Characterization of IL-4 and IL-13 signals dependent on the human IL-13 receptor α chain 1: redundancy of requirement of tyrosine residue for STAT3 activation

Ritsuko Umeshita-Suyama1,3, Rie Sugimoto1, Mina Akaiwa1, Kazuhiko Arima1, Bin Yu1, Morimasa Wada2, Michihiko Kuwano2, Koichi Nakajima4, Naotaka Hamasaki1 and Kenji Izuhara1

1Department of Clinical Chemistry and Laboratory Medicine, and 2Department of Medical Biochemistry, Graduate School of Medical Sciences, Kyushu University, 3-1-1 Maidashi, Higashi-ku, Fukuoka 812-8582, Japan
3R & D Institute, UNITIKA, Ltd, 23 Ujikozakura, Uji, 611-0021 Japan
4Department of Immunology, Osaka City University Medical School, 1-4-3 Asahi-machi, Abeno-ku, Osaka 545-8585, Osaka, Japan

Keywords: B cell, Jak1, Jak3, STAT6

Abstract

IL-4 and IL-13 are pleiotropic cytokines whose biological activities overlap with each other. IL-13 receptor α chain 1 (IL-13Rα1) is necessary for binding to IL-13, and the heterodimer composed of IL-13Rα1 and IL-4R α chain transduces IL-13 and IL-4 signals; however, the functional mapping of the intracellular domain of IL-13Rα1 is not fully understood. In this study, we constructed wild and mutated types of human IL-13Rα1, and analyzed IL-4 and IL-13 signals using an IL-13Rα1-transfected human B cell line. Expression of IL-13Rα1 evoked STAT3 activation by IL-4 and IL-13, and in stimulated human B cells, on which IL-13Rα1 was highly expressed, IL-4 and IL-13 induced STAT3 activation. Replacement of the two tyrosine residues completely abolished STAT3 activation, although replacing either tyrosine residue alone retained it. Furthermore, we found that the Box1 region and the C-terminal tail of IL-13Rα1 were critical for binding to Tyk2, and activation of Jak1, Tyk2, the insulin receptor substrate-1 and STAT6 respectively. These results suggest that STAT3 activation is involved with IL-4 and IL-13 signals in human B cells along with the activation of STAT6, and that there is a unique sequence in IL-13Rα1 to activate STAT3.

Introduction

IL-13 is a pleiotropic cytokine produced by Th2-type cells and mast cells (1,2). Its biological activities overlap with those of another cytokine, IL-4, and those include anti-inflammatory actions on monocytes, production of chemokines and induction of arachidonic acid-metabolizing enzymes on bronchial epithelial cells. However, IL-4, but not IL-13, induces expansion of Th2-type cells and proliferation of T cells in both the mouse and human systems (1,3,4). IL-4 acts on both mouse and human B cells in the same way, in that it induces class switching including IgE, and expression of CD23 and MHC class II. In contrast, IL-13 behaves on B cells differently in the mouse and human systems: human IL-13 induces class switching toward IgE and IgG4, as well as expression of CD23 and MHC class II on B cells, whereas mouse IL-13 does not exert such actions (1). It still remains undetermined whether this is because mouse IL-13R is not expressed on mouse B cells or because the machinery for mouse IL-13 is impaired in mouse B cells. It has been recently shown that IL-13 has a pivotal role in the defense mechanism against parasite infection (5,6) and that it is involved in the pathogenesis of bronchial asthma (7–9) in mice. We and others have recently proved that a polymorphism located in the human IL-13 gene is correlated with asthma (10,11).

To date, two IL-13-binding units have been identified, the IL-13 receptor α chain 1 (IL-13Rα1) and IL-13Rα2 in both the mouse and human systems (12–15). Both mouse and human
IL-13Rα1 have cytoplasmic domains 60 amino acids long, in which the Box1 region and two tyrosine residues exist (Y402 and Y405) (12,14). One of the tyrosine residues, Y405, is followed at the +3 position by Gln, which represents a consensus STAT3 recruitment and activation motif (16). The IL-13R is composed of the heterodimer consisting of IL-13Rα1 and IL-4 receptor α chain (IL-4Rα) (14). This heterodimer can also transduce IL-4 signals (17,18), so it is called type II IL-4R. IL-13Rα2 is assumed to act as a decoy receptor because it has a very short cytoplasmic domain (15); however, the details of the function of this molecule remain unclear.

