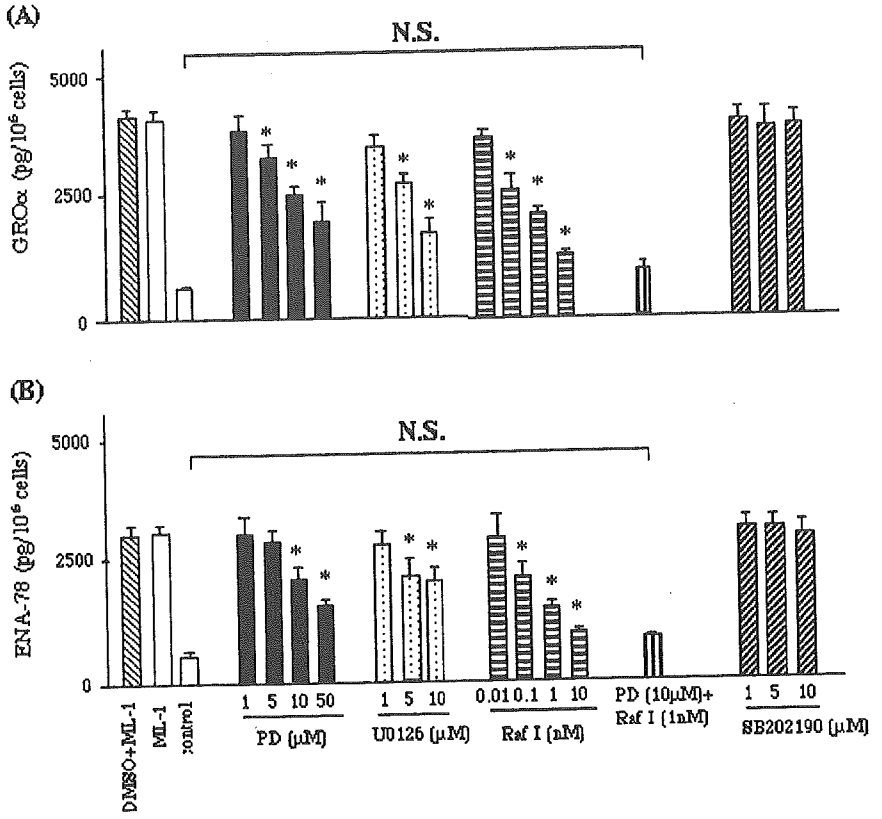

Fig. 4. Effect of PD98059, U0126, and Raf1 kinase inhibitor I on GROα and ENA-78 expression in NHBEs. NHBEs were preincubated with 1 to 50 μM PD98059 (PD), 1 to 10 μM U0126, 0.01 to 10 nM of Raf1 kinase inhibitor I (Raf I), 1 to 10 μM SB202190, or a combination of 10 μM PD and 1 nM Raf I for 1 h, followed by stimulation with 100 ng/ml ML-1 for 24 h. The results are expressed as the mean ± S.D. (n = 4 experiments). *, $p < 0.05$ was considered significant versus ML-1-stimulated cells.

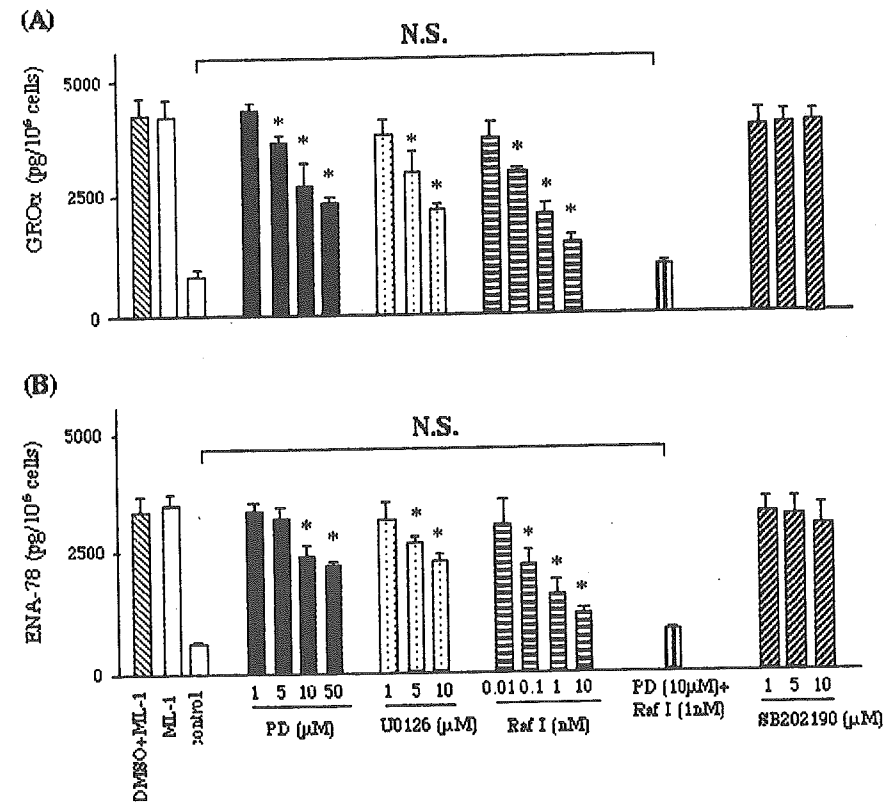

Fig. 5. Effect of PD98059, U0126, and Raf1 kinase inhibitor I on GROα and ENA-78 expression in HUVECs. The cells were preincubated with various inhibitors same as described in Fig. 4 legend, followed by stimulation with 100 ng/ml ML-1 for 24 h. The results are expressed as the mean ± S.D. (n = 4 experiments). *, $p < 0.05$ was considered significant versus ML-1-stimulated cells.

ited the production of these two chemokines in NHBEs and HUVECs.

We also investigated whether other signaling molecules, such as PKC and PI3K, are involved in upstream signaling pathway of the C-X-C chemokine expression. The results showed that no significant inhibitory effect on ML-1-induced

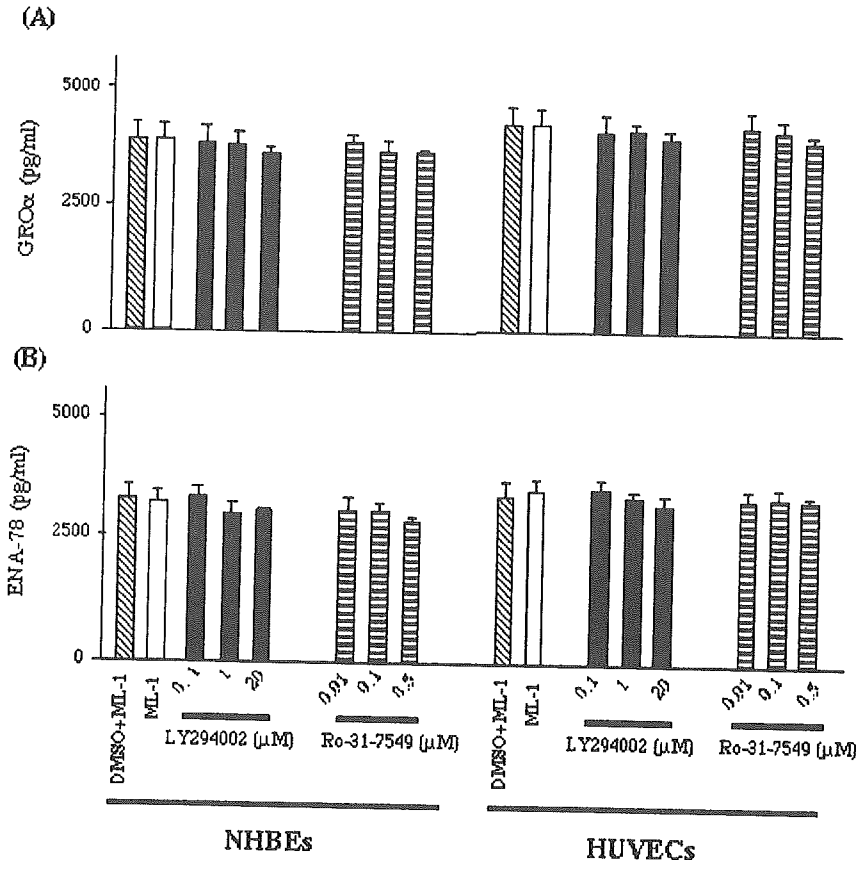

Fig. 6. Effect of Ro-31-7549 and LY294002 on GROα and ENA-78 expression. NHBEs and HUVECs were preincubated with 0.01 to 0.5 μM Ro-31-7549 and 0.1 to 20 μM LY294002 for 1 h, followed by stimulation with 100 ng/ml ML-1 for 24 h. The results are expressed as the mean ± S.D. ($n = 4$). *, $p < 0.05$ was considered significant versus ML-1-stimulated cells.

chemokine expression in NHBEs and HUVECs was found when a selective PKC inhibitor, Ro-31-7549 (0.01–0.5 μM), or a PI3K inhibitor, LY294002 (0.1–20 μM), was used (Fig. 6, A and B, respectively).

