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Cloning and expression of a gene encoding an interleukin 3 receptor-like protein: Identification of another member of the cytokine receptor gene family

(lymphokine/lymphokine receptor/hemopoietic growth factor/colony-stimulating factor)

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ABSTRACT **Using a monoclonal antibody to the interleukin 3 (IL-3) receptor (anti-Aic2), we isolated a cDNA (AIC2B) from a mouse mast cell line which is homologous to the previously characterized gene for the IL-3 receptor (AIC2A). This cDNA encodes a polypeptide of 896 amino acid residues and has 91% amino acid sequence identity with the IL-3 receptor. A consensus sequence defining an additional cytokine receptor family is present in this clone. Compared to the AIC2A clone, the AIC2B cDNA encodes a protein with amino acid substitutions, insertions, and deletions dispersed throughout the entire protein. Oligonucleotide probes specific for each cDNA hybridized with different genomic fragments, indicating that the AIC2A and AIC2B proteins are encoded by two distinct genes. Fibroblasts transfected with the AIC2B cDNA expressed the protein at the cell surface as determined by binding with the anti-Aic2 antibody but did not bind IL-3 or other cytokines, including IL-2, IL-4, granulocyte–macrophage colony-stimulating factor, erythropoietin, and IL-9 (p40) at concentrations between 1 and 10 nM. An S1 nuclease protection assay was used to discriminate between the AIC2A and AIC2B transcripts. We found that the AIC2B gene was coexpressed with the AIC2A gene. These results suggest a potential involvement of AIC2B in cytokine signal transduction.**

Interleukin 3 (IL-3) is a potent hemopoietic growth factor which stimulates multipotential hemopoietic stem cells; it stimulates the formation of multilineage colonies *in vitro* and also maintains spleen colony forming units (CFU-S) *in vitro*. In addition, it serves as a growth factor for committed cells, including mast cells, megakaryocytes, eosinophils, erythroblasts, pre-B cells, and potentially pre-T cells, and it induces the differentiation of osteoclasts (1, 2). Since IL-3 is produced mainly by activated T cells and is not produced by bone marrow stromal cells, it may be important for the expansion of hemopoietic cells in an inflammatory response rather than in constitutive hemopoiesis in the bone marrow (3).

IL-3 manifests its multiple biological activities through binding to the high-affinity IL-3 receptor expressed on various hemopoietic cells. We recently isolated a cDNA encoding a mouse IL-3-binding protein (4) by using a monoclonal antibody, anti-Aic2, raised against a multifactor-responsive immature mast cell line, IC2 (5). Fibroblasts transfected with the cloned cDNA bound IL-3 with low affinity, as do various IL-3-dependent cell lines that express the low-affinity IL-3 receptor (6). Although evidence suggests that the IL-3 receptor is linked to a protein tyrosine kinase in normal IL-3-responsive hemopoietic cells, neither kinase activity nor a consensus sequence for tyrosine kinases was found in the cloned IL-3 receptor (4). Thus, it is likely that the IL-3 receptor is a multiprotein complex, and the cloned cDNA encodes a binding component of the functional receptor.

A sequence comparison of the extracellular domains of the cytokine receptors [the IL-2 receptor β chain (7) and the receptors for IL-3 (4), IL-4 (8, 9), IL-6 (10), granulocyte–macrophage colony-stimulating factor (GM-CSF) (11), and erythropoietin (12)] revealed a common motif, establishing the existence of a distinct receptor gene family (4, 11, 13). The external domains of these receptors have two conserved regions separated by a sequence unique to each individual receptor. Interestingly, the external domain of the IL-3 receptor can be divided into two homologous units, each unit containing this common structural motif (4). In this report we describe the cloning of a cDNA homologous to the IL-3 receptor gene.** The protein encoded by this gene has the characteristics of the cytokine receptor family and the ligand for this protein is unknown.

MATERIALS AND METHODS

Transfection of COS-7 Cells and Binding Assays. COS-7 monkey cells were transfected with 10 μg of cDNA by the DEAE-dextran method or by electroporation at 220 V with 960 μF in a 0.4-cm cuvette. Three days after transfection, cells were detached by a 10-min incubation with Dulbecco's phosphate-buffered saline (PBS) containing 2 mM EDTA and washed with Hanks' balanced saline solution containing bovine serum albumin at 1 mg/ml and 10 mM Hepes buffer (pH 7.5). Cells were incubated with ^{125}I-labeled cytokines with or without unlabeled cytokines for 2 hr at 4°C. Cell-bound radioactivity was determined by centrifugation through oil as described previously (6).

