Characterization of the Interaction between Interleukin-13 and Interleukin-13 Receptors*

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Interleukin-13 (IL-13) possesses two types of receptor: the heterodimer, composed of the IL-13Ra1 chain (IL-13Ra1) and the IL-4Ra chain (IL-4Ra), transducing the IL-13 signals; and the IL-13Ra2 chain (IL-13Ra2), acting as a nonsignaling “decoy” receptor. Extracellular portions of both IL-13Ra1 and IL-13Ra2 are composed of three fibronectin type III domains, D1, D2, and D3, of which the last two comprise the cytokine receptor homology modules (CRHs), a common structure of the class I cytokine receptor superfamily. Thus far, there has been no information about the critical amino acids of the CRHs or the role of the D1 domains of IL-13Ra1 and IL-13Ra2 in binding to IL-13. In this study, we first built the homology modeling of the IL-13-hIL-13 receptor complexes and then predicted the amino acids involved in binding to IL-13. By incorporating mutations into these amino acids, we identified Tyr-207, Asp-271, Tyr-315, and Asp-318 in the CRH of human IL-13Ra2, and Leu-319 and Tyr-321 in the CRH of human IL-13Ra1, as critical residues for binding to IL-13. Tyr-315 in IL-13Ra2 and Leu-319 in IL-13Ra1 are positionally conserved hydrophobic amino acid residues. Furthermore, by using D1 domain-deleted mutants, we found that the D1 domain is needed for the expression of IL-13Ra2, but not IL-13Ra1, and that the D1 domain of IL-13Ra1 is important for binding to IL-13, but not to IL-4. These results provide the basis for a precise understanding of the interaction between IL-13 and its receptors.

Interleukin (IL)1–3 is a pleiotropic Th-2-type cytokine produced by CD4+ T cells, natural killer T cells, mast cells, basophils, and eosinophils (1). It is known that IL-13 plays a pivotal role in host defense against parasite infection and in the pathogenesis of allergic diseases (1–3). IL-13 exerts its actions by binding to the IL-13 receptor (IL-13R) on the cell surface, the heterodimer comprised of IL-13Ra1 chain (IL-13Ra1) and the IL-4Ra chain (IL-4Ra). IL-13 binds to IL-13Ra1 first with low affinity ($K_d = 2–10$ nM) and then recruits IL-4Ra to the complex, generating a high affinity receptor ($K_d = 0.03–0.4$ nM) (4–6). Heterodimerization of IL-13R causes activation of Janus kinases, TYK2 and JAK1, constitutively associated with IL-13Ra1 and IL-4Ra, respectively, followed by activation of the signal transducer and activator of transcription 6 (STAT6) (1). STAT6 is a transcription factor critical for IL-13 signals, causing expression of various IL-13-inducible genes together with other transcriptional factors (1, 7). There is another IL-13-binding unit, the IL-13Ra2 chain (IL-13Ra2), which binds to IL-13 with high affinity (0.25–1.2 nM) (8, 9). No other receptor molecule is known to be involved in the IL-13:IL-13Ra2 complex. IL-13Ra2 is thought to act as a nonsignaling “decoy” receptor because its cytoplasmic tail is short and does not contain any obvious signaling motif (10, 11). Consistent with this notion, IL-13Ra2-disrupted mice showed enhanced IL-13 responses (12, 13). Thus, the two IL-13-binding molecules, IL-13Ra1 and IL-13Ra2, have different affinities with IL-13 and opposite roles in signal transduction, which would cooperate with each other in tuning the IL-13 signals in the body.

