Characterization of the Murine Macrophage Mannose Receptor: Demonstration That the Downregulation of Receptor Expression Mediated by Interferon-γ Occurs at the Level of Transcription

By Neil Harris, Michael Super, Miriam Rits, Grace Chang, and R. Alan B. Ezekowitz

The macrophage mannose receptor (MMR) is a 175-Kd cell-surface transmembrane glycoprotein that is expressed on tissue macrophages where it functions both to mediate the uptake of mannose-rich glycoproteins and as a phagocytic receptor for bacteria, yeasts, and other pathogenic microorganisms. In this report we describe the cloning of the full-length cDNA of the mouse macrophage mannose receptor and we investigate the level at which interferon γ (IFN-γ) downregulates mannose receptor expression. The latter is a marker of the functional state of the cell as high levels are expressed on resident and inflammatory macrophages, whereas cells activated by treatment with IFN-γ have decreased-to-absent cell-surface mannose receptor expression. The murine MMR cDNA contains an open reading frame that predicts a protein of 1,456 amino acids. Transient expression of the protein in heterologous cells shows that this cDNA encodes a functional mannose receptor. The deduced amino acid sequence of this protein has an overall 82% homology with the human mannose receptor and as such, the ectodomain contains an N-terminus that is cysteine-rich followed by a fibronectin type II domain and eight carbohydrate recognition domains (CRDs). The ectodomain is linked to a hydrophobic transmembrane region and a 46-amino acid cytoplasmic tail. All of the eight CRDs are particularly well conserved, especially CRD4, which shows 92% homology with the equivalent region of the human protein. Steady-state levels of murine MMR mRNA were measured in the macrophage cell line J774E, which is known to express the protein at the cell surface. These levels were decreased by a 4- to 8-hour incubation with IFN-γ, but were almost abolished by overnight treatment with this cytokine. Nuclear run-on experiments showed that IFN-γ inhibits MMR gene transcription. Therefore, the regulation of mannose receptor expression by IFN-γ provides a novel system in which to study the mechanisms by which this cytokine represses gene expression.

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MACROPHAGES play a central role in host defense in collaboration with antibody, complement, and T lymphocytes.1-3 The macrophages function as effector cells and contribute to the induction of the immune response. Tissue macrophages often form a lattice beneath epithelial surfaces in the lung, gut, kidney, and skin and as such play a role in first line host defense.1,2 Direct recognition of foreign antigens is a primary function of tissue macrophages and it is these cells that express phagocytic receptors such as Fc3,4 and complement receptors,3 and high levels of mannose receptor.2 Although recognition of complex surfaces that decorate the cell walls of pathogens may well involve cooperation between a number of macrophage surface receptors, recent experiments have shown that each of the major phagocytic receptors, viz, Fc,5,6,7 complement,8 and mannose receptors,9 can function alone when expressed in heterologous cells. Transient transfection of the human mannose receptor into COS-1 cells showed that the receptor is sufficient to mediate the binding of Candida albicans and Pneumocystis carinii.9,10 These microorganisms, as well as other pathogens, have cell walls that contain mannose-rich glycans. The latter are rarely, if ever, present as exposed moieties in eukaryotic proteins. Binding of pathogens via the mannose receptor on the macrophage surface initiates secretion of a wide array of inflammatory mediators including arachidonic acid metabolites, reactive oxygen intermediates, and neutral proteinases.1,2

The cDNA of the human mannose receptor has been characterized9,11 and was found to encode a transmembrane protein of 1,438 amino acids after cleavage of the signal peptide. The receptor possesses a short transmembrane region and a C-terminal cytoplasmic tail of 28 and 45 residues, respectively. There are also eight tandem carbohydrate recognition domains (CRDs), each bearing the features of a calcium-dependent or C-type animal lectin.12 The C-type lectin family is very diverse.12,13 Some members are transmembrane proteins, e.g., the asialoglycoprotein receptor13-15 and the selectins16-18 while others are secreted humoral proteins, e.g., the serum mannose-binding proteins.5,19,20 The mannose receptor is the only member of this family in which a monomer contains more than one CRD. In this study we report the characterization of the murine macrophage mannose receptor cDNA. Both the cDNA and the predicted sequence of the murine protein display a high degree of homology with the human macrophage mannose receptor (MMR) sequence. The CRDs, the N-terminal cysteine-rich region, and the domain with the features of a fibronectin type II repeat are particularly well conserved. Because the latter two regions are known not to participate in the binding to carbohydrate,21 their sequence conservation suggests a hitherto unrecognized function. In addition to its physiologic role as a pinocytotic22,23 and phagocytic receptor,5,24,25 the expression of the MMR is a sensitive marker of the functional state of the macrophage in that high levels are expressed on nonactivated macrophages.

