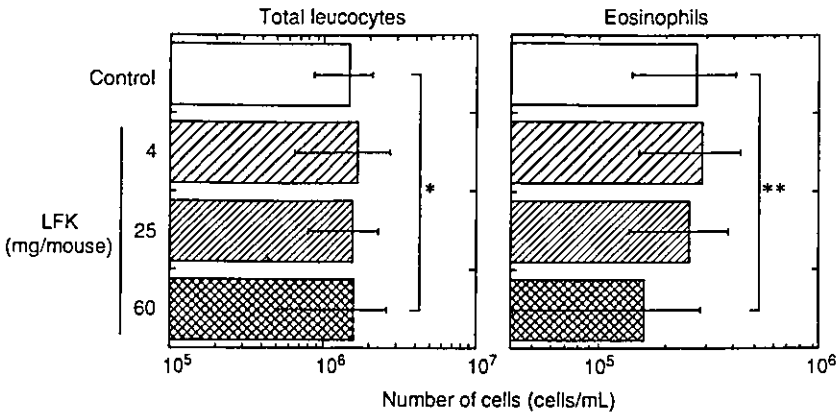

Fig. 3. Dose-dependent effect of LFK on peritoneal accumulation of total leucocytes and eosinophils in ragweed pollen allergen-sensitized mice. Each value represents mean ± SD of six mice. *$P = 0.96$, **$P = 0.14$, as compared with control.

cells/mL, respectively ($P = 0.78$). Interestingly, accumulation of eosinophils in control and LFK groups were 2.53×10^5 and 1.63×10^5 cells/mL, respectively ($P = 0.013$). Compared with a decrease in the number of eosinophils, the number of lymphocytes increased in LFK-treated mice (see Fig. 2).

Dose-dependent effect of LFK on peritoneal accumulation of eosinophils in ragweed pollen allergen-sensitized mice

As shown in Fig. 3, the total number of accumulated cells in control mice and 4 mg, 25 mg and 60 mg LFK-treated mice were 1.47×10^6, 1.66×10^6, 1.52×10^6 and 1.53×10^6 cells/mL, respectively. No significant difference was observed among all groups ($P = 0.96$). Accumulation of eosinophils in control mice and 4 mg, 25 mg and 60 mg LFK-treated mice was 2.76×10^5, 2.92×10^5, 2.55×10^5 and 1.60×10^5 cells/mL, respectively. It was inclined to be a dose-dependent fashion in combating eosinophil accumulation ($P = 0.14$), but LFK did not have any effect on the number of neutrophils, monocytes and lymphocytes among each group.

Discussion

Over the past few decades, the role of intestinal microflora, such as several strains of LAB in priming the immune system during ontogeny to limit allergy, has been brought to attention [1]. Epidemiological studies have demonstrated a higher incidence of allergy expression in early childhood among children who have low enteric populations of LAB, supporting the notion that appropriate microbial colonization of the gut can lower the risk of developing allergy [11, 12]. There is also clinical evidence that appropriate gut-colonizing microbes can control the development of atopy [13, 14]. Further studies, utilizing splenocytes derived from allergen-primed mice, have demonstrated that the inclusion of LAB cells in allergen-stimulated cell cultures can increase production of IFN-γ, but reduces levels of allergen-driven IL-4, IL-5 and IgE [15, 16].

Extensive experimental studies and clinical investigations have implicated eosinophils in the pathogenesis of allergic diseases. Eosinophil accumulation into inflammatory sites should be viewed as a characteristic phenomenon of allergic inflammation [9]. In the present study, it has been demonstrated that LFK, a preparation of lysozyme treated and heat-killed *E. faecalis* FK-23 strain, inhibited the ragweed pollen allergen- induced peritoneal accumulation of eosinophils and was inclined to be in a dose-dependent fashion in mice. However, FK23, a preparation of heat-killed *E. faecalis* FK-23 strain, did not show the same inhibition (data not shown). Interestingly, LFK and FK23 were differently treated preparations from the same strain of *E. faecalis* FK-23.

Although some attempts have been made to study the mechanism of LFK on inhibition of eosinophil accumulation, it remains uncertain. We have found that lysozyme treatment of the cell-wall compound of *E. faecalis* FK-23 could inhibit the peritoneal accumulation of eosinophils in mice [17]. In addition, LFK has an obvious effect on decreasing serum ovalbumin (OVA)-specific IgE levels, but not OVA-induced total IgE levels in experimental mice (data not shown). Furthermore, in a recent clinical study, we have observed that LFK could decrease the peripheral blood eosinophils and improve the tuberculin responses in patients with perennial allergic rhinitis (unpublished data).

Taken together, it is likely that LFK can prevent the overexpression of Th2-dominated immune responses. Also, alteration of gut mucosal immunity after LFK oral treatment might explain our finding. Further research on the molecular mechanism of LFK in combating eosinophilia and allergy is needed to clarify the association of orally delivered LAB and anti-allergy immunoregulation.

References

1 Cross ML, Gill HS. Can immunoregulatory lactic acid bacteria be used as dietary supplements to limit allergies? Int Arch Allergy Immunol 2001; 125:112–9.
2 Shimada T, Ohashi K, Yamamoto T. Effect of LFK (lysed *Enterococcus faecalis* FK-23) on active cutaneous anaphilaxis (ACA). Nippon Nogeikagaku Kaishi 1998; 72:293 (in Japanese).
3 Enomoto T, Dake Y, Shimada T, Kawai Y, Yamamoto T, Shirakawa T. Effect of LFK (lysed *Enterococcus faecalis* FK-23) on Japanese cedar pollinosis. ORL Tokyo 2000; 43:248–52 (in Japanese).
4 Abe S, Ohashi K, Uchida K, Ikeda T, Kimura S, Yamaguchi H. Antitumor and antimicrobial activities of enterococcal preparation orally administered to mice. Ann NY Acad Sci 1993; 685:372–4.
5 Ohashi K, Satonaka K, Yamamoto T et al. Antitumor activity of *Enterococcus faecalis* FK-23 preparation against murine syngeneic tumors. Yakugaku Zasshi 1993; 11:396–9 (in Japanese with English summary).

6 Hasegawa T, Ogura Y, Inomata T et al. Effect of orally administered heat-killed *Enterococcus faecalis* FK-23 preparation on leukopenia in mice treated with cyclophosphamide. J Vet Med Sci 1994; 56:1203–6.
7 Satonaka K, Ohashi K, Nohmi T et al. Prophylactic effect of *Enterococcus faecalis* FK-23 preparation on experimental candidasis in mice. Microbiol Immunol 1996; 40:217–22.
8 Shimda T, Kadowaki Y, Ohmiya K, Yamamoto T. Identification of an RNA fraction from *Enterococcus faecalis* FK-23 cells as the antihypertensive compound. J Ferment Bioeng 1996; 82:109–12.
9 Menzies-Gow A, Robinson DS. Eosinophils, eosinophilic cytokines (interleukin-5), and antieosinophilic therapy in asthma. Curr Opin Pulm Med 2002; 8:33–8.
10 Kaneko M, Yazumichi H, Takatsu K, Matsumoto S. Role of interleukin-5 in local accumulation of eosinophils in mouse allergic peritonitis. Int Arch Allergy Appl Immunol 1991; 96:41–5.
11 Bjorksten B, Naaber P, Sepp E, Mikelsaar M. The intestinal microflora in allergic Estonian and Swedish 2-year-old children. Clin Exp Allergy 1999; 29:342–6.
12 Kirjavainen PV, Gibson GR. Healthy gut microflora and allergy: factors influencing development of the microbiota. Ann Med 1999; 31:288–92.
13 Wheeler JG, Shema S, Bogle ML et al. Immune and clinical impact of *Lactobacillus acidophilus* on asthma. Ann Allergy Asthma Immunol 1997; 79:229–33.
14 Van de Water J, Keen CL, Gershwin ME. The influence of chronic yogurt consumption on immunity. J Nutr 1999; 129:1492S–5S.
15 Murosaki S, Yamomoto Y, Ito K et al. Heat-killed *Lactobacillus plantarum* L-137 suppresses naturally fed antigen-specific IgE production by stimulation of IL-12 production in mice. J Allergy Clin Immunol 1998; 102:57–64.
16 Shida K, Makino K, Morishita A et al. *Lactobacillus casei* inhibits antigen-induced IgE secretion through regulation of cytokine production in murine splenocyte cultures. Int Arch Allergy Immunol 1998; 115:278–87.
17 Ide M, Shimada T, Kawai Y, Enomoto T. Effective components of LFK in allergen-induced peritoneal accumulation of eosinophils [Abstract]. Jpn J Allergol 2002; 51:318 (in Japanese).

Polymorphism of *SLC11A1* (Formerly *NRAMP1*) Gene Confers Susceptibility to Kawasaki Disease

Kazunobu Ouchi,[1] Yoichi Suzuki,[2] Taro Shirakawa,[3] and Fumio Kishi[4]

[1]Department of Pediatrics, Kawasaki Medical School, Kurashiki, [2]Department of Medical Genetics, Tohoku University School of Medicine, Sendai, [3]Department of Health Promotion and Human Behavior, Kyoto University Graduate School of Public Health, Kyoto, and [4]Department of Microbiology and Immunology, Kagoshima University Dental School, Kagoshima, Japan

Since its first description in Japan >30 years ago, Kawasaki disease (KD) has been reported worldwide. Although an infectious etiology is suspected based on the epidemiology and clinical features, a causative agent has not been identified. The disease is more frequent in children of Japanese ancestry, and siblings of children with KD have a significantly greater risk of developing KD than do children of the same age in the general population. This suggests a possible genetic susceptibility to KD. Results of this study showed that allele 1 of the 5′ promoter (GT)n repeat in the *SLC11A1* (formerly *NRAMP1*) gene, which endows the gene with a weak promoter activity, was highly represented in patients with KD. This suggests possible explanations for both the infectious etiology of this disease and the genetic risk in the Japanese population.

Kawasaki disease (KD) is an acute multisystem vasculitis that occurs in infants and children [1]. The diagnostic criteria for KD include fever for ⩾5 days, typically with at least 4 of the following 5 clinical features: bilateral conjunctival injection without purulent discharge; inflammatory changes of the lips and "strawberry" tongue; changes of the peripheral extremities, particularly redness and swelling of the hands and feet with subsequent periungual desquamation; rash, primarily truncal and taking many forms, but nonvesicular; and cervical lymphadenopathy, usually unilateral.

Received 28 May 2002; revised 12 September 2002; electronically published 6 January 2003.

Informed consent was obtained from the parents of all patients and control subjects in accordance with institutional review board guidelines. Ethics committees of Saiseikai Shimonoseki (former affiliation of K.O.), Yamaguchi University, and Kagoshima University hospitals approved the study.

Reprints or correspondence: Dr. Kazunobu Ouchi, Dept. of Pediatrics, Kawasaki Medical School, 577 Matsushima, Kurashiki, 701-0192 Okayama, Japan (kouchi@med.kawasaki-m.ac.jp).

The Journal of Infectious Diseases 2003; 187:326–9

0022-1899/2003/18702-0020$15.00

Since 1970, nationwide epidemiologic surveys have been conducted biennially in Japan with the cooperation of medical facilities throughout the country. Three nationwide epidemics of KD occurred in Japan in 1979, 1982, and 1986 [2]. KD is clearly overrepresented in children of Asian background, both in Asia and North America. In 2001, Yanagawa et al. [3] reported an annual attack rate in Japan of 140/100,000 children aged <3 years, which is higher than the rate in US whites and blacks (~10/100,000 children aged <3 years) [4]. The KD attack rate for US Asians and for Pacific Islanders in San Diego County and Hawaii is 3 times higher than that in US white children [4, 5]. Siblings of children with KD have a significantly greater risk of developing KD than children of the same age in the general population, which suggests a possible genetic risk. However, the basis of genetic susceptibility remains unclear.

