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Molecular Cloning of Murine CC CKR-4 and High Affinity Binding of Chemokines to Murine and Human CC CKR-4

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We have cloned the murine homologue of human CC Chemokine Receptor-4 (CC CKR-4). In equilibrium competition binding assays performed in undifferentiated HL-60 cells transfected with human and murine CC CKR-4 cDNA, the IC_{50} values for the binding of [^{125}I]macrophage inflammatory protein-1α to human and murine CC CKR-4 were 14.5 ± 9.0 nM and 10.1 ± 3.0 nM, respectively, and the IC_{50} values for the binding of [^{125}I]RANTES to human and murine CC CKR-4 were 9.3 ± 3.0 nM and 5.7 ± 2.6 nM, respectively. The cDNA clone for murine CC CKR-4 is 1531 bp, and the largest open reading frame encodes a protein of 360 amino acids that is 85% identical to human CC CKR-4. Murine CC CKR-4 was detected in the thymus and T-cell lines by Northern blot analysis. This first report of direct binding of chemokines to CC CKR-4 demonstrates that the highly homologous human and murine receptors have similar binding characteristics and tissue distribution.

Inflammation is characterized by the infiltration of leukocytes from the blood into tissue affected by damage or infection. Understanding the molecular signals responsible for directing the leukocytes to the affected tissue would aid in identifying new targets for therapeutic intervention. Several members of the chemokine family have been implicated in the pathology of inflammatory diseases (reviewed in Ref. 1–3). Eighteen human chemokines and several species-homologues have been reported, and they are commonly divided into two subclasses, the α (or CXC) subclass, which have an intervening amino acid between the first two of four conserved cysteine residues, and the β (or CC) subclass, which lack this intervening residue. The CXC and CC subclasses are mainly associated with acute and chronic inflammation, respectively.

The interaction of the chemokines with specific leukocytes is mediated by G-protein-coupled seven transmembrane receptors. There are two known CXC chemokine receptors: an interleukin-8 (IL-8) receptor A which binds only IL-8, and IL-8 receptor B, which binds several CXC chemokines (4,5). Four CC chemokine receptors have been identified: CC CKR-1, cloned from the human monocytic cell line U937, binds the CC chemokines macrophage inflammatory protein-1α (MIP-1α) and RANTES (6,7); CC CKR-2, expressed in monocytes, binds monocyte chemotactic protein-1 and -3 (MCP-1 and 3) (8–10); CC CKR-3 is expressed in eosinophils, and MIP-1α and RANTES have been shown to mobilize calcium in cells transfected with this receptor (11); and CC CKR-4, expressed in basophils, monocytes, and peripheral blood T- and B-cells, is activated by MIP-1α, RANTES, and MCP-1 (12). In addition, a promiscuous erythrocyte receptor (13) and viral homologues of chemokine receptors have also been identified (14).

One approach to understanding the roles of each of these chemokine receptors in disease processes is to use murine models. To place confidence in these studies, however, it is necessary to not only isolate the murine homologues, but also to demonstrate that the murine and human molecules have the same characteristics. Three mouse CC chemokine receptor-like cDNAs have

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The abbreviations used are: CC CKR-1,2,3, or 4, CC Chemokine receptor-1,2,3, or 4; MIP-1α, or -2, macrophage inflammatory protein-1α or -2; RANTES, regulated on activation, normal T-cell expressed and secreted; MCP-1 or 3, monocyte chemoattractant protein-1 or 3; PCR, polymerase chain reaction; IL-8, interleukin-8; IL-2, interleukin-2.

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been identified (15). One, named MIP-1α receptor, is 80% identical to the human CC CKR-1 and was shown to bind MIP-1α and RANTES. The ligands for the other two (MIP-1α receptor-like 1 and 2) have not been identified, but these sequences show the highest homology to human CC CKR-3 (11). We now report the molecular cloning of murine CC CKR-4, and demonstrate the binding of MIP-1α and RANTES to both human and murine CC CKR-4. The high degree of homology at the amino acid level, in ligand binding characteristics, and in tissue distribution suggests that murine CC CKR-4 is the functional homologue of human CC CKR-4, and can therefore be useful in elucidating the role of CC CKR-4 in murine models of human disease.

