We report the cloning of a novel macrophage-restricted C-type lectin by differential display polymerase chain reaction. This molecule, named mouse macrophage C-type lectin, is a 219-amino acid, type II transmembrane protein with a single extracellular C-type lectin domain. Northern blot analysis indicates that it is expressed in cell lines and normal mouse tissues in a macrophage-restricted manner. The cDNA and genomic sequences of mouse macrophage C-type lectin indicate that it is related to the Group II animal C-type lectins. The mcl gene locus has been mapped between the genes for the interleukin-17 receptor and CD4 on mouse chromosome 6, the same chromosome as the mouse natural killer cell gene complex.

Compared with most cells in the body, macrophages (Mφ) display an unparalleled diversity of distribution and function. As constitutive components of most normal tissues, resident Mφ mediate or regulate the clearance of senescent or apoptotic cells, cytokine production, hemopoiesis, bone resorption, antigen transport, and neuro-endocrine regulation. Activated Mφ recruited to sites of infection or injury by immune or inflammatory stimuli play a crucial role in acute and chronic inflammation, tissue repair, immunopathology, and in the pathogenesis of metabolic diseases such as atherosclerosis (1–4).

The distribution and functional heterogeneity of Mφ derive in part from their specialized plasma membrane receptors. Cell surface markers such as F4/80, sialoadhesin, mannose receptor, and the scavenger receptor (SR-AI/SR-AII) have all contributed significantly to current understanding of Mφ ontogeny and function (5, 6). Yet, in comparison with other immune cells such as B and T lymphocytes, relatively few Mφ-restricted cell surface molecules have been identified, and much regarding their various physiological and pathological roles remains unknown.

Differential display-PCR (DD-PCR), the random amplification of differentially expressed mRNA species, provides a means of isolating cell-specific genes without relying upon the initial detection of a particular protein or cellular activity (7). This technique has already been successfully used to clone Mφ-specific molecules, including a rat homologue of mouse Mφ galactose/N-acetylgalactosamine-specific lectin (8); Mpg-1, a protein expressed on Mφ that shows localized homology to perforin (9); and mouse Emr1, which was found to be identical to the Mφ-restricted cell-surface glycoprotein F4/80 (10, 11). In this paper we document the identification and cloning of another novel putative Mφ-restricted C-type lectin using DD-PCR.

**MATERIALS AND METHODS**

**Cells**

Media and supplements were purchased from Life Technologies Inc. Primary Mφ—Primary Mφ were harvested in sterile, endotoxin-free phosphate-buffered saline (PBS) (Sigma-Aldrich) by peritoneal lavage of Balb/c mice at 10–12 weeks of age. Four days before harvest the mice were injected intraperitoneally with 1 ml of 2% Bio-Gel (Bio-Rad) or 1 ml of thioglycollate broth (Difco) or not injected (for resident Mφ). 3 × 10⁶ cells were plated/well of a 6-well plate in 3 ml of Opti-MEM (Life Technologies Inc.) and allowed to adhere for 3 h. The cells were then washed twice in Opti-MEM to remove all nonadherent cells and incubated overnight in Opti-MEM. The following day the cells were washed in PBS before lysis.

**Cell Lines**—L929, NS0, EL-4, J558L, PG19, MEL707, RAW 264.7 and COS-7 cells were cultured in Dulbecco’s modified Eagle’s medium. WEHI-231 cells were cultured in Dulbecco’s modified Eagle’s medium plus 2 × 10⁻⁵ M 2-β-mercaptoethanol (Sigma). J774.2 and BW5147 cells were maintained in RPMI 1640. CTLL-2 cells were grown in RPMI 1640 with 10 IU/ml interleukin-2. CHO-K1 cells were grown in nutrient mixture F-12 (Ham’s). All cells were cultured at 37 °C with 5% CO₂. All media were supplemented with 10% fetal calf serum, 2 mM l-glutamine, 50 μg/ml penicillin, 50 μg/ml streptomycin.

