sidered as one of the chromosome instability syndromes since the fibroblasts or lymphocytes of BS patients show excessive spontaneous chromosome instability. The causative gene of BS (*BLM*) was identified as a RecQ helicase homologue. In this review, we showed the characteristic phenotypes of BS, especially two Japanese siblings. In the latter of the review, the functional domains of BLM, those are nuclear localization signal and the interacting proteins such as ATM, are shown. Several lines of reports indicates that BLM helicase is involved in the re-initiation of DNA replication at sites where replication forks have arrested or collapsed. To elucidate the precise function of RecQ helicase in DNA repair and replication aims not only to improve our understanding of the molecular basis for tumorigenesis, but also to extend the range of potential therapeutic targets.

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

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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

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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

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(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 $[^3H]$ thymidine per well 4 h before harvesting onto glass-fiber filters. $[^3H]$ 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 nucleotide 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.

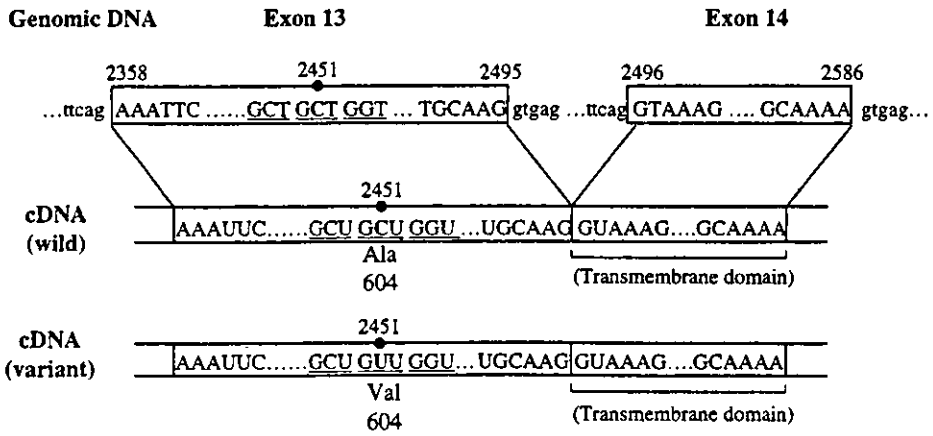

Fig. 1. Sequence analysis of genomic DNA and the cDNA of IL-12R β2 exons 13 and 14 and their flanking regions in non-atopic healthy controls and atopic patients. Sequence analysis of genomic DNA of the IL-12R β2 chain from any of the non-atopic healthy controls and any of the atopic patients showed no 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 IL-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.

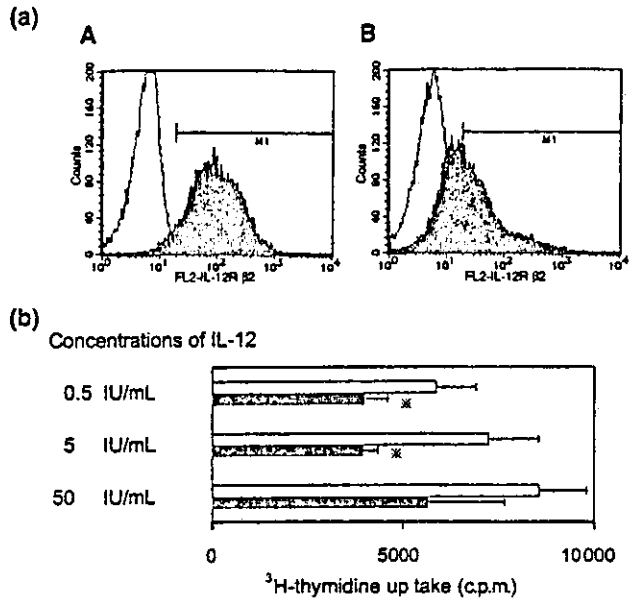

Fig. 2. (a) Expression of the IL-12R β2 chain without or with the C-to-U conversion at nucleotide 2451 (Ala 604 Val) in Ba/F3 cell clones transfected with wild-type (A) or variant-type (B) IL-12R β2 cDNA. 10^6 cells in 100 μL of staining buffer were incubated with 1 μg/mL rat anti-hu IL-12R β2(2B6) mAb (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