MATERIALS AND METHODS

Materials. Restriction enzymes and DNA-modifying enzymes were from New England Biolabs or Pharmacia. All cell culture reagents were from Gibco-BRL. Recombinant proteins IL-2, IL-8, MIP-1α, RANTES, and MCP-1 were expressed in *E. coli* and prepared at the Glaxo Institute for Molecular Biology (16–18). MCP-3 was purchased from Peprotech. Radiolabeled chemokines were either purchased from DuPont-NEN or iodinated by Amersham International (specific activity ≈ 2200 Ci/mmol). DIG-11-UTP, Blocking reagent, random-primed DNA labeling kit, CDP-Star chemiluminescent substrate, and proteinase K were from Boehringer Mannheim. RNAsin, T3 RNA polymerase, RQ1 DNase, and 5X transcription buffer were from Promega.

Cell lines. Cell lines were maintained in RPMI-1640 containing 10% FCS, 2 mM glutamine, and 50 IU/ml penicillin and 50 μg/ml streptomycin unless otherwise indicated. The media for some leukocytic cell lines contained 1 mM sodium pyruvate, 50 μM β-mercaptoethanol, and 5 ng/ml Interleukin-2. All cells were maintained at 37°Cin humidified incubators containing 5% CO_2.

Library amplification, degenerate PCR, and cDNA library screening. A mouse thymus cDNA library in the Lambda ZAP vector (Stratagene) was amplified, subjected to degenerate PCR, and the reaction products were screened and subcloned exactly as described by Power, *et al.* (12). CsCl gradient-purified plasmid for one clone obtained from the degenerate PCR (MT5) was digested with restriction enzymes *Hind*III and *Eco*RI. The resultant insert DNA which corresponded to the sequenced PCR product was gel-purified, labeled with [^{32}P]dCTP (Amersham International) using a random-primed DNA-labeling kit, and used to screen 5×10^5 pfus of the murine thymus cDNA library by plaque hybridization. Duplicating positive plaques were rescreened until pure positive phage plaques were obtained. *In vivo* rapid excision was used to isolate the Bluescript SK (-) phagemids from seven independent positive plaques according to the manufacturer's instructions. The DNA sequence was determined from both strands.

Digoxigenin-labeled riboprobe preparation. Clone MT5 plasmid DNA (5 μg) was linearized by *Hin*dIII digestion overnight at 37°C, followed by treatment with 20 μg of proteinase K for 30 min at 37°C. DNA was extracted twice with phenol and once with chloroform and precipitated with 0.1 volume 3 M sodium acetate, pH 5.5 and 3 volumes ethanol overnight at −20°C. The DNA was pelleted by centrifugation (14000 rpm, 4°C, Eppendorf microfuge, 30 min), washed with 70% ethanol, and resuspended in H_2O. An antisense digoxigenin-labeled riboprobe was prepared from 1 μg *Hind*III linearized DNA using the method outlined by Boehringer Mannheim.