The epidemic nature, seasonal distribution, and clinical features suggest an infectious etiology of this disease. Multiple agents have been implicated, including *Streptococcus*, *Staphylococcus*, *Yersinia*, and *Rickettsia* organisms, viruses, and environmental chemicals. However, none has been conclusively demonstrated to be causative. The activation of T cells, B cells, and monocytes and/or macrophages is well known in KD and suggests a generalized immune activation. Previous studies suggest that immunoregulatory abnormalities may contribute to its pathogenesis, and one of the significant clinical features of KD is local inflammatory reactivation of a previous bacillus Calmette-Guérin (BCG) inoculation site, a specific and early manifestation of KD [2, 6], which shows an erythematous indurated plaque with activated macrophages at the skin lesion, although no evidence of active mycobacterial infection has been observed.

In laboratory strains of inbred mice, resistance and/or susceptibility to the growth of BCG is controlled by locus *Bcg*. Subsequently, a gene called *SLC11A1* (formerly *NRAMP1*) was identified in the locus that controls resistance to BCG and other intracellular parasites, and the human homologue was subsequently isolated [7, 8]. *SLC11A1* regulates the cascade of gene-inductive events that follow interaction of macrophages with bacterial lipopolysaccharide (LPS) and/or natural killer cell– or T cell–derived interferon (IFN)–γ. The gene has multiple pleiotropic effects, including regulation of interleukin-1β, tumor necrosis factor–α, and major histocompatibility complex class II molecules [9]. Several polymorphic variants have been found in the human *SLC11A1* gene promoter, which may change many different aspects of macrophage function and many cytokine responses [10, 11]. KD has unique properties of macrophage and cytokine responses in the acute phase. Here we

present evidence that the polymorphism at the human *SLC11A1* gene promoter confers susceptibility to KD.

Materials and methods. Blood samples were obtained from 71 Japanese patients with KD and 110 age- and sex-matched healthy Japanese volunteers residing in the same regional area (Yamaguchi Prefecture, Japan). The diagnosis of KD was based on criteria described elsewhere [2].

Leukocyte DNA was extracted by use of the Wizard genomic DNA purification kit (Promega), and 50 ng was used in a polymerase chain reaction (PCR). The 5′-promoter (GT)n was genotyped by PCR with primers 5′-GTC TTG GAA CTC CAG ATC AAA G-3′ and 5′-TTG CAT ATT CAT GTC AAT ACC C-3′. PCR was done under the following conditions: denaturation at 95°C for 2 min followed by 30 cycles of denaturation at 95°C for 1 min, annealing at 60°C for 1 min, and extension at 72°C for 2 min. PCR was terminated by extension at 72°C for 7 min followed by cooling to 4°C.

Three alleles, 134 bp (designated as "allele 1"), 132 bp ("allele 2"), and 130 bp ("allele 3"), were identified in the Japanese population and imaged by use of an automated DNA sequencer. Exon15, Asp543Asn (D543N), and the deletion of the 3′-untranslated region (3′-UTR D/I) were genotyped by PCR with the primers 5′-GCA TCT CCC CAA TTC ATG GT-3′ and 5′-AAC TGT CCC ACT CTA TCC TG-3′ with the following conditions: denaturation at 95°C for 2 min followed by 35 cycles of denaturation at 95°C for 1 min, annealing at 58°C for 1 min, and extension at 72°C for 2 min. PCR was terminated by extension at 72°C for 7 min followed by cooling to 4°C. Restriction-endonuclease digestions were then done by using *Ava*II and *Fok*I under conditions recommended by the supplier (New England Biolabs). Restriction enzyme digestion products were resolved by electrophoresis on 12% polyacrylamide gels stained with ethidium bromide. *Ava*II cleaves the Asp alleles of exon 15 into 3 products of 126, 79, and 39 bp and the Asn alleles into 2 products of 201 and 39 bp. *Fok*I cleaves the −TGTG allele of the 3′-UTR region into 211 and 33 bp and does not cleave the +TGTG allele.

We estimated the power of this study by using the formula described by Ohashi et al. [12]. Our study had about 80% power (at the .05 level) to detect an allelic association with a sample size of 71 cases and 110 control subjects, assuming that the frequency of risk allele in control subjects is 0.03 and its relative risk is 3.35. (Under these conditions the incidence of KD is calculated to be 0.0014 in dominant model.) We used the Fisher's exact test to assess the association between the polymorphisms and the disease. Contingency table analysis, odds ratios, 95% confidence intervals, and significance values were estimated by use of computerized methods (SPSS program vers. 10.1J). In the 5′-promoter polymorphism, we also estimated empirical *P* values by use of the Monte Carlo method, using the CLUMP program with 100,000 stimulations [13]. Corrected *P* values with the number of alleles (Pc) <.02 were considered to be statistically significant.

Results. We conducted a genetic association study in a Japanese population to determine whether variants of *SLC11A1* relate to KD. We studied 71 Japanese children (48 boys and 23 girls) who were diagnosed with KD at ages 0–5 years and 110 healthy children (control subjects) without a history of KD who were matched by age and sex. No significant difference was observed in age or ratio of sexes between patients and control subjects. Table 1 shows gene frequency of genotypes of the 3′-UTR region, exon 15 region, and 5′-promoter (GT)n region in children with KD and in control subjects. No association was found between KD and distribution of the variants 3′-UTR D/I ($P = .834$) and exon 15 Asn543Asp ($P = .834$). However, the association of the distribution of the 5′-promoter (GT)n region and KD was significant, and the gene frequency of allele 1 of the 5′-promoter (GT)n was significantly higher in patients with KD than in control subjects ($P = .0074$).

Discussion. We investigated genetic associations in a Japanese population to determine whether variants of *SLC11A1* relate to KD, a disease for which the etiology and genetic susceptibility remain unclear. To date, 11 variants including 5 coding regions, 3 in the introns, 2 in the 3′-UTR, and 1 in the 5′ promoter region) have been identified in *SLC11A1*. Among these, only 2 of the polymorphisms were predicted to cause amino acid substitution in A318V, an alanine→valine substitution, and in D543N, an aspartic acid→asparagine substitution [8].

We genotyped the D543N polymorphism because this could affect protein function by substituting a negatively charged amino acid with an uncharged residue in the cytoplasmic carboxy terminal domain and could possibly alter the macrophage function. Another polymorphism on which we focused is a Z DNA-forming polymorphic (GT)n repeat 250–300 bp upstream of the transcription start site, designated here as the 5′-promoter (GT)n 14, which may be a functional polymorphism at the transcription level [10]. In addition to these possibly functional polymorphisms, a 4-bp deletion polymorphism in the 3′-UTR region, designated here as 3′-UTR D/I, was genotyped. The physiologic effects of sequence polymorphisms in the 3′-UTR are not fully understood, although there are regulatory elements for several genes within the 3′-UTR.

D543N and 3′-UTR D/I appeared to be in absolute linkage disequilibrium, and no association was found between KD and D543N and 3′-UTR D/I. In contrast, in this study, the gene frequency of allele 1 of 5′-promoter (GT)n was 0.032 in the control children and 0.113 in the KD patients, which indicates a significant association of the 5′-promoter (GT)n with susceptibility of KD. Allele 1 frequency was previously reported to be 0.022 in adults in an area of Japan remote from the region in our study [14]. Elsewhere the allele 1 frequency is 0.001, 0.021,

of the host is regulated by level of *SLC11A1* gene expression. The high incidence of KD in Asians may be due to a high genetic susceptibility to this pathogen.

Acknowledgments

We thank T. Yoshida, M. Fujiwara, T. Matsubara, and S. Furukawa of the Japan Kawasaki Disease Research Group for help with sample collection, clinical information, and critical review of the study proposal.

References

1. Kawasaki T. Acute febrile mucocutaneous syndrome with lymphoid involvement with specific desquamation of the fingers and toes in children: clinical observations of 50 cases [in Japanese]. Arerugi 1967; 16:178–222.
2. Yanagawa H, Nakamura Y, Ojima T, Yashiro M, Tanihara S, Oki I. Changes in epidemic patterns of Kawasaki disease in Japan. Pediatr Infect Dis J 1999; 18:64–6.
3. Yanagawa H, Yashiro M, Oki I, Nakamura Y, Tuohong Z. Thirty-year observation of the incidence rate of Kawasaki disease in Japan [abstract SA11] In: Program and abstracts of the 7th International Kawasaki Disease Symposium (Hakone, Japan), 2001:31.
4. Bronstein DE, Dille AN, Austin JP, Williams CM, Palinkas L, Burns JC. Relationship of climate, ethnicity and socioeconomic status to Kawasaki disease in San Diego County, 1994 through 1998. Pediatr Infect Dis J 2000; 19:1087–91.
5. Holman RC, Shahriari A, Effler PV, Belay ED, Schonberger LB. Kawasaki syndrome hospitalization among children in Hawaii and Connecticut. Arch Pediatr Adolesc Med 2000; 154:804–8.
6. Hsu YH, Wang YH, Hsu WY, et al. Kawasaki disease characterized by erythema and induration at the bacillus Calmette-Guérin and purified protein derivative inoculation sites. Pediatr Infect Dis J 1987; 6:576–8.
7. Kishi F. Isolation and characterization of human *NRAMP* cDNA. Biochem Biophys Res Commun 1994; 204:1074–80.
8. Liu J, Fujiwara TM, Buu NT, et al. Identification of polymorphisms and sequence variants in the human homologue of the mouse natural resistance-associated macrophage protein gene. Am J Hum Genet 1995; 56: 845–53.
9. Skamene E. The Bcg gene story. Immunobiology 1994; 191:451–60.
10. Blackwell JM, Barton CH, White JK, et al. Genomic organization and sequence of the human *NRAMP* gene: identification and mapping of a promoter region polymorphism. Mol Med 1995; 1:194–205.
11. Searle S, Blackwell JM. Evidence for a functional repeat polymorphism in the promoter of the human *NRAMP1* gene that correlates with autoimmune versus infectious disease susceptibility. J Med Genet 1999; 36: 295–9.
12. Ohashi J, Yamamoto S, Tsuchiya N, et al. Comparison of statistical power between 2 × 2 allele frequency and allele positivity tables in case-control studies of complex disease genes. Ann Hum Genet 2001; 65:197–206.
13. Sham PC, Curtis D. Monte Carlo test associations between disease and allele at highly polymorphic loci. Ann Hum Genet 1995; 59:97–105.
14. Gao PS, Fujishima S, Mao XQ, et al. Genetic variants of *NRAMP1* and active tuberculosis in Japanese populations. Clin Genet 2000; 58:74–6.
15. Yang YS, Kim SJ, Kim JW, Koh EM. *NRAMP* gene polymorphisms in patients with rheumatoid arthritis in Koreans. J Korean Med Sci 2000; 15:83–7.

Table 1. *SLC11A1* gene polymorphisms in Japanese children with Kawasaki disease ($n = 71$) and in control subjects ($n = 110$).

Characteristic	Kawasaki disease (frequency)	Control subjects (frequency)	P[a]
No. of boys/no. of girls	48/23	69/41	
Mean age, years	1.80	2.09	
Genotype			
3′-UTR D/I			
D/D	2	2	
D/I	13	18	
I/I	56	90	.834
Exon15 Asn543Asp			
Asn/Asn	2	2	
Asn/Asp	13	18	
Asp/Asp	56	90	.834
5′-promoter (GT)n repeat allelic distribution			
Allele 1	16 (0.113)	7 (0.032)	
Allele 2	19 (0.134)	39 (0.177)	
Allele 3	107 (0.754)	174 (0.790)	.00739; .00598[b]
Genotypic distribution			
Allele (1,1)	1	0	
Allele (1,2)	4	2	
Allele (1,3)	10	5	
Allele (2,2)	4	3	
Allele (2,3)	7	31	
Allele (3,3)	45	69	.00270; .00340[c]
Allele (1,1) + allele (1,2) + allele (1,3)	15	7	.0136[d]
Allele (2,2) + allele (2,3) + allele (3,3)	56	103	Reference

NOTE. UTR, untranslated region.