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Supported by National Institutes of Health Grant No. R01 AI23786-05 (R.A.B.E.), the South African Medical Research Council (N.H.), and R.A.B.E. is an Established Investigator of the American Heart Association.

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Treatment of macrophages with interferon γ (IFN-γ) induces the activated phenotype which, while it includes the upregulation of many genes, causes downregulation of MMR cell surface expression. Although much attention has focused on studying IFN-γ inducible genes, little is known about IFN-γ-modulated gene repression. To investigate whether decreased mannose receptor surface expression is regulated transcriptionally or posttranscriptionally, we chose to examine the murine macrophage cell line J774E, which is known to express cell-surface mannose receptor. We first established, by means of a nuclease protection assay, that IFN-γ treatment results in a decrease in the stable accumulation of MMR-specific mRNA. A nuclear run-on analysis then demonstrated that this decrease can be accounted for by a decrease in the transcription of the mannose receptor gene.

MATERIALS AND METHODS

Identification of Murine cDNA

An oligo-dT-primed mouse peritoneal macrophage Agt11 cDNA library (Clonetech Laboratories Inc, Cat. No. ML1005b; Palo Alto, CA) was plated on *Escherichia coli* strain Y1088 and was screened as previously described by plaque hybridization with a 1.9-kb *Pst*I fragment derived from the full-length human macrophage mannose receptor cDNA. Briefly, prehybridization and overnight hybridization of the nylon filters (Amerham, Hybond N; Arlington Heights, IL) were performed at 42°C in 6X SSC, 5X Denhardt’s solution, 0.5% sodium dodecyl sulfate (SDS), and 100 μg/mL denatured salmon sperm DNA. Filters were washed at 42°C in 1X SSC, 0.1% SDS.

The screening of approximately 500,000 plaques from the murine cDNA library yielded a single positive phage clone (agt11.1) that was isolated by two rounds of amplification. This library was rescreened at a higher stringency using a radiolabeled 3' 274-bp fragment derived from the single positive phage. This procedure identified a second positive overlapping cDNA phage clone (agt11.2).

A 292-bp *Bam*HI fragment of agt11.1 provided a probe to screen a *zAP2* random-primer mouse peritoneal macrophage cDNA library (MACZAP3, kindly provided by Dr Paul Crocker). This was performed according to a modification (K. Sastry, personal communication, 1990) of the protocol of Frohman et al. First-strand murine mannose receptor cDNA was tailed with poly-G as follows: 10 μL of the cDNA preparation was added to 4.6 μL of 5X Terminal Transferase Buffer (Boehringer Mannheim), 4 μL of 1 mmol/L dGTP, and 2 μL of terminal transferase (Boehringer Mannheim). The final reaction volume was 20 μL. The reaction was incubated at 37°C for 15 minutes and then inactivated at 65°C for 5 minutes. Finally, the reaction mixture was diluted to 500 μL with Tris-EDTA (TE) pH 7.5. Ten microliters of this product were then amplified with 3 oligonucleotide primers by means of the polymerase chain reaction (PCR). Two of the primers, KS1 and KS2, were sense “adaptor” primers. These primers both possess a number of useful restriction sites (including EcoRI) for the purposes of subcloning. KS1 was the “adaptor-dC” primer while KS2 was the “adaptor” primer (KS1, 5′-GCAATGCCTCACGCGGCGGGCCAATCTCCCATACCCTCCC; KS2, 5′-GCATGCGGCGGCGGCGGCGCAAATCCCATACCCTCCC). The third primer was the antisense 26-mer complementary to nucleotides 509 to 534 (primer A). This primer contained a 5′ *Bam*HI site.