**Differential Display PCR**

mRNA—Messenger RNA (mRNA) from 3 × 10⁶ cells from the cell lines listed above was harvested using the QuickPrep micro mRNA purification kit (Amersham Pharmacia Biotech).

cDNA—0.5 μg of mRNA was resuspended in 12 μl of sterile water, 2.5 μM oligo (dT) primer T₇ MA (M represents an equimolar mixture of A, C, G) and incubated at 70 °C for 10 min. After snap-cooling on ice, the reaction was continued in 20 μl containing 10 mM dithiothreitol (Life Technologies), 20 μM dNTPs (Amersham), 200 units of Superscript RNase H⁻ reverse transcriptase (Life Technologies) for 1 h at 37 °C before denaturing for 5 min at 95 °C.

PCR—2 μl of a 1:10 dilution of each cDNA were randomly amplified in a 20-μl reaction containing 1.2 mM MgCl₂ (Life Technologies), 0.05% W-1 detergent (Life Technologies), 2 μM dNTPs (Amersham), 5 units of Taq DNA polymerase (Life Technologies), 2 μCi [³²P]dATP (Amersham), 2.5 μM T₇ MA primer, 0.5 μM random 10-mer primer 4158 (TGTTAAAAGGG). Cycling parameters were 94 °C for 30 s, then 40
cycles of 94 °C for 30 s, 40 °C for 2 min, and 72 °C for 30 s followed by a final extension step of 72 °C for 5 min. 5 μl of each reaction product were run on a 6% polyacrylamide–8% urea gel. After drying, the gel was exposed for 16 h to Hyperfilm-HP (Amersham). Selected bands were excised from the gel, eluted in 100 μl of sterile water, precipitated, and resuspended in 10 μl of sterile water. 4 μl of each PCR product were reamplified in a 40 μl reaction volume with conditions similar to the original PCR except for the use of 20 μM dNTPs and 10 units of Taq polymerase. Reamplified PCR products were subcloned into the pGEM-T vector (Promega UK, Southampton, UK).

5’ Rapid Amplification of cDNA Ends (5’-RACE)

Adapter-ligated cDNA, synthesized from 1 μg of murine spleen poly(A)+ RNA (CLONTECH Laboratories UK Ltd., Basingstoke, UK) using the Marathon cDNA amplification kit (CLONTECH), was resuspended in sterile water. A 5’-RACE reaction was performed in a 50-μl volume containing 50 mM Tris-HCl (pH 9.2 at 25 °C), 16 mM (NH4)2SO4, 2.25 mM MgCl2, 0.2 mM dNTPs (Amersham), 1 μl of DNA polymerase mix (a 20-μl stock solution consisted of 14.3 μl of Expand Long Template PCR system enzyme mix (Boehringer Mannheim) plus 5.7 μl of TaqStart antibody (CLONTECH)), 200 nm Marathon adapter primer AP-1, 200 nm mMCL-specific primer complementary to residues 57–81 of the DD-PCR fragment (residues 856–880 of the cDNA sequence reported here in Fig. 2A), and 5 μl of adapter-ligated cDNA diluted 1:250 in sterile water. Reactions were incubated at 94 °C for 3 min, followed by 35 cycles at 94 °C for 45 s, 60 °C for 45 s, and 72 °C for 3 min. The resulting 871-bp base pair (bp) product was purified using a QIAquick PCR purification kit (Qiagen Ltd., Crawley, UK) and subcloned into the pGEM-T vector and sequenced.

