Monoclonal Antibodies Specific for Low-Affinity Interleukin-3 (IL-3) Binding Protein AIC2A: Evidence That AIC2A Is a Component of a High-Affinity IL-3 Receptor

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Mouse interleukin-3 (IL-3) binds to its receptor with high and low affinities. Using anti-Aic2 antibody, two distinct cDNAs (AIC2A and AIC2B) were isolated. The AIC2A gene encodes a protein of 120 Kd that binds IL-3 with low affinity, whereas the AIC2B gene encodes a protein that is 91% identical to AIC2A at the amino acid level, but which does not bind IL-3. To study the structure of the functional high-affinity IL-3 receptor (IL-3R), we generated specific monoclonal antibodies against the AIC2A protein. We produced a soluble AIC2A protein by inserting a termination codon at the beginning of the transmembrane domain of the AIC2A cDNA. Soluble AIC2A protein expressed in COS7 cells was purified to homogeneity and three anti-AIC2A monoclonal antibody-dependent proliferation. These results indicate that the AIC2A protein is a binding component of a high-affinity IL-3R.

MATERIALS AND METHODS

Cell lines, media, and cytokines. The IL-3-dependent cell lines, MC/9, FDCP2, B6SUtA, and PT18, were cultured in RPMI-1640 supplemented with 10% fetal calf serum (FCS), 50 μmol/L 2-mercaptoethanol (2-ME), and 100 U/mL IL-3. An IL-4-dependent subline of MC/9, MC/9(IL-4), was kindly provided by Dr. N. Harada (DNAX, Palo Alto, CA), and was cultured in RPMI-1640 supplemented with 10% FCS, 50 μmol/L 2-ME, and 10 ng/mL IL-4. PT18 and B6SUtA cells were transferred to RPMI-1640 media containing 10% FCS, 50 μmol/L 2-ME, and 25 U/mL GM-CSF before the IL-3 binding assays and flow cytometry. FDCP2(−) is an IL-3-independent variant derived from FDCP2 cells, and these cells were maintained in RPMI-1640 supplemented with 10% FCS and 50 μmol/L 2-ME. COS7 cells were

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cultured in Dulbecco’s modified Eagle’s medium (DMEM) containing 10% FCS. The L-cell transfectant (clone 26), which stably expresses AIC2A, was established previously. The L-cell transfectants were cultured in DMEM supplemented with 10% FCS and G418 (1 mg/mL). Recombinant mouse IL-3 was produced in silkworms infected with baculovirus containing an IL-3 cDNA. Mouse GM-CSF was produced in yeast as described previously. Escherichia coli-derived mouse IL-4 was provided by Drs N. Harada and R. Kastelein (DNAX). IL-3 was radioiodinated using Iodogen (Pierce, Rockford, IL) as described previously. Specific activity of [125I] IL-3 used for binding assays was 0.25 μCi/pmol for the high-affinity binding assay of PT18 cells. As background increases at high concentrations of radioligand, we used 0.025 μCi/pmol [125I]-IL-3 for the low-affinity binding assays of the L-cell transfectant.

Construction of the aIC2A protein. Fifty plates (10 cm) of COS7 cells (30% of confluence) were transfected with the sAIC2A cDNA by the diethyl aminoethyl (DEAE) dextran method. One day after transfection, cells were washed and cultured in DMEM without FCS for 48 hours. Supernatant was concentrated with an Amicon (Danvers, MA) membrane concentrator, PM5. One plate of transfected COS7 cells was metabolically labeled with [35S]methionine as described, and its supernatant was used as a tracer. A radiolabeled band at the molecular weight of 54 Kd appeared in the supernatant of the sAIC2A cDNA-transfected COS7 cells, but did not appear in the supernatant of mock transfected cells in reducing sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE). The 54-Kd protein was bound to IL-3–coupled resin. This 54-Kd band was monitored during chromatography steps to find fractions containing the sAIC2A protein by SDS-PAGE and autoradiography. The concentrated supernatant was applied on a Blue-affigel (20 mL) column (BioRad, Richmond, CA). The flow-through fraction was applied on Mono Q (10 mL) column (HR5/5, Pharmacia, Piscatway, NJ) eluted with 10 mmol/L Tris-HCl (pH 7.5) containing 1 mmol/L EDTA at a flow rate of 0.6 mL/min. Proteins were eluted by a linear gradient of 0 to 500 mmol/L NaCl. The pooled fractions were concentrated to 50 μL by Centricon 10 (Amicon). The concentrated fraction was applied to a Superose 12 HR10/30 (Pharmacia) eluted with 10 mmol/L Tris-HCl (pH 7.5) containing 150 mmol/L NaCl and 1 mmol/L EDTA, and eluted at a flow rate of 0.5 mL/min. Approximately 150 μg of the purified protein was obtained from 500 mL of the supernatant.

