Molecular Cloning and Expression of the Human Interleukin 5 Receptor

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Summary

Human interleukin 5 (IL-5) plays an important role in proliferation and differentiation of human eosinophils. We report the isolation of cDNA clones from cDNA libraries of human eosinophils by using murine IL-5 receptor α chain cDNA as a probe. Analysis of the predicted amino acid sequence indicated that the human IL-5 receptor has ~70% amino acid sequence homology with the murine IL-5 receptor and retains features common to the cytokine receptor superfamily. One cDNA clone encodes a glycoprotein of 420 amino acids (M_r 47,670) with an NH_2-terminal hydrophobic region (20 amino acids), a glycosylated extracellular domain (324 amino acids), a transmembrane domain (21 amino acids), and a cytoplasmic domain (55 amino acids). Another cDNA encodes only the extracellular domain of this receptor molecule. Other cDNA clones encode molecules having diversified cytoplasmic domains. COS7 cells transfected with the cDNA expressed a ~60-kD protein and bound IL-5 with a single class of affinity (K_d = 250–590 pM). The K_d values were similar to that observed in normal human eosinophils. In contrast to the murine 60-kD α chain, which binds IL-5 with low affinity (K_d = ~10 nM), the human α chain homologue can bind IL-5 with much higher affinity by itself. RNA blot analysis of human cells demonstrated two transcripts (~5.3 and 1.4 kb). Both of them were expressed in normal human eosinophils and in erythroleukemic cell line TF-1, which responds to IL-5. The human IL-5 receptor characterized in this paper is essential for signal transduction, because expression of this molecule in murine IL-3-dependent cell line FDC-P1 allowed these cells to proliferate in response to IL-5.

IL-5 is a glycoprotein secreted by T cells and mast cells (1, 2), originally identified by its ability to stimulate proliferation and differentiation in vitro of murine activated B cells and $CD5^+$ B cells, and to induce proliferation and differentiation of eosinophils from bone marrow proliferation (3–9). In the human system, IL-5 also promotes proliferation and differentiation of eosinophils from bone marrow progenitors (10–12). There are contradictory reports, however, describing the role of IL-5 on human B cells. It was reported that it induces maturation of human peripheral B cells stimulated with *Staphylococcus aureus* Cowan I into IgM-secreting cells (13), and was also reported to be an IgA-enhancing factor (14). However, it was also reported that human IL-5 (hIL-5)[1] does not show any activity on human B cells in a conventional B cell assay (15).

To clarify the role of IL-5 in the human immune system and hematopoiesis, we and others attempted to explore the characteristics of hIL-5R (16–19). It was reported that human eosinophils in peripheral blood and eosinophilic sublines from promyelocytic leukemia (HL-60) cells express a single class of high affinity binding sites for IL-5 with K_ds of 170–330 and 10–50 pM, respectively (16, 18). By cross-linking experiments, we detected a 55–60-kD protein on normal eosinophils (18). Others reported 60- and 120–130-kD proteins in eosinophilic sublines from HL-60 and in an erythroleukemic cell line, TF-1 (16, 19).

We have shown from the series of binding and cross-linking studies that murine IL-5 (mIL-5) binds to mIL-5R with both high (K_d = ~150 pM) and low affinity (K_d = ~30 nM) and that at least two polypeptide chains comprise the functional mIL-5R (20). We recently isolated cDNA clones encoding the mIL-5R α chain (60 kD) that binds IL-5 with low affinity (21). It is very suggestive that there is a significant homology between h- and mIL-5Rs, because mIL-5 was shown to bind hIL-5R as efficiently as hIL-5, and they are ~70% homologous in terms of amino acid sequences (13, 14). Then,

[1] *Abbreviations used in this paper:* FBN III, fibronectin type III; GM-CSF, granulocyte/macrophage colony-stimulating factor; h, human; m, murine; ORF, open reading frame; UTR, untranslated region.

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we tried to fish out a cDNA clone for hIL-5R by cross-species hybridization using mIL-5R α chain cDNA as a probe.

In this report, we describe the characterization of hIL-5R by isolating the cDNA clones for it. rhIL-5R expressed on COS7 cells showed similar binding properties and biochemical characteristics to those of the native receptor on human eosinophils. Analysis of the sequence of hIL-5R demonstrates that the hIL-5R is also a member of a cytokine receptor superfamily and contains three fibronectin type III (FBN III) modules (22–26).

Materials and Methods

Reagents and Cell Lines. rmIL-5 and ^{35}S-methionine labeled mIL-5 were prepared according to procedures described previously (27). rhIL-5 was purified using anti-IL-5 mAb NC17 (28) from the culture supernatant of *Spodoptera frugiperda* (Sf21) cells transfected with hIL-5 cDNA in baculovirus expression vector (unpublished results). rhIL-5 was labeled with ^{125}I using diiode-Bolton-Hunter reagent (4,000 Ci/mmol; DuPont-New England Nuclear, Boston, MA) as described (29). rhIL-2 was a kind gift from Takeda Central Research Institute (Osaka, Japan). rhIL-3, rhIL-4, rhIL-6, recombinant human granulocyte colony-stimulating factor (G-CSF), and human recombinant granulocyte/macrophage colony-stimulating factor (GM-CSF) were from Japan Roche Research Institute (Kamakura, Japan), Ono Pharmaceutical Co., Ltd. (Osaka, Japan), Dr. T. Hirano (Osaka University, Osaka, Japan), Chugai Pharmaceutical Co., Ltd. (Tokyo, Japan), and Sandoz Pharmaceutical Co., Ltd. (Basel, Switzerland), respectively. Human erythroleukemic cell line TF-1 (30) was a generous gift from Dr. T. Kitamura (DNAX Research Institute of Molecular and Cellular Biology, Palo Alto, CA). Human eosinophilic leukemia cell line EoL-3 and human adult T cell leukemia cell line ATL-2 were kindly provided from Drs. M. Hirashima and N. Asou (Kumamoto University Medical School, Kumamoto, Japan), respectively. All human cell lines were maintained in RPMI 1640 containing 10% FCS, 5×10^{-5} M 2-ME, 100 U/ml penicillin, and 100 μg/ml streptomycin. A mIL-3-dependent cell line, FDC-P1, was maintained in the same medium in the presence of 10 U/ml of mIL-3.