Isolation of DNA Clones

A cDNA library was constructed in the λZAP II vector (Stratagene Ltd., Cambridge, UK) using oligo(dT)-primed cDNA from the J774.2 cell line. A Sv/129 mouse liver genomic DNA library in the λFix II vector was purchased from Stratagene. Approximately 1 × 107 plaques from each library were screened with a [32P]-labeled cDNA probe corresponding to the 871-bp RACE product. Positive plaques from the cDNA library were enriched after a further two rounds of screening, resulting in seven independent cDNA clones that were isolated in pBlueScript II-SK(−) (Stratagene). All seven cDNA clones were sequenced to obtain unambiguous overlapping readings from both strands. Positive plaques from the genomic library were enriched after three further rounds of screening. DNA from two independent clones was purified using the Wizard prep kit (Promega) and digested with NotI to release the full-length genomic DNA inserts. The inserts were then subcloned into NotI-digested pBlueScript SK(−) and sequenced to obtain unambiguous overlapping readings from both strands.

Sequence Analysis

DNA sequence reactions were performed using the PRISM Ready Reaction DyeDeoxy Terminator sequencing kit (PE Applied Biosystems, Foster City, CA). Samples were subjected to electrophoresis on an ABI 373A DNA sequencer, read automatically, and recorded using ABI Prism Model Version 2.1.1 software (PE Applied Biosystems). Brookhaven Protein Data Bank, GenBank®, and EMBL data bases were searched for homologous sequences using the BLAST algorithm (12). Protein alignment, alignment consensus sequence, and percent identity were calculated by the Pileup, the Prettybox, and the Gap programs, respectively, included in the EGCG extensions to the Wisconsin Package Version 8.1.0, (13, 14). A gap penalty value of 3.0 and a gap length weight of 0.1 were used with the Pileup and Gap programs.

Northern Blot Analysis

15 μg (cell lines) or 20 μg (tissues) of total RNA were subjected to electrophoresis through a denaturing 1.2% agarose, 6% formaldehyde gel and transferred to a Genescreen Plus nylon membrane (NEN Life Science Products, Boston, MA). The membrane was hybridized overnight to a [32P]-labeled cDNA probe corresponding to the 871-bp RACE product. Hybridization, washing, and autoradiography were performed as described above. Equal amounts of each reaction product were run on a 6% polyacrylamide, 8 M urea gel and exposed for 16 h. The closed arrowhead indicates a gene expressed in all cell lines. The open arrowhead indicates a differentially expressed gene but one that is not Mø-restricted. The arrow shows the differentially expressed, Mø-restricted gene (mMCL) selected for further study. B and C, Northern blot analysis of mMCL expression in mouse cell lines and mouse tissue. 15 μg of total RNA from mouse cell lines (B) and 20 μg of total RNA from mouse tissues (C) were electrophoresed through a 1.2% agarose gel, transferred to a Genescreen Plus membrane, and probed with the 871-bp mMCL 5’-RACE fragment (top panels). Ethidium bromide staining of the gels (bottom panels) demonstrates equal loading of the samples. The relative positions of RNA markers are shown. Northern B shows an overnight exposure. Northern C shows a 21-day exposure. The band corresponding to expression in lymph nodes (L. NODE) is only faintly visible after a 40-day exposure, not after the 21-day exposure shown. The lane labeled Res. Mb. (4 HR) is a 4-h exposure of the adjacent overexposed Res. Mb lane. SM. INT., small intestine; S. MUSCLE, skeletal muscle; F. LIVER, fetal liver.
Hydropathy plot of the 219-amino acid mMCL sequence. The amino acids comprising the putative N-linked glycosylation sites are underlined with two solid lines, and those of the transmembrane region with one solid line. In-frame stop codons are highlighted with asterisks. The start of the first exon of the C-type lectin domain is identified with an open arrow. Intron-exon splice sites are indicated with closed arrowheads. The deleted amino acid Gly-77 is circled.

