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Structure of IL-13 Receptor: Analysis of Subunit Composition in Cancer and Immune Cells

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The structure of IL-13 receptor (IL-13R) is currently under investigation. Recently, two different human IL-13R chains, termed here IL-13Rα and -α' have been cloned. We have examined various cancer and normal cell lines for the presence of mRNA for IL-13Rα and α', as well as IL-4R p140 (termed β chain) and IL-2Rγ_c chains. In renal cell carcinoma, glioblastoma and ovarian carcinoma (IGROV-1) cell lines, both IL-13Rα and α' chains were expressed (type I IL-13R). In epidermoid, colon, ovarian adenocarcinoma (PA-1) and normal mouse fibroblast (COS7) cell lines, only IL-13Rα' was expressed (type II IL-13R). In hematopoietic TF-1 and EBV-immortalized normal B cell lines only IL-13Rα' but not α chain was expressed along with γ_c (type III or type IV IL-13R). IL-13Rα' chain was faintly detected in human T cells. All cells expressed the IL-4Rp140 β chain. These data provide a direct support for our model of IL-13R which consists of three different forms composed of different subunits.

Key Words: **Interleukin-13, Interleukin-4, Cytokine receptor, RT-PCR, Immunoprecipitation.**

Interleukin 13 (IL-13) is a pleiotropic immune regulatory cytokine and shares many characteristics with Interleukin 4 (IL-4) (1). Both cytokines are predominantly produced by activated Th2 cells and mast cells (2). Both cytokines mediate identical effects on many cell types including monocytes and B cells. However, unlike IL-4, IL-13 has no effects on T cells (3). The effect of these cytokines are mediated through cell surface receptors (4). The structure of IL-4R has been extensively studied. The first subunit of IL-4R was identified as a 140 kDa protein (5). Based on its similarities with the β chain of receptors for IL-4 family of cytokines (IL-3, IL-5 and GM-CSF) and the β chain of the IL-2R system, we have termed this chain as IL-4Rβ (6). In addition, since this protein also binds IL-13 in some cells, we have termed this chain IL-13/4 β_c (7). The second subunit of IL-4R system was shown to be the IL-2Rγ chain (termed γ_c) (8, 9) and recently we (6, 10) and other groups (11) have proposed that the 60-70 kDa protein form of IL-13R may also participate in mediating IL-4 effects and thus may constitute the third subunit of IL-4R system (termed here IL-4R α).

Unlike IL-4R system, the structure of IL-13R has not been fully delineated. Our previous observations indicate that the characteristics of IL-13R in human renal cell carcinoma (RCC) (10), colon carcinoma cells (12, 13), EB virus-immortalized B cells (14), brain tumor cells (15, 16), and other cell types are quite different from each other. Thus, we have hypothesized that the IL-13 receptor may be classified into three or four types (type I$\sim$IV) based on the characteristics of IL-4 and IL-13 binding (10, 14). Recently, two different human IL-13 binding proteins were cloned. The human IL-13 receptor which was cloned from human RCC cell line, Caki-1, is a $\sim$70 kDa protein and has a 50% homology to IL-5R α chain on DNA level (termed here, IL-13R α) (17). As in RCC cell lines, ^{125}I-IL-13 binding to its receptor in COS7 cells transfected with cloned IL-13R gene could not be inhibited by IL-4 (10). Furthermore, this 70 kDa protein did not require IL-4Rβ to be a high affinity IL-13 receptor in IL-13R cDNA transfected COS7 cells. On the other hand, Aman et al. (18) have cloned another type of IL-13 R from HTLV-1 infected MT-2 cell line, using the sequence of the murine IL-13R cDNA (termed here, IL-13Rα') (19). Unlike Caki-1 IL-13R, MT-2 IL-13R has no homology with any other human cytokine receptor. ^{125}I-IL-13 binding to its receptor could be inhibited by both IL-13 and IL-4, and IL-4Rβ is required for high affinity IL-13 binding. In spite of this knowledge, the exact structure of the various IL-13R remains unresolved.

