Shimizu M, Matsuda A, Yanagisawa K, Hirota T, Akahoshi M, Inomata N, Ebe K, Tanaka K, Sugiura H, Nakashima K, Tamari M, Takahashi N, Obara K, Enomoto T, Okayama Y, Gao PS, Huang SK, Tominaga S, Ikezawa Z, Shirakawa T	Functional SNPs in the distal promoter of the *ST2* gene are associated with atopic dermatitis.	**Hum Mol Genet.**	14(19)	2919-27	2005
Matsuda A, Hirota T, Akahoshi M, Shimizu M, Tamari M, Miyatake A, Takahashi A, Nakashima K, Takahashi N, Obara K, Yuyama N, Doi S, Kamogawa Y, Enomoto T, Ohshima K, Tsunoda T, Miyatake S, Fujita K, Kusakabe M, Izuhara K, Nakamura Y, Hopkin J, Shirakawa T.	Coding SNP in tenascin-C Fn-III-D domain associates with adult asthma.	**Hum Mol Genet.**	14(19)	2779-2786	2005
Meng J, Thongngarm T, Nakajima M, Yamashita N, Ohta K, Bates CA, Grunwald GK, Rosenwasser LJ	Association of transforming growth factor- β 1 single nucleotide polymorphism C-509T with allergy and immunological activities.	**Int Arch Allergy Immunol.**	138(2)	151-60	2005

研究成果の刊行に関する一覧表（平成 18 年度）

発表者氏名	論文タイトル	発表誌名	巻号	ページ	出版年
Kaneko H, Matsui E, Shinoda S, Kawamoto N, Nakamura Y, Uehara R, Matsuura, N Morita M, Tada H, Kondo N.	Effects of dioxins on the quantitative levels of immune components in infants.	Tox Ind Health.	22	131-136	2006
Kaneko H, Matsui E, Asano T, Kato Z, Teramoto T, Aoki M, Kawamoto N, Lian LA, Kasahara K, Kondo N.	Suppression of IFN-gamma production in atopic group at the acute phase of RSV infection.	Pediatr Allergy Immunol.	17	370-375	2006
Kato Z, Asano T, Kondo N.	Inosiplex affects the spectra of proton magnetic resonance spectroscopy in subacute sclerosing panencephalitis.	J Child Neurol.	21	177-178	2006
Kaneko H, Isogai K, Kondo M, Hosoi K, Asono T, Funato M, Kondo N.	Autologous peripheral blood stem cell transplantation in a patient with relapsed pleuropulmonary blastoma.	J Pediatr Hematol Oncol.	28	383-385	2006
Teramoto T, Fukao T, Tomita Y, Terauchi Y, Hosoi K, Matsui E, Aoki M, Kondo N, Mikawa H.	Pharmacokinetics of Beclomethasone Dipropionate in an Hydrofluoroalkane-134a Propellant System in Japanese Children with Bronchial Asthma.	Allergology International	55	317-320	2006
Kondo N, Katsunuma T, Odajima Y, Morikawa A.	A Randomized Open-Label Comparative Study of Montelukast Versus Theophlline Added to Inhaled Corticosteroid in Asthmatic Children.	Allergology International.	55	287-293	2006
松井永子、金子英雄、深尾敏幸、寺本貴英、近藤直実	気管支喘息領域におけるオーダーメイド治療と遺伝子多型	International Review of Asthma	8	64-72	2006
釣木澤尚実、秋山一男	小児喘息の成人へのキャリーオーバーの予防	小児科	48	25-35	2007
釣木澤尚実、秋山一男、他	成人喘息受診中断例の予後の検討一無治療無症状継続群と有症状群との比較検討	アレルギー	55	115-125	2006
Meng J, Thongngarm T, Nakajima M, Yamashita N, Ohta K, Bates CA, Grunwald GK, Rosenwasser LJ.	Association of Transforming Growth Factor-beta1 Single Nucleotide Polymorphism C-509T with Allergy and Immunological Activities.	Int Arch Allergy Immunol.	138	151-160	2005

発表者氏名	論文タイトル	発表誌名	巻号	ページ	出版年
Yamashita N, Tashimo H, Matsuo Y, Ishida H, Yoshiura K, Sato K, Yamashita N, Kakiuchi T, Ohta K.	Role of CCL21 and CCL19 in allergic inflammation in the ovalbumin-specific murine asthmatic model.	J Allergy Clin Immunol.	117	1040-1046	2006
Kobayashi M, Nasuhara Y, Kamachi A, Tanino Y, Betsuyaku T, Yamaguchi E, Nishihira J, Nishimura M.	Role of macrophage migration inhibitory factor in ovalbumin-induced asthma in rats.	Eur Respir J	27	1-9	2006
Maeda Y, Hizawa N, Jinushi E, Honda A, Takahashi D, Fukui Y, Konno S, Shimizu T, Shimizu H, Yamaguchi E, Nishimura M.	Polymorphisms in the muscarinic receptor 1 gene confer susceptibility to asthma in Japanese subjects.	Am J Respir Crit Care Med.	174	1119-1124	2006
Motohiro Ebisawa.	Management of Food Allergy : "Food Allergy Management Guideline 2005"	by National Food Allergy Research Group Supported by the Ministry of Health, Welfare, and Labor : Korea Journal of Asthma, Allergy and Clinical Immunology	26	177-185	2006
海老澤元宏	食物アレルギーへの対応について －厚生労働科学研究班による「食物アレルギーの診療の手引き 2005」－	アレルギー	55	107-114	2006
池松かおり、田知本寛、杉崎千鶴子、宿谷明紀、海老澤元宏	乳児期発症食物アレルギーに関する検討（第１報）－乳児アトピー性皮膚炎と食物アレルギーの関係－	アレルギー	55	140-150	2006
池松かおり、田知本寛、杉崎千鶴子、宿谷明紀、海老澤元宏	乳児期発症食物アレルギーに関する検討（第２報）－卵・牛乳・小麦・大豆アレルギーの３歳までの経年的変化－	アレルギー	55	533-541	2006
池田有希子、今井孝成、杉崎千鶴子、田知本寛、宿谷明紀、海老澤元宏	食物アレルギー除去食中の保護者に対する食生活のＱＯＬ調査および食物アレルギー児の栄養評価	日本小児アレルギー学会誌	20	119-126	2006
海老澤元宏	誤解されやすい子どものアレルギー 食物アレルギーの正しい診断に向けて －厚生労働科学研究班による「食物アレルギーの診療の手引き 2005」－	小児保健研究	65	165-170	2006

発表者氏名	論文タイトル	発表誌名	巻号	ページ	出版年
海老澤元宏、今井孝成	食物アレルギー診療ガイドライン2005 解説（I）	日本小児アレルギー学会誌	20	178-180	2006
向山徳子、西間三馨、有田昌彦、伊藤節子、宇理須厚雄、海老澤元宏、小倉英郎、河野陽一、近藤直実、柴田瑠美子、古庄巻史、眞弓光文（日本小児アレルギー学会食物アレルギー委員会）	食物アレルギー診療ガイドライン	日本小児科学会雑誌	110	904-911	2006
井口正道、宿谷明紀、小俣貴嗣、田知本寛、海老澤元宏	入院加療した食物アレルギー合併乳児重症アトピー性皮膚炎患者に関する検討（第1報）	日本小児科学会雑誌	110	1534-1539	2006
井口正道、宿谷明紀、小俣貴嗣、田知本寛、海老澤元宏	入院加療した食物アレルギー合併乳児重症アトピー性皮膚炎患者に関する検討（第2報）	日本小児科学会雑誌	110	1540-1544	2006
杉井京子、田知本寛、宿谷明紀、鈴木誠、海老澤元宏	小児の口腔アレルギー症候群（Oral Allergy Syndrome）と、小児アレルギー疾患患児の各種花粉への感作状況	アレルギー	55	1400-1408	2006
富川盛光、鈴木直仁、宇理須厚雄、粒来崇博、伊藤節子、柴田瑠美子、伊藤浩明、海老澤元宏	日本における小児から成人のエビアレルギーの臨床像に関する検討	アレルギー	55	1536-1542	2006
Tamaki K, Kakinuma T, Saeki H, Horikawa T, Kataoka Y, Fujisawa T, et al.	Serum levels of CCL17 / TARC in various skin diseases.	J Dermatol	33	300-302	2006
藤澤隆夫	乳幼児のケモカインーアレルギー疾患発症メカニズムとのかかわり	アレルギー科	21	612-616	2006
藤澤隆夫	小児における上気道アレルギーと下気道アレルギー	三重県小児科医会報	71	20-23	2006
長尾みづほ、藤澤隆夫	DSCG の適応と使い方	小児アレルギーシリーズ「喘息」		106-110	2006
藤澤隆夫	吸入ステロイド薬投与による局所副作用	Pharma Medica	24 (Suppl)	55-60	2006
藤澤隆夫	乳幼児喘息治療の新しい展開	大宮医師会報	610	58-61	2007

Ⅳ. 研究成果の刊行物・別冊

Clin Exp Allergy 2004; 34:363–368

RNA editing of interleukin-12 receptor β2, 2451 C-to-U (Ala 604 Val) conversion, associated with atopy

N. Kondo, E. Matsui, H. Kaneko, M. Aoki, Z. Kato, T. Fukao, K. Kasahara and N. Morimoto
Department of Pediatrics, Gifu University School of Medicine, Tsukasa-machi 40, Gifu 500-8705, Japan

Summary

Background The production of IgE in B lymphocytes is down-regulated by IFN-γ. IL-12 induces IFN-γ production by T lymphocytes and natural killer cells by binding to its specific receptor. RNA editing is a post-transcriptional modification.
Objective Here we show that the RNA editing of IL-12 receptor (R) β2 is associated with atopy.
Methods Atopic patients and non-atopic healthy controls were studied. Fragments of IL-12R β2 cDNA and genomic DNA were amplified and sequenced. Furthermore, the function of the IL-12R β2 chain was investigated.
Results Sequence analysis of the cDNA clones representing IL-12R β2 mRNA transcripts revealed a C-to-U conversion at nucleotide 2451 (Ala 604 Val) on exon 13 in some atopic patients. Surprisingly, sequence analysis of their genomic DNA showed no 2451 C-to-T (Ala 604 Val) mutation. We concluded that the observed C-to-U mismatch in the cDNA clone is due to a post-transcriptional modification, RNA editing. The C-to-U conversion was observed in 21 (20.6%) of 102 atopic patients, whereas this conversion was observed in only 4 (3.8%) of 104 non-atopic subjects ($P<0.001$). IFN-γ production by peripheral blood mononuclear cells (PBMCs) stimulated with IL-12 in the subjects with the C-to-U conversion was significantly lower than that in the subjects without the C-to-U conversion. In atopic patients with the C-to-U conversion, PBMCs faintly showed the tyrosine phosphorylation of Stat4, and the IgE production by PBMCs was not suppressed by IL-12 whereas it was suppressed by IFN-γ.
Conclusions The RNA editing of IL-12R β2, 2451 C-to-U (Ala 604 Val) conversion causes impairment of the IL-12 signal cascade and the subsequent reduction in IFN-γ production, resulting in the impaired down-regulation of IgE production. This is the first report indicating that atopy is associated with RNA editing.

