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Cloning and characterization of a binding subunit of the interleukin 13 receptor that is also a component of the interleukin 4 receptor

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ABSTRACT **Interleukins 4 (IL-4) and 13 (IL-13) have been found previously to share receptor components on some cells, as revealed by receptor cross-competition studies. In the present study, the cloning is described of murine NR4, a previously unrecognized receptor identified on the basis of sequence similarity with members of the hemopoietin receptor family. mRNA encoding NR4 was found in a wide range of murine cells and tissues. By using transient expression in COS-7 cells, NR4 was found to encode the IL-13 receptor α chain, a low-affinity receptor capable of binding IL-13 but not IL-4 or interleukins 2, -7, -9, or -15. Stable expression of the IL-13 receptor α chain (NR4) in CTLL-2 cells resulted in the generation of high-affinity IL-13 receptors capable of transducing a proliferative signal in response to IL-13 and, moreover, led to competitive cross-reactivity in the binding of IL-4 and IL-13. These results suggest that the IL-13 receptor α chain (NR4) is the primary binding subunit of the IL-13 receptor and may also be a component of IL-4 receptors.**

Interleukin (IL) 13 (IL-13) is a cytokine that shares a number of structural characteristics with IL-4 (for review, see refs. 1 and 2). The genes for IL-4 and IL-13 have a related intron/exon structure and are adjacent on both human chromosome 5 and the syntenic region of mouse chromosome 11 (1, 2). At the protein level, IL-4 and IL-13 share ≈30% amino acid identity, including four cysteine residues (1, 2). Biologically, IL-13 and IL-4 are also similar, being produced by activated T cells and being able to act on macrophages to induce differentiation and suppress the production of inflammatory cytokines (1, 2). Additionally, human IL-13 but not murine IL-13 may act as a costimulatory signal for B-cell proliferation and affect immunoglobulin isotype switching (1, 2). Unlike IL-4, however, IL-13 has not been found to exhibit biological actions on T cells (1, 2).

Two subunits of the IL-4 receptor have been identified: the IL-4-receptor α chain (IL-4Rα; ref. 3) and the γ chain of the interleukin 2 receptor (IL-2Rγ; ref. 4); however, the components of the IL-13 receptor remain less well defined. Many studies have provided evidence that IL-4 and IL-13 share receptor components, although their relationship appears quite complex (5–13). Several observations need to be considered when formulating a model of IL-4 and IL-13 receptors. (*i*) The IL-4Rα appears specific for IL-4 because unlabeled IL-4 but not IL-13 can compete for ^{125}I-labeled IL-4 binding to COS-7 cells in which the IL-4Rα is overexpressed (3, 5). Moreover, soluble IL-4Rα can inhibit the biological action of IL-4 but not of IL-13 (12). (*ii*) The binding component of the IL-13 receptor, the IL-13-receptor α chain (IL-13Rα), also appears specific because unlabeled IL-13 but not unlabeled IL-4 can compete for the cross-linking of ^{125}I-labeled IL-13 to a M_r 56,000–68,000 protein expressed by renal cells (10). (*iii*) Despite binding to specific receptor components, cross-competition by IL-4 and IL-13 for receptors is observed in certain cell types (5, 9, 10, 13). The extent of competition varies greatly, and on the basis of analogies with receptors, such as the IL-3, IL-5, and granulocyte/macrophage colony-stimulating factor receptors, a likely explanation for cross-competition is that IL-4 and IL-13 may share one or more receptor components. (*iv*) Although the IL-2Rγ is required for IL-4 signal transduction in some cells (14–20), other IL-4-responsive cell lines, such as the plasmacytoma B9 and renal cell lines, do not express the IL-2Rγ (10, 11). This result has led to the suggestion that there are two classes of functional IL-4 receptor (12, 13). The IL-2Rγ is unlikely to be the component shared by the IL-4 and IL-13 receptors because IL-13 is capable of transducing a signal in humans with X chromosome-linked severe combined immunodeficiency resulting from a mutation in the IL-2Rγ gene (20) and some cell lines in which IL-4 and IL-13 cross-compete for receptor binding do not express the IL-2Rγ (10, 11). (*v*) Although binding to the IL-4Rα is specific, neutralizing antibodies to the IL-4Rα inhibit both IL-4 and IL-13 action, suggesting that the IL-4Rα is a component of the IL-13 receptor (12, 13). (*vi*) Two observations suggest that a second receptor component, possibly the putative IL-13Rα, may be shared between IL-4 and IL-13 receptors. First, although IL-4 does not compete for binding of ^{125}I-labeled IL-13 to the M_r 56,000–68,000 IL-13Rα expressed by renal cells (10), competition is observed on other cell types (13). Second, a mutant of IL-4, Y124D, binds to isolated IL-4Rα with the expected affinity but binds to IL-4 receptors expressed by IL-4 responsive cell lines with a 100-fold reduced affinity. Y124D also antagonizes IL-4 and IL-13 action, suggesting that the former is defective in its ability to interact with a shared component of the IL-4 and IL-13 receptors required for generation of functional high-affinity receptors.