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TABLE 1

PCR Primers for the Detection of Cytokine Receptor Transcripts Used in This Study

PCR product	GenBank Accession no.	Position of 5′ residue	Sequence (5′-3′)	Ref.
IL-13R α	X95302	52	AATGGCTTTCGTTTGCTTGG	11
		489	ACGCAATCCATATCCTGAAC	
IL-13R α'	U62858	957	GGAGAATACATCTTGTTTCATGG	12
		1104	GCGCTTCTTACCTATACTCATTTCTTGG	
IL-4R β	X52425	598	GACCTGGAGCAACCCGTATC	14
		913	CATAGCACAACAGGCAGACG	

To directly investigate the exact structure of IL-13R in cancer cells and compare it with immune cell IL-13R, we have utilized specific primers for IL-13Rα, α' and IL-4Rβ chains and antibody to γ_c to perform RT-PCR, dot blot and immunoprecipitation analyses on a variety of cell types. We demonstrated that the structural composition of IL-13R is dramatically different in different cell types and this fully corroborates our recently proposed model of hIL-13R.

MATERIALS AND METHODS

Cells. PM-, and ML-RCC cell lines were established in our laboratory. Ovarian cancer cell lines IGROV-1, PA-1, human colon carcinoma cell line HT-29, glioblastoma cell line U251, human epidermoid carcinoma A431, monkey fibroblast COS7, human T cell line H9, gibbon ape T cell line MLA-144 and human myeloid TF-1 cell lines were purchased from American Type Culture Collection (Rockville, MD). Epstein-Barr virus-immortalized B cell line (EBV-B) Tory was established in Dr. G. Tosato's laboratory (CBER, FDA). These cells were maintained in medium containing DMEM, EMEM or RPMI1640 respectively according to the cell types with gentamicin, 10 % FBS and 10 mM HEPES.

RT-PCR. Total RNA was isolated using TRIZOL reagent (GIBCO BRL, Gaithersburg, MD). For RT-PCR analysis, we used GeneAmp RNA PCR kit (Perkin-Elmer, Branchburg, NJ) with 0.1 μg of each specific primers (see Table 1) according to manufacture's instructions. 10 μl of various PCR products were run on 2% Nusieve 3:1 agarose (FMC, Rockland, ME) for UV analysis.

Hybridization of PCR products. The identity of the PCR products of IL-13R α, and α' chains were confirmed by dot blot hybridization of the amplified products with specific internal probes complementary to sequence +445 to +461 of IL-13Rα and +988 to +1005 of IL-13Rα' cDNA. The membranes were washed in 2 × SSC, 0.1% SDS at 37°C for 30 min and 0.2 × SSC and 0.1% SDS at 42°C for 15 min and exposed to X-ray film for 24 hrs in −70 °C.

Immunoprecipitation and Western blotting. This experiment was performed as described previously (12). Briefly, cells were lysed in cold lysing buffer and incubated with protein A sepharose beads conjugated anti-γ_c antibody (Santa Cruz Inc. Santa Cruz, CA) for 2 h. Immunoprecipitates were separated by 8% SDS-PAGE, transfered to PVDF membrane, probed with anti-γ_c antibody and visualized by chemiluminesant reagent (DuPont NEN, Boston, MA).

RESULTS AND DISCUSSION

IL-13Rα and α' chains are expressed in PM-, ML-RCC, U251 and IGROV-1 cell lines. To determine structure of IL-13R and to confirm our previous hypothesis (7), we performed RT-PCR analysis in various cell lines. As shown in Figure 1A, both IL-13R chains α, α' were expressed in PM-, ML-RCC, U251 and IGROV-1 cell lines (lane 1-4, row a-b). Our RT-PCR findings were confirmed by using internal probe for IL-13R α or α' chain by dot blot hybridization. Again, both IL-13R α and α' were detected in these cell lines (Figure 1B, lane