Northern blot analysis. Snap-frozen tissues from normal adult mice were homogenized with a Polytron. RNA was extracted from the homogenized tissues or from cultured cells using Trizol (Gibco-BRL). Poly(A)+ RNA was prepared from the tissue total RNA using Oligotex-dT (QIAgen). RNA was fractionated on a denaturing formaldehyde-agarose gel, transferred to an Electran membrane (British Drug Houses) by capillary elution, and UV-crosslinked using a Stratalinker (Stratagene). Membranes were prehybridized for 4 h at 68°C in hybridization buffer (50% formamide, 5X SSC, 2% Blocking Reagent, 0.02% SDS, 0.1% N-lauroylsarcosine), and hybridized overnight at 68°C in hybridization buffer containing 100 μg/ml DIG-labeled riboprobe. Membranes were washed twice with 2X SSC, 0.1% SDS at 68°C for 5 min, twice with 0.1X SSC, 0.1% SDS at 68°C for 20 min, and once with buffer B1 (0.15 M NaCl, 0.1 M maleic acid, pH 7.5) at 25°C for 5 min. The membranes were blocked with 1% Blocking Reagent in buffer B1 at 25°C for 1 h, followed by three washes (5 min, 15 min, and 60 min) with buffer B1 containing 0.3% Tween-20 at 25°C. The membranes were then equilibrated for 5 min with buffer B3 (0.1 M NaCl, 5 mM $MgCl_2$, 0.1 M Tris-HCl, pH 9.5) and incubated with CDP-Star (I:100 dilution in buffer B3) at 25°C for 5 min. The membranes were exposed to Hyperfilm-ECL (Amersham).