Ten microliters of the poly-G-tailed first-strand cDNA was mixed with 10 pmol KS1, 25 pmol KS2, and 25 pmol of primer A. PCR was performed in a final reaction volume of 50 μL in the presence of 250 μmol/L of each dNTP, 2 mmol/L dTTT, 1X PCR buffer (Boehringer Mannheim), and 2.5 U Taq polymerase (Boehringer Mannheim). Amplification was performed over 30 cycles. Melting was at 94°C for 30 seconds, annealing at 55°C for 30 seconds, and extension at 72°C for 3 minutes. The PCR product was purified and was digested with EcoRI and *Bam*HI and was subcloned into the polylinker site of pUC18.

Mammalian Cell Cultures

Cell cultures were performed at 37°C in 5% CO₂/95% humidified air. J774E cells were obtained as a kind gift of Dr Philip Stahl (Washington University School of Medicine, St Louis, MO). These cells were maintained in RPMI-1640 (GIBCO BRL, Grand Island, NY) containing 10% fetal calf serum (FCS) (GIBCO). COS-1 cells were grown in Dulbecco’s Modification of Eagle’s Minimal Essential Medium (DMEM; GIBCO) and supplemented with either 10% FCS or 10% newborn calf serum. The former was used to maintain the cells in culture while the latter was used in the transfection protocol.
Construction of a Full-length Mannose Receptor cDNA

The 5-kb hZAP3.3 cDNA clone was the longest that was isolated and it contained the nucleotides that corresponded to in number from 175 to 5,100 of the full-length cDNA. A unique BspHII site was present 121 bp from the 5' end of this clone. This corresponded to the BspHI site at position 296 of the full-length cDNA. The hZAP3.3 clone was excised from the pUC18 vector and was subcloned into the EcoRI site of a mammalian expression vector (obtained as a kind gift of Dr Brian Seed, Massachusetts General Hospital, Boston). This vector contained a unique MluI site in the polylinker immediately upstream of the EcoRI cloning site. Restriction digest analysis of these plasmids containing subcloned hZAP3.3 allowed selection of those with the correct 5' to 3' orientation. These constructs were then sequentially digested with BspHII and MluI.

The region encompassing nucleotides 1 to 489 of the 534 bp 5' RACE product was amplified by a PCR. The 5' PCR sense primer contained a unique MluI site that was absent from the RACE template. Melting was at 94°C for 1 minute, annealing at 55°C for 2 minutes, and extension was performed at 72°C for 3 minutes. The PCR proceeded over 30 cycles. The product of this reaction was sequentially digested with BspHII (which cuts at position 296) and with MluI that would cut at the new 5' MluI site. The 296-bp fragment that was generated by this digest was then vectorially subcloned into the BspHII/MluI-cut pZAP3 expression vector. This generated a full-length cDNA construct termed pMMR. Partial sequence analysis confirmed that the ends of the construct were as predicted.

Expression of the Mannose Receptor in Heterologous Cells

COS-1 cells were transfected as previously described with the full-length murine mannose receptor construct pMMR. The day after the transfection, the cells were trypsinized and were replated into 6-well 25-mm diameter cluster plates (Costar). The next day, a suspension of zymosan particles conjugated with the fluorescent probe BODIPY (BODIPY-Zymosan; Molecular Probes Inc, Eugene, OR) that was added to a final concentration of 50 μg/mL. The cells were incubated with the zymosan particles for 30 to 60 minutes, and extension was performed at 72°C for 3 minutes. The PCR proceeded over 30 cycles. The product of this reaction was sequentially digested with BspHII and MluI.

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Isolation of Total RNA From Cells

This was performed according to the single-step acid guanidium thiocyanate phenol-chloroform extraction procedure as described. RNA samples were stored at −80°C.