RNA Isolation and Construction of cDNA Library. Human eosinophils were isolated from peripheral blood of healthy volunteers or from a patient with idiopathic eosinophilic syndrome. In brief, eosinophils were purified from the 0.5% polyvinylpyrrolidone-sedimented erythrocyte/leukocyte fraction of peripheral blood through discontinuous layers (density, 1.080–1.100) of Percoll (Pharmacia LKB Biotechnology, Uppsala, Sweden) as described (18). Total RNAs were prepared from eosinophils by the guanidium isothiocyanate/CsTFA method (31), and poly(A)$^+$ RNA was selected by oligo(dT)-cellulose column chromatography. cDNA library from eosinophils from healthy volunteers was constructed in the vector with chicken β-actin promoter, pAGS-3 (32). Double-stranded cDNA was synthesized using random primer according to the method by Gubler and Hoffman (33) using 5 μg of poly(A)$^+$ RNA with the manufacturer's kit (Bethesda Research Laboratories, Bethesda, MD). The blunt-end cDNA was modified with BstXI linkers, size fractionated by 5–20% potassium acetate gradient centrifugation, and cDNA $\sim$>1.0 kb was cloned into pAGS-3 as described (34). Transformation of *Escherichia coli* strain MC1061 was carried out by electroporation (2.5 kV, 25 μF, 200 Ω) in 0.2-cm cuvettes using Gene Pulser apparatus (Bio-Rad Laboratories, Richmond, CA) (35). Approximately 10^6 independent colonies were obtained by transforming MC1061 with pAGS-3 cDNA library. cDNA library from a patient with eosinophilia was constructed in the λgt10 vector using 5 μg of poly(A)$^+$ RNA. 1.6×10^6 independent plaques were obtained.

Screening of cDNA Libraries. Screening of the pAGS-3 library was performed using a ^{32}P-labeled 1.2-kb HindIII-PstI fragment of mIL-5R α chain cDNA clone pIL-5R.8 (21) as a hybridization probe under low-stringency conditions. Colonies were blotted on nitrocellulose membranes (Millipore, Bedford, MA) and hybridization was performed at 65°C with 10× Denhart's solution, 6× SSC, 0.5% SDS, 100 μg/ml of denatured salmon sperm DNA for 20 h, and washed with 1× SSC, 0.1% SDS at 45°C (36). Subsequently, a 1.0-kb XhoI fragment of positive clone (ph5R.1, see Results) was used as a probe to isolate other clones from the λgt10 library. Plaques were transferred to nylon membranes (Colony/Plaque Screen; DuPont-New England Nuclear) and hybridization was performed according to the manufacturer's recommendation and washed with 2× SSC, 1% SDS at 65°C. All hybridization probes were labeled with α-[^{32}P]dCTP by random primer labeling method using a kit from Pharmacia LKB Biotechnology.

DNA Sequencing. The cDNA inserts of clones, ph5R.1, λh5R.12, λh5R.27, and λh5R.25, were sequenced by the dideoxy chain termination method (37) using the modified T7 polymerase (Sequenase; U.S. Biochemical Corp., Cleveland, OH) after being subcloned into pBluescript KS(−) vector (Stratagene, La Jolla, CA).

Expression of the hIL-5R cDNA. A transient expression system with COS7 cells was used together with the mammalian expression vector pCAGGS (38) (generously provided by Dr. J.-I. Miyazaki, University of Tokyo, Tokyo, Japan), which is a derivative of pAGS-3 (32). An EcoRI fragment of either λh5R.12 or λh5R.27 clones was inserted into a unique EcoRI site of pCAGGS, resulting in pCAGGS-h5R.12 or pCAGGS-h5R.27. Each plasmid DNA was transfected into COS7 cells by electroporation as described (39). COS7 transfectants were cultured in DME containing 10% FCS, 100 U/ml penicillin, and 100 μg/ml streptomycin. After a 3-d culture, cells were harvested with the treatment of PBS containing 0.5 mM EDTA and subjected to further analysis.

For FDC-P1 cells, pSV2-neo and pCAGGS-5R.12 (1 and 20 μg, respectively) were cotransfected by electroporation at 1.5 kV and 25 μF. Stable transfectants were selected with 400 μg/ml of G418 (geneticin; Sigma Chemical Co., St. Louis, MO).

Binding Assay and Scatchard Analysis. For competition experiments, COS7 transfectants were harvested and suspended at 4×10^5 cells per 100 μl of the binding medium (RPMI 1640, 25 mM Hepes, pH 7.2, 0.1% BSA), and incubated with 500 pM ^{125}I-hIL-5 (2.0×10^{13} cpm/mmol) in the presence of >1,000-fold molar excess of unlabeled various cytokines at 4°C for 1 h (18, 29). For binding assay, COS7 transfectants were resuspended at $5–10 \times 10^4$ per 100 μl of the binding medium, and incubated with increasing concentrations of ^{125}I-hIL-5 or ^{35}S-labeled mIL-5 (2.3×10^{15} cpm/mmol) at 4°C for 1 h. Specific binding was defined as the difference between total binding and nonspecific binding obtained in the presence of a 250-fold molar excess of unlabeled IL-5. The K_d was calculated by Scatchard plot analysis (40) of the binding data.

Chemical Cross-linking. COS7 transfectants were cultured for 3 d and resuspended at 2.5×10^6 cells in 1 ml of binding medium. Cells were incubated with either 5.5 nM ^{35}S-labeled mIL-5 or 1 nM ^{125}I-hIL-5 at 4°C for 1 h in the absence or presence of a 250-fold molar excess of unlabeled IL-5 and subsequently cross-linked with 1 mM BS^3 (Bis[sulfosuccinimidyl] suberate) (Pierce Chemical Co., Rockford, IL) at 4°C for 30 min (16). Cells were then washed and lysed with lysis buffer containing 1% Triton X-100 in the presence of protease inhibitors (2 mM EGTA, 2 mM EDTA,

2 mM PMSF, 10 μM pepstatin, 10 μM leupeptin, 2 mM *O*-phenanthroline, and aprotinin at 200 KIU/ml) as described (29). The detergent extraction mixture was subjected to SDS-PAGE with 7.5% polyacrylamide under nonreducing conditions, and then analyzed by a Bio-Analyzer (model 100; Fuji Photo Film, Tokyo, Japan).