Subcloning human and murine CC CKR-4 into pcDNA1neo. Murine CC CKR-4 Clone 10A was excised from Bluescript with *Eco*RI, gel-purified, blunt-ended by treatment with T4 polynucleotide kinase and Klenow enzymes, and ligated into the *Eco*RV site of pcDNA1neo (Invitrogen). The coding sequence of human CC CKR-4 (12) was amplified using PCR primers containing a consensus Kozak sequence (19) and *Bam*HI recognition sequence on the 5′ end (sense, 5′ TCG GGA TCC GCC ACC ATG AAC CCC ACG GAT A) and an *Eco*RV recognition sequence on the 3′ end (antisense, 5′ TAT CGA TAT CTT ACT ACA GAG CAT CAT GAA). Thirty cycles of PCR (95°C, 2 min; 55°C, 2 min; 72°C) were performed with 1 μM of each primer and 100 ng human CC CKR-4 pBluescript plasmid DNA (12). PCR products were gel-purified, cut with restriction enzymes *Bam*HI and *Eco*RV, and T4 DNA ligase was used to ligate the products into appropriately-digested pcDNA1neo. Ligation products were electroporated into electrocompetent bacteria (MC1061/P3, Clontech) using a Bio Rad

```
   1  CGCTGCCTGCTGGTACCCGGAGCGCGACGGCATTGCTTCATAGACTGTCCTCAGGATCAC   60
  61  TTTCAGAAGAGCAAGGCAGCTCAACTGTTCTCATTGGCTTCTCCTGCTGGTACCCGGAGC  120
 121  GCGACGATTCCAAAGATGAATGCCACAGAGGTCACAGACACCACCCAGGATGAAACTGTG  180
                    M  N  A  T  E  V  T  D  T  T  Q  D  E  T  V

 181  TACAATAGTTATTACTTCTACGAAAGCATGCCAAAGCCTTGCACCAAGGAAGGTATCAAG  240
      Y  N  S  Y  Y  F  Y  E  S  M  P  K  P  C  T  K  E  G  I  K

 241  GCATTTGGGGAGGTCTTCCTGCCTCCTCTCTACTCCTTGGTCTTCTTGTTGGGTCTGTTT  300
      A  F  G  E  V  F  L  P  P  L  Y  S  L  V  F  L  L  G  L  F

 301  GGAAATTCTGTTGTGGTTCTGGTCCTGTTCAAATACAAGAGGCTCAAGTCCATGACGGAC  360
      G  N  S  V  V  V  L  V  L  F  K  Y  K  R  L  K  S  M  T  D

 361  GTGTACCTGCTGAACCTGGCCATCTCGGATTTGCTGTTCGTCCTGTCCCTCCCATTCTGG  420
      V  Y  L  L  N  L  A  I  S  D  L  L  F  V  L  S  L  P  F  W

 421  GGCTACTACGCCGCCGACCAGTGGGTTTTTGGACTAGGTCTGTGCAAGATCGTTTCATGG  480
      G  Y  Y  A  A  D  Q  W  V  F  G  L  G  L  C  K  I  V  S  W

 481  ATGTACCTGGTGGGCTTCTACAGCGGCATCTTCTTCATCATGCTCATGAGCATAGACAGA  540
      M  Y  L  V  G  F  Y  S  G  I  F  F  I  M  L  M  S  I  D  R

 541  TACCTGGCCATCGTGCACGCGGTATTCTCCTTGAAGGCAAGGACCCTGACCTATGGGGTC  600
      Y  L  A  I  V  H  A  V  F  S  L  K  A  R  T  L  T  Y  G  V

 601  ATCACCAGCCTGATCACGTGGTCAGTGGCTGTGTTTGCCTCCCTCCCAGGCCTCTTGTTC  660
      I  T  S  L  I  T  W  S  V  A  V  F  A  S  L  P  G  L  L  F

 661  AGCACTTGCTACACAGAGCACAACCACACGTACTGCAAAACCCAGTACTCGGTCAACTCG  720
      S  T  C  Y  T  E  H  N  H  T  Y  C  K  T  Q  Y  S  V  N  S

 721  ACGACGTGGAAAGTCCTCAGCTCCCTGGAGATCAACGTCCTGGGGCTGCTTATCCCCCTG  780
      T  T  W  K  V  L  S  S  L  E  I  N  V  L  G  L  L  I  P  L

 781  GGCATCATGCTGTTTTGGTATTCCATGATCATTAGGACTCTGCAACACTGCAAGAATGAG  840
      G  I  M  L  F  W  Y  S  M  I  I  R  T  L  Q  H  C  K  N  E

 841  AAGAAGAACAGAGCAGTGCGCATGATCTTCGGCGTGGTGGTCCTCTTCCTCGGCTTCTGG  900
      K  K  N  R  A  V  R  M  I  F  G  V  V  V  L  F  L  G  F  W

 901  ACGCCGTACAACGTGGTGCTTTTCCTGGAGACGCTGGTGGAGCTTGAAGTCCTTCAGGAC  960
      T  P  Y  N  V  V  L  F  L  E  T  L  V  E  L  E  V  L  Q  D

 961  TGCACCTTGGAGAGGTACCTAGACTACGCCATCCAGGCTACAGAAACCCTGGGCTTCATT 1020
      C  T  L  E  R  Y  L  D  Y  A  I  Q  A  T  E  T  L  G  F  I

1021  CACTGCTGCCTTAACCCCGTCATTTACTTCTTTCTCGGGGAGAAATTCCGCAAGTACATC 1080
      H  C  C  L  N  P  V  I  Y  F  F  L  G  E  K  F  R  K  Y  I

1081  ACCCAACTCTTCAGAACATGCCGGGGTCCCCTCGTGCTCTGCAAACACTGTGACTTCCTC 1140
      T  Q  L  F  R  T  C  R  G  P  L  V  L  C  K  H  C  D  F  L

1141  CAGGTCTACTCGGCTGACATGTCCAGCTCCTCTTACACGCAGTCCACTGTGGATCATGAC 1200
      Q  V  Y  S  A  D  M  S  S  S  S  Y  T  Q  S  T  V  D  H  D

1201  TTCCGTGACGCTTTGTAAGGTGTGAGTGGGGGTAACATGGCGTTAACAAGCTCCACACAC 1260
      F  R  D  A  L

1261  CCAGCACCTGCTCGCCTTGTTTCAGTCAGGGTGCCCTGAACAGGGCTCTGAGGAAGAAAA 1320
1321  CAAGTAAAACCAAGACCATGGCAAGATGGCTTCTCACCCTGCAGGTGGCTCCCAAGAGGT 1380
1381  TCAGAGCCCTGCTGGGTGGAGGAAATCACCCCTTCATGACAATGAGCCCTTGAGTGGATC 1440
1441  TCTAGTTTGGTTGAACTACCTAGAATTCTTGGACATGCTGTATTCCATAAAGCCAGATGT 1500
1501  CTGGAGAAAAAAAAAAAAAAAAAAAAAAAAA 1531
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FIG. 1. Nucleotide sequence of murine CC CKR-4 clone 10A cDNA and deduced amino acid sequence. Murine CC CKR-4 clone 10A was isolated from a thymus cDNA library. The nucleotide sequence of MT5, obtained by degenerate PCR, is underlined. The sequence for murine CC CKR-4 is available on the GenBank/EMBL/DDBJ databases with the accession number X90862.