IFN-γ

Recombinant murine IFN-γ was obtained as a kind gift of Genentech Inc (South San Francisco, CA). Just before use, the IFN was diluted in RPMI containing 10% fetal bovine serum (FBS). The IFN was used at concentrations of 200 to 500 U/mL in all experiments with J774E cells.

Ribonuclease Protection Assay

cDNA clone agt11.1 in pUC18 was digested with Hind III and Pst I. A 299-bp fragment of this digest was subcloned into the vector pGEM-4Z (Promega). This 299-bp fragment encompassed the area corresponding to nucleotides 3660 to 3925 (264 bp) of the full-length cDNA (the extra 35 bp consisted of a fragment of the pUC18 polylinker). The orientation of the subcloned fragment was determined by partial sequencing. PvulII digestion of the construct yielded a linear 681-bp blunt-ended fragment that contained both the insert as well as the SP6 and T7 RNA polymerase promoter sites. This fragment was used as a template for in vitro transcription. Radiolabeled 570-bp antisense mannose receptor RNA transcripts were generated from 0.5 μg of template in the presence of 15 U of SP6 polymerase (Promega). In a similar fashion, a human γ-actin control riboprobe was transcribed off a pSP6 γ-actin vector. In vitro transcription was performed in a final reaction volume of 20 μL and in the presence of 1X transcription buffer (Promega), 10 mmol/L L DTT, 30 U ribonuclease inhibitor (Promega), 0.5 mmol/L of ATP/GTP/UTP, 0.015 mmol/L CTP, and 100 μCi of α32P CTP (Amersham PB 40382). Transcription proceeded at 37°C for 1 hour and was terminated by the addition of 1 U of DNase for an additional 15 minutes at 37°C (RQDNAse, RNase-free; Promega). An extra 30 U RNase inhibitor was added at this stage to protect the riboprobe.

The riboprobes were then extracted in phenol/chloroform plus isoamyl-alcohol and were ethanol precipitated in the presence of ammonium acetate (1 vol extract, 1 vol 4 mol/L ammonium acetate, 4 vol 95% ethanol). The probes were then washed several times in 70% ethanol and dried briefly before being finally resuspended in 100 μL sterile RNase-free water.

For the protection assay, 20 μg of each sample of total cellular RNA was resuspended in 24 μL of hybridization buffer comprising 40 mmol/L PIPES pH 6.4, 400 mmol/L NaCl, 1 mmol/L L EDTA, and 80% (vol/vol) formamide. Freshly prepared mannose receptor and γ-actin riboprobes, 2 to 5 x 106 cpm, were added to each of the RNA suspensions. The samples were heated at 95°C for 5 minutes and were immediately transferred to 37°C for overnight hybridization. The total volume of the reaction mix was 25 μL. After hybridization, 350 μL of RNase buffer (RNase A 40 μg/mL, RNase T1 2 U/mL, 10 mmol/L Tris-HCl pH 7.5, 5 mmol/L L EDTA, 300 mmol/L NaCl) was added to each sample. Digestion was allowed to proceed at 37°C for 20 minutes. This digestion was terminated by the addition of SDS and proteinase K (Boehringer Mannheim) to final concentrations of 0.5% (wt/vol) and 0.27 mg/mL, respectively, with a further 15-minute incubation at 37°C. Samples were then extracted by phenol/chloroform plus isoamyl-alcohol, ethanol precipitated, and dried without additional ethanol washes. They were then reconstituted in 10 μL of gel loading buffer (US Biochemicals, Sequencing Stop Buffer), heated at 90°C for 5 minutes, and snap-cooled on ice. The samples were electrophoresed on a 3% polyacrylamide gel containing 7 mol/L urea in the presence of Tri-Borate EDTA buffer (TBE, 89 mmol/L Tris-Borate, 2 mmol/L L EDTA). The gel was dried and was subjected to autoradiography at −80°C. 4x174 DNA (GIBCO-BRL) that had been digested with HaeIII and labeled with α32P dGTP in the presence of Klenow fragment DNA polymerase served as the molecular size standards in the electrophoresis.
Transcription Run-on Assay

DNA, 2 to 5 μg, was used at each position of the dot-blot. The appropriate plasmid was denatured by boiling for 10 minutes in 200 μL of TE pH 7.4 containing 0.2 mol/L NaOH. After boiling, the sample was made up to 400 μL with 10X SSC and was neutralized with 44 μL of 2 mol/L HEPES. The sample was loaded onto a prewetted nylon membrane (Amersham, Hybond N) using a dot-blot apparatus (Schleicher & Schuell, Minifold I; Keene, NH). Each well of the apparatus was rinsed with 6X SSC. The membrane was dried and the DNA was linked to the nylon by UV irradiation.

