

[Original Paper]

Age-related changes in allergic symptoms and serum TARC concentration in school children

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SUMMARY

Background Thymus and activation-regulated chemokine (TARC) is a Th2-related chemokine that is associated with allergic diseases. We investigated growth-related changes in allergic symptoms and TARC concentration in serum during later childhood to examine how Th2-related factors are associated with allergic symptoms.

Methods This study was performed in 146 school children living in a suburban Japanese city. At the 1st grade in 1997 and the 5th grade in 2001, these children underwent questionnaire investigation on respiratory and allergic symptoms and blood collection for determining total IgE, mite IgE and TARC concentrations in serum.

Results TARC concentration in serum significantly decreased at the 5th grade (424.1 pg/mL) compared with at the 1st grade (489.2 pg/mL) in the entire child population. In children with no allergic symptom at either grade, serum TARC concentration was significantly decreased at the 5th grade (410.7 pg/mL) compared with at the 1st grade (521.4 pg/mL), while there was no decrease in the serum concentration of TARC in allergic children at either or both of the two grades. There was also a difference in the decrease of serum TARC concentration at the 5th grade according to total IgE and mite IgE in addition, the presence of allergic symptom affected the decrease of serum TARC concentration.

Conclusion Serum TARC concentration decreased in association with growth in children, suggesting possible age-related decrease of Th2 cell activity. TARC concentration in serum significantly decreased in children with no allergic symptom, but no significant decrease was observed in allergic children, showing that TARC is associated with the onset of allergic symptoms. It is also considered that serum TARC concentration had strong relation to especially atopic dermatitis.

Key words: thymus and activation-regulated chemokine (TARC), bronchial asthma, allergic rhinitis, atopic dermatitis, IgE

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平野好絵^{1,3}, 鳥正之², 羽田明¹, 栗山喬之³: 児童のアレルギー症状及び血清中TARC濃度の経年変化について.

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I. Introduction

The increased prevalence of allergic diseases such as bronchial asthma and allergic rhinitis has gathered the attention of researchers worldwide in recent years[1]. An epidemiological study is necessary to evaluate the incidence of allergic diseases with time for elucidation of changes in the prevalence of allergic symptoms and individual symptoms and associated factors[2]. It is known that cytokines that are released from Th2 cells play important roles in the onset of allergic diseases. Th2-type cytokines promote IgE production and affect the function and life of eosinophils[3]. It is pointed out that TARC, a CC chemokine, is relevant to allergic diseases because its receptor, CCR4, is specifically expressed in Th2 cells and involved in the activity of Th2 cytokines[4]. High blood TARC concentration has been reported in allergic diseases[5-7].

There have been few studies of successive follow-up of children with allergic diseases, and no paper is available concerning the follow-up investigation of allergy-related cytokines and chemokines. It is important for better understanding of the cause of allergic disorders to examine how Th2-related factors are associated with allergic symptoms in school age.

The present study investigated the prevalence of allergic symptoms in primary school children at the 1st and 5th grades, and determined serum total IgE, mite IgE and TARC concentrations. Based on these results, we examined growth-related changes in the prevalence of allergic symptoms in later childhood. We also studied changes in TARC concentration with time to examine how the changes were associated with allergic diseases.

II. Materials and methods

Subjects

This study was performed in children from three primary schools in a suburban city of Chiba Prefecture, Japan. All children were at the 1st grade (6 to 7 years old) in 1997 at any of the three schools and at the 5th grade (10 to 11 years old) in 2001 at the same school, and lived in a neighborhood of these schools. In the autumn of 1997 and 2001, a standardized respiratory symptom questionnaire (ATS-DLD-78-C) [8] and the International Study of Asthma and Allergies in Childhood (ISAAC) [9] programme-compatible questionnaire on allergic symptoms were administered to investigate the presence of symptoms of bronchial asthma, wheeze, allergic rhinitis and atopic dermatitis. Blood samples were collected at the same time points for serological investigation. Both parents of each child were well explained how to respond to the questionnaires and the details of blood sampling, and written informed consent was obtained. The study protocol was approved by the Ethics Committee of Graduate School of Medicine, Chiba University.