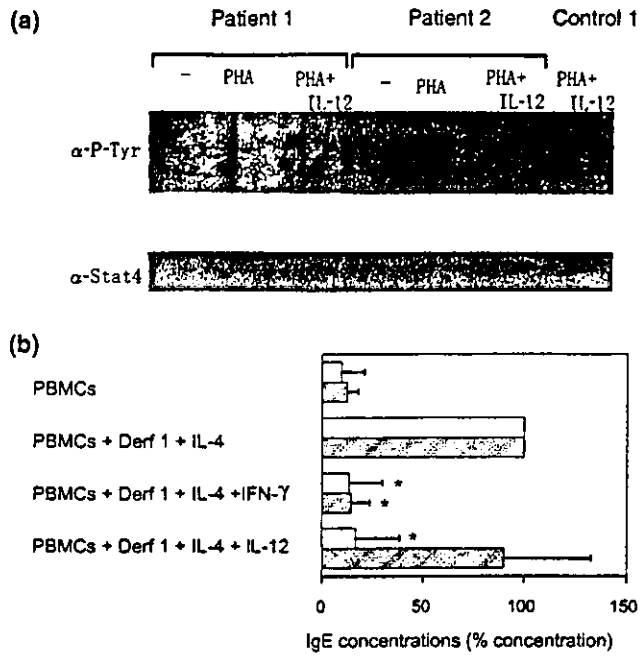

Fig. 3. (a) Tyrosine phosphorylation of Stat4 in PBMCs. PBMCs from the patients with or without the C-to-U conversion at nucleotide 2451 (Ala 604 Val) on the IL-12R β2 chain and the control subject were cultured with PHA and IL-12. Cell lysates were immunoprecipitated with anti-Stat4, resolved by SDS-PAGE, 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.

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Review Article

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

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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.

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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]

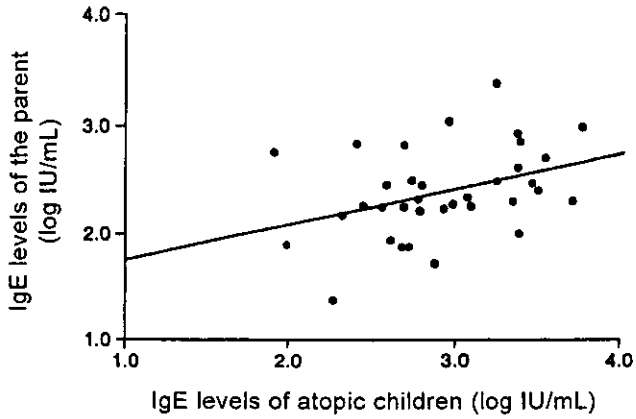


Fig. 1 Relationship between serum total IgE levels of atopic children and the IgE levels of their parents (the highest IgE level of two spouses was used). Children older than 6 years were selected. $y = 1.38 + 0.3461x$; $P < 0.016$.