An attractive model that incorporates these observations was recently proposed by Leonard and colleagues (12). The central thesis of this model is that there are two types of IL-4 receptor but a single type of IL-13 receptor. IL-4 receptors are proposed to be formed by an initial interaction between IL-4 and the IL-4Rα, followed by recruitment of either the IL-2Rγ or the putative IL-13Rα to yield a complex capable of signal transduction. Conversely, IL-13 is proposed to bind directly to the putative IL-13Rα, leading to recruitment of the IL-4Rα to form a functional receptor.

The major barrier to validation of this model has been the lack of a cDNA encoding the IL-13-binding subunit (IL-13Rα). The present study describes the cloning of a cDNA for

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Abbreviations: IL, interleukin; IL-4Rα, interleukin 4-receptor α chain; IL-13Rα, interleukin 13-receptor α chain; IL-2Rγ, interleukin 2-receptor γ chain.

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a previously undescribed member of the hemopoietin receptor family termed NR4. NR4 is shown to be the IL-13Rα, and evidence is presented that both the IL-13Rα (NR4) and the IL-4Rα are common components of the IL-13 and IL-4 receptors.

MATERIALS AND METHODS

Isolation of Genomic and cDNAs Encoding Murine NR4. *Apo* I-digested genomic DNA, extracted from an embryonal stem cell line, was cloned into the λ ZAP II bacteriophage (Stratagene). Plaques (10^6) from this library were screened with a ^{32}P-labeled oligonucleotide corresponding to the sequence WSDWS (21). Positively hybridizing clones were sequenced by using an automated DNA sequencer according to the manufacturer's instructions (Applied Biosystems). One clone appeared to encode for part of a previously undescribed member of the hemopoietin receptor family. Oligonucleotides were designed on the basis of this genomic DNA sequence and were used in the conventional manner to isolate clones from mouse peritoneal macrophage (Clontech), mouse skin, mouse lung, mouse kidney, and WEHI-3B (Stratagene) λ-bacteriophage cDNA libraries.

Construction of Expression Vectors and Transfection of Cells. Using the PCR, a derivative of the NR4 cDNA was generated that encoded the IL-3 signal sequence (MVLASSTTSIHTMLLLLLMLFHLGLQASIS) and an N-terminal FLAG epitope tag (DYKDDDDK) preceding the mature coding region of NR4 (Thr-27 to Pro-424; Fig. 1). The PCR product was cloned into the mammalian expression vector pEF-BOS (22). Constructs were sequenced in their entirety before use. Cells were transfected and selected as described (21, 23).

RNA Extraction and Northern Blots. Northern blots were performed as described (24). The sources of hybridization probes were as follows: NR4—a PCR product from nt 1477 to 1824 (Fig. 1)—and glyceraldehyde-3-phosphate dehydrogenase—a 1.2-kbp *Pst* I fragment of chicken glyceraldehyde-3-phosphate dehydrogenase cDNA (25).

Cytokines and Binding Experiments with Radioiodinated Cytokines. Purified recombinant murine IL-2, IL-4, IL-7, IL-9, IL-13, and IL-15 were obtained commercially (R&D Systems). For radioiodination cytokines were dissolved at 50 μg/ml in 20 mM sodium phosphate/150 mM NaCl (phosphate-buffered saline)/0.02% (vol/vol) Tween 20 at pH 7.0. Two micrograms of IL-13 was radioiodinated by using the iodine monochloride method (26, 27), and 2 μg of IL-4 was radiolabeled using dioiodo-Bolton–Hunter reagent (14). Binding studies and determination of specific radioactivity and bindability of labeled cytokines were done as described (27).