It is known that engagement of the IL-13R by IL-13 induces activation of a variety of signal-transducing molecules (19,20). These molecules converge into two pathways: the Jak–STAT pathway and the phosphatidylinositol-3 (PI3) kinase pathway. The former includes activation of Jak1, Jak2 and Tyk2, followed by activation of STAT6 (17,21–25). The latter includes tyrosine phosphorylation of the insulin receptor substrate (IRS)-1/2, followed by activation of PI3-kinase (17,21,25,26). Analyses of STAT6 knockout mice have verified the necessity of STAT6 for class switching on B cells and expansion of Th2-type cells induced by IL-4, as well as anti-inflammatory actions on monocytes induced by IL-4 and IL-13 (19,20). It has been recently demonstrated that expression of mouse IL-13Rα1 induces activation of STAT3, in addition to STAT6 (27); however, the necessity for tyrosine residues in STAT3 activation has remained unclear. To date, no study has been reported for human IL-13Rα1.

In this study, we constructed wild and mutated types of human IL-13Rα1, and analyzed IL-13 and IL-4 signals in these types of IL-13Rα1-transfected human B cell line. It turned out that the C-terminal tail of IL-13Rα1 is important for activation of Jak1, Tyk2, IRS-1 and STAT6. We furthermore demonstrated that STAT3 activation by IL-4 and IL-13 occurred when IL-13Rα1 was expressed, which is also the case in primary human B cells. Replacement of either tyrosine residue can still cause STAT3 activation; however, replacement of both tyrosine residues diminished STAT3 activation. These results indicate that there exists a novel signal transduction pathway of IL-13 and IL-4 in human B cells, and that there is a redundancy for requirement of tyrosine residue for STAT3 activation via IL-13Rα1.

**Methods**

**Plasmid construction**

Full-length human IL-13Rα1 cDNA was cloned from a cDNA library of HeLa cells, as described before (28). Three different constructs were made: IL-13Rα1 fullo (wild type), IL-13Rα1 CP (C-terminal truncated), IL-13Rα1 CT (C-terminal truncated, Y405 mutated to F), and IL-13Rα1 F (both tyrosine residues mutated to phenylalanine). These constructs were transfected into human B cell line, and the expression of STAT3 was analyzed by Western blotting. The results showed that STAT3 was activated in both wild type and mutated types of IL-13Rα1, indicating that IL-13Rα1 can still activate STAT3 even when the tyrosine residues are mutated.

**Fig. 1.** Schematic model of the mutated types of IL-13Rα1 and expression of the transfected IL-13Rα1. (A) A schematic model of the structures of wild and mutated types of IL-13Rα1. ssIL-4R, TM, Y and F denote the signal sequence of IL-4Rα, transmembrane domain, tyrosine residue and phenylalanine residue respectively. (B) Expression of wild and mutated types of IL-13Rα1 on the transfecteds. Each transfecant was incubated with (solid line) or without (dashed line) anti-Flag M2 antibody, followed by FITC-conjugated anti-mouse Ig antibody.
nucleotides existed between ours and that published before (14); T → C at 473, C → T at 833 and A → G at 1157 from the starting point of translation. A survey of nucleotide sequencing from peripheral blood cells of five healthy Japanese donors showed C, C and G respectively in all cultured in RPMI 1640 medium with 10% FCS, 100 U/ml of penicillin and 100 µg/ml of streptomycin either in the absence or presence of 10 µg/ml of anti-IgM antibody (Cappel, Aurora, OH) and 0.5 µg/ml of anti-CD40 antibody (Immunotech, Marseille, France) for the indicated period.

Harvested cells were stimulated with the indicated concentration of IL-4 (provided by Bayer Yakuhin, Kyoto, Japan) or IL-13 (PeproTech, Rocky Hill, NJ) for the indicated period.

Flow cytometry
The washed cells were incubated with anti-Flag M2 antibody (Sigma, St Louis, MO), followed by FITC-conjugated anti-mouse Ig antibody (Zymed, San Francisco, CA). Quantitation was performed using FACScan flow cytometry (Becton Dickinson, San Jose, CA).

Luciferase activity assay
Procedures of luciferase activity assay were performed as described before (31). The cells were incubated with the indicated concentrations of either IL-4 or IL-13 for 24 h. After the cells were washed once by PBS and lysed with reporter lysis buffer (Toyoink, Tokyo, Japan), cell lysates were mixed with luciferase assay reagent (Toyoink).

Immunoprecipitation and Western blotting
Procedures of immunoprecipitation and Western blotting were conducted as previously described (24). For immunoprecipitation, the cells were lysed in lysis buffer containing 1 or 0.5% Triton X-100, followed by incubation with the indicated antibodies. Proteins eluted by boiling with SDS-PAGE sample buffer or cell lysates were applied to SDS-PAGE and transferred electrophoretically to a PVDF membrane (Amersham, Arlington Heights, IL). Proteins were probed with the indicated antibodies and visualized by enhanced chemiluminescence (Amersham).

The antibodies used were anti-STAT3, anti-STAT6, anti-Jak1, anti-Jak3, anti-Tyk2 (Santa Cruz Biotechnology, Santa Cruz, CA), anti-phosphotyrosine (PY) (4G10), anti-IRS-1 (Upstate Biotechnology, Lake Placid, NY), anti-phospho STAT3 (New England Biolabs, Beverly, MA) and anti-Flag M2. Anti-STAT5 was kindly provided from Dr H. Wakao (Helix Research Institute, Kisarazu, Japan).