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

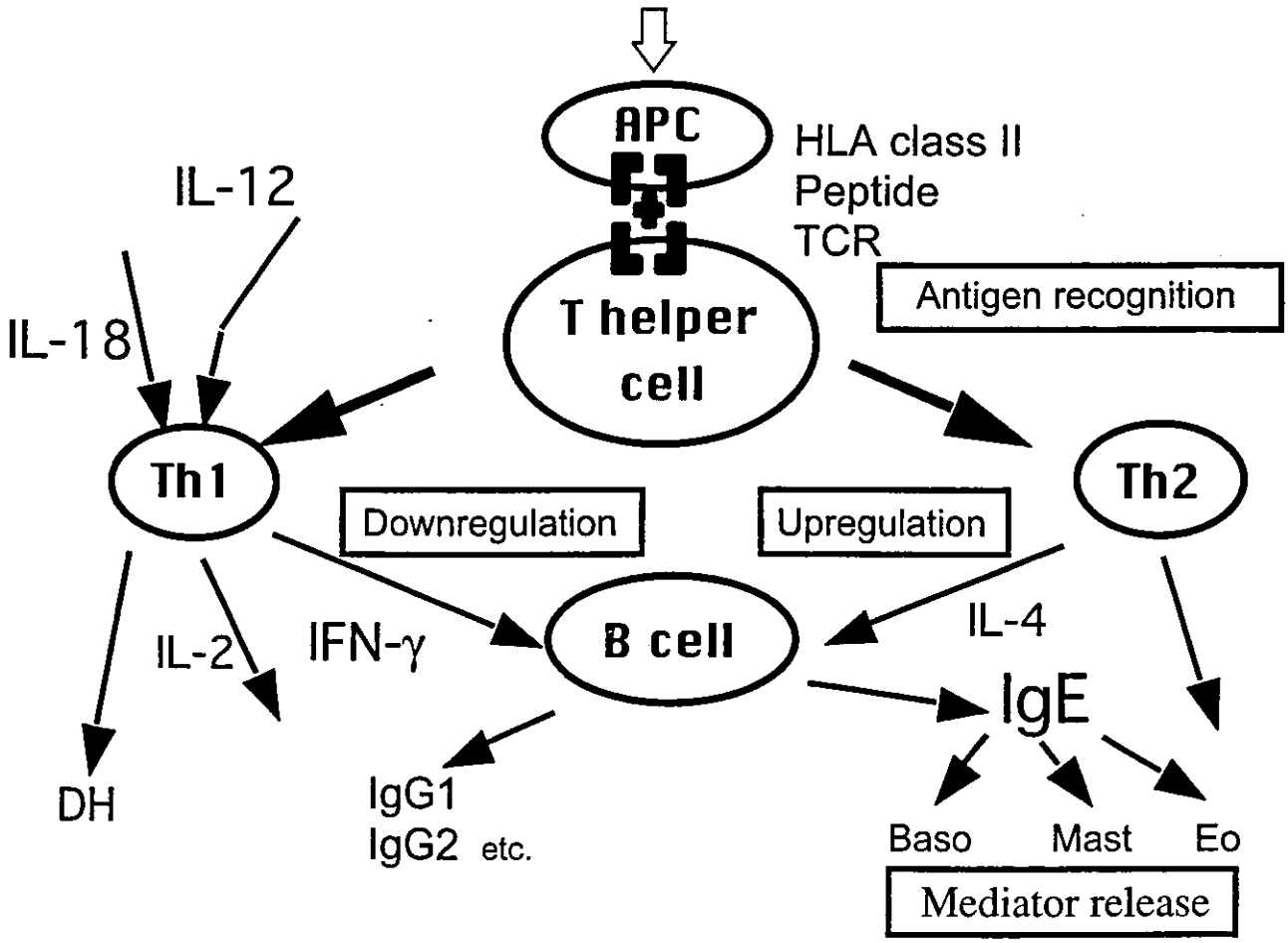


Fig. 2 The Th1 and Th2 lymphocyte balance and upregulation and downregulation of IgE production. IL, interleukin; DH, delayed-type hypersensitivity; IFN, interferon; APC, antigen-presenting cell; HLA, human leukocyte antigen; TCR, T cell receptor; Baso, basophils; Mast, mast cells; Eo, eosinophils.

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-1 complex is composed of a binding chain termed I 18Rα, a member of the IL-1R family previously identifi as the IL-1R-related proteins, and a signaling chain, als a member of the IL-1R family. The IL-18R compl recruits the IL-1R-activating kinase and tumor necros factor (TNF)-associated factor 6, which phosphorylat nuclear factor (NF)-κB-inducing kinase, with subseque activation of NF-κB[26–28] (Fig. 4).

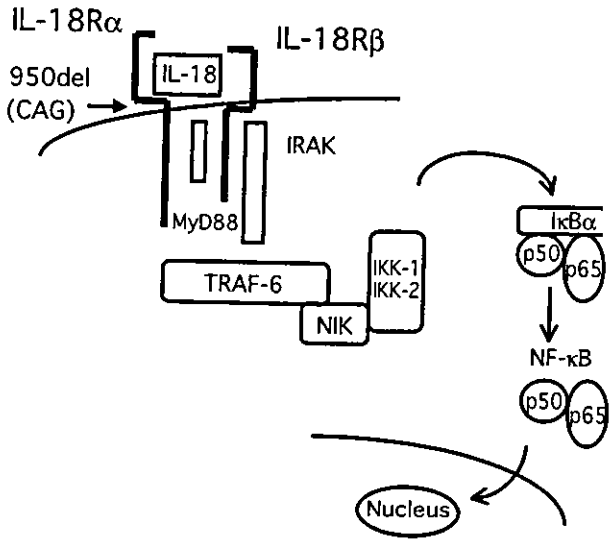


Fig. 4 Interleukin (IL)-18 signaling. IL-18Rα, IL-18Rβ, IL-18 receptor α and β chains, respectively; IKK-1, Ikk-2, IκBα kinases 1 and 2, respectively; NF-κB, nuclear factor-κB; NIK, NF-κB-inducing kinase; TRAF-6, tumor necrosis factor receptor-associated factor 6; IRAK, IL-1 receptor-associated kinase.

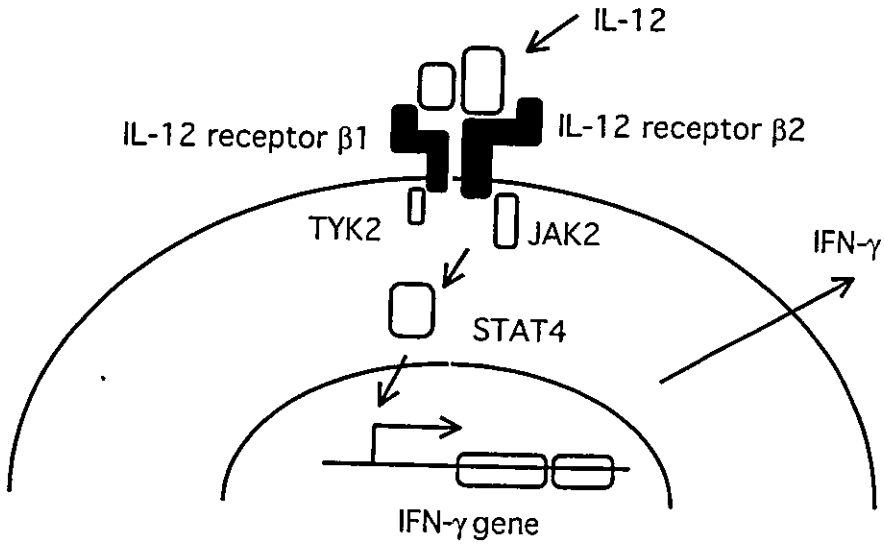


Fig. 3 Interleukin (IL)-12 signaling. TYK2, tyrosine kinase 2; JAK2, Janus kinase 2; STAT4, signal transducers and activators of transcription 4; IFN, interferon.

