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REVIEW

Atopy and mutations of IL-12 receptor β2 chain gene

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Introduction

Atopy, which is characterized by enhanced IgE responses to environmental antigens, leads to clinical disorders such as asthma, eczema and rhinitis. These atopic disorders develop due to the interactions between genetic and environmental factors. The class switch to IgE and production of IgE in B lymphocytes are regulated by cytokines [1–6]. IL-12 is one of the most important cytokines which down-regulate IgE production. In this paper, the relationship between atopy and aberrant IL-12 signal transduction, particularly mutations in the IL-12 receptor (IL-12R) chain gene is focused upon.

Helper T lymphocytes and IgE production

Several reports suggest that the $CD4^+$ helper-inducer T lymphocyte subset is in itself heterogeneous [7–9]. Mosmann *et al*. [8,9] reported that mouse helper T cell clones fall into two main groups (Thl and Th2), defined primarily by differences in the pattern of lymphokines synthesized. Thl clones synthesize IL-2, IFN-γ and lymphotoxin, whereas these lymphokines are not detectably expressed in Th2 clones. Conversely, only Th2 clones synthesize detectable amounts of IL-4 and IL-5. Thl clones also effectively cause delayed types of hypersensitivity reactions. Wierenga *et al*. [10] presented evidence for the presence of distinct functional subsets of $CD4^+$ T lymphocytes in human beings on the basis of the production of IL-4 and IFN-γ.

IgE plays an important role in immediate hypersensitivity. T lymphocyte- or monocyte-derived cytokines play an important role in the regulation of immunoglobulin isotype switching. IL-4 promotes class switching to IgE in B lymphocytes and Th2 lymphocyte differentiation [1]. Isotype switching occurs through transcriptional activation of germline genes prior to switch recombination. The production of IgE in B lymphocytes is down-regulated by IFN-γ which is one of the Th1 cytokines [4]. Several investigators have shown that, upon mitogen stimulation, peripheral blood mononuclear cells (PBMCs) from patients with atopic diseases produce less IFN-γ but more IL-4 than PBMCs from healthy subjects [11,12]. It has been reported that IFN-γ levels showed an inverse association with the serum IgE levels [13]. We previously reported that the serum IgE levels were negatively correlated with the amount of IFN-γ produced by phytohemagglutinin (PHA)-stimulated PBMCs [14]. We also measured the levels of IFN-γ, IL-4 and IgE production by PBMCs stimulated with ovalbumin [15]. In patients with allergic symptoms who were sensitive to hen's egg, there was an inverse correlation between the levels of IFN-γ and IgE production ($P < 0.05$), and a significant positive correlation was found between the levels of IL-4 and IgE production ($P < 0.01$) [15]. Furthermore, IgE production by PBMCs stimulated with IL-4 and pokeweed mitogen was suppressed by recombinant IFN-γ [16]. These results suggest that IFN-γ regulates IL-4-induced IgE production, and that when the level of IFN-γ production is low, IgE production is enhanced. Next, we investigated the reason for insufficient IFN-γ production by the MIMIC method (semi-quantitative PCR). The results reveal that the IFN-γ concentrations are well correlated with the amounts of IFN-γ mRNA, suggesting that reduced IFN-γ production is caused by reduced expression of IFN-γ mRNA [14].

IL-12 and IL-12 receptor

IL-12 is a heterodimeric cytokine that consists of two disulphide-linked subunits, p35 and p40, and is produced by activated antigen-presenting cells, particularly in response to infection with intracellular microbes. IL-12 plays a central role in promoting Th1-type immune responses and thus cell-mediated immunity [17–22]. IL-12 also induces IFN-γ production by T lymphocytes and natural killer cells [23–25].

The biological activities of IL-12 are mediated via binding to a specific cell surface receptor. The receptor for IL-12 (IL-12R) is composed of two distinct subunits;

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β1 and β2, that assemble to form a high-affinity IL-12R complex [26] (Fig. 1). 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, it is the expression of both the β1 and β2 chains that accounts for the responsiveness of T lymphocytes to IL-12 and mediates Th1 lymphocyte differentiation [27]. Each of the two IL-12R subunits is more closely related to gp130 (IL-6 receptor β chain). IL-12Rβ1 chain does not contain any cytoplasmic tyrosine residues, whereas the cytoplasmic region of IL-12Rβ2 chain contains three tyrosine residues. This suggests that the β2 subunit plays an important role in IL-12 signal transduction. IL-12 induces activation of specific members of the Stat family of transcription factors. Stat4-deficient mice manifest impaired production of IFN-γ [28] and the phenotype of the IL-12-p40-deficient mouse is similar to that of the Stat4-deficient mouse [22]. Therefore, Stat4 is particularly important. IL-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 [28,29].