Anti-AIC2A monoclonal antibodies. Three rats were injected intraperitoneally with 10 μg of the purified sAIC2A protein with Freund’s complete adjuvant every 3 weeks. Seven weeks after the first immunization, a rat was reinmunized and its spleen cells were fused with an equal number of P3X63 cells using 50% polyethylene glycol. Fused cells were selected by hypoxanthine-aminopterin-thymidine media supplement (HAT; Sigma, St Louis, MO) in RPMI-1640 supplemented with 10% heat-inactivated FCS; after 1 week, medium containing hypoxanthine-thymidine (HT; Sigma) was used instead of HAT. Hybridoma supernatants were assayed by the solid-phase radioimmunoassorbent method. Briefly, flexible vinyl 96-well trays (Dynatech, Alexandria, VA) were coated with affinity-purified rabbit anti-rat IgG + IgM antibody (Zymed Laboratories, South San Francisco, CA) (1 μg/well). Excess binding site was blocked with bovine serum albumin (BSA). The supernatants of hybridoma cells (100 μL) were added to the wells and incubated for 30 minutes at 37°C. Plates were washed and [35S]-methionine–labeled proteins, secreted from COS7 cells transfected with the sAIC2A cDNA, were added (20,000 cpm/well). Plates were incubated at 37°C for 1 hour and washed with phosphate-buffered saline (PBS). Bound [35S]-labeled proteins were dissolved by incubation with 20 μL/well of a solution containing 2% SDS and 0.125 mmol/L Tris-HCl, pH 6.8. A 10-μL aliquot of the sample was used to measure the radioactivity by the LKB Betaplate scintillation counter (LKB Instruments, Uppsala, Sweden). The other 10 μL from the sample that showed positive signal (>50 cpm/50 μL of the supernatant) was stored and analyzed by SDS-PAGE. Autoradiography was performed using Enlighting (NEN, Boston, MA) and a set of Cronex Lightning Plus (Du Pont, Wilmington, DE) screens for 2 weeks. Three positive clones were purified by limiting dilution and transferred to RPMI-1640 media supplemented with 1% Nutridoma by stepwise substitution of 10% FCS with Nutridoma as instructed by the supplier (Boehringer Mannheim, Indianapolis, IN). The subclass of the immunoglobulin was determined by an immunodiffusion assay using the rat monoclonal typing kit (ICN Biomedicals, Costa Mesa, CA). All three monoclonal antibodies described in this report were classified as IgG2a. Immunoglobulins were purified by ammonium sulfate fractionation (50% of saturation) followed by immunofinity column chromatography with affinity-purified rabbit anti-rat IgG + IgM antibody (Zymed Laboratories) coupled with Carbolink (Pierce).