```
            1*                                                      50
HCCCKR4     MNPTDIADTT LDESIYSNYY LYESIPKPCT KEGIKAFGEL FLPPLYSLVF
MCCCKR4     MNATEVTDTT QDETVYNSYY FYESMPKPCT KEGIKAFGEV FLPPLYSLVF

            51                      ‡                              100
HCCCKR4     VFGLLGNSVV VLVLFKYKRL RSMTDVYLLN LAISDLLFVF SLPFWGYYAA
MCCCKR4     LLGLFGNSVV VLVLFKYKRL KSMTDVYLLN LAISDLLFVL SLPFWGYYAA

            101                                                 †   150
HCCCKR4     DQWVFGLGLC KMISWMYLVG FYSGIFFVML MSIDRYLAIV HAVFSLRART
MCCCKR4     DQWVFGLGLC KIVSWMYLVG FYSGIFFIML MSIDRYLAIV HAVFSLKART

            151                                 *           *   200
HCCCKR4     LTYGVITSLA TWSVAVFASL PGFLFSTCYT ERNHTYCKTK YSLNSTTWKV
MCCCKR4     LTYGVITSLI TWSVAVFASL PGLLFSTCYT EHNHTYCKTQ YSVNSTTWKV

             ‡                                                      250
HCCCKR4     LSSLEINILG LVIPLGIMLF CYSMIIRTLQ HCKNEKKNKA VKMIFAVVVL
MCCCKR4     LSSLEINVLG LLIPLGIMLF WYSMIIRTLQ HCKNEKKNRA VRMIFGVVVL

            251                                                    300
HCCCKR4     FLGFWTPYNI VLFLETLVEL EVLQDCTFER YLDYAIQATE TLAFVHCCLN
MCCCKR4     FLGFWTPYNV VLFLETLVEL EVLQDCTLER YLDYAIQATE TLGFIHCCLN

            301                     †                               ‡
HCCCKR4     PIIYFFLGEK FRKYILQLFK TCRGLFVLCQ YCGLLQIYSA DTPSSSYTQS
MCCCKR4     PVIYFFLGEK FRKYITQLFR TCRGPLVLCK HCDFLQVYSA DMSSSSYTQS