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.

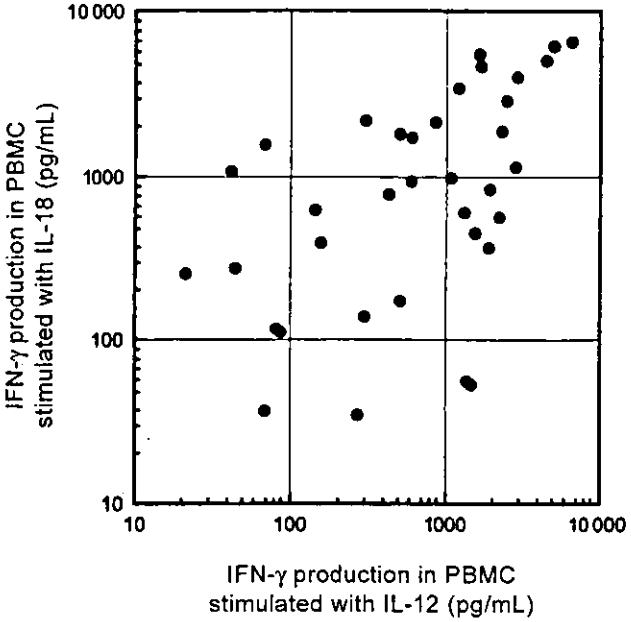


Fig. 5 Interferon (IFN)-γproduction in peripheral blood mononuclear cells (PBMC) stimulated with interleukin (IL)-12 or IL-18 (see text for details).

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

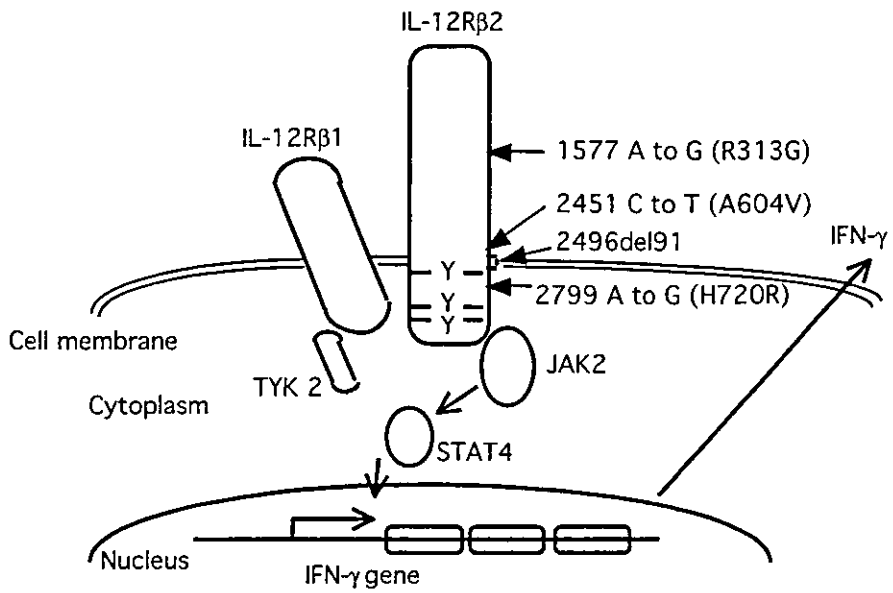


Fig. 6 Interleukin (IL)-12 signaling and mutations of IL-12 receptor β (IL-12Rβ) 2 chain gene. R, arginine; G, glycine; H, histidine; Y, tyrosine (2451 C to T: by RNA editing); TYK2, tyrosine kinase 2; JAK2, Janus kinase 2; STAT4, signal transducers and activators of transcription 4.