Keywords atopy, IL-12 receptor β2, RNA editing
Submitted 30 December 2002; revised 8 August 2003; accepted 17 November 2003

Introduction

Atopy is characterized by enhanced immunoglobulin E (IgE) responses to common environmental antigens and leads to clinical disorders such as asthma, eczema and rhinitis. IL-4 promotes a class switch to IgE in B lymphocytes and Th2 $CD4^{+}$T lymphocyte differentiation [1]. IgE production by B lymphocytes is down-regulated by IFN-γ that is one of the Th1 cytokines [1]. IL-12 induces IFN-γ production by T lymphocytes and natural killer (NK) cells by binding to its specific receptor [2–4].

The receptor of IL-12 is composed of two distinct subunits, β1 and β2, that assemble to form a high-affinity IL-12 receptor (R) complex [5]. While the β2 chain of the IL-12R is expressed only in Th1 lymphocytes, the β1 chain is expressed in both Th1 and Th2 lymphocytes. Thus, the expression of both β1 and β2 chains accounts for the responsiveness of T lymphocytes to IL-12 and mediates Th1 lymphocyte differentiation [6]. On binding to its receptor, IL-12 induces activation of specific members of the Stat family of transcription factors, which then translocate to the nucleus and bind to genomic promoter regions. Stat4 is particularly important in this respect, since Stat4-deficient mice manifest impaired IFN-γ production [7]. Furthermore, the phenotype of the IL-12p40-deficient mouse is similar to that of the Stat4-deficient mouse [8]. We reported that reduced IFN-γ production by peripheral blood mononuclear cells (PBMCs) following stimulation with IL-12 but not with phytohemagglutinin (PHA) is associated with the heterozygous IL-12 β2 chain gene mutations, 1577 A-to-G (Arg 313 Gly), 2496 del 91, and 2799 A-to-G (His 720 Arg), in some atopic subjects [9].

RNA editing is a post-transcriptional modification that results in the generation of nucleotides within an RNA transcript that do not match the bases present within the genome [10]. Mammalian RNA editing events, often represented by cytidine-to-uridine (C-to-U) and adenosine-to-inosine

Correspondence: Prof. Naomi Kondo, Department of Pediatrics, Gifu University School of Medicine, Tsukasa-machi 40, Gifu 500-8705, Japan. E-mail: nkondo@cc.gifu-u.ac.jp

(A-to-I) conversions, are predominantly mediated by base deaminations [10]. Here we show that the RNA editing of the IL-12R β2, 2451 C-to-U (Ala 604 Val) conversion on exon 13 is associated with the reduction in the extent of IL-12 signalling, leading to insufficient IFN-γ production and atopy. This is the first report indicating that atopy is associated with RNA editing.

Methods

Atopic and control subjects

One hundred and two atopic patients (9.3 ± 8.2 years old) with major allergic diseases such as bronchial asthma and/or atopic dermatitis having elevated levels of serum IgE and/or specific IgE antibodies, were studied. The diagnosis of bronchial asthma was made according to the criteria of the American Thoracic Society, and that of atopic dermatitis was made according to the criteria of Hanifin. The levels of specific IgE antibodies against house dust, mite, hen's egg, and cow's milk were measured by fluoroenzyme immunoassay. Scores of 3+ to 6+ were considered positive. None of the patients had been receiving systemic steroids. One hundred and four healthy controls (11.5 ± 13.7 years old) had no history of atopic diseases and their serum IgE levels were within normal limits for their age. The ethics committee of Gifu University School of Medicine approved the research project, and informed consent was obtained from all the subjects or their parents.

Cell preparation and culture

PBMCs were isolated from the heparinized blood of the controls and atopic subjects by Ficoll-Paque (Pharmacia, Sweden) gradient centrifugation. The cells were suspended to give a density of 10^6 cells/mL in the culture medium which consisted of RPMI1640 supplemented with 10% heat-inactivated fetal calf serum. PBMCs were cultured at 2 mL per tube in culture test tubes in the presence or absence of 5 IU/mL IL-12 (R&D systems, Germany), 400 ng/mL IL-18, or 10 μg/mL PHA for 24 h at 37 °C in a humidified atmosphere containing 5% CO_2 [9].

Assays for cytokines

The culture supernatants incubated for 24 h in test tubes were spun to remove the cells after the cultures. The IFN-γ concentrations of the supernatants were measured with a human enzyme-linked immunosorbent assay (ELISA) kit (Ohtsuka, Japan). The detection limit was 20 pg/mL [9].

Sequence analysis of cDNA and genomic DNA of IL-12R β2 chain

Total cellular RNA was extracted from PBMCs cultured with PHA for 24 h using an Isogen kit (Nippon Gene, Japan). Fragments of IL-12R β2 cDNA were amplified by reverse transcription-polymerase chain reaction (RT-PCR), ligated to a T-vector (Novagen) and sequenced using an autosequencer [5]. The conditions for RT-PCR were 94 °C for 1 min, 54 °C for 1 min and 72 °C for 1 min, for 40 cycles. For amplification of exon 13 of IL-12R β2 cDNA, the sense primer 5′-GATGACAGCTCTGACAGCTG-3′ and the anti-sense primer 5′-GGCCTGATGACCTTGGAIT-3′ were used. Genomic DNA was extracted from leukocytes. Exon 13 and the flanking region of IL-12R β2 genomic DNA were amplified by PCR with the sense primer 5′-GATGA CAGCTCTGACAGCTG-3′ and the anti-sense primer 5′-CATTGTCTCCAGGAAGATAG-3′ [11]. The conditions used for PCR were 94 °C for 1 min, 57 °C for 1 min and 72 °C for 1 min, for 30 cycles.

Expression constructs encoding IL-12R β2 and transfected Ba/F3 cell clones

The expression constructs encoding human wild-type IL-12R β2 or variant-type (2451 C-to-U conversion) IL-12R β2 were prepared in the PEF-BOS expression vector, as described elsewhere [5]. Ba/F3 cells were transfected by electroporation with the expression constructs encoding either the wild-type IL-12R β2 or the variant-type IL-12R β2. Then, the transfected Ba/F3 cells were cloned.

Flow cytofluorometric analysis

IL-12R β2 expressing Ba/F3 cell clones were detected by indirect immunofluoresence analysis using flow cytometry. Briefly, 10^6 cells in 100 μL of staining buffer were incubated with 1 μg/mL rat anti-hu IL-12R β2(2B6) mAb or isotype control Ab for 30 min, followed by incubation with biotinylated anti-rat-Ig $F(ab)^2$ fragments for 30 min, and finally incubated with streptavidin conjugated to PE (PharMingen) for 30 min. All incubations were performed at 4 °C in a staining buffer, and the cells were washed twice between incubations. The stained cells were analysed on a FACScan flow cytometer (Becton Dickinson, Mountain View, CA, USA).

Proliferative responses

The Ba/F3 cell clones were cultured with IL-12 (0.5, 5 or 50 IU/mL) for 24 h. DNA synthesis was measured by adding 0.5 μCi [^{3}H] thymidine per well 4 h before harvesting onto glass-fiber filters. [^{3}H] thymidine incorporation (c.p.m.) was measured by liquid scintillation counting, and the results were expressed as the means of triplicate.

Immunoprecipitation assay for phosphorylated Stat4

PBMCs from the patients and controls were stimulated with PHA and IL-12 (5 IU/mL) or the control culture medium for 15 min. The cells were lysed in 1% Triton X-100, 150 mM NaCl, 20 mM Na_2PO_4, 1% aprotinin, 5 mM PMSF, 100 mM NaF and 2 mM Na_3VO_4, and were immunoprecipitated with rabbit antisera for Stat4 (Santa Cruz Biotechnology). Precipitates were resolved by SDS-polyacrylamide gel electrophoresis (SDS-PAGE). After transferring to a nylon membrane, the blots were probed with antibody for phosphotyrosine. Equal loading of Stat4 was confirmed by stripping the same membranes and reprobing them with the antiserum for Stat4.

Suppression of in vitro *IgE production*

PBMCs (10^6 cells/mL) from the atopic patients with or without the 2451 C-to-U conversion were cultured at 2 mL per tube in culture test tubes with Derf 1 (5 μg/mL, Asahi, Tokyo, Japan) and IL-4 (500 U/mL, Genzyme/Techne, USA) for 14 days at 37 °C in a humidified atmosphere containing 5% CO_2. For suppression of IgE production, IFN-γ (1000 U/mL, Genzyme/Techne, USA) or IL-12 (5 IU/mL) was added to the culture. The IgE concentrations of the culture supernatants were measured by ELISA.

Statistics

The significance of difference between groups was analysed by the Mann–Whitney's *U* test or χ^2-test.

Results

RNA editing of IL-12R β2, 2451 C-to-U (Ala 604 Val) conversion associated with atopy

In this study, we found that IL-12R β2 mRNA editing modifies cytidine in an alanine codon (GCU) at nucleotide 2451 in the extracellular domain to a uridine (GUU), converting to a valine codon (Ala 604 Val) in atopic patients. Fragments of IL-12R β2 cDNA were amplified by RT-PCR, ligated to a T-vector and sequenced using an autosequencer. Interestingly, sequence analysis of the cDNA clones representing IL-12R β2 mRNA transcripts revealed the C-to-U conversion at nucleotide 2451 (Ala 604 Val) on exon 13 in some of the atopic patients (Fig. 1). Very recently, van Rietschoten et al. [11] reported the genomic organization of the human IL-12R β2 chain gene. Therefore, we determined the sequence of the genomic DNA of the IL-12R β2 chain. Surprisingly, sequence analysis of the genomic DNA of the IL-12R β2 chain from the atopic patients showed neither 2451 C-to-T (Ala 604 Val) mutation on exon 13 nor mutation in the flanking region of exon 13 (Fig. 1). Therefore, it was suggested that the C-to-U mismatch observed upon comparison of IL-12R β2 genomic DNA with cDNA clones had arisen at the RNA level. RNA editing is formally defined as any RNA-processing event (excluding RNA splicing) that generates an RNA transcript with a primary nucleotide sequence different from that of its gene. Therefore, we concluded that the observed C-to-U mismatch in the cDNA clone of the IL-12R β2 chain is due to the RNA editing of this transcript.

To determine whether the C-to-U conversion at nucleotide 2451 in IL-12R β2 chain cDNA is associated with atopy, we conducted a genetic association study on atopy. The C-to-U conversion was observed in 21 (20.6%) of the 102 atopic patients, whereas this conversion was observed in only 4 (3.8%) of the 104 non-atopic subjects. There was a significant ($P<0.001$, by χ^2-test) difference in the C-to-U conversion frequency between the non-atopic subjects and the atopic subjects (Table 1). The subjects exhibited neither this conversion nor any mutations in the flanking region of exon 13 in the genomic DNA of the IL-12R β2 chain.