Genomic Southern Analysis. Chromosomal DNA digested with *Eco*RI was separated on a 1% agarose gel and transferred to a nylon membrane filter (Schleicher & Schuell). The filter was prehybridized at 42°C in a solution containing 6× SSC, 5 mM EDTA (pH 8.0), 10 mM Tris·HCl (pH 7.5), 5× Denhardt's solution, 0.1% SDS, denatured calf thymus DNA at 100 μg/ml, and yeast tRNA at 100 μg/ml (1× SSC = 0.3 M NaCl/0.03 M sodium citrate; 1× Denhardt's solution = 0.02% bovine serum albumin/0.02% Ficoll/0.02% polyvinylpyrrolidone). Hybridization was performed at 42°C for 20 hr in the same solution with an oligonucleotide probe labeled

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Abbreviations: IL, interleukin; GM-CSF, granulocyte-macrophage colony-stimulating factor.

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with [γ-^{32}P]ATP by the T4 polynucleotide kinase and purified by a Sephadex G-25 NAP column (Pharmacia). The filter was washed at 40°C with 6× SSC.

Isolation of RNA and S1 Nuclease Protection Assays. Total cellular RNA was isolated by the guanidinium isothiocyanate method as described previously (14). The S1 nuclease protection assays were performed according to the method of Berk and Sharp (15). Briefly, the *Hin*dIII fragment of the AIC2-19 cDNA or the AIC2-5 cDNA in the pCEV4 expression vector (4), which covers the 5′ end of the simian virus 40 promoter to the unique *Hin*dIII site of the cDNA (see Fig. 2), was isolated. The fragment was dephosphorylated by alkaline phosphatase and the 5′ end of the *Hin*dIII cleavage site was labeled with [γ-^{32}P]ATP and T4 polynucleotide kinase to a specific activity of 10^6 cpm/μg of DNA. Five micrograms of total RNA was hybridized with 10^4 cpm of either the A probe or the B probe in a 15-μl reaction mixture containing 80% (vol/vol) formamide, 0.4 M NaCl, 40 mM Pipes (pH 6.4), 70 μg of yeast tRNA, and 1 mM EDTA for 16 hr at 45°C. Then, 150 μl of the S1 solution, which contains S1 nuclease at 0.3 unit/ml, 250 mM NaCl, 30 mM sodium acetate (pH 4.5), and 1 mM $ZnCl_2$, was added and the mixture was incubated at 37°C for 1 hr. The protected DNA fragments were analyzed by electrophoresis on a 5% polyacrylamide gel.

RESULTS

Cloning of cDNAs Encoding the IL-3 Receptor and Its Homologue. From the IL-3-dependent mouse mast cell line MC/9 we isolated a cDNA encoding an IL-3 receptor by using the rat IgM anti-IL-3 receptor antibody (anti-Aic2) as described previously (4). During this process, we isolated additional cDNA clones homologous to the clones encoding the IL-3 receptor. To screen the cDNA library we employed an expression cloning procedure using COS-7 cells as developed by Seed and Aruffo (16). Briefly, the MC/9 cDNA library made in the simian virus 40-based mammalian expression vector pCEV4 (4) was introduced into COS-7 cells. COS-7 cells expressing the Aic-2 antigen were enriched by panning using the anti-Aic2. Plasmid DNA was recovered from the selected COS-7 cells and subjected to the same selection procedure. After three cycles of enrichment, individual plasmids were analyzed. Out of 24 plasmids analyzed, 13 plasmids had the same 1.6-kilobase (kb) cDNA insert (AIC2-2), 3 plasmids had the same 2.7-kb cDNA insert (AIC2-19), and 1 had a 2.8-kb insert (AIC2-5). All these plasmids expressed the Aic2 antigen in COS-7 cells upon transient transfection. Interestingly, while COS-7 cells transfected with either AIC2-2 or AIC2-19 bound IL-3, COS-7 cells transfected with the AIC2-5 cDNA did not (Fig. 1). Restriction analysis as well as hybridization with the AIC2-2 cDNA as a probe indicated that AIC2-5 was closely related to but not identical with AIC2-2 or AIC2-19. To distinguish these two types of cDNA, we designate the cDNA encoding the IL-3-binding protein as AIC2A and the other cDNA, encoding a non-IL-3-binding protein, as AIC2B.