Immunopurification of the 120-Kd protein using 3D1 antibody. Purified 3D1 antibody was coupled with Carbolink (Pierce) resin at a concentration of approximately 1 mg/mL. PT18 cells (4 × 10⁴) were lysed by lysis buffer containing 1% Triton X-100, 10 mmol/L sodium phosphate buffer, pH 7.0, 100 mmol/L NaCl, 1 mmol/L EDTA, 1 mmol/L benzamide, 0.5 mmol/L phenylmethylsulfonyl fluoride (PMSF), 0.5 μg/mL pepstatin, and 5.0 μg/mL leupeptin, chymostatin, antipain, and aprotinin. The lysate was centrifuged at 8,000 g for 20 minutes, and the supernatant was applied to 4 mL of the 3D1 column at a flow rate of 2 mL/min for 2 hours at 4°C. After washing the column by lysis buffer containing 0.1% Triton X-100, proteins were eluted with 100 mmol/L glycine-HCl, pH 2.5. Protein sequencing of the 120-Kd protein. The fractions from the 3D1 column were collected and neutralized by addition of 1/5 vol of 1 mol/L Tris-HCl, pH 8.0. The proteins were precipitated by sodium deoxycholic acid and trichloroacetic acid (TCA). Proteins were separated by SDS-PAGE using MINI-PROTEAN II Ready Gel (4% to 15% gradient; BioRad) in the presence of 100 μmol/L thiglycolic acid in the upper buffer, and were then electrophoresed onto Immobilon-P membrane (Millipore, Bedford, MA) in the buffer containing 25 mmol/L Tris and 192 mmol/L glycine, pH 8.3, at 100 V for 1 hour. The membrane was stained with 0.025% Cooomasie Brilliant Blue R-250 in 40% methanol and 5% acetic acid, and destained with 30% methanol and 5% acetic acid. The band at 120 Kd was excised and sequenced on the Applied Biosystems (Foster City, CA) Protein Sequencer Model 477A with an on-line phenylthiohydantoin (PTH)-derivative analyzer Model 120A as described at the Protein Structure Laboratory, University of California, Davis, CA.

Flow cytometric analysis. Three days after transfection of COS7 cells with the receptor cDNAs, cells were detached by PBS containing 1 mmol/L EDTA. Cells incubated with anti-AIC2A antibodies were stained with fluorescein isothiocyanate (FITC)-conjugated anti-rat IgG second antibody. Cells were analyzed by
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FACScan (Becton Dickinson, San Jose, CA). For staining of factor-dependent cells, PT18 and B6SUtA, cells grown in medium containing GM-CSF were used. Cells incubated with biotinylated antibody and biotinylated IL-3 for 1 hour on ice were stained with fluorescein isothiocyanate (FITC)-conjugated antibiotin antibody. Biotinylation of IL-3 and the 9D3 antibody was performed using biotin-hydrazide (Pierce) and Enzotin (Enzo Biochem, New York, NY), respectively.

Radioligand binding assay. Iodination of IL-3 and binding assays were described previously. Briefly, cells (5 x 10^6) were incubated with [^125]IL-3 (10 nmol/L for low-affinity binding and 200 pmol/L for high-affinity binding) for 2 to 4 hours at 4°C. Cells were spun through an oil layer and cell-bound radioactivity was measured. Serial dilutions of [^125]IL-3 were mixed with PT18 cells in the presence or absence of 40 nmol/L 9D3 antibody. The value for Kd and number of the binding sites per cell were calculated by the Scatchard analysis.

Cell proliferation assay. Effects of the antibodies on the proliferation of cells were examined using 3-[4,5-DimethylthiazoIe-2-yl]-2,5-diphenyltetrazorium bromide (MTT, Sigma) as originally described by Mosmann. MC/9 cells (1 x 10^5) were incubated for 48 hours with 4 µg of purified antibody in the presence of different amounts of IL-3.

RESULTS

Purification of the sAIC2A protein. Termination codons were inserted at the beginning of the transmembrane domain of the AIC2A cDNA to produce the sAIC2A protein. COS7 cells transiently transfected with the sAIC2A cDNA secreted a protein of 54 Kd (sAIC2A protein), which was absent in COS7 cells transfected with the control vector plasmid. We purified this 54-Kd protein by sequential column chromatography on Blue-affigel, Mono Q HR5/5, and Superose 12 HR10/30 as described in Materials and Methods. The purified protein showed a single band at a molecular weight of 54 Kd when analyzed by reducing SDS-PAGE followed by either staining with Coomassie Brilliant Blue R-250 (Fig 1A) or autoradiography (Fig 1B, left lane). In the absence of 2-ME, an additional 114-Kd band was observed (Fig 1B, right lane). This 114-Kd protein

![Fig 1](image-url)

