levels of (a) serum chemokine (C-C motif) ligand (CCL)17/thymus and activation regulated chemokine (TARC), (b) CCL22/macrophage-derived chemokine (MDC) and (c) total IgE were significantly increased in children with current AD. (d,e) CCL22/MDC levels declined with age, but this was not observed in children with AD. CCL22/MDC was significantly correlated with CCL17/TARC in children from Ishigaki. \*\* $P < 0.01$ , \*\*\* $P < 0.001$ .

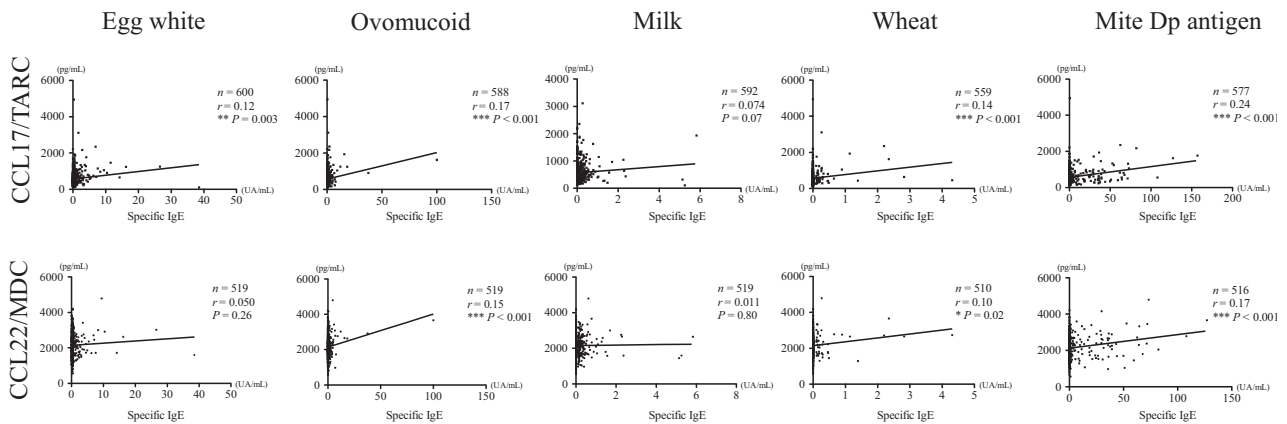


**Figure 2.** Levels of immunoglobulin (Ig)E specific to the major food allergens in children from Ishigaki. The levels of (a) specific IgE to egg white (EW), (b) ovomucoid (OVM), (c) wheat and (d) mite *Dermatophagoides pteronyssinus* (Dp) antigen were significantly higher in atopic dermatitis (AD) children than in non-AD children. (e) There was not a statistical difference in the level of specific IgE to milk between AD and non-AD children. \* $P < 0.05$ , \*\* $P < 0.01$  and \*\*\* $P < 0.001$ .

## DISCUSSION

In this cohort study, serum Th2 chemokine (CCL17/TARC and CCL22/MDC) levels and the levels of IgE specific to major food allergens in children with and without AD were examined. The

levels of these two Th2 chemokines in children with AD were significantly higher than those in children without this disease, in accordance with previous reports on adult<sup>17,18</sup> and childhood AD.<sup>19,20</sup> Our recent genome-wide association study revealed



**Figure 3.** Associations between T-helper (Th)2 chemokines and immunoglobulin (Ig)E specific to the major food allergens. There were relatively strong correlations of ovomucoid with chemokine (C-C motif) ligand (CCL)17/thymus and activation regulated chemokine (TARC) and CCL22/macrophage-derived chemokine (MDC) in children from Ishigaki; those for mite *Dermatophagoides pteronyssinus* (Dp) antigen were almost as strong. \* $P < 0.05$ , \*\* $P < 0.01$ , \*\*\* $P < 0.001$ .