[a] Fisher's exact test.

[b] P value for T1 statistic obtained with Monte Carlo simulation [13]. $\chi^2 = 10.053$.

[c] P value for T1 statistic obtained with Monte Carlo simulation [13]. $\chi^2 = 16.028$.

[d] Fisher's exact test corrected for multiple testing (3 times). Odds ratio, 3.94 (95% confidence interval, 1.52–10.2).

and 0.039 in white persons [11], Brazilians [10], and Koreans [15], respectively. These findings show that Asians have a higher genetic frequency and incidence of KD than persons elsewhere. These results are suggestive of the importance of *SLC11A1* expression level in the difference of susceptibility of KD.

Of the 3 (GT)n alleles, allele 3 was observed to drive more than 10-fold higher levels of gene expression than either allele 1 or allele 2 [11]. Addition of IFN-γ as an exogenous stimulus causes a 1–2-fold enhancement of the gene expression in all 3 alleles, and bacterial LPS as a second signal results in a further ~2-fold enhancement of allele 3–driven gene expression. In contrast, no enhancement by LPS was observed in the expression levels of alleles 1 and 2. Moreover, allele 1 differed significantly from allele 2, and its baseline expression level was less than one-half that of allele 2. Therefore, there might be a biologic difference in phenotypic expression between allele 1 and allele 2. The significance of these biologic differences in human disease remains to be investigated.

KD is characterized by monocyte and/or macrophage activation and high circulating cytokine levels of all of the proinflammatory cytokines in response to IFN-γ, LPS, and other stimuli in the acute phase. It seems inconsistent that allele 1, a poor promoter of *SLC11A1*, which regulates the cascade of gene-inductive events that follow interaction of macrophages or many procytokines, increased patients with KD than in control subjects. However, no data showed polymorphisms of the *SLC11A1* gene; therefore, circulating cytokine levels in homozygous KD patients or control subjects should be analyzed further with each allele of the *SLC11A1* gene.

In conclusion, we show that 1 variant of the *SLC11A1* gene is associated with KD in a group of Japanese children. The skewed ethnic distribution and seasonality are consistent with the hypothesis that KD is an infectious disease that is influenced by environmental and genetic factors. Our results suggest that KD might be caused or induced by a certain infection with a yet unknown intracellular parasite to which innate resistance

of the host is regulated by level of *SLC11A1* gene expression. The high incidence of KD in Asians may be due to a high genetic susceptibility to this pathogen.

Acknowledgments

We thank T. Yoshida, M. Fujiwara, T. Matsubara, and S. Furukawa of the Japan Kawasaki Disease Research Group for help with sample collection, clinical information, and critical review of the study proposal.

References

1. Kawasaki T. Acute febrile mucocutaneous syndrome with lymphoid involvement with specific desquamation of the fingers and toes in children: clinical observations of 50 cases [in Japanese]. Arerugi 1967; 16:178–222.
2. Yanagawa H, Nakamura Y, Ojima T, Yashiro M, Tanihara S, Oki I. Changes in epidemic patterns of Kawasaki disease in Japan. Pediatr Infect Dis J 1999; 18:64–6.
3. Yanagawa H, Yashiro M, Oki I, Nakamura Y, Tuohong Z. Thirty-year observation of the incidence rate of Kawasaki disease in Japan [abstract SA11] In: Program and abstracts of the 7th International Kawasaki Disease Symposium (Hakone, Japan), 2001:31.
4. Bronstein DE, Dille AN, Austin JP, Williams CM, Palinkas L, Burns JC. Relationship of climate, ethnicity and socioeconomic status to Kawasaki disease in San Diego County, 1994 through 1998. Pediatr Infect Dis J 2000; 19:1087–91.
5. Holman RC, Shahriari A, Effler PV, Belay ED, Schonberger LB. Kawasaki syndrome hospitalization among children in Hawaii and Connecticut. Arch Pediatr Adolesc Med 2000; 154:804–8.
6. Hsu YH, Wang YH, Hsu WY, et al. Kawasaki disease characterized by erythema and induration at the bacillus Calmette-Guérin and purified protein derivative inoculation sites. Pediatr Infect Dis J 1987; 6:576–8.
7. Kishi F. Isolation and characterization of human *NRAMP* cDNA. Biochem Biophys Res Commun 1994; 204:1074–80.
8. Liu J, Fujiwara TM, Buu NT, et al. Identification of polymorphisms and sequence variants in the human homologue of the mouse natural resistance-associated macrophage protein gene. Am J Hum Genet 1995; 56: 845–53.
9. Skamene E. The Bcg gene story. Immunobiology 1994; 191:451–60.
10. Blackwell JM, Barton CH, White JK, et al. Genomic organization and sequence of the human *NRAMP* gene: identification and mapping of a promoter region polymorphism. Mol Med 1995; 1:194–205.
11. Searle S, Blackwell JM. Evidence for a functional repeat polymorphism in the promoter of the human *NRAMP1* gene that correlates with autoimmune versus infectious disease susceptibility. J Med Genet 1999; 36: 295–9.
12. Ohashi J, Yamamoto S, Tsuchiya N, et al. Comparison of statistical power between 2 × 2 allele frequency and allele positivity tables in case-control studies of complex disease genes. Ann Hum Genet 2001; 65:197–206.
13. Sham PC, Curtis D. Monte Carlo test associations between disease and allele at highly polymorphic loci. Ann Hum Genet 1995; 59:97–105.
14. Gao PS, Fujishima S, Mao XQ, et al. Genetic variants of *NRAMP1* and active tuberculosis in Japanese populations. Clin Genet 2000; 58:74–6.
15. Yang YS, Kim SJ, Kim JW, Koh EM. *NRAMP* gene polymorphisms in patients with rheumatoid arthritis in Koreans. J Korean Med Sci 2000; 15:83–7.

Allergology International (2003) **52**: 1–12

Review Article

Regulatory mechanisms of human IgE synthesis

Yukiyoshi Yanagihara

Clinical Research Center, National Sagamihara Hospital, Sagamihara, Japan

Abstract

The induction of allergen-specific IgE synthesis requires the cognate interactions between B and T helper (Th) 2 cells. The B cell-activating signal for IgE synthesis is delivered through interleukin (IL)-4 or IL-13 and CD40 ligand, which are provided by activated Th2 cells. Signaling through the IL-4 receptor α chain (IL-4Rα) triggers IL-4- or IL-13-dependent germline Cε transcription by activating signal transducer and activator of transcription (STAT)-6 through members of the Janus kinase (JAK) family. In addition to the known JAK–STAT pathway, two adaptor molecules associated with the IL-4Rα, which include Src homologous and collagen (Shc) and a product of the *fes* proto-oncogene family, are involved in the induction of germline Cε transcription. These adaptor molecules transmit the downstream signaling, leading to activation of PU.1, a product of the *ets* proto-oncogene family, which cooperates functionally with STAT-6 for germline Cε transcription. Ligation of CD40 in the presence of IL-4 or IL-13 leads to expression of activation-induced cytidine deaminase (AID). This novel RNA-editing enzyme plays a role upstream of the putative switch recombinase, activation of which results in IgE isotype switching, mature Cε transcription and IgE synthesis. Although CD40 signaling activates multiple pathways that are critical for the activation of the switch recombination machinery, none of the known second messengers and transcription factors generated by CD40 ligation is involved in AID expression and isotype switching. Elucidation of the merging point of IL-4Rα and CD40 signaling pathways required for IgE switching will provide potential new strategies for the isotype-specific regulation of IgE synthesis.

Key words: activation-induced cytidine deaminase, adaptor molecule, germline Cε transcription, IgE isotype switching, switch recombination machinery.

Introduction

The human immunoglobulin family consists of nine isotypes, each of which is involved in humoral immunity. Of these isotypes, IgE plays a key role in the pathogenesis of allergic disease. The production of IgE by B cells requires interactions with T cells and is induced through cytokines and cell surface molecules provided by activated T cells. Increasing understanding of the cellular and molecular events underlying IgE synthesis has allowed the identification of new targets for IgE regulation. The present article provides an up-to-date overview of the regulatory mechanisms of human IgE synthesis.

Immunoglobulin expression during B cell differentiation

The primary function of B cells is to produce antibodies, including the IgE isotype, against a vast array of environmental antigens. The production of such a large spectrum of antibodies is due to the generation of a large repertoire of B cells, each of which expresses antibodies of a different specificity.

Antibody molecules are composed of paired heavy and light chains and their variable regions have a unique antigen-binding specificity. Of clonally diverse B cells, a cell bearing a particular Ig receptor responds to the complementary antigen with proliferation and differentiation into antibody secreting plasma cells.

The B-lineage cells, which derive from hemopoietic stem cells that give rise to cells of other blood lineages, can be divided into five general stages of differentiation represented by pro-B cells, pre-B cells, immature B cells,

Correspondence: Dr Yukiyoshi Yanagihara, Clinical Research Center, National Sagamihara Hospital, 18-1 Sakuradai, Sagamihara 228-8522, Japan.
Email: y-yanagihara@sagamihara-hosp.gr.jp
Received 28 October 2002.

mature B cells and plasma cells (Fig. 1). The generation of B cells and their maturation in the bone marrow are antigen-independent processes, whereas the terminal differentiation of mature B cells into plasma cells in lymphoid organs is antigen dependent.

The production of B cells by the bone marrow is a multifocal process that involves the generation of an extensive repertoire of Ig specificities. Such a repertoire is acquired by a series of gene rearrangements of the variable regions of the Ig heavy and light chains.[1,2] Although pro-B cells start to synthesize cytoplasmic μ heavy chains, but not the conventional light chains of the κ or λ type necessary for the formation of a complete IgM molecule, the cells constitutively express recombination-activating gene-1 and -2, the products of which (RAG-1 and RAG-2, respectively) initiate V (variable)–D (diversity)–J (joining) recombination at the μ chain locus. Subsequently, these two gene products expressed in pre-B cells, which express both μ chains and surrogate light chains, initiate V-J recombination at the light chain loci, thereby leading to the formation of completed IgM molecules and pre-B cell transition into immature B cells bearing surface IgM. These cells further differentiate into mature B cells that coexpress IgD, having identical binding specificity.

Mature B cells respond to antigens by undergoing plasma cell differentiation. This terminal differentiation process involves isotype switching, which allows expression of IgG, IgA or IgE.[3] The generation of switched B cells and their differentiation are dependent on T cell help, which is mediated through cytokines and cell surface molecules provided by activated T cells. Re-expression of RAG-1 and RAG-2 is also inducible in mature B cells under suitable conditions before isotype switching, which is predicted to allow secondary rearrangements leading to Ig receptor revision.[4] Isotype switching results from Ig gene rearrangement that involves the deletion of constant region genes upstream of the one to be expressed. Thus, individual plasma cells produce antibodies of a single isotype.