Proliferation Assays. The proliferation of CTLL-2 cells in response to cytokines was measured in Lux 60 microwell HL-A plates (Nunc). Cells were washed three times in Dulbecco's modified Eagle's medium/20% (vol/vol) newborn calf serum and resuspended at 2×10^4 cells per ml in the same medium. Samples of 10 μl of the cell suspension were placed in the culture wells with 5 μl of various concentrations of purified recombinant cytokines. After 2 days of incubation at 37°C in a fully humidified atmosphere of 10% CO_2/90% air, viable cells were counted by using an inverted microscope.

RESULTS

Cloning and Characterization of Murine NR4. A library was constructed in λ ZAP II using *Apo* I-digested genomic DNA from embryonal stem cells and screened using a pool of ^{32}P-labeled oligonucleotides encoding the amino acid sequence Trp-Ser-Asp-Trp-Ser common to many members of the hemopoietin receptor family. One hybridizing clone was found to contain a sequence that appeared to encode part of a previously undescribed member of the hemopoietin receptor family. This putative receptor was given the operational name NR4. The sequence of the partial genomic clone was used to isolate cDNAs encoding NR4 from WEHI-3B cell, peritoneal macrophage, bone marrow, skin, and kidney libraries. A composite of the nucleotide and predicted amino acid sequence of these cDNAs is shown in Fig. 1. The NR4 cDNA is predicted to encode a protein of 424 amino acid residues,

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-60   tgaaaagatagaataaatggcctcgtgccgaattcggcacgagccgaggcgagggcctgc

1     ATGGCGCGGCCAGCGCTGCTGGGCGAGCTGTTGGTGCTGCTACTGTGGACCGCCACCGTG
1      M  A  R  P  A  L  L  G  E  L  L  V  L  L  L  W  T  A  T  V

61    GGCCAAGTTGCCGCGGCCACAGAAGTTCAGCCACCTGTGACGAATTTGAGCGTCTCTGTC
21     G  Q  V  A  A  A  T  E  V  Q  P  P  V  T  N  L  S  V  S  V

121   GAAAATCTCTGCACGATAATATGGACGTGGAGTCCTCCTGAAGGAGCCAGTCCAAATTGC
41     E  N  L  C  T  I  I  W  T  W  S  P  P  E  G  A  S  P  N  C

181   ACTCTCAGATATTTTAGTCACTTTGATGACCAACAGGATAAGAAAATTGCTCCAGAAACT
61     T  L  R  Y  F  S  H  F  D  D  Q  Q  D  K  K  I  A  P  E  T

241   CATCGTAAAGAGGAATTACCCCTGGATGAGAAAATCTGTCTGCAGGTGGGCTCTCAGTGT
81     H  R  K  E  E  L  P  L  D  E  K  I  C  L  Q  V  G  S  Q  C

301   AGTGCCAATGAAAGTGAGAAGCCTAGCCCTTTGGTGAAAAAGTGCATCTCACCCCCTGAA
101    S  A  N  E  S  E  K  P  S  P  L  V  K  K  C  I  S  P  P  E

361   GGTGATCCTGAGTCCGCTGTGACTGAGCTCAAGTGCATTTGGCATAACCTGAGCTATATG
121    G  D  P  E  S  A  V  T  E  L  K  C  I  W  H  N  L  S  Y  M

421   AAGTGTTCCTGGCTCCCTGGAAGGAATACAAGCCCTGACACACACTATACTCTGTACTAT
141    K  C  S  W  L  P  G  R  N  T  S  P  D  T  H  Y  T  L  Y  Y

481   TGGTACAGCAGCCTGGAGAAAAGTCGTCAATGTGAAAACATCTATAGAGAAGGTCAACAC
161    W  Y  S  S  L  E  K  S  R  Q  C  E  N  I  Y  R  E  G  Q  H

541   ATTGCTTGTTCCTTTAAATTGACTAAAGTGGAACCTAGTTTTGAACATCAGAACGTTCAA
181    I  A  C  S  F  K  L  T  K  V  E  P  S  F  E  H  Q  N  V  Q

601   ATAATGGTCAAGGATAATGCTGGGAAAATTAGGCCATCCTGCAAAATAGTGTCTTTAACT
201    I  M  V  K  D  N  A  G  K  I  R  P  S  C  K  I  V  S  L  T