RT-PCR
Total RNA was extracted from purified human B cells stimulated with 10 µg/ml of anti-IgM antibody and 0.5 µg/ml of anti-CD40 antibody for the indicated period by Isogen (Nippongene, Funakoshi). Plasmids containing wild and mutated types of IL-13Rα were transduced to DND-39 cells by electroporation. The resulting stable transfectant was named DND-39/Ge. These cells were maintained in a culture medium containing 6 µg/ml of blasticidin S hydrochloride (Funakoshi). Plasmids containing wild and mutated types of IL-13Rα were transduced to DND-39/Ge cells by electroporation, and stable transfectants were named DND-39/Ge/IL-13Rα1Full, DND-39/Ge/IL-13Rα1ΔCP, DND-39/Ge/IL-13Rα1ΔCT, DND-39/Ge/IL-13Rα1Y402F, DND-39/Ge/IL-13Rα1Y406F and DND-39/Ge/IL-13Rα1FF. These cells were maintained in a culture medium containing 6 µg/ml of blasticidin S hydrochloride and 250 µg/ml of hygromycin B (Wako, Osaka, Japan).

COS7 cells were maintained in DMEM supplemented with 10% FCS, 100 µg/ml streptomycin and 100 U/ml penicillin.

Procedures to purify human B cells were described before (30). Human B cells were isolated from buffy coats provided by Kyushu University Hospital. Mononuclear cells were separated over Ficoll-Hypaque. B cells were isolated from mononuclear cells with magnetic beads conjugated with anti-CD19 antibody (Dynal, Oslo, Norway). Isolated B cells were cultured in RPMI 1640 medium with 10% FCS, 100 U/ml of penicillin and 100 µg/ml of streptomycin either in the absence or presence of 10 µg/ml of anti-IgM antibody (Cappel, Aurora, OH) and 0.5 µg/ml of anti-CD40 antibody (Immunotech, Marseille, France) for the indicated period.

Flow cytometry
The washed cells were incubated with anti-Flag M2 antibody (Sigma, St Louis, MO), followed by FITC-conjugated anti-mouse Ig antibody (Zymed, San Francisco, CA). Quantitation was performed using FACScan flow cytometry (Becton Dickinson, San Jose, CA).

Luciferase activity assay
Procedures of luciferase activity assay were performed as described before (31). The cells were incubated with the indicated concentrations of either IL-4 or IL-13 for 24 h. After the cells were washed once by PBS and lysed with reporter lysis buffer (Toyoink, Tokyo, Japan), cell lysates were mixed with luciferase assay reagent (Toyoink).

Immunoprecipitation and Western blotting
Procedures of immunoprecipitation and Western blotting were conducted as previously described (24). For immunoprecipitation, the cells were lysed in lysis buffer containing 1 or 0.5% Triton X-100, followed by incubation with the indicated antibodies. Proteins eluted by boiling with SDS-PAGE sample buffer or cell lysates were applied to SDS-PAGE and transferred electrophoretically to a PVDF membrane (Amersham, Arlington Heights, IL). Proteins were probed with the indicated antibodies and visualized by enhanced chemiluminescence (Amersham).

The antibodies used were anti-STAT3, anti-STAT6, anti-Jak1, anti-Jak3, anti-Tyk2 (Santa Cruz Biotechnology, Santa Cruz, CA), anti-phosphotyrosine (PY) (4G10), anti-IRS-1 (Upstate Biotechnology, Lake Placid, NY), anti-phospho STAT3 (New England Biolabs, Beverly, MA) and anti-Flag M2. Anti-STAT5 was kindly provided from Dr H. Wakao (Helix Research Institute, Kisarazu, Japan).

RT-PCR
Total RNA was extracted from purified human B cells stimulated with 10 µg/ml of anti-IgM antibody and 0.5 µg/ml of anti-CD40 antibody for the indicated period by Isogen (Nippongene, Tokyo, Japan), and then treated with DNase I (Stratagene, La Jolla, CA) and RNase inhibitor (Roche Diagnostics, Tokyo, Japan). Reverse transcription was performed using a TaKaRa RNA PCR kit (Takara Shuzo, Otsu, Japan).