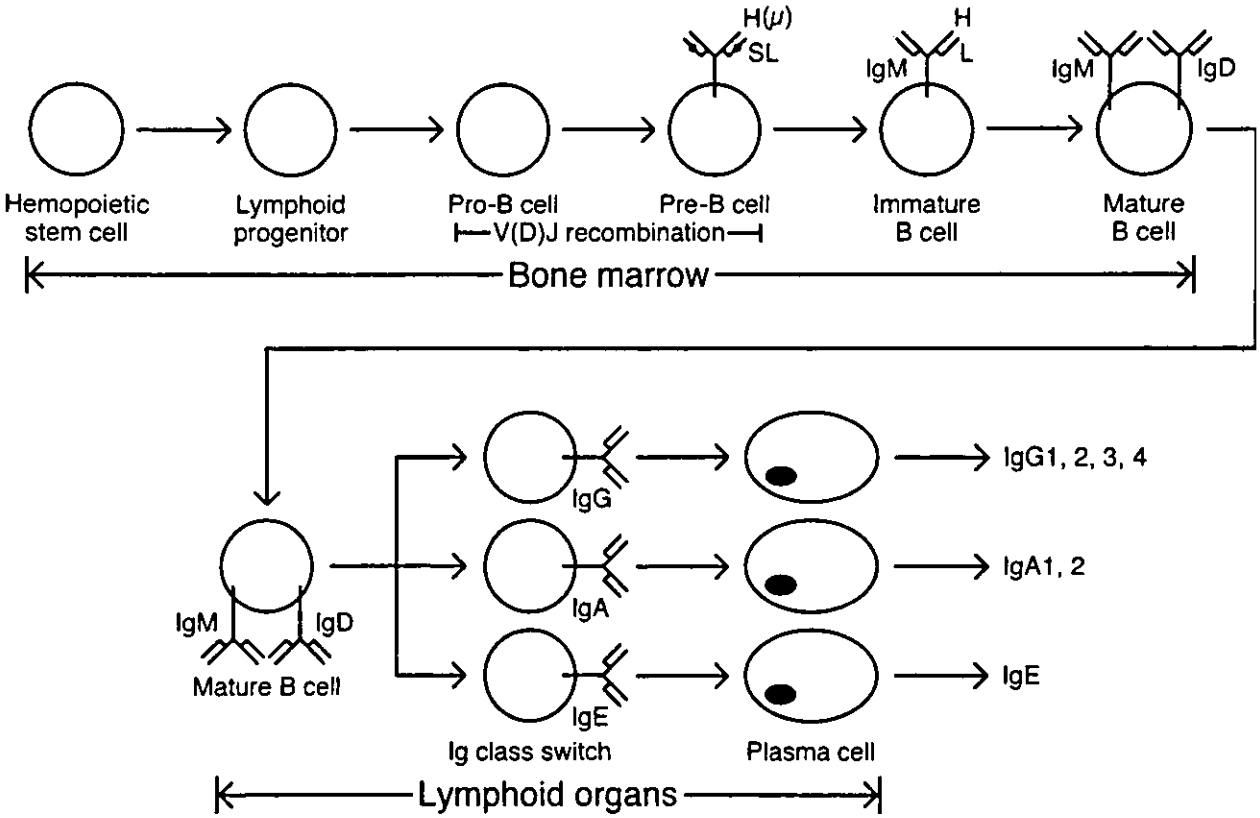

Fig. 1 B cell differentiation pathway and immunoglobulin gene rearrangements. Cell surface molecules indicate immunoglobulins expressed during B cell differentiation. H, heavy chain; L, light chain; SL, surrogate light chain; V(D)J, variable (diversity) joining.

Cellular events in the induction of IgE synthesis

Cognate interactions between B and T cells are required to induce allergen-specific IgE synthesis (Fig. 2). When a mature B cell recognizes a particular allergen via B cell receptor, the cell processes the allergen and presents its T cell epitope, together with major histocompatibility complex (MHC) class II molecules, to an allergen-specific $CD4^+$ T cell with a Th2 phenotype. Subsequently, the T cell is activated through engagement of the αβ T cell receptor–CD3 complex and is induced to produce Th2-type cytokines, such as interleukin (IL)-4 and IL-13, and to express CD40 ligand (CD40L, CD154), which belongs to the tumor necrosis factor superfamily. In the presence of T cell-derived IL-4 or IL-13, the B cell undergoes germline Cε transcription, which is a critical initiating step for switching from IgM to IgE. This cytokine-dependent transcription directs switching to the corresponding isotype.[5–7]

Signaling through the IL-4 receptor α chain (IL-4Rα, CD124), which is a component of both the heterodimeric IL-4R consisting of the IL-4Rα and the common γ chain (γc, CD132) and the heterodimeric IL-13R consisting of the IL-4Rα and IL-13Rα1 (CD213a1), plays a key role in the induction of germline Cε transcription.[8–10] In support of this is the finding that B cells of X-linked severe combined immunodeficiency patients with mutations in the γc gene can express germline Cε transcripts in response to IL-4 or IL-13.[11,12] CD40L, a non-covalent trimer expressed on the activated T cell, in conjunction with IL-4 or IL-13, not only enhances cytokine-dependent germline Cε transcription, but also induces IgE isotype switching through cross-linking of CD40 constitutively expressed on the B cell. Engagement of CD40 by CD40L also mediates rescue from apoptosis, proliferation and terminal differentiation into IgE antibody secreting plasma cells.[13,14] Thus, IL-4Rα and CD40 signaling pathways are integrated to induce IgE isotype switching and IgE synthesis.

The central role of the interaction of CD40 with CD40L in switching to T cell-dependent isotypes has been shown in patients with X-linked hyper-IgM syndrome who have mutations in the CD40L gene that result in defective isotype switching.[15] Although CD40 on B cells prompts endocytosis of surface CD40L expressed on activated T cells, engagement of CD40L by CD40 is able to increase IL-4 production by T cells, thereby leading to enhanced germline Cε transcription.[16,17] This may

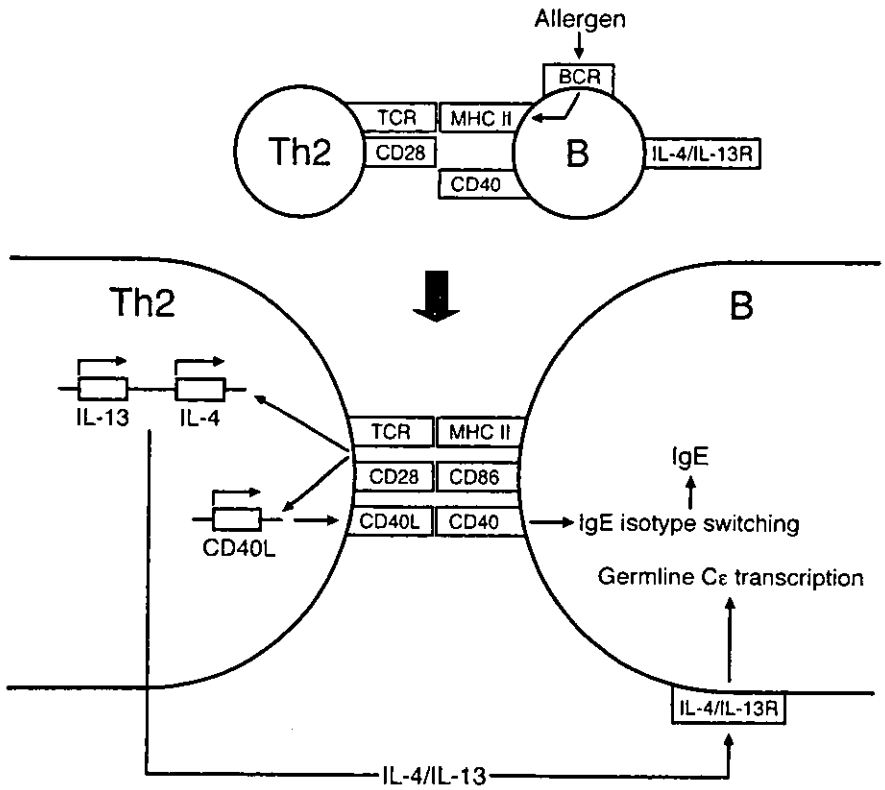

Fig. 2 Induction of allergen-specific IgE synthesis by cognate interactions between B and T cells. BCR, B cell receptor; CD40L, CD40 ligand; IL, interleukin; IL-4/IL-13R, receptor for IL-4 or IL-13; MHC II, major hisotocompatibility complex class II molecules; TCR, α/β T cell receptor.

contribute to upregulation of IgE isotype switching and IgE synthesis.

In addition to CD4+ T cells with a Th2 phenotype, CD8+ T cells with a Tc2 phenotype, CD3+ T cells bearing the γδ T cell receptor, mast cells, basophils and eosinophils produce IL-4 and/or IL-13 and express CD40L after immunologic or non-immunologic stimulation.[18-22] Such cellular responses allow adjacent B cells to induce IgE isotype switching and differentiation into IgE-secreting plasma cells.

More recently, glucocorticoids have been shown to upregulate CD40L expression both in T and B cells.[23] Thus, several cell types are involved in the induction of IgE synthesis, although the production of specific IgE antibody by a given B cell clone is critically dependent on the interaction with an allergen-specific CD4+ T cell. In contrast, other lineage cells may participate mainly in polyclonal IgE production by different B cells.

Molecular mechanisms of IgE isotype switching

A mature B cell can switch the Ig class while retaining the same antigen specificity. This event results from isotype switching that occurs by a DNA rearrangement in the CH (constant region of the heavy chain) gene locus.[3,24] The human CH gene family consists of nine functional genes and two pseudogenes. The organization of the CH locus located at the 3′ side of a given VH segment, a D segment and a JH segment that complete a VH region sequence is as follows: 5′-JH-Cμ-Cδ-Cγ3-Cγ1-Cψε-Cα1-Cψγ-Cγ2-Cγ4-Cε-Cα2-3′ (Fig. 3a). A DNA recombination involved in isotype switching takes place between two switch (S) regions located at the 5′ side of each CH gene, except Cδ and Cψγ.[25] The S regions include Sμ, Sγ, Sα and Sε, each of which is composed of tandem repeats of short unit sequences. Although the Sε region is also present before the Cψε gene, this region is not involved in recombination because of the defect in a part of the exon. Furthermore, the germline IH exons (Iμ, Iγ, Iα and Iε) are located 5′ to each functional S region. With the exception of constitutive activation of the Iμ promoter, the other IH promoter is activated in response to appropriate cytokines, resulting in transcription of the IH exon, the S region and the CH exons. Because transcripts of the S region are spliced out by splicing factors, the resultant IH and CH transcripts are germline CH transcripts, expression of which directs