**Fig 1.** Molecular properties of purified sIL-3R. (A) Coomassie Brilliant Blue R-250 staining of the purified sAIC2A protein. One microgram of the purified sAIC2A protein was analyzed by SDS-PAGE under reducing conditions. (B) Autoradiography of the [^35S]-methionine-labeled purified sAIC2A protein (10,000 cpm). Proteins were analyzed by SDS-PAGE in the presence (+) or absence (-) of 2-ME (0.7 mol/L). (C) Gel filtration of the purified sAIC2A protein on Superose 12. Molecular weight standards used were: myoglobin, 17 Kd; carbonic anhydrase, 29 Kd; ovalbumin, 44 Kd; BSA, 66 Kd; γ-globulin, 158 Kd; thyroglobulin, 670 Kd.
may be a dimer of the 54-Kd protein. Interestingly, the purified protein was eluted at an apparent molecular weight of approximately 160 Kd from the Superose 12 gel filtration column (Fig 1C). These results suggest self-association of the sAIC2A monomers and possible involvement of intermolecular disulfide bonds.

**Generation of monoclonal antibodies against the sAIC2A protein.** The purified sAIC2A protein was used to immunize rats, and hybridomas were generated by fusing the spleen cells of the immunized rat with P3X63 cells. Hybridoma supernatants were screened by the solid-phase immunoadsorbent method using 35S-labeled proteins secreted from COS7 cells transfected with the sAIC2A cDNA as described in Materials and Methods. We found several wells that gave positive signals (>50 cpm/50 μL of the supernatant) in the initial screening and three of them (3D1, 3D4, and 9D3) were cloned (Fig 2A). 9G7 is shown as a representative negative clone (Fig 2A and B). Comparable amounts of 35S-labeled protein bound to all three antibodies and the polyclonal antibodies from the immunized rat in the solid-phase immunoadsorbent assays. The radioactive protein adsorbed on the plate was identified as the 54-Kd sAIC2A (Fig 2B).

**Specificity of the antibodies.** We examined the specificity of these antibodies using COS7 cells transiently transfected with either the AIC2A or AIC2B cDNA. The anti-Aic2 antibody, which was used to clone the AIC2 cDNAs, bound to COS7 cells transfected with either AIC2A or AIC2B cDNA equally well (indicated as A/A and B/B in Fig 3, respectively). Whereas all three antibodies bound to COS7 cells transfected with the AIC2A cDNA, none of them bound significantly to COS7 cells transfected with the AIC2B cDNA (Fig 3B).

We then made chimeric receptors using the AIC2A and AIC2B cDNAs to locate the approximate region responsible for binding of these antibodies. The extracellular portion of the AIC2A and AIC2B proteins can be divided into two domains and each contains common structural features of the cytokine receptor family (Fig 3A). The BA/A protein has the N-terminal portion of the first domain from AIC2B and the rest of the sequence from AIC2A. The B/A protein has the first domain from AIC2B and the second domain from AIC2A (Fig 3A). The 3D1 antibody bound to COS7 cells transfected with the BA/A and B/A cDNAs, but not the AIC2B cDNA, indicating that the 3D1 antibody recognizes the second domain of the AIC2A protein. The 3D4 antibody bound to COS7 cells transfected with the BA/A construct, but not with the B/A and AIC2B cDNAs, indicating that binding of this antibody requires the C-terminus of the first domain of AIC2A. Since the 9D3 antibody bound to COS7 cells transfected only with the AIC2A cDNA, 9D3 probably recognizes the N-terminus of the AIC2A protein (Fig 3A).

**N-terminal sequence of the 120-Kd immunoprecipitate by 3D1 antibody.** The three monoclonal antibodies immunoprecipitated a protein that migrates at a molecular weight of 120 Kd under reducing conditions on an SDS-PAGE. Judging from its molecular weight, this protein appeared to be an AIC2A gene product. To confirm that the 120-Kd protein was the AIC2A gene product, the protein was isolated from the gel and subjected to protein microsequencing. In the first cycle from the sequencer, three peaks of histidine (2 pmol), glycine (10 pmol), and serine (10 pmol) were observed (Fig 4, bottom). This glycine was thought to be a contaminant from the transfer buffer. After the second cycle, only one major peak was obtained in each cycle at the level of 5 pmol until the 15th cycle, except for the 11th and 13th cycles. The peptide sequence thus obtained was found to be identical to the sequence deduced from the AIC2A, but not AIC2B cDNA (Fig 4, top and bottom), confirming the specificity of the antibody to AIC2A. The N-terminal amino acid of the mature AIC2A protein is most likely