            351
HCCCKR4     TMDHDLHDAL
MCCCKR4     TVDHDFRDAL
```

FIG. 2. Alignment of human and murine CC CKR-4 deduced protein sequences. Conserved sites for potential N-linked glycosylation (*), Protein Kinase C (†), and Casein Kinase II phosphorylation (‡) are indicated.

Gene Pulser (2.5 kV, 25 μF, 200 ω, 0.2 cm gap cuvette), and the bacteria were plated on LB plates containing 15 μg/ml tetracycline. Miniprep DNA was isolated from individual tetracycline-resistant colonies after overnight growth at 37°C and sequenced to identify clones with error-free coding sequences.

HL-60 cell transfection and ligand binding assays. Thirty μg human CC CKR-4-pcDNA1neo, murine CC CKR-4-pcDNA1neo, or pcDNA1neo were electroporated into 500 μl HL-60 cells (2×10^7 cells/ml in 0.15 M NaCl, 20 mM HEPES, pH 7.3) using a Bio Rad Gene Pulser (260 volts, 960 μF, 0.4 cm gap cuvette). Cells were seeded into T-175 flasks containing 25 ml AIM-V serum-free media (GIBCO). On day 2 or 3 following transfection the cells were diluted in a total volume of 45 ml AIM-V media containing 600 μg/ml G418, and on day 6, cells were further diluted to 180 ml AIM-V media containing 600 μg/ml G418. On days 7–15 post-transfection cells were maintained in AIM-V media (+G418) at a density of 0.4–1.2 $\times 10^6$ cells/ml, and binding assays were performed during this time. Equilibrium competition binding was carried out by incubating 5×10^5 cells in 100 μl binding buffer (1 mM $CaCl_2$, 5 mM $MgCl_2$, 0.5% BSA, 50 mM HEPES, pH 7.2), 0.34 nM [^{125}I]radioligand, and 0.5–2000 nM cold ligand in Millipore -DV 96-well filter plates. After 1.5 h incubation at room temperature, cells were washed four times by vacuum filtration with binding buffer containing 0.5 M NaCl. Fifty μl Optiphase scintillant (Wallac) were added to each well, and the radioactivity was measured with a Wallac Microbeta Plate Reader. All binding data was normalized as the percentage of total binding. Total binding for a given ligand was defined as the radioactivity bound in the absence of competing ligand to 5×10^5 cells transfected with human CC CKR-4 (range: 1000–2500 cpm).

RESULTS

Molecular cloning of murine CC CKR-4. By using a degenerate PCR approach, we have isolated a murine homologue of the recently described human CC CKR-4 (12). Degenerate oligonucleotide primers corresponding to the intracellular loop between transmembrane domains 3 and 4 and to the transmembrane domain 7 were used to amplify a murine thymus cDNA library. The thymus library was chosen because human CC CKR-4 was shown to be highly expressed in the thymus (12). PCR products of the expected 500 bp size were gel-purified, subcloned into pBluescript II SK(-), and

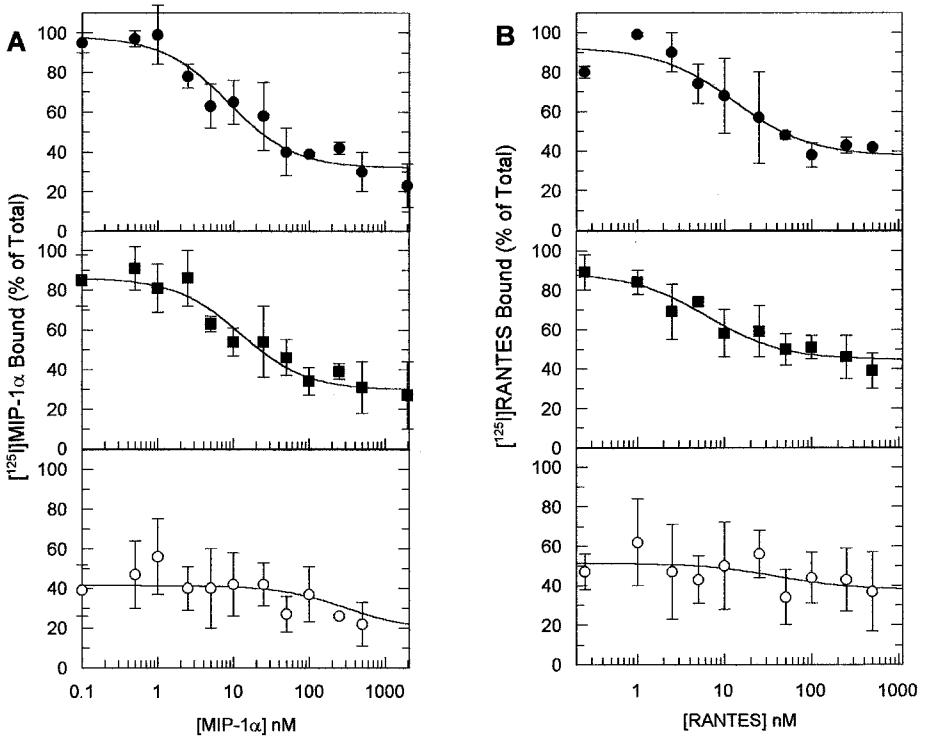