Discussion

GROα has been shown to be a potent neutrophil chemoattractant and activator in vitro, and this chemotactic activity is equivalent to that of IL-8 (Balentien et al., 1990). Similarly, ENA-78 is as equally potent as IL-8 in inducing neutrophil chemotaxis; however, it is consistently less active in inducing the release of granules from neutrophils (Walz et al., 1991). Besides eosinophils, it is reported that neutrophils are also involved in the features of bronchial asthma, airway hyper-reactivity, airway hypersecretion, and airway wall remodeling (Molet et al., 2001). In addition, pulmonary neutrophilia has also been found in severe asthmatic airways, and at sites of allergen challenge in asthmatic subjects (Ordonez et al., 2000). Several inflammatory stimuli such as tumor necrosis factor α, lipopolysaccharide, diesel exhaust exposure, and respiratory syncytial virus infection can induce GROα and/or ENA-78 (Lukacs et al., 1995; Matsukawa et al., 1999; Salvi et al., 2000; Nasu et al., 2001; Zhang et al., 2001). The expression of GROα and ENA-78 has been found in several inflammatory models and diseases (Luster, 1998), strongly implicating its role in the pathogenesis of inflammation.

ML-1 is derived from activated $CD4^+$ T cells, basophils, and mast cells, which are important regulatory cells for the inflammation (Kawaguchi et al., 2001). It is thus a strong possibility that ML-1-induced GROα and ENA-78 are involved in neutrophilic inflammation. It is of interest to note that ML-1 induces C-X-C chemokines, but not C-C chemokines, such as eotaxin and regulated on activation normal T cell expressed and secreted, which are potent chemoattractants for eosinophil (data not shown), suggesting a selective role of ML-1 in neutrophil recruitment and activation. As a corollary, a recent study has suggested an in vivo role of human IL17F in recruiting neutrophils into the pulmonary mucosa in mice after adenoviral gene transfer (Hurst et al., 2002), further suggesting a potential role of ML-1 in the pathogenesis of neutrophilic inflammation.

Raf-1 is a MAP kinase kinase kinase (MAP3K), which functions downstream of the Ras family of membrane associated GTPases, and is able to activate the dual specificity protein kinases MEK1 and MEK2, which in turn activate the serine/threonine-specific protein kinases ERK1 and ERK2 (English and Cobb, 2002). Our previous and current data demonstrated that ML-1-induced IL-8, GROα, and ENA-78 production is dependent on the activation of ERK1/2, but not p38 and JNK in the MAPK signaling pathway (Kawaguchi et al., 2002). ERK1/2 is known to be involved in the regulation of cell proliferation and apoptosis. In this study, the inhibitors used did not affect both the cell number and viability (data not shown), suggesting an effect on gene and protein expression.

Finally, we used inhibitors for other signal molecules, such as PKC and PI3K, because several studies have demonstrated both PKC and PI3K are linked to MAP kinase pathway. PKC is a key activator of the Raf1/MAP kinase cascade

at multiple steps. It is known that PKC can regulate Raf1 signaling through phosphorylation of Raf kinase inhibitory protein (Corbit et al., 2003) and also phosphorylates Raf1 at serine 499 (Kolch et al., 1993). On the other hand, Ras is likely to act through additional proteins besides Raf1. PI3K is a candidate Ras effector (Rodriguez-Viciana et al., 1997). Activation of PI3K by a variety of extracellular stimuli leads to the accumulation of the second messenger phosphatidylinositol 3,4,5-trisphosphate. Its final target is the serine/threonine kinase Akt/PKB. Activated Ras promotes cell survival in epithelial cell through activation of PI3K and Akt/PKB because at high dose, 20 μM, LY294002 induces apoptosis (Khwaja et al., 1997). In our study, however, LY294002 did not show any effect on the cell number and viability (data not shown). This is likely due to different stimuli and cell types used.

To date little is known about the upstream signaling pathway of GROα and ENA-78 expression. However, the results of current study suggest that Raf is predominantly associated with the activation of MEK-ERK1/2 pathway. Therefore, we concluded that the Raf-MEK-ERK1/2 pathway is a central upstream pathway of ML-1 induced GROα and ENA-78 expression in NHBEs and HUVECs. In fact, MAP kinases are important molecules in the airway epithelial activation in response to various stimuli such as tumor necrosis factor-α, IL-1, diesel exhaust particles, and influenza virus infection (Griego et al., 2000; Hashimoto et al., 2000a,b; Reibman et al., 2000). Also, MAP kinases, including ERK1/2, are involved in cytokine signaling in HUVECs (May et al., 1998; Goebeler et al., 1999; Surapisitchat et al., 2001). On the other hand, the downstream signaling pathway is currently unclear. IL-17 is known to activate transcription factor nuclear factor-κB in chondrocytes and intestinal epithelial cells (Shalo-Barak et al., 1998; Awane et al., 1999). Because of high homology between IL-17 and ML-1, it is possible that ML-1 is able to activate nuclear factor-κB in the downstream signaling pathway.

It is noted that a delay between the synthesis and release of both GROα and ENA-78 chemokines was observed. For example, the level of ML-1-induced GROα in cell lysate was noted at the 6-h time point, whereas significant increase of GROα in the supernatants was seen at 24 h after stimulation. The significance of this delay is at present unclear. It is noted, however, that a trend of increase for GROα secretion is seen at the 12-h time point, although it did not reach statistical significance. Additional time intervals between the 12- and 24-h time points will be needed to identify its release kinetics. Of interest, we have also found previously a similar "delay" phenomenon for ML-1-induced IL-6 secretion (Kawaguchi et al., 2002). The delay in protein expression may be as a result of the required time frame for protein modification and/or transport, or alternatively, but not mutually exclusively, a faster synthesis/secretion kinetic requires an additional factor induced by ML-1. It is also noted that chemokine gene expression is induced by ML-1 at the 2-h time point, suggesting a direct effect of ML-1 on de novo synthesis of transcripts. However, until a ML-1-inducible factor, if it exists, is found, a possible secondary (or perhaps additive) effect of ML-1 on the induction of chemokine gene and protein expression cannot be ruled out in the current study.

In conclusion, this study reports that ML-1 induces C-X-C chemokines GROα and ENA-78 via the activation of the Raf1-MEK-ERK1/2 pathway. These results suggest a potential role of ML-1 in the pathogenesis of the airway inflammatory diseases, such as chronic obstructive pulmonary disease, bronchial asthma, and bacterial pneumonia, and the Raf1-MEK-ERK1/2 pathway is a potential pharmacotherapeutical target for inhibition of ML-1-induced neutrophil recruitment and activation in the airway inflammatory diseases.

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Linkage and Association Studies of STAT6 Gene Polymorphisms and Allergic Diseases

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Key Words
Allergic disease · Atopy · IL-4 · IL-13 · STAT6 · G2964A variant · Dinucleotide repeat · Polymorphism

Abstract
Background: Signal transducer and activator of transcription 6 (STAT6) is a key transcription factor involved in both interleukin-4 (IL-4) and IL-13-mediated biological responses, such as allergies. Recently, we reported that the polymorphism of the STAT6 gene exon 1 was associated with allergic diseases, while another group studied the G2964A variant of the STAT6 gene's association with atopic asthma. We undertook an association study between these variants of the STAT6 gene and allergic diseases, including atopic dermatitis, bronchial asthma, and food-related anaphylaxis in a Japanese population. ***Methods:*** STAT6 gene polymorphisms were genotyped by polymerase chain reaction (PCR) fragment length polymorphism analysis, and PCR-SSCP analysis in 106 allergic and 66 control subjects. ***Results:*** The 2964A variant was in significant linkage disequilibrium with the dinucleotide repeat polymorphism, the 13-GT repeat allele of STAT6 exon 1 ($p < 0.0000000003$). There was no association between the STAT6 2964A variant and allergic subjects in a Japanese population ($p = 0.2724$). The genotype of 13/15-GT repeat allele heterozygosity was significantly associated with allergic subjects ($p = 0.0006$), as previously reported. In one major genotype of the STAT6 exon 1 (15 GT repeat homozygosity), wild-type 2964G allele homozygosity was significantly associated with allergic subjects ($p = 0.0382$). ***Conclusions:*** Our findings indicate that in combination the dinucleotide repeat polymorphism of the STAT6 exon 1 gene and the 2964A variant may be useful markers for predicting allergic diseases in a Japanese population.