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**Fig 2.** Solid-phase radioimmunoassay. Hybridoma supernatants were assayed using 20,000 cpm of the 35S-labeled proteins secreted from COS7 cells transiently transfected with the sAIC2A cDNA as described in Materials and Methods. Radioactivity of one half of the solubilized immunoprecipitate was measured by a Betaplate scintillation counter (A) and the other half was analyzed by SDS-PAGE (B). Names of clones are shown on the bottom (A) and top (B) of the figure. Rat serum, polyclonal antibody from the immunized rat; COS, 10,000 cpm of the supernatant from 35S-labeled COS7 cells transfected with the sAIC2A cDNA.
Fig 3. Chimeric receptors between AIC2A and AIC2B and their reactivity to the monoclonal antibodies. (A) The AIC2A cDNA, cl. 2 lacking most of the cytoplasmic domain, and the AIC2B cDNA, cl. 5 having most of the cytoplasmic domain, were used to make the chimeric receptors. (B) COS7 cells transiently transfected with various receptor cDNAs were incubated with the anti-Aic2, 3D1, 3D4, and 9D3 antibodies and stained with FITC-conjugated anti-rat IgG antibody. Fluorescence was analyzed by FACSscan as described in Materials and Methods. The shaded areas show the staining profiles of anti-AIC2A monoclonal antibodies with FITC-conjugated anti-rat IgG antibody, and the clear areas show the staining with FITC-conjugated anti-rat IgG alone.

histidine, which was predicted from the amino acid sequence of the AIC2A protein.

Expression of the AIC2A protein and IL-3 binding. Expression of the AIC2A protein in various IL-3-dependent and IL-3-independent cell lines was examined by FACS analysis using the biotinylated 9D3 antibody (biotin-9D3). Biotin-IL-3 was also used to detect the IL-3 binding. PT18 and B6SUtA1 expressed a large number of the high-affinity
Amino acid sequences of AIC2 gene products deduced from cDNAs

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\begin{align*}
\text{AIC2A: MDQQMA} & \text{LTWG LOYMA} \text{VALC} \\
\text{AIC2B:} & \text{TVPLKTL} \\
\text{Amino acid sequence of } & \text{p120} \\
\text{immunoprecipitated by 3D1 antibody} \\
& \text{G A}
\end{align*}
\]

Fig 4. Comparison of the N-terminal amino acid sequences of 120-Kd immunoprecipitate with the sequence deduced from the AIC2 cDNAs. The predicted N-terminal amino acid sequences from the AIC2A cDNA and AIC2B cDNA are compared with the amino acid sequence obtained from microsequencing of the 120-Kd protein that was immunopurified using the 3D1 antibody as described in Materials and Methods. Numbers indicate the amino acids from the N-terminus of the mature protein. X indicates an unidentified amino acid residue.

IL-3R (1 to 6 x 10^6 receptors/cell estimated by [32p]IL-3 binding). These cells were brightly stained with biotin-IL-3 at 2 nmol/L and biotin-9D3 at 10 µg/mL (Fig 5A). Interestingly, an IL-3-nonresponsive variant, FDCP2(−), did not bind 9D3 (Fig 5B). Binding of [32p]IL-3 under conditions permitting detection of high-affinity IL-3 binding sites (200 pmol/L) indicated that FDCP2(−) cells had no IL-3R (Fig 5D). This result was consistent with the mRNA level of AIC2A and AIC2B in FDCP2 and FDCP2(−). MC/9(II-4) is an IL-4-dependent variant, which does not respond to IL-3, but which is derived from an IL-3-dependent mast cell line, MC/9. Whereas parental MC/9 bound the anti-Aic2 and 9D3 antibodies, as well as [32p]IL-3 (200 pmol/L), neither of these antibodies nor [32p]IL-3 bound to MC/9(II-4) (Fig 5C and D). In addition, neither AIC2A nor AIC2B mRNA was detected in MC/9(II-4) cells by S1 protection assays using the AIC2A cDNA probe (data not shown). These results suggest that the 9D3 antigen (AIC2A protein) is required for the high-affinity IL-3R.