The competitor for β-actin was constructed with cDNA as a template using the primers 5'-CCCTCGCCCTTGGCCGAT-CCGGTATGTTGCGGATGGTTGCCGGAT-3' and 5'-ATGAGTGATGCTAATGGTTCGCGCTCG-3', corresponding to the portion between -49 and -32 attached to that between +126 and +143 (underlined) and that between +568 and +546 from the starting point.
of translation respectively. Consequently, the product of the competitor for β-actin was composed of the portion between −49 and −32 attached to that between +126 and +568. The PCR reaction was performed with cDNA as a template using the primers corresponding to the portion between −49 and −32 and that between +568 and +546. This was done in the presence of various amounts of competitors after an initial 5 min denaturation at 94°C, followed by 35 cycles of 94°C for 45 s, 57°C for 45 s and 72°C for 45 s. After the amount of total RNA was normalized based on that of β-actin, the competitive PCR for IL-13Rα1 was performed. The competitor for IL-13Rα1 was constructed with cDNA as a template using the primers 5′-CAGTGTAGCACCAGATGAGGAC-3′ and 5′-TCAGGGTAGTAAAG-3′, corresponding to the portion between +301 and +324 and that between +686 and +662 from the starting point of translation respectively. Then the portion between +353 and +404 was cleaved by NsiI. Consequently, the product of the competitor for IL-13Rα1 was composed of the portion between +301 and +352 attached to that between +405 and +686 (334 bp). The PCR reaction was performed with cDNA as a template using the above primers in the presence of various amounts of competitors after an initial 5 min denaturation at 94°C, followed by 45 cycles of 94°C for 45 s, 57°C for 45 s and 72°C for 45 s (386 bp).

Results

Generation of transfectants and expression of the transfected IL-13Rα1

To generate IL-13Rα1-expressing transfectants, we employed a human B cell line, DND-39. Flow cytometry confirmed that this cell line expressed IL-4Rα and γc, but RT-PCR showed that it did not express IL-13Rα1 (data not shown). Plasmids coding six kinds of IL-13Rα1 were transfected by electroporation, and several positive clones for each IL-13Rα1 were obtained. The expression levels of the transfected IL-13Rα1 were almost the same in each clone of DND-39/Ge/IL-13Rα1Full8C, DND-39/Ge/IL-13Rα1ACP, DND-39/Ge/IL-13Rα1ΔCP and DND-39/Ge/IL-13Rα1Y405F, although clones of DND-39/Ge/IL-13Rα1FF and DND-39/Ge/IL-13Rα1402F expressed comparatively lower levels of IL-13Rα1 (Fig. 1B). DND-39/Ge/IL-13Rα1Full8C was used for the following experiments as DND-39/Ge/IL-13Rα1Full. Another clone of DND-39/Ge/IL-13Rα1Full8C, DND-39/Ge/IL-13Rα1 FullE5, expressed almost the same level of IL-13Rα1 as DND-39/Ge/IL-13Rα1FF (Fig. 1B).

STAT6 activation of the transfectants

It has been confirmed that STAT6 has a pivotal role for IL-4 and IL-13 signals (19,20). To clarify the role of the C-terminal tail of IL-13Rα1 in STAT6 activation, we first analyzed it using four kinds of transfectants: mock-transfected DND-39, DND-39/Ge/IL-13Rα1Full, DND-39/Ge/IL-13Rα1ACP and DND-39/Ge/IL-13Rα1ΔCT. The mock-transfected DND-39 cells induced tyrosine phosphorylation of STAT6 by IL-4, but not by IL-13, whereas DND-39/Ge/IL-13Rα1Full did so by both IL-4 and IL-13, demonstrating that the transfectant IL-13Rα1 transduced the IL-13 signal (Fig. 2A). In contrast, tyrosine phosphorylation of STAT6 by IL-4 declined and that by IL-13 was not detected in either DND-39/Ge/IL-13Rα1ΔCP or DND-39/Ge/IL-13Rα1ΔCT (Fig. 2A). The amounts of immunoprecipitated STAT6 were almost the same among the transfectants (Fig. 2A). These results clearly indicated that the C-terminal tail of IL-13Rα1 was essential for STAT6 activation by IL-13 and affected that by IL-4.

As the le promoter region contains the binding site for STAT6, we next analyzed the transcription activity of IL-4 and IL-13 on the le promoter in the transfectants. The mock-transfected DND-39 cells displayed the transcription activity of IL-4 on the le promoter in a dose-dependent manner, but not that of IL-13, whereas DND-39/Ge/IL-13Rα1Full displayed that of both IL-4 and IL-13 in a dose-dependent manner (Fig. 2B). In contrast, the transcription activity of IL-4 on the le promoter decreased and that of IL-13 disappeared in both DND-39/Ge/IL-13Rα1ΔCP and DND-39/Ge/IL-13Rα1ΔCT, in line with the results of STAT6 activation (Fig. 2B). Several clones of each transfectant showed the same tendency (data not shown).
These results suggest that the transcription activity of IL-4 and IL-13 on the \( \varepsilon \) promoter parallels the STAT6 activation.