The run-on assay was performed according to previously described protocols. Subconfluent J774E mouse macrophage cells were cultured overnight in the presence or absence of 500 U/mL of appropriate plasmid was denatured sample was made up to 400 μL with 10X SSC. The sample was then digested sequentially with DNase1 and with Proteinase K. An extraction with phenol-chloroform was followed by TCA precipitation onto Millipore HA filters (Millipore, Bedford, MA). The samples were eluted from the filters, alkalinized briefly, and then neutralized according to the protocol. The RNA was then precipitated and partially dried before being resuspended in a hypotonic lysis buffer containing 1% NP-40 and 1% SDS. The sample was then digested sequentially with DNase1 and with Proteinase K. An extraction with phenol-chloroform was followed by TCA precipitation onto Millipore HA filters (Millipore, Bedford, MA). The samples were eluted from the filters, alkalinized briefly, and then neutralized according to the protocol. The RNA was then precipitated and partially dried before being resuspended in the hybridization solution.

The labeled nascent RNA transcripts were hybridized with nylon strips containing the various plasmids described in the legend to Fig 6. Hybridization and washing of the nylon strips was performed at 65°C.

RESULTS AND DISCUSSION

Mannose Receptor cDNA and Primary Sequence

A human mannose receptor cDNA fragment encompassing the region coding for carbohydrate recognition domains (CRDs) 3 through 7 was used as a probe to screen an oligo-dT primed mouse peritoneal macrophage λgt11 cDNA library. Two positive clones were selected from ~5 × 10⁵ plaques and were purified, subcloned, and sequenced. The two clones, λgt11.1 and λgt11.2, were found to contain an overlapping nucleotide sequence (Fig 1B). The combined sequence of 3.8 kb had a significant degree of homology (75% to 80%) with the nucleotide sequence downstream of position 1368 of the human mannose receptor cDNA. Clone λgt11.2 contained a long poly-A stretch consistent with a poly-A tail. A 5' fragment of clone λgt11.1 corresponding to nucleotides 1368 to 1660 of the human cDNA was used to screen a random-primed λZAP murine macrophage cDNA library. Four positive overlapping clones, λZAP3.2, λZAP3.3, λZAP3.5, and λZAP3.10, were selected from ~4 × 10⁵ plaques. λZAP3.5 of ~5 kb was both the longest clone as well as that with the greatest 5' extent (Fig 1B). This clone was homologous to nucleotides 179 through 5150 of the human cDNA and it contained most of the sequence of the previous two λgt11 clones, with the exception of the most 3' untranslated region and the poly-A tail.

Because further screening of the available mouse cDNA libraries yielded no further clones, we used the previously described PCR-based RACE procedure to obtain an additional 5' murine cDNA sequence. This approach (see Materials and Methods) generated a 538-bp product with 75% to 80% homology to the human mannose receptor cDNA from nucleotide 1 to 538 (Fig 1B). When the sequence of the RACE product was overlapped with the λZAP3.3 clone, the combined sequence had significant homology with the full-length human sequence. The first nucleotide of the RACE product corresponded to the most 5' extent of the human cDNA sequence. Translation of the derived full-length murine sequence showed a long

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Fig. 1. Murine mannose receptor cDNA. (A) Schematic view of the macrophage mannose receptor showing the structural domains. The numbering 1 to 8 refers to the carbohydrate recognition domains. Abbreviations: CYS RICH, cysteine-rich; FN II, fibronectin type II repeat; TM, transmembrane segment. (B) Murine mannose receptor cDNA clones. The restriction map of the full-length cDNA is given at the top. The protein coding region is shown in this map as an open box. The extent of the cDNA clones are depicted as lines below the main restriction map. The arrowhead on clone λgt11.2 indicates a poly-A tail. The nomenclature of these clones is described in the text. The nucleotide sequence can be obtained via EMBL accession no. Z11974.
open reading frame that terminated with a stop codon at position 4467. The ATG at position 100 through 103 of the murine cDNA was determined to be the translation start site, based on the very close homology with the previously sequenced human cDNA.