661   TCCTATGTGAAACCTGATCCTCCACATATTAAACATCTTCTCCTCAAAAATGGTGCCTTA
221    S  Y  V  K  P  D  P  P  H  I  K  H  L  L  L  K  N  G  A  L

721   TTAGTGCAGTGGAAGAATCCACAAAATTTTAGAAGCAGATGCTTAACTTATGAAGTGGAG
241    L  V  Q  W  K  N  P  Q  N  F  R  S  R  C  L  T  Y  E  V  E

781   GTCAATAATACTCAAACCGACCGACATAATATTTTAGAGGTTGAAGAGGACAAATGCCAG
261    V  N  N  T  Q  T  D  R  H  N  I  L  E  V  E  E  D  K  C  Q

841   AATTCCGAATCTGATAGAAACATGGAGGGTACAAGTTGTTTCCAACTCCCTGGTGTTCTT
281    N  S  E  S  D  R  N  M  E  G  T  S  C  F  Q  L  P  G  V  L

901   GCCGACGCTGTCTACACAGTCAGAGTAAGAGTCAAAACAAACAAGTTATGCTTTGATGAC
301    A  D  A  V  Y  T  V  R  V  R  V  K  T  N  K  L  C  F  D  D

961   AACAAACTGTGGAGTGATTGGAGTGAAGCACAGAGTATAGGTAAGGAGCAAAACTCCACC
321    N  K  L  W  S  D  W  S  E  A  Q  S  I  G  K  E  Q  N  S  T

1021  TTCTACACCACCATGTTACTCACCATTCCAGTCTTTGTCGCAGTGGCAGTCATAATCCTC
341    F  Y  T  T  M  L  L  T  I  P  V  F  V  A  V  A  V  I  I  L

1081  CTTTTTTACCTGAAAAGGCTTAAGATCATTATATTTCCTCCAATTCCTGATCCTGGCAAG
361    L  F  Y  L  K  R  L  K  I  I  I  F  P  P  I  P  D  P  G  K

1141  ATTTTTAAAGAAATGTTTGGAGACCAGAATGATGATACCCTGCACTGGAAGAAGTATGAC
381    I  F  K  E  M  F  G  D  Q  N  D  D  T  L  H  W  K  K  Y  D

1201  ATCTATGAGAAACAATCCAAAGAAGAAACGGATTCTGTAGTGCTGATAGAAAACCTGAAG
401   I  Y  E  K  Q  S  K  E  E  T  D  S  V  V  L  I  E  N  L  K

1261  AAAGCAGCTCCTTGAtggggagaagtgatttctttcttgccttcaatgtgaccctgtgaa
421    K  A  A  P  *

1321  gatttattgcattctccatttgttatctgggggacttgttaaatagaaactgaaactact
1381  cttgaaaaacaggcagctcctaagagccacaggtcttgatgtgacttttgcattgaaaac
1441  ccaaacccaaaggagctccttccaagaaaagcaagagttcttctcgttccttgttccaat
1501  ccctaaaagcagatgttttgccaaatccccaaactagaggacaaagacaaggggacaatg
1561  accatcaattcatctaatcaggaattgtgatggcttcctaaggaatctctgcttgctctg
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FIG. 1. Nucleotide and predicted amino sequence of NR4. The untranslated region is shown in lowercase letters, and the translated region is shown in uppercase letters. The conventional one-letter code for amino acids is used, potential asparagine-linked glycosylation sites are underlined, and the conserved cysteine residues and WSXWS motif of hemopoietin receptor family members are shown in boldface type. The predicted signal sequence is underlined in boldface; the transmembrane domain is underlined with dashes. This sequence is a composite derived from an analysis of eight cDNA clones derived from three libraries. The 5′ end of the sequence (nt −60 to 351) is derived from a single cDNA clone but is also present in genomic DNA clones that have been isolated (data not shown).

containing a putative signal sequence and transmembrane domain. The extracellular region of the protein contains an immunoglobulin-like domain (amino acids 27–117), in addition to a typical hemopoietin receptor domain (amino acids 118–340), which includes four conserved cysteine residues and the characteristic WSXWS motif (Fig. 1). The cytoplasmic tail of the receptor is 60 amino acids in length.