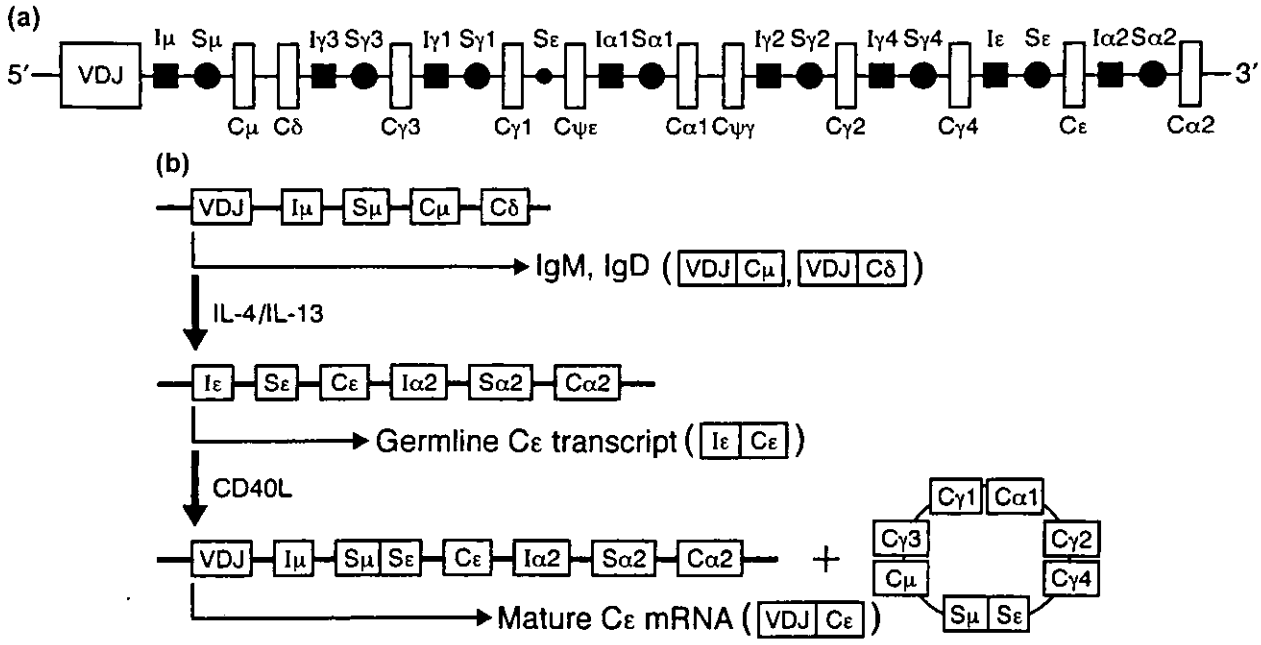

Fig. 3 Molecular events involved in IgE isotype switching. (a) Organization of nine functional genes and two pseudogenes of CH isotypes. The functional Cε gene is located between the Cγ4 gene and the Cα2 gene. (b) Interleukin (IL)-4- or IL-13-dependent germline Cε transcription and CD40 ligand (CD40L)-dependent switching from IgM to IgE. See text for details.

isotype switching by regulating the accessibility of a particular S region to a putative common recombinase system.[5,26] This type of recombination involves the Sμ region as one of the pair and the Sγ, Sα or Sε region is involved as the other partner, resulting in the activation of switch recombination responsible for the induction of switching from Cμ to Cγ, Cα or Cε.

Engagement of CD40 by CD40L in the presence of a particular cytokine plays a crucial role in a given switch recombination.[3,14,27] During recombination, the DNA segment between the expressed *VH* and *CH* genes is looped out as a circle and deleted from the chromosome. Although the switch recombinase has not as yet been identified, activation-induced cytidine deaminase (AID), a B cell-specific RNA-editing enzyme, has recently been reported to be expressed after cytokine and CD40L stimulation and to be involved in regulation or catalysis of the DNA modification step of isotype switching.[28–30] Actually, AID deficiency causes the autosomal recessive form of the hyper-IgM syndrome[30] characterized by defective DNA switch recombination.

During an IgE response, IL-4- or IL-13-dependent induction of germline Cε transcription precedes CD40-mediated Sμ–Sε recombination (Fig. 3b). Activation of the Iε promoter, which contains an IL-4 or IL-13 response element, initiates transcription of the Iε exon, the Sε region and the Cε1–4 exons. Subsequently, splicing cuts the transcript of the Sε region, thereby allowing expression of germline Cε transcripts. Several studies have shown that spliced switch transcripts bind the DNA of the corresponding S region and induce stable RNA/DNA hybrids that are a target for both a ribonuclease and a switch recombinase.[5,6,26] Thus, processing of germline Cε transcripts may be of importance in directing Sμ–Sε recombination.

CD40 ligation not only upregulates IL-4- or IL-13-driven germline Cε transcription due to full activation of the Iε promoter, but also induces expression of AID, which plays a role downstream of the germline Cε transcription. Activation-induced cytidine deaminase expression is followed by activation of the recombination machinery that allows the deletion of the intervening DNA between the Sμ region and the targeted Sε region. This deletional recombination results in the juxtaposition of the Cε gene to the expressed gene of the variable region and in the subsequent induction of mature Cε transcription and IgE synthesis. In addition, alternative splicing is involved in the transition from the membrane to the secreted form of IgE.

Signal transduction of IL-4 and IL-13

The pleiotropic activities of IL-4 and IL-13 in B cells are ascribed to the ability of these cytokines to mediate a diverse array of functions, including induction of germline Cε transcription and enhanced expression of CD23, CD86 and MHC class II molecules.[9–11] Such overlapping activities arise from using the IL-4Rα that forms a heterodimeric complex with either the γc or the IL-13Rα1. Although these three receptor chains lack the intrinsic tyrosine kinase domain, IL-4 and IL-13 induce tyrosine phosphorylation of several cellular proteins. Many cytokines activate members of the Janus kinase (JAK) family, resulting in activation of members of the signal transducer and activator of transcription (STAT) family.[31] The IL-4Rα, γc and IL-13Rα1 associate with JAK1, JAK3 and TYK2, respectively. Ligand binding activates these JAK, which, in turn, induces phosphorylation of STAT-6 recruited to the IL-4Rα. Furthermore, the phosphorylated STAT-6 forms a homodimer via its Src homology (SH) 2 domain, translocates to the nucleus and binds to the consensus sequence present in the promoter regions of the IL-4- or IL-13-responsive genes. These regions include the Iε promoter, activation of which leads to induction of germline Cε transcription. Extensive studies have shown that the Iε promoter contains binding elements for STAT-6, CCAAT/enhancer-binding protein (C/EBP), nuclear factor (NF)-κB and B cell-specific activator protein (BSAP, Pax-5).[32–36] In addition, the upstream NF-κB site overlaps with a binding element for PU.1, a product of the *ets* proto-oncogene family.[36] The essential role of STAT-6 in germline Cε transcription and IgE isotype switching is well established. Interestingly, a genetic variant of IL-4Rα, namely Ile50Val, has been identified in relation to atopic asthma, associates with IL-4 or IL-13 activity and upregulates STAT-6 activation.[37,38]

In addition to the JAK–STAT pathway, other pathways are involved in the activation of the Iε promoter. The IL-4Rα associates with adaptor molecules, such as insulin recepor substrate (IRS)-1, IRS-2, Src homologous and collagen (Shc) and IL-4 receptor-interacting protein (FRIP) and the products of the *fes* proto-oncogene family (FES and FER),[39–42] and these molecules transmit the downstream signaling (Fig. 4). Although FRIP is not expressed in B cells, other molecules are constitutively expressed in many cell types, including B and T cells. Both IRS-1/2 and Shc bind to the insulin/IL-4R (I4R) region of the IL-4Rα. This region contains an Asn-Pro-X-Tyr (NPXY)

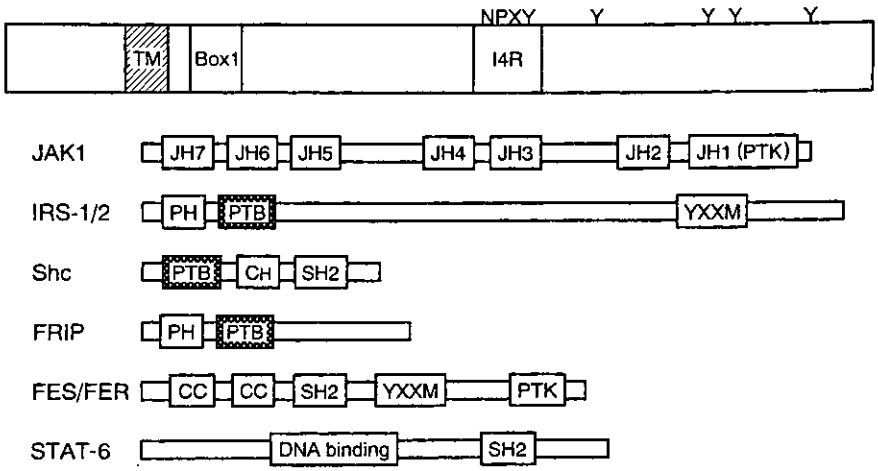

Fig. 4 Association of several molecules with the intracellular domain of the interleukin (IL)-4 receptor α chain. Of these molecules, insulin receptor substrate (IRS), Src homologous and collagen (Shc), IL-4 receptor interaction protein (FRIP) and the products of the *fes* proto-oncogene family (FES/FER) are adaptor molecules containing the phosphotyrosine-binding (PTB) domain that binds to an Asn-Pro-X-Tyr (NPXY) motif present in the insulin/IL-4 receptor (I4R) region. Only FRIP is not expressed in B cells. Both IRS-1/2 and FES/FER contain the conserved Tyr-X-X-M (YXXM) motif to which the Src-homology (SH) 2 domain of phosphatidylinositol 3-kinase binds. The Box1 region is critical for the constitutive association with Janus kinase (JAK) 1 containing the JAK homology (JH) domains and a protein tyrosine kinase (PTK) sequence. CC, coiled-coil region; CH, collagen homology domain; TM, transmembrane region; PH, pleckstrin homology (PH) domain.

motif that specifically interacts with the phosphotyrosine-binding (PTB) domain of IRS-1/2 or Shc.[39,44] In contrast, FES/FER contains the coiled-coil regions that are able to interact with the region located between the Box1 and the I4R.[41] Among these adaptor molecules, IRS-1/2 and FES/FER contain the conserved Tyr-X-X-Met (YXXM) motif to which the SH2 domain of phosphatidylinositol 3-kinase (PI3K) binds. Furthermore, FER, but not FES, is selectively expressed in mature B cells.[41] Although IRS-1/2-dependent activation of PI3K has been most extensively studied in many cell types, FER can mediate PI3K activation in mature B cells, independently of IRS-1/2.[43] Therefore, it is possible that, in B cells, Shc rather than IRS-1/2 preferentially binds to the NPXY motif of the I4R region. This possibility is supported by the finding that recombinant Shc can bind to a phosphopeptide identical to the I4R region, despite the negligible sequence homology between the PTB domain of IRS-1/2 and that of Shc.[44,45] Although Shc mediates activation of phospholipase Cγ1 (PLCγ1) through direct association, the initial production of inositol 1,4,5-trisphosphate is marginal, thus resulting in no significant change in intracellular Ca^{2+} levels.[43] This contrasts with the high and prolonged production of 1,2-diacylglycerol (DAG). Both the lipid product of PI3K activated through FER and DAG generated through Shc-dependent PLCγ1 activate isozymes of protein kinase C (PKC), the substrates of which include transcription factors that cooperate functionally with STAT-6 (Fig. 5).