As the AIC2-2 cDNA hybridized only with an RNA of approximately 4.5 kb (4), all the plasmids recovered from COS-7 cells were partial cDNAs. We therefore isolated larger cDNA clones by hybridization using the AIC2-2 cDNA insert as a probe. We obtained 18 cDNA clones from the original MC/9 cDNA library. Eight of these were AIC2A and 10 were AIC2B as determined by restriction analysis. The major form of AIC2B cDNAs including AIC2-5 has a long open reading frame. Comparison with the other clones showed that AIC2-5 lacked 66 amino acids from the C terminus. We therefore constructed a cDNA (AIC2-522) encoding the entire coding region by recombining AIC2-5 and AIC2-22, which contains the C-terminal region, and used this cDNA for further biochemical characterization.

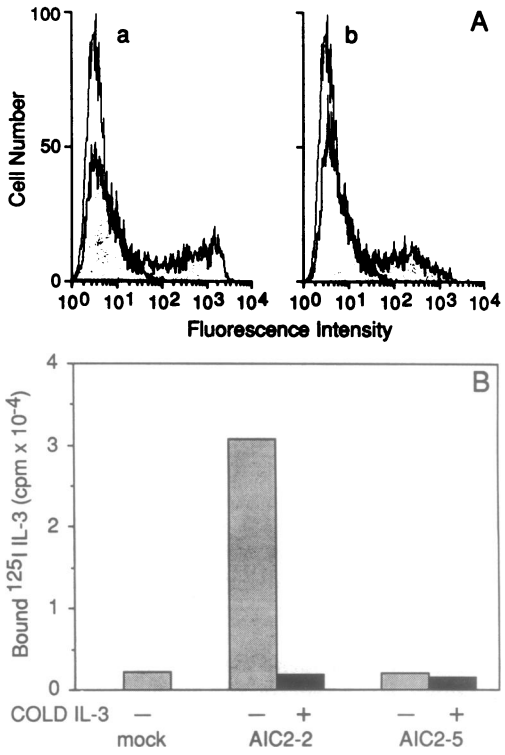

FIG. 1. Expression of AIC2-2 and AIC2-5 in COS-7 cells. (*A*) COS-7 cells transiently transfected with cDNA (*a*, AIC2-2; *b*, AIC2-5) were stained with anti-Aic2 and fluorescein isothiocyanate-conjugated antibody to rat IgM. The shaded area shows the staining profile with the anti-Aic2 and the blank area shows the staining profile with the second-stage antibody alone. (*B*) COS-7 cells transfected with cDNA were used for the IL-3-binding assay. Cells (5×10^4) were incubated with 2.75 nM ^{125}I-labeled IL-3 (^{125}I-IL-3) in the presence (+) or absence (−) of 10 μM unlabeled ("cold") IL-3 for 2 hr at 4°C and cell-bound radioactivity was measured.

The AIC2B Gene and Its Predicted Protein Structure. Nucleotide sequencing of the AIC2-5 and AIC2-22 cDNAs revealed a 95% nucleotide sequence identity with AIC2-26, including the 5′ noncoding region and the 3′ noncoding region sequence (Fig. 2). The protein encoded by the cDNA has a 91% amino acid sequence identity with AIC2-26 (Fig. 3). Nucleotide substitutions, deletions, and insertions were dispersed throughout the entire coding region, indicating that these two types of cDNA were derived from two distinct genes rather than created by alternative splicing. To confirm that AIC2A and AIC2B are encoded by two distinct genes, we performed genomic Southern analysis using oligonucleotide probes specific for each cDNA (Fig. 4). Probe A, which is specific to the AIC2A cDNA, hybridized with an 11-kb *Eco*RI fragment, whereas probe B, which is specific to the AIC2B cDNA, hybridized with a 15-kb *Eco*RI fragment (Fig. 4), indicating that the two types of AIC2 cDNA were derived from two different genes. Isolation of the two genes from a genomic library confirmed the existence of two distinct genes (unpublished work).