RNA Blot Analysis. Poly(A)$^+$ RNA (6 μg) prepared from human eosinophils in peripheral blood, TF-1, EoL-3, ATL-2, Raji, and U-937 were subjected to electrophoresis through 1% agarose gel containing 2.2 M formaldehyde as described (36), and transferred to a nylon membrane (Gene Screen; DuPont-New England Nuclear). Hybridization was carried out according to manufacturer's recommendation using a ^{32}P-labeled full-length EcoRI cDNA insert of λh5R.12 as a probe.

Proliferation Assay. FDC-P1 cells and FDC-P1 transfectants were inoculated in a 96-well microtiter plate at a concentration of 10^4/0.2 ml/well with various concentrations of hIL-5. They were pulse labeled with [^{3}H]thymidine (0.2 μCi per well) during the last 6 h of a 48-h culture period, and incorporated [^{3}H]thymidine was measured by a liquid scintillation counter.

Results

Isolation and Characterization of hIL-5R cDNA Clones. Two cDNA libraries of human eosinophils prepared from normal volunteers and from a patient with eosinophilia were used to isolate hIL-5R cDNA clones. At first, screening was performed by cross-species hybridization using mIL-5R α chain cDNA clone as a probe at low-stringent conditions. One clone, ph5R.1, that has a 931-bp insert was obtained from pAGS-3 library of normal human eosinophils (Fig. 1 *A*). This clone did not cover the entire coding region of hIL-5R. Then, three clones (λh5R.12, λh5R.27, and λh5R.25) were isolated from λgt10 library of eosinophils from an eosinophilia patient using ph5R.1 as a probe by plaque hybridization. cDNA inserts from λ5hR.12, λh5R.27, and λh5R.25 were found to be 2,006, 2,024, and 1,405 bp, respectively (Fig. 1 *A*). Both λh5R.12 and λh5R.27 have the open reading frame (ORF) that encodes a protein equivalent to mIL-5R α chain in molecular size. Because the nucleotide sequence of the ORF of λh5R.12 clone (1,260 bp) was the best match for the mIL-5R α chain (21) in terms of constitution, this clone was analyzed most in this paper. The nucleotide sequence of hIL-5R from λh5R.12 is shown in Fig. 1 *B*. A 3′ untranslated region (3′ UTR) of 685 nucleotides extends without the polyadenylation signal AATAAA. The ORF of λh5R.12 encodes a mature protein of 400 amino acids with a calculated molecular weight of 45,556 synthesized with a 20-amino acid signal sequence. The site of signal sequence was in agreement with the sites based on the predictive system of von Heijne (41). The mature hIL-5R protein of 400 amino acids can be divided into several regions according to a prediction by the Doolittle hydropathy plot (42); the extracellular domain was comprised of 324 amino acids, transmembrane domain was comprised of 21 amino acids, and cytoplasmic domain was comprised of 55 amino acids. The extracellular domain contains two pairs of cysteines and a "WSxWS" box, as seen in a cytokine receptor superfamily (22–24). Six N-linked glycosylation sites were seen.

There was a 57-bp insert in the cytoplasmic domain of the ph5R.1 clone, generating a 19-amino acid insertion in this domain (Fig. 1 *C*). The 3′ UTR of ph5R.1 has 511 nucleotides, which is the same as the 3′ UTR of λh5R.12, except for one base: the 1,714th T in λh5R.12 is changed into C in ph5R.1.

The nucleotide sequence of the λh5R.27 clone was identical to that of λh5R.12 in extracellular domain and transmembrane domain, except a point mutation at position 385 that changed G to A resulting in a change of Val to Ile. λh5R.27 was distinguished from λh5R.12 by having a totally different sequence of 737 bp, which starts from nucleotide 1185 (Fig. 1 *D*). Consequently, the cytoplasmic domain of λh5R.27 is 24 amino acids shorter than that of λh5R.12.

λh5R.25 clone encodes an hIL-5R that has neither a transmembrane domain nor a cytoplasmic domain. The site indicated by the open arrowhead (Fig. 1, *A* and *B*) is the predicted site of splicing that is followed by an unique sequence resulting in the addition of two amino acids before termination (Fig. 1 *E*). This predicted site of splicing is very similar to that observed in pIL-5R.2 encoding soluble mIL-5R (21). The sequence upstream from this arrow is identical to that of λh5R.27. Thus, λh5R.25 may code for a soluble form of hIL-5R. This predicted mature protein is 313 amino acids in length with a calculated M_r of 35,607.

Three Units of a FBN III Module in the Extracellular Domain and the Proline Cluster Region in the Cytoplasmic Domain. Alignment of the predicted amino acid sequence of the hIL-5R (λh5R.12) with that of the mIL-5R α chain (21) exhibited a high degree of homology (69% homology) throughout the length of the molecules (Fig. 2). In addition, all seven of the extracellular and four of the transmembrane and intracellular cysteine residues are conserved in the h- and mIL-5R molecules. Four of the six potential *N*-glycosylation sites are conserved in the mIL-5R α chain (Asn35, Asn131, Asn216, and Asn244) (data not shown). A part of the extracellular domain of hIL-5R (126–344 amino acid residues) contains two pairs of cysteine residues and the "WSxWS" box (Fig. 2 *A*). The hIL-5R, therefore, belongs to a cytokine receptor superfamily (22–24), as in the mIL-5R α chain (21). We examined the extracellular domain in view of sequence and structural pattern matching as others did (25, 26, 43–46). We detected FBN III motifs not only in domains that belong to the cytokine receptor superfamily, but also in the NH_2-terminal domain (21–125 amino acid residues) (Fig. 2 *A*). There were conserved motifs such as a pair of proline residues that precede an amphiphilic β strand and a conserved tryptophan. In the cytoplasmic domain, the proline cluster region (21) (from Leu371 to Asp384) is well conserved in the h- and mIL-5R molecules (Fig. 2 *B*).