Isozymes of PKC can be classified into four groups according to endogeneous and exogeneous activators: (i) conventional PKC (α, β1, β2 and γ), which depend on both Ca^{2+} and DAG; (ii) novel PKC (δ, ε, θ and η), which are Ca^{2+} independent and regulated by DAG; (iii) atypical PKC (ζ and ι/λ), which require neither Ca^{2+} nor DAG; and (iv) PKCμ that has a putative transmembrane domain.[46] Moreover, both novel and atypical PKC isozymes are activated by the lipid products of PI3K. Among these, the Ca^{2+}-independent isozymes PKCδ and PKCζ are specifically activated in response to IL-4 or IL-13 and can mediate threonine phosphorylation of PU.1.[24,47] Although binding elements for C/EBP, NF-κB and BSAP are also present in the Iε promotor, none of these transcription factors is susceptible to PKCδ and PKCζ. Thus, two such PKC isozymes appear to regulate transactivation by PU.1. Indeed, activation of the Iε promotor by IL-4 and IL-13 can be blocked not only by dominant negative mutants of PKCδ and PKCζ, but also by isozyme-specific inhibitors rottlerin and PKCζ pseudosubstrate peptide.[47] However, these mutants and inhibitors do not affect tyrosine phosphorylation and DNA

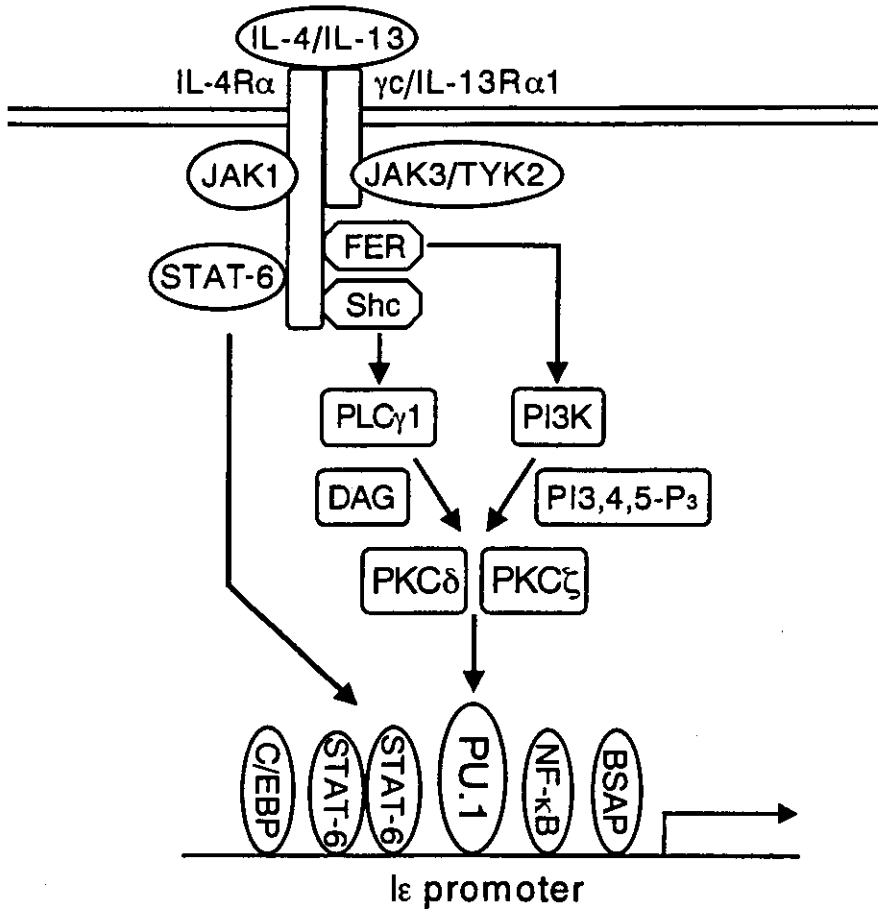

Fig. 5 Signal transduction pathways of interleukin (IL)-4 and IL-13 for the activation of the Iε promoter that results in germline Cε transcription. Ligand binding activates not only the Janus kinase (JAK)-dependent signal transducer and activator of transcription (STAT) pathway, but also the adaptor molecule-dependent pathway. BSAP, B cell-specific activator protein; C/EBP, CCAAT/enhancer-binding protein; DAG, 1,2-diacylglycerol; FER, a product of the *fes* proto-oncogene family; IL-4Rα, IL-4 receptor α chain; IL-13Rα1, IL-13 receptor α1 chain; NF-κB, nuclear factor-κB; PI3,4,5-P_3, phosphatidylinositol 3,4,5-triphosphate; PI3K, phosphatidylinositol 3-kinase; PLCγ1, phospholipase Cγ1; PKC, protein kinase C; PU.1, a product of the *ets* proto-oncogene family; γc, common γ chain; Shc, Src homologous and collagen; TYK2, tyrosine kinase 2.

binding activity of STAT-6. Several lines of evidence support the notion that PU.1, as well as NF-κB, cooparates with STAT-6 for the synergistic activation of the Iε promotor.[34,36,48,49]

Signal transduction through CD40

Ligation of CD40 on B cells upregulates IL-4- or IL-13-driven germline Cε transcription and activates DNA switch recombination that leads to IgE isotype switching, mature Cε transcription and IgE synthesis. The cytoplasmic domain of CD40, which lacks any motifs for transducing signals into B cells, associates not only with two tyrosine kinases (Lyn and JAK3), but also with four members of the six known tumor necrosis factor receptor-associated factor (TRAF) family proteins, namely TRAF2, TRAF3, TRAF5 and TRAF6.[50–53] These molecules can mediate activation of transcription factors, such as STAT-3 and NF-κB. In particular, NF-κB cooperates with STAT-6 and thereby contributes to the increased activity of the Iε promotor. However, none of the known transcription factors activated through CD40 ligation is critical for isotype switching that results from loop-out and deletional recombination. Isotype switching requires AID, expression of which is dependent on a combination of cytokine stimulation and CD40 ligation.[29,30] This novel enzyme appears to play a role upstream of the putative switch recombinase (Fig. 6). Furthermore, Ku70 and Ku80, which form a heterodimer and are associated with CD40, are required to perform switch recombination.[54–56] This heterodimer is dissociated from the CD40 following cytokine stimulation and CD40 ligation, translocates into the nucleus and binds to the DNA-dependent protein kinase. Such a heterotrimeric complex, as well as DNA ligase IV, is involved in the repair of double-strand breaks. Thus, CD40 signaling activates multiple pathways that are important for both the enhancement of germline Cε transcription and the induction of IgE switching.

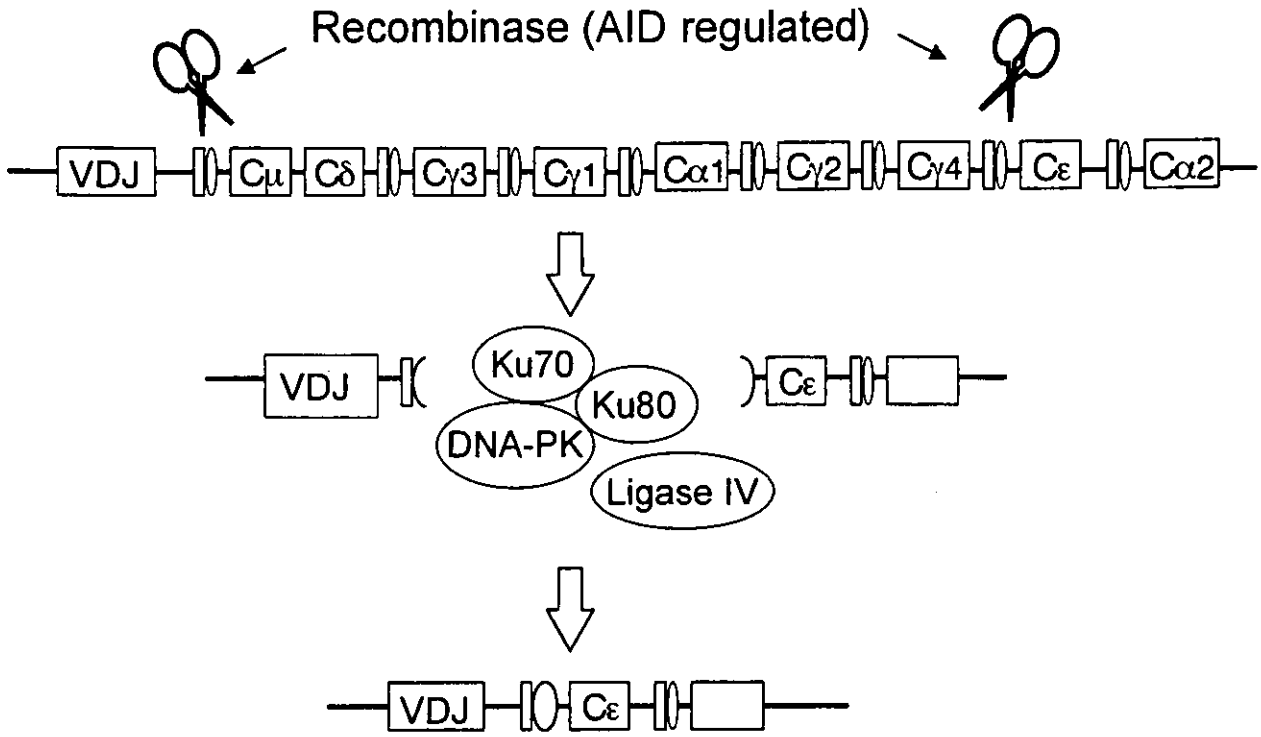

Fig. 6 Involvement of activation-induced cytidine deaminase (AID) in IgE isotype switching. Expression of AID induced by stimulation with interleukin (IL)-4/IL-13 and CD40 ligand is a critical step for the activation of the putative switch recombinase. The same stimulation also induces formation of a heterotrimeric complex composed of Ku70, Ku80 and DNA-dependent protein kinase (PK). This heterotrimer, as well as DNA ligase IV, is involved in the repair of double strand breaks, which is required to perform switch recombination. VDJ, variable–diversity–joining segment.

CD40 ligation-derived signals enhance IL-4- or IL-13-driven germline Cε transcription. One such signal is NF-κB, which synergizes with STAT-6 on the Iε promoter for enhanced DNA-binding affinity.[34,36,48] Among the TRAF proteins associated with CD40, TRAF2, TRAF5 and TRAF6 mediate activation of NF-κB through their ability to bind activators of the IκB kinase complex.[53,57] However, TRAF-dependent NF-κB activation appears to be cell type specific. In B cells, TRAF6 is of importance in activating NF-κB and exerts an enhancing effect on germline Cε transcription.[53] Furthermore, TRAF3 is involved in upregulating germline Cε transcription in a manner that is independent of NF-κB activation. This may be mediated through TRAF3-dependent activation of mitogen-activated protein kinase (MAPK)/extracellular signal-regulated kinase (ERK) kinase 1 (MEK1).[58] Actually, like PD98059, a specific inhibitor of MEK1, selective abrogation of constitutive expression of TRAF3 protein by antisense oligodeoxynucleotide for TRAF3 inhibits CD40-mediated ERK activation, resulting in decreased germline Cε transcription.[58] However, the downstream events that arise from TRAF3-dependent ERK activation remain unclear.

Although at least TRAF3 and TRAF6 are important for CD40-mediated enhancement of germline Cε transcription, none of the TRAF proteins is involved directly in CD40-mediated IgE switching. CD40 ligation also mediates activation of tyrosine kinases, including Lyn and JAK3. The finding that tyrosine kinase inhibitors, such as genistein and herbimycin A, inhibit CD40-mediated isotype switching suggests that tyrosine kinase activity may contribute to the activation of the switch recombination machinery system.[59] However, neither Lyn nor JAK3 is critical in isotype switching, because B cells of JAK3-deficient patients have the ability to switch to IgE and because Lyn, as well as JAK3, is also associated with other receptors.[59] Despite great efforts, the nature of the tyrosine kinase(s) involved in CD40-mediated switch recombination has not yet been defined. It is also currently unclear whether CD40 signaling pathway for the

upregulation of germline Cε transcription and that for the induction of AID expression and IgE switching are overlapping or totally different.

