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Review

Recent Advances in Understanding How Interleukin 13 Signals Are Involved in the Pathogenesis of Bronchial Asthma

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Abstract. The prevalence of allergic disease has dramatically increased in recent decades, especially in urban and industrialized areas. Allergic diseases are disorders of the immune system, the results of complex interactions among various genetic and environmental factors. Among them, the important role of interleukin 13 (IL-13), a Th2-type cytokine, has recently emerged in the pathogenesis of bronchial asthma. Based on studies using mice, great attention has been paid to the direct effects of IL-13 on bronchial tissues. In this review, we describe recent advances in understanding the signal transduction mechanism of IL-13, the involvement of IL-13 signal-related genes as genetic factors in the pathogenesis of bronchial asthma, and the expression of IL-13 receptor on bronchial tissues. We describe potential strategies for targeting IL-13 signals to improve allergic states.

Key words: allergy; interleukin 13; interleukin 4; bronchial asthma; receptor.

Introduction

Interleukin 4 (IL-4) and IL-13 are pleiotropic cytokines primarily produced by Th2-type cells, mast cells and basophils[6, 72]. IL-4 and IL-13 have overlapping biological activities, such as class-switching toward IgE and IgG4, expression of CD23 and MHC class II on B cells, anti-inflammatory actions on monocytes, production of chemokines and induction of arachidonic acid-metabolizing enzymes on bronchial epithelial cells[31, 39, 72]. On the other hand, IL-4, but not IL-13, induces expansion of Th2-type cells and proliferation of T cells[72].

Much evidence has accumulated indicating that IL-4 and IL-13 are involved in the pathogenesis of bronchial asthma. It has been shown that IL-4 and IL-13 are highly expressed in lung, and IL-4 and IL-13 levels are high in peripheral blood in bronchial asthma patients[10, 24, 25, 35, 46, 57, 66]. Furthermore, over-expression of IL-4 or IL-13 induces a bronchial asthma-like phenotype, whereas blockage of IL-4 or IL-13 signals impairs the induction of a bronchial asthma-like phenotype in

Abbreviations used: IL – interleukin, IL-4Rα – IL-4 receptor α chain, γ_c – the common cytokine receptor γ chain, IL-13Rα – IL-13R α chain, Jak – Janus tyrosine kinase, STAT – signal transducer and activator of transcription, PI3 – phosphatidylinositol 3, IRS – insulin receptor substrate, Val – valine, Ile – isoleucine, PBMC – peripheral blood mononuclear cells, Gln – glutamine, Arg – arginine, SNP – single nucleotide polymorphism.

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mice[5, 11, 21, 52, 70, 71]. Particularly, based on the results of administration of IL-13 in mice, it is assumed that IL-13 acts on non-hematopoietic cells, evoking a bronchial asthma-like phenotype, because murine IL-13 does not induce IgE synthesis in B cells nor expand Th2-type cells.

In this review, we describe progress in the analysis of IL-13 signals and their involvement in the pathogenesis of bronchial asthma as well as potential strategies for targeting IL-13 signals to treat allergic diseases.

Signal Transduction of IL-4 and IL-13

It had been believed that functional IL-4R was composed of the IL-4 receptor α chain (IL-4Rα) and the common cytokine receptor γ chain (γ_c), which is now called type I IL-4R[29]. γ_c, which was originally identified as the IL-2R γ chain, has been shown to be involved in the cytokine receptors of IL-2, IL-4, IL-7, IL-9 and IL-15[34, 38] (Fig. 1). Thereafter, two IL-13-binding units were identified, the IL-13R α chain 1 (IL-13Rα1) and IL-13Rα2, in both the mouse and human systems[1, 7, 16, 23]. In addition to type I IL-4R, the heterodimer consisting of IL-4Rα and IL-13Rα1 has been recently shown to act as a functional IL-4R[1, 45, 49], so that it is called type II IL-4R. Although the homodimer of IL-4Rα is able to transduce IL-4 signals[18, 37, 53], formation of the heterodimer consisting of IL-4Rα and either γ_c or IL-13Rα1 would be required to transduce optimal IL-4 signals[28, 44, 45, 62]. It is controversial whether the heterotrimer type of IL-4R composed of IL-4Rα, γ_c and IL-13Rα1 exists[45, 50]. The IL-13 receptor is also composed of the heterodimer consisting of IL-13Rα1 and IL-4Rα[1]. IL-4Rα is necessary for IL-13Rα1 to exert substantial binding activity to IL-13[1]. These results indicate that IL-4 and IL-13 transduce the same signals when they engage type II IL-4R/IL-13R. IL-13Rα2 is assumed to act as a decoy receptor, because it has a very short cytoplasmic domain[7, 16]; however, the details of the function of this molecule remain unclear.