Introduction

Allergic diseases may be based on an inflammatory mechanism involving T-helper type 2 (Th2) cytokines, such as IL-4 and IL-13, through their receptors [1, 2]. Upon release of IL-4 or IL-13, binding of the cytokine to its receptor at the cell surface induces receptor dimeriza-

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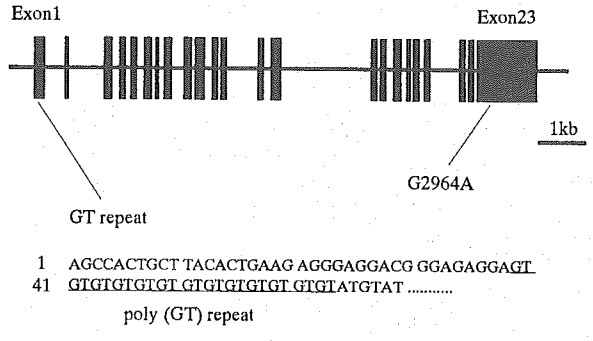

Fig. 1. Exon-intron organization of the human STAT6 gene. Solid black boxes represent exons and horizontal lines indicate introns. The polymorphisms are shown. The nucleotide sequences of exon 1 of the human STAT6 gene and the GT repeat polymorphisms are shown. The number of (GT)n repeats was determined by a DNA sequencer.

tion and activation of the Janus tyrosine kinase (JAK) 3 and the transcription factor STAT6 [3–5]. Tyrosine phosphorylation of signal transducer and activator of transcription 6 (STAT6) mediates homodimerization, triggering movement to the nucleus, and leading to its specific binding to promoter sequences in the DNA [6, 7]. STAT6 DNA-binding sites have been identified in the promoter regions of several IL-4-inducible genes. This can result in transactivation of the target genes, which have central roles in IgE synthesis and the development of bronchial hyperresponsiveness [2, 8–10].

Th2 cytokines, such as IL-4 or IL-13, have been suggested as candidate genes for atopy, and several groups have studied the association of these genetic variants with atopy/allergic diseases in western or Japanese populations [11–18]. Recently, we reported the association between the dinucleotide repeat polymorphism of the STAT6 exon 1 and allergic subjects in a Japanese population [19]. Another group showed that the G2964A variant of the STAT6 gene was associated with mild atopic asthma, also in a Japanese population [18]. Since the study of genetic association is difficult, and can be confounded by small sample size, sample selection, and genetic admixture, it would be worth examining the association of the polymorphism to other phenotypes in allergic diseases. Therefore, we performed an association study between STAT6 gene polymorphisms and allergic subjects, including those with atopic dermatitis (AD), bronchial asthma (BA) and food-related anaphylaxis (FA).

Material and Methods

Allergic and Control Subjects

Japanese children (n = 102) with a major allergic disease including AD, BA, and/or FA, were studied. Among these allergic subjects, 71 children had AD, 47 had BA, and 14 had FA. A physical examination of each subject was carried out by a pediatrician. The serum IgE levels were measured by enzyme-linked immunosorbent assays in almost all subjects.

The diagnosis of AD was based on the appearance of an active skin disease, the distribution of skin lesions, and the clinical course of the disease according to the diagnostic criteria of Hanifin and Rajka [20]. BA was diagnosed according to the criteria of the National Institutes of Health of the United States [21] with minor modifications. Briefly, the diagnosis of BA was based on the appearance of the two following characteristics: two or more episodes of wheezing and shortness of breath during the past year, and reversibility of the wheezing and dyspnea, either spontaneously or after treatment with a bronchodilator. Since wheezing is often associated with viral respiratory infections in young children, subjects more than 3 years old were evaluated for the asthma phenotype [15]. The diagnosis of FA was based on recognition of typical manifestations and the patient's history, with regard to the reproducibility of the reaction, the timing of the reaction, and response to elimination of particular foods from the diet. All FA patients had a positive RAST response to food to which they were susceptible.

Control subjects were 66 individuals with no history of major atopic disease. Informed consent was obtained from all subjects or their parents, and the study was approved by the Committee of Ethics at the Department of Pediatrics, University of Gunma.

Molecular Methods

DNA was extracted from peripheral blood leukocytes. Genotyping of the dinucleotide repeat polymorphism of the first exon of the STAT6 gene was done as previously described [19]. Genotyping of the G2964A variant of the STAT6 gene was done by PCR-SSCP analysis. All PCR primer sets were designed to amplify the fragment containing each of the polymorphisms. The polymorphisms are shown in figure 1.

PCR Primer Sequences

Primers for STAT6 exon 1
Forward: 5′-GAGGGACCTGGGTAGAAGGA-3′
Reverse: 5′-CACCCCCATGCACTCATAG-3′
Primers for STAT6 G2964A variant
Forward: 5′-GGAGCCAATCCACTCCTTCC-3′
Reverse: 5′-CAGACTCCTCCTATGCTCCC-3′

PCR Conditions

PCR were performed in a volume of 25 μl containing 50 ng of genomic DNA, 125 μmol of each dNTP, 2 U of *Taq* polymerase, *Taq* buffer, and 10 pmol of forward and reverse primer. For STAT6 exon 1, 6-FAM-labeled forward primer was used. Cycle conditions were 95 °C for 5 min, and then 40 cycles at 95 °C for 30 s, at 66 °C (STAT6 exon 1) for 30 s or at 60 °C (G2964A variant), and at 72 °C for 30 s, with a final extension step of 7 min at 72 °C, in a GeneAmp 2400 thermocycler (Perkin Elmer, Calif., USA). After PCR, 1 μl of the products plus 0.5 μl of Genescan 400HD molecular weight standard (Applied Biosystems, Calif., USA) were denatured in 12 μl of formamide, separated in an Applied Biosystems Prism Genetic Analyz-

er (ABI PRISM™310) with POP6 polymer, and the fragment lengths determined.

PCR-SSCP Analysis

The amplified products of the G2964A variant were fractionated by electrophoresis on polyacrylamide gels, GeneGel Clean 15/24 (Amersham Pharmacia Biotech, Uppsala, Sweden), at 20°C for 2 h. The gels were visualized by silver staining. Samples from two known homozygotic individuals and one heterozygotic individual as confirmed by sequencing were included in each reaction. Sequencing was performed using an automated sequencer (ABI PRISM™310).

Statistical Analysis

The data were analyzed by Fisher's direct method. Significant values were taken based on the numbers of tests.

Results

The distribution of the G2964A variant of the STAT6 gene is shown in table 1. There was no association between allergic subjects and this variant (p = 0.2724), nor was any association seen with any allergic subgroups in a Japanese population. There was no significant difference in the levels of total serum IgE in allergic individuals, comparing the 2964A variant and wild-type groups, as shown in figure 2. We examined the association with the dinucleotide repeat polymorphism of STAT6 exon 1, and then polymorphic PCR products were classified into four alleles (fig. 1): 13-, 14-, 15- and 16-GT repeat alleles. There was a significant linkage disequilibrium between the 2964A variant and the 13-GT repeat allele of the STAT6 exon 1 (p = 0.0000000003) (table 2). The distribution of the GT repeat polymorphism of the STAT6 exon 1 in a Japanese population is shown in table 3. The 13/15-

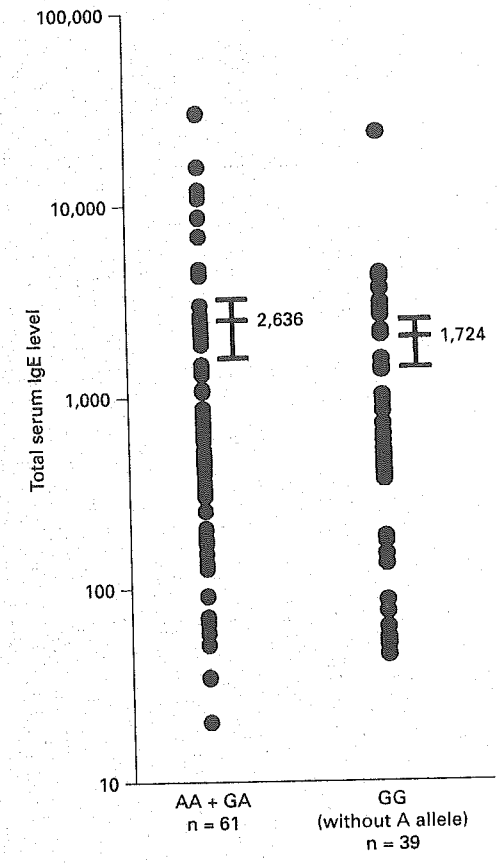

Fig. 2. Comparison of total IgE levels between the group of allergic subjects that carried an 2964A allele and the group without 2964A allele. The level of total IgE is plotted. Values are means ± SE, n = 61 and n = 39.