IFN-γ production by PBMCs stimulated with IL-12, IL-18 or PHA

To determine whether the C-to-U conversion at nucleotide 2451 in IL-12R β2 chain cDNA affects the IL-12 signal

Table 1. An association study of the C-to-U conversion at nucleotidc 2451 (Ala 604 Val) in IL-12R β2 chain cDNA responsible for atopy

	C-to-U conversion at nucleotide 2451 in IL-12R β2 chain cDNA			
	n	–	+	*P*-value
Non-atopic subjects	104	100	4 (3.8%)	<0.001
Atopic patients	102	81	21 (20.6%)	

P-value was calculated by χ^2-test.
Sequence analysis of genomic DNA of the IL-12R β2 chain showed no 2451 C-to-T (Ala 604 Val) mutation in any of the subjects tested.

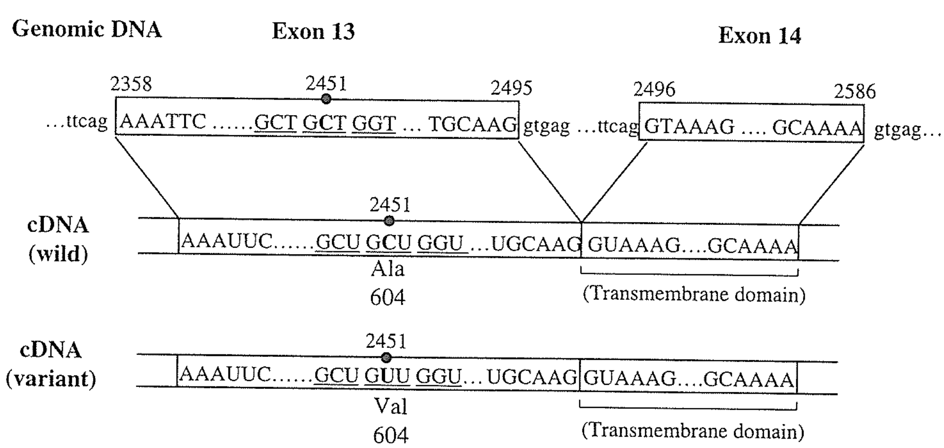

Fig. 1. Sequence analysis of genomic DNA and the cDNA of IL-12R β2 exons 13 and 14 and their flanking regions in non-atopic healthy controls and atopic patients. Sequence analysis of genomic DNA of the IL-12R β2 chain from any of the non-atopic healthy controls and any of the atopic patients showed no 2451 C-to-T (Ala 604 Val) mutation in exon 13. Sequence analysis of cDNA (variant) clones representing IL-12R β2 mRNA transcripts indicates the C-to-U conversion at nucleotide 2451 (Ala 604 Val) in some of the atopic patients. Therefore, it is indicated that the observed C-to-U mismatch in the cDNA clone of 1L-12R β2 chain is due to RNA editing of this transcript. The number above each sequence indicates the number of the nucleotide according to the Genbank database U 64198, and the number under each sequence indicates the number of amino acid.

Table 2. IFN-γ production by PBMCs stimulated with IL-12, IL-18 or PHA in the subjects (atopic patients and non-atopic subjects) with or without the C-to-U conversion at nucleotide 2451 (Ala 604 Val) in IL-12R β2 chain cDNA

	IFN-γ concentration (pg/mL)*		
	Without C-to-U conversion† ($n = 169$)	With C-to-U conversion† ($n = 25$)	*P*-value‡
Stimulated			
with IL-12	154.7 (34.9 ~ 685.9)	69.3 (16.0 ~ 266.5)	0.013
with IL-18	68.6 (11.8 ~ 398.7)	57.3 (9.0 ~ 364.5)	0.962
with PHA	1568.2 (582.1 ~ 4224.5)	1578.1 (711.8 ~ 3498.6)	0.970

*Genometric means are shown, and the ranges of SD are shown in parentheses. †at nucleotide 2451 (Ala 604 Val) in IL-12R β2 chain cDNA. ‡*P*-values were calculated by Mann–Whitney's *U* test.

cascade, we, next, conducted an association study on IFN-γ production by PBMCs following stimulation with IL-12, IL-18 or PHA. After PBMCs were cultured with IL-12, IL-18 or PHA for 24 h, the IFN-γ concentration in the culture supernatants was measured (Table 2). The results revealed that IFN-γ production by PBMCs stimulated with IL-12 in the subjects with the C-to-U conversion was significantly ($P<0.013$) lower than that in the subjects without the C-to-U conversion. In contrast, there was no significant difference in IFN-γ production by PBMCs stimulated with IL-18 or PHA between the subjects with the C-to-U conversion and those without the conversion.

Expression of IL-12R β2 chain

To investigate the expression of the wild-type or variant-type IL-12R β2 chain, Ba/F3 cells were transfected by electroporation with wild-type IL-12R β2 cDNA or variant-type IL-12R β2 cDNA containing the C-to-U conversion at nucleotide 2451 in the PEF-BOS expression vector and then cloned [5]. The IL-12R β2-chain-expressing cells were detected by flow cytometry using the anti-IL-12R β2 antibody. The results revealed that the staining intensity and the percentage of cells expressing the IL-12R β2 chain in the clone with the C-to-U conversion were lower than those in the clone without the conversion (Fig. 2a). Furthermore, the degree of proliferative responses of the cells was measured. As a result, the proliferative response of the Ba/F3 cell clones transfected with variant-type IL-12R β2 cDNA containing the C-to-U conversion at nucleotide 2451 to IL-12 (0.5, 5 IU/mL) was lower than that of the Ba/F3 cells transfected with wild-type IL-12R β2 chain cDNA (Fig. 2b).

Tyrosine phosphorylation of Stat4

Furthermore, to investigate the functional aspects of the C-to-U conversion at nucleotide 2451 in IL-12R β2 chain cDNA, we examined the tyrosine phosphorylation of Stat4. Although PBMCs from the patient without the C-to-U conversion (patient 2) and the control (control 1) cultured with IL-12 and PHA showed the tyrosine phosphorylation of Stat4, PBMCs from the patient with the C-to-U conversion (patient 1) cultured with IL-12 and PHA faintly showed the tyrosine phosphorylation of Stat4 (Fig. 3 a). These results suggest that the C-to-U conversion at nucleotide 2451 in IL-12R β2 chain cDNA is associated with reduced signal transduction of IL-12 for IFN- γ production by PBMCs.

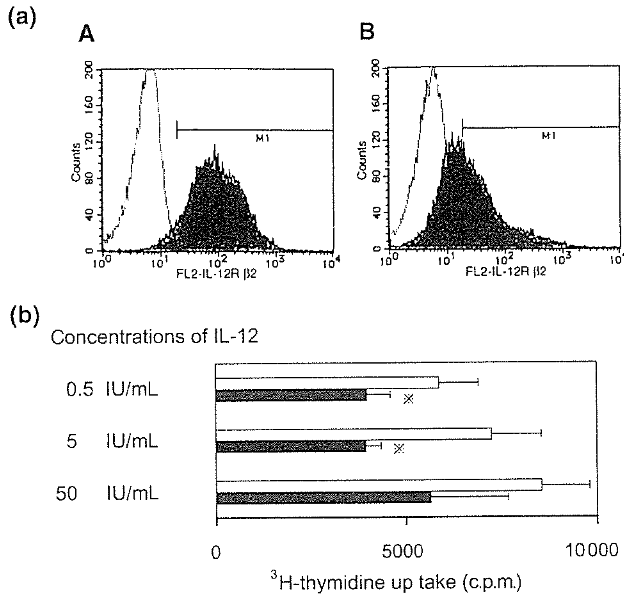

Fig. 2. (a) Expression of the IL-12R β2 chain without or with the C-to-U conversion at nucleotide 2451 (Ala 604 Val) in Ba/F3 cell clones transfected with wild-type (A) or variant-type (B) IL-12R β2 cDNA. 10^6 cells in 100 μL of staining buffer were incubated with 1 μg/mL rat anti-hu IL-12R β2(2B6) mAb (black) or isotype control Ab (white) for 30 min, followed by incubation with biotinylated anti-rat-Ig F(ab)^2 fragments for 30 min, and finally incubated with streptavidin conjugated to PE (PharMingen) for 30 min. IL-12R β2 expressing cells were detected by flow cytometry. The staining intensity and the percentage (50.1%) of cells expressing the IL-12R β2 chain in the clone with the C-to-U conversion were lower than those (the percentage: 94.4%) in the clone without the conversion. (b) Proliferative responses of the Ba/F3 cell clones transfected with wild-type or variant-type IL-12R β2 cDNA containing the C-to-U conversion at nucleotide 2451. Ba/F3 were stimulated with IL-12 (0.5, 5 or 50 IU/mL) for 24 h. The proliferative responses of the Ba/F3 cell clones transfected with variant-type IL-12R β2 cDNA (closed column) to IL-12 (0.5 IU/mL, 5 IU/mL) were significantly (*$P<0.05$ for each) lower than those of the Ba/F3 cell clones transfected with wild-type IL-12R β2 chain cDNA (open column). Means+SD (c.p.m.) of triplicate are shown.

In vitro *IgE production suppressed by IL-12*

Next, we examined the effects of IL-12 on *in vitro* IgE production by PBMCs from the atopic patients with the C-to-U conversion at nucleotide 2451 in IL-12R β2 chain cDNA (Fig. 3 b). IgE production by PBMCs cultured with IL-4 and Derf 1 for 14 days was suppressed by IL-12 as well as by IFN-γ in the atopic patients without the C-to-U conversion at nucleotide 2451 in IL-12R β2 chain cDNA. In contrast, in the