The extracellular domains of all members of the class I cytokine receptor superfamily, including IL-13Ra1 and IL-13Ra2, contain the cytokine receptor homology module (CRH), composed of two fibronectin type III (FnIII) domains (14). Each domain consists of 100 amino acid residues, generating a β-sandwich structure where seven β-strands are arranged in the Greek key topology analogous to an immunoglobulin-constant domain. Four positionally conserved cysteine residues in the first FnIII domain form two disulfide bonds, and a WSXWS sequence locates in the F′-G′ loop in the second FnIII domain, both of which are critical for the receptors to position correctly and bind to ligands (14). Crystal structural analyses of the ligand-receptor complexes of growth hormone (GH), erythropoietin, IL-4, IL-6, IL-12, and the granulocyte colony-stimulating factor (G-CSF) have demonstrated that several loops of the CRHs of these receptors provide binding interfaces composed of hydrophobic and polar amino acids (15–20).

The extracellular domains of a subgroup of the class I cytokine receptor superfamily (gp130, G-CSFR, the granulocyte/macrophage colony-stimulating factor receptor α chain, the leukemia inhibitory factor receptor (LIFR), IL-3Ra, and IL-
The extracellular domains of human IL-13Rα1 (hIL-13Rα1) and hIL-13Rα2 have ∼33% homology and ∼21% identity (Fig. 1A). Both are composed of three FnIII domains, D1, D2, and D3 (numbering from the N terminus), of which D2 and D3 comprise the CRHs. The most homologous receptor molecules of hIL-13Rα1 and hIL-13Rα2 in their extracellular domains are hIL-5Ra (44% for hIL-13Rα1 and 45% for hIL-13Rα2, respectively). Mutagenesis analyses of IL-13 have been performed extensively, identifying the amino acid residues important for binding to IL-13 receptors (29–31). However, there has been no information about the critical amino acids of the CRHs of IL-13Rα2, IL-13Rα1 complexes. A, homology modeling of the D2 and D3 domains of the IL-13-hIL-13Rα2 complex was based on the x-ray structure of the GH-PRLR 1:1 complex (PDB code 1BP3) as a template. The E-F, B'-C', D'-E', and F'-G' loops of hIL-13Rα2 are colored in red, yellow, orange, and green, respectively. Residues that were replaced by Ala in 4A-mut (Tyr-207, Asp-271, Tyr-315, and Asp-318) are depicted. The pink portion represents α-helix D of IL-13. B, homology modeling of the IL-13-hIL-13Rα1 complex was based on the x-ray structure of the viral IL-6-gp130 complex (PDB code 111R) and the IL-13-hIL-13Rα2 modeling as templates. The E-F, B'-C', and F'-G' loops of hIL-13Rα1 are colored in red, yellow, and green, respectively. Leu-319 and Tyr-321 of hIL-13Rα1 are depicted. The pink portion represents α-helix D of IL-13.

The extracellular domains of human IL-13Rα2 (hIL-13Rα2) and hIL-13Rα2 have ∼33% homology and ∼21% identity (Fig. 1A). Both are composed of three FnIII domains, D1, D2, and D3 (numbering from the N terminus), of which D2 and D3 comprise the CRHs. The most homologous receptor molecules of hIL-13Rα1 and hIL-13Rα2 in their extracellular domains are hIL-5Ra (44% for hIL-13Rα1 and 45% for hIL-13Rα2, respectively). Mutagenesis analyses of IL-13 have been performed extensively, identifying the amino acid residues important for binding to IL-13 receptors (29–31). However, there has been no information about the critical amino acids of the CRHs of IL-13Rα1 and hIL-13Rα2 for binding to IL-13, and the role of the D1 domain in IL-13Rα1 and IL-13Rα2 has been unknown.

In this study, we first built the homology modeling of the IL-13-hIL-13R receptor complexes and then identified critical residues in the CRHs of hIL-13Rα1 or hIL-13Rα2 by mutagenesis analyses, based on these models. Furthermore, we analyzed the roles of the D1 domains in IL-13Rα1 and IL-13Rα2 in their expression and binding to IL-13 and IL-4.