Table 1. Association between 2964G/A variant of STAT6 gene and allergic subjects in a Japanese population

Group	No.	STAT6 2964G/A		
		AA+GA	GG (without A allele)	
Control subjects	66	44	22	
Allergic subjects	102	62	40	0.2724
Atopic dermatitis	47	34	13	0.3327
Bronchial asthma	73	41	32	0.1369
Food-related anaphylaxis	14	10	4	0.4974

Significance was taken at the level of $p < 0.0125$, giving an overall significant level for the four tests of 0.05. The G allele corresponds to 2964G and the A allele corresponds to 2964A.

GG denotes homozygosity for the wild-type allele, GA heterozygosity, and AA homozygosity for the mutant allele.

Table 2. Linkage disequilibrium between STAT6 2964G/A and STAT6 exon 1 (GT)n polymorphisms

Genotype of the STAT6 exon 1	STAT6 2964G/A	
	AA+GA	GG (without A allele)
With 13-GT repeat allele	65	8
Without 13-GT repeat allele	41	54

p = 0.0000000003. The G allele corresponds to 2964G and A allele corresponds to 2964A.

GG denotes homozygosity for the wild-type allele, GA heterozygosity, and AA homozygosity for the mutant allele.

Table 4. Association between 2964G/A variant of STAT6 gene and allergic subjects in 15-GT repeat homozygosity of the STAT6 exon 1 in a Japanese population

Group	n	STAT6 2964G/A	
		GG (without A allele)	AA+GA
Allergic subjects	29	21	8
Control subjects	32	15	17

p = 0.0382. The G allele corresponds to 2964G and A allele corresponds to 2964A.

GG denotes homozygosity for the wild-type allele, GA heterozygosity, and AA homozygosity for the mutant allele.

Table 3. Frequencies of the GT dinucleotide repeat polymorphisms in Japanese populations with allergic disease and controls

STAT6 exon 1 genotype	n	Allergic subjects	Control subjects	p value[1]
13GT/13GT	10	6 (0.0588)	4 (0.0606)	0.6545
/15GT	53	42 (0.4118)	11 (0.1667)	0.0006
/16GT	10	5 (0.0490)	5 (0.0758)	0.3455
14GT/15GT	3	2 (0.0196)	1 (0.0152)	0.6592
15GT/15GT	61	29 (0.2843)	32 (0.4848)	0.0068
/16GT	27	16 (0.1569)	11 (0.1667)	0.6525
16GT/16GT	4	2 (0.0196)	2 (0.0303)	0.5134
		n = 102	n = 66	

[1] Significance value was taken at the level of $p < 0.007$, giving an overall significant level for the seven tests of 0.05.

GT repeat allele heterozygosity was significantly associated with allergic subjects (p = 0.0006).

In one major genotype of the GT repeat polymorphism (15-GT repeat allele homozygosity), the 2964G wild-type allele homozygosity was significantly associated with allergic subjects (odds ratio = 2.98, 95% confidence interval 1.02–8.68, p = 0.0382) (table 4).

Discussion

We showed a strong linkage disequilibrium between the 2964A variant allele and the 13-GT repeat allele of the STAT6 gene. Both the genotype of the 13/15-GT repeat allele heterozygosity, and the genotype of the 15-GT repeat allele homozygosity without the 2964A variant were significantly associated with allergic subjects in our Japanese population.

STAT6 is a key transcription factor involved in IL-4- and IL-13-mediated biological responses. The human STAT6 gene, approximately 19 kb in length, is located at 12q13.3-q14.1 and consists of 23 exons interrupted by 22 introns. The AUG translation initiation codon encoding the first amino acid is located within exon 3 [22]. Genome-wide linkage studies have shown several candidate loci linked to atopy and asthma [23]. Strong evidence for linkage of atopic asthma to chromosomal region 12q13-24 was found in ethnically diverse populations [24–26]. A number of candidate genes for the atopic and asthmatic phenotypes have been mapped to this chromosomal re-

gion, including interferon-γ and STAT6 [22]. Association studies between the polymorphism of the STAT6 gene and atopy/allergic diseases have recently been reported [18, 19]. In the present study, there was a significant association between the 13/15-GT repeat allele heterozygosity and allergic subjects, confirming our previous finding [19]. Gao et al. [18] studied G2964A polymorphism in Japanese asthmatics and found an association with mild atopic asthma. We tried to extend the association study to confirm the role of this variant in the development of any allergic diseases, including atopic/nonatopic BA, AD and FA. However, in the present study, we could not confirm a significant association between the 2964A variant and any other allergic subjects. It remains a possibility that the allergic phenotypes in our study are different from the phenotypes reported by Gao et al. [18]. Furthermore, we could not undertake an association study between the 2964A variant and mild asthma, since the sample size was too small to classify the severity of asthma. However, our results might be partly confirmed by the recent study of Duetsch et al. [27] which showed no linkage/association to asthma in a Caucasian sib-pair study.

In the association study with the GT repeat polymorphism of the STAT6 exon 1, we showed significant linkage disequilibrium between the 2964A variant and the genotype with the 13-GT repeat allele of the STAT6 exon 1. Moreover, in one major genotype of the GT repeat polymorphism (15-GT repeat allele homozygosity), we found a significant association between the 2964G wild-type allele homozygosity and allergic subjects. The two genotypes, the 13/15-GT repeat heterozygosity and the 15GT repeat homozygosity without the 2964A variant, were found in more than 60% of allergic subjects in this study (63/102), retrospectively.

Our results indicate that a combination of STAT6 gene polymorphisms may be a useful marker for predicting allergic diseases in a Japanese population. However, the sample size of this study was relatively small, so there is a need to extend the sample size in this association study to confirm the role of these variants in the development of each of the allergic diseases investigated. We intend to expand epidemiological or genetic studies on the signal transducer. Furthermore, to elucidate the role of the STAT6 gene in the development of allergic diseases, functional analyses of STAT6 may be needed.

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1歳児のアレルギー疾患の発症に関与する因子に関する検討

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key words：アレルギー性疾患の発症，母体の感染，母親のアレルギー，家族歴

要 旨

1歳児のアレルギー性疾患の発症に影響する要因を解明するため，問診票により母親の妊娠分娩歴，栄養，予防接種歴，ペット，喫煙環境，さらに児の罹患歴を調査した．母親の妊娠中の感染症状の中では発熱，咳，咽頭炎を有する場合アトピー性皮膚炎または喘息（喘鳴），喘息（喘鳴），アトピー性皮膚炎がそれぞれ有意に多く発症した．さらに，母体の感染頻度が高いほどアレルギー疾患の発症が増加した．妊娠中の母体のアレルギー症状数が多い程，児のアレルギー発症率は高くなった．生後，母親がアレルギーをもつ場合，児が高率にアレルギーを発症し，それは母親が多数のアレルギー症状を持つ場合著しかった．家族のうちアレルギーを有する人数と児のアレルギー発症率は相関した．予防接種に関しては三種混合の接種回数が少ないほどアレルギーが発症しやすく，BCG非接種群では接種群に比べてアレルギーの発症が有意に多かった．以上より，出生前後の母親の感染症状・アレルギー症状や児の予防接種のほか，遺伝的要因が児のアレルギー発症に関与することが示唆された．

はじめに

近年認められるアレルギー疾患の増加の背景には生活様式の変化やhygiene hypothesis[1]により提唱されている感染症の減少などがあることが示唆されている．一方でアレルギー発症に関与するアレルゲン特異的Tリンパ球は出生前からすでに認められる[2]ことから，出生前の母体の状態も児のアレルギー発症に影響を与えることが予想される．これらのことから，本研究では出生前後の病歴，環境要因についてアンケート調査を行ない，1歳児のアレルギー発症との関連について検討を行った．