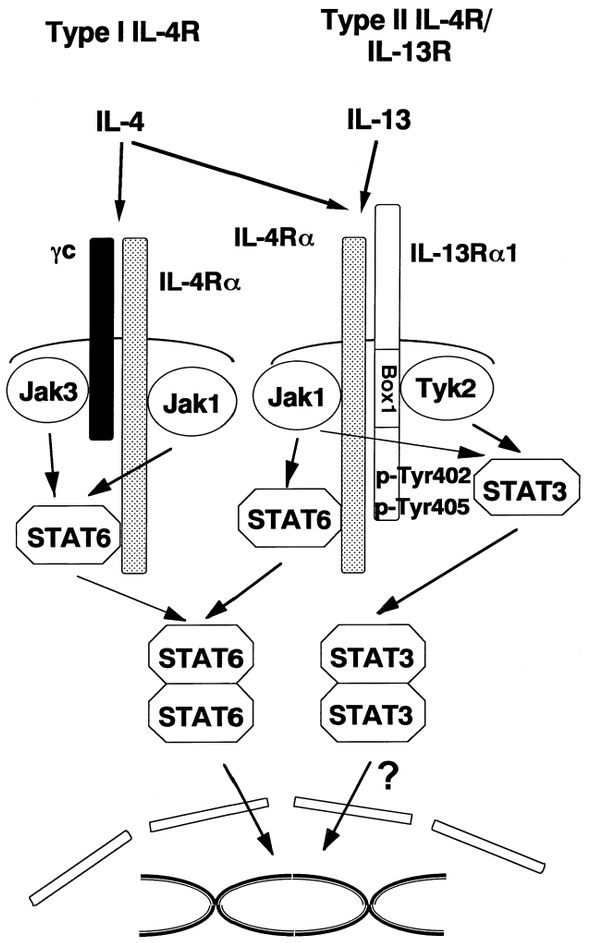

Fig. 1. Signal transduction pathways of IL-4 and IL-13. A schematic model of signal transduction pathways of IL-4 and IL-13 is depicted. Note that type II IL-4R/IL-13R transduces the STAT3 pathway in addition to the STAT6 pathway

It is known that engagement of IL-4 and IL-13 receptors by the ligands induces activation of a variety of signal-transducing molecules[29, 47]. These molecules converge into two pathways: the Jak-signal transducer and activator of transcription (STAT) pathway and the phosphatidylinositol 3 (PI3)-kinase pathway. The Jak-STAT pathway includes activation of Jak1, Jak3 and Tyk2 by IL-4, and Jak1 and Tyk2 by IL-13 in hematopoietic cells, followed by activation of STAT6[29, 47], which explains the similar biological activities of IL-4 and IL-13. It has been confirmed that STAT6 has a central role in the signal transduction of IL-4 and IL-13, including IgE synthesis, Th2 differentiation, proliferation of B cells and T cells and deactivation of monocytes, based on analyses of STAT6-deficient mice and those of the promoter region of the cytokine-targeted genes[15, 32, 41, 56, 60, 61]. We and others recently demonstrated that, in addition to STAT6, STAT3 is also activated by IL-4 and IL-13, dependent on expression of IL-13Rα1[50, 64]. Tyrosine residues located in the C terminus of IL-13Rα1 are the binding sites for STAT3. These results demonstrated that the signal pathways are different between type I and type II IL-4R, even though both receptors are engaged by IL-4. It is important which type of receptor is expressed on the targeted cells to elucidate the signal transduction mechanism of IL-4 in those cells. To date, the physiological role of STAT3

on IL-4 and IL-13 signals remain to be resolved. It is of interest to clarify what kinds of genes are induced by STAT3. The PI3-kinase pathway includes tyrosine phosphorylation of the insulin receptor substrate 1/2 (IRS-1/2) by Jak molecules, followed by recruitment of PI3-kinase to the IRS/IL-4R complex[29, 47]. PI3-kinase is assumed to be important for the growth signal of IL-4[58, 68].