MATERIALS AND METHODS

Reagents and Cells—Recombinant human IL-13 was expressed in Escherichia coli transformant-harborning pET28a vector (Novagen, Madison, WI) in which the DNA encoding human IL-13 was inserted. The expressed IL-13 was purified by nickel-nitrilotriacetic acid resin (Qiagen) followed by refolding in 0.1 M Tris-HCl, pH 8.5, containing 3 M

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In this study, we first built the homology modeling of the IL-13-hIL-13R receptor complexes and then identified critical residues in the CRHs of hIL-13Rα1 or hIL-13Rα2 by mutagenesis analyses, based on these models. Furthermore, we analyzed the roles of the D1 domains in IL-13Rα1 and IL-13Rα2 in their expression and binding to IL-13 and IL-4.

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urea, 30% glycerol, 5 mM cysteine, and 5 mM cystamine for 4 days at 4 °C. IL-13 was further purified by SP-Sepharose Fast Flow column chromatography (Amersham Biosciences) and then TSKgel SP5-PW column chromatography (Tosoh, Tokyo, Japan). Detailed expression results of human IL-13 will be published elsewhere. Recombinant human IL-4 was purchased from PeproTech (Rocky Hill, NJ). A human Burkitt’s B-lymphoma cell line, DND-39, and DND-39 cells transfected with the germ line promoter-luciferase gene (DND-39/G9280 cells) were prepared and cultured, as described before (32, 33). HEK 293T cells were cultured in Dulbecco’s modified Eagle’s medium (Invitrogen) supplemented with 10% fetal calf serum, 100 μg/ml streptomycin, and 10 units/ml penicillin G.

**Plasmids and Transfection—**Plasmids encoding wild types of hIL-13Rx1 (pIRES1hyg-FLAG-hIL-13Rx1) and hIL-13Rx2 (pIRESneo2-HA-hIL-13Rx2) were prepared as described previously (32, 33). The FLAG and HA sequences were attached to the N termini of the D1 regions of hIL-13Rx1 and hIL-13Rx2 cDNA, respectively. The mutated types of IL-13Rx1 and IL-13Rx2 were generated by the QuikChange method (Stratagene, La Jolla, CA) or the modified inverse PCR method using mutation-incorporated oligonucleotides as the primers and pIRES1hyg-FLAG-hIL-13Rx1 and pIRESneo2-HA-hIL-13Rx2 as the templates.

The plasmids were transfected into DND-39 cells by electroporation. Stable transfected cells were maintained with the culture medium containing 250 μg/ml hygromycin B (Wako, Osaka, Japan) for the hIL-13Rx1 mutants or 1.25 mg/ml G418 (Sigma) for the hIL-13Rx2 mutants, respectively. Expression of the receptors was confirmed by flow cytometry (FACSCalibre, BD Biosciences) using anti-FLAG antibodies (Abs, Sigma) for the IL-13Rx1 mutants and anti-HA Abs (Sigma) for the IL-13Rx2 mutants. Transient transfection of the plasmids into HEK 293T cells was performed by Lipofectamine 2000 (Invitrogen), according to the manufacturer’s protocol.

**Sequence Alignment and Homology Model Building—**Alignment of the amino acid sequences of hIL-13Rx2 and hIL-13Rx1 without signal peptides (1 to 26, and 1 to 22, respectively) and prediction of FnIII domains (D1, D2, and D3), the transmembrane regions, and several β-strand sequences of the D2 and D3 domains of both receptors were executed through the MyHits data base (34). The sequence alignments of extracellular portions of human prolactin (PRL) receptor-hIL-13Rx2 and human gp130-hIL-13Rx1 were performed by FUGUE (35) with slight manual modification based on experimental results.