Analysis of the deduced primary amino acid sequence of the murine macrophage mannose receptor protein shows a striking homology with its human counterpart. The two molecules show an overall 82% identity at the amino acid level. The first 18 amino acids following the initiator methionine have the features of a signal sequence, viz, a hydrophobic core and a charged residue following the methionine. A leucine residue 18 amino acids downstream of this methionine was deduced to be the N-terminus of the mature protein. This again was based on the homology between the human and the mouse sequence.

The mature murine macrophage mannose receptor possesses 1,438 amino acids and is therefore identical in this regard to the human receptor. The sequence displays seven potential N-glycosylation sites while the human equivalent has eight.10 Like the human protein,311 the murine mannose receptor possess an N-terminal cysteine-rich domain followed by a domain with close homology to the type II repeat found in fibronectin (see below). The latter is in turn followed by eight CRDs, a transmembrane region, and a C-terminal cytoplasmic tail (Fig 1A). The extent of the eight CRDs, as well as their alignment, was mapped using previous data from the human receptor sequence and with the assistance of the recently published crystallographic analysis of the rat MBP-A carbohydrate recognition domain.39

This allowed us firstly to define the position of each of the domains together with their connecting stalks. Secondly, we were able to align the eight murine CRDs both with one another as well as with the single CRD of rat MBP-A (Fig 2). Weis et al39 have demonstrated that when a number of animal C-type lectins are aligned, 37 amino acid positions are generally conserved. In some cases, a particular type of amino acid is conserved, while in other cases it is the class of amino acid that is conserved, eg, aliphatic or aromatic. All the CRDs of the mouse mannose receptor have at least 27 of these conserved residues (Fig 2). CRD4 has 34, CRD2 has 33, while CRD4 and CRD6 each have 31.

In Fig 3 the close homology between the murine and human mannose receptors is displayed. These data are summarized in Table 1. Almost all regions of the two proteins show a high degree of amino acid identity and many of the substitutions are conservative. CRD4 is the most highly conserved of the carbohydrate recognition domains with 92% identity between the human and mouse proteins. This is consistent with the recent observation that CRD4 is important in determining both the carbohydrate-binding affinity and specificity of the intact mannose receptor.21 Indeed, CRD4 appears to be the only CRD that can bind to carbohydrate in isolation. However, our data demonstrate significant conservation of all the carbohydrate recognition domains despite evidence that not only are CRDs 1 through 3 nonessential for carbohydrate recognition,21 they also fail to bind to mannose-Sepharose affinity columns, unlike CRDs 4 through 8 or 5 through 8.

The issue of functional importance also arises when one

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**Fig 2.** The alignment of the eight CRDs of the murine mannose receptor. The eight CRDs of the murine macrophage mannose receptor have been aligned with one another and with the single CRD of rat MBP-A according to the criteria of conserved amino acid positions set out in ref 39. There are 37 such positions, some of which refer to a single invariant amino acid while others encompass a particular class of amino acids. The conservation is indicated by the single letters on the bottom line of the figure. The predicted secondary structures within the CRDs, viz, α-helices, β-sheets, and loops (L) are depicted on the line below the human domains. Note that no single domain fulfils all 37 criteria, which were based on an analysis of a number of C-type lectin carbohydrate recognition domains. The following code is used (see ref 39): α, aliphatic; φ, aromatic; χ, aliphatic or aromatic; Z, E or Q, B, D or N; Ω, D, N, E, or Q (carbonyl oxygen atom in side chain). Amino acids that have been conserved in five or more mannose receptor CRDs have been shaded. These have been selected independently of the abovementioned criteria.
Fig 3. Alignment of the murine and human mannose receptor domains. Selected regions of the mature murine and human mannose receptor amino acid sequences have been aligned with respect to one another. The murine sequence is shown above the human sequence and conserved amino acids are shaded. The regions shown include the two N-terminal domains of the mature protein (cysteine-rich and fibronectin type II) as well as the eight CRDs together with the transmembrane region and the C-terminal cytoplasmic tail. The stalks refer to the segments which connect the CRDs. The amino acid numbering is given at the beginning of each domain. Both the mouse and human numbering are given at the points where these differ.