Genetic Factors Involved in Bronchial Asthma among IL-4- and IL-13- Signal-Transducing Molecules

Allergic diseases are the results of complex interactions between genetic factors and environmental influences. Many attempts have been made to identify allergy or asthma-causing genes[2]. We here describe genetic factors involved in bronchial asthma among IL-4 and IL-13-signal-transducing molecules which we have identified.

IL-4Rα

Atopy is characterized by an inherited tendency to heighten IgE levels and it leads to clinical symptoms of asthma, eczema and rhinitis. As it has been proved that the IL-4Rα gene shows a strong genetic linkage with atopy through maternal alleles in a German population[14], we applied the candidate gene approach for the first time to identify functional polymorphism in the IL-4Rα gene. A variant of human IL-4Rα, which substitutes isoleucine (Ile) for valine (Val), has been identified[9, 26]. To test whether this variant might promote

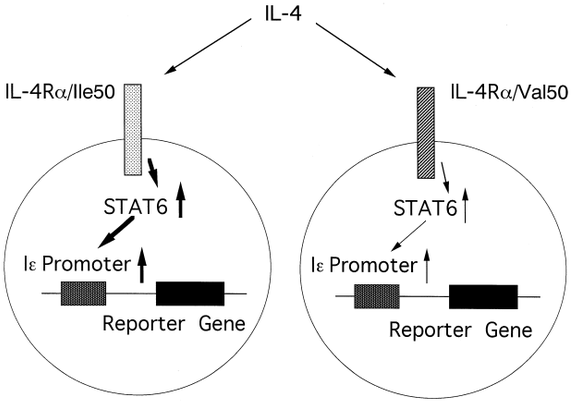

Fig. 2. Ile50Val effects on IL-4 signals. A schematic model of Ile50Val effects on IL-4 signals is depicted. Upon stimulation of IL-4, Ile50-typed IL-4Rα transduces stronger signals than Val50--typed IL-4Rα

dysregulation of IgE synthesis, we conducted genetic association studies of serum IgE levels in Japanese and British populations[42] (SHIRAKAWA et al., unpublished data). There was a significant difference in Ile/Val50 genotype frequencies between control and atopic subjects in a Japanese population: Ile50 associated with atopic asthma but not non-atopic asthma; Ile50 associated specifically and highly significantly with raised total serum IgE levels and mite-specific IgE. However, this variant did not show significant association with atopy in a British population (SHIRAKAWA et al., unpublished data).

To evaluate the functional effect of Ile50Val on IgE synthesis, we transfected the plasmid encoding human IL-4Rα carrying either Ile50 or Val50 into a mouse pro-B cell line, Ba/F3, and a human Burkitt lymphoma cell line, Jijoye[42] (Fig. 2). The plasmid encoding the IL-4 responsive element in the promoter region of Iε and the reporter gene had been incorporated into these cell lines. In both transfectants, human IL-4 enhanced the transcription activity of Iε in the Ile50-type three times as much as in the Val50-type. Activation of STAT6, which is critical for the transcription of Iε, was augmented in the Ile50-type almost two times as much as in the Val50-type, judged by electrophoretic mobility-shift assay. We next compared IL-4 responsiveness in peripheral blood mononuclear cells (PBMC) whose IL-4Rα genes were homozygotes of either Ile50 or Val50[43]. Ile50-carrying PBMC produced IgE upon stimulation of IL-4 almost three times as much as Val50-carrying ones did. Taken together, these findings indicate that the Ile50-type of IL-4Rα can transduce higher IL-4 signals than the Val50-type. It had been assumed that, because Ile50Val is located at the extracellular domain of IL-4Rα, the Ile50-type would enhance the affinity with IL-4 compared with the Val50--type; however, there was no difference in the affinity with IL-4 between the Ile50-type and the Val50-type[42, 43]. To date, the mechanism by which the Ile50-type up--regulates the ability to transduce IL-4 signals has not been described. As IL-4Rα is utilized also by functional IL-13R, it would be possible that Ile50Val affects not only IL-4 signals, but also IL-13 signals. Work is currently under way to explore such a possibility.