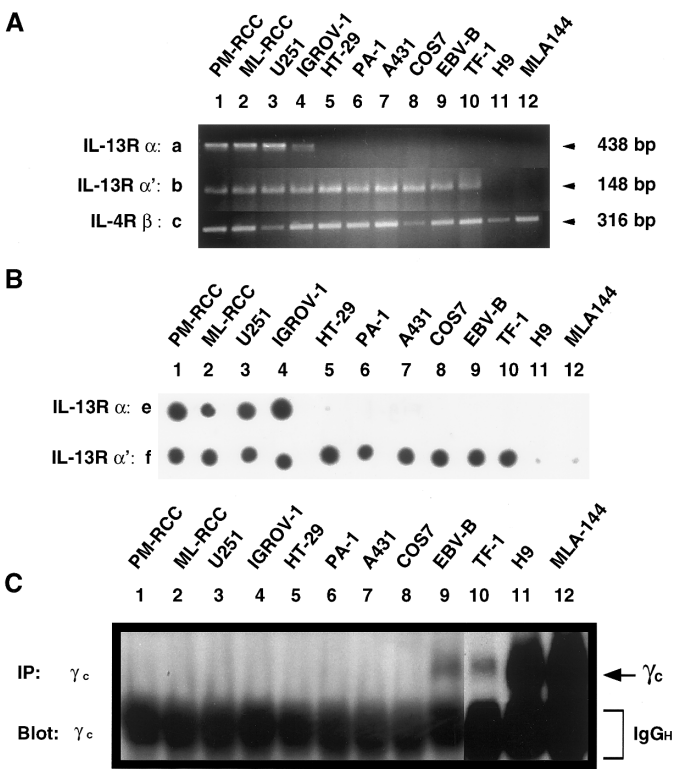

FIG. 1. Detection of mRNA for IL-13 receptors by RT-PCR. The PCR reaction mixture containing specific primers were amplified as follows: after an initial denaturation step at 95°C for 2 min; 35 cycles at 95°C 15 sec; 60°C 30 sec, respectively (A). Dot blot hybridization for the amplified products probed with specific internal probes (IL-13Rα probe: ACCACAAGGAATTCCAG; IL-13Rα' probe: GTTCTTCCTGATACTTTG) (B). Detection of γ_c protein by immunoprecipitation and western blotting. Cells were lysed in cold lysing buffer for 30 min. γ_c protein was immunoprecipitated from the lysates, transfered to PVDF membrane and probed with anti-γ_c antibody (arrow) (C).

1-4). We also examined the expression of IL-4Rβ and IL-2R γ chain (γ_c). In all cell types, the IL-4Rβ chain was expressed (Fig. 1A, row c). Because of the high background with reported primers of γ_c (18, 19), we immunoprecipitated γ_c chain and performed western blot analysis for an ultimate test of γ_c expression. As shown in Fig. 1C, γ_c was not expressed by any of the tumor or COS7 fibroblast cell lines. On the other hand, hematopoietic cell lines (e.g. EBV-B, TF-1, H9 and MLA-144) significantly expressed γ_c as expected.

Previously, we have reported that a large number of intermediate to high affinity IL-13R are expressed in these RCC (10), brain tumor (7, 15) and IGROV-1 (21) cell lines and binding of ^{125}I-IL-13 to its receptor was inhibited by IL-13 but not by IL-4. Upon ^{125}I-IL-13 crosslinking only a broad ~70 kDa band was observed, but crosslinking to IL-4R p140 protein was not observed. In addition, anti-γ_c anti-body failed to immunoprecipitate any protein in RCC (10), or U251 and IGROV-1 cell lines (22). Taken together, our data indicate that type I IL-13R consist of homo-dimer or heterodimer of ~70 kDa IL-13R chains and it does not involve IL-4Rβ or γ_c chains.

