Molecular Characterization of the Mouse Interleukin-3 Receptor

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Introduction

Interleukin-3 (IL-3), also known as a multi-colony stimulating factor, is a potent hemopoietic growth factor which acts on the multi-potential hemopoietic stem cells as well as committed progenitor cells such as erythroblasts, eosinophils, megakaryocytes, mast cells, and pro-B cells [1]. Whereas IL-3 is capable of regulating proliferation and differentiation of various hemopoietic cell lineages, production of IL-3 is limited to antigen-stimulated T cells and activated mast cells and so far there is no evidence indicating that IL-3 is produced in normal bone marrow. Therefore, the major role of IL-3 may be in antigen-induced hemopoiesis rather than normal hemopoiesis in the bone marrow [2].

IL-3 manifests its multiple biological activities through specific cell surface receptors and signal transduction pathways in target cells. Information about the structure and function of the receptor should contribute to an understanding of the multiple biological activities of IL-3. Here we describe the biochemical characterization and molecular cloning of the mouse IL-3 receptor gene.

The IL-3 Receptor and Signal Transduction

Previous reports indicated that IL-3 bound only to a single class of high affinity receptor. However, our binding data clearly shows the presence of two distinct binding sites on various IL-3 dependent cells: high affinity ($Kd \sim 100\ pM$) and low affinity ($Kd \sim 10\ nM$) binding sites. In addition, two binding sites have also been distinguished by dissociation kinetics. The dissociation rate from the low affinity site ($T_{1/2} = 4$ min) is much faster than that from the high affinity site ($T_{1/2} = 4$ h) [3]. Cross-linking experiments using ^{125}I-labeled IL-3 have revealed the presence of 140, 120, and 70 kDa proteins. The interrelation between these proteins and two distinct binding sites was unknown.

While the structure of the IL-3 receptor is unclear, it has been well established that IL-3 induces rapid tyrosine phosphorylation of a specific set of proteins including 140, 95, 90, 70, and 55 kDa proteins [4, 5]. Evidence indicates that the 140 kDa IL-3 binding protein is tyrosine phosphorylated [6]. In addition, it is known that, in IL-3 dependent cells, the IL-3 requirement is abrogated by oncogenes having a tyrosine kinase. In particular, *v-abl* carrying a temperature-sensitive tyrosine kinase abrogates the IL-3 requirement in a temperature dependent manner (Fig. 1 A, B) [7, 8], suggesting the involvement of tyrosine phosphorylation in the IL-3 signal transduction pathway. These results indicate that the IL-3 receptor is directly or indirectly coupled to a tyrosine kinase.

If the IL-3 receptor is linked to a signal transduction system similar to the growth factor receptors containing a tyrosine kinase, such as the epidermal growth factor (EGF) receptor, the requirement

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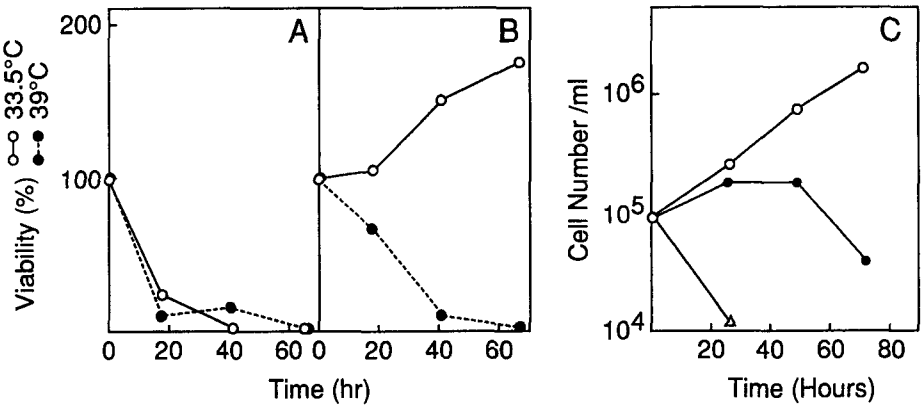