対象及び方法

対象は北里大学小児科，国立療養所南福岡病院小児科，JR仙台病院小児科，東京医科歯科大学小児科，東邦大学大森病院小児科，横浜赤十字病院小児科の6施設の小児科外来を受診した生後1歳以上，2歳未満の児207名で，家族の理解が充分に得られたものを対象とした．家族により記載された問診票により，対象の性別，妊娠分娩歴，栄養，予防接種歴，ペット，喫煙環境，さらに0歳から現在までの間に経験した症状および罹患した疾患名を確認し，それぞれの因子とアレルギー疾患の発症の関係を調査した．

児のアレルギー疾患は皮膚症状（アトピー性皮膚炎）と呼吸器症状（喘息および喘鳴）からなり，それぞれ問診票の記載に基づいて診断した．耳の下が切れる，かゆみ，かさつき，赤いぶつぶつ，の4症状のうち，耳切れを含む2項目以上陽性のものまたは医師によりアトピー性皮膚炎と診断されているものをアトピー性皮膚炎とした．また，ヒューヒューまたはゼーゼーを示すもの，または医師により喘息性気管支炎及び気管支喘息と診断されたものを喘息および喘鳴とした．母親のアレルギー疾患については気管支喘息・アトピー性皮膚炎・アレルギー性鼻炎・花粉症・アレルギー性結膜炎・蕁麻疹・食物アレルギーの有無及び罹患時の妊娠月数について質問した．母親の感染症状については下痢・発熱・鼻汁・咳・咽頭炎・肺炎・気管支炎について同様に質問した．

統計学的検討はカイ2乗検定（必要に応じてYates補正を施行）とSpearmannの相関係数を用いた．$P<0.05$で有意差ありとした．

結　果

母体感染の影響について

妊娠中の母親の感染症状の検討では以下の項目が有意であった．発熱は児のアトピー性皮膚炎及び喘息(喘鳴)を起こしやすくし（図1a），母体の咳は児の喘息および喘鳴の発症と有意に関連した（図1b）．また，母体の咽頭炎は児のアトピー性皮膚炎の発症と関連していた（図1c）．更に，下痢・発熱・鼻汁・咳・咽頭炎・肺炎・気管支炎を含めた，母体の感染頻度を検討すると，感染頻度が高いほど児のアレルギー疾患を起こしやすくすることが判った（図2）．($r=0.82299$, $P<0.01$, Spearman）しかしながら，下痢，鼻汁，肺炎・気管支炎の有無について，個々に検討するとそれぞれは単独では児のアレルギー発症に影響を与えなかった．

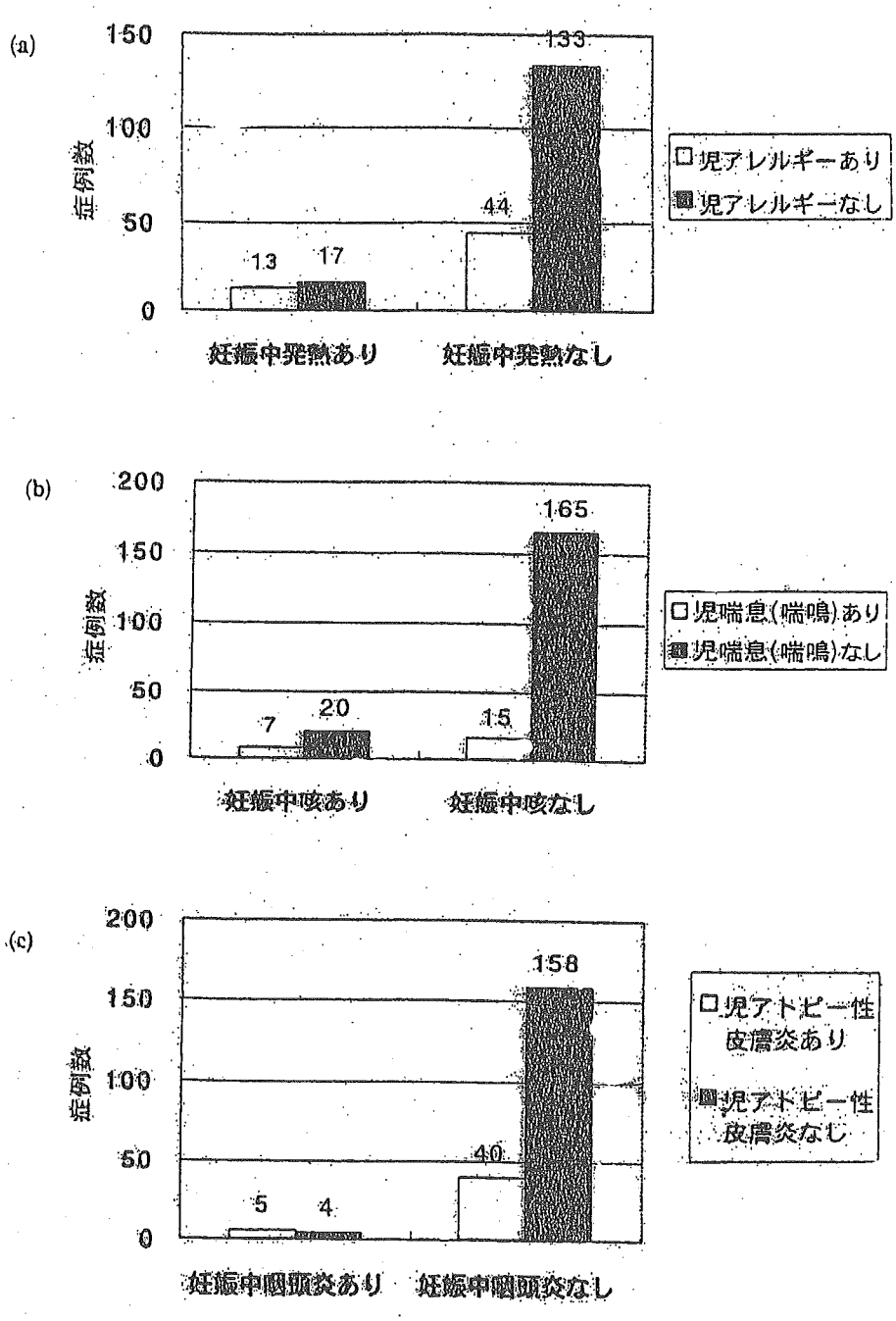


図1　妊娠中の母体感染症と児のアレルギー発症

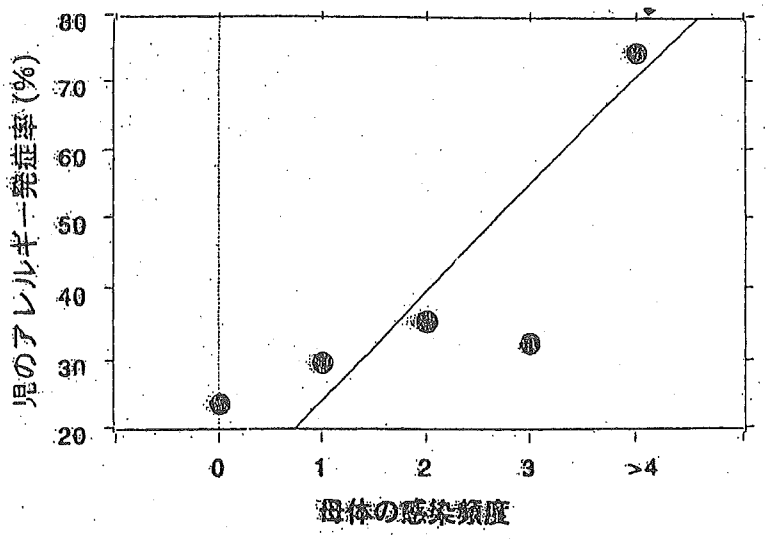


図2 母体の感染症の頻度と児のアレルギー発症の相関

妊娠中の母体のアレルギーの及ぼす影響について

母体（妊娠中）のアレルギー症状数の多い程，児のアトピー性皮膚炎と喘息(喘鳴)を合わせたアレルギー発症率は高くなり，またアトピー性皮膚炎発症率も高くなった．しかし，喘息および喘鳴の発症率には影響しなかった．(図3)

生後の母親のアレルギーの及ぼす影響について

児の出生後，母親がアレルギーをもつ場合，児が高率にアレルギーを発症する（図4)．また，母親が多数のアレルギー症状を持つ程児が高率にアレルギー（アトピー性皮膚炎，喘息および喘鳴それぞれ単独の場合，及び両者を合わせた総アレルギーの場合）を発症する（図5)