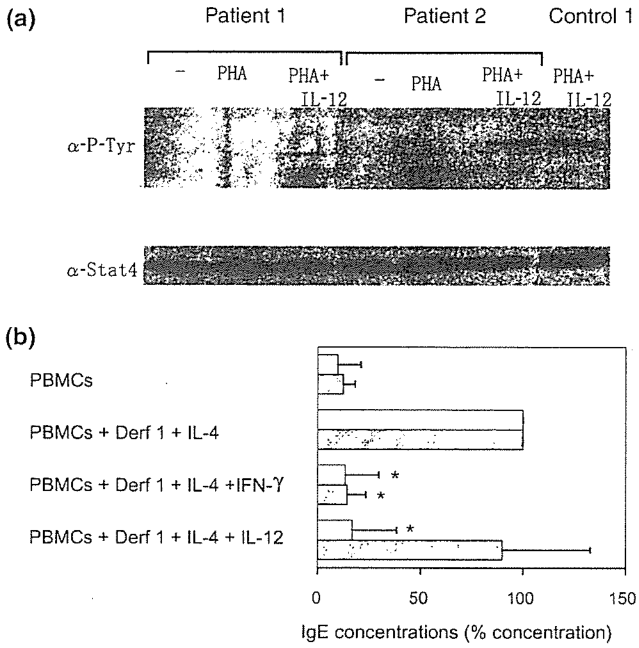

Fig. 3. (a) Tyrosine phosphorylation of Stat4 in PBMCs. PBMCs from the patients with or without the C-to-U conversion at nucleotide 2451 (Ala 604 Val) on the IL-12R β2 chain and the control subject were cultured with PHA and IL-12. Cell lysates were immunoprecipitated with anti-Stat4, resolved by SDS-PAGE, transferred to a nylon membrane and blotted sequentially with anti-phosphotyrosine (α-P-Tyr, upper panel) or anti-Stat4 (a Stat4, lower panel). No band was shown on blotting with α-P-Tyr in PBMCs from the patients cultured with or without PHA. Although PBMCs from the patient without the C-to-U conversion (patient 2) and the control (control 1) cultured with both IL-12 and PHA showed a band on blotting with α-P-Tyr, indicating phosphorylation of Stat4, PBMCs from the patient with the C-to-U conversion (patient 1) cultured with both IL-12 and PHA showed a very faint band. (b) Suppression of *in vitro* IgE production. IgE production by PBMCs was induced by Derf 1 and IL-4. Suppression by IFN-γ (1000 U/mL) and IL-12 (5 IU/mL) was represented by % concentration. % concentration = 100 X IgE concentration in the culture with IFN-γ or IL-12/IgE concentration in the culture without both IFN-γ and IL-12. In the atopic patients (open column, mean +SD, $n = 3$) without the C-to-U conversion at nucleotide 2451 in IL-12R β2 chain cDNA, IgE production was significantly (* $P < 0.05$ for each) suppressed by IL-12 as well as by IFN-γ. In contrast, in the atopic patients (shadow column, mean+SD, $n = 3$) with the C-to-U conversion at nucleotide 2451 in IL-12R β2 chain cDNA, IgE production was not suppressed by IL-12, whereas it was significantly (* $P < 0.05$) suppressed by IFN-γ.

atopic patients with the C-to-U conversion, IgE production by PBMCs was not suppressed by IL-12 whereas it was suppressed by IFN-γ.

Discussion

Our results showed that RNA editing of IL-12R β2, 2451 C-to-U (Ala 604 Val) conversion in atopic subjects caused impairment of the IL-12 signal cascade, and then reduced IFN-γ production by PBMCs following IL-12 stimulation, resulting in impaired down-regulation of IgE production.

Recently, it has been reported that a homozygous nonsense mutation of the IL-12R β1 chain gene causes impairment of salmonella and mycobacterial immunity [12, 13]. The development of Th1 lymphocytes is disturbed in IL-12 or IL-12R β1 knockout mice [8, 14]. In this study, atopic subjects with RNA editing of IL-12R β2, 2451 C-to-U (Ala 604 Val) conversion, did not exhibit any impairment of salmonella and mycobacterial immunity. The IL-12R β2 subunit, similar to the IL-12R β1 subunit, is a member of the gp130-type subgroup of the cytokine receptor superfamily. However, each of the two IL-12R subunits itself is more closely related to gp130 than to each other. In contrast to IL-12R β1, which does not contain any tyrosine residues, the cytoplasmic region of IL-12R β2 contains three tyrosine residues, suggesting an important role of the β2 subunit in IL-12 signal transduction [5]. Presky et al. [5] reported that Ba/F3 cells transfected with IL-12R β2 alone proliferates in response to human IL-12 although the role of endogenous mouse IL-12R β1 in IL-12 signal transduction in these transfectants cannot be ruled out. Thus, IL-12R β2 is different from IL-12 β1 in both structure and function.

It has been noted that RNA editing, a post-transcriptional modification, plays an important role in achieving molecular diversity [10]. The forms of RNA editing are classified into two categories, namely, C-to-U and A-to-I conversions that occur by nucleotide deamination. The best example of C-to-U editing occurs within RNA transcripts encoding apolipoprotein B RNA [15, 16] and is mediated by the activity of cytidine deaminase. Recently, A-to-I conversions have been observed within a growing number of RNAs, including those encoding several glutamate receptor subunits [17–19] and the G-protein-coupled serotonin 2C receptor [20]. Moreover, RNA editing of WT1, that is thought to be a susceptibility gene for Wilm's tumour, converts U-to-C at nucleotide 839, transforming genomically encoded leucine into proline [21]. The leucine, non-edited form of the protein, is a more potent transcriptional repressor than the proline-containing isoform, suggesting that this editing might be associated with the development of Wilm's tumour. The neurofibromatosis type-1 gene product neurofibromin, associated with an increased risk of neurofibromatosis type 1 (NF1), is thought to serve as a tumour suppressor [10]. Although editing of C 2914 of this gene occurs at low levels (<2%) in control subjects, patients with NF1 show almost eight times the level of editing at this position. The editing at this site converts a CGA (Arg) codon into a UGA (stop) codon, suggesting that NF1 patients lack sufficient quantities of neurofibromin [22]. Furthermore, it is suggested that a reduction in the amount of this potential tumour suppressor may prevent appropriate regulation of the Ras signalling pathway, leading to unchecked cellular proliferation and cancer [22].

The expression levels of IL-12R β2 and the proliferative responses to IL-12 in variant-type IL-12R β2 transfected Ba/F3 were lower than those of wild-type. The RNA editing of IL-12R β2, 2451 C-to-U (Ala 604 Val) conversion, associated with atopy in this study may disturb conformational binding of IL-12 to IL-12R, although the possibility that the antibody is affected by the conformational change cannot be excluded.

In the immunological system, RNA editing has not been reported. An immunological system as well as a neurological system require molecular diversity. From this viewpoint, it is natural that lympocytes utilize RNA editing for the regulation of the function on the cytokine and cytokine receptor. Activation induced cytidine deaminase (AID), which is the causative gene for the hyper-IgM syndrome, is homologous to that of mammalian RNA editing deaminase, APOBEC1.

AID had deaminase activity when tested for deoxycytidine deamination. Therefore, AID may be another RNA editing deaminase although its substrate has not yet been identified. AID or other enzymes may be the candidate for RNA editing in an immunological system. It is indicated that the regulatory spacer and mooring sequences (such as UGAUAC, AAUU, UGAUCAGUAUA, respectively in human apolipoprotein B) may provide binding sites for distinct components of the cellular editing machinery: once bound, the factor(s) would be correctly positioned to edit nucleotides within a certain distance upstream from the binding site [10, 23]. Therefore, we investigated these sequences in the IL-12R β2 chain gene. However, we were not able to find any motifs.

Atopic disorders develop by a combination of genetic risk factors and environmental factors. Very recently, Karcher et al. found the temperature sensitivity of RNA editing reaction in the plastid ndhB transcript [24]. Therefore RNA editing, one of post-transcriptional modifications, in atopic patients may be induced by a combination of genetic factors and environmental factors. Experiments along these lines are now under way. Our results indicate that several candidate genes that have failed to show association should be investigated at the mRNA level. Although several polymorphisms or mutations of the genes associated with atopy have been reported [9, 25–27], this study is the first report indicating that atopy is associated with RNA editing, a post-transcriptional modification.

References

1 Pene J, Rousset F, Briere F et al. IgE production by normal human lymphocytes is induced by interleukin 4 and suppressed by interferons gamma and alpha and prostaglandin E2. Proc Natl Acad Sci USA 1988; 85:6880–4.

2 Kobayashi M, Fitz L, Ryan M et al. Identification and purification of natural killer cell stimulatory factor (NKSF), a cytokine with multiple biologic effects on human lymphocytes. J Exp Med 1989; 170:827–45.

3 Wolf SF, Temple PA, Kobayashi M et al. Cloning of cDNA for natural killer cell stimulatory factor, a heterodimeric cytokine with multiple biologic effects on T and natural killer cells. J Immunol 1991; 146:3074–81.

4 Gately MK, Renzetti LM, Magram J et al. The interleukin-12/interleukin-12-receptor system: role in normal and pathologic immune responses. Annu Rev Immunol 1998; 16:495–521.

5 Presky DH, Yang H, Minetti LJ et al. A functional interleukin 12 receptor complex is composed of two β-type cytokine receptor subunits. Proc Natl Acad Sci USA 1996; 93:14002–7.

6 Barbulescu K, Becker C, Schlaak JF, Schmitt E, Meyer zum Buschenfelde KH, Neurath MF. IL-12 and IL-18 differentially regulate the transcriptional activity of the human IFN-gamma promoter in primary CD4+ T lymphocytes. J Immunol 1998; 160:3642–7.

7 Thierfelder WE, Deursen JM, Yamamoto K et al. Requirement for Stat4 in interleukin-12-mediated responses of natural killer and T cells. Nature 1996; 382:171–4.

8 Magram J, Connaughton SE, Warner RR et al. IL-12-deficient mice are defective in IFN gamma production and type 1 cytokine responses. Immunity 1996; 4:471–81.

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

10 Niswender CM. Recent advances in mammalian RNA editing. Cell Mol Life Sci 1998; 54:946–64.

11 van Rietschoten JGI, Smits HH, Westland R, Verweij CL, den Hartog MT, Wierenga EA. Genomic organization of the human interleukin-12 receptor beta2-chain gene. Immunogenet 2000; 51:30–6.

12 Altare F, Durandy A, Lammas D et al. Impairment of mycobacterial immunity in human interleukin-12 receptor deficiency. Science 1998; 280:1432–5.

13 de Jong R, Altare F, Haagen IA et al. Severe mycobacterial and Salmonella infections in interleukin-12 receptor-deficient patients. Science 1998; 280:1435–8.

14 Wu C, Ferrante J, Gately MK, Magram J. Characterization of IL-12 receptor beta1 chain (IL-12Rbeta1)-deficient mice: IL-12Rbeta1 is an essential component of the functional mouse IL-12 receptor. 1997; 159:1658–65.

15 Chen SH, Habib G, Yang CY et al. Apolipoprotein B-48 is the product of a messenger RNA with an organ-specific in-frame stop codon. Science 1987; 238:363–6.

16 Powell LM, Wallis SC, Pease RJ, Edwards YH, Knott TJ, Scott J. A novel form of tissue-specific RNA processing produces apolipoprotein-B48 in intestine. Cell 1987; 50:831–40.

17 Sommer B, Kohler M, Sprengel R, Seeburg PH. RNA editing in brain controls a determinant of ion flow in glutamate-gated channels. Cell 1991; 67:11–9.

18 Kohler M, Burnashev N, Sakmann B, Seeburg PH. Determinants of Ca2+ permeability in both TM1 and TM2 of high affinity kainate receptor channels: diversity by RNA editing. Neuron 1993; 10:491–500.

19 Lomeli H, Mosbacher J, Melcher T et al. Control of kinetic properties of AMPA receptor channels by nuclear RNA editing. Science 1994; 266:1709–13.

20 Burns CM, Chu H, Rueter SM et al. Regulation of serotonin-2C receptor G-protein coupling by RNA editing. Nature 1997; 387:303–8.

21 Sharma PM, Bowman M, Madden SL, Rauscher FJ 3rd, Sukumar S. RNA editing in the Wilms' tumor susceptibility gene, WT1. Genes 1994; 8:720–31.