Definition of allergic symptoms

The presence of allergic symptoms was evaluated from responses to the questionnaires. Bronchial asthma, wheeze, allergic rhinitis, and atopic dermatitis were defined in the same fashion as in our previous investigation[10] and the ISAAC programme[2]. Briefly, a child had bronchial asthma if at least two events of wheezing and/or whistling in the chest and acute onset of breathing difficulty, and he/she was diagnosed with asthma by his/her physician at the time of onset; a child had wheeze if he/she presented with wheezing and/or whistling respiration on at least two occasions when he/she caught cold within 2 years; a child had allergic rhinitis if symptoms of sneezing, nasal

discharge and/or nasal obstruction occurred within 1 year even when he/she did not suffer from cold; and a child had atopic dermatitis if he/she had itchy eczema continuing for 6 months or longer and occurred in the elbow, knee, ankle, hip, neck, ear, and/or periocular area within 1 year. A child was defined as allergic if he/she corresponded to any of the above criteria, and not allergic if he/she did not correspond to any of the above criteria.

Sample collection and laboratory measurements

Children with findings indicative of acute infection on the day before or the day of the investigation were excluded and blood was collected from the remaining children. Blood samples were collected in the morning and centrifuged on the same day. Serum thus separated was analyzed for total IgE concentration according to the Latex-Nephelometry (Dade Behring, Marburg, Germany) and for mite IgE according to the Uni-CAP methods (Pharmacia, Uppsala, Sweden), and then kept frozen at -80°C . Frozen serum samples were subsequently analyzed for TARC concentration using a commercially available enzyme-linked immunosorbent assay (ELISA) kits (R&D systems, Minneapolis, MN, USA).

Data analysis

Serum TARC concentration data showed a distribution similar to the logarithmic normal type. These data thus underwent logarithmic transformation and were analyzed for geometric means and 95% confidence intervals to examine the presence of allergic symptoms and the relationship with total IgE and mite IgE concentrations in serum. Data on serum concentration of total IgE was classified into high and low groups of ≥ 250 IU/mL and < 250 IU/mL in conformity with the clinical criteria in Japanese children[11]. Similarly, serum

mite IgE concentration data were classified into high and low groups of ≥ 0.35 U_A/mL and < 0.35 U_A/mL, respectively. Serum TARC concentration was compared between the 1st and 5th grades within each child for the time profile. Children were divided into four groups according to the presence of symptoms at the 1st and 5th grades, and the relationship with changes in serum TARC concentration were examined. Children were also classified into groups according to high and low total IgE and mite IgE concentrations in serum at the 1st and 5th grades, and the relationship with changes in serum TARC concentrations was examined. In addition, children were divided into groups according to allergic symptoms at the 1st and 5th grades for cross-sectional comparison of serum TARC concentration.

The Stat View (SAS Institute, Cary, NC, USA) was used for statistical procedures.

III. Results

It was possible to administer the questionnaires and hematological examination in 146 children at both the 1st and 5th grades. Table 1 shows symptoms in the 146 children. Of males and females combined, at least one allergic symptom was observed in 34.9% at the 1st grade and 34.2% at the 5th grade. The prevalence of allergic symptoms was significantly higher in males than in females at the 1st grade. The most frequent allergic symptom was rhinitis in both males and females at both the 1st and 5th grades.

As for the relationship between allergic symptoms and total IgE, the percentage of allergic children was significantly higher in the high total IgE group compared with the low group at both the 1st and 5th grades. Similarly, children were allergic more frequently in the high mite IgE group than in the low group at both the 1st and 5th grades (Table 2).