The human and murine N-terminal domains are extensively conserved (Table 1) with the fibronectin type II repeat showing the greatest sequence conservation of the entire protein (93% identity). Although this suggests some essential function, none has yet been assigned to these N-terminal domains. Like the first three CRDs, neither the N-terminal cysteine-rich domain nor the fibronectin type II repeat are required for carbohydrate binding. The cysteine-rich domain appears to be unique to the mannose receptor in that a similar region has not been identified in any other known protein sequence. The fibronectin type II repeat, which is found in a number of proteins (see below), was named for its presence in the extracellular matrix protein fibronectin. The latter is a dimer of two large 250-Kd subunits, each containing multiple copies of three types of homology units, types I, II, and III. Each subunit contains two type II repeats and these are present in the region of the molecule known to interact with collagen. The latter is not a member of the C-type lectin family. It functions to direct lysosomal enzymes tagged with mannose-6-phosphate to the appropriate lysosomal compartment and also serves as a receptor for the growth factor IGF II. The only protein domain common to the macrophage mannose receptor and the cation-independent mannose-6-phosphate receptor appears to be the fibronectin type II repeat. However, the extensive conservation of this domain in the macrophage mannose receptor as well as its presence in a variety of secreted, transmembrane, and extracellular proteins suggests that it may have an important function. One postulated function is that this domain plays a role in macrophage adherence to the extracellular matrix and in the subsequent spreading of the cell. In addition, it is possible that the interaction with certain classes of pathogens may be enhanced by this region of the ectodomain. The high degree of conservation between the mouse and the human receptors provide support for this concept.

Expression of the Mannose Receptor in Heterologous Cells

The human mannose receptor has been shown to retain its phagocytic properties when expressed in heterologous cells. Therefore, we investigated whether the same was true for the murine equivalent. A full-length murine mannose receptor cDNA construct was generated by joining the 5' RACE product to the longest cDNA clone XZAP3.3 (see...
### Table 1. Comparison of the Amino Acid Sequences of the Individual Domains of the Murine and Human Mannose Receptors

<table>
<thead>
<tr>
<th>Domain</th>
<th>% Identity</th>
</tr>
</thead>
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<tr>
<td>Signal peptide</td>
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<tr>
<td>Cysteine-rich</td>
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<tr>
<td>Fibronectin type II</td>
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<td>Carbohydrate Recognition Domains</td>
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</tr>
<tr>
<td>CRD 1</td>
<td>88</td>
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<tr>
<td>CRD 2</td>
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<td>84</td>
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The identities between the human and murine sequences were determined using the alignment function of the Microgenie sequence analysis program (SciSoft, Inc, Distributed by Spinco Division, Beckman Instruments, Inc, Palo Alto, CA).
Fig 4. Transient expression of the murine receptor in COS-1 cells. A full-length murine mannose receptor cDNA construct was subcloned into a mammalian expression vector, which was then transfected into COS-1 cells. Two days posttransfection, the cells were treated for 30 to 60 minutes with zymosan particles conjugated with the fluorescent probe BODIPY. The cells were trypsinized and spun onto microscope slides using a cytocentrifuge. The preparations were stained and examined under a fluorescence microscope (set up to observe fluorescein isothiocyanate) using dual-transmitted light and epi-fluorescence. The photographs labeled (A) and (B) show two representative sections of the slide. The arrow indicates a number of adherent zymosan particles that appear to be undergoing phagocytosis. Original magnification: (A) x650; (B) x400.