22 Skuse GR, Cappione AJ, Sowden M, Metheny LJ, Smith HC. The neurofibromatosis type I messenger RNA undergoes base-modification RNA editing. Nucleic Acids Res 1996; 24:478–85.

23 Seeburg PH. A-to-I editing; new and old sites, functions and speculations. Neuron 2002; 35:17–20.

24 Karcher D, Bock R. Temperature sensitivity of RNA editing and intron splicing reactions in the plastid ndhB transcript. Curr Genet 2002; 41:48–52.

25 Shirakawa T, Li A, Dubowitz M et al. Association between atopy and variants of the beta subunit of the high-affinity immunogloblin E receptor. Nature Genetics 1994; 7:125–9.

26 Mitsuyasu H, Izuhara K, Mao XQ et al. I150Val variant of IL4R α upregulates IgE synthesis and associates with atopic asthma. Nature Genetics 1998; 19:119–20.

27 Shirakawa T, Deichmann KA, Izuhara I, Mao I, Adra CN, Hopkin JM. Atopy and asthma: genetic variants of IL-4 and IL-13 signalling. Immunol Today 2000; 21:60–4.

Allergology International (2004) **53**: 77–85

Review Article

Genetic defects in downregulation of IgE production and a new genetic classification of atopy

Naomi Kondo, Eiko Matsui, Hideo Kaneko, Zenichiro Kato, Toshiyuki Fukao, Takahide Teramoto, Hiroaki Shikano, Minako Aoki, Hidenori Onishi, Koji Tatebayashi, Kentaro Omoya, Masashi Kondo, Eiji Matsukuma, Kimiko Kasahara and Naoko Morimoto

Department of Pediatrics, Graduate School of Medicine, Gifu University, Gifu, Japan

ABSTRACT

Atopic disorders, such as asthma, eczema and rhinitis, develop due to the interactions between genetic and environmental factors. Atopy is characterized by enhanced IgE responses to environmental antigens. The production of IgE is upregulated by Th2 cytokines, in particular interleukin (IL)-4, and downregulated by Th1 cytokines, in particular interferon (IFN)-γ. In the present review, we present the genetic factors responsible for IgE production and genetic defects in the downregulation (brake) of IgE production, especially in terms of IL-12 and IL-18 signaling, mutations of the IL-12 receptor β2 chain gene and mutations of the IL-18 receptor α chain gene in atopy. Moreover, we newly present a genetic classification of atopy. There are four categories of genes that control the expression of allergic disorders, which include: (i) antigen recognition; (ii) IgE production (downregulation = brake; and upregulation); (iii) the production and release of mediators; and (iv) events on target organs. In the near future, this genetic classification will facilitate the development of tailor-made treatment.

Key words: atopy, IgE production downregulation, interferon-γ, interleukin-12 receptor β2, interleukin-18 receptor α.

Correspondence: Dr Naomi Kondo, Department of Pediatrics, Graduate School of Medicine, Gifu University, 1-1 Yanagido, Gifu 501-1193, Japan. Email: nkondo@cc.gifu-u.ac.jp
Received 28 December 2003.

INTRODUCTION

Atopic disorders, such as asthma, eczema and rhinitis, develop due to the interactions between genetic and environmental factors. Atopy is characterized by enhanced IgE responses to environmental antigens. The production of IgE is upregulated by Th2 cytokines, in particular interleukin (IL)-4, and is downregulated by Th1 cytokines, in particular interferon (IFN)-γ.[1] Interleukin-12, which is a cytokine that promotes cell-mediated Th1 responses and the production of IFN-γ, is one of the important cytokines that downregulates IgE production. Interleukin-18, originally known as an IFN-γ-inducing factor, is a recently cloned cytokine of approximately 18 kDa secreted by Kupffer cells of the liver and activated macrophages.[2] Interleukin-18 strongly augments IFN-γ production by T lymphocytes, natural killer (NK) cell cytotoxicity and T lymphocyte proliferation.

In the present review, we discuss the genetic factors responsible for IgE production and the genetic defects in the downregulation (brake) of IgE production in atopy. Moreover, we newly present a genetic classification of atopy.

DEVELOPMENT OF ALLERGIC DISEASES

A questionnaire was distributed in March 1991 to children under 16 years of age who were attending kindergarten or elementary or junior high school in two Japanese cities, namely Gifu, with a temperate climate, and Itoman, with a subtropical climate. The number of subjects analyzed was 1243 in Gifu and 1953 in Itoman. Multiple logistic regression analysis was performed using SAS (SAS Institute, Cary, NC, USA).

Multiple logistic regression analysis showed that, in both cities, children of families with a history of allergy have a significantly higher risk (relative risk 3.58 and 4.22 for Gifu and Itoman, respectively) of contracting an allergic disease (Table 1). These results show that there is a genetic accumulation in the development of allergic disorders. Therefore, the development of allergic disorders is correlated with some genes. We think that multiple causative genes, but not a single gene, are correlated, because there are multiple pathogeneses of allergic reactions.

GENETIC FACTORS OF ENHANCED IGE PRODUCTION AND ATOPY

Serum IgE levels of atopic children were plotted against serum IgE levels of their parents (Fig. 1) and a good correlation was found ($P < 0.016$). Therefore, this indicates that IgE production shows genetic accumulation.[3] Several linkage analyses and mutations for candidate genes of atopy (i.e. enhanced IgE production) have been reported. In 1989, Cookson *et al.*[4] reported a linkage between IgE responses underlying asthma and rhinitis and chromosome 11q. Moreover, Shirakawa *et al.*[5]

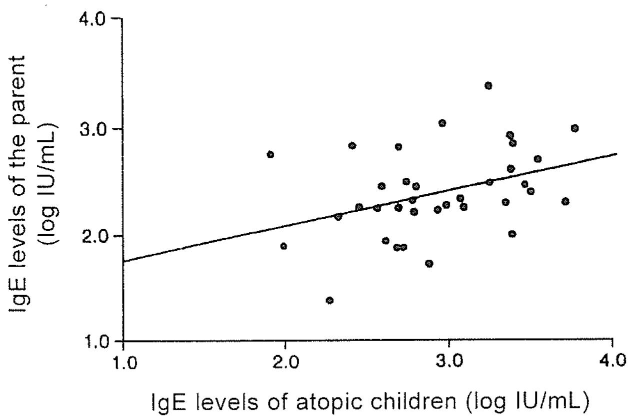

Fig. 1 Relationship between serum total IgE levels of atopic children and the IgE levels of their parents (the highest IgE level of two spouses was used). Children older than 6 years were selected. $y = 1.38 + 0.3461x$; $P < 0.016$.

Table 1 Genetic and environmental factors in relation to any allergic diseases as analyzed by multiple logistic regression

Independent variables	Relative risk (95% confidence interval)	
	Gifu (n = 1243)	Itoman (n = 1953)
Family history		
No	1	1
Yes	3.58 (2.17–5.91)	4.22 (2.91–6.12)
Sex		
Male	1	1
Female	0.93 (0.69–1.27)	0.60 (0.45–0.79)
Age (years)		
0–3	1.72 (0.87–3.40)	0.70 (0.27–1.82)
4–6	1.47 (0.93–2.31)	0.80 (0.44–1.46)
7–9	1.30 (0.81–2.07)	1.10 (0.75–1.62)
10–12	1.15 (0.71–1.85)	1.06 (0.72–1.56)
13–15	1	1
Structure of house		
Made of wood	1	1
Made of reinforced concrete	1.22 (0.87–1.72)	1.15 (0.75–1.78)
Apartment house	1.27 (0.66–2.42)	0.94 (0.60–1.48)
Flooring		
Wooden floor	1	1
Tatami	0.98 (0.64–1.49)	1.91 (1.08–3.38)
Carpet on tatami	1.17 (0.79–1.72)	1.65 (0.75–3.63)
Carpet on wooden floor	2.00 (1.17–3.42)	1.71 (0.91–3.23)
Pets		
No	1	1
Yes	0.88 (0.62–1.23)	0.81 (0.58–1.14)

reported that a common variant of the β-subunit of the high-affinity IgE receptor (FcεRIβ) on chromosome 11, Ile181Leu within the 4th transmembrane domain, shows significant association with positive IgE responses. Several associations have been noted between atopy and genes on the chromosome 5 cytokine cluster, including IL-4.[6,7] In 1998, Mitsuyasu *et al.*[8] reported that the Ile50Val variant of the IL-4 receptor α (IL-4Rα) chain upregulates IgE synthesis and is associated with atopic asthma. Moreover, Shirakawa *et al.*[9] noted genetic variants of IL-13. Very recently, we found that reduced IFN-γ production by peripheral blood mononuclear cells (PBMC) following stimulation with IL-12 or IL-18 is associated with heterozygous IL-12 receptor β2 (IL-12Rβ2) chain gene or IL-18 receptor α (IL-18Rα) chain gene mutations in atopic subjects.[10,11]

Genetic defects in the downregulation of IgE production in atopy

The production of IgE is upregulated by Th2 cytokines, in particular IL-4, and is downregulated by Th1 cytokines, in particular IFN-γ.[1] Interleukin-12 and IL-18 are the important cytokines that induce IFN-γ and downregulate IgE production (Fig. 2).

In this section, the genetic defects in the downregulation (brake) of IgE production, especially, in terms of IL-12 and IL-18 signaling, are discussed.

Interleukin-12 and IL-12R

Interleukin-12, which is produced by activated antigen-presenting cells, is a cytokine that consists of two disulfide-linked subunits, p35 and p40. Interleukin-12