Inhibition of [32p]IL-3 binding to PT18 cells. The possible inhibitory effect of these antibodies on the IL-3 binding
was examined under conditions permitting the detection of high-affinity IL-3 binding (200 pmol/L of $[^{125}I] \text{IL-3}$) to PT18 cells. The 9D3 antibody inhibited $[^{125}I] \text{IL-3}$ binding dose-dependently, and the maximum inhibition was approximately 50%. In contrast, no inhibition was observed with the 3D1 and 3D4 antibodies under the same conditions (Fig 6A). Similar results were obtained with another IL-3-dependent cell line, B6SUtA, (data not shown). To examine the effect of antibodies on the low-affinity IL-3 binding, L cells stably transfected with the AIC2A cDNA (cl. 26) that exhibited only low-affinity IL-3 binding were used for the binding assays. The 9D3 and 3D1 antibodies inhibited specific binding up to 87% and 37%, respectively (Fig 6B). The 9D3 antibody abolished most of the low-affinity binding sites. To determine the effect of the 9D3 antibody on the IL-3 binding more quantitatively, Scatchard analysis was performed in the presence or absence of the 9D3 antibody (Fig 6C). In the absence of the 9D3 antibody, $[^{125}I] \text{IL-3}$ bound to PT18 cells with high and low affinities: $k_d$ values were 138 pmol/L and 20 nmol/L, respectively. In the presence of the 9D3 antibody, the numbers of both high- and low-affinity binding sites decreased substantially, suggesting that the 9D3 antigen (AIC2A protein) is a binding component of a high-affinity IL-3R.

Inhibition of IL-3 activity by anti-AIC2 antibodies. Effects of the 3D1 and 9D3 antibodies on IL-3-dependent proliferation of MC/9 were examined to evaluate their potential to inhibit IL-3 function. Whereas the 3D1 antibody had no effect on IL-3-dependent proliferation of MC/9, the 9D3 antibody inhibited proliferation from 20% to 50% (Fig 7), consistent with the binding results (Fig 6). Maximal inhibition seemed to be reversed by increased IL-3 concentrations. In addition, neither 9D3 nor 3D1 antibodies (4 μg/mL) showed any growth stimulatory effect on MC/9 cells (data not shown).

**DISCUSSION**

The molecular weight of the sAIC2A protein produced in COS7 cells was estimated to be 160 Kd by gel filtration, whereas the purified protein migrated at 54 Kd on reducing SDS-PAGE and an additional 114-Kd protein was detected under nonreducing conditions (Fig 1). These results suggest that the sAIC2A protein secreted from transfected COS7 cells forms a dimer or oligomer. Association of receptors by ligands is observed in various growth factor receptors such as the epidermal growth factor (PDGF) receptors. Moreover, truncated soluble IL-2R p55 chain was secreted as a homodimer of two noncovalently associated 40-Kd subunits. Although higher molecular weight protein (> 200 Kd) is sometimes detectable by cross-linking of the IL-3R with $[^{125}I] \text{IL-3}$ (data not shown), it is not clear at present whether the membrane-bound natural IL-3R forms oligomers. The physiologic