Expression Pattern of NR4 cDNA. The pattern of NR4 mRNA expression was examined by Northern blot analyses of RNA extracted from a range of adult mouse tissues. Two hybridizing species of 5.2 and 2.2 kb in length were detected in mRNA from most tissues (Fig. 2), consistent with the diversity of cDNA libraries from which NR4 cDNAs were cloned. Notably, NR4 mRNA was not detectable in skeletal muscle (Fig. 2).

NR4 Encodes IL-13Rα—A Specific Binding Subunit of IL-13 Receptor. By using an anti-FLAG antibody as a probe for an immunoblot, an estimate of 55,000–65,000 was obtained for the apparent M_r of NR4 expressed in COS-7 cells (data not shown). This estimate was similar to the size of the IL-13Rα measured in cross-linking studies (10, 13), suggesting that NR4 might encode the binding subunit of the IL-13 receptor. To address this issue, binding of IL-4 and IL-13 was examined with NR4 over-expressed in COS-7 cells. Untransfected COS-7 cells expressed low levels of IL-4 and IL-13 receptors (data not shown). Upon transfection with a plasmid containing the NR4 cDNA, the number of IL-13 receptors but not IL-4 receptors expressed by COS-7 cells was dramatically increased (Fig. 3*A*; 100,000–500,000 receptors per cell). The affinity of IL-13 for NR4 expressed by COS-7 cells was low ($K_D \approx$ 2–10 nM), and binding was specific because it was competed for by unlabeled IL-13 but not by other cytokines, including IL-2, IL-4, IL-7, IL-9, or IL-15 (Fig. 4*A*; data not shown). These results suggest that NR4 is the IL-13Rα.

IL-13Rα (NR4) and IL-4Rα Are Shared Components of IL-4 and IL-13 Receptors. To investigate the relationship between IL-4 and IL-13 receptors, the IL-4-responsive cell line CTLL-2 was examined. Parental CTLL-2 cells expressed a single class of IL-4 receptor ($K_D \approx$ 660 pM; ≈3600 receptors per cell) but no detectable IL-13 receptors (Fig. 3*B*). The IL-4 receptors expressed by CTLL-2 cells appeared specific because binding of ^{125}I-labeled IL-4 was inhibited by unlabeled IL-4 but not by IL-13 (Fig. 4*B*).

Upon expression of IL-13Rα (NR4) in CTLL-2 cells no change was seen in the number or affinity of IL-4 receptors,

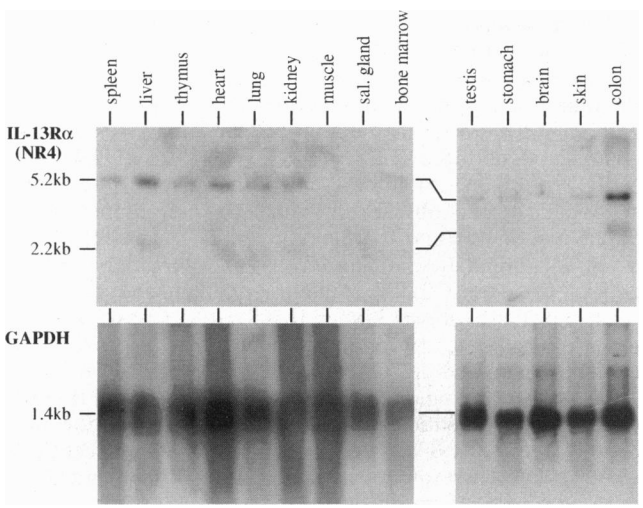

FIG. 2. Northern analysis of NR4 mRNA expression in adult mouse tissues. Membranes were hybridized with an IL-13Rα (NR4) probe, stripped, and rehybridized with a glyceraldehyde-3-phosphate dehydrogenase (GAPDH) probe. sal., Salivary.

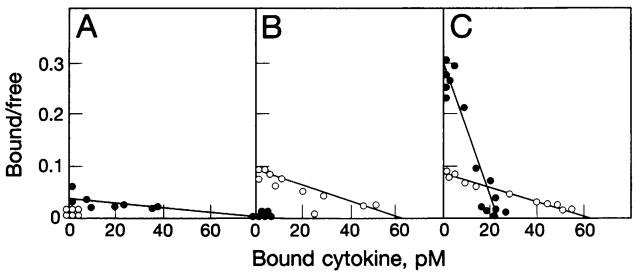

FIG. 3. Saturation isotherms of ^{125}I-labeled IL-13 and ^{125}I-labeled IL-4 binding. Saturation isotherms depicted as Scatchard plots of IL-4 (○) and IL-13 (●) binding to COS-7 cells expressing IL-13Rα (NR4) (*A*), CTLL-2 cells (*B*), and CTLL-2 cells expressing IL-13Rα (NR4) (*C*). Data have been normalized to 1×10^4 COS-7 cells and 1×10^6 CTLL-2 cells, and binding was done at 4°C for 2–4 hr.