家族のアレルギーの及ぼす影響について

家族のうちアレルギーを有する人数が多い程児がアレルギー（アトピー性皮膚炎，喘息および喘鳴それぞれ単独の場合，及び両者を合わせた総アレルギーの場合）を発症しやすいことが判った（図6)

児自身の感染の及ぼす影響について

児自身の感染症状はアレルギー発症に関して明らかな影響を認めなかった．

児の栄養の及ぼす影響について

児の栄養（母乳か人工乳か）は児のアレルギー発症と明らかな関連を認めなかった．

児の予防接種歴の及ぼす影響について

三種混合の接種回数が多いほどアレルギーは発症しにくかった($p<0.01$)（図7)．接種回数とアレルギー発症率は有意に逆相関した（$rs=1$, Spearmann, $p<0.01$）アトピー性皮膚炎・喘息および喘鳴の個々の疾患についての検討では有意差がでなかった．BCG接種群では非接種群に比べてアレルギー全体・アトピー性皮膚炎・喘息および喘鳴の発症が有意に少なかった（$p<0.05$）（図8)．ポリオ・麻疹・風疹・水痘・ムンプス・インフルエンザなどの予防接種の有無は影響しなかった．二種混合・日本脳炎については接種症例数が少なく，検討できなかった．

ペットの及ぼす影響について

ペットの有無は児のアレルギー発症に有意な影響をもたらさなかった．

喫煙環境の及ぼす影響について

家族の喫煙の有無および喫煙本数は児のアレルギー発症と関連が無かった．

考　察

これまでの報告から，小児期のアレルギー疾患発症に関与するとされている因子は，栄養・環境（ダニ・ネコ・煙草など）生活様式（家の職業・経済状態など）・遺伝・乳児期の感染など多様である[3]．一方，アレルゲン特異的Tリンパ球は出生前の胎児期から既に認められ，アレルゲンに対する胎内感作の可能性も指摘されている[2]．

本研究では出生前後の母体の症状，生後の児の罹患した疾患・症状，栄養，予防接種歴，及びペット・喫煙などの環境要因とアレルギー発症との関連を分析した．本調査は病院受診者のうち，本研究に理解の得られた対象者をできる限り多く含むよう努めた．本調査は後方視的なアンケート調査であるが，ISAAC（international study of asthma and allergic diseases）の診断基準に準じた．

妊娠中の母体の下痢・発熱・鼻汁・咳嗽・咽頭炎・気管支炎・肺炎などの感染症状の頻度と児のアレルギー発症率が有意に相関し，母体の感染が胎児の免疫系に影響を与えている事が示唆された．このことは母体の発熱を認めた場合児が高率にアレルギーを発症したことと一致する．

母体の咳嗽症状があった場合児の喘息および喘鳴の発症率が高くなる原因については以下のふたつが挙げられる．即ち，母体の咳嗽を感染症状のひとつとみなせば，母体の感染による胎児の免疫系の修飾の結果，児が喘息（喘鳴）を発症したとすることもできる．しかしながら，妊娠の経過中に喘息の症状が増悪することは知られており[4]，母体自身が喘息と診断されていなくても，軽度のアレルギー素因をもつ妊婦の場合，妊娠によりもたらされるTh2優位の免疫系[5,6]が喘息様症状の発現閾値を低下させ，軽微なアレルギー症状として咳嗽が認められた可能性がある．つまり，これは母親がアレルギー素因をもつ場合，児がアレルギーを発症しやすいという遺伝的要因の現れに過ぎないともいえる．

妊娠中の母体の咽頭炎とアトピー性皮膚炎の関連については明らかな証拠はないが，妊婦の免疫系に影響を与える要因が児のアレルギー発症に影響を与えるとする報告もあり[7]，母体の咽頭炎が免疫系を変化させた可能性が考えられる．このことから，感染も含め，妊娠中の母体の免疫系に影響を与える要因が児のアレルギー発症にも関わると考えられる．母体の咽頭に存

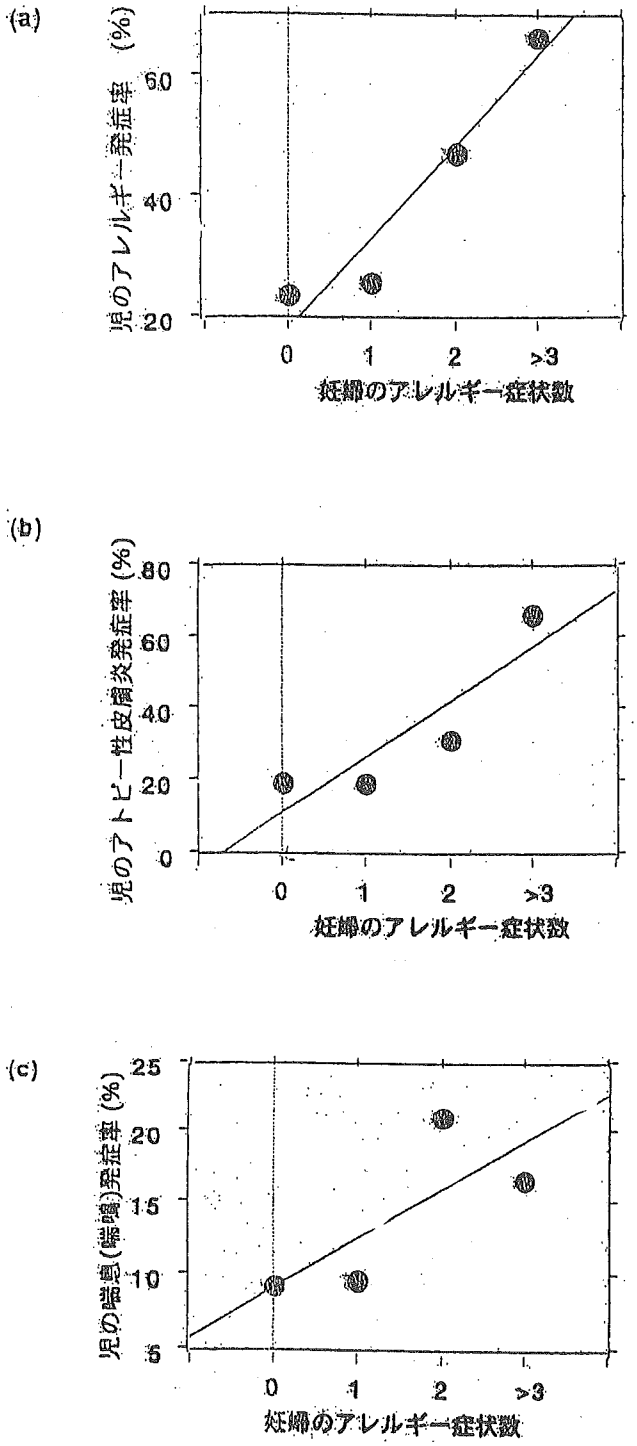


図3　妊娠中の母体のアレルギー発症と児のアレルギー発症

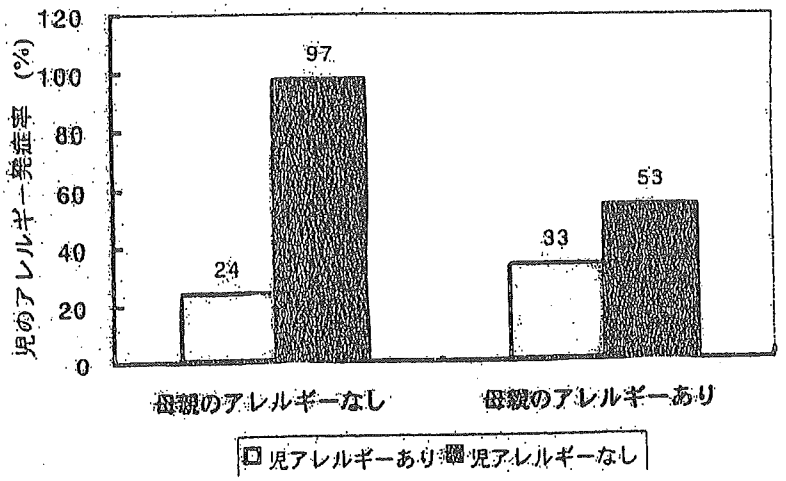


図4 生後の母親のアレルギー症状と児のアレルギー発症

在するリンパ球が炎症により活性化され，種々のサイトカイン・メディエーターを産生し，これらの産物が成熟段階にある胎児のリンパ球に作用し，胎児リンパ球が皮膚へのホーミング機能を獲得した可能性が考えられる．皮膚への遊走を誘導する，ケモカイン及びその受容体などの発現がサイトカイン・メディエーターにより修飾された可能性があげられる[8]．