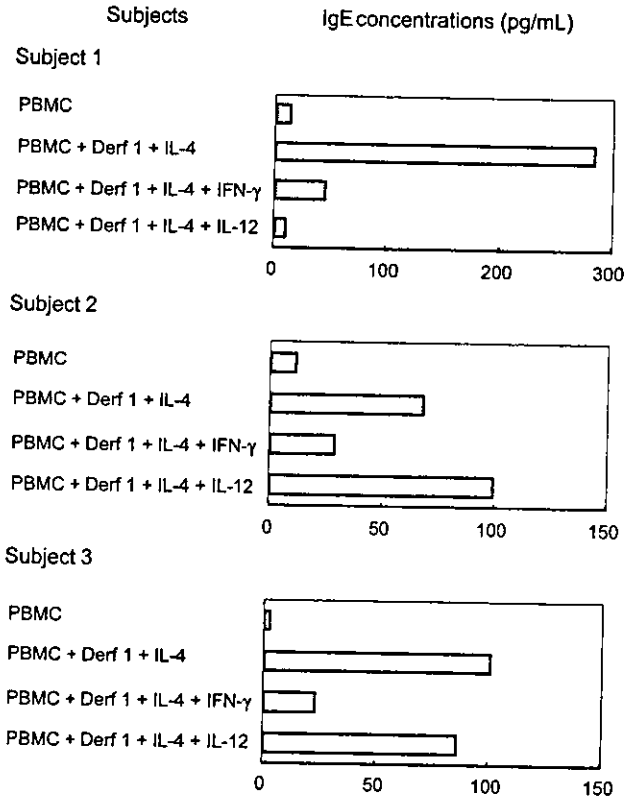
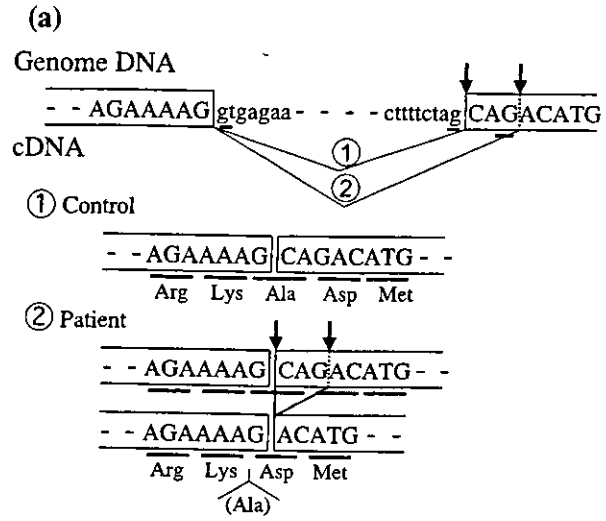


Fig. 7 Suppression of IgE production of peripheral blood mononuclear cells (PBMC; see text for details). The PBMC (10^{-6} /mL) were cultured with Derf 1 (0.5 μg/mL; Asahi, Tokyo, Japan) and interluekin (IL)-4 (500 U/mL; Genzyme, Cambridge, MA, USA) in culture test tube for 14 days. Moreover, interferon (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.

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



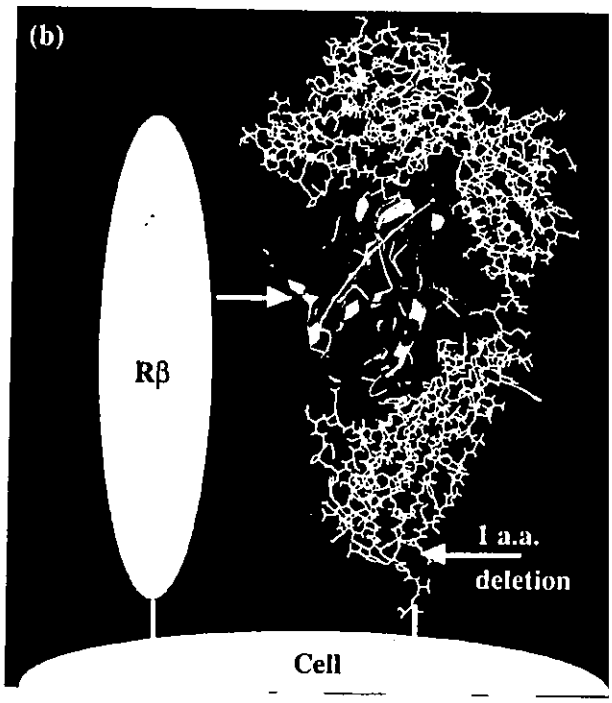


Fig. 8 (a) 950delCAG in interluekin (IL)-18 receptor (IL-18Rα) chain cDNA and (b) model of the ternary complex o IL-18Rβ : IL-18 : IL-18Rα.

impairment of the downregulation (brake) of IgE production, whereas homozygous mutations of IL-12β1 o 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