whereas a single class of high-affinity IL-13 receptors was detected (Fig. 3*C*; $K_D \approx$ 75 pM; 1350 receptors per cell). The affinity of IL-13 for IL-13Rα (NR4) expressed in CTLL-2 cells was higher than in COS-7 cells, suggesting that the former expressed a protein capable of interacting with the IL-13Rα (NR4) to increase the affinity for IL-13. A likely candidate based on previous studies is the IL-4Rα. To explore this possibility we assessed the ability of IL-4 to compete with ^{125}I-labeled IL-13 for binding to CTLL-2 cells expressing the IL-13Rα (NR4). Fig. 4*B* shows that IL-4 and IL-13 were equally effective in competing for ^{125}I-labeled IL-13 binding to CTLL-2 cells expressing NR4 ($IC_{50} \approx$ 300 pM; Fig. 4*C*) and, in addition, could compete with ^{125}I-labeled IL-4 for binding ($IC_{50} \approx$ 300 pM; Fig. 4*D*).

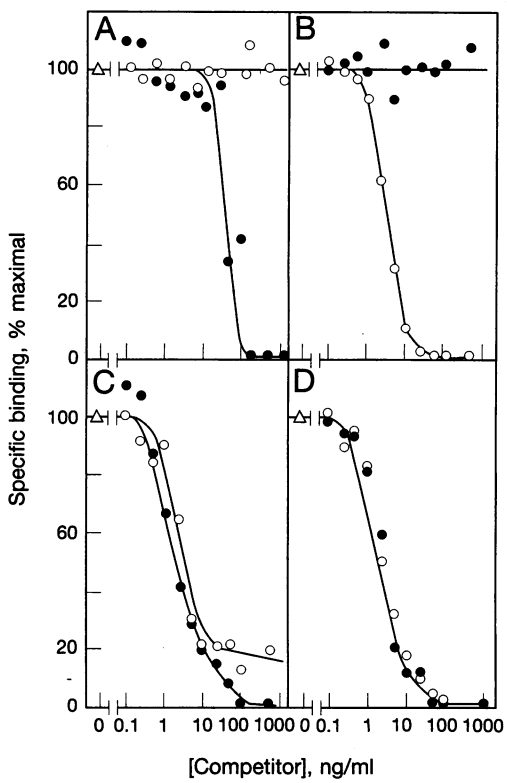

FIG. 4. Specificity of IL-4 and IL-13 binding. The ability of IL-4 (○) and IL-13 (●) to compete for ^{125}I-labeled IL-13 binding to COS-7 cells expressing IL-13Rα (NR4) (*A*) and CTLL-2 cells expressing IL-13Rα (NR4) (*C*) or to compete for ^{125}I-labeled IL-4 binding to CTLL-2 cells (*B*) and CTLL-2 cells expressing IL-13Rα (NR4) (*D*). Binding was done at 4°C for 2–4 hr, and data have been expressed as percentage of specific binding seen without a competitor (△).

Expression of IL-13Rα (NR4) Permits Transduction of a Proliferative Signal by IL-13. CTLL-2 cells require the addition of exogenous cytokines for survival and proliferation. IL-2 was found to be a potent proliferative stimulus for CTLL-2 cells ($EC_{50} \approx 100$–200 pM), whereas IL-4 was relatively weak (EC_{50} 2–7 nM), and IL-13 was inactive (Fig. 5*A*). Expression of the IL-13Rα (NR4) in CTLL-2 cells conferred on them ability to survive and proliferate weakly in response to IL-13 ($EC_{50} \approx 700$ pM) and to proliferate somewhat more strongly than parental cells in response to IL-4 ($EC_{50} \approx 700$ pM; Fig. 5*B*). The response observed by CTLL-2 cells expressing NR4 to IL-4 and IL-13 was far weaker than observed to IL-2.