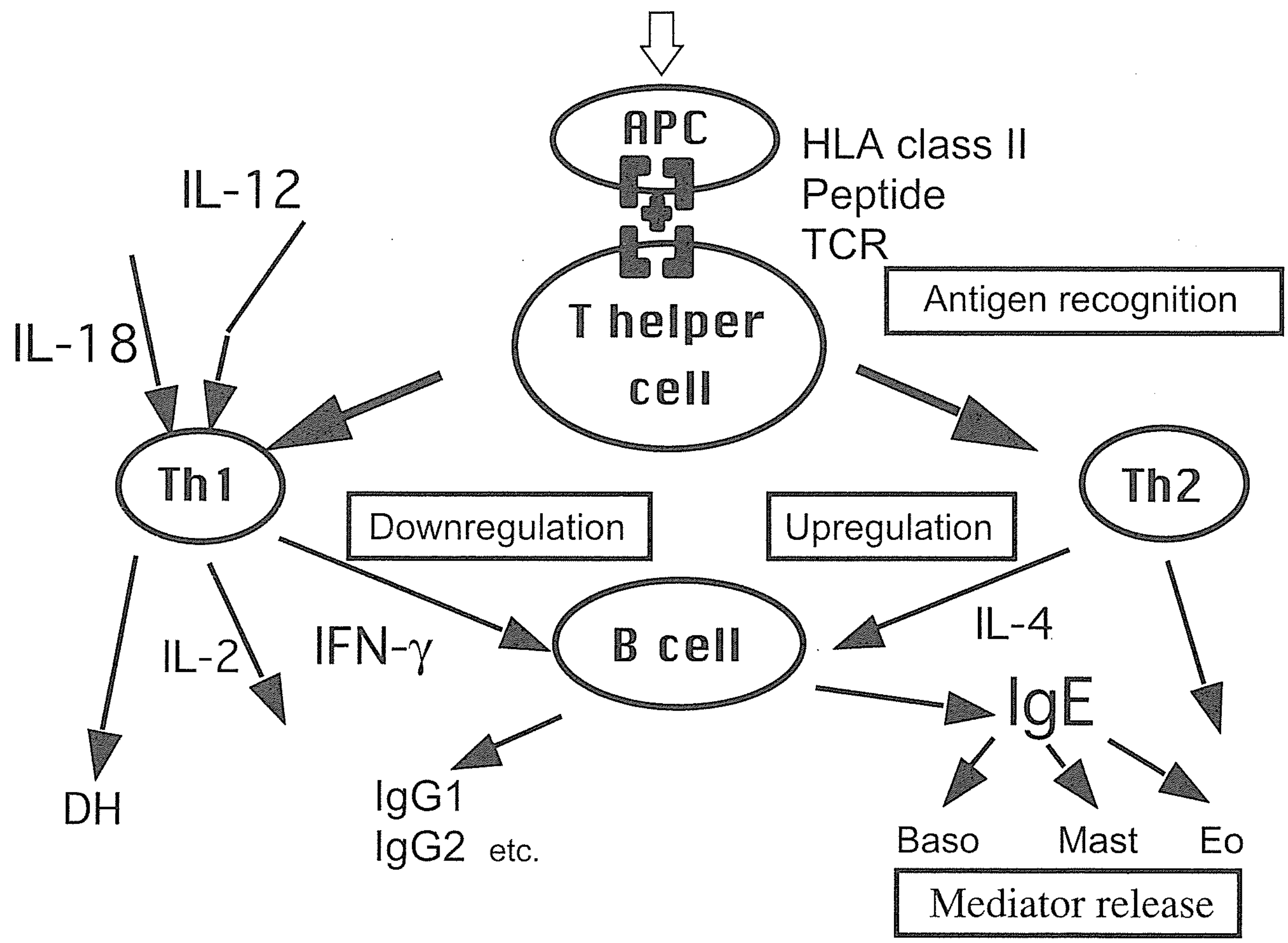

Fig. 2 The Th1 and Th2 lymphocyte balance and upregulation and downregulation of IgE production. IL, interleukin; DH, delayed-type hypersensitivity; IFN, interferon; APC, antigen-presenting cell; HLA, human leukocyte antigen; TCR, T cell receptor; Baso, basophils; Mast, mast cells; Eo, eosinophils.

plays a central role in promoting Th1-type immune responses and, thus, cell-mediated immunity.[12–15] Interleukin-12 also induces IFN-γ production by T lymphocytes and NK cells.[16–18] The receptor for IL-12 (IL-12R) is composed of two distinct subunits, β1 and β2[19] (Fig. 3). Although the β2 chain of the IL-12R is expressed only in Th1 lymphocytes, the β1 chain is expressed in both Th1 and Th2 lymphocytes.[20] The IL-12Rβ1 chain does not contain any cytoplasmic tyrosine residues, whereas the cytoplasmic region of the IL-12Rβ2 chain contains three tyrosine residues. This suggests that the β2 subunit plays an important role in IL-12 signal transduction. Interleukin-12 induces activation of specific members of the signal transducers and activators of transcription (Stat) family of transcription factors and it has been shown that Stat4-deficient mice manifest impaired production of IFN-γ[21] and the phenotype of the IL-12-p40-deficient mouse is similar to that of the Stat4-deficient mouse.[15] Therefore, Stat4 is particularly important. Interleukin-12 induces rapid tyrosine phosphorylation of Stat4 and the formation of nuclear complexes capable of binding to DNA sequences, such as the Stat4-binding site.[21,22]

Interleukin-18 and IL-18R

A variety of biological functions have been associated with human IL-18, including the induction of the proliferation of activated T lymphocytes, enhancement of NK cytotoxity, induction of the production of IFN-γ and granulocyte-macrophage colony stimulating factor (GM-CSF), and promotion of a Th1 response.[2,23–25] The activity of IL-18 is via an IL-18R complex. This IL-18R complex is composed of a binding chain termed IL-18Rα, a member of the IL-1R family previously identified as the IL-1R-related proteins, and a signaling chain, also a member of the IL-1R family. The IL-18R complex recruits the IL-1R-activating kinase and tumor necrosis factor (TNF)-associated factor 6, which phosphorylates nuclear factor (NF)-κB-inducing kinase, with subsequent activation of NF-κB[26–28] (Fig. 4).

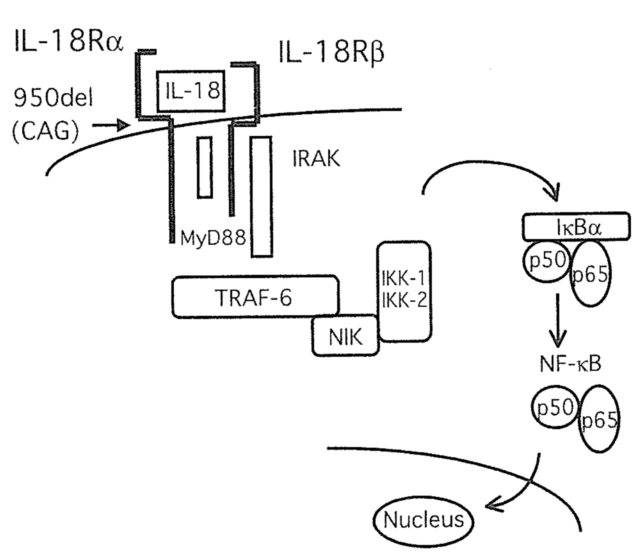

Fig. 4 Interleukin (IL)-18 signaling. IL-18Rα, IL-18Rβ, IL-18 receptor α and β chains, respectively; IKK-1, Ikk-2, IκBα kinases 1 and 2, respectively; NF-κB, nuclear factor-κB; NIK, NF-κB-inducing kinase; TRAF-6, tumor necrosis factor receptor-associated factor 6; IRAK, IL-1 receptor-associated kinase.

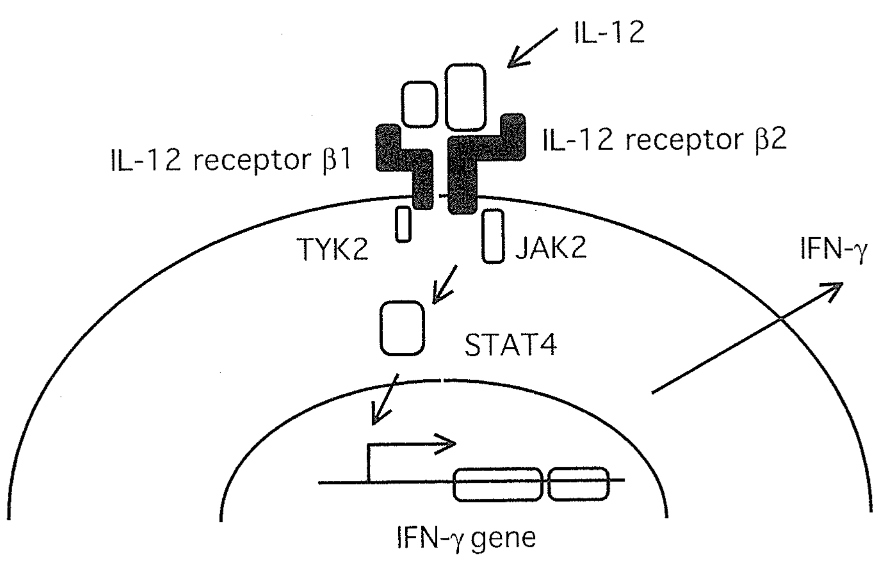

Fig. 3 Interleukin (IL)-12 signaling. TYK2, tyrosine kinase 2; JAK2, Janus kinase 2; STAT4, signal transducers and activators of transcription 4; IFN, interferon.

Interferon-γ production by IL-12 or IL-18 in atopy

We examined the production of IFN-γ in PBMC of atopic patients and healthy controls following stimulation with IL-12 or IL-18.[10,11] The PBMC of non-atopic healthy controls showed adequate IFN-γ production following stimulation with either IL-12 or IL-18. Although the concentrations of IFN-γ in IL-18-stimulated PBMC were correlated with those of IL-12-stimulated PBMC in atopic patients, there were cases showing different responses to IL-12 and IL-18, as shown in Fig. 5. The production of IFN-γ following stimulation with IL-12 (or IL-18) was poor, but IL-18 (or IL-12) stimulation elicited detectable IFN-γ production in some atopic patients. The discrepancy in IFN-γ production following stimulation with IL-12 or IL-18 suggests a disturbance in the IL-12 or IL-18 signal cascade in these patients.

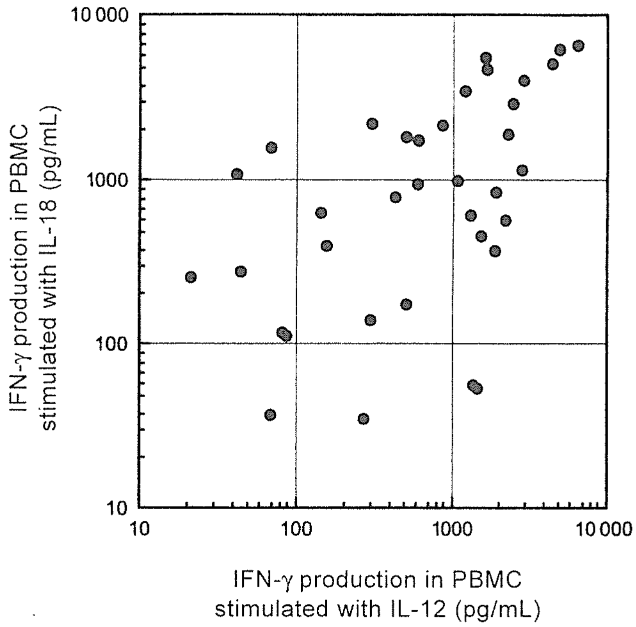

Fig. 5 Interferon (IFN)-γproduction in peripheral blood mononuclear cells (PBMC) stimulated with interleukin (IL)-12 or IL-18 (see text for details).