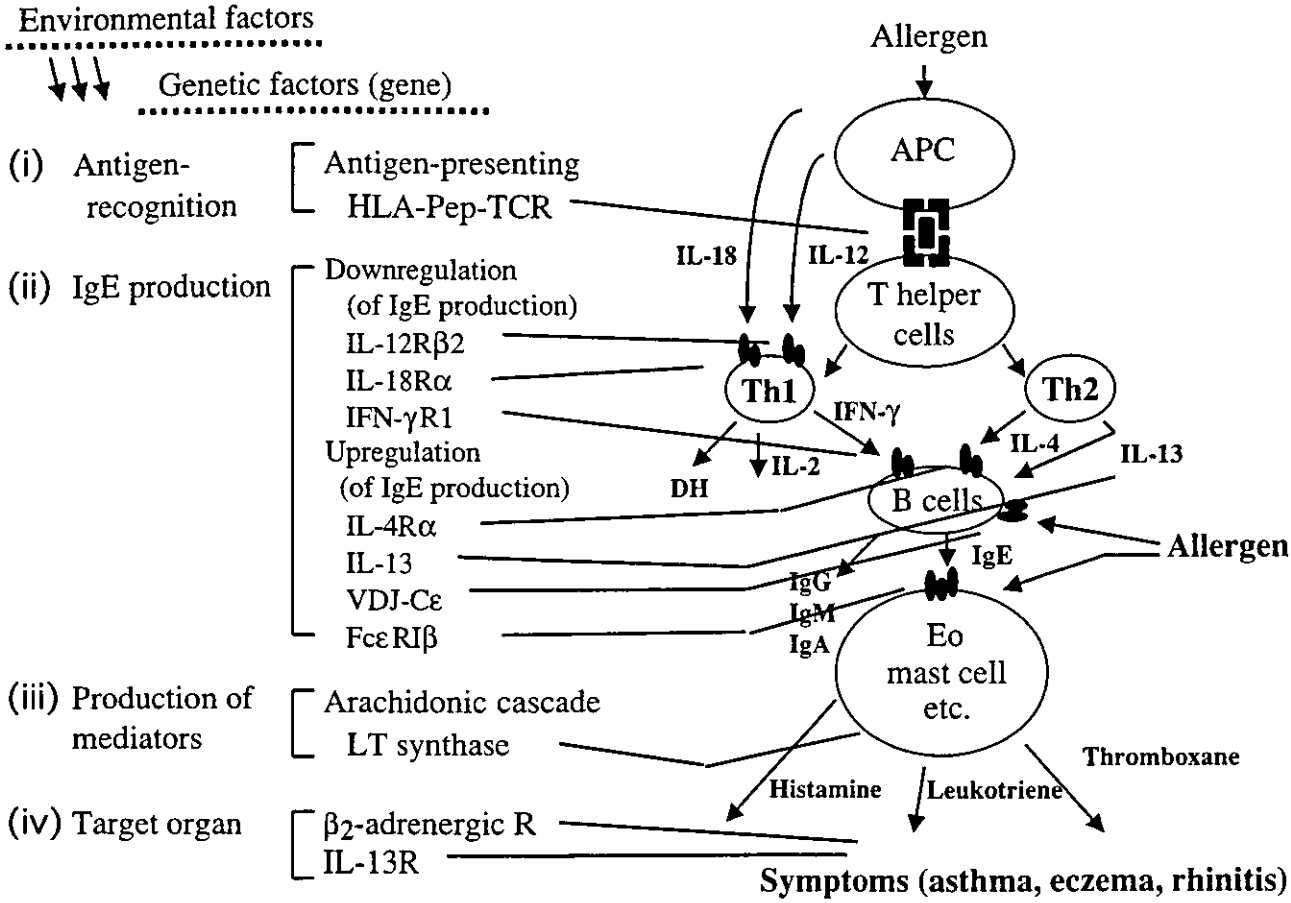


Fig. 9 A new genetic classification of atopy and genetic ecologic medicine in allergy (designed by N Kondo, 2002). HLA-Pep-TCR, human leukocyte antigen–peptide–T cell receptor; IL, interleukin; IFN, interferon; VDJ-Cε, variable diversity joining-ε constant region; LT, leukotriene; R, receptor; APC, antigen-presenting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

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Original Article

Urinary leukotriene E_4 and 11-dehydro-thromboxane B_2 excretion in children with bronchial asthma

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Abstract

Background: Cysteinyl leukotrienes (CysLTs) and thromboxane (TX) A_2 have been implicated in the pathogenesis of bronchial asthma. Urinary leukotriene E_4 (LTE_4) and 11-dehydro-TXB_2 ($11DTXB_2$) levels are often used to assess the production of CysLTs and TXA_2. However, few studies have examined the products of these two mediators in the same asthmatic patients. To define the potential roles of CysLTs and TXA_2 in the pathogenesis of bronchial asthma in children, their urinary levels were measured in the present study.

Methods: Urinary LTE_4 and $11DTXB_2$ levels were measured by enzyme immunoassay (EIA) and radioimmunoassay (RIA), respectively. Urine samples from asthmatic children were measured during the stable condition and during an acute attack.

Results: Urinary LTE_4 levels during an acute attack (median 476 pg/mg creatinine; range 191–1100 pg/mg creatinine) and during the stable condition (median 332 pg/mg creatinine; range 128–965 pg/mg creatinine) were significantly higher ($P < 0.05$) than those of controls (median 233 pg/mg creatinine; range 103–389 pg/mg creatinine). Urinary $11DTXB_2$ levels during an acute attack and during the stable condition (median 1666 (range 110–5105) and 1009 (range 46–6070) pg/mg creatinine, respectively) were significantly higher ($P < 0.05$) than those of controls (median 252 pg/mg creatinine; range 41–716 pg/mg creatinine). Comparing different stages of asthma, LTE_4 levels during an acute attack were significantly higher ($P < 0.05$) than during the stable condition; however, there was no difference in urinary TXB_2 levels.