Binding and Biochemical Characteristic of hIL-5Rs. We examined the binding specificity of the protein coded by the cDNA clones, by expressing the cDNA in COS7 cells. We inserted λh5R.12 and λh5R.27 cDNA fragments into the expression vector pCAGGS (38), resulting in pCAGGS-h5R.12 and pCAGGS-h5R.27, respectively. These two recombinant clones were transfected into COS7 cells. The binding of ^{125}I-hIL-5 to the COS7 cells transfected with pCAGGS-h5R.12 was specific for IL-5. Other human cytokines such as IL-2,

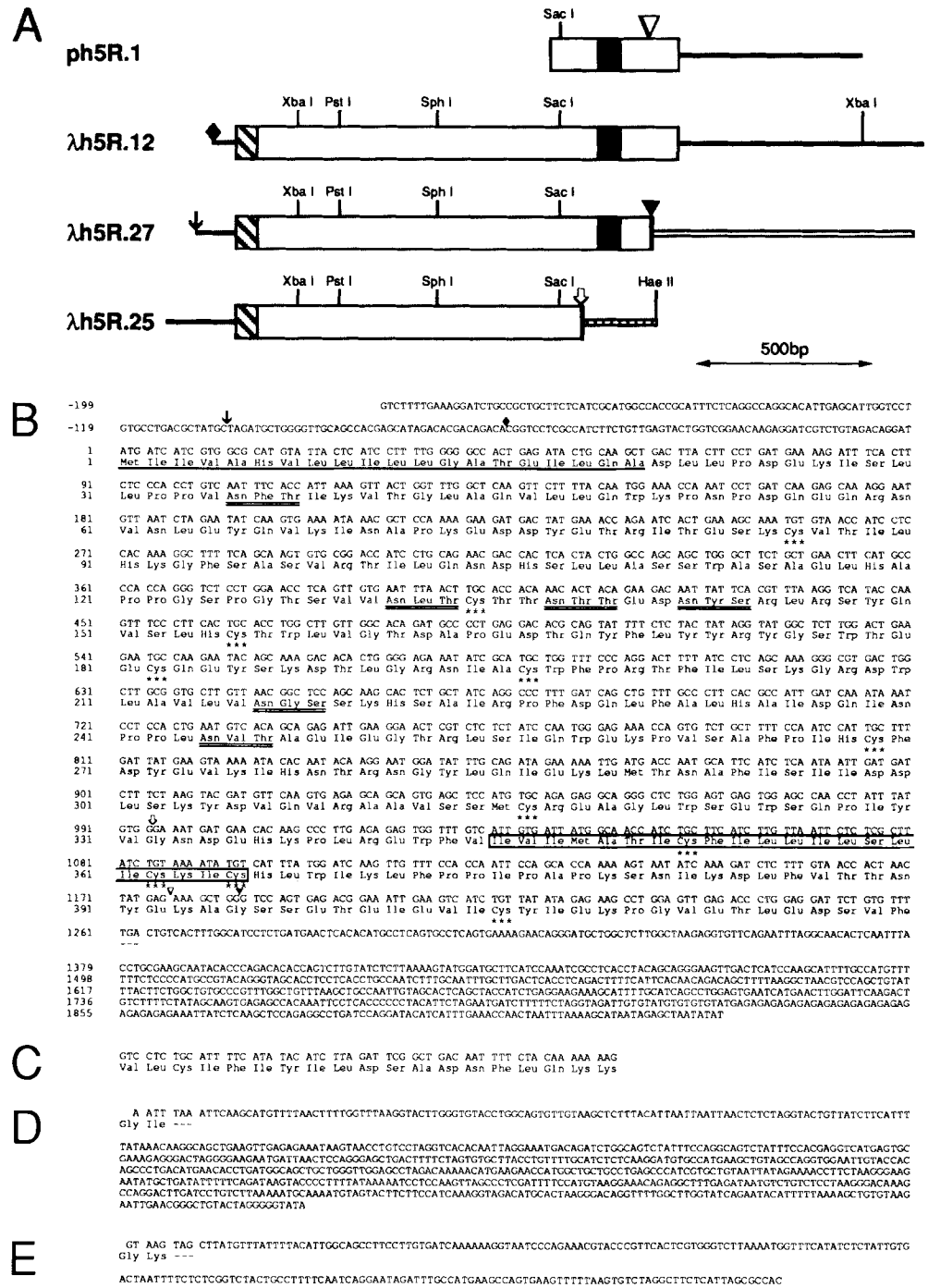

Figure 1. Structure of the hIL-5R cDNAs. (*A*) Schematic representation and restriction maps of hIL-5R cDNAs. The coding regions are boxed. The hatched boxes represent the signal sequence; solid boxes represent the transmembrane domain. The open triangle indicates regions where there is an insertion in the cytoplasmic domain of ph5R.1, taking the structure of λh5R.12 as a standard. The thinner open box at the 3′ end of λh5R.27 represents a different nucleotide sequence from that of λh5R.12. The striped thinner box in λh5R.25 indicates a unique 3′ UTR among four clones. The filled diamond and the plain arrow represent the 5′ terminal of λh5R.12 and λh5R.27, respectively. (*B*) The nucleotide sequence and deduced amino acid sequence of the hIL-5R cDNA. Nucleotides and deduced amino acids are numbered on the left. The nucleotide sequence of 5′ UTR is from λh5R.25. λh5R.27 and λh5R.12 start from the thin arrow and the filled diamond, respectively. The first underlined 20 amino acid residues represent the signal sequence. The transmembrane domain is marked by a filled box. The potential N-linked glycosylation sites are shown by double underlines. Cysteine residues are marked by asterisks. (*C*) The nucleotide sequence and deduced amino acid sequence of the insertion present in ph5R.1. Insertion occurs after nucleotide 1176, designated by the open triangle in *A* and *B*. (*D*) The nucleotide sequence and deduced amino acid sequence in λh5R.27, which occur after the filled triangle in *A* and *B*. (*E*) The nucleotide sequence and deduced amino acid sequence in λh5R.25, which occur after the open arrow in *A* and *B*. These sequence data are available from EMBL/GenBank/DDBJ under accession numbers X61176 (clone λh5R.12), X61177 (clone λh5R.27), X61178 (clone ph5R.1), and X62156 (clone λh5R.25).