Only IL-13 α' chain is expressed in COS7, A431, PA-1 and HT-29 cell lines. We next investigated the expression of IL-13R chains by RT-PCR in other non-hematopoietic (HT-29, PA-1, A431 and COS7) cell lines which were hypothesized to express type II IL-13R (7). IL-13R α' chain was expressed in all cell types examined (Fig. 1A, lane 5-8, row b). However, no mRNA for IL-13R α chain was detected (row a). We also confirmed these findings by using an internal probe for dot blot hybridization. Similar to UV analysis, IL-13R α' was strongly expressed in these cell lines but faint dots of IL-13α were also observed (Fig. 1B, lane 5-6). Low level IL-13Rα chain expression has been reported in TF-1, A431, U937 and IM9 cell lines (17). Thus, it is possible that either a low level IL-13α chain mRNA expression, detectable by a sensitive technique, was observed in these cells or this was a technical artifact due to non-specific binding of template cDNA. IL-4Rβ mRNA was strongly expressed in all of these four cell line examined. However, γ_c was not detected in any of these cell lines either by immunoprecipitation (Fig. 1C) or Northern analysis (12, 22).

In A431 (7), COS7 (7), PA-1 (22) and HT-29 cells (12), receptors for IL-13 were expressed but ^{125}I-IL-13 binding was limited and both IL-13 and IL-4 cross-neutralized this binding. Interestingly, IL-4 was a better competitor for IL-13 binding on these cells indicating that IL-4 has higher affinity for IL-13R α' when it is expressed along with IL-4R β chain. Moreover, ^{125}I-IL-4 binding to its receptor was also inhibited by both IL-4 and IL-13. These data suggest that in type II receptor, IL-13R α' and IL-4Rβ may form a heterodimer which is utilized in both IL-4R and IL-13R systems.

TABLE 2

IL-13Rα, IL-13Rα', IL-4Rβ, γ_c Chains Expression in Various Cell Lines

Cells	IL-13R α	IL-13R α'	IL-4Rβ	IL-2R γ (γ_c)[1]
PM-RCC	++[2]	++	++	−
ML-RCC	++	++	++	−
WS-RCC	+	++	++	−
HL-RCC	++	++	++	−
Caki-1 (RCC)	+	++	++	−
RC-2 (RCC)	+	++	++	−
U251	++	++	++	−
A172 (brain)	++	++	++	−
IGROV-1	++	++	++	−
PA-1	±	++	++	−
OVCA429 (ovarian)	−	++	++	−
HT-29	±	++	++	−
UMMEL (melanoma)	+	++	++	−
A431	−	++	++	−
COS7	−	++	++	−
TF-1	−	++	++	+
Tory (EBV-B)	−	++	++	+
Raji (Burkitt)	−	+	++	+
H9	−	±	++	+
MLA-144	−	±	++	+

1: γ_c protein expression was detected by immunoprecipitation and western bloting.

2: ++; detected by UV analysis and dot blot hybridization, +; detected by dot blot hybridization only, ±; detected by dot blot hybridization faintly, −; not detected by both experiments.

IL-13R α' chain is expressed in TF-1 and EBV- B cells, but not H9 and MLA144 cell lines. Since IL-13 has various effects on TF-1 (1) and B lymphocytes (23), we examined which type of IL-13R chain is expressed in these cells. In TF-1 cell line only IL-13 α' but not α chain was expressed (Fig. 1A, lane 10, row a and b). In addition, IL-4Rβ and γ_c were also detected (Fig. 1A, lane 10, row c and Fig. 1C, lane 10). In these cells IL-13 cross-links to IL-4Rβ and ~70 kDa protein (7). Both IL-4 and IL-13 bind to these cells and both interleukins compete for the binding of both interleukins (type III IL-13R) (7). In EBV-immortalized B cells, IL-13 α' chain was expressed along with IL-4Rβ and γ_c chains (Fig. 1A and Fig. 1C, lane 9) but α chain was not detected. In these cells IL-13 binding was limited (Murata and Puri, unpublished data) and thus IL-13 crosslinking could not be performed while IL-4 binding was prominent (14). However, contrary to what was observed in every cell line examined, IL-13 only minimally inhibited IL-4 binding to these cells (type IV IL-13R). Taken together our data suggest that at least IL-13Rα' and IL-4Rβ chains are utilized in IL-13R system in hematopoietic cells and γ_c may influence IL-13 binding. Thus, in hematopoietic cells, IL-13R can be classified as type III or type IV receptors. In human and gibbon ape T cell lines, H9 and MLA144, although IL-4Rβ and γ_c chains were expressed (Fig. 1A, row c and