DISCUSSION

The ability to identify members of the hemopoietin receptor family on the basis of sequence similarity (13) enabled identification of the gene and cDNA clones encoding an additional member of this receptor family (Fig. 1). This putative receptor was given the working name NR4. The apparent molecular weight of NR4, expressed by COS-7 cells, was estimated to be 55,000–65,000 (data not shown) and was similar to the likely size of the IL-13Rα, as estimated from cross-linking studies using ^{125}I-labeled IL-13 (9, 10, 13). We therefore assessed the ability of IL-13 to bind to NR4 and found that NR4 bound IL-13 specifically and with low affinity and was, therefore, the IL-13Rα (Figs. 3*A* and 4*A*). Additionally, evidence was found that IL-13Rα (NR4) and IL-4Rα are likely to be shared components of both IL-4 and IL-13 receptors (Figs. 3*C* and 4 *C* and *D*).

Expression in COS-7 cells demonstrated that the primary binding specificity of the IL-13Rα (NR4) is for IL-13 (Fig. 4*A*), a conclusion also reached in analyses of IL-13 binding to renal carcinoma cells (10). Furthermore, CTLL-2 cells bind and respond to IL-4 but not IL-13 (Fig. 5*A*), suggesting that the IL-13Rα (NR4) is not an obligatory component of all IL-4 receptors. Because CTLL-2 cells also respond to IL-2 (Fig. 5*A*), it is likely that IL-2Rγ interacts with IL-4Rα in these cells. Expression of the IL-13Rα (NR4) in CTLL-2 cells generated an IL-13 receptor that had a higher affinity than observed for the IL-13Rα (NR4) expressed in COS-7 cells (75 pM vs. 2–10 nM). This result suggests that CTLL-2 cells express a second nonbinding component of the IL-13 receptor responsible for affinity conversion. On the basis of previous studies (refs. 5–13; see the introduction), the IL-4Rα appeared to be a good candidate for this second component, and this hypothesis was further strengthened by the fact that IL-4 could compete for binding of IL-13 to CTLL-2 cells expressing IL-13Rα (NR4). The reciprocal was also observed with IL-13 competing with IL-4 for binding, further suggesting that IL-13Rα (NR4), like IL-2Rγ, can interact with IL-4Rα. No increase in affinity of the IL-4 receptor expressed by CTLL-2 was seen upon transfection with NR4, suggesting that IL-2Rγ and the IL-13Rα are equally effective in generating high-affinity IL-4 receptor complexes. This proposition will be better tested by measuring the affinity of IL-4 for the IL-4Rα expressed in the presence or absence of IL-13Rα and IL-2Rγ, in a cell type, such as L-cells, that normally expresses none of these components.

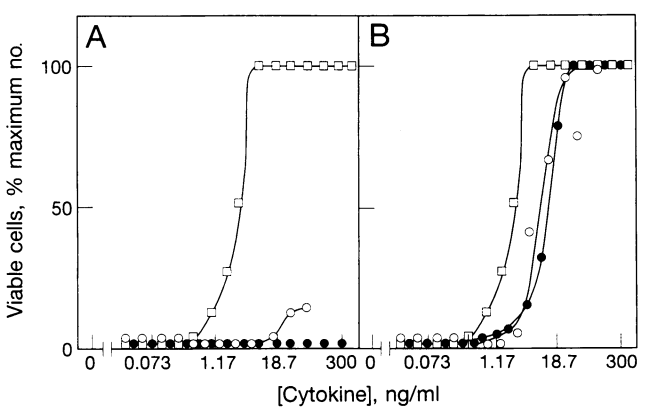

FIG. 5. Factor-dependent proliferation of cells expressing NR4. Two hundred CTLL-2 cells (*A*) or CTLL-2 cells expressing IL-13Rα (NR4) (*B*) were incubated in the absence of cytokine or with various concentrations of IL-2 (□), IL-4 (○), or IL-13 (●). After 48 hr, viable cells were counted, and data were expressed as a percentage of the number of viable cells seen with maximal IL-2 concentration.

The degree to which the IL-4Rα and IL-13Rα can transmit a proliferative stimulus is unclear. Although CTLL-2 cells expressing these receptor components survive and proliferate weakly in response to IL-4 and IL-13, preliminary experiments, in which the IL-13Rα (NR4) was expressed in the IL-3- and IL-4-responsive cell line Ba/F3, failed to yield evidence of the ability of IL-13 to stimulate cell survival or division (data not shown). The inability of Ba/F3 cells expressing the IL-13Rα and IL-4Rα to proliferate in response to IL-13 highlights the complexity of this receptor system and suggests that other proteins, whether signaling molecules or receptor subunits, are required for a robust response to IL-13.