IL-3, IL-4, IL-6, G-CSF, and GM-CSF did not compete with IL-5, as shown in Fig. 3. The results of binding assays for ^{125}I-hIL-5 to these COS7 transfectants are shown in Fig. 4, *A* and *B*. Transfectants with pCAGGS-h5R.12 or pCAGGS-h5R.27 expressed a single affinity class of IL-5R with an apparent K_d of 590 and 410 pM, respectively. Because of the low specific radioactivity of this ligand, we could not measure the specific binding under ~100 pM. Since we found that the ^{35}S-labeled mIL-5 binds to human eosinophils and this binding can compete with either cold mIL-5 or hIL-5 in a similar manner (18) and ^{35}S-labeled mIL-5 had much higher specific radioactivity, we applied ^{35}S-labeled mIL-5 in this binding assay. The binding assays for ^{35}S-labeled mIL-5 to the COS7 transfectants with these cDNA clones are shown in Fig. 4, *C* and *D*. Transfectants with pCAGGS-h5R.12 or pCAGGS-h5R.27 expressed a single affinity class of IL-5R with an apparent K_d of 355 and 250 pM, respectively. These K_d values are quite similar to those of normal human eo-

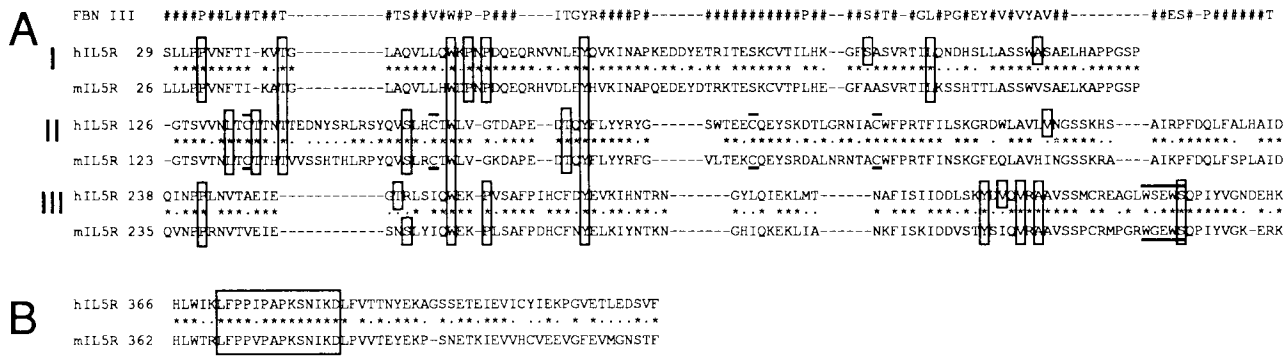

Figure 2. Alignment of human and murine IL-5R amino acid sequence. (*A*) Alignment of human and murine IL-5R extracellular region with a consensus sequence of a FBN III module. The consensus sequence of FBN III was distilled by Hibi et al. (46). Nonconserved amino acid residues and gaps are indicated by # and −, respectively. The residues identical with those of the FBN III module are boxed. Four conserved cysteine residues and "WSxWS" box, commonly seen in the cytokine receptor superfamily (22–24), are indicated by thick bars. (*B*) Comparison of the amino acid sequences from the cytoplasmic domain of IL-5R between human and mouse. The conserved proline cluster region is boxed. Numbers at the left indicate the amino acid number starting from the first methionine. Identical and related amino acids between the hIL-5R and mIL-5R α chain (21) are shown by asterisks and dots, respectively.

sinophils, which we reported in our previous paper (K_d = 170–330 pM) (18).

The molecular mass of the hIL-5R on transfected COS7 cells was estimated by chemical cross-linking experiments. When ^{35}S-labeled mIL-5 was cross-linked with the COS7 cells transfected with pCAGGS-h5R.12, a single band of ~100 kD was observed. After subtraction of the molecular mass of ^{35}S-labeled mIL-5 (~45 kD), estimated molecular mass of hIL-5R was ~55–60 kD (Fig. 5 *A*). In the case of the cross-linking experiment with ^{125}I-hIL-5, whose molecular mass is ~30 kD, the molecular mass of the major band was ~85 kD. The expected molecular mass was ~55–60 kD, as shown in Fig. 5 *B*. The molecular weight of the mature hIL-5R calculated from the predicted amino acid sequence is 45,556, and it is likely that the higher molecular weight observed for the expressed receptor molecule is due to glycosylation or other posttranslational modifications. The estimated molecular weight of hIL-5R derived from the cDNA cloned in this study was very similar to that of hIL-5R of normal eosinophils in human peripheral blood (18).

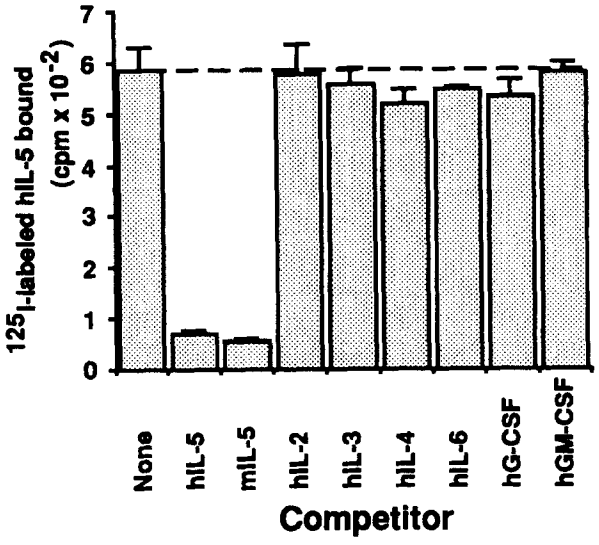

Figure 3. Specificity of ^{125}I-hIL-5 binding to rhIL-5R expressed in COS7 cells transfected with pCAGGS-h5R.12. Radioligands were added at 500 pM, and the competitors were at >1,000-fold molar excess. The bars represent the SD of three experiments.

