

Review

The Role of Interleukin-4 and Interleukin-13 in the Non-Immunologic Aspects of Asthma Pathogenesis¹⁾

Kenji Izuhara*

Division of Medical Biochemistry, Department of
Biomolecular Sciences,
Division of Medical Research, Center for Comprehensive
Community Medicine,
Saga Medical School, Saga, Japan

Bronchial asthma is a complex disease characterized by airway inflammation involving a Th2-cytokine, interleukin (IL)-13. A substantial body of evidence has accumulated pointing to the pivotal role of IL-13 in the pathogenesis of bronchial asthma. The evidence is categorized as (i) analyses of mouse models, (ii) expression of these cytokines in the bronchial lesions, and (iii) genetic association of the signaling molecules of these cytokines. In addition, the molecular mechanism of the signal transduction of IL-13 has also been well characterized. We have applied microarray analyses to human bronchial epithelial cultures to search for genes regulated by IL-13 and have identified a subset of disease-relevant genes by comparison with cDNA libraries derived from normal and asthmatic bronchial biopsies. Expression of squamous cell carcinoma antigen-1 (SCCA1) and SCCA2, the cysteine and serine protease inhibitors, respectively, was the highest in the bronchial epithelial cells stimulated by IL-4 and IL-13 and was augmented in the asthmatic cDNA library. Furthermore, serum levels of SCCA were also elevated in asthmatic patients. Taken together, it was supposed that SCCA may play some role in the pathogenesis of bronchial asthma, and measuring its serum level may be relevant for diagnosing or monitoring the status of bronchial asthma. Clin Chem Lab Med 2003; 41(7):860–864

Key words: Bronchial asthma; Allergy; Interleukin-13; Receptor; Squamous cell carcinoma antigen.

Abbreviations: AHR, airway hyperresponsiveness; BECs, bronchial epithelial cells; Ig, immunoglobulin; IL, interleukin; IL-4R α , the IL-4 receptor α chain; IL-13R α 1, the IL-13 receptor α 1 chain; IL-13R α 2, the IL-13 receptor α 2 chain; serpin, serine proteinase inhibitor; SCCA, squamous cell carcinoma antigen; SNP, single nuclear polymorphism.

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*E-mail of the corresponding author:

kizuhara@post.saga-med.ac.jp

Introduction

The incidence of allergic diseases has dramatically increased in recent decades, especially in urban and industrialized areas. It has been reported that, at present, up to half of the population in Japan suffers from bronchial asthma, atopic dermatitis, or allergic rhinitis (1). The medical cost for treating such patients is huge and on the increase. Thus, it is important socially as well as medically to establish more useful strategies to overcome allergic disorders.

Bronchial asthma is a complex disorder involving a combination of genetic and environmental factors (2). Together, these result in infiltration of Th-2 lymphocytes, mast cells, and eosinophils into asthmatic airways with downstream mediator release and disordered airway function. Cytokines derived from the Th-2 lymphocytes are considered to orchestrate the asthmatic phenotype, irrespective of atopy (2).

Although among Th-2 cytokines, paradigmatic ones, interleukin (IL)-4 and IL-5, had been thought to be the primary regulators of bronchial asthma, it has been recently suggested that IL-13 is a central mediator of bronchial asthma. In this Review, we summarize the pathophysiological roles of IL-13 in bronchial asthma and the trials to clarify the molecular mechanism for IL-13 to induce bronchial asthma.

1. Significance of IL-13 in the Pathogenesis of Bronchial Asthma

Key roles for IL-13 in the pathogenesis of bronchial asthma are supported mainly based on analyses of mouse models; however, some evidence to prove that IL-13 is also involved in the pathogenesis of human asthma patients has been demonstrated.

1.1 Analyses of mouse models

Analyses of mice null for components of the IL-13 signal transduction pathways, including IL-13, the IL-4 receptor α chain (IL-4R α), and STAT6, have revealed that IL-13, as well as IL-4, plays a pivotal role in induction of airway hyperresponsiveness (AHR), a characteristic feature of asthma (3–5). Furthermore, epithelial overexpression of an IL-13 transgene or administration of IL-13 in mice have shown that IL-13 induces an asthma-like phenotype independent of lymphocytes (6–8). It is of importance to note that these analyses indicated that IL-13 acts directly on non-immune cells in bronchial tissue, evoking the phenotypic changes. Actually,

it has been very recently reported that reconstitution of STAT6 only in bronchial epithelial cells into STAT6-disrupted mice restores IL-13-induced AHR and mucous production, but not inflammation or fibrosis (9), showing the importance of IL-13's direct action on bronchial epithelial cells (BECs).