The data presented in this study are consistent with previous work (5–13) and the model proposed by Leonard and colleagues (12) in which high-affinity IL-4 receptors may be composed of the IL-4Rα and either the IL-13Rα (NR4) or the IL-2Rγ, whereas high-affinity IL-13 receptors are formed by the combination of IL-13Rα (NR4) with IL-4Rα. Cross-competition would then occur if a single receptor component can act either as a primary binding subunit or a secondary affinity subunit component but cannot perform both functions simultaneously.

The observation that IL-4 and IL-13 compete to different extents for binding to different cell types is consistent with the model outlined above and is consistent with data presented in this study. When the IL-13Rα (NR4) is expressed in the absence of the IL-4Rα, as observed in COS-7 cells expressing IL-13Rα (NR4) and presumably certain renal cell lines, binding will be specific for IL-13, and IL-4 will be unable to compete (Fig. 4*A*; ref. 10). Conversely, when IL-4Rα is expressed in the absence of the IL-13Rα (NR4), as in CTLL-2 cells (Fig. 4*B*) and, presumably, SP-21 cells (5), IL-13 cannot compete for IL-4 binding. When both IL-13Rα (NR4) and IL-4Rα are coexpressed, cross-competition for binding will occur, and the extent of competition will depend on the relative expression levels of each receptor subunit.

The IL-4 mutant Y124D binds to IL-4Rα with the expected affinity but with a 100-fold reduced affinity to IL-4 receptors expressed by responsive cells (5, 13). This result suggests that the mutation abrogates interaction with a secondary receptor component required for generation of high-affinity IL-4 receptors, possibly the IL-2Rγ and/or IL-13Rα (NR4). That Y124D is unable to interact with the IL-13Rα is further suggested by the observation that the mutant acts as an antagonist of IL-4 and IL-13 action, on TF-1 cells (5, 13) and anti-CD40-stimulated human B cells (6). Interestingly, Y124D acts as an agonist in the stimulation of murine T-cell line CT.h4S, which expresses human IL-4Rα (28). Considering that murine T-cells are not known to be IL-13 responsive, an explanation of these results may be that the mutation Y124D affects the IL-4 region responsible for binding to IL-13Rα (NR4) more dramatically than the region required for interaction with IL-2Rγ. Whether this is a consistent effect needs to be clarified by examining other IL-4-responsive but IL-13-unresponsive cell lines.

The sharing of receptor subunits by IL-4 and IL-13 is most similar to the situation for leukemia inhibitory factor and oncostatin M. Leukemia inhibitory factor binds first to the leukemia inhibitory factor receptor α chain and uses gp130 to

generate a high-affinity receptor capable of signal transduction, whereas oncostatin M binds directly to gp130 and subsequently recruits the leukemia inhibitory factor receptor α chain to generate a functional high-affinity receptor (for review, see refs. 29 and 30). Moreover, just as there appear to be IL-4 receptors that do not involve the IL-13Rα (NR4) but rather use the IL-2Rγ, evidence exists that there is a second class of oncostatin M receptors that do not contain the leukemia inhibitory factor receptor α chain (30, 31).

Several issues remain unresolved. The IL-13Rα (NR4) appears to encode a low-affinity IL-13 receptor, whereas coexpression of IL-13Rα (NR4) and IL-4Rα yields a high-affinity complex. The degree to which IL-13Rα (NR4) and IL-2Rγ are responsible for generating a high-affinity IL-4 receptor complex is unclear and will require reconstitution of receptor complexes in cells thought to express none of the components, such as L-cells. Likewise, although IL-4 receptors apparently use either the IL-13Rα (NR4) or IL-2Rγ, it is not known whether both IL-2Rγ and IL-13Rα (NR4) are used at the same time or whether IL-2Rγ sometimes contributes to the function of IL-13 receptors. Furthermore we have not investigated whether other cytokines, such as IL-2, IL-7, IL-9, and IL-15, use the IL-13Rα (NR4), rather than the IL-2Rγ, in some circumstances. Availability of the IL-13Rα cDNA should now allow these questions to be more easily addressed.

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