Expression of the hIL-5R Transcripts. Expression of the hIL-5R mRNA was examined by Northern hybridization with a cDNA insert from λh5R.12 as a probe. RNA blot analysis of human cells demonstrated the presence of two mRNA transcripts of ~5.3 and ~1.4 kb in normal eosinophils and human erythroleukemic cell line TF-1 (Fig. 6, lanes *1* and *2*). These bands were absent in the RNA prepared from eosinophilic leukemia EoL-3, adult T cell leukemia ATL-2, Burkitt lymphoma cell line Raji, and histiocytic lymphoma cell line U-937 (Fig. 6, lanes *3–6*). In general, this pattern of expression of hIL-5R mRNA correlates with the expression of hIL-5R estimated from the binding assay (18). Although the 1.4-kb mRNA is specifically expressed on the cells that have IL-5Rs and is hybridized with the probe containing only the coding region of λh5R.12 (data not shown), it may be too small to code for the 1,260-bp ORF and UTR. This size of hIL-5R mRNA may code for a soluble form of hIL-5R. In PCR analysis, we could hardly detect the transcripts that correspond to λh5R.27 in normal eosinophils (data not shown).

Expression of hIL-5R in IL-3-dependent Cells. pCAGGS-h5R.12 was cotransfected with pSV2-neo into mIL-3-dependent cells, FDC-P1, and the cells resistant to G-418 were selected. We picked up two clones, FDC-h5R.12 no. 2 and FDC-h5R.12 no. 4, expressing hIL-5R by binding assay and checked their responsiveness to hIL-5. Both clones acquired responsiveness to hIL-5 as shown in Fig. 7. In contrast, parental cell line FDC-P1 or its transfectant with pSV2-neo alone (data not shown) did not respond to hIL-5. It was confirmed that this hIL-5R is essential to transduce signals.

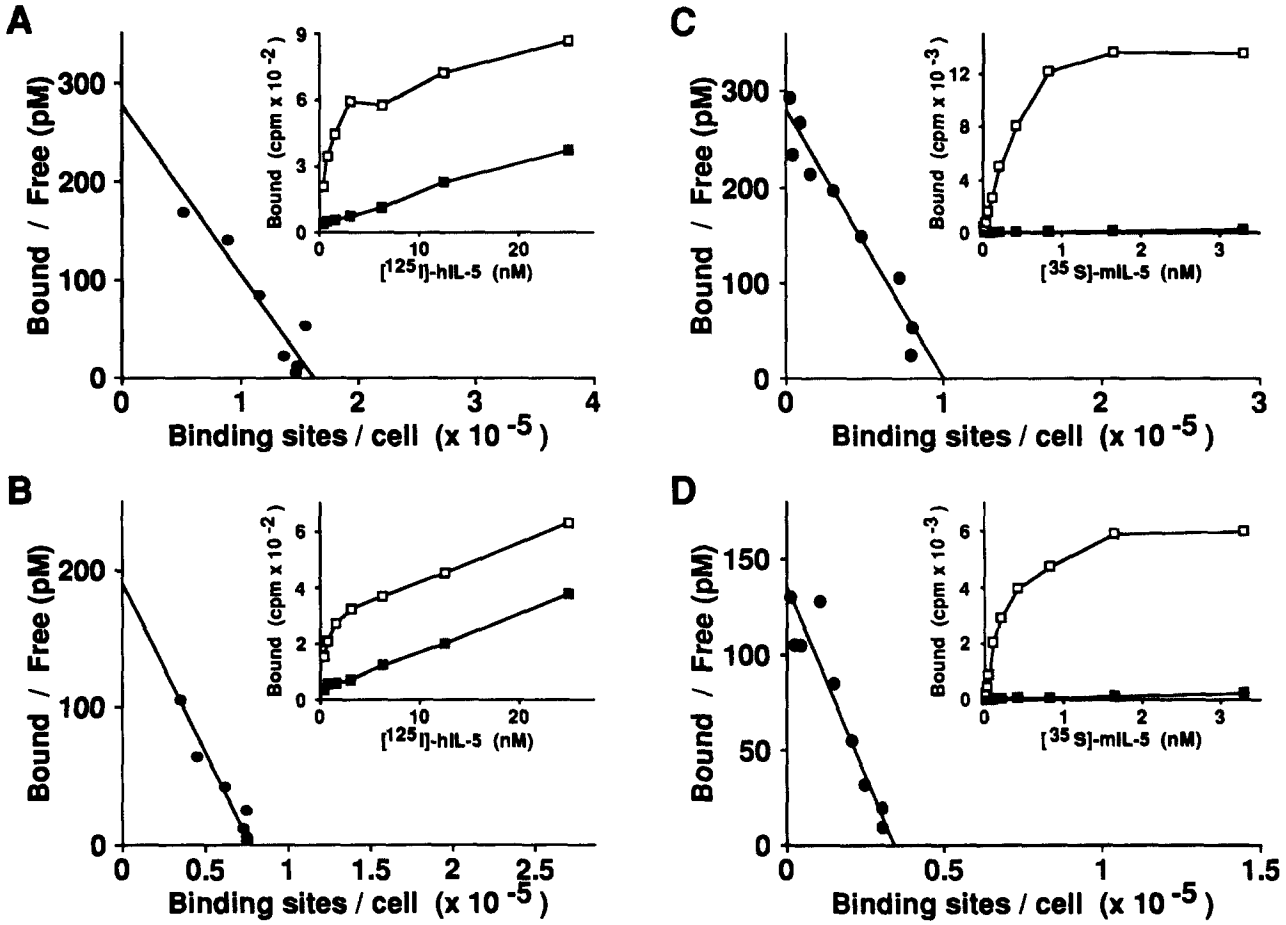

Figure 4. Scatchard plot analysis of ^{125}I-hIL-5 or ^{35}S-labeled mIL-5 binding to the COS7 transfectants expressing the cloned cDNA. The insets show the direct binding data (□, total binding; ■, nonspecific binding). Points are means of duplicate determinants. (*A* and *B*) Binding of ^{125}I-hIL-5 to COS7 cells transfected with pCAGGS-h5R.12 (*A*) or with pCAGGS-h5R.27 (*B*). (*C* and *D*) Binding of ^{35}S-labeled mIL-5 to COS7 cells transfected with pCAGGS-h5R.12 (*C*) or with pCAGGS-h5R.27 (*D*).