Atopy and mutations of IL-12Rβ2 chain gene

Recently, it was shown that homozygous nonsense mutation of the IL-12Rβ1 chain gene resulted in impairment of immunity against salmonella and mycobacteria [30,31]. Moreover, IL-12Rβ1 knock out mice showed impaired development of Th1 [32].

Very recently, we found that reduced IFN-γ production by PBMCs following stimulation with IL-12, but not that with PHA, is associated with the heterozygous mutations of IL-12Rβ2 chain cDNA in atopic subjects [33]. In our study, IFN-γ production from PBMCs was measured following stimulation with a non-specific stimulator (PHA), a specific stimulator (Der f 1) and IL-12 in healthy controls and atopic patients with various serum IgE levels. As a result, the serum IgE level was negatively correlated with the IFN-γ production by PBMCs stimulated with IL-12 ($P < 0.001$), and with IL-12 production by PBMCs stimulated with Der f 1 ($P < 0.001$). Although the IFN-γ concentrations by PHA-stimulated PBMCs were correlated with those by IL-12-stimulated PBMCs, there were several cases showing different responses to PHA and IL-12 [34]. Twenty-four of 75 atopic individuals with high levels of IgE showed insufficient IFN-γ production by PBMCs following stimulation with IL-12, but not that with PHA [33]. Most of these atopic patients showed sufficient IFN-γ production by PBMCs stimulated with PHA. Furthermore, in separation experiment, IFN-γ was produced dominantly by $CD4^+$ T lymphocytes. These findings show that Th1 lymphocytes exist in these atopic patients. Interestingly, 12 of the above 24 subjects were found to be a heterozygous deletion (2496 del 91) or heterozygous missense mutations (1577 A to G, 2451 C to T and 2799 A to G) of the IL-12Rβ2 chain cDNA (Fig. 1). The frequencies of these heterozygous mutations in non-atopic subjects and atopic subjects are shown in Table 1. These mutations were not detected in any of the 62 healthy controls. Very recently, van Rietschoten *et al*. reported the genomic organization of the human IL-12Rβ2 chain gene [35]. The missense mutation, 1577 A to G, observed in the cDNA of IL-12Rβ2 has also been identified by the sequence analysis of the genomic DNA. However, the deletion, 2496 del 91, and the missense mutations 2451 C to T and 2799 A to G, have not been identified in the genomic DNA. Insufficient phosphorylation of Stat4 was also demonstrated in these atopic patients [33].

Taken together, in these atopic patients, a heterozygous IL-12Rβ2 chain gene 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 IgE levels. The heterozygous missense mutations, 1577 A to G (R313G), 2451 C to T (A 604V) and 2799 A to G (H720R), may lead to changes in the conformational structure. The mutations 2451 C to T and 2799 A to G, in the cDNA may be caused by a RNA editing because this mutation has not been identified in the genomic DNA. It has been reported that the loss of exon 15 of exactly 100 bp, due to an alternative splicing, results in a frameshift leading to an in-frame stop codon, 13 codons downstream [35]. The 2496 del 91 of IL-12Rβ2 chain cDNA, which is whole exon 14 and is found all over the transmembrane portion, causes a frameshift that introduces a premature stop codon at nucleotide positions to 2601–2603 (TAG) in the intracellular domain of the IL-12Rβ2. This deletion of the IL-12Rβ2 chain may be caused by an aberrant alternative splicing of the gene, since the deletion has not been identified in the genomic DNA of IL-12Rβ2 chain. Moreover, the heterozygous mutations in our study may play a role via a dominant negative effect. At least, the 12 patients with the heterozygous mutations of IL-12Rβ2 chain gene have not exhibited impairment of immunity against salmonella and mycobacteria. We could not identify any individuals with a homozygous IL-12Rβ2 chain gene mutation. It would be of great interest to examine whether subjects with the homozygous mutation of IL-12Rβ2 chain gene show impairment of salmonella and mycobacterial immunity, as do those with homozygous IL-12Rβ1 chain gene mutations. Experiments along these lines are now under way. The results of our study indicate that atopic diseases are caused, in part, by impairment of