Table 1 Allergic characteristics in study subjects

		male (%) (n = 82)	female (%) (n = 64)	total (%) (n = 146)
1st grade	not allergic	57.3	75.0	65.1
	allergic	42.7*	25.0	34.9
	asthma	3.6	3.1	3.4
	wheeze	14.6	4.7	10.3
	rhinitis	28.0	20.3	24.7
	A.D.	10.9	3.1	7.5
5th grade	not allergic	64.6	67.2	65.8
	allergic	35.4	32.8	34.2
	asthma	4.9	3.1	4.1
	wheeze	3.7	3.1	3.4
	rhinitis	31.7	31.3	31.5
	A.D.	7.3	3.1	5.4

A.D.; atopic dermatitis

*p = 0.025 compared with females

Table 2 Serum concentrations of total IgE and mite IgE in children

		n	allergic (%)	p value
1st grade	total IgE	250 ≥	114	28.1
	(IU/mL)	250 <	32	59.4
	mite IgE	0.35 ≥	97	24.7
	(U _A /mL)	0.35 <	49	55.1
5th grade	total IgE	250 ≥	96	21.9
	(IU/mL)	250 <	50	58.0
	mite IgE	0.35 ≥	77	20.8
	(U _A /mL)	0.35 <	69	50.7

IgE; Immunoglobulin E

IU; International unit

Table 3 Allergic symptoms at the 1st and 5th grades

		1st grade		total
		not allergic	allergic	
5th grade	not allergic	78 (53.4%)	18 (12.3%)	96
	allergic	17 (11.6%)	33 (22.6%)	50
total		95	51	

Table 3 shows changes in allergic symptoms between the 1st and 5th grades. Of all children, 53.4% were not allergic either at the 1st or 5th grade, 22.6% were allergic at the 1st and 5th grades, 12.3% were allergic only at the 1st grade, and 11.6% were allergic only at the 5th grade.

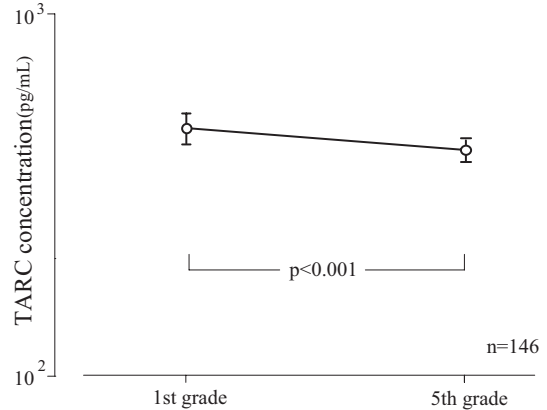


Fig. 1 Changes in serum TARC concentration in all children.

TARC; thymus and activation-regulated chemokine

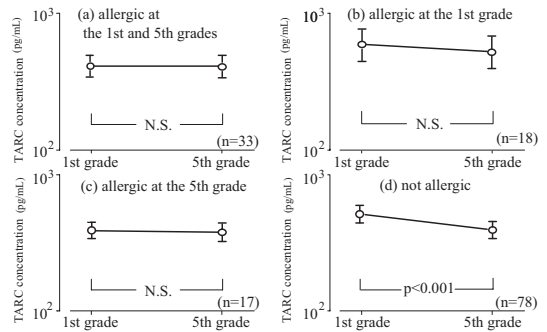


Fig. 2 Changes in serum TARC concentration according to the presence of allergic symptoms. Changes in serum TARC concentration are compared among four groups of allergic children at both the 1st and 5th grades, allergic children at the 1st grade, allergic children at the 5th grade, and children who were not allergic at either of the 1st or 5th grade.

N.S.; not significant

As for changes in serum TARC concentration from the 1st to 5th grades, it was decreased at the 5th grade compared with at the 1st grade within each child (Fig. 1). According to the presence of allergic symptoms, TARC concentration in serum decreased only in children who were not allergic either at the 1st or 5th grade. Serum TARC concentration did not significantly decrease in children who were allergic at both the 1st and 5th grades or only at the 1st or 5th grade (Fig. 2), and the almost

same results were obtained when we studied about each allergic disease.