Discussion

In this report, we described the isolation of hIL-5R cDNA from human eosinophil libraries by cross-species hybridization using the mIL-5R α chain cDNA that encodes IL-5 binding protein of mIL-5R (21). The predicted mature hIL-5R has ~70% homology with that of its murine counterpart. It has conserved two pairs of cysteines and a "WSxWS" box that are the characteristics of the cytokine receptor superfamily (22–24). Recently, it has been reported that the sequence and structural pattern of the FBN III module are conserved in a cytokine receptor superfamily (25, 26, 43–46). We found not only domains with characteristics of a cytokine receptor superfamily, but also the NH_2-terminal domain of IL-5R (21–125 and 18–122 amino acid residues for hIL-5R and mIL-5R, respectively) has the sequence and structural pattern of FBN III (Fig. 2 *A*). The role of the FBN III module in this cytokine receptor superfamily is discussed in two ways. One possibility is that the region containing the FBN III module contributes to the interaction of the receptors with ligands or with other components of the receptor complex (26). In fact, mIL-5R consists of two chains, α and β (47–49). It might simply imply that the ligand binding moiety of a cytokine receptor superfamily consisting of the V-shaped crevice between linked FBN III modules is evolved from a primitive adhesion molecule (25). The other hypothesis is that it is involved in cell adhesion. Fukunaga et al. (43, 44) discussed that the similarity of the extracellular domain of the G-CSF receptor with contactin may explain the role of this region in the communication of neutrophilic progenitor cells with stromal cells. Of course, these two possibilities do not exclude each other. These FBN III modules of a cytokine receptor superfamily may be involved in cell-to-cell communication and ligand binding at the same time (45, 46).

The cytoplasmic domain of this hIL-5R does not have any kinds of protein kinase domain (50), or serine-rich region observed in other cytokine receptors (39, 43–46, 51–57). However, the proline cluster region (from Leu371 to Asp384) that is shared among receptors for growth hormone (58, 59), prolactin (60, 61), and GM-CSF (24) is conserved as in the mIL-5R α chain (21). This fact suggests the existence of a certain common interaction mechanism among these receptors. The existence of a second chain of hIL-5R is also suggested because the cytoplasmic domain of this ligand binding moiety

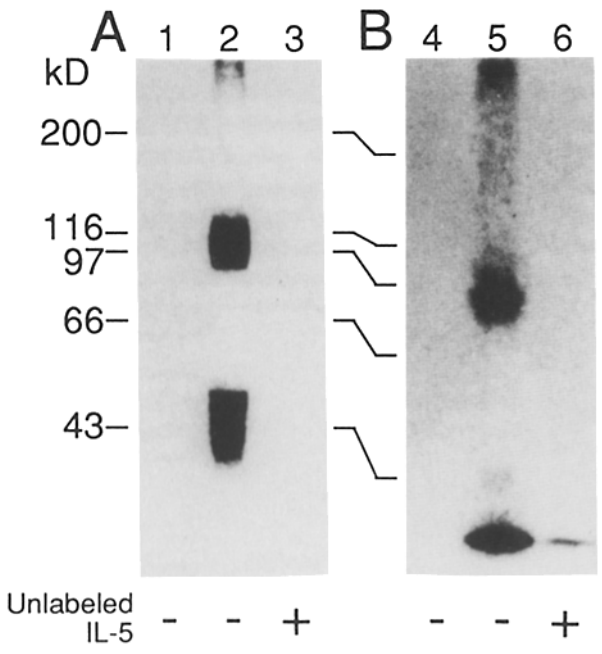

Figure 5. Affinity cross-linking. COS7 cells transfected with pCAGGS-h5R.12 (lanes *2*, *3*, *5*, and *6*) or a control vector pCAGGS (lanes *1* and *4*) were incubated with 5.5 nM ^{35}S-labeled mIL-5 (*A*) or 1 nM ^{125}I-hIL-5 (*B*) in the absence (lanes *1*, *2*, *4*, and *5*) or presence (lanes *3* and *6*) of 250-fold molar excess of unlabeled IL-5. The cell lysates were subjected to SDS-PAGE under nonreducing conditions, and were analyzed by a Bio-Analyzer 100.

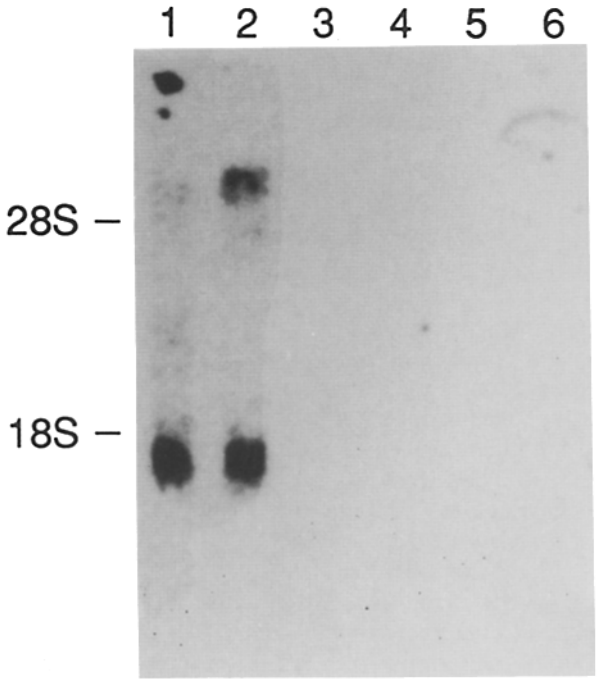

Figure 6. Detection of hIL-5R mRNA expression in normal human eosinophils and other hematopoietic cell lines. Poly(A)$^+$ RNA (6 μg) from human eosinophils in peripheral blood (lane *1*), TF-1 (lane *2*), EoL-3 (lane *3*), ATL-2 (lane *4*), Raji (lane *5*), and U-937 (lane *6*) were subjected to each lane. An EcoRI fragment of λh5R.12 was used as a probe. The 28 S and 18 S ribosomal RNAs are indicated.

of the hIL-5R is too short to transduce signals. The cytoplasmic region of hIL-5R also has similarity with β-spectrin as suggested (62). It is possible that the IL-5R complex may be anchored in the F-actin-containing structure.