The complex structure of IL-13 and the D2 and D3 domains of hIL-13Rx2 was modeled based on the crystal structure of the GH-PRLR complex (PDB code 1BP3). The hIL-13Rx1 was modeled from the crystal structure of the viral IL-6-gp130 complex (PDB code 1I1R), and the resulting structure was overlaid onto the IL-13-hIL-13Rx2 complex structure model. Homology modelings of hIL-13Rx2 and hIL-13Rx1

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**FIG. 3. Characterization of the mutated types of hIL-13Rx2.** A, schematic model of mutated types of hIL-13Rx2 and their Kd values with IL-13 using stable DND-39 transfants. Experiments were done at least twice for one clone in at least two different clones, and statistical differences with p < 0.05 (*) are indicated. The white bands in the D2 domain and the gray band in the D3 domain represent the conserved cysteine residues and the WSXWS sequence. B, expression of hIL-13Rx2 on the surface of DND-39 cells stably transfected with hIL-13Rx2 (wild type, 4A-mut, Y207A, D271A, Y315A, D318A) by flow cytometry. The shaded and open areas represent the counts with or without the first Ab, respectively. C, binding assay of DND-39 cells stably transfected with hIL-13Rx2 (wild type, 4A-mut, Y207A, D271A, Y315A, D318A). The white bands in the D2 domain and the gray band in the D3 domain represent the conserved cysteine residues and the WSXWS sequence.

2 E. Honjo, unpublished data.
were generated with MODELLER6v2 (36) followed by energy minimization and simulated annealing with Amber. The figures were drawn with DS Viewer Pro 5.0 (Accelrys, CA).

![Image](http://www.jbc.org/)

**Fig. 4. Expression of D1-deleted types of IL-13Rα2.** A schematic model of D1-deleted type of hIL-13Rα2. EC, TM, and CY represent the extracellular domain, transmembrane domain, and cytoplasmic domain, respectively. Either wild type or the D1-deleted type of hIL-13Rα2 was transfected into HEK 293T cells. Their protein expression was analyzed by Western blotting (B) and flow cytometry (C). In B, the arrow represents the expressed hIL-13Rα2. In C, the shaded and open areas represent the counts with or without the first Ab, respectively.

Trp-104 of GHR, Phe-169 of gp130, and Phe-93 of the erythropoietin receptor, known as a "hot spot" of the ligand binding (Refs. 15, 16, 18, 39, and Fig. 1B).

**Identification of Critical Residues of the D2 and D3 Domains of hIL-13Rα2 for Binding to IL-13—**To address the importance of the amino acids in the D2 and D3 domains of hIL-13Rα2, predicted based on the homology modeling for binding of IL-13, we generated several kinds of hIL-13Rα2 mutants in these amino acids and analyzed their binding affinity for IL-13. We first analyzed the mutagenized hIL-13Rα2 in which Tyr-207, Asp-271, Tyr-315, and Asp-318 were replaced with Ala (4A-mut, Fig. 3A). In the modeling, Asp-271 on the strand C was assumed to locate close to Asp-318 (Fig. 2A). When 4A-mut was transfected into DND-39 cells, although the expression level of the cell surface was invariable with that of the wild type of hIL-13Rα2 (Fig. 3B), no binding activity was detected (Fig. 3, A and C), demonstrating the critical roles of these four amino acids in binding to IL-13. To delineate the contribution of each of these four residues in binding to IL-13, we next analyzed the mutants in which Tyr-207, Asp-271, Tyr-315, or Asp-318 was replaced with Ala (4A-mut, Fig. 3A). When any of these four amino acids was mutated with Ala, the affinity of the mutated hIL-13Rα2 was significantly decreased. Expression of these four single mutated hIL-13Rα2 on the cell surface was invariable with the wild type (Fig. 3B). When Asp-318 was replaced with Ala together with Asp-271, the affinity was lowered more than in the single exchange of Asp-271 or Asp-318 (Fig. 3A). The replacement of amino acids that were either adjacent or close to these four critical amino acids (Asp-206, Ala-267, Arg-268, Ser-317, and Asp-318) affected the affinity with IL-13 (Fig. 3A). Double mutations of Glu-289 and Glu-291 with Ala, but not with Gln, slightly decreased the affinity, indicating that a hydrogen bond may be formed between Glu-289 and/or Glu-291 and IL-13. These results coincided with the molecular model in that the E-F loop and the F′-G′ loop of hIL-13Rα2 generates the main binding surface to IL-13 and that the critical amino acids in these loops (Tyr-207, Asp-271, Tyr-315, and Asp-318) are involved in comprising the binding interface at focal contacts. Particularly, it turned out that Tyr-207 is a hot spot for the ligand binding as well as Trp-72 of
PRLR, Trp-104 of GHR, Phe-169 of gp130, and Phe-93 of the erythropoietin receptor. Although Asp-271 in the B'-C' loop is not exposed to the main binding interface, this amino acid would act cooperatively with Asp-318 for the binding.