According to total IgE concentration in serum, serum TARC concentration showed no significant decrease in the group with high total IgE only at the 5th grade (23 children). Serum TARC concentration significantly decreased, however, in the groups with high total IgE at the 1st and 5th grades (27 children) and low total IgE at the 1st and 5th grades (91 children) (Fig. 3-a). There were only 5 subjects whose total IgE were high at the 1st grade and low at the 5th grade, and they were thus excluded from comparison.

According to mite IgE concentration in serum, serum TARC concentration did not significantly decreased in the group of high mite IgE at both the 1st and 5th grades (47

children). Serum TARC concentration was significantly decreased, however, in the group of low mite IgE at both the 1st and 5th grades (75 children) and the high mite IgE group only at the 5th grade (22 children) (Fig. 3-b). There were only 2 subjects whose mite IgE were high at the 1st grade and low at the 5th grade, and they were thus excluded from comparison.

The above groups were further examined according to the presence of allergic symptoms. In children with high total IgE at both the 1st and 5th grades, TARC concentration in serum showed no significant decrease either in the asymptomatic group, the group of symptomatic relief and onset, or the persistently

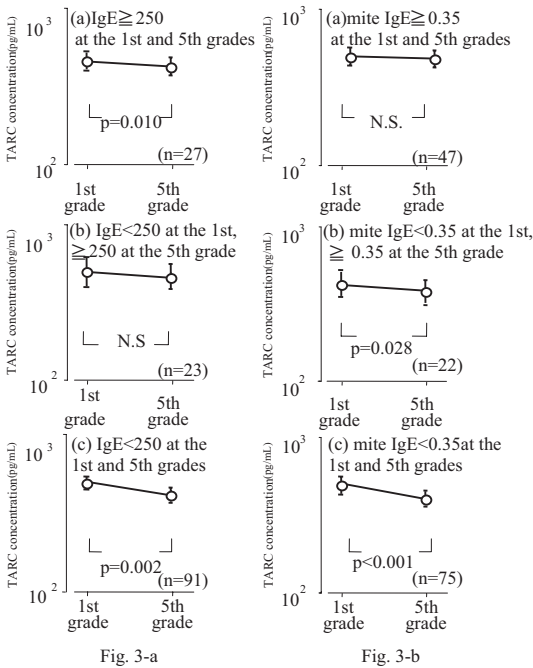


Fig. 3-a Comparison of changes in serum TARC concentration according to total IgE concentration (IU/mL).

3-b Comparison of changes in serum TARC concentration according to mite IgE concentration (U_A/mL).

IgE; Immunoglobulin E
N.S.; not significant

Table 4-a Changes in serum TARC concentration in children with high serum total IgE at the 1st and 5th grade

allergic symptoms	n	1st grade		5th grade		p value
		GM	95%CI	GM	95%CI	
none	7	455.5	358.1-579.5	405.0	313.4-523.5	N.S.
both or either grades	20	492.5	409.4-592.4	449.1	383.2-526.4	N.S.

The group of children with total IgE concentration of ≥ 250 IU/ml at both the 1st and 5th grades is divided into subgroups of children who were not allergic at the either grade and children who were allergic at the 1st and/or 5th grades for comparison of TARC changes.

Table 4-b Changes in serum TARC concentration in children with high serum mite IgE at the 5th grade

allergic symptoms	n	1st grade		5th grade		p value
		GM	95%CI	GM	95%CI	
none	4	406.1	269.6-611.6	343.2	242.1-486.6	0.039
both or either grades	13	425.4	325.5-556.0	397.1	318.4-495.2	N.S.

GM; geometric mean
CI; confidence interval

The group of children with serum mite IgE of ≥ 0.35 U_A/mL at the 5th grade is divided into subgroups of children who were not allergic at the either grade and children who were allergic at the 1st and/or 5th grades for comparison of TARC changes.

symptomatic group (Table 4-a). In children with high mite IgE only at the 5th grade, serum TARC concentration significantly decreased in the asymptomatic group, with no significant decrease in the group of symptomatic relief and onset or the persistently symptomatic group (Table 4-b).