Expression of the Mannose Receptor Message in J774E Murine Macrophage Cells

The cytokine IFN-γ is known to effect a number of significant changes in both the morphology and biology of tissue macrophages. The expression of the high affinity Fc receptor Fcγ R1 (CD64) is enhanced and there is increased production of reactive oxygen intermediates. The ability of the macrophage to kill intracellular parasites is significantly augmented. However, mannose receptor expression has been reported to decrease in the presence of IFN-γ. Therefore, an RNase protection assay was performed on J774E cells to investigate the response of the mannose receptor messenger RNA to IFN-γ (see Materials and Methods).
It was anticipated that if the 570-bp mannose receptor riboprobe was digested with RNAses A and T1 in the presence of total cellular RNA, a protected band of 264 bp would be seen if mannose receptor-specific mRNA was present. A band of the correct size was observed in assays performed with RNA from both thioglycollate-elicited mouse peritoneal macrophages and J774E cells (Fig 5). A second larger protected band of 310 bp was consistently observed. Because the riboprobe comprised 264 bp of mannose receptor-specific sequence and 306 bp of vector sequence, the presence of a larger protected band could not be readily explained. Therefore, account was taken only of the 264-bp band.

Preliminary experiments indicated that brief periods of treatment (<4 hours) with IFN-γ had no apparent effect on the steady-state levels of mannose receptor mRNA in J774E cells (data not shown). If the cells are incubated with the IFN-γ for 1½ hours and are then washed and allowed to grow in medium free of IFN-γ for a further 6½ hours, a slight downregulation of the message is observed (Fig 5). Longer incubations with the cytokine have a more pronounced effect. However, overnight treatment of J774E cells with recombinant IFN-γ markedly downregulates mannose receptor mRNA levels (Fig 5).

The data presented above are consistent with previous evidence that IFN-γ downregulates the expression of the mannose receptor at the cell surface.26,37 Although both the
The full-length cDNA construct was used. These filters were hybridized at 65°C in the presence of nascent 3H-labeled RNA from J774E cells either untreated (A) or treated (B) overnight with IFN-γ.

Fig 6. The effect of IFN-γ on mannose receptor expression in J774E cells appears to be at the level of transcription. cDNA probes for the murine mannose receptor, pMMR (1) or murine β-actin (2) were attached to nylon filters using a dot-blot apparatus as described in Materials and Methods. In the case of the mannose receptor, the full-length cDNA construct was used. These filters were hybridized at 65°C in the presence of nascent 3H-labeled RNA from J774E cells either untreated (A) or treated (B) overnight with IFN-γ.

mannose receptor and FcγRII appear to mediate phagocytosis, their opposite response to IFN-γ makes it plausible that while the high-affinity Fc receptor interacts with opsonised microorganisms as part of the specific immune response, the mannose receptor functions at an earlier stage during the innate immune response, i.e., in the absence of specific antibodies or activated T-helper cells.

To address the question of the level of action of the IFN in the above experiments, a nuclear run-on assay (see Materials and Methods) was performed with J774E cells that were either untreated or treated overnight with IFN-γ at 500 U/mL (Fig 6). This assay demonstrates that the rate of transcription of the mannose receptor gene is significantly decreased in the presence of IFN-γ whereas transcription of actin is unaltered. Repression of mannose receptor gene expression by IFN-γ appears to be cycloheximide sensitive (results not shown), and this implies that this effect may be mediated via a newly synthesized IFN-γ-inducible protein that would be a candidate repressor. We are at present attempting to map the cis-acting elements that may be targets for this putative trans-acting protein to test this hypothesis.

ACKNOWLEDGMENT

We thank the members of the laboratory for helpful discussions and Marsha Kartzman for her assistance in preparing this manuscript. We are grateful to Dr Celeste Simon for providing the human γ-actin probe and to Dr Nancy Andrews for the murine β-actin probe.

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Characterization of the murine macrophage mannose receptor: demonstration that the downregulation of receptor expression mediated by interferon-gamma occurs at the level of transcription

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