Transfection of CHO.K1 Cells

DNA—A PCR fragment encoding the entire mMCL open reading frame was amplified in a 100-μl reaction containing 200 μM dNTP (Amersham), 1 mM MgSO₄, 1 mM KCl, 20 mM Tris-HCl, pH 8.8 at 25 °C, 10 mM (NH₄)₂SO₄, 0.1% w/v Triton X-100, 1 μM primer 8354, 1 μM primer 8534, 3 units of Vent DNA polymerase (New England Biolabs UK Ltd., Hitchin, UK), and 100 ng of the 871-bp 5’-RACE product. Cycling parameters were 94 °C for 5 min followed by 30 cycles at 94 °C for 1 min, 60 °C for 30 s, 72 °C for 1 min, and a final extension step at 72 °C for 10 min. Primer 7745 (sense), 5’-TGTTGATACCTCTTTCCCTCTCT-3’ corresponding to the first 4 amino acids of the 871-bp 5’ RACE open reading frame, excluding the BclI restriction enzyme site (underlined). The PCR product was gel-purified, digested with BclI, and subcloned into BamHI-digested pcdNA3 (Invitrogen BV, Leek, The Netherlands), and sequenced to confirm its orientation and integrity. The pcDNA3-mMCL-FLAG construct was stably transfected into CHO.K1 cells by the calcium phosphate precipitation technique. FLAG-tagged mMCL-expressing cells were selected with 2 μg/ml G418 (GIBCO BRL), 1 mg/ml geneticin (Life Technologies). Briefly, 20 μg of the construct were resuspended in 1 ml of 0.25 M CaCl₂ (BDH Laboratory Supplies, Poole,
UK) and precipitated by slowly adding it to an equal volume of 2 × HBS (1.64% w/v NaCl (BDH), 1.18% HEPES (free acid) (Sigma), 0.04% Na2HPO4 (anhydrous) (BDH), pH 7.05). The precipitate was added to 1 × 10^6 CHO.K1 cells cultured in an 80-cm^2 flask in medium and supplements as described above. After 3.5 h, the medium was removed, and the cells were treated with 2 ml of 15% glycerol, 1 M NaCl, 10 mM EDTA, 10 mM NaN3, 10 mM Tris-HCl, pH 8, 1 mM iodoacetamide. Lysates were cleared by centrifugation at 12,000 × g for 10 min, 37 °C. Cells were allowed to recover overnight in fresh medium. Geneticin, 2 mg/ml, was added the following morning.

Enrichment—FLAG-tagged mMCL-expressing cells were enriched 1 week after transfection by selection with the Anti-FLAG M2 (Sigma) monoclonal antibody (mAb) and sheep anti-mouse IgG magnetic Dynabeads (Dynal (UK) Ltd., Wirral Merseyside, UK). Transfected cells were stripped with 0.5 mM EDTA before fixation. Cells were lysed at 4 °C in a solution of 1% v/v Nonidet P-40 (Sigma), and incubated on ice for 40 min in 200 μl of PBS/BSA and 3 μl of Dynabeads. Cells selected by magnetic attraction during subsequent extensive PBS/BSA washes were allowed to recover overnight in a 25-cm^2 flask in geneticin-free medium. The medium and detached beads were removed following morning and replaced with fresh medium containing 2 mg/ml geneticin. Two rounds of limiting dilution clonal and subsequent fluorescence-activated cell sorting (FACS) analysis with the anti-FLAG M2 mAb yielded a stable FLAG-tagged, mMCL-expressing clone.

Rabbit Antiserum

200 μg of keyhole limpet hemocyanin-conjugated peptide corresponding to amino acids 2–16 of mMCL (H-N-WLEESQMKGTKHRP-COOH) (Multiple Sclerosis Peptide Laboratory, Oxford Brookes University) were diluted in 500 μl of PBS, combined with an equal volume of Freund’s incomplete adjuvant (Sigma), and injected subcutaneously into adult New Zealand White rabbits. Booster injections were given as above, except using Freund’s incomplete adjuvant (Sigma), at weeks 3, 5, and 10. Pre-immune serum was designated as sample 16P. A test bleed was taken at 8 weeks to check for anti-peptide antibody activity, and a final bleed (50 ml) was collected 3 weeks after the final injection. 5 ml of the serum were absorbed with 4 × 10^6 CHO.K1 cells, which had been fixed with 4% paraformaldehyde (BDH) and permeabilized with 0.2% v/v Triton X-100 (Sigma). This absorbed serum was designated rabbit antiserum 16T.