**Activation of Jak molecules and tyrosine phosphorylation of IRS-1 of the transfectants**

It is widely known that Jak molecules locate upstream of STAT molecules and that activation of Jak molecules is followed by activation of STAT molecules (32,33). To elucidate the effect of the C-terminal tail of IL-13R\( \alpha_1 \) for STAT6 activation, we next analyzed activation of Jak molecules induced by IL-4 and IL-13 in the transfectants. Jak1 has been shown to be activated by both IL-4 and IL-13, and associate with IL-4R\( \alpha \) upon stimulation of IL-4 (34–37). The mock-transfected DND-39 cells induced tyrosine phosphorylation of Jak1 by IL-4, but not by IL-13, whereas DND-39\( / \Gamma_1 \) did so by both IL-4 and IL-13 (Fig. 3A). In contrast, tyrosine phosphorylation of Jak1 by IL-13 was not detected and that by IL-4 was markedly inhibited in both DND-39\( / \Gamma_1 \) and DND-39\( / \Gamma_1 \)\( \Delta \)CP and DND-39\( / \Gamma_1 \)\( \Delta \)CT (Fig. 3A). It has been shown that Jak3 constitutively associates with \( \gamma_c \), and is tyrosine phosphorylated upon stimulation of IL-4 (36,38). Activation of Jak3 occurred by IL-4 stimulation in the mock-transfected DND-39 cells and activation of Jak3 by IL-4 was slightly decreased in DND-39\( / \Gamma_1 \) or DND-39\( / \Gamma_1 \)\( \Delta \)CP or DND-39\( / \Gamma_1 \)\( \Delta \)CT, whereas IL-13 did not cause tyrosine phosphorylation of Jak3 in any of the four transfectants (Fig. 3B). It has been demonstrated that Tyk2 is tyrosine phosphorylated by both human and mouse IL-13 (17,21,23,25). Activation of Tyk2 was induced by IL-4 and IL-13 only in DND-39\( / \Gamma_1 \)Full; however, it was not induced by IL-4 or IL-13 in the mock-transfected DND-39, DND-39\( / \Gamma_1 \)\( \Delta \)CP or DND-39\( / \Gamma_1 \)\( \Delta \)CT, whereas IL-13 did not cause tyrosine phosphorylation of Jak3 in any of the transfectants (Fig. 3B). It has been demonstrated that Tyk2 was also activated in the same manner as Jak1 in the transfectants (data not shown).

It has been already shown that stimuli of both IL-4 and IL-13 lead to tyrosine phosphorylation of IRS-1/2, important for recruitment of PI3-kinase to IL-4R\( \alpha \) (20). To examine the effect of the C-terminal tail of IL-13R\( \alpha_1 \) for tyrosine phosphorylation of IRS-1/2, we analyzed tyrosine phosphorylation of IRS-1/2 in the transfectants. Neither expression nor tyrosine phosphorylation of IRS-2 by IL-4 or IL-13 was detected in these transfectants (data not shown). The mock-transfected DND-39 cells induced tyrosine phosphorylation of IRS-1 by IL-4, but not by IL-13, whereas DND-39\( / \Gamma_1 \) Full did so by both IL-4 and IL-13 (Fig. 3D). In contrast, tyrosine phosphorylation of IRS-1 significantly decreased with either IL-4 or IL-13 in both DND-39\( / \Gamma_1 \)\( \Delta \)CP and DND-39\( / \Gamma_1 \)\( \Delta \)CT as well as Jak1 activation (Fig. 3D), which is compatible with previous reports that IRS-1/2 is a substrate of Jak1 (37,39,40). The amounts of immunoprecipitated Jak1, Jak3 and Tyk2 were almost the same among the transfectants. In (A–C), Jak2 was also activated as the same manner as Jak1 in the transfectants (data not shown).

**Association of Tyk2 with IL-13Rα1**

It has been shown that Box1 regions located at the proximal regions of the cytoplasmic domains of the cytokine receptors...
are necessary for binding to Jak molecules (41). The result that expression of IL-13Rα1 was essential for activation of Tyk2 prompted us to investigate whether the Box1 region in the cytoplasmic domain of IL-13Rα1 is required for association of Tyk2 with IL-13Rα1. We co-transfected Tyk2 and either IL-13Rα1Full or IL-13Rα1ΔCP or IL-13Rα1ΔCT into COS7 cells. The constitutive association of Tyk2 with IL-13Rα1Full and IL-13Rα1ΔCT, but not IL-13Rα1ΔCP, was detected and stimulation of IL-13Rα1Full enhanced the association of Tyk2 with IL-13Rα1Full, but not IL-13Rα1ΔCT (Fig. 4). The expression levels of Tyk2 and the receptors were invariant (Fig. 4). These results demonstrated that the Box1 region in IL-13Rα1 was critical for the association with Tyk2 and that engagement of IL-13Rα1 by IL-13Rα1 enhanced this association.