The amino acid sequence indicated that the AIC2B protein as compared to the AIC2A protein has one additional potential N-linked glycosylation site in the external domain and has 18 extra amino acids in the cytoplasmic domain. The AIC2B protein retains the unique feature of the IL-3 receptor (AIC2A protein); i.e., the external domain of the AIC2B protein can be divided into two homologous units sharing 24% identity and 42% similarity and each unit contains the common motif of the cytokine receptors such as four conserved cysteine residues and the unique WSXWS motif found near the transmembrane domain (4) (Fig. 3). The cytoplasmic

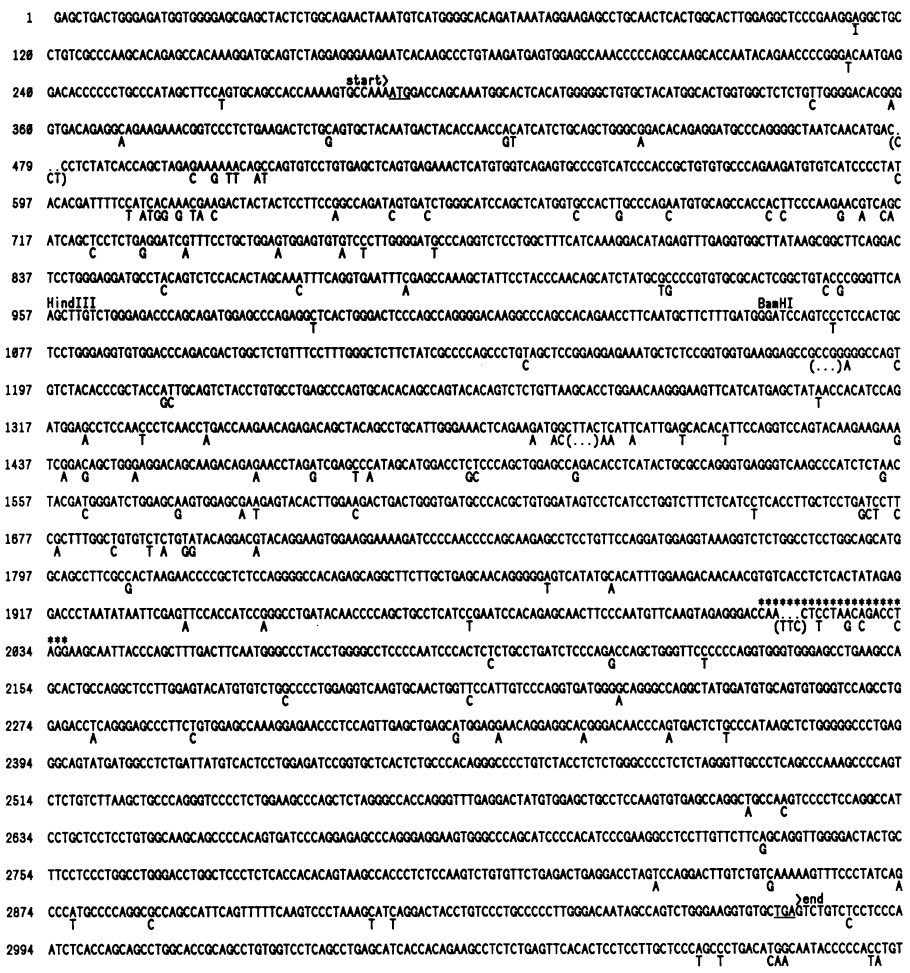

FIG. 2. Nucleotide sequence of the AIC2B cDNA. The AIC2B sequence is shown in the upper rows and the substitutions, deletions, and insertions in the AIC2A sequence are shown in the lower rows. The initiation codon and the termination codon are marked by >. The *Hin*dIII restriction site used for the S1 protection assays is indicated. A stretch of nucleotides marked by * indicates the region that oligonucleotide probes were made for in the Southern analysis in Fig. 4.

domain of the AIC2B protein has features similar to those of the IL-3 receptor such as proline-rich and serine-rich motifs (Fig. 3). Moreover, the secondary structure as predicted by the Chou–Fasman method (17) is also well retained because most of the changes which occur in the AIC2B protein are conservative. However, several changes in the sequence potentially affect local secondary structure. For example, the addition of a helix-disrupting residue, glycine, at amino acid