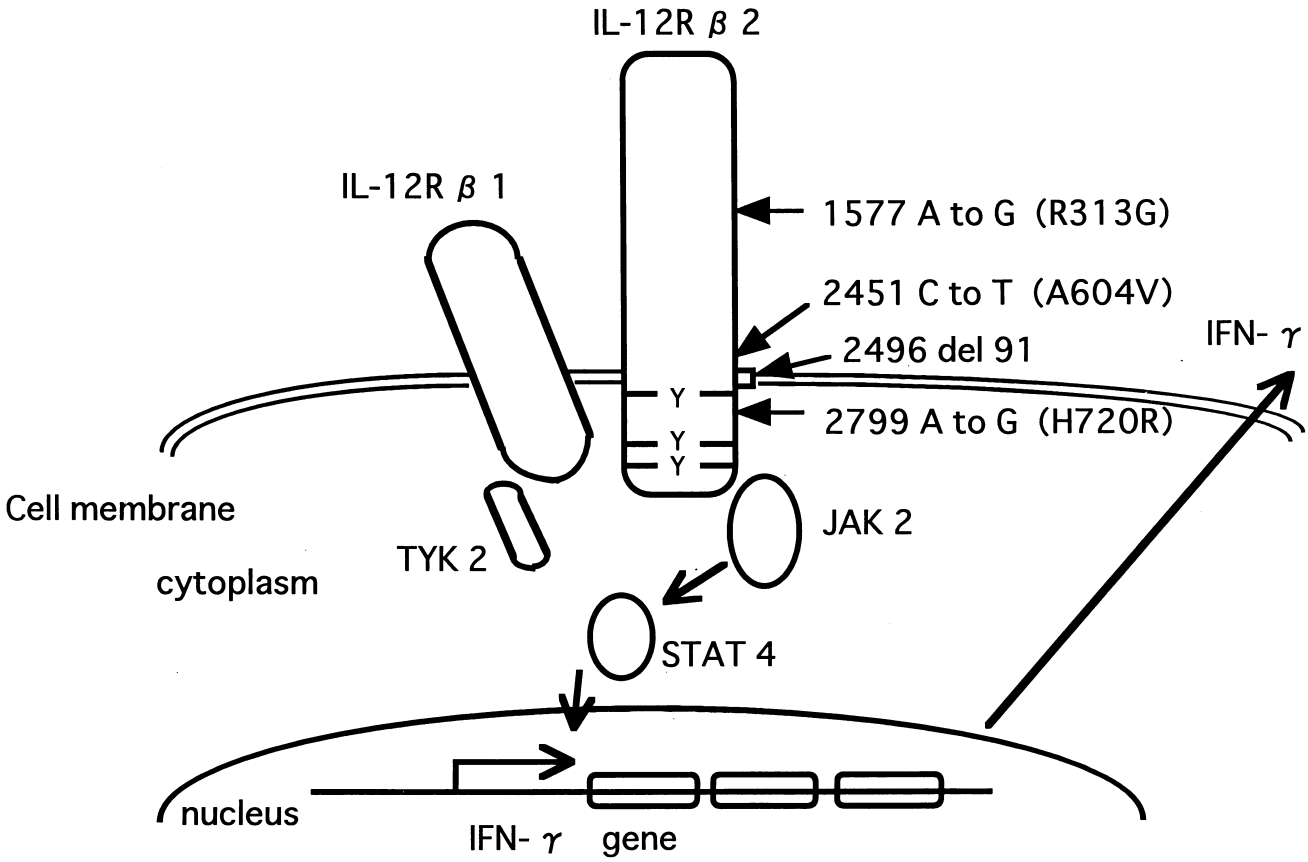

Fig. 1. IL-12 signalling and mutations of IL-12 receptor β2 chain gene.

the IL-12 signal cascade, which down-regulates IgE production.

Conclusion

We conclude that the mutation of the IL-12Rβ2 chain gene is one of the causative genes of atopy. Recently, mutations or genetic polymorphisms of several genes, such as those encoding the FcεRIβ, IL-4 receptor (IL-4R)α and IL-13, etc. have been reported as the probable causative genes of atopy [36–39]. Based on these reports and further findings, atopy can be genetically classified in the near future.

Table 1. The frequencies of heterozygous mutations of IL-12Rβ2 chain cDNA in non-atopic subjects and atopic subjects*

Group	Number of subjects	Heterozygous mutations				
		1577 A to G	2451 C to T	2496 del 91	2799 A to G	Total
Non-atopic subjects	62	0	0	0	0	0
Atopic subjects	75	1	2	8	1	12
IFN-γ production from PBMCs stimulated with IL-12						
> 20pg/mL	24	1	2	8	1	12
⩽ 20pg/mL	51	0	0	0	0	0

*[33] and our recent findings.

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