Fig. 1A–C. Conditional abrogation of IL-3 requirement from IC2 cells. **A, B**: Cell viability after depletion of IL-3 at 39 °C (*dashed line*) and 33.5 °C (*solid line*). **A** IC2 parental cells. **B** IC2 cells infected with *v-abl* containing a temperature-sensitive tyrosine kinase. **C** IC2 cells transfected with the human EGF receptor cDNA were cultured in IL-3 medium. Exponentially growing cells were washed, resuspended in medium in the absence (△) or presence of IL-3 (○) or EGF (●) and viable cell numbers were counted

for IL-3 might be substituted by those growth factors in IL-3 dependent cells. To examine this possibility we expressed the human EGF receptor complementary deoxyribonucleic acid (cDNA) in an IL-3 dependent mouse immature mast cell line, IC2 [9]. The cells expressing the EGF receptor proliferated continuously in response to IL-3, whereas EGF only transiently sustained cell viability (Fig. 1C). Both IL-3 and EGF maintained the level of c-*myc* RNA which is necessary for maintenance of cell viability [8]. This indicates that the signal transduction pathways of the IL-3 receptor and the EGF receptor overlap only partially and that IL-3 apparently induces an additional signal(s), not induced by EGF, which leads to long-term proliferation [9].

Molecular Cloning of the IL-3 Receptor

To better understand the structure and function of the IL-3 receptor, we cloned the IL-3 receptor cDNA by using the anti-Aic2 antibody [10]. This antibody was raised against an IL-3 dependent cell line, IC2, and partially inhibits IL-3 binding. A cDNA library from an IL-3 dependent mast cell line, MC/9, was made in the SV40-based mammalian expression vector and was introduced into COS7 cells. After 3 days, COS7 cells expressing the Aic2 antigen were collected by panning using the anti-Aic2 antibody. Plasmid DNA was recovered from the COS 7 cells into *E. coli*. After three cycles of this enrichment, individual plasmids were analyzed and we obtained two different plasmids (AIC2A and AIC2B). Both plasmids expressed the Aic2 antigen on COS7 cells equally well. However, only AIC2A cDNA conferred IL-3 binding following transfection in COS7 cells (Fig. 2) [10, 11].

The AIC2A cDNA encodes a protein of 878 amino acids composed of a signal sequence, an extracellular domain, a transmembrane domain, and a cytoplasmic domain of 22, 417, 26, and 413 amino acid residues, respectively. Fibroblasts transfected with the AIC2A cDNA specifically bind IL-3 and the binding is not competed with by other cytokines, including GM-CSF. IL-3 binds to the AIC2A protein with low affinity ($Kd \sim 10$ nM) and dissociates rapidly ($T_{1/2} \sim 3$ min at 15 °C). These binding characteristics are identical to those of the low affinity binding site in IL-3 responsive cells. Cross-linking of ^{125}I-labeled IL-3 to the AIC2A transfectants has revealed similar protein as to the IL-3 dependent MC/9 cells, suggesting that

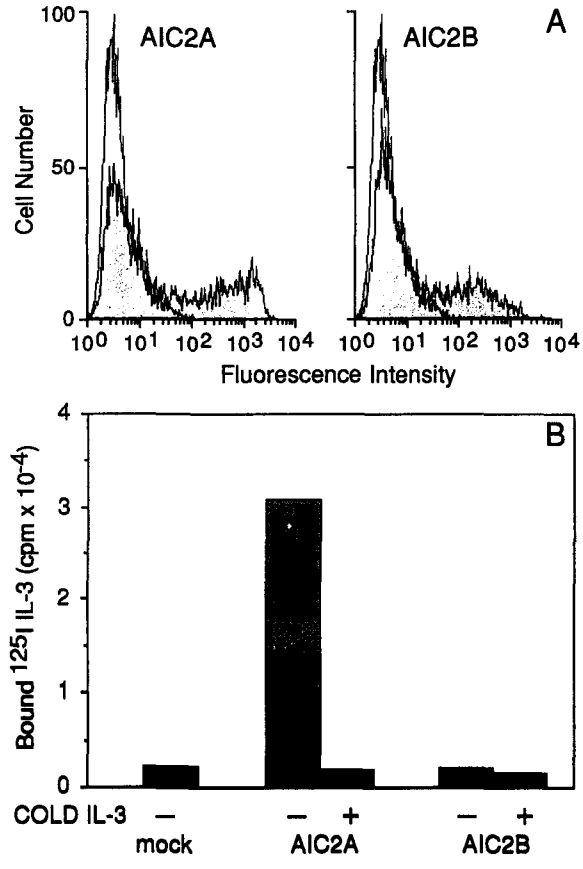

Fig. 2A,B. Expression of AIC2A and AIC2B cDNA in COS7 cells. **A** Anti-Aic2 antibody staining of COS7 cells transfected with AIC2 cDNAs. **B** IL-3 binding to COS7 cells transfected with AIC2 cDNAs

AIC2A is a major IL-3 binding protein [10].