**STAT3 activation in the transfectants**

To explore the possibility that IL-4 and IL-13 induce activation of STAT molecules other than STAT6, we next studied tyrosine phosphorylation of STAT molecules induced by either IL-4 or IL-13 in the transfectants. Neither expression nor tyrosine phosphorylation of STAT1, 2, and 4 induced by either IL-4 or IL-13 was observed in these transfectants (data not shown). Expression of STAT5 was detected; however, neither IL-4 nor IL-13 caused tyrosine phosphorylation of STAT5 in these transfectants (Fig. 5A). In contrast, tyrosine phosphorylation of STAT3 was induced by IL-4 and IL-13 only in DND-39/Gε/IL-13Rα1Full, although it was not evoked by IL-4 or IL-13 in the mock-transfected DND-39, DND-39/Gε/IL-13Rα1ΔCP and DND-39/Gε/IL-13Rα1ΔCT (Fig. 5B). The amounts of immunoprecipitated STAT3 were almost the same among the samples (Fig. 5B). These results clearly suggest that expression of IL-13Rα1 was required for both IL-4 and IL-13 to activate STAT3 and that the C-terminal tail of IL-13Rα1 was essential.

**Activation of STAT3 by IL-4 and IL-13 in stimulated human B cells**

We next explored the possibility that STAT3 is activated by IL-4 and IL-13, dependent on expression of IL-13Rα1, in not only the human B cell line, but also in primary human B cells. It has been revealed that co-stimulation of Ig and CD40 receptors markedly induces expression of IL-13Rα1 in human B cells (42). We first confirmed that stimulation of purified B cells with anti-IgM antibody and anti-CD40 antibody augmented the amount of IL-13Rα1 mRNA. After the amount of cDNA was normalized based on that of β-actin, competitive PCR for IL-13Rα1 was performed. The amount of cDNA for IL-13Rα1 started to increase 6 h after stimulation of anti-IgM antibody and anti-CD40 antibody by ~10-fold, and then the amount reached levels up 100-fold 24 h after the stimulation (Fig. 6A). Using purified B cells unstimulated and stimulated with anti-IgM antibody and anti-CD40 antibody, we investigated whether IL-4 and IL-13 activate STAT3. In the stimulated, but not unstimulated B cells, IL-4 and IL-13 induced tyrosine phosphorylation of STAT3 by Western blotting (Fig. 6B). The amounts of STAT3 themselves were increased in the stimulated B cells, compared to unstimulated B cells (Fig. 6B). These results clearly showed that both IL-4 and IL-13 activated STAT3 in human B cells stimulated with anti-IgM antibody and anti-CD40 antibody.

**Effects of the tyrosine residues of IL-13Rα1 on IL-4 and IL-13 signals**

It is well known that most cytokine receptors are phosphorylated on tyrosine residues by stimuli and it is important for many signal-transducing molecules to be recruited to the receptors (32). IL-13Rα1 possesses two tyrosine residues in the cytoplasmic portion, at 402 and 405 amino acids. As both of these tyrosine residues were absent in IL-13Rα1ΔCT, it was assumed that impairment of the IL-13 signal in DND-39/Gε/IL-13Rα1ΔCT is due to the lack of these tyrosine residues, although we could not detect tyrosine phosphorylation of IL-13Rα1 (data not shown). Particularly, the sequence around Y405 was compatible with the YXXQ motif, the consensus sequence for binding and activation of STAT3 (16). To test these possibilities, we investigated the IL-4 and IL-13 signals in...
DND-39/Ge/IL-13Rα1Y402F, DND-39/Ge/IL-13Rα1Y405F and DND-39/Ge/IL-13Rα1FF. The transcription activity on the Iε promoter and activation of STAT6 by IL-4 and IL-13 were almost the same between DND-39/Ge/IL-13Rα1Full and these three transfectants (Fig. 7A and B). Tyrosine phosphorylation of Tyk2 by IL-4 and IL-13 in DND-39/Ge/IL-13Rα1FF was invariant compared to DND-39/Ge/IL-13Rα1FF (Fig. 7C). In contrast, activation of Jak1 by IL-4 was slightly diminished and that by IL-13 was significantly attenuated in DND-39/Ge/IL-13Rα1FF, whereas it was invariant in DND-39/Ge/IL-13Rα1Y402F and DND-39/Ge/IL-13Rα1Y405F (Fig. 7D). Activation of IRS-1 by IL-13 was also blocked in DND-39/Ge/IL-13Rα1FF as well as Jak1 (data not shown). Furthermore, it is noteworthy that tyrosine phosphorylation of STAT3 induced by either IL-4 or IL-13 completely disappeared in DND-39/Ge/IL-13Rα1FF, although IL-4 and IL-13 could activate STAT3 in both DND-39/Ge/IL-13Rα1Y402F and DND-39/Ge/IL-13Rα1Y405F (Fig. 7E). The comparisons with another clone of DND-39/Ge/IL-13Rα1Full, DND-39/Ge/IL-13Rα1FullE5 expressing lower IL-13Rα1 and longer exposure of the membranes showed the same results, indicate that these results were not due to relatively lower expression of the receptor in DND-39/Ge/IL-13Rα1FF (data not shown). These results suggest that these tyrosine residues were essential for activation of STAT3 and Jak1, but not Tyk2 and STAT6, and that the existence of either tyrosine residue alone was enough to activate these molecules.