Immunofluorescence

Transfected and wild-type CHO.K1 cells were grown on glass cover-slips, fixed with 4% paraformaldehyde in PBS at 4 °C for 1 h, quenched in 10% fetal calf serum for 10 min, washed, blocked in PBS, 15% normal goat serum solutions. To permeabilize cells, 0.2% v/v Triton X-100 (Sigma) was added for 5 min at 4 °C. Cells were incubated at 4 °C for 1 h with a 1:300 dilution of either rabbit serum 16P or 16T in PBS, 15% normal goat serum. After additional washes, cells were incubated at 4 °C for 1 h with a 1:300 dilution of fluorescein isothiocyanate-conjugated goat anti-mouse IgG (Chemicon International, Inc., Temecula, CA) or goat anti-rabbit IgG (Sigma), then analyzed by fluorescent microscopy or FACS. Cells analyzed by FACS were detached from the culture flasks with 0.5 mM EDTA before fixation. To permeabilize cells, 0.2% v/v Triton X-100 was added to all PBS, 15% normal goat serum solutions.

Western blot Analysis

Cells were lysed at 4 °C in a solution of 1% w/v Nonidet P-40 (Sigma), 150 mM NaCl, 10 mM EDTA, 10 mM Na2HPO4, 150 mM Tris-HCl, pH 8, 1 mM phenylmethylsulfonyl fluoride, 5 mM iodoacetamide. Lysates were cleared by centrifugation at 12,000 × g for 30 min at 4 °C. Samples were boiled for 3 min in 5% SDS sample buffer and subjected to SDS-polyacrylamide gel electrophoresis. Samples were reduced by boiling and subjected to SDS-polyacrylamide gel electrophoresis. Samples were reduced by boiling.
them in a sample buffer containing a final concentration of 5% 2-mercaptoethanol (Sigma) before electrophoresis. Proteins were transferred to Hybond-C extra nitrocellulose membrane (Amersham). The membrane was treated with blocking solution (5% milk protein, 0.1% v/v Tween 20, 15% normal goat serum) and blotted for 1 h in blocking solution containing 10 mg/ml anti-FLAG M2 or a 1:500 dilution of either rabbit serum 16P or 16T. After washing with blocking solution, a second 1-h incubation was performed, again in blocking solution, using a 1:1000 dilution of goat anti-mouse IgG (Sigma) or donkey anti-rabbit IgG (Chemicon) coupled to horseradish peroxidase. The bound antibody was detected by enhanced chemiluminescence (Amersham). For competition assays, saturating amounts of either mMCL peptide or FLAG peptide was combined with the primary antibody before its application to the blot.

**RESULTS AND DISCUSSION**

DD-PCR analysis was performed on cDNA from the following mouse cell lines: J774.2 (Mφ), RAW 264.7 (Mφ), EL-4 (thymoma), NS0 (myeloma), and L929 (fibroblast). This panel of cell lines was chosen to provide a range of immune and nonimmune cell types as well as two independent Mφ and three non-Mφ internal controls to facilitate the selection of Mφ-restricted genes. DD-PCR products amplified from both Mφ cell lines but absent from the non-Mφ cell lines (Fig. 1A) were eluted, reamplified, subcloned into the pGEM-T vector, and sequenced. A 272-bp cDNA fragment amplified exclusively from both Mφ cDNA samples was selected for further study because it contained a putative polyadenylation signal site downstream of a TGA stop codon, indicating that it might be a segment of a functional gene. Subsequently, 5'-RACE-PCR was performed on adapter-ligated mouse spleen cDNA using adapter-specific sense and DD-PCR fragment-specific antisense primers. This reaction generated an 871-bp product.