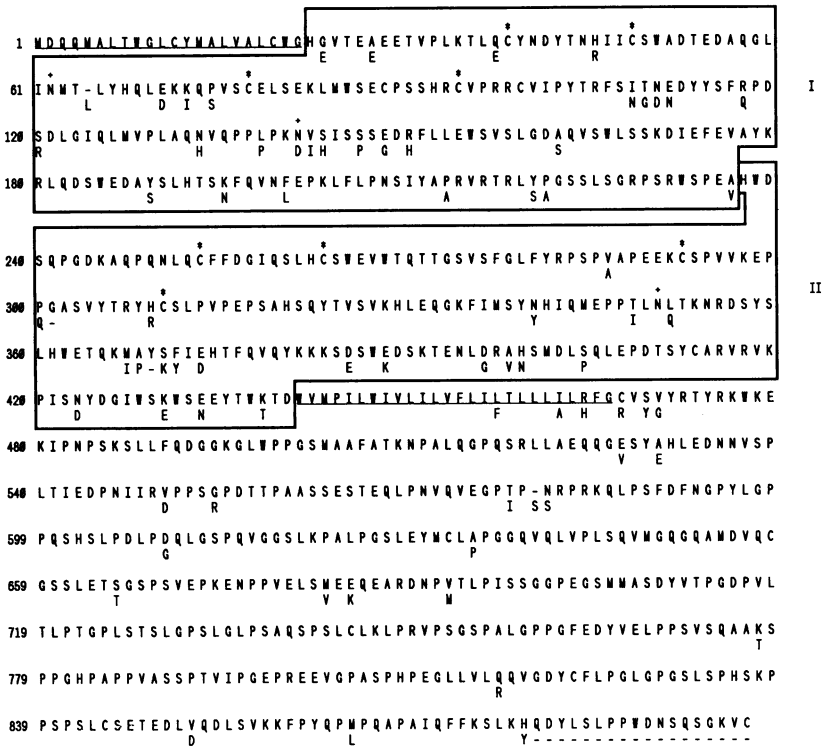

FIG. 3. Amino acid sequence of AIC2B. The amino acid sequence of the AIC2B protein is shown in the upper rows and the substitutions, insertions, and deletions in the AIC2A protein are shown in the lower rows. Amino acid deletions are marked by dashes. The signal sequence and the transmembrane domain are marked by an underline. The potential N-linked glycosylation sites are shown by +. The conserved cysteine residues are shown by *. Two homologous segments in the external domain are boxed.

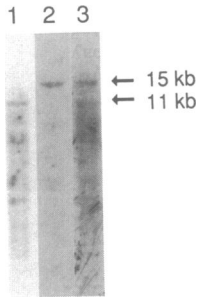

FIG. 4. Southern hybridization with oligonucleotide probes specific to AIC2A and AIC2B. *Eco*RI-digested mouse gemonic DNA was separated on an agarose gel and transferred to a nylon membrane filter (Schleicher & Schuell). The oligonucleotide probes used are as follows: lane 1, AIC2A-specific 21-mer (CTGGGTCGGCTAGAAGGAATT) (see Fig. 2); lane 2, AIC2B-specific 20-mer (CCTAGGTCTGTTAGGAGTTG) (see Fig. 2); and lane 3, a mixture of two probes.

301 may lead to a loss of α-helical structure. Between residues 367 and 371 there are several amino acid changes that appear to increase the local hydrophobicity. In addition, other changes which may cause the loss of IL-3 binding include (*i*) charge switches at amino acid positions 388 and 430, (*ii*) addition of helix breakers at positions 214 and 222, and (*iii*) a switch from glycine at 396 to a charged aspartic residue. The relationship of the receptor structure to IL-3 binding remains to be determined.

Expression of the AIC2B cDNA in Fibroblasts. To test whether the AIC2B protein binds any known cytokines, we expressed the AIC2-5 and AIC2-522 cDNAs in COS-7 cells by using the expression vector pCEV4 (4), and we examined the cells for specific ^{125}I-labeled cytokine binding. Although COS-7 cells expressed the AIC2B protein, which was confirmed by staining with anti-Aic2, they did not bind ^{125}I-IL-3 at a concentration of ligand (2.8 nM) shown to give appreciable binding to the AIC2A protein (Fig. 1*B*). Calculations indicate that if AIC2B itself binds IL-3, it must do so with an extremely low affinity, not accurately measurable with the given techniques. If AIC2B is involved in the formation of a high-affinity IL-3 receptor complex by interaction with AIC2A, a known IL-3-binding protein, then the affinity of IL-3 binding would be altered upon coexpression of AIC2A and AIC2B in the same cells. However, no such change was noted (data not shown). We have therefore concluded that AIC2B is not a binding protein for IL-3, and we have examined its ability to bind other cytokines. However, IL-2 (7.6 nM), IL-4 (0.7 nM), GM-CSF (4.5 nM), erythropoietin (12.7 nM), and IL-9 (p40) (4.1 nM) at the indicated concentrations all failed to show any significant binding (data not shown).