A cytoplasmic variant of IL-4Rα, in which glutamine (Gln) at amino acid 551 is replaced with arginine (Arg), has been found in excess in Caucasian subjects with hyper-IgE syndrome, severe eczema and bronchial asthma[33, 54]; however, Arg/Gln551 genotype frequencies between control and atopic subjects were invariant in both Japanese and British populations[59] (SHIRAKAWA et al., unpublished data) and the frequency

of Arg551 tended to be much lower in atopics than in controls in a German population[36]. We and others, furthermore, demonstrated that Arg551Gln does not affect IL-4 signals[43, 67].

IL-13

We have recently identified that a polymorphism, G to A, exists at the 4464 base of the IL-13 gene from the starting point of translation, which leads to replacement of Arg residue at the 110th amino acid with Gln[22]. The incidence of homozygous for Gln and heterozygous type was higher than that of homozygous for Arg in bronchial asthma patients in both Japanese and British populations. The incidence is almost the same between atopic and non-atopic asthma. Furthermore, asthmatic patients homozygous for Gln110 had significantly higher levels of serum IL-13 than those homozygous for Arg110. It is noteworthy that this polymorphism is correlated with bronchial asthma, irrespective of ethnic difference. These results indicate that the IL-13 gene is a genetic factor of bronchial asthma, but not of atopy.

Investigation of how this polymorphism affects IL-13 signals is now under way. A computer modeling analysis suggests that the 110th amino acid is close to Arg at the 10th amino acid sterically, and that the substitution of Gln for Arg would change electrostatistic strength, which may affect the conformational position of the 10th amino acid[22]. This positional change at the 10th amino acid may lead to conformational changes of aspartic acids at the 11th and 14th amino acids. As the portion between the 10th and 14th amino acids is assumed to be important for binding to the receptor, based on the analogy with IL-4[63], it would be possible that this polymorphism influences the binding affinity of the ligand.

IL-13Rα1

We have identified a novel single nucleotide polymorphism (SNP), A1398G, in the coding region of the IL-13Rα1 gene, numbered based on the cDNA[22]. As the IL-13Rα1 gene locates on the X chromosome, the incidence of this variant was calculated for atopy in male and female subjects[22]. In the British population, the incidence was significantly higher in males; however, there was no significant difference in the Japanese population. As this is a silent variant, it would be possible that there is another linkage disequilibrium SNP, functionally correlated with atopy, or alternatively, that this variant affects expression of IL-13Rα1.

STAT6

A G2964A variant in a 3' untranslated region of the STAT6 gene, numbered based on the cDNA, has been identified. This variant was correlated with mild atopic asthma in the Japanese population, but not in the British population[20]. We analyzed the expression level of STAT6 in peripheral B and T cells from Japanese donors whose genotypes are homozygous for A or G; however, there was no difference (IZUHARA et al., unpublished data). It would be important to search for another variant that influences the activity or expression level of STAT6. Such a variant and G2964A may result in linkage disequilibrium.

Expression of the IL-4R and IL-13R in Bronchial Tissues

It has been shown that the functional IL-13R expresses on the surface of B cells and monocytes in hematopoietic cells[48, 72]; however, it is not fully understood what kinds of cells express the functional IL-13R in non-hematopoietic cells (Fig. 3). The results, i.e that IL-13 evokes a bronchial asthma-like phenotype in mice and that the polymorphism on the IL-13 gene is correlated with bronchial asthma, suggest that IL-13 acts directly on non-hematopoietic cells in bronchial tissues, leading to bronchial asthma. To address this question, we recently generated anti-IL-13Rα1 antibody and analyzed the expression of IL-13Rα1 by histochemistry[22]. It turned out that bronchial epithelial cells and smooth muscle cells highly express IL-13Rα1. IL-4Rα, another component of the functional IL-13R, was also highly expressed on the same cells, which is in line with a previous report[65]. These results indicate that type II IL-4R/IL-13R can be expressed on these cells and that both IL-4 and IL-13 exert their actions on these cells. The specimens derived from bronchial asthma patients showed the same distribution of