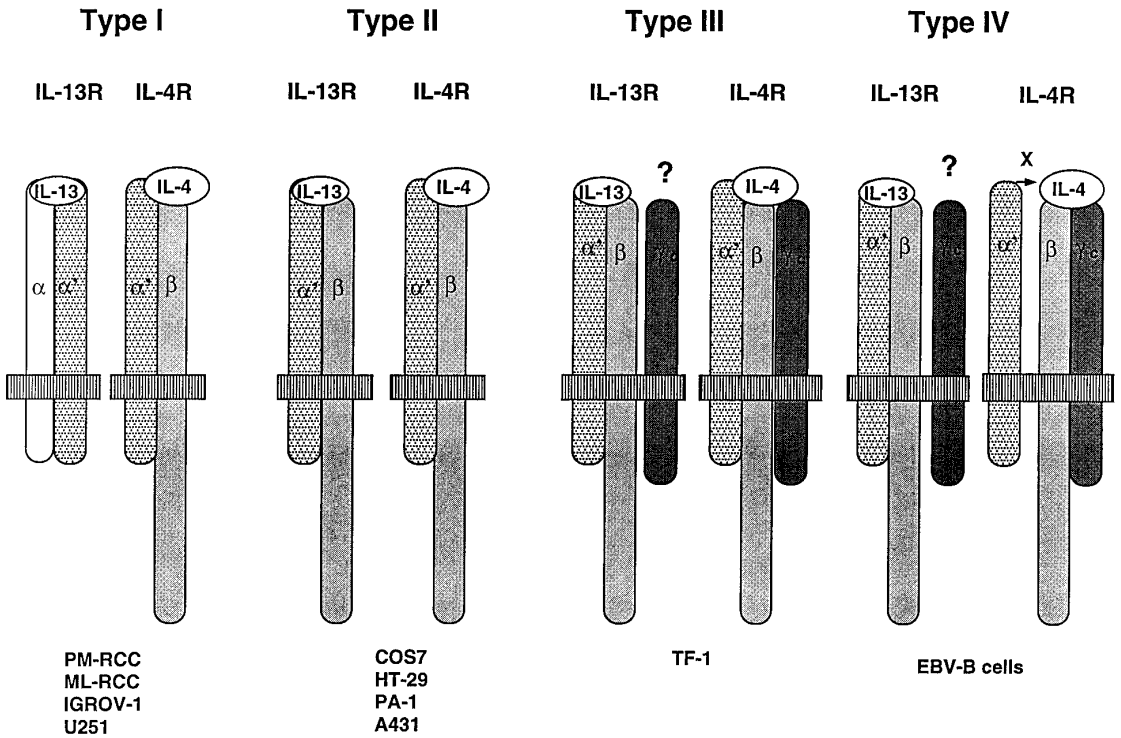

FIG. 2. IL-13 receptor structure model. Type I IL-13R are expressed on RCC, U251 and IGROV-1 cells and consist of two chains termed IL-13R α and IL-13R α'. Type I IL-4R consist of IL-13Rα' plus IL-4Rβ. Because IL-13Rα and α' form an isodimer, they have higher affinity for IL-13 than IL-4 and thus IL-4 can not compete for the binding of IL-13 on these cells. Type II IL-13R are expressed on other non-hematopoietic cells and consist of only IL-13R α' and IL-4Rβ while IL-4R structure is similar as in type I receptor. Thus, IL-13 binding to its complex can be inhibited by both cytokines and IL-13R binding is limited. Type III IL-13R are expressed in hematopoietic such as TF-1 cells and may consist of three chains, IL-13R α', β and γ_c (IL-2R γ) although γ_c does not appear to bind IL-13 (10). Type IV receptor may also include all three chains as type III IL-13 receptor, but it may include an additional chain or show different characteristics because IL-13 does not compete for the binding of IL-4.