Expression of the Two Types of the AIC2 mRNA in Various Cells. Northern blotting of MC/9 RNA using the AIC2-2 cDNA as a probe showed only one broad band at about 4.5 kb, although two types of cDNA were isolated from MC/9 (4). This result indicates that the sizes of the AIC2A and AIC2B mRNAs are very similar and cannot be distinguished by Northern blotting. To examine expression of the AIC2A and AIC2B genes we performed S1 nuclease protection assays using RNA prepared from COS-7 cells transfected with either the AIC2A or AIC2B cDNA. An end-labeled AIC2A cDNA probe was protected by RNA prepared from the AIC2A cDNA-transfected COS-7 cells, but it was only partially protected by the RNA of AIC2B-transfected COS-7 cells because of the base substitutions (Figs. 2 and 5). Conversely, when the end-labeled AIC2B cDNA probe was used, AIC2A RNA only partially protected the probe, whereas the larger DNA probe was protected by the AIC2B RNA (data not shown). Under the same conditions using the AIC2A probe, RNA from the IL-3-dependent cell lines (IC2, MC/9, PT18, FDCP2, NFS60) generated two types of protected cDNA probe, indicating that both genes are expressed in these cells (Fig. 5). Interestingly, an IL-3-nonresponsive variant, FDCP2(−), derived from the IL-3-dependent FDCP2 cell line, had no detectable IL-3 binding (ref. 27; unpublished results) and did not express a detectable level of either transcript (Fig. 5). This result suggests that the AIC2A and

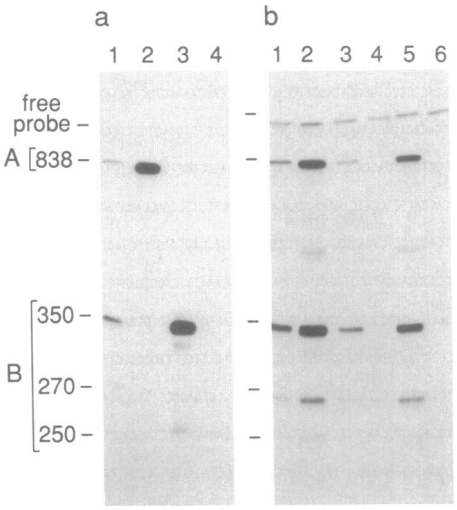

FIG. 5. S1 nuclease protection assays using the AIC2A probe. RNAs added were as follows: (*a*) Lane 1, IC2; lane 2, COS-7 cells transfected with the AIC2A cDNA; lane 3, COS-7 cells transfected with AIC2B cDNA; lane 4, tRNA. (*b*) Lane 1, IC2; lane 2, PT18; lane 3, FDCP2; lane 4, FDCP2(−); lane 5, MC/9; lane 6, tRNA. A and B indicate AIC2A and AIC2B RNA protected bands.

AIC2B genes may be coregulated. Neither the A- nor the B-type AIC2 RNA was detectable in T cells (HT-2, D10, HDK), stromal cells (ALC8, 30E, and 30R.7), or a fibroblast cell line (L). However, a macrophage cell line (P388) and CD5$^+$ B-cell lines (CH12, CH44), which are IL-3-nonresponsive in proliferation assays, expressed a relatively high level of the AIC2A and AIC2B RNA (Table 1). In all cells tested the level of the AIC2B RNA was always higher than that of AIC2A.

DISCUSSION

Further characterization of the cDNAs cloned with the anti-Aic2 antibody has revealed that two distinct yet homologous cDNAs are present in the cDNA library of the mouse mast cell line MC/9. The differences in the nucleotide sequences are dispersed throughout the entire coding region, and oligonucleotide probes specific to each cDNA hybridized to two different genomic fragments, indicating the existence of two distinct genes. Moreover, our isolation of two distinct genomic clones has proved the existence of two separate genes (unpublished work).

Several genes with extensive homology to known receptor genes have been isolated: the c-*erbB2* protooncogene (*neu*) and c-*erbB3* are homologues of the epidermal growth factor receptor gene and the c-*kit* protooncogene has a stucture similar to the structures of the genes for the platelet-derived growth factor receptor and the macrophage colony-stimulating factor receptor. The c-*erbB2* gene was shown to be activated as a transforming gene by a point mutation (18), and gene amplification of c-*erbB2* was observed in some tumors (19), suggesting a functional importance for the c-*erbB2* gene. The c-*kit* protooncogene is included in the mouse *W* locus (20), and mutations in this locus cause a defect in embryonic development and hemopoiesis (21), indicating an important function in development. However, in neither case has the ligand for the putative receptor been identified. The AIC2B gene is an example of such a homologue in the cytokine receptor family.