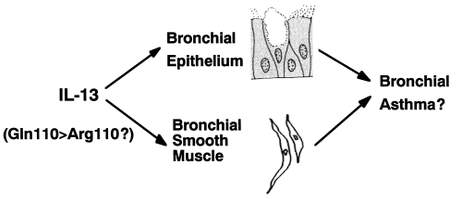

Fig. 3. IL-13-targeted cells in bronchial tissues. IL-13 mainly acts on bronchial epithelial cells and smooth muscle cells in bronchial tissues, which may be closely correlated with the pathogenesis of bronchial asthma. Gln110-typed IL-13 may transduce stronger signals than Arg110-typed IL-13

IL-13Rα1 as those derived from healthy donors. It has been shown that IL-13 causes induction of eotaxin production and expression of 15-lipoxygenase from bronchial epithelial cells[31, 39], both assumed to be important steps for the pathogenesis of bronchial asthma. On the other hand, it is uncertain what kinds of biological effects IL-13 triggers in bronchial smooth muscle cells. As it is well-known that hypertrophy and proliferation of bronchial smooth muscle cells are characteristic of bronchial asthma, it is of great interest to explore whether IL-13 is involved in such a phenotypic change of bronchial smooth muscle cells.

Potential Therapeutic Strategies for Targeting IL-13 Signals

The importance of IL-13 signals in the pathogenesis of bronchial asthma prompts us to speculate that IL-13 signals are potential therapeutic targets for improving allergic states. Several reagents are now listed for such a purpose, as described below. Furthermore, much attention has been paid to the development of low molecular weight compounds to block the IL-13/IL-13R interaction, IL-13 synthesis, and the intracellular signal pathway of IL-13[30].

Soluble IL-13Rα2

As soluble IL-4Rα is a potent reagent to block IL-4 signals[30], and administration of soluble IL-4Rα is effective for bronchial asthma patients[4], it is reasoned that soluble IL-13R is also a good candidate to block the binding of IL-13 to the receptor. As the affinity of IL-13Rα2 to IL-13 is higher than that of IL-13Rα1[1, 7, 16, 23], soluble IL-13Rα2 would be a better candidate. As a matter of fact, soluble IL-13Rα2 has been shown to be effective to block IL-13 signals in mice[8, 9, 21, 70]. The next step is to apply the humanized molecule or the human counterpart to allergic patients.

IL-4 mutein

The IL-4 mutein whose tyrosine residue at the 124th amino acid is replaced with aspartic acid (Y124D) has been developed, and it has been shown that it acts as a competitor for IL-4, resulting in the blockage of IL-4 signals[30]. In addition, Y124D prevents the binding of IL-13 to the IL-13R and inhibits its signals by occupying IL-4Rα, a functional component of the IL-13R[3, 17, 73]. These results suggest to us that the advantage of this reagent will be that it has a wider spectrum to block both IL-4 and IL-13 signals.

IL-13-toxin

Cell-surface receptor-specific fusion toxins have been widely applied as highly selective reagents to eliminate specific receptor-bearing cells. A fusion protein of IL-13 and *Pseudomonas* exotoxin displays specific cytotoxicity on IL-13R-expressing malignant cells[12, 13, 51]; however, it is yet to be shown whether such a fusion protein is effective for allergic states.

Conclusions and Future Prospects

The molecular basis for the involvement of IL-13 signals in the pathogenesis of bronchial asthma is beginning to be elucidated. A framework for the signal transduction mechanism of IL-13, definition of IL-13--targeting cells in bronchial tissues, and several genetic factors correlating with IL-13 signals have been revealed. It is hoped that, by clarifying the detailed mechanism of IL-13 signals in such targeted cells, identification of genetic and environmental factors involved in IL-13 signals will progress. Based on such knowledge, we should be able to predict the risk of developing bronchial asthma by combining these factors and to try to decrease the incidence of bronchial asthma by prevention. Furthermore, it is also expected that one or more reagents will be developed to target IL-13 signals specifically and will be found effective for bronchial asthma patients.

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