Role of mutations of the IL-12Rβ2 chain gene in atopy

Recently, it was shown that homozygous nonsense mutation of the IL-12Rβ1 chain gene resulted in impairment of immunity against *Salmonella* and mycobacteria.[29] Moreover, IL-12Rβ1-knock out mice showed impaired development of Th1.[30] In a previous study,[10] sequence analysis of the cDNA of IL-12Rβ2 revealed three types of distinct genetic mutations (2496del91, 1577 A to G (R313G), 2799 A to G (H720R)) in some atopic patients (Fig. 6). Reduced production of IFN-γ by PBMC following stimulation with IL-12, but not IL-18, is associated with heterozygous IL-12Rβ2 chain cDNA mutations in atopic subjects. In these atopic patients, a heterozygous IL-12Rβ2 chain cDNA mutation results in decreased tyrosine phosphorylation of Stat4 and subsequently reduced production of IFN-γ following stimulation with IL-12. Such reduced production of IFN-γ could cause insufficient suppression of accelerated IgE production in B lymphocytes by IL-4, resulting in the elevation of serum

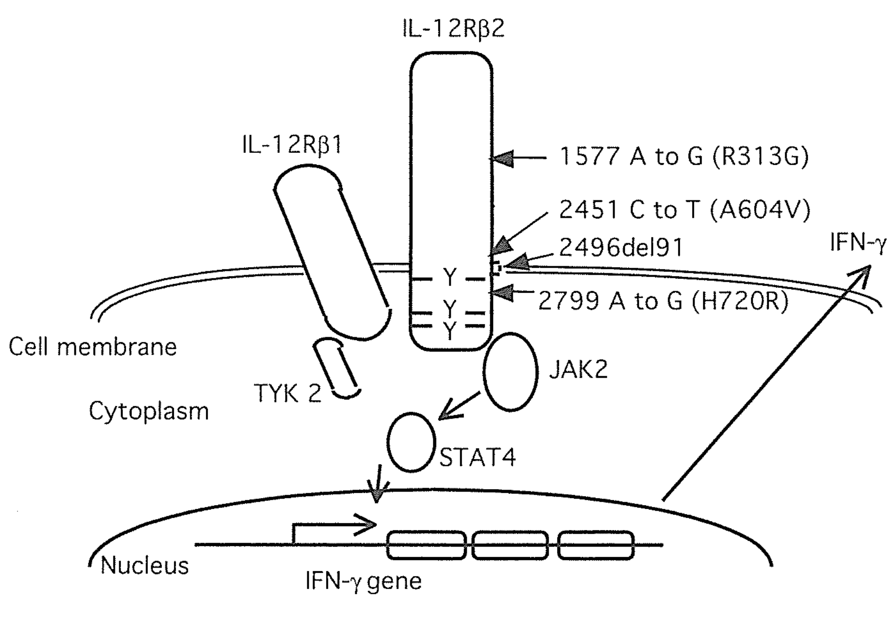

Fig. 6 Interleukin (IL)-12 signaling and mutations of IL-12 receptor β (IL-12Rβ) 2 chain gene. R, arginine; G, glycine; H, histidine; Y, tyrosine (2451 C to T: by RNA editing); TYK2, tyrosine kinase 2; JAK2, Janus kinase 2; STAT4, signal transducers and activators of transcription 4.

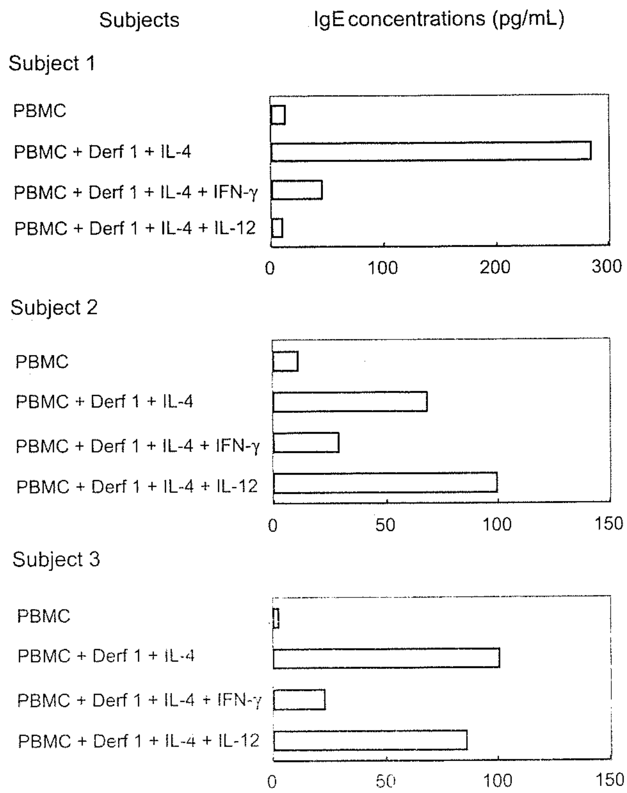

Fig. 7 Suppression of IgE production of peripheral blood mononuclear cells (PBMC; see text for details). The PBMC (10^{-6} /mL) were cultured with Derf 1 (0.5 μg/mL; Asahi, Tokyo, Japan) and interluekin (IL)-4 (500 U/mL; Genzyme, Cambridge, MA, USA) in culture test tube for 14 days. Moreover, interferon (IFN)-γ (100 U/mL) or IL-12 (5 IU/mL) was added to these PBMC cultures. Culture supernatants were obtained after the cultures. The concentration of IgE in culture supernatants was measured by ELISA. Subject 1, an atopic patient without mutations of the IL-12Rβ2 chain gene; subject 2, an atopic patient with 91del of the IL-12Rβ2 chain gene; subject 3, an atopic patient with a missense mutation of the IL-12Rβ2 chain gene.

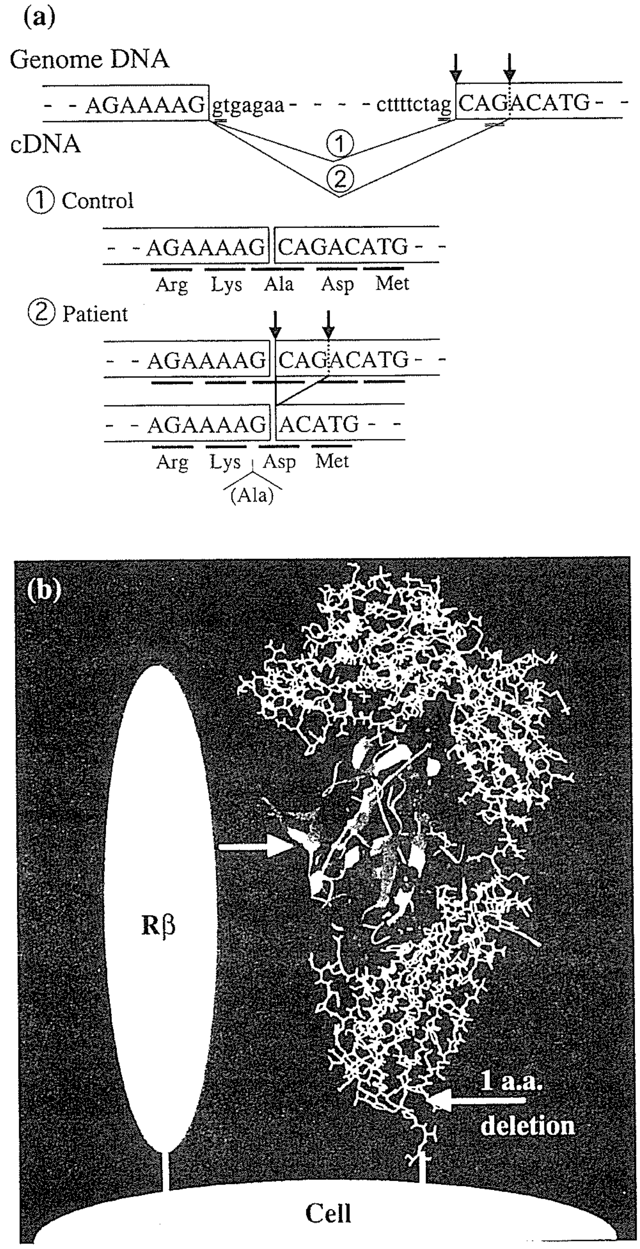

Fig. 8 (a) 950delCAG in interluekin (IL)-18 receptor α (IL-18Rα) chain cDNA and (b) model of the ternary complex of IL-18Rβ : IL-18 : IL-18Rα.

IgE levels (Fig. 7). The 2496del91 mutation of IL-12Rβ2, which is found all over the transmembrane portion, causes premature termination. The heterozygous missense mutations, 1577 A to G (R313G) and 2799 A to G (H720R), may lead to changes in the conformational structure. Moreover, these heterozygous mutations may play a role via a dominant negative effect. At least, these patients with heterozygous mutations of IL-12Rβ2 chain cDNA have not exhibited impairment of immunity against *Salmonella* and mycobacteria.

The balance between IFN-γ-producing Th1 lymphocytes and proallergic Th2 lymphocytes is important. Heterozyous mutations of IL-12β1 or β2 may result in impairment of the downregulation (brake) of IgE production, whereas homozygous mutations of IL-12β1 or β2 may lead to an obvious impairment of Th1-type cell-mediated immunity in addition to impairment of the downregulation of IgE production. The results of our study[10] indicate that atopic diseases are caused, in part, by impairment of the IL-12 signal cascade, which downregulates IgE production, and that the mutation of