Fig. 1C, lane 11 and 12), both IL-13R chains were not detected by UV analysis (Fig. 1A, lane 11-12). However, IL-13R α' chain was very faintly detected in both cell lines by dot blot analysis (Fig. 1B lane 11-12, row f). IL-13 did not bind to these cells nor did IL-13 compete for the IL-4 binding on these cell lines (10).

IL-13R model. The results of the expression of various chains of IL-13R are summarized in Table 2. From these experiments, we affirm a model for IL-13R (7) which was hypothetically conceived on the basis of our binding, susceptibility to cytotoxin and crosslinking experiments. Our data suggest that IL-13R may be classified into four forms according to the combination of subunits utilized (Figure 2). In this figure, we have also included for purposes of comparison, the model for IL-4R structure previously reported (12). IL-13R type I which is expressed on RCC, U251 and IGROV-1 cells consist of two chains termed IL-13R α and IL-13R α'. We hypothesize that IL-13Rα alone binds only IL-13 with high affinity but not IL-4. On the other hand, IL-13 α' binds both IL-13 and IL-4 with low affinity. These cells also express IL-4Rβ but IL-13 does not bind to this protein. Thus, in type I IL-13R, because IL-13 may form an iso-dimer, it would have a higher affinity for IL-13R α than for the IL-4Rβ and IL-13 α' complex and the numbers of the former are much higher than the later. Because of the high affinity of IL-13 binding to IL-13Rα and α', IL-4 could not inhibit IL-13 binding to its receptors (10, 17). On the other hand, IL-4R may consist of β and IL-13R α', so that IL-4 binding to its receptor can be inhibited by both cytokines (10, 13, 17). In type II IL-13R which is expressed in other non-hematopoietic cells, IL-13R consist of only IL-13Rα' and IL-4Rβ. Thus, IL-13 binding to this complex can be inhibited by both cytokines and IL-13R binding affinity is lower than type I receptor (12, 19). Type III IL-13 receptor which is expressed in the TF-1 myeloid cell line may consist of three chains, α', β and γ_c. Previously, it has been proposed that γ_c is not a component of IL-13R (10). However, it has been shown that γ_c decreased the IL-13R binding affinity in α, β and γ_c transfected COS7 cells (17). We also found that ^{125}I-IL-13 binding to its receptor was significantly decreased in γ_c transfected RCC and COS7 cells (25, 7). For this reason, IL-13 binding to the γ_c positive TF-1 cells may be naturally limited. Consistent with this hypothesis,

only ~100 IL-13 binding sites/cell were observed in these cells (10). Moreover, IL-13R signaling in hematopoietic cells is different from type I or II IL-13R (12, 14, 22, 24). These data suggest that γ_c affects IL-13R binding affinity and its signaling mechanism.

Finally, type IV receptor also include IL-13R α', IL-4Rβ and γ_c. But unlike type III receptor, IL-13 does not compete for the binding of IL-4 (10). These data suggest that in these cells IL-13R α' expression is so much lower than IL-4Rβ and γ_c that IL-4R practically consist of IL-4Rβ and γ_c. Because of this arrangement, IL-13 does not compete for the binding of IL-4. Alternatively, additional component(s) may be present in type IV IL-13R which does not allow IL-13 binding to IL-4R. It is still unclear whether γ_c is a part of the IL-13R complex. Availability of antibodies to both chains of IL-13R will help in further deciphering the structure of IL-13R.

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