Sequence analysis of the 5'-RACE product showed that it included 601 bp of novel sequence and 257 bp of sequence corresponding to the amplified portion of the original DD-PCR fragment. The novel sequence included a second in-frame TAA stop codon 678 bp upstream of the original DD-PCR stop codon, suggesting that the 5'-untranslated region, two potential in-frame start codons were found 20 bp apart. Although neither of these codons lies in a perfect context for translation initiation, the codon at position 161–163 is believed to be the translation start site. It is the first initiation codon downstream of an in-frame stop codon and the only initiation codon with an adenosine in the -3 position (18, 19).

As a means of testing the expression specificity of this putative gene, Northern blot analysis was performed on an expanded range of 11 mouse cell lines including CTLL-2 (interleukin-2-dependent T cell), J558L (myeloma), BW5147 (thymoma), WEHI-231 (B cell lymphoma), PG19 (melanoma), NS0, RAW 264.7, L929, MEL 707 (erythroleukemia), J774.2, and NIH-3T3 (fibroblast). Total RNA from these cell lines probed with the 32P-labeled 5'-RACE product showed a pair of 1.1-kb and 700-bp bands solely in the J774.2 and RAW 264.7 cell lines (Fig. 1B) and confirmed that the gene was expressed in a Mφ-restricted manner. Using gene-specific sense and an-
tisense primers, a more sensitive reverse transcription-PCR assay of cDNA from J774.2, N50, L929, EL-4, RAW 264.7, MEL 707, and P388-D1 (Mφ) cell lines was also conducted. Again, specific amplified bands were found only in the three Mφ cell lines (data not shown). To determine whether the same Mφ-restricted expression pattern exists in vivo, Northern blot analysis was conducted on RNA isolated from 12 different normal mouse tissues. The 871-bp 5'-RACE probe recognized a 1.1-kb transcript in resident peritoneal Mφ \( \gg \) bone marrow \( \gg \) spleen = lung \( \gg \) lymph nodes (Fig. 1C). The intensity and distribution pattern of the bands were in accordance with known Mφ populations and supported the evidence of Mφ-restricted expression as seen in the cell lines. In particular, the strong expression in bone marrow correlated well with previous studies, which showed that mice bone marrow is the richest source of Mφ as determined by the Mφ-restricted marker F4/80 (20). The presence of the 1.1-kb band alone (tissues) or at a higher intensity (cell lines) on both Northern blots strongly suggests that the 1.1-kb transcript is the predominant form of the modified mRNA in tissue Mφ.

The possibility that alternatively spliced transcripts might account for the 700-bp band, which appeared only in the murine Mφ cell lines, was investigated by screening a J774.2 cDNA library with the 5'-RACE probe. Sequence analysis of seven independent clones isolated from the library revealed no alternatively spliced variants. Two of the clones were found to lack three consecutive base pairs (390–392), leading to an in-frame deletion of Gly-77. Three of the seven clones were also found to have matching 3'-untranslated regions, which extended an additional 74 bp past the site of the start of the poly(A) tail in the original DD-PCR clone. These three longer clones included a second, rarer AUAUA polyadenylation signal sequence (21) 25 bp upstream of the start of their poly(A) tails. The physiological abundance and importance of the Gly-77 deletion and the extended 3'-untranslated sequence are yet to be determined.

A 918-bp cDNA consensus sequence, including Gly-77, was constructed from the overlapping regions of each cDNA clone using a minimum of two independent cDNA clones to confirm each nucleotide. In total, the consensus sequence consists of 160 bp of 5' noncoding sequence, a 660-bp open reading frame, and 98 bp of 3'-untranslated sequence (Fig. 2A). No discrepancies were found between the open reading frame of this consensus sequence and that of the 5'-RACE PCR product amplified from mouse spleen, thereby confirming that the gene expressed in the cell lines was the same gene expressed in normal mouse tissue and the same gene that was amplified in the original DD-PCR.