FIG. 3. High affinity binding of [^{125}I]MIP-1α and [^{125}I]RANTES to human and murine CC CKR-4. HL-60 cells were transfected with human CC CKR-4 (●), murine CC CKR-4 (■), or an empty vector (○) and maintained in AIM-V media containing G418 for 7–15 days. Equilibrium competition assays were performed as described under Materials and Methods with [^{125}I]MIP-1α (**A**) and [^{125}I]RANTES (**B**). Each point represents the mean ± S.D. of duplicate points from four (A) or three (B) separate experiments. Data were curve-fitted with GraFit 3.01 software (20) using the equation $B/Bmax^{app} = 1/(1 + ([L]/IC_{50}))$, where B = cpm bound, $Bmax^{app}$ = cpm bound in the absence of competing ligand, L = competing ligand, and the IC_{50} = [radioligand] + K_d (21).

sequenced. One clone, designated MT5, was 67% identical to human CC CKR-4 cDNA over 500 bp. This clone was used to screen the murine thymus library by conventional plaque hybridization. Of seven independent clones isolated which closely corresponded to human CC CKR-4, four were full length clones. One clone, designated 10A, contained 5′ untranslated sequence and a poly(A)+ tail and is 78% identical to Human CC CKR-4 (**Figure 1**). The longest open reading frame encodes a polypeptide of 360 amino acids that is 85% identical to human CC CKR-4 (**Figure 2**). Like the human CC CKR-4, the three potential *N*-linked glycosylation sites and potential phosphorylation sites are all conserved. The murine CC CKR-4 receptor shows a higher degree of homology to human CC CKR-4 than to the other murine CC chemokine receptors recently published. It shares 41–48% identity with the murine MIP-1α receptor (homologue of human CC CKR-1) and murine MIP-1α receptor-like 1 and 2 (15), and 41% identity to the murine chemokine receptor homologous to IL-8 receptor B (22).

Equilibrium competition binding assays. Human CC CKR-4 was previously shown to encode a functional chemokine receptor since MIP-1α, MCP-1, and RANTES stimulated Ca^{2+} activated chloride channels in *Xenopus laevis* oocytes injected with human CC CKR-4 cRNA. Equilibrium competition binding assays were performed 7–15 days following the transfection of undifferentiated HL-60 cells with an expression vector containing human CC CKR-4 cDNA, murine CC CKR-4 cDNA, or with an empty vector. Reverse transcriptase PCR on undifferentiated HL-60 cells demonstrated that human CC CKR-1, -2, -3, and -4 were not present (data not shown). Following

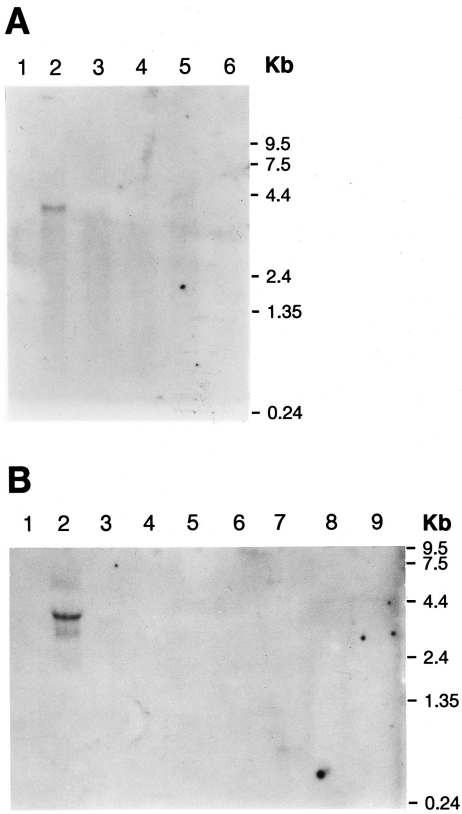

FIG. 4. Expression of murine CC CKR-4 mRNA in normal adult tissues and leukocytic cell lines. Northern blotting with antisense DIG-riboprobes was performed as described under Materials and Methods. A. 15 min exposure of a Northern Blot performed with 1 μg poly A+RNA from normal adult mice: 1, Spleen; 2, Thymus; 3, Lung; 4, Heart; 5, Kidney; 6, Liver. B. 2 min exposure of a Northern Blot performed with 10 μg total RNA from murine cell lines: 1, A20 B-lymphoma; 2, CTLL Cytotoxic T lymphocyte CD40+; 3, EL4 T cell lymphoma; 4, L929 fibrosarcoma; 5, L1210 B cell leukemia; 6, NIH3T3 fibroblast; 7, P3X myeloma; 8, RSW leukemia; 9, WEHI B cell.