アレルギーの家族歴と児のアレルギー発症についてはこれまでの報告と同様有意な関連を認め，遺伝的要因及び環境的要因の関与が示唆された．

児の感染症状とアレルギー発症については感染症の有無よりもむしろ抗生物質の使用がアレルギー疾患発症を増加させるとする研究[9]もあり，今後の検討課題と考えられた．

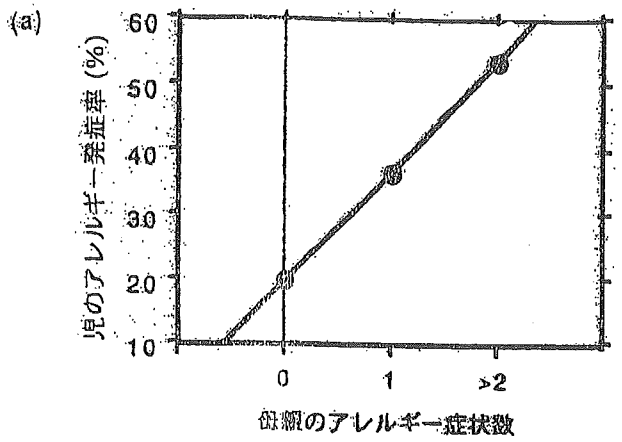
母乳栄養はアレルギー発症を低くすると考えられてきた[10]が，一方で母乳哺育はむしろアレルギー発症の危険因子とする報告もあり[11]，一定の見解に至っていない．本研究では母乳栄養は明らかな影響を与えなかったが，今後多方面での研究が待たれるところである．

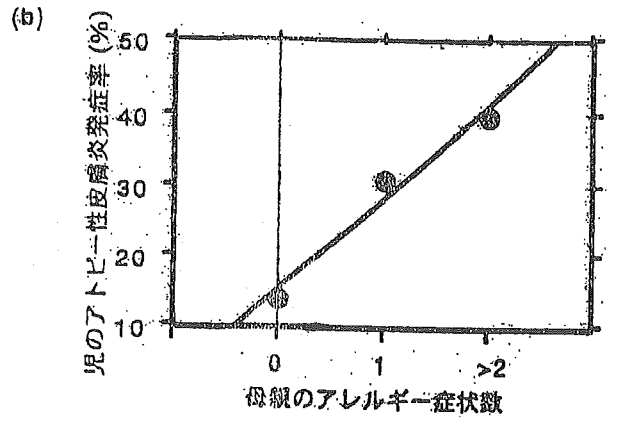
BCG接種群でアレルギー発症率が低下していた事実は既に報告されているとおりであったが[12,13]，三種混合ワクチン接種回数がアレルギー発症者で少なかったことは以前の研究[13]と合致しない．この理由として対象となった集団の年齢の違いを考慮する必要がある．また，筆者らはアレルギー疾患を有する児に対し予防接種を遅らせる指導はしていないが，近隣の医療機関にてアレルギー児が三種混合接種を遅らせるよう指導された可能性もある．

今回の結果ではペット及び受動喫煙のアレルギー発症に与える影響は認められなかった．ネコ・受動喫煙とアレルギー発症の関連を示唆する報告[3]もあるが，これは，本研究が対象を2歳未満としたため，2歳以降に喘息を発症する症例が含まれていないためであろう．

以上より，これらの出生前後の児の免疫系に影響を与えうる因子が児のアレルギー発症に関わることが示唆された．これらの要因を可能な限り回避することでアレルギー発症を予防することができれば幸いである．

なお本研究の一部は厚生科学研究費補助金，感覚器障害及び免疫・アレルギー等研究によった．





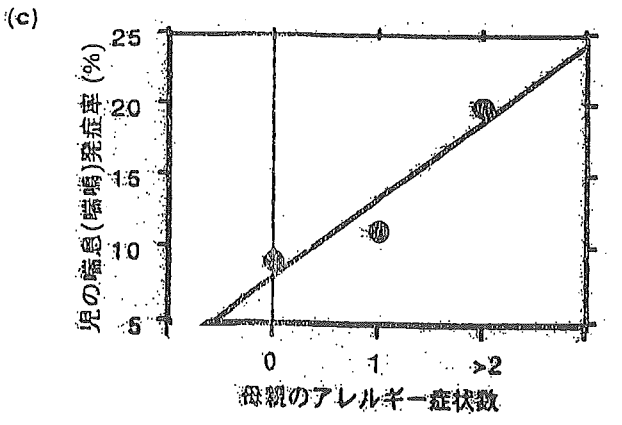
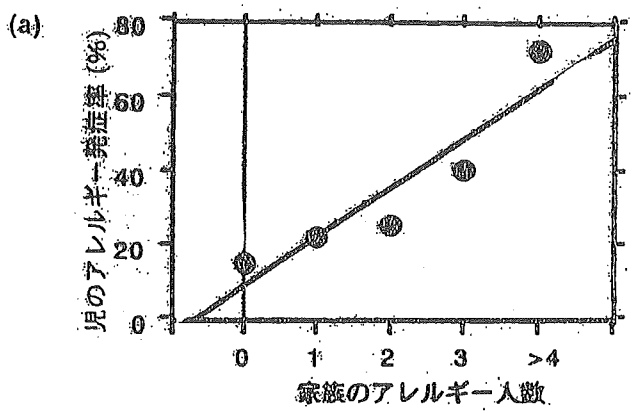
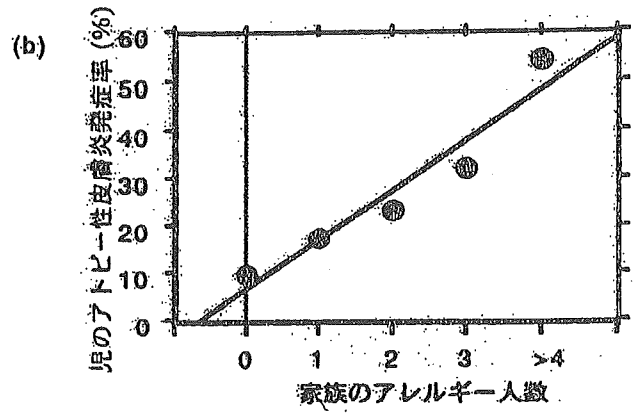