Critical Role of the D1 Domain of hIL-13Rα2 in Its Expression—We next analyzed the functional role of the D1 domains of hIL-13Rα1, which could not be modeled because of a lack of information about homologous structure. For this purpose, we
generated a truncated type of hIL-13Rα2 lacking its D1 domain (hIL-13Rα2ΔD1, Fig. 4A). When hIL-13Rα2ΔD1 was transfected in HEK 293T cells, its expression was not detected by either Western blotting or flow cytometry analysis (Fig. 4, B and C). These results showed that the D1 domain of hIL-13Rα2 is critical for its expression.

**Homology Modeling of the IL-13-hIL-13Rα1 Complex**—Because the extracellular portions of IL-13Rα1 and IL-13Rα2 are homologous (Fig. 1A; ~33%) and several common amino acids in α-helix D of IL-13 are important for binding to both receptors (30), it is reasoned that amino acid residues involved in IL-13 binding are topologically conserved between these two receptors. We built a homology model of the IL-13-hIL-13Rα1 complex using the x-ray structures of the viral IL-6gp130 complex (PDB code 111R) and the IL-13/hIL-13Rα2 modeling as templates (Fig. 2B). In this model, it was assumed that the E-F loop, the B'-C' loop, and the F'-G' loop are composed of Thr-190 to Glu-198, Glu-248 to Arg-256, and Asn-317 to Glu-333 amino acids, respectively (Fig. 1C). Furthermore, it was predicted that Phe-197 in the E-F loop would correspond to Tyr-207 in hIL-13Rα1 and that Leu-319 and Asp-323 in the F'-G' loop would correspond to Tyr-315 and Asp-318 of hIL-13Rα2, respectively (Fig. 1A). The E-F loop of hIL-13Rα1 was two residues longer than that of hIL-13Rα2, and Ser-195 to Ser-196 was assumed to be the unique sequence to hIL-13Rα1 because no amino acid in hIL-13Rα2 corresponds to these amino acids (Fig. 1A).

**Identification of Critical Residues of the D2 and D3 Domains of hIL-13Rα1 for Binding to IL-13**—We explored whether the E-F loop and the F'-G' loop of hIL-13Rα1 generate the main binding interface to IL-13 as well as hIL-13Rα2 and whether the amino acids in those loops of hIL-13Rα1 corresponding to the critical amino acids in hIL-13Rα2 contribute to the binding. To address this possibility, we generated several kinds of mutated hIL-13Rα1 and analyzed their binding affinity for IL-13. Because DND-39/G 4Ro cells express endogenous hIL-4Ro, but not hIL-13Rα1, the mutated hIL-13Rα1 transfected on the cells comprises IL-13Rα1/type II IL-4Rα together with endogenous hIL-4Ro (1). We first analyzed the involvement of the E-F loop of hIL-13Rα1 for binding to IL-13. When both Ser-195 and Ser-196, unique amino acids in hIL-13Rα1, were exchanged with Ala, the affinity was decreased, although the deleted mutant of both amino acids showed only a slight decrease (Fig. 5A). Mutations of Phe-197 corresponding to Tyr-207 in hIL-13Ro2 or adjacent Glu-198 did not show any difference. We next analyzed the involvement of the F'-G' loop. When either Leu-319 or Asp-323 corresponding to Tyr-315 and Asp-318 in hIL-13Rα2 was replaced with Ala, the affinity of the mutated hIL-13Rα1 to IL-13 was dramatically decreased in L319A, but there was no change in D323A (Fig. 5, A and C). Replacement of Asp-324 adjacent to Asp-323 showed only a slight decrease of the affinity. In contrast, when Tyr-321 was exchanged with Ala, the affinity was decreased significantly. Replacement of Glu-322 also attenuated the affinity, but less than Leu-319 or Tyr-321. Expression of these mutated types of hIL-13Rα1 was invariable with the wild type (Fig. 5B and data not shown). Substitution at Phe-259 corresponded to Asp-271 in hIL-13Ro2 on the strand C' and did not influence the affinity.