**Discussion**

In this study, we transfected various kinds of human IL-13Rα1 to a human B cell line, and then analyzed activation of Jak molecules, IRS-1 and STAT molecules in the transfectants to clarify the functional role of the cytoplasmic domain, particularly the tyrosine residues of human IL-13Rα1 on IL-4 and IL-13 signals in human B cells.

It had been controversial whether IL-4 and IL-13 evoke STAT3 activation (23, 43–46). Very recently, it was reported that almost the same between DND-39/Ge/IL-13Rα1Full and these three transfectants (Fig. 7A and B). Tyrosine phosphorylation of Tyk2 by IL-4 and IL-13 in DND-39/Ge/IL-13Rα1FF was invariant compared to DND-39/Ge/IL-13Rα1FF (Fig. 7C). In contrast, activation of Jak1 by IL-4 was slightly diminished and that by IL-13 was significantly attenuated in DND-39/Ge/IL-13Rα1FF, whereas it was invariant in DND-39/Ge/IL-13Rα1Y402F and DND-39/Ge/IL-13Rα1Y405F (Fig. 7D). Activation of IRS-1 by IL-13 was also blocked in DND-39/Ge/IL-13Rα1FF as well as Jak1 (data not shown). Furthermore, it is noteworthy that tyrosine phosphorylation of STAT3 induced by either IL-4 or IL-13 completely disappeared in DND-39/Ge/IL-13Rα1FF, although IL-4 and IL-13 could activate STAT3 in both DND-39/Ge/IL-13Rα1Y402F and DND-39/Ge/IL-13Rα1Y405F (Fig. 7E). The comparisons with another clone of DND-39/Ge/IL-13Rα1Full, DND-39/Ge/IL-13Rα1FullE5 expressing lower IL-13Rα1 and longer exposure of the membranes showed the same results, indicate that these results were not due to relatively lower expression of the receptor in DND-39/Ge/IL-13Rα1FF (data not shown). These results suggest that these tyrosine residues were essential for activation of STAT3 and Jak1, but not Tyk2 and STAT6, and that the existence of either tyrosine residue alone was enough to activate these molecules.

Fig. 6. Activation of STAT3 by IL-4 and IL-13 in stimulated human B cells. (A) RT-PCR for IL-13Rα1. Competitive PCR was performed in the presence of the indicated amounts of competitors with cDNA from purified human B cells unstimulated and stimulated with 10 ng/ml of anti-IgM antibody and 0.5 μg/ml of anti-CD40 antibody for the indicated period. (B) Tyrosine phosphorylation of STAT3 induced by IL-4 and IL-13 in unstimulated and stimulated human B cells. Cell lysates of unstimulated and stimulated human B cells activated by either 10 ng/ml of IL-4 or 100 ng/ml of IL-13 for 15 min were blotted by anti-phospho STAT3 antibody (upper panel) or anti-STAT3 antibody (lower panel).
that as Y402 and Y405 are located close together, the SH2 domain of STAT3 can sterically recognize Y402 the same as Y405. Analyses of the crystal structure of intracellular domain of IL-13Rα1 would clarify this point.

STAT3 has been shown to be activated by various kinds of cytokines and growth factors (32), and several target genes of STAT3 have been already identified, such as junB, IRF1, STAT3, p19INK4D and c-myc (48–51). The physiological roles of STAT3 in B cells are not fully understood, but it has been shown that STAT3 is involved in Ig production, an anti-apoptotic effect, and a proliferation effect in B cell lines and myeloma cells (50,52–54), and it is assumed that STAT3 activation is correlated with induction of CD23, ICAM-1 and lymphotoxin-α by engagement of CD40 in human B cells (55). To date, the functional role of STAT3 on IL-4 and IL-13 signals in stimulated human B cells remains to be resolved, whereas that of STAT6 is well investigated (19,20). It would be possible that STAT3 and STAT6 independently correlate with IL-4 and IL-13 signals or alternatively that STAT3 and STAT6 co-operatively act to transduce IL-4 and IL-13 signals. Further studies aimed at clarifying this point are awaited.