1.2 Expression of IL-13 in the bronchial lesions

Expression of IL-13 is higher at the baseline and greatly up-regulated by allergen challenge in bronchial tissues or bronchoalveolar lavage fluids derived from asthma patients (10, 11). Thus, expression of IL-13 is predominant, compared to IL-4. It is well-known that IL-4 and IL-13 have common activities (12); however, these results suggest that the biological activities of IL-4 and IL-13 are skewed toward immune cells and peripheral tissues, respectively, *in vivo*.

1.3 Genetic association of the IL-13 signaling molecules

Genetic predisposition to allergic diseases is thought to be polygenic and the genetic factors are thought to exist among polymorphisms (13). Extensive efforts have been made to identify such factors by genome-wide search and candidate-gene study (14). Based on candidate-gene study, it has been reported that several polymorphisms of genes encoding IL-13 signaling molecules such as *IL13*, *IL4RA*, *IL13RA1*, *STAT6*, and *BCL6* are genetically associated with asthma or atopy (15). Among the single nuclear polymorphisms (SNPs) that are demonstrated to be genetically associated with bronchial asthma or atopy, we have analyzed the functional properties of variants of *IL4RA* (Ile50Val) and *IL13* (Gln110Arg). The variant of IL-4R α , whose amino acid at 50 is isoleucine, up-regulates receptor responses to IL-4, leading to increased STAT6 activation and immunoglobulin E (IgE) synthesis, compared to the valine type (16, 17). It remains undetermined whether this SNP affects the IL-13 signal. The variant of IL-13 whose amino acid at 110 is glutamine has less affinity with the IL-13 receptor α 2 chain (IL-13R α 2), a decoy receptor, and an enhanced stability, compared to the arginine type, causing up-regulation of the IL-13 concentration *in vivo* (Figure 1) (18, 19).

2. Structure of the IL-13 Receptor and Signal Transduction Mechanism of IL-13

IL-13 exerts its biological activities by binding to its receptor on the cell surface as well as other cytokines (Figure 2). IL-13R is composed of two components, IL-4R α and the IL-13R α 1 chain (IL-13R α 1) (20–23). IL-4 can also bind to this receptor, denoted as type II IL-4R (12, 24). There exists another IL-13-binding unit, IL-13R α 2 (25, 26). As the cytoplasmic domain of IL-13R α 2 is short, IL-13R α 2 is thought not to transduce the IL-13 signal but to block it, acting as a decoy receptor. Actually, we have recently observed that IL-13 completely diminished STAT6 phosphorylation in the

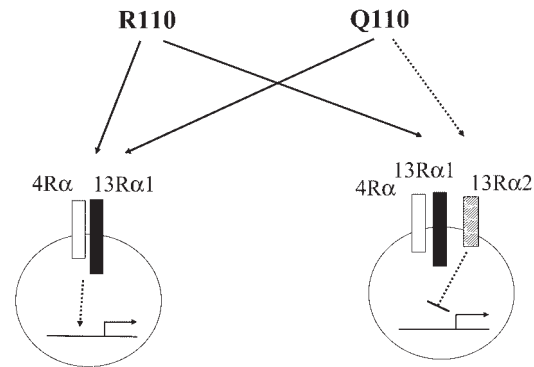


Figure 1 Association between the SNP on the *IL13* gene (Arg110Gln, R110Q) and the IL-13 receptors. Q110 and R110 have the same affinity with the heterodimer composed of IL-13R α 1/IL-4R α , whereas Q110 has lower affinity with IL-13R α 2 than R110, transducing stronger IL-13 signal.

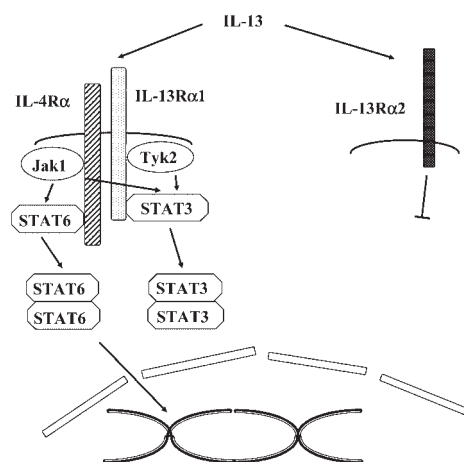


Figure 2 The receptor structure and signal transduction mechanism of IL-13. IL-13 binds to either IL-13R, composed of IL-13R α 1 and IL-4R α , or IL-13R α 2. Engagement of IL-13R causes signal transduction, mainly through the JAK-STAT pathway.

IL-13R α 2-expressing BEAS-2B cells, a human bronchial epithelial cell line, whereas IL-4 did not influence it, supporting this idea (Yasunaga S, *et al.*, unpublished data).