We next tested whether the lowered affinities of the mutated hIL-13Rα1 would lead to reduction in the IL-13 signal. DND-39/G 4Ro cells expressing endogenous hIL-4Ro and the transfected hIL-13Rα1 are able to transduce the IL-13 signal by engagement of the ligand, augmenting expression of the reporter gene. When we performed a reporter gene assay using DND-39/G 4Ro cells expressing all kinds of the mutated hIL-13Rα1 investigated for the binding assay, only the mutant types of Leu-319 and Tyr-321 significantly impaired the IL-13 response (Fig. 5D and data not shown). The double mutation type at Ser-195 and Ser-196 and the single mutation types at Glu-322 or Asp-324 showed normal IL-13 responses. The responses of all transfectants to IL-4 were invariable. To identify the amino acid residues critical for binding to hIL-13Rα1, we generated five more mutants and analyzed their IL-13 responses in HEK 293T cells. In this experiment, we stimulated HEK 293T cells with 0.01 ng/ml IL-13 in which expression of the reporter gene through transfected hIL-13Rα1, but not endogenous hIL-13Rα1, was detected (Fig. 5D). We confirmed that the responses to IL-13 could be detected as the same as in the system using DND-39/G 4Ro cells and that again L319A and Y321A showed lower activities. However, none of the additionally investigated mutants, single mutations of Val-192, Lys-195, or Asp-194 in E-F loop and Lys-318 or Lys-325 in F'-G' loop with Ala, changed the IL-13 responses. These results suggested that both the E-F loop and more dominantly the F'-G' loop contribute to binding to IL-13 in hIL-13Rα1 as well as hIL-13Rα2 and that particularly, Leu-319 and Tyr-321 in the F'-G' loop are critical residues for the binding to transduce the IL-13 signal. Leu-319 in hIL-13Rα1 corresponding to Tyr-315 in hIL-13Rα2 is a positionally conserved hydrophobic residue for binding to IL-13.

**Critical Role of the D1 Domain of hIL-13Rα1 in Its Binding to IL-13 but Not to IL-4**—We next analyzed the functional role of the D1 domain of hIL-13Rα1, which could not be modeled because of a lack of information about homologous structure as well as hIL-13Rα2. For this purpose, we generated a truncated type of hIL-13Rα1 lacking its D1 domain (hIL-13Rα1ΔD1; Fig. 6A). When hIL-13Rα1ΔD1 was transfected in HEK 293T cells, its expression was detected by both Western blotting and flow cytometry analysis at the same level as the wild type in contrast to hIL-13Rα2ΔD1, confirming the ability of this mutated type to be expressed on the cell surface (Fig. 6, B and C).