The cytoplasmic domain does not contain any consensus sequence for kinases, phosphatases, or nucleotide binding proteins. Moreover, the Aic2 antigen expressed on L cells is neither down regulated nor induced to phosphorylate by IL-3 [10]. These results suggest that the high affinity IL-3 receptor is composed of multiple subunits and that the AIC2A protein is a major binding protein. This possibility is further supported by the isolation of a high affinity IL-3 receptor from IL-3 depentent cells [19]. Since the high affinity receptor has a tight association with IL-3, cells preincubated with biotin-IL-3 were washed to remove IL-3 bound to the low affinity sites. The biotin-IL-3-receptor complex was then isolated by streptavidin-agarose from a detergent extract. Proteins eluted from the agarose beads by acid were analyzed by SDS gel electrophoresis. We detected a major protein of 120 kDa and additional proteins of 70 and 50 kDa. Western blotting using the antibody against an AIC2A peptide revealed that the 120 kDa protein is the AIC2A protein. In addition, the 120 kDa protein was also recognized by an anti-phosphotyrosine antibody. These results indicate that the 120 kDa AIC2A protein is a major IL-3 binding component of the high affinity IL-3 receptor and that the AIC2A protein can be tyrosine phosphorylated [19]. Additional proteins found in the acid eluate from the agarose beads may involve in formation of the high affinity receptor.

AIC2B, an IL-3 Receptor-Like Molecule

A second cDNA clone, AIC2B, encodes another Aic2 antigen composed of 896 amino acids. Despite its unusually high degree of sequence homology (91% identity) to the AIC2A protein, the AIC2B protein does not bind IL-3 [11]. Nor did we find any specific binding of the AIC2B protein to other cytokines including IL-2, IL-4, IL-5, IL-9, GM-CSF, and erythropoietin.

Amino acid substitutions, deletions, and insertions are dispersed throughout the entire proteins, suggesting that two proteins are encoded by two distinct genes. This is supported by genomic Southern analysis using oligonucleotide probes specific for either AIC2A or AIC2B cDNA [11]. Moreover, cloning of two distinct genomic genes has confirmed the presence of two AIC2 genes. Genetic analysis has localized the AIC2 gene on mouse chromosome 15 and no other locus has been found, suggesting that the two genes are closely linked. In addition, the exon-intron structure of these two genes is very similar. These results indicate that the AIC2A and AIC2B genes were created by gene duplication [20].

Table 1. Expression of the AIC2 RNA

Cell lines	Cell types	AIC2A	AIC2B
IC2	Mast	++	+++
MC/9	Mast	+++	++++
PT18	Mast	++++	+++++
FDCP2	Myeloid	+	++
FDCP2(−)	Myeloid	−	−
NFS60	Myeloid	+	++
M1	Macrophage	+/−	+/−
P388	Macrophage	++	+++++
J174	Macophage	+/−	+
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	−	−

RNA prepared from various cells were used to evaluate the expression of the AIC2 RNA using the SI protection assays.

Since two genes are so similar, the transcripts of each gene cannot be distinguished by northern analysis. We have developed S1 nuclease protection assays to study the expression of the AIC2A and AIC2B genes. Among various cell lines examined, the AIC2 RNA was detected in all the IL-3 responsive cells and in some IL-3 non-responsive cells. We did not find any AIC2 RNA in T cells, stromal cells, and fibroblasts. Interestingly, IL-3 non-responsive FDCP2(−) cells derived from IL-3 dependent FDCP2 cells do not express any IL-3 binding site nor AIC2A and AIC2B RNA (Table 1). These results suggest that both AIC2 genes are regulated under the same mechanism [11]. Although, the AIC2B protein does not bind any cytokine by itself, there remains the possibility that AIC2B is a component of a cytokine receptor. Recent evidence suggests this possibility [12].