In the cross-sectional investigation of serum TARC concentration at the 1st and 5th grade, there was no difference in the decrease of TARC concentration in serum according to the presence of allergic symptoms. As for individual allergic symptoms, serum TARC concentration was significantly higher in 5th grade children with atopic dermatitis compared with non-allergic children or children with other allergic

Table 5 Serum TARC concentration at the 1st and 5th grades

		TARC concentration (pg/mL)		
		n	GM	95%CI
1st grade	not allergic	95	500.3	452.8-552.7
	all allergic students	51	469.4	405.1-543.8
	asthma	5	463.0	414.3-517.4
	allergic without asthma	46	470.1	399.4-553.3
	wheeze	15	420.6	318.2-555.9
	allergic without wheeze	36	491.3	413.1-584.4
	rhinitis	36	451.1	381.0-534.2
	allergic without rhinitis	15	516.2	383.6-694.6
	A.D.	11	546.7	416.3-718.0
	allergic without A.D.	40	450.1	379.2-534.2
5th grade	not allergic	96	432.4	391.9-477.1
	all allergic students	50	408.7	355.4-470.1
	asthma	6	447.7	342.9-584.4
	allergic without asthma	44	403.7	345.7-471.4
	wheeze	5	567.4	339.0-949.7
	allergic without wheeze	45	394.1	341.7-454.5
	rhinitis	46	389.5	341.3-444.5
	allergic without rhinitis	4	711.3	338.1-1496.4
	A.D.	8	604.9*	361.3-1012.9 [#]
	allergic without A.D.	42	379.3	334.4-430.3

A.D.; atopic dermatitis

GM; geometric mean

CI; confidence interval

[#]p = 0.048 compared allergic without A. D.

*p = 0.007 compared with not allergic

symptoms. There was no difference in serum TARC concentration in terms of other allergic symptoms (Table 5).

IV. Discussion

The prevalence of allergic diseases such as bronchial asthma and allergic rhinitis is increasing in recent years[1,2]. In Sweden, the number of pediatric allergic patients has approximately doubled during the last 12 years [12]. Such increase in the number of allergic children is of global concern. Various research institutions and other organizations conducted other epidemiological studies, many of which were cross-sectional targeting single regions and populations[13,14]. Few follow-up investigations of the same individuals are available except for a study of Kulig et al.[15]. In the present study, we investigated the same individuals twice with a 4-year interval for allergic symptoms and determined serum concentrations of total IgE, mite IgE and the allergy-related chemokine TARC. There is no other study where any chemokine was determined at multiple time points with intervals of several years during the later childhood where allergic symptoms are apt to change.

TARC is a CC chemokine. CC chemokines chemically induce eosinophils, monocytes and T lymphocytes in asthmatic patients and are thus indicated to play important roles in the pathogenesis of bronchial asthma[16,17]. In addition, the preferential expression of CC chemokine receptors in Th2 cells gathers attention of researchers for the relationship with allergic disorders[5,6]. Serum TARC is thus expected to be a novel potential marker for allergic disorders.

It is reported that the overexpression of Th2 cells leads to the occurrence of allergic disorders, and imbalance of Th1 and Th2 cells plays a partial role[1,18-20]. Th2 cells are predominant

in newborns[1] because antenatal predominance of Th2 cells is preferable for pregnancy[21,22]. It has been indicated that, in individuals without hereditary atopic predisposition and people with predominant presence of Th1 cells due to such reasons as infection, Th2 cell activity subsides and allergic diseases are less likely to occur[23,24]. A research article describes that children have the same balance of Th1 and Th2 cells as adults within 5 years after birth[25]. It has also been reported that imbalance of Th1 and Th2 cells in infants will specify the onset of allergic symptoms[26].

In the present study in school children that examined changes in serum TARC concentration between 6 and 11 years of age, the activity of Th2 chemokine decreased with age. This finding suggests that TARC may induce changes in Th2 cell activity even during the later childhood. In addition, Th2 chemokines such as TARC might be a cause of age-related changes in allergic symptoms in school children. Specifically, in children with persistent allergic symptoms, high TARC activity continued even at the 5th grade, suggesting that TARC may play a role in the onset and persistence of allergic symptoms. It is necessary to further study other cytokines and chemokines that are associated with allergic diseases.