We next analyzed the involvement of the D1 domain of hIL-13Rα1 in binding to IL-13. hIL-13Rα1ΔD1 completely lost the binding affinity to IL-13, although it was expressed on the cell surface at the same level as the wild type (Fig. 7, A and B). In concordance with the results of the binding assay, hIL-13Rα1ΔD1 failed to induce the transcription of the reporter gene.
gene and activate both STAT6 and TYK2 by engagement of IL-13 (Fig. 7, C and D). In contrast, expression of hIL-13Rα1ΔD1 did not prevent the reporter gene activity or STAT6 activation by IL-4 (Fig. 7, C and D). IL-4 could activate STAT6 through either type I IL-4R composed of IL-4Ra and the common γ chain (γc) or type II IL-4R composed of IL-4Ra and IL-13Rα1. It would be possible that even though the type II IL-4R composed of IL-4Ra and hIL-13Rα1ΔD1 was nonfunctional, IL-4 could activate STAT6 through the type I IL-4R. However, activation of TYK2, a specific signaling event of type II IL-4R/IL-13R, was detected, when hIL-13Rα1ΔD1-expressed cells were stimulated with IL-4 (Fig. 7D), indicating that the type II IL-4R composed of hIL-4Ra and hIL-13Rα1ΔD1 was functional for the IL-4 binding and its signaling. These results suggested that the D1 domain of hIL-13Rα1 is critical for binding to IL-13, but not to IL-4.

**DISCUSSION**

In this article, we identified for the first time critical residues in the CRHs of hIL-13Rα1 and hIL-13Rα2 in binding to IL-13 by the mutagenesis approach based on homology modeling of the IL-13-hIL-13 receptor complexes. In our findings, Tyr-207, Asp-271, Tyr-315, and Asp-318 in the CRH of hIL-13Rα2, and Leu-319 and Tyr-321 in the CRH of hIL-13Rα1 are critical residues for binding to IL-13 (Figs. 3 and 5). Leu-319 in hIL-13Rα1 and Tyr-315 in hIL-13Rα2 are positionally conserved hydrophobic amino acids. Tyr-207, Tyr-315, and Asp-318 in hIL-13Rα2, and Leu-319 in hIL-13Rα1 are conserved in all known species, whereas Tyr-321 in hIL-13Rα1 is conserved in porcine and canine IL-13Rα1 but is replaced by Phe in rat and mouse IL-13Rα1. It has been assumed that the binding site on IL-13 to IL-13Rα1 is α-helix A and α-helix D because the α-helix A and α-helix D of IL-4 interact with either γc or IL-13Rα1, based on the structure of the IL-4-IL-4Ra complex (20), and IL-13 has a significant similarity in folding topology with IL-4 (40, 41). Consistent with this assumption, it has been demonstrated that several amino acid residues in α-helix D of IL-13 (Lys-90, Ile-91, His-103, Leu-104, Lys-105, Lys-106, Arg-109, Gln-110, and Arg-112) are important for binding to hIL-13Rα1 and/or hIL-13Rα2, although α-helix A of IL-13 is predicted to interact with IL-4Ra (29–31). Some of these amino acids would interact with those identified in our present study, involved in the binding between IL-13 and hIL-13Rα1/IL-13Rα2. It is of note that Asp-318 of hIL-13Rα2, contributing most to the binding among the investigated amino acid residues, was assumed to interact with Lys-105 of IL-13, forming a salt bridge, in our present model (data not shown).

We furthermore demonstrated that the D1 domain is necessary for expression of hIL-13Rα2, but not for that of hIL-13Rα1 (Figs. 4 and 6), whereas the D1 domain of hIL-13Rα1 is critical for binding to IL-13 (Fig. 7). Thus far, it is unclear how the D1 domain of hIL-13Rα2 is involved in the expression mechanism of the receptor. When the D1 domain is deleted, the D2/D3 domains of hIL-13Rα2 may be unable to keep their conformations. Mutagenesis analyses of the extra FnIII domain have already shown its importance in binding to ligands and the signal transduction in gp130 (21, 22), G-CSFR (23, 24), LIFR (25, 26), and IL-5Rα (27). Our present finding is the first evidence showing involvement of the D1 domain of hIL-13Rα1 in binding to IL-13. Structural analyses of the IL-6/IL-6R/gp130 complex show that this complex is a 2:2:2 hexamer, in which the extra FnIII domains interact with the binding epitopes on IL-6 in the opposite trimers (18, 28). Although the precise structure of the IL-13-hIL-13Rα1-hIL-4Ra complex remains undetermined, this complex may also form a 2:2:2 hexamer via the D1 domain of hIL-13Rα1 (Fig. 8A).