Cytokine Receptor Gene Family

Comparison of the amino acid sequences of the external domains of the AIC2A and AIC2B proteins with other cytokine receptors (IL-2 receptor β chain, IL-4, IL-6, IL-7, GM-CSF, G-CSF, erythropoietin receptors) has revealed significant structural homologies in a stretch of about 200 amino acids (Fig. 3). Two highly conserved motifs are present: one at the N-terminal half of these segments contains four conserved cysteine residues and the other at just upstream of the transmembrane domains contains a Trp-Ser-X-Trp-Ser (WSXWS) motif. The AIC2A and AIC2B proteins have two such segments of the conserved sequences [10, 11]. In addition to the cytokine receptors, growth hormone and prolactin receptors have similar structural motifs [13]. The C-terminal half of the conserved motif of the cytokine receptor family has homology with the type III domain of fibronectin [14].

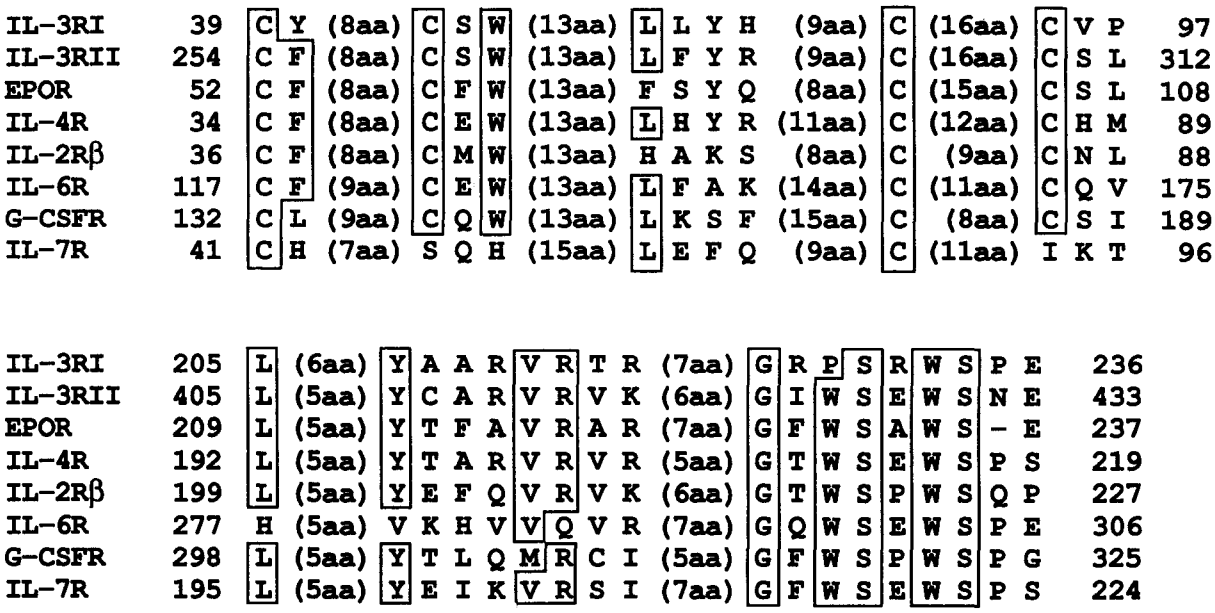

Fig. 3. Common motif of mouse cytokine receptor family. Amino acid sequence comparison of the external domains. Two homologous regions are shown

Another interesting feature of the cytokine receptors is multi-subunit structure. It is well known that the high affinity IL-2 receptor is composed of multiple subunits [15]. The high affinity IL-6 receptor is also composed of at least two subunits [16]. As described above, the high affinity IL-3 receptor requires multiple proteins. Similarly, the GM-CSF receptor cloned by Gearing et al. [17] has a low affinity. We recently isolated a cDNA encoding a second component of the GM-CSF receptor which confers high affinity GM-CSF binding with the low affinity receptor [12]. Interestingly, whereas several cytokines, whose receptor belongs to the cytokine rceptor family, clearly induce protein tyrosine phosphorylation [5, 18], none of those cytokine receptors has a kinase consensus sequence in the cytoplasmic domains nor kinase activity. Therefore functional receptors require additional components, possibly including protein kinase.

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