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**Fig 6. Inhibition of $[^{125}I] \text{IL-3}$ binding by the antibodies.** Cell-bound radioactivity was measured as described in Materials and Methods. (A) PT18 cells (5 × 10⁶ cells/100 μL) were incubated with $[^{125}I] \text{IL-3}$ (200 pmol/L) and various concentrations of unlabeled IL-3 (----), 9D3 (----), 3D1 (-----), or 3D4 (-----) at 4°C for 2 hours. (B) L-cell stable transfectants expressing AIC2A (5 × 10⁶ cells/100 μL) were incubated with 10 nmol/L $[^{125}I] \text{IL-3}$ in the presence or absence of 40 nmol/L of 3D1, 3D4, 9D3, or unlabeled IL-3 at 4°C for 4 hours. Each point represents a mean value of duplicate experiments. (C) PT18 cells (5 × 10⁶ cells/100 μL) were incubated with various concentrations of $[^{125}I] \text{IL-3}$ in the presence (○) or absence (●) of 40 nmol/L of the 9D3 antibody at 4°C for 2 hours. Values for $k_d$ and binding sites per cell were analyzed by the method of Scatchard.
Significance of the oligomerization of the sAIC2A protein must be carefully evaluated, since the sAIC2A protein was abundantly expressed in COS7 cells, which usually do not express IL-3R.

The three monoclonal antibodies we have produced in this study specifically recognize the AIC2A protein. They did not bind to the AIC2B protein despite its high degree of sequence homology to the AIC2A protein. They recognize different regions of the extracellular domain of the AIC2A protein as determined by binding to chimeric receptors between AIC2A and AIC2B (Fig 2). Using the 9D3 antibody, which appears to recognize the N-terminal region of the AIC2A protein, we examined binding on two sets of IL-3-dependent cell lines and their IL-3-independent variant cell lines: FDCP2 versus FDCP(−) and MC/9 versus MC/9(IL-4). Binding of the 9D3 antibody was consistent with the IL-3 binding that was measured by either biotin-[125I]IL-3 or [125I]IL-3: loss of IL-3 binding in IL-3-independent variants, FDCP2(−) and MC/9(IL-4), was coincident with the loss of the AIC2A protein detected by the 9D3 antibody (Fig 5). These results support the idea that the AIC2A protein is a binding component of the high-affinity IL-3R. This notion is further supported by the observation that the 9D3 antibody inhibits both high- and low-affinity IL-3 binding and also partially inhibits IL-3–dependent proliferation (Figs 6 and 7).

Recently, we have cloned the cDNA for the human IL-3R α-subunit and reconstituted a high-affinity human IL-3R with the β-subunit of the human GM-CSF receptor (a human homologue of AIC2A and AIC2B). Interestingly, although the human IL-3R α-subunit binds IL-3 with extremely low affinity and the β-subunit has no binding activity by itself, a combination of these two subunits confers high-affinity IL-3 binding. Moreover, reconstitution experiments clearly indicate that the IL-3R and GM-CSF receptor share the same β-subunit in humans. In addition, the high-affinity IL-5R may also use the same β-subunit in humans. In contrast to man, the mouse has two genes, AIC2A and AIC2B, which are homologous to the human common β-subunit of the IL-3R and GM-CSF receptor. Recent evidence indicates that the AIC2B protein is the β-subunit for both mouse IL-5R and GM-CSF receptor.

As described above, the 9D3 antibody specific for the AIC2A protein inhibits the IL-3 binding, indicating that AIC2A is a component of the mouse IL-3R. Because of the structural similarity to the human common β-subunit, AIC2A appears to be the β-subunit. Therefore, it is likely that a putative mouse α-subunit in combination with the AIC2A protein binds IL-3 with high affinity. If there are residual low-affinity IL-3 binding sites in PT18 cells in the presence of 9D3 antibody (Fig 6), it may be due to the loss of the putative mouse IL-3R α-subunit. Because the human β-subunit is shared by both IL-3R and GM-CSF receptor, the putative mouse IL-3R α-subunit may also form a high-affinity IL-3R with the AIC2B protein. This hypothesis may explain the observation that the 9D3 antibody did not completely inhibit high-affinity IL-3 binding in PT18 cells (Fig 6). Identification of the putative mouse IL-3R α-subunit and reconstitution of a high-affinity IL-3R is necessary to clarify this issue.

Because the antibodies described in this report are capable of immunoprecipitating the AIC2A protein and can be used for staining IL-3–dependent cells, these antibodies will help to identify proteins associated with AIC2A and to study the expression of the IL-3R.

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Monoclonal antibodies specific for low-affinity interleukin-3 (IL-3) binding protein AIC2A: evidence that AIC2A is a component of a high-affinity IL-3 receptor

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