Analysis of rhIL-5Rs confirmed that their ligand binding characteristics and their features in chemical cross-linking patterns are very similar to those of their naturally occurring molecules. This is quite contradictory to the constitution of mIL-5Rs in view of the role of a second chain in the formation of a high affinity receptor. A functional mIL-5R consists of an α and β chain (47–49). While the 60-kD α chain has IL-5 binding moiety, the 130-kD β chain does not bind IL-5 by itself. The α chain itself binds IL-5 with low affinity. The α/β heterodimer forms a high affinity mIL-5R. The ~60-kD protein encoded by the cloned hIL-5R cDNA, which is the counterpart of the mIL-5R α chain, bound IL-5 with an affinity almost similar to native hIL-5R-bearing cells. In terms of affinity, there are significant differences among reports published, including ours. Plaetinck et al. (16) reported a single class of binding sites with a K_d of 22 pM on human eosinophilic subline from HL-60. Chihara et al. (17) reported two types of hIL-5Rs; one is for hypodense eosinophils with a K_d of 518 pM, and the other is for normodense cells with a K_d of 2.6 nM. Kitamura et al. (19) also reported two types of hIL-5R on TF-1 cells at the basal level; one is high affinity sites (K_d = 120 pM), and the other is low affinity sites (K_d = 2 nM). On normal human eosinophils, we reported a single class of high affinity receptors with K_ds of 170–330 pM. We described that the 55–60-kD protein was cross-linked with ^{35}S-labeled mIL-5 (18). This protein is probably encoded by the cDNA cloned in this report. Besides this molecule, Plaetinck et al. (16) reported the 130-kD protein cross-linked with IL-5 on an eosinophilic subline from HL-60. Kitamura et al. (19) also reported 55- and 120-kD components on TF-1

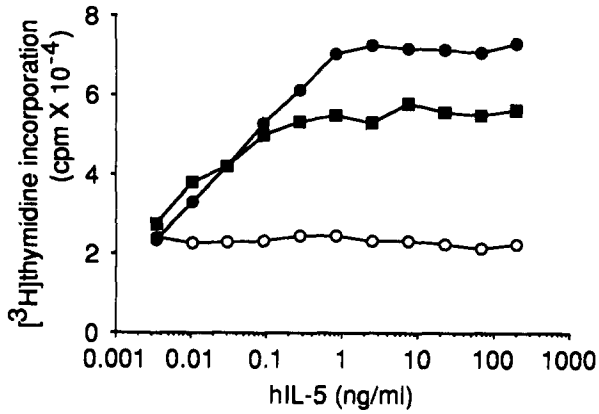

Figure 7. IL-5-dependent proliferation of FDC-P1 transfectants expressing the hIL-5R. FDC-P1 cells were cotransfected with pCAGGS-h5R.12 and pSV2-neo, and two clones were isolated. [^{3}H]Thymidine incorporations by FDC-P1 cells (○), FDC-h5R.12 no. 2 clone (●), and FDC-h5R.12 no. 4 clone (■) were determined as in Materials and Methods. Each result expresses the average cpm of three wells.

cells. It is still not clear whether hIL-5R on normal eosinophils consists of two chains as in the murine system.

In the mIL-5R system, the β chain is proved to be IL-3R homologue AIC2B (48, 49), and is probably shared with GM-CSF receptor (GM-CSFR) (63). We can expect the hIL-5R to also use AIC2B homologue KH97 (the β chain of human GM-CSFR) (39, 57) for the construction of a high affinity receptor. In light of these studies, our results can be interpreted in two different ways concerning the contribution of β chain to the formation of the high affinity hIL-5R. The first possibility is that the hIL-5R does not use the β chain of human GM-CSFR. It may not have a second chain as its constituent or may use a different protein. However, this is unlikely because of the short cytoplasmic domain and conserved amino acid sequences of hIL-5R α chain with mouse homologue. The second possibility is that hIL-5R also uses the β chain of GM-CSFR, but it is engaged in the hIL-5R system in a way that does not contribute to its affinity as much as in the murine system. We favor the latter possibility, because IL-3, IL-5, and GM-CSF seem to work coordinately in hematopoiesis. Expression of human GM-CSFR in murine FDC-P1 cells allowed these cells to proliferate in response to human GM-CSF, though only low affinity receptors were expressed (64). Kitamura et al. (63) reported that the expression of the α chain of human GM-CSFR together with AIC2B (β chain of mIL-5R) in CTLL-2 (IL-2-dependent mouse T cell line) cells conferred a growth response to human GM-CSF on these cells. We observed similar phenomena in the case of IL-5, as shown in Fig. 7. In this case, the hIL-5R α chain probably forms a heterodimer with the β chain of mIL-5R like the GM-CSFR α chain. We are in the process of checking the possibility that the β chain of human GM-CSFR is shared with the hIL-5R by cotransfecting appropriate cells with cDNAs of hIL-5R and KH97 (57), the β chain of human GM-CSFR.

We detected two classes of mRNAs ($\sim$5.3 and $\sim$1.4 kb) encoding hIL-5R. 1.4-kb transcripts may code for soluble form of hIL-5R. In addition to membrane-bound receptor forms, we isolated a clone, λh5R.25, that encodes a soluble form of the hIL-5R. The soluble form has been also identified in the mouse (21, 62) and a common splicing mechanism seems to be used in two species. The biochemical characterization and the examination of the expression of this molecule in various tissues at different stages may shed light on its biological role. It was clear that λh5R.27-type mRNA are not abundant, because this mRNA type could not be detected in normal eosinophils by PCR technique (data not shown). It is possible that the λh5R.27-type message is restricted to the eosinophilia patient from whom we constructed the library. This may imply a change in the genome or in the processing of the messages.

The expression of this hIL-5R gene is restricted in eosinophils or related cell lines as shown in this paper or in our previous paper (18). In the mouse, however, mIL-5 selectively supports the growth of $CD5^+$ B cells besides eosinophils in vivo (4). Indeed, IL-5R is expressed in the $CD5^+$ B cell subpopulation, and this B cell subpopulation is more responsive than conventional B cells are to IL-5 (65, 66). We have to wait before we draw any conclusions until an extensive survey in the detection of the hIL-5R message at various developmental and activated stages of human B cells is done. The molecular cloning of hIL-5R cDNA also enabled us to study the possible role of the IL-5R in human disease with an increased level of eosinophils.

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