Translation of the open reading frame of the cDNA beginning at the 161–163 start codon yielded a deduced 219-amino acid protein sequence (Fig. 2A). The lack of an identifiable signal peptide and a hydrophobic profile displaying a hydrophobic anchor sequence near the amino terminus (Fig. 2B) suggested that the gene encoded a type II integral membrane protein. The complete protein sequence was unique insofar as it displayed no overall sequence homology or identity to any other protein sequence entered in a variety of protein data bases. The final 130 carboxyl-terminal amino acids showed similarity to a wide range of carbohydrate-binding proteins, namely C-type lectins, with the greatest degree of homology to chicken hepatic lectin. The carboxyl terminus of the putative protein sequence was aligned with the carbohydrate recognition domains (CRDs) of four of the lectins with which it had the highest similarity scores: mouse Kupffer cell fucose receptor, human macrophage lectin, human gp120 binding lectin, and chicken hepatic lectin. The sequence of rat mannose binding lectin (rMBL-A), although apparently more distantly related to the novel protein, was included because it has an extensively studied C-type lectin CRD (22–25). The alignment showed that the putative sequence is 29, 37, 36, 39, and 31 percent identical to the CRDs of these proteins, respectively. In addition it indicated that apart from Gly-158, Pro-173, Gly-191, and Arg-204, the novel protein shares 11 of the 14 invariant and 17 of the 18 highly conserved amino acids used to define C-type lectins (26) (Fig. 3). Sequence data from two independent clones isolated from a \( \lambda \)FixII mouse liver genomic DNA library screened with the 5'-RACE probe also revealed that the protein is encoded within six exons. As is characteristic of Group-II C-type lectins, the region corresponding to its CRD is encoded by three exons, and the amino-terminal cytoplasmic tail and anchor sequence are encoded by two exons (26). The final two introns of this protein precisely match the position of introns found in the CRDs of other Group-II C-type lectins: CD23 (27), the major form of the rat asialoglycoprotein receptor (28), the Kupffer cell fucose receptor (29), and chicken hepatic lectin (30). The number, position, and phasing of all the intron/exon splice sites of the putative protein are analogous to those of chicken hepatic lectin (30). Collectively, this evidence suggested that the novel protein is indeed a C-type lectin. It has a predicted structure of a 20-amino acid amino-terminal cytoplasmic domain, a 20-amino acid transmembrane domain attached to an extracellular region comprising a 49-amino acid stalk and a 130-amino acid transmembrane domain.
mMCL exhibits the highest protein sequence similarity to the members of Group II C-type animal lectins, a diverse set of type-II transmembrane receptors, including rat, mouse, and chicken hepatic lectins (38). Generally these proteins are thought to mediate glycoprotein endocytosis and degradation. mMCL might therefore possess a similar function. However, the characterization of the tumor binding capabilities of the Mφ-specific Group II lectin mouse macrophage galactose/N-acetylgalactosamine-specific lectin (39–41) might imply that mMCL performs a comparable immune surveillance role. The Mφ-restricted expression and tissue distribution of mMCL, particularly in bone marrow, similarly suggests a hemopoietic function for this protein. Likewise, considering its lectin structure, mMCL may play a role in cell-cell recognition (42).

To determine whether this novel Mφ-restricted gene co-localized with other known lectins or Mφ-specific genes, the chromosomal localization of the mouse mcl gene was investigated. A panel of DNA samples from an interspecific cross that has been characterized for over 1000 genetic markers throughout the genome was analyzed. The genetic markers included in this map span between 50 and 80 centimorgans on each mouse autosome and the X chromosome.2 Initially, DNA from the two parental mice (C3H/HeJ-gld (C3H/HeJ-gld × Mus spretus)F1) were digested with various restriction endonucleases and hybridized with a mMCL cDNA probe to determine restriction fragment length variants to allow haplotype analyses. Informative MspI restriction fragment length variants were detected: C3H/HeJ-gld, 7.0 kb, 3.8 kb; Mus spretus, 8.6 kb. Comparison of the haplotype distribution of the mcl restriction fragment length variants and those previously defined in this interspecific cross indicated that this locus co-segregated in 112 of the 114 meiotic events examined with the CD4 and CD9 loci on mouse chromosome 6. The haplotype distribution among the other genes localized to mouse chromosome 6 is shown in Fig. 4. The best gene order ± the S.D. indicated the gene order: (centromere)II-17r-4.4 centimorgan ± 1.9 centimorgan-mcl-1.8 centimorgan ± 1.2 centimorgan-Cd9/Cd4.