Upon engagement of the receptor by IL-13, its signal is transduced intracellularly mainly *via* the JAK-STAT and the phosphatidylinositol-3 kinase/insulin receptor substrate-1/2 pathways (12). The JAK-STAT pathway activated by IL-13 corresponds to activation of JAK1 and TYK2 followed by activation of STAT6, a critical transcription factor for IL-13 to exert its biological activities, and STAT3 (23, 27); however, thus far, the biological role of STAT3 in the IL-4 and IL-13 signals remains to be clarified.

3. Tissue Distribution of the Receptors of IL-13

In hematopoietic and immune cells, B cells and monocytes are the main target cells for IL-13 (28). Although IL-13R α 1 is not expressed on the cell surface at the rest-

ing stage of B cells, activation by anti-IgM antibodies and anti-CD40 antibodies enhances expression of IL-13R α 1, which enabled the cells to respond to IL-13, causing class switching to IgE and CD23 expression (23, 29). Thus, the IL-13 signal is regulated by expression of IL-13R α 1 in B cells. In contrast, we revealed that IL-13R α 1 is widely expressed in non-hematopoietic cells, including human BECs (18, 30). It has been reported that fibroblasts and smooth muscle cells, in addition to BECs, express the functional IL-13R, so that IL-13 induces production of eotaxin, C3, and β 1-integrin in these cells (31–35).

Tissue distribution and expression regulation of IL-13R α 2 had remained obscure. It has been confirmed that immune cells do not express IL-13R α 2 (29). We have recently observed that either IL-4 or IL-13 induced expression of IL-13R α 2, but not of IL-13R α 1 or IL-4R α in BECs (Yasunaga S *et al.*, unpublished data). Induction of IL-13R α 2 by IL-4 or IL-13 was STAT6-dependent, although it required *de novo* protein synthesis. Furthermore, IL-13R α 2 was expressed in lung tissues of ovalbumin-induced asthma model mice, in which both IL-4 and IL-13 were highly expressed. These results suggested that IL-13R α 2 is induced by its ligand, down-regulating the IL-13 signal in BECs, which functions as a negative-feedback system for the cytokine signal (Figure 3).

4. Identification of IL-13-Inducible Genes in Human BECs

To study potential downstream consequences of IL-13 on human BECs, we first employed microarrays (HuGeneFL Array, Affymetrix, Santa Clara, CA, USA) to identify genes induced by IL-13 in human BECs (36). Human BECs derived from three different donors were exposed to IL-13 for 24 h and subjected to microarray analysis. We limited target identification to those genes whose expression was up-regulated > 2-fold by IL-13. Of the 5600 annotated genes present on the arrays, IL-13 affected 47, 63, and 49 genes. Expression of 12 genes was up-regulated also by IL-4 (Table 1). Among these genes, two members of the serine proteinase inhibitor (serpin) family of protease inhibitors, squamous cell carcinoma antigen-1 (SCCA1) (SERPINB3) and SCCA2 (SERPINB4), showed the highest increase (~20-fold). No other investigated cytokine, in-

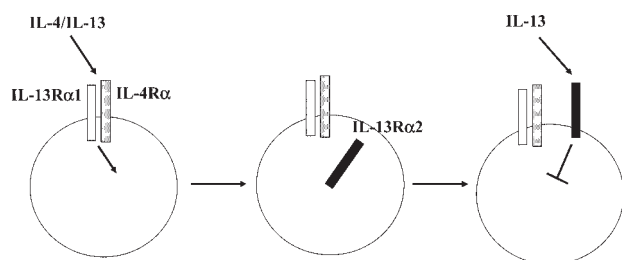


Figure 3 The negative feedback system of IL-13 signal in bronchial epithelial cells. Either IL-4 or IL-13 induces IL-13R α 2 in bronchial epithelial cells, which self-limits IL-13 signal.

Table 1 Identified IL-4/IL-13-inducing genes.

SCCA1*
SCCA2*
DD96 (MAP17)*
KAL1*
Periostin
Tenascin-C
Carboxypeptidase M
IL-13R α 2
Cathepsin C
CYP1B1
Carbonic anhydrase II
Endothelin-A receptor

*Up-regulated in bronchial tissues of asthma patients.

cluding IL-5, IL-9, tumor necrosis factor- α (TNF- α), and IL-1 β , induced expression of these two genes.

To verify induction of expression of SCCA1 and SCCA2, we used quantitative reverse transcriptase-polymerase chain reaction (RT-PCR) (36). While the fold change varied between individual cultures, increased expression of SCCA1 and SCCA2 was confirmed. Kinetic analysis showed that the expression profile of SCCA1 and SCCA2 was augmented within 6 h of stimulation by IL-13 and continued to be enhanced for up to 48 h.

