

RNA editing of interleukin-12 receptor $\beta 2$, 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) $\beta 2$ is associated with atopy.

Methods Atopic patients and non-atopic healthy controls were studied. Fragments of IL-12R $\beta 2$ cDNA and genomic DNA were amplified and sequenced. Furthermore, the function of the IL-12R $\beta 2$ chain was investigated.

Results Sequence analysis of the cDNA clones representing IL-12R $\beta 2$ 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 $\beta 2$, 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 $\beta 2$, 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, $\beta 1$ and $\beta 2$, that assemble to form a high-affinity IL-12 receptor (R) complex [5]. While the $\beta 2$ chain of the IL-12R is expressed only in Th1 lymphocytes, the $\beta 1$ chain is expressed in both Th1 and Th2 lymphocytes. Thus, the expression of

both $\beta 1$ and $\beta 2$ 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 $\beta 2$ 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 β 2, 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 μ g/mL PHA for 24 h at 37 °C in a humidified atmosphere containing 5% CO₂ [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 β 2 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 β 2 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 β 2 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 β 2 genomic DNA were amplified by PCR with the sense primer 5'-GATGACAGCTCTGACAGCTG-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 β 2 and transfected Ba/F3 cell clones

The expression constructs encoding human wild-type IL-12R β 2 or variant-type (2451 C-to-U conversion) IL-12R β 2 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 β 2 or the variant-type IL-12R β 2. Then, the transfected Ba/F3 cells were cloned.

Flow cytofluorometric analysis

IL-12R β 2 expressing Ba/F3 cell clones were detected by indirect immunofluorescence analysis using flow cytometry. Briefly, 10^6 cells in 100 μ L of staining buffer were incubated with 1 μ g/mL rat anti-hu IL-12R β 2(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 μ Ci [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 Na₂PO₄, 1% aprotinin, 5 mM PMSF, 100 mM NaF and 2 mM Na₃VO₄, 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% CO₂. 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

	<i>n</i>	C-to-U conversion at nucleotide 2451 in IL-12R β2 chain cDNA		<i>P</i> -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.

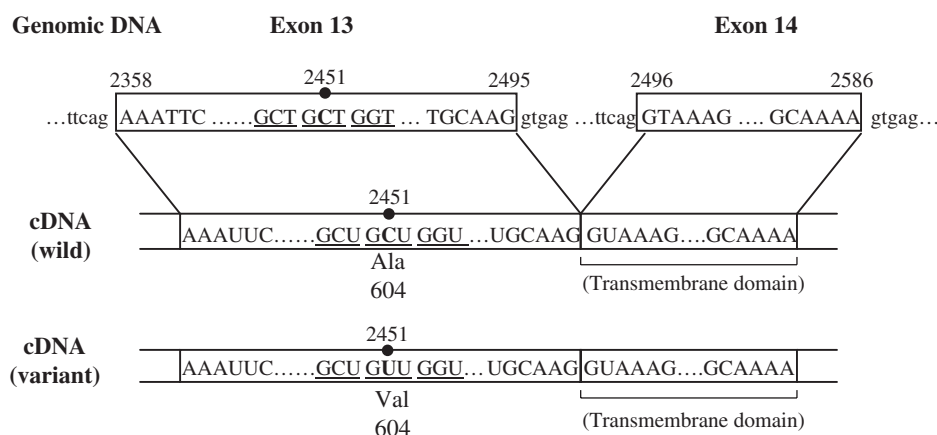


Fig. 1. Sequence analysis of genomic DNA and the cDNA of IL-12R β2 exons 13 and 14 and their flanking regions in non-atopic healthy controls and atopic patients. Sequence analysis of genomic DNA of the IL-12R β2 chain from any of the non-atopic healthy controls and any of the atopic patients showed no 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 β 2 chain cDNA

	IFN- γ concentration (pg/mL)*		P-value‡
	Without C-to-U conversion† (n = 169)	With C-to-U conversion† (n = 25)	
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 β 2 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 β 2 chain

To investigate the expression of the wild-type or variant-type IL-12R β 2 chain, Ba/F3 cells were transfected by electroporation with wild-type IL-12R β 2 cDNA or variant-type IL-12R β 2 cDNA containing the C-to-U conversion at nucleotide 2451 in the PEF-BOS expression vector and then cloned [5]. The IL-12R β 2-chain-expressing cells were detected by flow cytometry using the anti-IL-12R β 2 antibody. The results revealed that the staining intensity and the percentage of cells expressing the IL-12R β 2 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 β 2 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 β 2 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 β 2 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 β 2 chain cDNA is associated with reduced signal transduction of IL-12 for IFN- γ production by PBMCs.