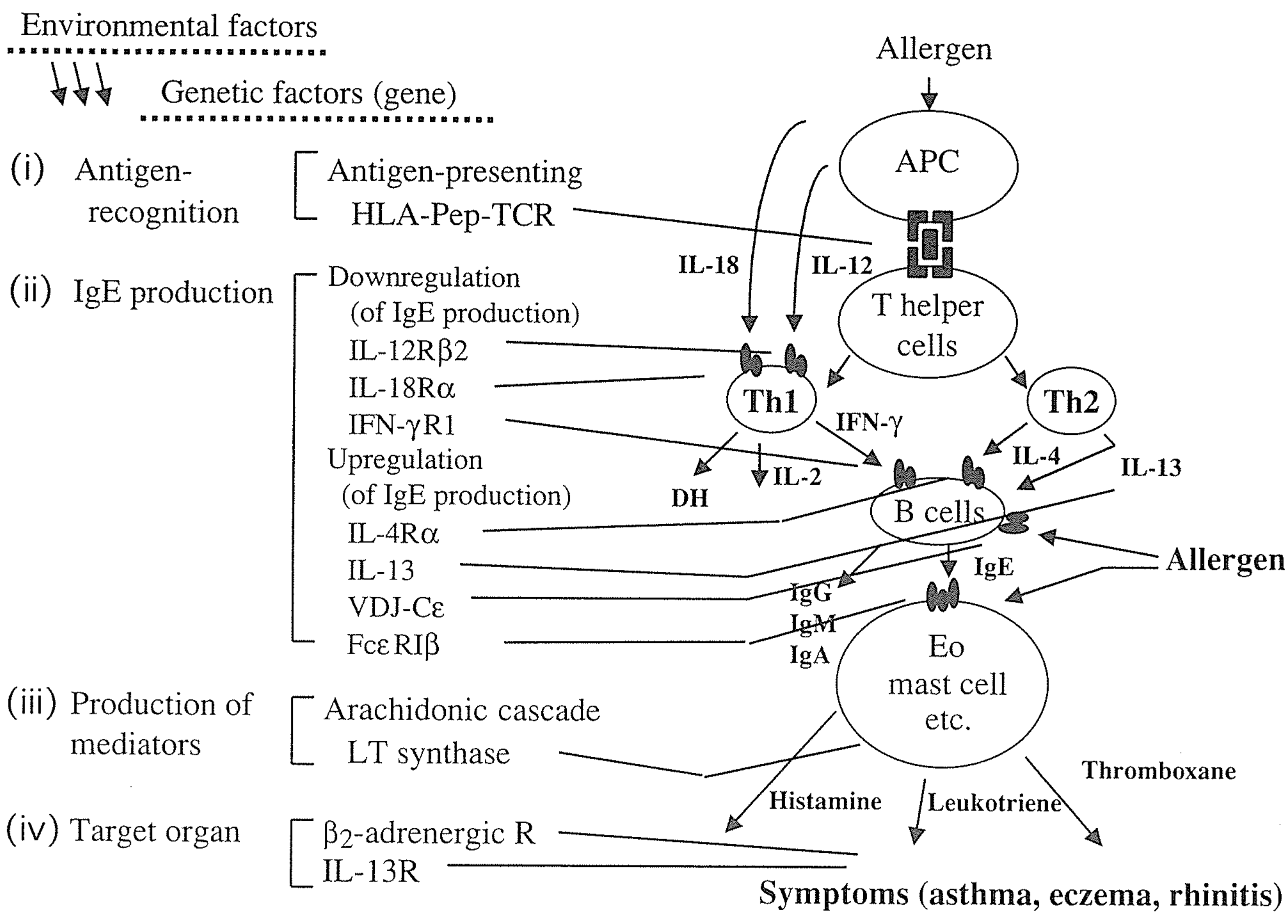

Fig. 9 A new genetic classification of atopy and genetic ecologic medicine in allergy (designed by N Kondo, 2002). HLA-Pep-TCR, human leukocyte antigen–peptide–T cell receptor; IL, interleukin; IFN, interferon; VDJ-Cε, variable diversity joining-ε constant region; LT, leukotriene; R, receptor; APC, antigen-presenting cell; DH, delayed-type hypersensitivity; Eo, eosinophils.

the IL-12β2 chain gene is one of the causative genes for atopy.

Role of mutation of the IL-18Rα chain gene in atopy

The IL-18Rα chain cDNA of atopic patients was sequenced.[11] We identified a three-base deletion of the IL-18Rα chain cDNA (950delCAG), which was generated by alternative splicing, as determined on the basis of genomic sequence data for the IL-18Rα chain gene (Fig. 8). Peripheral blood mononuclear cells with the predominant expression of 950delCAG significantly showed reduced IFN-γ production after IL-18 stimulation. There was a significant difference in the expression pattern of the IL-18Rα chain transcript between atopic patients and non-atopic controls. According to these results, the dominant expression of the 950delCAG transcript of IL-18Rα chain cDNA, which was associated with reduced IFN-γ production following IL-18 stimulation and high serum IgE levels, predisposes to some atopic diseases.

Role of mutation of the IFN-γR1 chain gene in atopy

We identified a novel heterozygous single-nucleotide substitution 1400 T to C (Leu467Pro) in the seventh exon of the IFN-γR1 chain gene.[31] This substitution was detected in six of 89 allergic patients, but not in 72 non-allergic subjects. There was a difference in the Leu467Pro frequency between allergic and non-allergic subjects ($P < 0.05$). Serum IgE levels of allergic patients with Leu467Pro were higher than those of non-allergic subjects ($P < 0.001$). These results suggest that Leu467Pro in the IFN-γR1 chain gene is one of the candidate susceptibility genes for atopic diseases.

Genetic classification of atopy

Recently, mutations or genetic polymorphisms of several genes, such as those encoding the FcεRIβ,[5] IL-4Rα subunit[8] and IL-13,[9] have been reported as the probable causative genes of atopy, which is characterized by enhanced IgE production. Based on these reports and our results, we present a new genetic classification of atopy in Fig. 9. There are four categories of genes that control the expression of allergic disorders, which include: (i) antigen recognition; (ii) IgE production (downregulation = brake; and upregulation); (iii) the production and release of mediators; and (iv) events on target organs. In the near future, this genetic classification will facilitate the development of tailor-made treatment.

References

1 Snapper CM, Paul WE. Interferon-gamma and B cell stimulatory factor-1 reciprocally regulate Ig isotype production. *Science* 1987; **236**: 944–7.

2 Okamura H, Tsutsi H, Komatsu T *et al.* Cloning of a new cytokine that induces IFN-gamma production by T cells. *Nature* 1995; **378**: 88–91.

3 Fujii H, Kondo N, Agata H *et al.* Genetic analysis of IgE and the IGHE, IGHEP1 and IGHEP2 genes in atopic families. *Int. Arch. Allergy Immunol.* 1995; **106**: 62–8.

4 Cookson WO, Sharp PA, Faux JA, Hopkin JM. Linkage between immunoglobulin E responses underlying asthma and rhinitis and chromosome 11q. *Lancet* 1989; **i**: 1292–5.

5 Shirakawa T, Li A, Dubowitz M *et al.* Association between atopy and variants of the beta subunit of the high-affinity immunoglobulin E receptor. *Nat. Genet.* 1994; **7**: 125–30.

6 Marsh DG, Neely JD, Breazeale DR *et al.* Linkage analysis of IL4 and other chromosome 5q31.1 markers and total serum immunoglobulin E concentrations. *Science* 1994; **264**: 1152–6.

7 Rosenwasser LJ. Genetics of atopy and asthma: Promoter-based candidate gene studies for IL-4. *Int. Arch. Allergy Immunol.* 1997; **113**: 61–4.

8 Mitsuyasu H, Izuhara K, Mao XQ *et al.* Ile50Val variant of IL4R alpha upregulates IgE synthesis and associates with atopic asthma. *Nat. Genet.* 1998; **19**: 119–20.

9 Shirakawa T, Deichmann KA, Izuhara I, Mao I, Adra CN, Hopkin JM. Atopy and asthma: Genetic variants of IL-4 and IL-13 signalling. *Immunol. Today* 2000; **21**: 60–4.

10 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.

11 Watanabe M, Kaneko H, Shikano H *et al.* Predominant expression of 950delCAG of IL-18R alpha 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.

12 Hsieh CS, Macatonia SE, Tripp CS, Wolf SF, O'Garra A, Murphy KM. Development of TH1 CD4+ T cells through IL-12 produced by *Listeria*-induced macrophages. *Science* 1993; **260**: 547–9.

13 Manetti R, Parronchi P, Giudizi MG *et al.* Natural killer cell stimulatory factor (interleukin 12 [IL-12]) induces T helper type 1 (Th1)-specific immune responses and inhibits the development of IL-4-producing Th cells. *J. Exp. Med.* 1993; **177**: 1199–204.

14 Trinchieri G. Interleukin-12: A cytokine produced by antigen-presenting cells with immunoregulatory functions in the generation of T-helper cells type 1 and cytotoxic lymphocytes. *Blood* 1994; **84**: 4008–27.

15 Magram J, Connaughton SE, Warrier RR *et al.* IL-12-deficient mice are defective in IFN gamma production and type 1 cytokine responses. *Immunity* 1996; **4**: 471–81.

16 Chan SH, Perussia B, Gupta JW *et al.* Induction of interferon gamma production by natural killer cell stimulatory factor: Characterization of the responder cells and synergy with other inducers. *J. Exp. Med.* 1991; **173**: 869–79.

17 Murphy EE, Terres G, Macatonia SE *et al.* B7 and interleukin 12 cooperate for proliferation and interferon gamma production by mouse T helper clones that are unresponsive to B7 costimulation. *J. Exp. Med.* 1994; **180**: 223–31.

18 Gately MK, Warrier RR, Honasoge S *et al.* Administration of recombinant IL-12 to normal mice enhances cytolytic lymphocyte activity and induces production of IFN-gamma *in vivo*. *Int. Immunol.* 1994; **6**: 157–67.

19 Presky DH, Yang H, Minetti LJ *et al.* A functional interleukin 12 receptor complex is composed of two β-type cytokine receptor subunits. *Proc. Natl Acad. Sci. USA* 1996; **93**: 14 002–7.

20 Barbulescu K, Becker C, Schlaak JF, Schmitt E, Meyer zum Buschenfelde KH, Neurath MF. IL-12 and IL-18 differentially regulate the transcriptional activity of the human IFN-γ promoter in primary CD4+ T lymphocytes. *J. Immunol.* 1998; **160**: 3642–7.

21 Thierfelder WE, Deursen JM, Yamamoto K *et al.* Requirement for Stat4 in interleukin-12-mediated responses of natural killer and T cells. *Nature* 1996; **382**: 171–4.

22 Jacobson NG, Szabo SJ, Weber-Norde RM *et al.* Interleukin 12 signaling in T helper type 1 (Th1) cells involves tyrosine phosphorylation of signal transducer and activator of transcription (Stat) 3 and Stat4. *J. Exp. Med.* 1995; **181**: 1775–62.

23 Ushio S, Namba M, Okura T *et al.* Cloning of the cDNA for human IFN-gamma-inducing factor, expression in *Escherichia coli*, and studies on the biologic activities of the protein. *J. Immunol.* 1996; **156**: 4274–9.

24 Micallef MJ, Ohtsuki T, Kohno K *et al.* Interferon-gamma-inducing factor enhances T helper 1 cytokine production by stimulated human T cells: Synergism with interleukin-12 for interferon-gamma production. *Eur. J. Immunol.* 1996; **26**: 1647–51.
25 Kohno K, Kataoka J, Ohtsuki T *et al.* IFN-gamma-inducing factor (IGIF) is a costimulatory factor on the activation of Th1 but not Th2 cells and exerts its effect independently of IL-12. *J. Immunol.* 1997; **158**: 1541–50.
26 Parnet P, Garka KE, Bonnert TP, Dower SK, Sims JE. IL-1Rrp is a novel receptor-like molecule similar to the type I interleukin-1 receptor and its homologues T1/ST2 and IL-1R AcP. *J. Biol. Chem.* 1996; **271**: 3967–70.
27 Torigoe K, Ushio S, Okura T *et al.* Purification and characterization of the human interleukin-18 receptor. *J. Biol. Chem.* 1997; **272**: 25 737–42.
28 Dinarello CA. Interleukin-18. *Methods* 1999; **19**: 121–32.
29 de Jong R, Altare F, Haagen IA *et al.* Severe mycobacterial and *Salmonella* infections in interleukin-12 receptor-deficient patients. *Science* 1998; **280**: 1435–8.
30 Wu C, Ferrante J, Gately MK, Magram J. Characterization of IL-12 receptor beta1 chain (IL-12Rbeta1)-deficient mice: IL-12Rbeta1 is an essential component of the functional mouse IL-12 receptor. *J. Immunol.* 1997; **159**: 1658–65.
31 Aoki M, Matsui E, Kaneko H *et al.* A novel single-nucleotide substitution, Leu467Pro, in the interferon-gamma receptor 1 gene associated with allergic diseases. *Int. J. Mol. Med.* 2003; **12**: 185–91.