transfection the HL-60 cells were maintained in AIM-V serum free media. Cells transfected with human or murine CC CKR-4 bound [^{125}I]MIP-1α, whereas the cells transfected with the empty vector only bound the chemokine at background levels (**Figure 3A**). The IC_{50} values for the competition with cold ligand were 14.5 ± 9.0 nM and 10.1 ± 3.0 nM for human and murine CC CKR-4, respectively. **Figure 3B** shows the competition binding curves obtained with the transfected cells and [^{125}I]RANTES. The IC_{50} values for the binding of [^{125}I]RANTES to human and murine CC CKR-4 were 9.3 ± 3.0 nM and 5.7 ± 2.6 nM, respectively. No binding of [^{125}I]MCP-1 or [^{125}I]MCP-3 was observed to cells maintained under the same conditions, and neither was there specific binding of [^{125}I]IL-8 (data not shown). Thus, we demonstrate the high affinity binding of MIP-1α and RANTES to cells transfected with human and murine CC CKR-4, and the absence of binding of MCP-1, MCP-3, and IL-8. Both the human and murine receptors bound the same ligands with similar affinity, confirming that the murine clone obtained is a true homologue of the human receptor. The absence of binding of MCP-1 to the transfected cells was surprising, since in previous studies in *Xenopus laevis* oocytes transfected with human CC CKR-4, a Ca^{2+} activated chloride current was stimulated in response to this ligand (12). This apparent absence of observed binding may be due to a low affinity interaction of receptor and ligand, or to alteration in the affinity of MCP-1 for the receptor upon iodination of the ligand. Since both the murine and human CC CKR-4

bound the iodinated human MIP-1α and RANTES, and neither the murine or human CC CKR-4 bound human MCP-1, the lack of binding to the murine receptor is probably not due to species differences in the ligand.

Tissue expression of murine CC CKR-4. Northern blots were performed in order to determine the cell and tissue expression of CC CKR-4 in the mouse. **Figure 4A** shows the Northern blot performed on poly(A)+ RNA from normal adult mouse tissues. A transcript of approximately 4 kb was observed only in the thymus, a leukocyte-rich organ. Since individual murine leukocyte populations are difficult to obtain in quantities necessary for Northern blotting, we used several leukocytic cell lines to examine the mRNA expression. Of the cell lines examined, expression of the mRNA was only observed in the T-cell line CTLL, with a major 4 kb transcript and minor transcripts of 3.4 kb and 7.0 kb (**Figure 4B**). The identification of multiple transcripts suggests that alternatively spliced transcripts are present. The transcript size of 4 kb and tissue distribution in the thymus and T-cells are similar to that observed for human CC CKR-4.

In summary, we have cloned the murine homologue of the human CC chemokine receptor-4 and show that it is highly homologous with respect to amino acid identity (85%), ligand binding characteristics, and in tissue distribution. This high level of correlation between the murine and human genes suggests that this gene has been conserved during evolution and gives confidence in studying the function of this gene in mouse models of chronic human diseases.

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