Intriguingly, these studies place the mcl gene locus just proximal of the nkrp1 cluster of the natural killer cell gene complex (NKC), in close proximity to the NKC linkage group (hchp/cd4/lag3)-a2-m-cd69-prp (43). The NKC contains the

2 Data can be accessed through the internet: http://www.informatics.jax.org/crossdata.html. Enter DNA Mapping Panel Data Sets from MGD, then select the Seldin cross and chromosome.

![Diagram](image-url)
genes encoding numerous C-type lectins, including Ly-49 family members involved in “missing self” recognition and natural killer cell inhibition, CD161 homologues involved in NK cell activation, and CD69, a lymphoid activation marker (44, 45). Each of these lectins is a type II transmembrane, disulfide-linked homodimer approximately 200–300 amino acids in size (46–49). Together they belong to the animal C-type lectin Group V and are evolutionarily distinct from the Group II lectins that they resemble structurally (26). This gross structural resemblance between Group V and Group II lectins and the propensity of the NKC and mcl gene loci might suggest a similar self-recognition function for mMCL in Mø biology. However, excluding the core C-type lectin-conserved residues, mMCL bears very little sequence similarity to any of the lectins found on natural killer cells and other lymphocytes. mMCL lacks, for example, the distinctive cytoplasmic immunoreceptor tyrosine-based inhibitory motifs (50) and activation motifs (51) found in the proteins encoded within the NKC.

Concurrent with the sequence and genomic analysis of this molecule, biological investigations of mMCL were also undertaken in order to study its ligand binding, distribution characteristics, and possible functions. The protein was expressed in the presence of microsomes using the rabbit reticulocyte lysate system to confirm its nature as an integral membrane protein. A single specific product approximately 30 kDa in size was transcribed (data not shown). Multiple, ultimately unsuccessful attempts were made to generate CHO.K1 and COS-7 cells transiently expressing various soluble and membrane forms of the protein. Eventually a stable line of CHO.K1 cells expressing a carboxyl-terminal FLAG-tagged full-length version of this protein was isolated. Staining of permeabilized and nonpermeabilized transfected cells with the anti-FLAG M2 mAb revealed variable surface expression of mMCL (Fig. 5, A and C). The majority of the overexpressed protein appeared to be trapped within the endoplasmic reticulum of the cells (Fig. 5B). A rabbit antisem (rabbit 16T) raised against the cytoplasmic tail of a single specific product approximately 30 kDa in size was reduced protein runs at approximately 30 kDa again, verifying the presence of microsomes using the rabbit reticulocyte system lysates suggests that these forms might be artifacts of overexpression or misfolding of the recombinant protein. The possible formation of higher order oligomers and misfolded monomers may also explain why so much of the expressed protein is retained within the endoplasmic reticulum of the stably transfected CHO.K1 cells rather than being transported to the cell surface.

The low and variable surface expression and possible misfolding of the recombinant form of the mMCL molecule have hitherto prevented the identification and study of its ligand and possible function. Current studies are under way to isolate a mMCL-specific mAb and a functional form of recombinant mMCL protein. Additional investigations into the nature of this lectin have the potential of furthering our understanding of this relatively new and growing field of cell-surface proteins. It may also increase our knowledge of the activities Mø perform, how these cells are regulated, and how they regulate the cells around them.

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