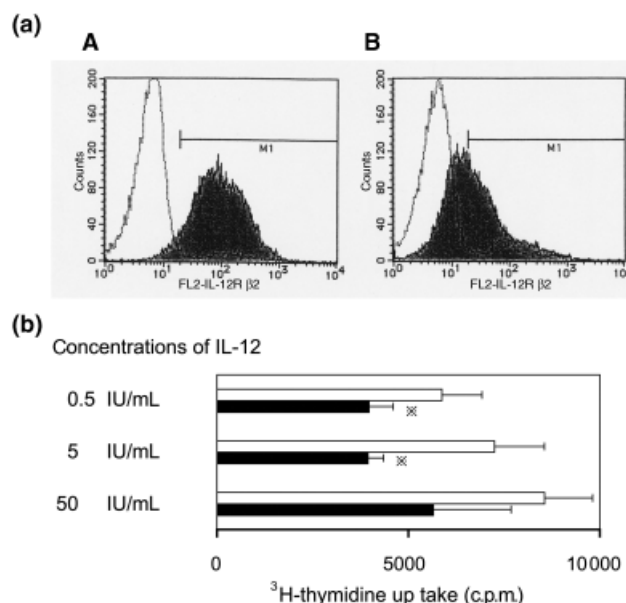


Fig. 2. (a) Expression of the IL-12R β 2 chain without or with the C-to-U conversion at nucleotide 2451 (Ala 604 Val) in Ba/F3 cell clones transfected with wild-type (A) or variant-type (B) IL-12R β 2 cDNA. 10^6 cells in 100 μ L of staining buffer were incubated with 1 μ g/mL rat anti-hu IL-12R β 2 (2B6) 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 β 2 expressing cells were detected by flow cytometry. The staining intensity and the percentage (50.1%) of cells expressing the IL-12R β 2 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 β 2 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 β 2 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 β 2 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 β 2 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 β 2 chain cDNA. In contrast, in the

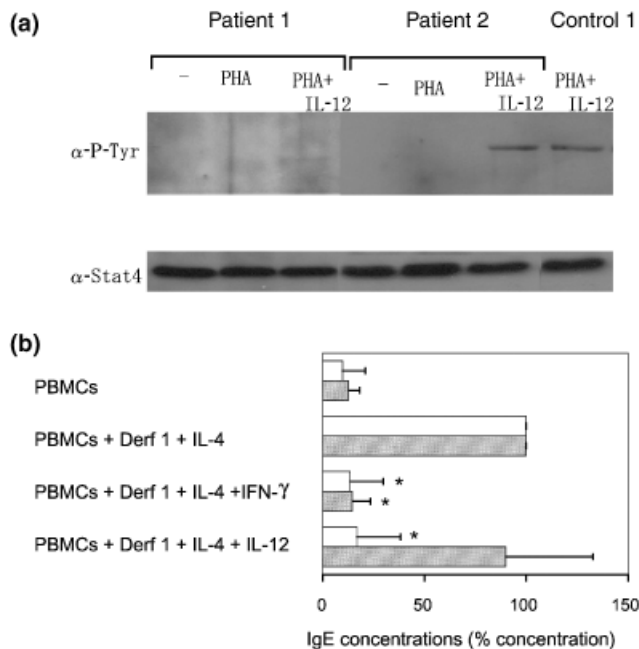


Fig. 3. (a) Tyrosine phosphorylation of Stat4 in PBMCs. PBMCs from the patients with or without the C-to-U conversion at nucleotide 2451 (Ala 604 Val) on the IL-12R β 2 chain and the control subject were cultured with PHA and IL-12. Cell lysates were immunoprecipitated with anti-Stat4, resolved by SDS-PAGE, 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 $\pm$ SD, $n = 3$) without the C-to-U conversion at nucleotide 2451 in IL-12R β 2 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 $\pm$ SD, $n = 3$) with the C-to-U conversion at nucleotide 2451 in IL-12R β 2 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 β 2, 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 β 1 chain gene causes impairment of salmonella and mycobacterial immunity [12, 13]. The development of Th1 lymphocytes is disturbed in IL-12 or IL-12R β 1 knockout mice [8, 14]. In this study, atopic subjects with RNA editing of IL-12R β 2, 2451 C-to-U (Ala 604 Val) conversion, did not exhibit any impairment of salmonella and

mycobacterial immunity. The IL-12R β 2 subunit, similar to the IL-12R β 1 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 β 1, which does not contain any tyrosine residues, the cytoplasmic region of IL-12R β 2 contains three tyrosine residues, suggesting an important role of the β 2 subunit in IL-12 signal transduction [5]. Presky et al. [5] reported that Ba/F3 cells transfected with IL-12R β 2 alone proliferates in response to human IL-12 although the role of endogenous mouse IL-12R β 1 in IL-12 signal transduction in these transfectants cannot be ruled out. Thus, IL-12R β 2 is different from IL-12R β 1 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 β 2 and the proliferative responses to IL-12 in variant-type IL-12R β 2 transfected Ba/F3 were lower than those of wild-type. The RNA editing of IL-12R β 2, 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 lymphocytes 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 β 2 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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