We found that the D1 domain of hIL-13Rα1 is critical for the binding and signal transduction of IL-13, but not IL-4 (Fig. 7). This finding suggests that the binding modes of IL-4 and IL-13 with IL-13Rα1 are different, although both ligands utilize the common heterodimeric complex composed of IL-4Ra and IL-13Rα1. Similarly, it has been already shown that the D1 domain of gp130 is needed for binding to IL-6, but not to LIF, IL-11, ciliary neurotrophic factor, or oncostatin-M (21, 42). IL-4 first binds to IL-4Ra and then recruits IL-13Rα1 or γc to the complex, forming the high affinity receptor. IL-13Rα1 alone has almost no binding activity to IL-4 (4, 43). Mutagenesis experiments have suggested that Arg-121 and Tyr-124 located at the α-helix D of IL-4 are important for the interaction with IL-13Rα1 (44, 45), which overlaps with the interaction site to γc (46, 47). Our present finding indicates that α-helix D of IL-4 probably interacts with the D2 and D3 domains of IL-13Rα1, independently from its D1 domain (Fig. 8B). Involvement of the D1 domain of hIL-13Rα1 in binding to IL-13, but not to IL-4, at
least partially explains the different affinities of hIL-13Rα1 to IL-13 and IL-4. We found previously that there exists a variant of the IL13 gene, in which arginine residue at 110 (Arg-110; numbering from the starting residue of the mature protein at Gly-1) is replaced by glutamine (Gln-110); this variant is associated with bronchial asthma in both Japanese and British populations (48). The same variant was thereafter reported to be positively correlated with high IgE levels and atopic dermatitis (49–51). We furthermore demonstrated that the Gln-110 type has a lower affinity with hIL-13Rα2 than the Arg-110 type, whereas both types show the same affinity with hIL-13Rα1, which would cause up-regulation of the IL-13 concentration in the body (33). We assumed that the interaction between Arg-110 and hIL-13Rα2 might be disrupted by the substitution of the glutamine residue, although the alanine scanning approach showed only a slight involvement of this residue in binding to hIL-13Rα2 (30). If such an amino acid in hIL-13Rα2 interacting with Arg-110 in IL-13 were displaced by another amino acid, the affinities of the mutant hIL-13Rα2 Arg-110 and Gln-110 types would become the same. All of the investigated mutant hIL-13Rα2 showed lower affinities with the Gln-110 type than the Arg-110 type or the wild type (data not shown). These results implied the possibility that R110Q may change the conformation of IL-13 itself, not the direct interaction with hIL-13Rα2.

Considering the importance of IL-13 in the pathogenesis of allergic diseases, particularly bronchial asthma, several IL-13 antagonists have been developed as means of improving allergic states (1). Our present finding would be useful in these strategies. The D1 domain is a particularly good target to develop a neutralizing Ab or a low molecular weight compound to block specifically the interaction between hIL-13Rα1 and IL-13, but not IL-4. It has been already shown that the monoclonal Abs against the D1 domains of gp130 (21, 22, 42) or G-CSF-R (24) inhibit binding to ligands and their signals. It will be of great interest to analyze the effects of a neutralizing Ab or a low molecular weight compound targeting the D1 domain of hIL-13Rα1 on the development of allergic diseases.

In conclusion, we for the first time identified the critical residues in the CRH of hIL-13Rα1 and hIL-13Rα2 for binding to IL-13 and clarified the roles of the D1 domains of these receptors by the mutagenesis approach. These results provide the basis for a precise understanding of the interaction between IL-13 and its receptors.

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Characterization of the Interaction between Interleukin-13 and Interleukin-13 Receptors
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