Conclusions: The present findings suggest that high levels of CysLTs and TXA_2 are associated with the pathogenesis of bronchial asthma. The measurement of urinary LTE_4 and $11DTXB_2$ would be useful in understanding the individual pathogenesis of asthmatic children.

Key words: bronchial asthma, cysteinyl leukotrienes, 11-dehydro-thromboxane B_2, leukotriene E_4, thromboxane A_2.

Introduction

Cysteinyl leukotrienes (CysLTs), namely leukotrienes C_4, D_4 and E_4, and thromboxane (TX) A_2 are considered to play important roles in bronchial asthma.[1–10] Cysteinyl leukotrienes are derived from arachidonic acid by the action of 5-lipoxygenase and increase vascular permeability, stimulate mucus secretion and induce bronchial hyperreactiveness and bronchoconstriction. Moreover, increased production of CysLTs in asthmatic patients *in vivo* has been observed in several studies.[1–5,11,12] A potent bronchoconstrictor, TXA_2 is generated from arachidonic acid by cyclooxygenase. Enhanced TXA_2 release has also been reported in asthmatic patients after allergen challenge.[7] Owing to the significant roles of CysLTs and TXA_2, their inhibitors or receptor antagonists have been developed extensively and recently some drugs have become available.[13]

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Although these mediators of asthma have been discussed previously, few studies have examined the products of these two mediators in the same asthmatic patients. In addition, few studies have compared the TXA_2 products of asthmatic patients with those of healthy control subjects. Leukotriene E_4 is a stable product of CysLTs and is considered an index of the systemic production of CysLTs production in humans.[14] 11-Dehydro-thromboxane B_2 ($11DTXB_2$) is the most abundant degradation product of TXB_2 and is also considered an index of systemic TXA_2 production.[15–17] The measurement of urinary LTE_4 and $11DTXB_2$ is a non-invasive method for assessing the production of CysLTs and TXA_2 *in vivo*, respectively. Oosaki *et al.*[18] previously established sensitive and selective methods of determination of urinary LTE_4 by enzyme immunoassay (EIA) and Ruitta *et al.*[16] established the method of determining urinary $11DTXB_2$ by radioimmunoassay (RIA). In the present study, using these methods, the products of CysLTs and TXA_2 were assessed *in vivo* in control subjects and in children with bronchial asthma during the stable condition and during an acute attack.

METHODS

Subjects

Twenty-nine children with bronchial asthma (19 males, 10 females) and nine control subjects (six males, three females) were enrolled in the study (Table 1). The mean age of asthmatic children and control subjects was 7 years (range 1–15 years) and 8 years (range 1–15 years), respectively. None of the patients had a history of aspirin sensitivity. Twenty-six of the asthmatic patients were receiving theophylline, all were using inhaled disodium cromoglycate (DSCG) and β_2-adrenergic receptor agonists, 10 were receiving inhaled corticosteroids and 17 were using a leukotriene receptor antagonist (LTRA). All patients were classified into one of four categories (intermittent, mild persistent, moderate persistent and severe persistent) according to Glolabal Initiative for Asthma (GINA) guidelines (http://www/ginasthma.com/ Table 1). None of the patients had been treated previously with oral prednisolone (PSL) prior to their enrollment in the study. In the present study, the urinary excretion of LTE_4 or $11DTXB_2$ was not influenced by the usage of LTRA or corticosteroids, because the patients kept the treatments unmodified throughout the duration of the study. Informed consent to participate in the study was obtained from all subjects or their parents.

Urine samples from children with bronchial asthma were measured during the stable condition and during an acute attack. Urine samples from nine asthmatic children selected at random (Table 1, patients 1–9) were also measured 2 days after treatment. The 'stable condition' refers to the condition in which the patients did not complain of any symptoms with or without receiving their usual medications. The 'acute attack' refers to a condition in which the patients complained of some active symptoms, cough and/or wheezing and/or chest tightness, which were occurring repeatedly on waking and/or disturbing sleep at night; therefore, they needed additional treatment to their usual treatments.[19] Patients were treated with steroid and/or theophylline by injection and/or inhaled β_2-adrenergic receptor agonists and, 2 days after treatment, they felt better but were still complaining slightly of some asthmatic symptoms, such as cough and/or wheezing and/or chest tightness.

Urine samples were collected when the asthmatic children visited our hospitals during the stable condition maintaining their usual treatments. Urine samples from asthmatic children were also collected on arrival at hospital when they had acute asthma attacks. Patients were treated with theophylline and/or β_2-adrenergic receptor agonists and/or corticosteroid drip infusion. Urine samples from nine asthmatic children were collected 2 days after treatment.