PHARMACOLOGIC REGULATION OF B CELL DIFFERENTIATION INTO IgE-SECRETING PLASMA CELLS

Several attempts have been made to regulate germline Cε transcription and IgE synthesis. Cytokine-dependent induction of germline Cε transcription is inhibited by neutralizing antibodies against IL-4 or IL-13, a soluble form of the IL-4Rα or IL-13Rα1/α2 and a single or double mutant of IL-4.[60,61] In addition to these antibodies and antagonists, agents that prevent IL-4Rα signaling inhibit germline Cε transcription. Such agents include not only interferon (IFN)-γ itself or inducers of IFN-γ production, but also inhibitors of PKCδ and PKCζ that are activated through adaptor molecules associatd with the IL-4Rα.[47,62] Activation of IFN-γR induces expression of a negative regulator of JAK-dependent STAT-6 activation.[62] As for IL-12- or IL-18-dependent IFN-γ production, the predominant expression of a 91 base deletion of the IL-12Rβ2 cDNA or a three base deletion of the IL-18Rα cDNA is associated with reduced IFN-γ production in some allergic patients with high serum IgE levels.[63,64] Thus, therapy with inducers of IFN-γ production will limit their usefulness in allergic patients without such a deletion of the cytokine receptor cDNA. Although abrogation of CD40 signaling can be targeted by inhibiting switch recombination, this abrogation leads to non-specific suppression of isotype switching. Thus, strategies that target the merging point of IL-4Rα and CD40 signaling pathways would be desirable. Because allergic individuals have some B cells that have already switched to IgE *in vivo*, therapy directed towards IgE-expressing B cells also needs to regulate the terminal differentiation into IgE-secreting plasma cells. In this respect, a therapeutic approach using potent IgE-binding agents, such as a soluble form of the high-affinity IgE receptor α subunit (soluble FcεRIα) and anti-IgE antibodies, may be useful in inactivating or eliminating IgE-expressing B cells.

Both soluble FcεRIα and anti-IgE antibodies can selectively modulate IgE synthesis by binding to IgE-expressing B cells.[65,66] Although the membrane-bound form of IgE is a common target for these agents, regulation of IgE synthesis by soluble FcεRIα differs entirely from that by anti-IgE antibodies. For instance, soluble FcεRIα inhibits IgE synthesis via monovalent recognition of membrane IgE, whereas $F(ab')_2$ but not Fab fragments of anti-IgE antibodies have an inhibitory effect.[65,66] The latter finding indicates that inhibition of IgE synthesis by anti-IgE antibodies requires divalent recognition of membrane IgE. Furthermore, there is a marked difference between mechanisms for soluble FcεRIα- or anti-IgE antibody-induced inhibition of IgE synthesis.[67] Binding of soluble FcεRIα to IgE-expressing B cells leads to a decrease in the autocrine production of IL-6, which provides a late amplification signal for IgE synthesis.[67] In contrast, anti-IgE antibodies or their $F(ab')_2$ fragments induce apoptosis in IgE-expressing B cells, although neither their Fab fragments nor soluble FcεRIα have such apoptotic activity. Thus, cross-linking of membrane IgE is able to induce apoptosis, which accords with a report describing that anti-IgE antibodies were effective in downregulating expression of Bcl-2, known to inhibit apoptotic cell death.[68] These data suggest that both soluble FcεRIα and anti-IgE antibodies may be useful in inhibiting the terminal differentiation of IgE-expressing B cells, including memory cells, into IgE-secreting plasma cells. In particular, non-anaphylactogenic humanized or chimeric anti-IgE monoclonal antibodies have been produced that bind to free IgE and membrane IgE but not to IgE bound to the cell surfact FcεRI.[69–72] These properties are similar to those of soluble FcεRIα, which traps IgE via its Cε3 domain responsible for receptor binding.

CONCLUSIONS

Allergen-specific IgE synthesis contributes to the induction and maintenance of allergic symptoms. The expotential increase in the understanding of the cellular mechanisms of IgE synthesis has led to the development and clinical trials of agents capable of regulating the differentiation of B cells into IgE-secreting plasma cells. However, the molecular mechanisms involved in IgE isotype switching are incompletely understood. Although AID appears to be an essential part of the switch recombination machinery, the switch recombinase has not, as yet, been identified. Elucidation of the merging point of IL-4Rα and CD40 signaling pathways that are required for AID expression and IgE switching, as well as identification and characterization of the switch recombinase, are the next challenge for future studies and should provide potential new strategies for the isotype-specific regulation of IgE synthesis.

REFERENCES

1 Schwartz RS. Jumping genes and the immunoglobulin V gene system. *N. Engl. J. Med.* 1995; **333**: 42–4.
2 Schwarz K, Gauss GH, Ludwig L *et al.* RAG mutations in human B cell-negative SCID. *Science* 1996; **274**: 97–9.
3 Vercelli D, Geha RS. Regulation of isotype switching. *Curr. Opin. Immunol.* 1992; **4**: 794–7.
4 Kelsoe G. V (D) J hypermutation and receptor revision: Coloring outside the lines. *Curr. Opin. Immunol.* 1999; **11**: 70–5.
5 Lorenz M, Jung S, Radbruch A. Switch transcripts in immunoglobulin class switching. *Science* 1995; **267**: 1825–8.
6 Stavnezer J. Immunoglobulin class switching. *Curr. Opin. Immunol.* 1996; **8**: 199–205.
7 Fujieda S, Lin YQ, Saxon A, Zhang K. Multiple types of chimeric germ-line Ig heavy chain transcripts in human B cells: Evidence for trans-splicing of human Ig RNA. *J. Immunol.* 1996; **157**: 3450–9.
8 Kondo M, Takeshita T, Ishii N *et al.* Sharing of the interleukin-2 (IL-2) receptor γ chain between receptors for IL-2 and IL-4. *Science* 1993; **262**: 1874–7.
9 Hilton DJ, Zhang JG, Metcalf D, Alexander WS, Nicola NA, Wilson TA. Cloning and characterization of a binding subunit of the interleukin 13 receptor that is also a component of the interleukin 4 receptor. *Proc. Natl Acad. Sci. USA* 1996; **93**: 497–501.
10 Gauchat JF, Schlagenhauf E, Feng NP *et al.* A novel 4 kb interleukin-13 receptor α mRNA expressed in human B, T, and endothelial cells encoding an alternate type-II interleukin-4/interleukin-13 receptor. *Eur. J. Immunol.* 1997; **27**: 971–8.
11 Matthews DJ, Clark PA, Herbert J *et al.* Function of the interleukin-2 (IL-2) receptor γ-chain in biologic responses of X-linked severe combined immunodeficient B cells to IL-2, IL-4, IL-13, and IL-15. *Blood* 1995; **85**: 38–42.
12 Izuhara K, Heike T, Otsuka T *et al.* Signal transduction pathway of interleukin-4 and interleukin-13 in human B cells derived from X-linked severe combined immunodeficiency patients. *J. Biol. Chem.* 1996; **271**: 619–22.
13 Tsubata T, Wu J, Honjo T. B-cell apoptosis induced by antigen receptor crosslinking is blocked by a T-cell signal through CD40. *Nature* 1993; **364**: 645–8.
14 Banchereau J, Bazan F, Blanchard D *et al.* The CD40 antigen and its ligand. *Annu. Rev. Immunol.* 1994; **12**: 881–922.
15 Allen RC, Armitage RJ, Conley ME *et al.* CD40 ligand gene defects responsible for X-linked hyper-IgM syndrome. *Science* 1993; **259**: 990–3.
16 Blotta MH, Marshall JD, DeKruyff RH, Umetsu DT. Cross-linking of the CD40 ligand on human $CD4^+$ T lymphocytes generates a costimulatory signal that up-regulates IL-4 synthesis. *J. Immunol.* 1996; **156**: 3133–40.
17 Koshio T, Kajiwara K, Ikizawa K, Nakagami K, Yanagihara Y. Blocking the CD154–CD40 interaction with anti-CD154 antibody differentially regulates interleukin-4 synthesis in T cells and IgE production in B cells. *Allergol. Int.* 2001; **50**: 35–41.
18 Paganelli R, Scala E, Ansotegui IJ *et al.* CD8+ T lymphocytes provide helper activity for IgE synthesis in human immunodeficiency virus-infected patients with hyper-IgE. *J. Exp. Med.* 1995; **181**: 423–8.
19 Horner AA, Jabara H, Ramesh N, Geha RS. γ/δ T lymphocytes express CD40 ligand and induce isotype switching in B lymphocytes. *J. Exp. Med.* 1995; **181**: 1239–44.
20 Gauchat JF, Henchoz S, Fattah D *et al.* CD40 ligand is functionally expressed on human eosinophils. *Eur. J. Immunol.* 1995; **25**: 863–5.
21 Pawankar R, Okuda M, Yssel H, Okumura K, Ra C. Nasal mast cells in perennial allergic rhinitics exhibit increased epression of the FcεRI, CD40L, IL-4, and IL-13, and can induce IgE synthesis in B cells. *J. Clin. Invest.* 1997; **99**: 1492–9.
22 Yanagihara Y, Kajiwara K, Basaki Y *et al.* Cultured basophils but not cultured mast cells induce human IgE synthesis in B cells after immunologic stimulation. *Clin. Exp. Immunol.* 1998; **111**: 136–43.
23 Jabara HH, Brodeur SR, Geha RS. Glucocorticoids upregulate CD40 ligand expression and induce CD40L-dependent immunoglobulin isotype switching. *J. Clin. Invest.* 2001; **107**: 371–8.
24 Yanagihara Y. Molecular regulation of human IgE synthesis. *Allergol. Int.* 1999; **48**: 111–19.
25 Kinoshita K, Tashiro J, Tomita S, Lee CG, Honjo T. Target specificity of immunoglobulin class switch recombination is not determined by nucleotide sequences of S regions. *Immunity* 1998; **9**: 849–58.
26 Tracy RB, Hsieh CL, Lieber MR. Stable RNA/DNA hybrids in the mammalian genome: Inducible intermediates in immunoglobulin class switch recombination. *Science* 2000; **288**: 1058–61.
27 van Essen D, Kikutani H, Gray D. CD40 ligand-transduced co-stimulation of T cells in the development of helper function. *Nature* 1995; **378**: 620–3.
28 Muramatsu M, Sankaranand VS, Anant S *et al.* Specific expression of activation-induced cytidine deaminase (AID), a novel member of the RNA-editing deaminase family in germinal center B cells. *J. Biol. Chem.* 1999; **274**: 18 470–6.
29 Muramatsu M, Kinoshita K, Fagarasan S, Yamada S, Shinkai Y, Honjo T. Class switch recombination and somatic hypermutation require activation-induced cytidine deaminase (AID), a potential RNA editing enzyme. *Cell* 2000; **102**: 553–63.
30 Revy P, Muto T, Levy Y *et al.* Activation-induced cytidine deaminase (AID) deficiency causes the autosomal recessive form of the hyper-IgM syndrome (HIGM2). *Cell* 2000; **102**: 565–75.
31 Heim MH, Kerr IM, Stark GR, Darnell Jr JE. Contribution of STAT SH2 groups to specific interferon signaling by the Jak–STAT pathway. *Science* 1995; **267**: 1347–9.
32 Albrecht B, Peiritsch S, Woisetschlager M. A bifunctional control element in the human IgE germline promoter involved in repression and IL-4 activation. *Int. Immunol.* 1994; **6**: 1143–51.

33 Thienes CP, De Monte L, Monticelli S, Busslinger M, Gould HJ, Vercelli D. The transcription factor B cell-specific activator protein (BSAP) enhances both IL-4- and CD40-mediated activation of the human ε germline promoter. *J. Immunol.* 1997; **158**: 5874–82.

34 Messner B, Stütz AM, Albrecht B, Peiritsch S, Woisetschäger M. Cooperation of binding sites for STAT6 and NF-κB/rel in the IL-4-induced up-regulation of the human IgE germline promoter. *J. Immunol.* 1997; **159**: 3330–7.

35 Mikita T, Kurama M, Schindler U. Synergistic activation of the germline ε promoter mediated by Stat6 and C/EBPb. *J. Immunol.* 1998; **161**: 1822–8.

36 Stütz AM, Woisetschäger M. Functional synergism of STAT6 with either NF-κB or PU.1 to mediate IL-4-induced activation of IgE germline gene transcription. *J. Immunol.* 1999; **163**: 4383–91.