Davies and co-workers (36) analyzed the expression profiles of cDNA libraries constructed from atopic normal or asthma-derived bronchial biopsies using Life-SeqTM Gold from Incyte Genomics Inc. (Palo Alto, CA, USA). Comparison of the 12 differentially identified genes induced by both IL-4 and IL-13 (Table 1) with sequence data from these cDNA libraries revealed that four of the genes, *SCCA1/2*, *KAL-1*, and *DD96* were represented in at least one of the two libraries. The number of SCCA-related clones derived from SCCA was significantly higher in bronchial tissues of asthma patients than those of normal donors (11 clones vs. 3 clones; $p < 0.005$). One clone derived from *KAL-1* and one from *DD96* existed in the atopic asthmatic library but none were detected in the atopic, non-asthmatic library.

5. High Serum Levels of SCCA in Asthma Patients

SCCA1 and SCCA2 are members of the ovalbumin-serpin (ova-serpin) proteinase inhibitor family (37). They are 92% identical at the amino acid level and have probably arisen by gene duplication. SCCA was originally isolated from human cervical squamous carcinoma cells and is produced by various squamous tumors, as well as normal bronchial epithelium (38). Thus far, no normal cell except BECs is known to produce SCCA1 and SCCA2. It has recently been revealed that SCCA1 and SCCA2 have distinct properties and substrates: SCCA1 inhibits cysteine proteinases such as cathepsins K, L, S, whereas SCCA2 inhibits serine proteinases including cathepsin G and mast cell chymase (37). At present, it remains to be determined

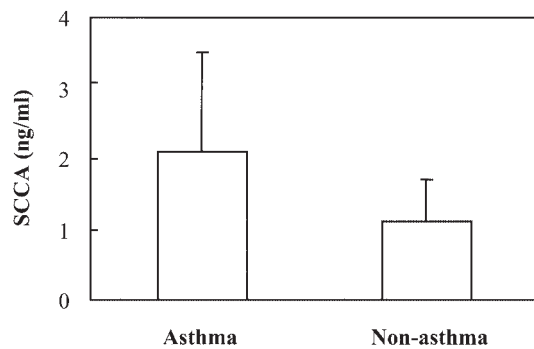


Figure 4 Serum levels of SCCA in asthma patients. Serum levels of SCCA in asthmatic and non-asthmatic children are shown. The medians are depicted as bars.

whether SCCA1 and SCCA2 are acting as protective or exacerbating molecules to modify the asthmatic phenotype by inhibiting these intrinsic proteinases. Alternatively, SCCA1 and SCCA2 may act on extrinsic proteinases derived from mites or parasites, because it is known that mite- or parasite-derived cysteine proteinases have an important role for their responsiveness or virulence (39–41). Further studies are now underway aiming to clarify this point.

While most serpins are secreted proteins that work extracellularly, the ov-serpins also function as intracellular proteinase inhibitors (37). We next analyzed the localization of SCCA proteins in human BECs. Immunocytochemical analysis using an antibody that recognizes both SCCA1 and SCCA2 (mAb27, Dainabot, Tokyo, Japan) showed positive staining only when cells were cultured in the presence of IL-4 or IL-13. Kinetic analysis of cellular extracts and supernatants by ELISA (IMx, Dainabot) showed that intracellular expression of SCCA protein increased within 24 h of IL-4 or IL-13 stimulation and was slightly augmented thereafter. SCCA secretion was not detected at 24 h but was observed 48 h after stimulation and reached a plateau thereafter. These results suggest that SCCA1 and SCCA2 could act both intracellularly and extracellularly in BECs.

It has been reported that secreted SCCA can be detected in serum of certain cancer patients (42) and that increased serum levels of SCCA are also observed in children suffering from atopic dermatitis (with or without asthma), with levels correlating with disease severity but not with serum IgE or eosinophils (43). Based on these observations, we explored the possibility that expression of SCCA in bronchial tissue of asthmatic patients was also reflected in the circulation. Assessment of serum concentrations of SCCA in asthmatic and non-asthmatic children by ELISA showed that levels were significantly higher in the asthmatic subjects (2.1 (1.4–3.3) ng/ml, median (interquartile range), $n = 32$) than in control, non-asthmatic subjects (1.1 (0.6–1.9) ng/ml, $n = 23$, $p < 0.005$; Figure 4). In a small group of patients ($n = 8$) who were studied during, and 4–5 days after, an exacerbation, SCCA levels were found to be higher during the attack phase (2.4 (1.7–3.8) ng/ml) than during recovery (1.4 (0.9–1.7) ng/ml), although

this failed to reach statistical significance ($p = 0.09$). These results suggest that measuring the serum level of SCCA may be relevant for diagnosing or monitoring the status of bronchial asthma.

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Corresponding author: Dr. Kenji Izuhara, Division of Medical Biochemistry, Department of Biomolecular Sciences, Saga Medical School, 5-1-1 Nabeshima, Saga 849-8501, Japan
Phone: +81 952 34 2261, Fax: +81 952 34 2058,
E-mail: kizuhara@post.saga-med.ac.jp