図5 母親のアレルギー症状数と児のアレルギー発症率の相関





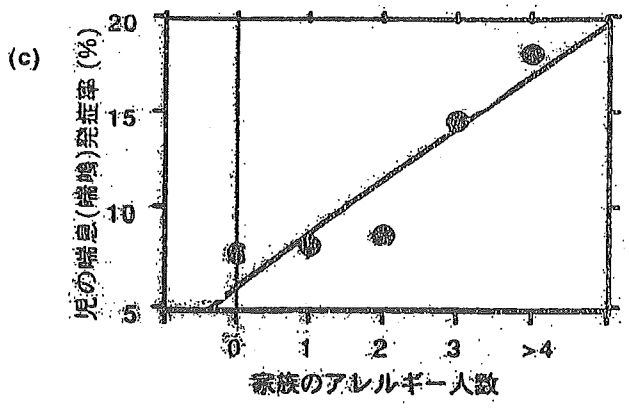


図6 アレルギーを有する家族の数と児のアレルギー発症

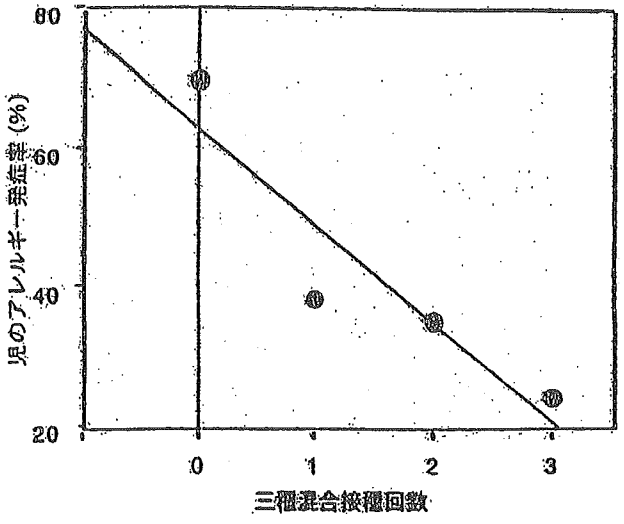


図7 三種混合の接種回数とアレルギー発症

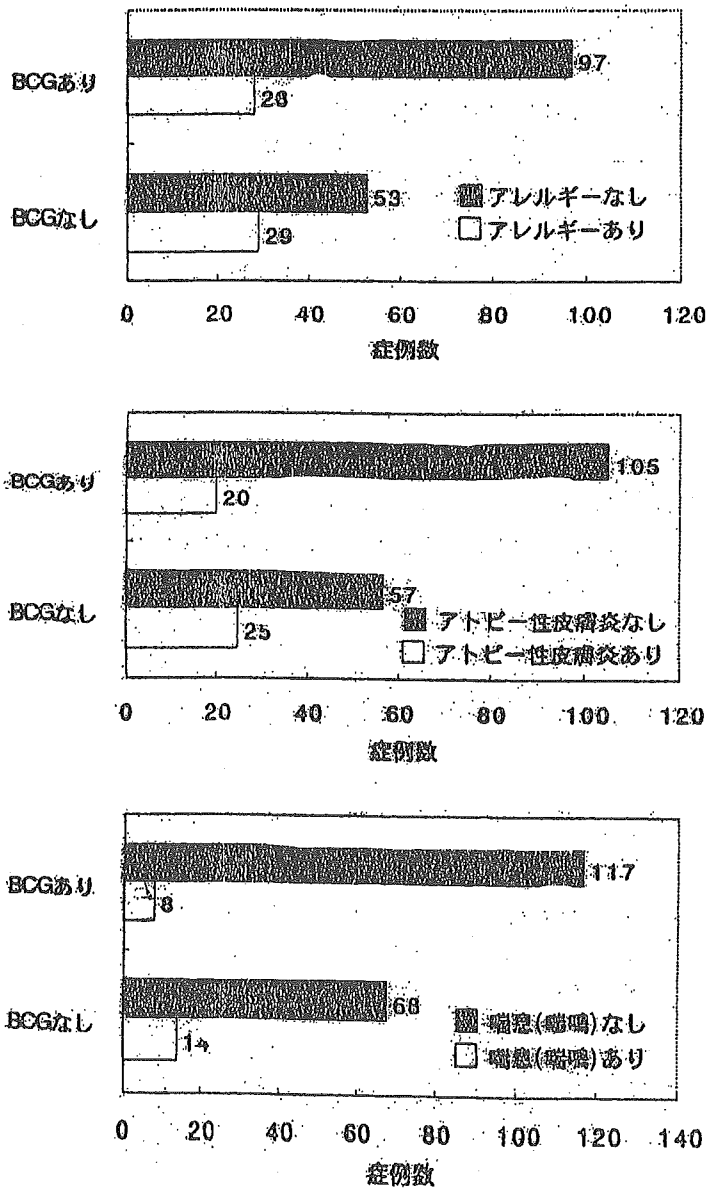


図8 BCG接種とアレルギー発症

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特集

小児気管支喘息のリモデリングとアーリーインターベンション

-Airway remodeling and it's early intervention in childhood asthma-

―特集に寄せて―

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1979年金沢大学医学部医学科卒業。88年英国National Heavt & Lung Institute留学，2002年群馬大学助教授，現在に至る。研究テーマは小児喘息における気道炎症・リモデリングの機序の解明。

Key words：小児喘息，リモデリング，予防，病態生理

喘息治療の概念は，その病態が気道の慢性炎症に基づく疾患であると認識されるようになってから大きく変化し，対症療法中心から発作予防中心の治療へと向っている。更に最近では，喘息の早期治療介入（アーリーインターベンション）に関心が向けられている。アーリーインターベンションは，喘息発症を予知・予防する一次予防と，既に発症した喘息の増悪を防止する二次予防とに分けられる。一次予防を適切に行うには喘息発症機序の解明が不可欠である。また，二次予防においては発症機序に加えて，喘息悪化の進展経路や最終的な転帰の病態生理学的な解明が行なわれている必要がある。しかしながら，小児喘息の病態はいずれも不明な点が多いため，適切なアーリーインターベンションの方法は確立されていないのが実状である。

成人喘息では気道生検など検査手技の進歩に伴ない，その病態は，“気道の慢性炎症性疾患”と認識され，病理学的には気道上皮下の線維性肥厚，平滑筋の肥大，粘膜下腺の過形成・気道上皮細胞の杯細胞化生といった，気道組織の再構築，いわゆるリモデリング remodeling を伴う疾患であるとの概念が確立されている。気道リモデリングは慢性気道炎症の持続により２次的に惹起されると考えられ，慢性患者において見られる一部不可逆的で進行性の呼吸機能低下の原因であると認識されている。このため，吸入ステロイドを中心とした抗炎症薬による治療がアーリーインターベンションとしても重要視されている。

一方，小児喘息では気道の慢性炎症やリモデリングの有無，あるいはそれらの病態に果す役割については不明な点が多い。気道局所の情報からは，小児喘息においても特に重症患者では慢性の気道炎症とリモデリングが存在することが判明している。しかしながら，慢性の気道炎症がリモデリングの原因であるとのエビデンスはない。更に，小児喘息では慢性の気道炎症の持続・リモデリングの存在は必ずしも不可逆的な呼吸機能の低下を招来しないという報告がある。また，小児喘息では少なくとも過半数の患児が成人になるまでにアウトグローするという，成人喘息では見られない高い寛解率がある。このため，小児喘息における慢性気道炎症とリモデリングの役割を，成人喘息の理論でそのまま説明するのは難しく，新たな知見の集積が待たれている。

本特集では，小児喘息における気道リモデリングの意義とアーリーインターベンションの方法について，現時点での考え方を整理し，今後解明すべき方向性を明らかにしたいと考えた。そこでまず，小児喘息における気道リモデリングの役割についての総説をお願いした。次いで，アトピー素因，アレルゲンによる感作と曝露，ウイルス感染，といった小児喘息の発症や増悪にかかわる重要な要因について，気道リモデリング形成の側面から，各分野のエキスパートの先生方に解説をお願いした。更に気道リモデリングのアーリーインターベンションの観点から，吸入ステロイドを始めとした各種抗炎症薬による治療法の有用性と限界について解説をいただいた。最後に，気道リモデリングに対する新たな治療法についての展望をお示しいただいた。本特集が，小児喘息の長期管理を行う上での参考になれば幸いである。

粘液分泌細胞

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1．気道粘液，特にムチン産生と喘息

喀痰の組成は，**図1**に示すように大きく唾液と気管支分泌液に分けられ，気管支分泌液は気道粘液産生細胞から分泌される粘液と組織からの滲出液に分けられる。粘液の主成分はムチンであり，ムチンはさらに酸性糖蛋白であるシアロムチンとスルホムチン，中性糖蛋白であるフコムチンに分かれる。ムチンは，ヒトにおいては気道上皮に存在する杯細胞（goblet cell）と，粘膜下腺の構成細胞である粘液細胞（mucus cell）が産生する。

ムチンの発現については，中心に存在する蛋白成分に対してはMUC geneが，また糖鎖に対してはglycosyltransferase geneが制御している。現在ムチンはMUC13まで明らかにされており，分泌型ムチンと膜結合型ムチンに分けられる。前者ではMUC5ACが最も多く発現しており，そのgeneは染色体の11番目に存在するといわれている。一方，後者のうちMUC3，MUC4，MUC12の3つのムチンはepidermal growth factor（EGF）様のドメインをもつことが知られている（**図2**）。

ムチン産生部位のうち粘膜下腺については，軽症から中等症の喘息では明らかな変化が認められないが，死亡例など重篤な症例では含量の増加が認められる。また，気道上皮の杯細胞はすでに軽

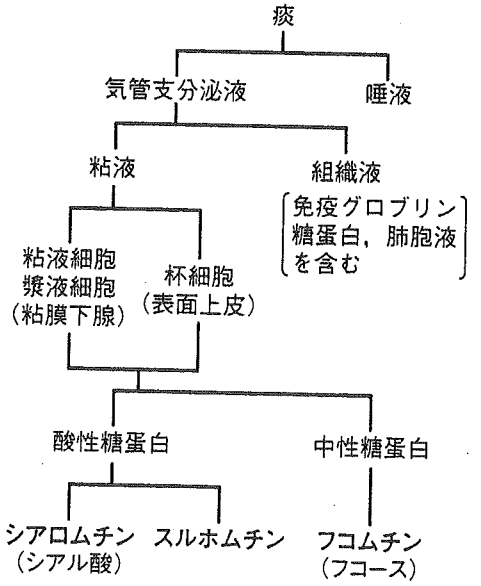

図1　喀痰の組成