Measurement of LTE_4

Urine samples were stored at –80°C and analyzed within 1 month of collection. An aliquot of urine was removed to determine creatinine concentration. The urinary creatinine level was determined using a Creatinine test kit (Pure Auto S CRE-L; Daiichi-kagaku, Tokyo, Japan).

Approximately 3000 d.p.m. [^{3}H]-LTE_4 was added to each urine sample as an internal standard and the urine was applied to a Sep-Pak C18 cartridge (Waters, Milford, MA, USA) that had been preconditioned by the serial addition of methanol and distilled water. Then, the cartridge was washed with distilled water, followed by 40% methanol; LTE_4 was eluted with 80% methanol. This elution was dried with nitrogen gas and was dissolved in the elution buffer used in high-performance liquid chromatography (HPLC; 486 Tunable Absorbance Detector; Waters) and the solution was injected onto a C18 reverse-phase column (CAPPCELL PAC UG 120; Shiseido, Tokyo, Japan). The fractions that contained peak [^{3}H]-LTE_4 radioactivity and also corresponded to the

Table 1 Characteristics of patients and urinary levels of leukotriene E_4 and 11-dehydro-thromboxane B_2

Patient no.	Gender	Age (years)	Serum IgE (U/mL)	HD score	Mite score	Severity (GINA)	Steroid treatment during attack	Theophylline	LTRA	DSCG	β_2-Adrenergic receptor agonist	Steroid inhalant	Stable condition LTE_4	Stable condition 11DTXB$_2$	Acute attack LTE_4	Acute attack 11DTXB$_2$	After treatment LTE_4	After treatment 11DTXB$_2$
1	M	7	1784	5	6	Moderate	+	+	−	+	+	−	183	861	671	1285	156	2130
2	M	5	200	4	5	Mild	+	+	−	+	+	−	212	2166	611	1188	295	820
3	M	14	900	6	6	Moderate	+	−	−	+	+	+	482	745	1100	563	443	785
4	M	1	21	0	0	Severe	+	+	+	+	+	−	373	282	280	3122	524	2708
5	M	9	70	0	0	Moderate	+	+	+	+	+	−	480	1059	546	2096	771	2137
6	F	10	199	2	2	Moderate	+	+	+	+	+	−	253	1515	437	2247	231	842
7	F	5	82	6	6	Mild	+	+	−	+	+	−	188	2150	409	589	340	492
8	F	9	294	4	4	Severe	+	+	−	+	+	+	484	131	797	166	473	533
9	F	7	1281	4	4	Moderate	+	+	+	+	+	−	556	1009	483	2087	872	1515
10	M	10	653	2	3	Moderate	+	+	+	+	+	+	378	904	615	1847		
11	M	4	208	4	5	Moderate	+	+	+	+	+	−	341	1842	468	3254		
12	M	5	2892	4	4	Moderate	+	+	+	+	+	+	274	163	553	2461		
13	M	3	401	5	5	Moderate	+	+	+	+	+	−	519	2462	567	2352		
14	M	9	265	5	5	Mild	+	+	+	+	+	−	254	1476	369	2499		
15	M	3	180	5	6	Moderate	+	+	+	+	+	+	332	1392	424	480		
16	M	3	1381	3	4	Moderate	+	+	−	+	+	+	606	167	191	379		
17	M	9	890	2	1	Mild	+	+	+	+	+	−	165	873	269	1259		
18	M	9	703	6	5	Severe	+	+	+	+	+	+	278	804	334	939		
19	M	2	1417	6	6	Moderate	+	+	+	+	+	−	755	3535	743	4075		
20	M	9	578	5	6	Mild	+	+	+	+	+	−	286	793	284	1700		
21	M	8	62	2	0	Intermittent	+	−	−	+	+	−	365	1992	371	258		
22	M	4	334	3	3	Moderate	+	+	+	+	+	+	328	3483	394	2548		
23	M	2	394	5	6	Mild	+	+	+	+	+	−	610	6070	980	5104		
24	F	15	301	0	0	Moderate	+	+	−	+	+	+	284	46	1100	311		
25	F	5	866	6	6	Intermittent	−	−	−	+	+	−	727	196	245	110		
26	F	7	1300	5	6	Mild	+	+	−	+	+	−	259	1837	476	2413		
27	F	7	1000	2	1	Mild	−	+	−	+	+	−	128	903	289	1318		
28	F	13	597	5	5	Mild	−	+	−	+	+	−	332	1026	504	1666		
29	F	3	600	6	6	Moderate	+	+	+	+	+	+	965	261	528	429		
Median			577.5										332	1009	476	1666	443	842
Average		7	684.5	4	4								393.0	1384.2	518.6	1680.9	456.1	1329.2

GINA, Global Initiative for Asthma (GINA) guideline (http://www.ginasthma.com/).

HD, house dust; LTRA, leukotriene receptor antagonist; DSCG, disodium cromoglycate; LTE_4, leukotriene E_4; 11DTBX$_2$, 11-dehydro-thromboxane B_2.