The AIC2B protein has all the structural features common to the external domains of the cytokine receptors such as the IL-2 receptor β chain (7), IL-3 (4), IL-4 (8, 9), IL-6 (10), GM-CSF (11), and erythropoietin (12) receptors. These elements include four conserved cysteines and the unique

Table 1. Expression of the AIC2 RNA

Cell line	Cell type	Band intensity	
		AIC2A	AIC2B
IC2	Mast	++	+++
MC/9	Mast	+++	++++
PT18	Mast	++++	+++++
FDCP2	Myeloid	+	++
FDCP2(−)	Myeloid	−	−
NFS60	Myeloid	+	++
M1	Macrophage	±	±
P388	Macrophage	++	+++++
J174	Macrophage	±	+
B5B3C4	Pre-B	+	++
BCL-1	B	−	+
CH12	B	+	++++
CH44	B	+	+++
CH32	B	−	+
K23Tr	T	−	−
HT2	T	−	−
D10	T	−	−
ALC8	Stromal	−	−
30E	Stromal	−	−
30R.7	Stromal	−	−
L	Fibroblast	−	−

RNAs prepared from various cells were used to evaluate the expression of the AIC2 RNA by using the S1 nuclease protection assay. Intensity of the protected bands on the autoradiogram was visually classified.

WSXWS sequence which is located close to the transmembrane domain. It is likely that AIC2B is a receptor for a known or an unknown cytokine. The amino acid sequence identity of AIC2B to the IL-3 receptor (AIC2A) is unusually high (91%) compared to a 30% identity in the external domains of the α and β platelet-derived growth factor (PDGF) receptors (22), both of which bind the BB form of PDGF (23). The receptors for growth hormone and prolactin also have a structural motif similar to the cytokine receptor family and have a 33% identity (24). Although the similarity between these two hormones is weak, they compete with each other for binding to both receptors (24). Therefore a specific ligand for the AIC2B protein, if it is present, may have a similar structure to IL-3. The external domains of AIC2A and AIC2B can be divided into two relatively homologous segments, each containing the conserved motifs of the cytokine receptor gene family. Preliminary results indicate that the second segment of the AIC2A protein is important for IL-3 binding (H.-M. Wang and A.M., unpublished data), suggesting an alternative possibility, that the first segment is responsible for the binding to the unidentified ligand. Since the cytoplasmic domains of both proteins are also well conserved, a putative ligand for AIC2B may induce a biological response similar to IL-3.

The AIC2B protein did not bind any cytokine (IL-2, IL-3, IL-4, GM-CSF, erythropoietin, or IL-9) at concentrations between 1 and 10 nM when expressed in COS-7 cells. However, this experiment still does not exclude the possibility that AIC2B is a component of one of these cytokine receptors. The observation that the IL-2 receptor β chain did not bind IL-2 when expressed in COS-7 cells but bound IL-2 when coexpressed with the IL-2 receptor α chain (7) led us to speculate that AIC2B may be a component of a cytokine receptor complex. Because these two genes are coexpressed in many cases, the possibility that the AIC2B protein is a component of the IL-3 receptor was examined. However, as described above, cotransfection of the AIC2A and AIC2B cDNAs into COS-7 fibroblasts did not change the affinity of IL-3 binding, suggesting that the AIC2B protein is not involved in the formation of the high-affinity IL-3 receptor by itself. Alternatively, the AIC2B gene might have been created by gene duplication of the AIC2A and have lost its function during evolution. Further studies are required for discrimination between these possibilities.

Coexpression of the AIC2A and AIC2B RNA in various cells suggests the possibility that expression of these two genes is coordinately regulated under the same mechanism. However, some cells, such as P388, CH12, and CH44, expressed the AIC2 RNA abundantly (Table 1) yet expressed the Aic2 antigen poorly (unpublished results). There may be some post-transcriptional regulatory mechanism for the expression of the Aic2 antigen. During the screening of the AIC2 cDNA by hybridization, we found several minor forms of cDNAs in both AIC2A and AIC2B. Since it is common that multiple forms of mRNA are produced by differential splicing in various cell surface proteins such as CD45 (25), CD8 (26), and IL-4 receptor (8), AIC2 may also have such a mechanism to produce multiple forms of mRNA. It would be interesting to study how the expression of the AIC2 genes is regulated.