37 Mitsuyasu H, Izuhara K, Mao XQ *et al.* Ile50Val variant of IL-4Rα upregulates IgE synthesis and associates with atopic asthma. *Nat. Genet.* 1998; **19**: 119–20.

38 Mitsuyasu H, Yanagihara Y, Mao XQ *et al.* Dominant effect of Ile50Val variant of the human IL-4 receptor α-chain in IgE synthesis. *J. Immunol.* 1999; **162**: 1227–31.

39 Keegan AD, Nelms K, White M, Wang LM, Pierce JH, Paule WE. An IL-4 receptor region containing an insulin receptor motif is important for IL-4-mediated IRS-1 phosphorylation and cell growth. *Cell* 1994; **76**: 811–20.

40 Patti ME, Sun XJ, Bruening JC *et al.* 4PS/insulin receptor substrate (IRS)-2 is the alternative substrate of the insulin receptor in IRS-1-deficient mice. *J. Biol. Chem.* 1995; **270**: 24 670–3.

41 Izuhara K, Feldman RA, Greer P, Harada N. Interleukin-4 induces association of the c-*fes* proto-oncogene product with phosphatidylinositol-3 kinase. *Blood* 1996; **88**: 3910–18.

42 Nelms K, Snow AL, Hu-Li J, Paul WE. FRIP, a hematopoietic cell-specific rasGAP-interacting protein phosphorylated in response to cytokine stimulation. *Immunity* 1998; **9**: 13–24.

43 Ikizawa K, Yanagihara Y. Possible involvement of Shc in IL-4-induced germline ε transcription in a human B cell line. *Biochem. Biophys. Res. Commun.* 2000; **268**: 54–9.

44 Wolf G, Trüb T, Ottinger E *et al.* PTB domains of IRS-1 and Shc have distinct but overlapping binding specificities. *J. Biol. Chem.* 1995; **270**: 27 407–10.

45 Zhou MM, Huang B, Olenjniczak ET *et al.* Structural basis for IL-4 receptor phosphopeptide recognition by the IRS-1 PTB domain. *Nat. Struct. Biol.* 1996; **3**: 388–93.

46 Newton AC. Regulation of protein kinase C. *Curr. Opin. Cell. Biol.* 1997; **9**: 161–7.

47 Ikizawa K, Kajiwara K, Izuhara K, Yanagihara Y. PKCδ and ζ mediate IL-4/IL-13-induced germline ε transcription in human B cells: A putative regulation via PU.1 phosphorylation. *Biochem. Biophys. Res. Commun.* 2001; **288**: 34–41.

48 Shen CH, Stavnezer J. Interaction of Stat6 and NF-κB: Direct association and synergistic activation of interleukin-4-induced transcription. *Mol. Cell. Biol.* 1998; **18**: 3395–404.

49 Pesu M, Takaluoma K, Aittomaki S *et al.* Interleukin-4-induced transcriptional activation by Stat6 involves multiple serine/threonine kinase pathways and serine phosphorylation of Stat6. *Blood* 2000; **95**: 494–502.

50 Ren CL, Morio T, Fu SM, Geha RS. Signal transduction via CD40 involves activation of lyn kinase and phosphatidylinositol-3-kinase, and phosphorylation of phospholipase Cγ2. *J. Exp. Med.* 1994; **179**: 673–80.

51 Hanissian SH, Geha RS. Jak3 is associated with CD40 and is critical for CD40 induction of gene expression in B cells. *Immunity* 1997; **6**: 379–87.

52 Pullen SS, Miller HG, Everdeen DS, Dang TT, Crute JJ, Kehry MR CD40-tumor necrosis factor receptor-associated factor (TRAF) interactions. Regulation of CD40 signaling through multiple TRAF binding sites and TRAF heterooligomerization. *Biochemistry* 1998; **37**: 11 836–45.

53 Bradley JR, Pober JS. Tumor necrosis factor receptor-associated factors (TRAFs). *Oncogene* 2001; **20**: 6482–91.

54 Manis JP, Gu Y, Lansford R *et al.* Ku70 is required for late B cell development and immunoglobulin heavy chain class switching. *J. Exp. Med.* 1998; **187**: 2081–9.

55 Casellas R, Nussenzweig A, Wuerffel R *et al.* Ku80 is required for immunoglobulin isotype switching. *EMBO J.* 1998; **17**: 2404–11.

56 Morio T, Hanissian SH, Bacharier LB *et al.* Ku in the cytoplasm associates with CD40 in human B cells and translocates into the nucleus following incubation with IL-4 and anti-CD40 mAb. *Immunity* 1999; **11**: 339–48.

57 Dadgostar H, Cheng G. An intact zinc ring finger is required for tumor necrosis factor receptor-associated factor-mediated nuclear factor-κB activation but is dispensable for c-Jun N-terminal kinase signaling. *J. Biol. Chem.* 1998; **273**: 24 775–80.

58 Basaki Y, Ikizawa K, Kajiwara K, Yanagihara Y. CD40-mediated tumor necrosis factor receptor-associated factor 3 signaling upregulates IL-4-induced germline Cε transcription in a human B cell line. *Arch. Biochem. Biophys.* 2002; **405**: 199–204.

59 Bacharier LB, Jabara H, Geha RS. Molecular mechanisms of immunoglobulin E regulation. *Int. Arch. Allergy Immunol.* 1998; **115**: 257–69.

60 Holgate ST. Asthma therapy in the new millennium. *Allergol. Int.* 2000; **49**: 231–6.

61 Chung KF. Current and potential improvements in the treatment of asthma from increased understanding of airway pathophysiology. *Allergol. Int.* 2002; **51**: 153–66.

62 Yoshimura A. The CIS family: Negative regulators of JAK–STAT signaling. *Cytokine Growth Factor Rev.* 1998; **9**: 197–204.

63 Matsui E, Kaneko H, Fukao T *et al.* Mutations of the IL-12 receptor β2 chain gene in atopic subjects. *Biochem. Biophys. Res. Commun.* 1999; **266**: 551–5.

64 Watanabe M, Kaneko H, Shikano H *et al.* Predominant expression of 950delCAG of IL-18R α chain cDNA is associated with reduced IFN-gamma production and high serum IgE levels in atopic Japanese children. *J. Allergy Clin. Immunol.* 2002; **109**: 669–75.

65 Yanagihara Y, Kajiwara K, Ikizawa K, Koshio T, Okumura K, Ra C. Recombinant soluble form of the human high-affinity immunoglobulin E (IgE) receptor inhibits IgE production through its specific binding to IgE-bearing B cells. *J. Clin. Invest.* 1994; **94**: 2162–5.

66 Stämpfli MR, Miescher S, Aebischer I, Zürcher AW, Stadler BM. Inhibition of human IgE synthesis by anti-IgE antibodies requires divalent recognition. *Eur. J. Immunol.* 1994; **24**: 2161–7.

67 Kajiwara K, Ra C, Yanagihara Y. Recombinant soluble form of the high-affinity IgE receptor α subunit and anti-IgE antibody inhibit IgE synthesis by IgE-expressing B cells through distinct pathways. *Allergol. Int.* 2002; **51**: 175–84.

68 Stadler BM, Stümpfli MR, Miescher S, Rudolf M, Vogel M. Cloning of human anti-IgE autoantibodies and their role in the regulation of IgE synthesis. *Int. Arch. Allergy Immunol.* 1995; **107**: 48–50.

69 Casale TB, Bernstein IL, Busse WW *et al.* Use of an anti-IgE humanized monoclonal antibody in ragweed-induced allergic rhinitis. *J. Allergy Clin. Immunol.* 1997; **100**: 110–21.

70 Corne J, Djukanovic R, Thomas L *et al.* The effect of intravenous administration of a chimeric anti-IgE antibody on serum IgE levels in atopic subjects: Efficacy, safety, and pharmacokinetics. *J. Clin. Invest.* 1997; **99**: 879–87.

71 Fahy JV, Fleming HE, Wong HH *et al.* The effect of an anti-IgE monoclonal antibody on the early- and late-phase responses to allergen inhalation in asthmatic subjects. *Am. J. Respir. Crit. Care Med.* 1997; **155**: 1828–34.

72 Soler M, Matz J, Townley R *et al.* The anti-IgE antibody omalizumab reduces exacerbations and steroid requirement in allergic asthmatics. *Eur. Respir. J.* 2001; **18**: 254–61.

喘息におけるケミカルメディエーター受容体の遺伝子多型

Genetic Variants of Chemical Mediator Receptors in Relation to Asthma

はじめに

喘息の発症や病態形成には環境要因に加えて，遺伝要因も関与している．遺伝要因に関しては，全ゲノムに存在する一塩基多型(SNP)を用いて喘息関連遺伝子の同定が試みられている．喘息はTh2優位な疾患であることから，今までにIL-4/IL-13とその受容体の遺伝的多型がアトピー素因や喘息と関連している結果が示されている[1)-4)]．IL-4/IL-13は気道の上皮細胞や平滑筋細胞などの組織構成細胞にも作用するので，両サイトカインは粘液過分泌，過敏性亢進およびリモデリングなどの病態形成にも関与していることが近年明らかにされつつある．また，各種のケミカルメディエーター受容体の発現調節にもIL-4/IL-13が関与していることを示唆する知見も得られつつある．

本稿では，各種ケミカルメディエーター受容体の遺伝子多型と喘息との関連およびIL-4/IL-13によって発現調節されるケミカルメディエーター受容体や酵素などについて，最近の知見を中心に述べてみたい．

Ⅰ．ケミカルメディエーター受容体

喘息の病態修飾因子である各種ケミカルメディエーター受容体の発現部位・作用については**表1**に，またこれらの受容体の遺伝子多型については**表2**に示した．

1) ヒスタミン受容体

ヒスタミン受容体(HR)にはH1R-H4Rの4種類が同定されている．これらのうち，H1Rがヒスタミンによる気道収縮，粘液分泌亢進および血管透過性亢進などの反応に関与していることはよく知られている．気道平滑筋はH1R，H2RおよびH4Rを発現しているが，いずれの発現もIL-4/IL-13刺激によって影響を受けない．一方，T細胞に関しては，ヒスタミンはH1Rの活性化を介してTh1反応を促進するが，H2Rの活性化はTh1とTh2の両反応を抑制する[5)]．H1RとH2Rの遺伝子はそれぞれ3p25と5q35に存在し，日本人においてはH1Rの-17C/T多型や1045G/A多型が報告されている[6)]．また，H2Rにもいくつかの SNPs が同定されているが，両遺伝子と喘息との関連は認められていない．しかし，3p25の染色体座位は日本人ではアトピー素因と関連していることが示されているマーカーの1つでもあるので[7)]，H1RやH2Rの遺伝的多型は喘息よりもむしろアトピーと連鎖していると考えられる．H4R遺伝子(18q11)に関しては，喘息やアトピーとの関連を検討した報告はまだみられない．

2) システイニルロイコトリエン受容体

システイニルロイコトリエン受容体(CysLTR)にはCysLT1RとCysLT2Rの2種類が同定されている．LTC_4やLTD_4などのCysLTsによる気道収縮作用，血管透過性亢進作用および粘液分泌亢進作用は，LT1Rを介して発現され，実際臨床においても治療薬としてLT1R拮抗薬が用いられている．気道平滑筋細胞はCysLT1RとCysLT2Rを発現しているが，IL-4/IL-13はCysLT1Rの発現を選択的に増強する(山本ら，投稿中)．この増強作用が機能的であることについては，LTD_4による細胞内Ca^{2+}濃度がさらに増加することからも確認できる．また，IL-13にはLTD_4による気道平滑筋細胞の増殖促進作用も報告されている[8)]．一方，CysLT2Rは抗炎症シグナルを媒介すると考えられているが，その詳細については不明な点が多い．CysLT1RやCysLT2Rは喘息の病態修飾