We also investigated the relationships of serum TARC concentration with total IgE and mite IgE concentrations in serum. Decrease of serum TARC concentration depended not only on serum IgE concentration but also on the presence of allergic symptoms. In the group of high serum concentration of total IgE at both the 1st and 5th grades, there was no significant difference in the decrease of serum TARC concentration in asymptomatic children. This was probably due to the small number of such subjects. Generally, there is a strong association between serum IgE concentration and allergic diseases[27], but serum IgE is not enough to

explain some allergic disorders[28]. Chemokines such as TARC regulate IgE production by activating Th2 cells via B cells in allergic diseases[18]. It is considered from the results of the present study that TARC may be involved in the occurrence of allergic symptoms while this chemokine directly affected IgE production.

In this cross-sectional investigation, there was no difference in serum TARC concentration other than the difference according to the presence of allergic symptoms at either the 1st or 5th grade. This was probably due to the involvement of various cytokines and chemokines in addition to TARC in the onset of allergy. In children with atopic dermatitis at the 5th grade, serum TARC concentration was significantly higher than in non-allergic children, and the concentration was also higher compared with children with other allergic diseases. It has been reported that TARC concentration in serum differs according to the severity of atopic dermatitis with higher serum TARC concentration in severer cases[5], showing that the serum concentration of TARC may be higher even in milder cases of atopic dermatitis compared with other allergic diseases. Subjects of the present study, even allergic children, can perform the normal activities of daily living, and this may explain the non-significant difference in children with allergic symptoms except for atopic dermatitis in contrast to a previous report[6]. It is necessary to continue this type of follow-up investigation in a wider range and increased number of subjects.

In conclusion, we determined serum concentrations of total IgE, mite IgE and TARC and investigated their time profiles in primary school children in the present study. It is considered from the results of the present study that changes in serum TARC concentration may alter the balance of Th1 and Th2 cells even during the later childhood. Changes in serum TARC concentration differed according to the

presence of allergic symptoms, suggesting that there may be a close relationship between TARC and allergic symptoms. It is also considered that serum TARC concentration had strong relation to especially atopic dermatitis.

Th2 cell activation by chemokines other than TARC and cytokines should be further examined in future research.

要 旨

【目的】 Thymus and activation-regulated chemokine (TARC) はアレルギー疾患に関与するTh2関連ケモカインの一つである。学童期におけるアレルギー症状と血清TARC濃度の成長に伴う変化を調査し、Th2関連因子がどのようにアレルギー症状に関連しているかを検討した。

【方法】 日本の地方都市に在住する学童146人を対象として、小学校1年生時と5年生時の2回、呼吸器及びアレルギー症状に関する質問紙調査と共に、採血を行って血清総IgE、ダニ特異IgE及びTARCの血清中濃度を測定した。

【結果】 対象児童全体において血清TARC濃度は1年時(489.2pg/ml)に比べ、5年時(424.1pg/ml)には、有意に低下していた。いずれの学年でもアレルギー症状がなかった児童では血清TARC濃度は1年時(521.4pg/ml)に比べ、5年時(410.7pg/ml)で有意に低下していたが、いずれかの学年または両学年でアレルギー症状があった児童では血清TARC濃度の低下が見られなかった。総IgE値及び、ダニ特異IgE値によっても血清TARC濃度の低下の差が認められたが、これらに加えアレルギー症状の有無が更に血清TARC濃度低下に影響していた。

【結論】 小児の血清TARC濃度は成長に伴い低下することから、Th2細胞の活性は年齢と共に低下する可能性が示唆された。アレルギー症状の無い児童ではTARC濃度の低下が有意であったのに対し、アレルギー症状のある児童では有意な低下は見られず、TARCがアレルギー症状の発現に関与しているものと考えられた。

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