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1. Schrader, J. W. (1986) *Annu. Rev. Immunol.* **4**, 205–230.
2. Barton, B. E. & Mayer, R. (1989) *J. Immunol.* **143**, 3211–3216.
3. Miyajima, A., Miyatake, S., Schreurs, J., De Vries, J., Arai, N., Yokota, T. & Arai, K. (1988) *FASEB J.* **2**, 2462–2473.
4. Itoh, N., Yonehara, S., Schreurs, J., Gorman, D. M., Maruyama, K., Ishii, A., Yahara, I., Arai, K. & Miyajima, A. (1990) *Science* **247**, 324–327.
5. Yonehara, S., Ishi, A., Yonehara, M., Koyasu, S., Miyajima, A., Schreurs, J., Arai, K. & Yahara, I. (1990) *Int. Immunol.* **2**, 143–150.
6. Schreurs, J., Arai, K. & Miyajima, A. (1990) *Growth Factors* **2**, 221–234.
7. Hatakeyama, M., Tsudo, M., Minamoto, S., Kono, T., Doi, T., Miyata, T., Miyasaka, M. & Taniguchi, T. (1989) *Science* **244**, 551–556.
8. Mosley, B., Beckmann, P., March, C. J., Idzerda, R. L., Gimpel, S. D., VandenBos, T., Friend, D., Alpert, A., Anderson, D., Jackson, J., Wignall, J. M., Smith, C., Gallis, B., Sims, J. E., Urdal, D., Widmer, M. B., Cosman, D. & Park, L. S. (1989) *Cell* **59**, 335–348.
9. Harada, N., Castle, B. E., Gorman, D. M., Itoh, N., Schreurs, J., Barrett, R. L., Howard, M. & Miyajima, A. (1990) *Proc. Natl. Acad. Sci. USA* **87**, 857–861.
10. Yamasaki, K., Taga, T., Hirata, Y., Yawata, H., Kawanishi, Y., Seed, B., Taniguchi, T., Hirano, T. & Kishimoto, T. (1988) *Science* **241**, 825–828.
11. Gearing, D. P., King, J. A., Gough, N. M. & Nicola, N. A. (1989) *EMBO J.* **8**, 3667–3676.
12. D'Andrea, A. D., Lodish, H. F. & Wong, G. G. (1989) *Cell* **57**, 277–285.
13. Bazan, J. F. (1989) *Biochem. Biophys. Res. Commun.* **164**, 788–795.
14. Chirgwin, J. M., Przybyla, A. E., MacDonald, R. J. & Rutter, W. J. (1978) *Biochemistry* **18**, 5294–5299.
15. Berk, A. J. & Sharp, P. A. (1978) *Proc. Natl. Acad. Sci. USA* **75**, 1274–1278.
16. Seed, B. & Aruffo, A. (1987) *Proc. Natl. Acad. Sci. USA* **84**, 3365–3369.
17. Chou, P. & Fasman, G. (1978) *Adv. Enzymol.* **47**, 45–148.
18. Bargmann, C. I., Hung, M.-C. & Weinberg, R. A. (1986) *Cell* **45**, 649–657.
19. Semba, K., Kamata, N., Toyoshima, K. & Yamamoto, T. (1985) *Proc. Natl. Acad. Sci. USA* **82**, 6497–6501.
20. Geissler, E. N., Ryan, M. A. & Housman, D. E. (1988) *Cell* **55**, 185–192.
21. Russell, E. S. (1979) *Adv. Genet.* **20**, 357–459.
22. Claesson-Welsh, L., Eriksson, A., Westermark, B. & Heldin, C.-H. (1989) *Proc. Natl. Acad. Sci. USA* **86**, 4917–4921.
23. Heldin, C.-H., Backstrom, G., Ostman, A., Hammacher, A., Ronnstrand, L., Rubin, K., Nister, M. & Westermark, B. (1988) *EMBO J.* **7**, 1387–1393.
24. Boutin, J. M., Jolicoeur, C., Okamura, H., Gagnon, J., Edery, M., Shirota, M., Banville, D., Dusanter-Fourt, I., Djiane, J. & Kelly, P. A. (1988) *Cell* **53**, 69–77.
25. Streuli, M., Hall, L. R., Saga, Y., Schlossman, S. F. & Saito, H. (1987) *J. Exp. Med.* **166**, 1548–1566.
26. Giblin, P., Ledbetter, J. A. & Kavathas, P. (1989) *Proc. Natl. Acad. Sci. USA* **86**, 998–1002.
27. Sugawara, M., Hattori, C., Tezuka, E., Tamura, S. & Ohta, Y. (1988) *J. Immunol.* **140**, 526–530.