**Table 1.** Questionnaire-based food allergy

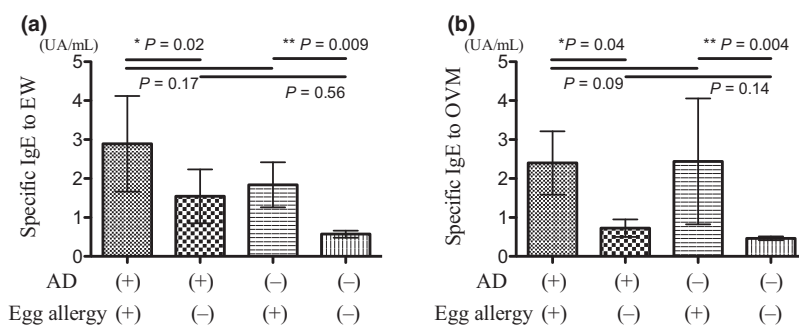
	Yes	No	Total	%
AD group				
Egg	7	24	31	22.6
Milk	2	29	31	6.5
Wheat	0	31	31	0
Non-AD group				
Egg	23	463	489	4.7
Milk	3	486	489	0.61
Wheat	2	487	489	0.41
Total				
Egg	30	490	520	5.8
Milk	5	515	520	0.96
Wheat	2	518	520	0.38

that the CCL22/MDC is a potential risk factor for AD,<sup>21</sup> and significantly higher levels of CCL22/MDC in children with AD were confirmed in this study. The levels of CCL22/MDC were extremely high even in children without AD compared with normal

adult levels, as reported previously.<sup>17,18</sup> The combination of high Th2 chemokine levels and less effective barrier functions of the skin in infants with high transepidermal water loss and low cutaneous capacitance<sup>22</sup> may explain their AD-prone nature via upregulation of thymic stromal lymphopoietin<sup>23–25</sup> and impaired filaggrin expression,<sup>26</sup> as well as their ability to recover naturally from the disease as they grow older.<sup>10</sup>

Significantly higher disease severity was associated with a history of egg allergy in children with AD in our recent study.<sup>13</sup> We could confirm this questionnaire-based finding in a more objective manner using the degree of food sensitization since IgE specific to OVM (or EW) were mildly, but significantly, related to the levels of CCL17/TARC or CCL22/MDC, both of which have been known as disease severity markers for AD (Fig. 3).

Regardless of whether or not egg allergy promotes disease severity, a severer disease condition or higher levels of these Th2 chemokines would induce repeated percutaneous sensitization via the easy entry of allergens through the impaired skin barrier.<sup>27</sup> A dysfunctional epithelial barrier due to skin inflammation may lead to the direct activation of antigen-sampling dendritic



**Figure 4.** Levels of immunoglobulin (Ig)E specific to egg allergens in conjunction with the presence of egg allergy history in atopic dermatitis (AD) and non-AD children from Ishigaki. The levels of IgE specific to (a) egg white (EW) and (b) ovomucoid (OVM) were significantly increased in children with egg allergy history than those without it both in AD and non-AD group. \* $P < 0.05$ , \*\* $P < 0.01$ .

cells by allergens and their maturation in a Th2-permissive milieu.<sup>28,29</sup> Indeed, our recent study showed that the level of CCL17/TARC, a disease severity marker for AD, reflects the impairment of epidermal functions even in healthy subjects and those with mild AD.<sup>30</sup> An interesting finding is that, although such easy entry and sensitization to allergens could occur for any allergens in AD patients, the degree of OVM-specific IgE as well as that of the mite Dp antigen was well associated with the levels of CCL17/TARC and CCL22/MDC. This may simply be due to the relatively small AD population examined in this cohort study; alternatively, egg allergens (particularly OVM) could possess the potential to cause slightly more allergic inflammation, thereby increasing disease severity compared with other food allergens.

In conclusion, both CCL17/TARC and CCL22/MDC levels were significantly increased in children with AD in the KIDS cohort. Markedly increased CCL22/MDC in children as compared with those reported in adults may partly explain their AD-prone nature and spontaneous remission afterwards. Increased chance of exposure could occur to any major food allergens through the impaired skin barrier in children with AD. However, considering the degree of correlations with disease severity markers (CCL17/TARC and CCL22/MDC), the comorbidity of egg allergy may somewhat contribute to the degree of skin inflammation.

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**CONFLICT OF INTEREST:** None declared.

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