Jak1 has been shown to be activated by both IL-4 and IL-13, and associate with IL-4Rα upon stimulation of IL-4 (34–37). The present results—that tyrosine phosphorylation of Jak1 induced by IL-13 was completely abolished in DND-39/Gε/IL-13Rα1ΔCT and DND-39/Gε/IL-13Rα1ΔCP, and severely impaired in DND-39/Gε/IL-13Rα1FF (Figs 3A and 7D)—suggested that IL-13Rα1 was involved in the activation mechanism of Jak1 and that its C-terminal tail, particularly the tyrosine residues, was important for the activation. The existence of either tyrosine residue alone was enough for Jak1 activation as well as STAT3 activation (Fig. 7D). Thus far, a mechanism requiring the tyrosine residues of IL-13Rα1 for activation of Jak1 remains unknown. It may be important for the binding of

![Fig. 7. Effects of the tyrosine residues of IL-13Rα1 on IL-4 and IL-13 signals.](image-url)
Jak1 to IL-13Rα1 or, alternatively, some molecule, which is correlated with Jak1 activation, may be recruited to this site. STAT3 was not co-immunoprecipitated with Jak1 (data not shown). The present result—that tyrosine phosphorylation of Jak1 by IL-4 was impaired in DND-39/Ge/IL-13Rα1ΔCT and DND-39/Ge/IL-13Rα1ΔCP compared to DND-39/Ge mock cells—suggested that competition between IL-13Rα1 and γc for IL-4Rα occurred (Fig. 3A). This notion was supported by the results that STAT6 or Jak3 activation was higher in DND-39/Ge mock cells than DND-39/Ge/IL-13Rα1ΔCT and DND-39/Ge/IL-13Rα1ΔCP or the other three transfectants respectively (Figs 2A and B, and 3B).

It had been already demonstrated that Tyk2 is tyrosine phosphorylated by both human and mouse IL-13 (17,21,23,25); however, it was controversial whether IL-4 also induces tyrosine phosphorylation of Tyk2 (17,21,23,25,35,37). The present result showed that expression of IL-13Rα1 enabled both IL-4 and IL-13 to activate Tyk2 by engaging IL-4 and IL-13 receptors containing IL-13Rα1 as a component (Fig. 3C), in line with the previous results that in IL-13R-expressing cells, IL-4 can activate Tyk2 (17,21,23,25). The present results—that IL-13Rα1Full and IL-13Rα1ΔCT, but not IL-13Rα1ΔCP, constitutively associated with Tyk2 (Fig. 4)—clearly showed that the Box1 region of IL-13Rα1 was the binding site for Tyk2, so that Tyk2 was not tyrosine phosphorylated in DND-39/Ge/IL-13Rα1ΔCP (Fig. 3C). The result that the association of Tyk2 with IL-13Rα1 was enhanced by IL-13 and that this enhancement decreased in IL-13Rα1ΔCT (Fig. 4) may indicate that the C-terminal tail of IL-13Rα1 is required for its optimal conformational change by the ligands, important for association and activation of Tyk2, so that Tyk2 activation may be diminished in DND-39/Ge/IL-13Rα1ΔCT (Fig. 3C).

It has been shown that Jak3 constitutively associates with γc and is tyrosine phosphorylated upon stimulation of IL-4 (36,38). The present result—that tyrosine phosphorylation of Jak3 was not induced by IL-13 (Fig. 3B)—was in line with the previous results that γc is not involved in IL-13R (21,24,56), and that a heterotrimer composed of IL-13Rα1, IL-4Rα and γc does not exist (18,27). Analyses of cross-linking using IL-4 labeled with 125I in DND-39/Ge/IL-13Rα1Full also showed the existence of the heterodimers, but not the heterotrimer (data not shown).

It is well known that upon stimulation, STAT6 is recruited to phosphorylated tyrosine residues of IL-4Rα and then is tyrosine phosphorylated, followed by localization to the nucleus (20,57). It has been already reported that Jak1 and Jak3 are correlated with STAT6 activation when stimulated by IL-4 (19,40,58,59); however, it remained to be resolved whether Tyk2 is also involved in STAT6 activation. The results that activation of STAT6 by IL-13 was retained as well as that of Tyk2 in DND-39/Ge/IL-13Rα1ΔCT, whereas activation of Jak1 by IL-13 was significantly blocked in this transfectant (Fig. 7B–D), strongly supported the notion that Tyk2 is also involved in STAT6 activation. In DND-39/Ge/IL-13Rα1ΔCT and DND-39/Ge/IL-13Rα1ΔCP, in which activation of both Tyk2 and Jak1 by IL-13 was completely blocked, STAT6 activation was not detected (Fig. 2), suggesting that the C-terminal tail of IL-13Rα1 was critical for tyrosine phosphorylation of not only Jak1, Tyk2 and IRS-1, but also of STAT6 induced by IL-13.

Acknowledgments

We thank Dr Toshikiko Akimoto for critical support of this study and Dr Kazuya Shimoda for giving us the plasmid. We also thank Dr Dovie R. Wylie for critical review of this manuscript.

Abbreviations

IL-13Rα IL-13 receptor α chain
IL-4Rα IL-4 receptor α chain
IRS insulin receptor substrate
Fli3 phosphodiesterinosil 3
Py phosphotyrosine

References


IL-4 and IL-13 signals dependent on human IL-13Rα1


