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Molecular cloning of a human macrophage lectin specific for galactose

(Mac-2 antigen/cDNA sequence/laminin binding)

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ABSTRACT **The murine Mac-2 protein is a galactose- and IgE-binding lectin secreted by inflammatory macrophages. We describe here the cloning and characterization of a cDNA representing the human homolog of Mac-2 (hMac-2). The amino acid sequence derived from the hMac-2 cDNA indicates that the protein is evolutionarily highly conserved, with 85% of its amino acid residues being similar to those in the murine homolog. This conservation is especially marked in the carboxyl-terminal lectin domain. The amino-terminal half of the protein is less conserved but still contains the repetitive proline-glycine-rich motif seen in the mouse protein. hMac-2 synthesized *in vitro* is recognized by the M3/38 monoclonal antibody to Mac-2 and binds to the desialylated glycoprotein asialofetuin and to laminin, a major component of basement membranes. These findings are discussed in the context of the potential functions of hMac-2.**

Macrophages activated during the course of acute or chronic inflammation release a number of soluble proteins which mediate or regulate the effects of the inflammatory response. Understanding the function and mode of regulation of these monokines is critical to devising strategies for therapeutic intervention in inflammatory disorders. The murine Mac-2 antigen was originally described by Ho and Springer (1) and was shown to be expressed at a high level on the surface of inflammatory macrophages. We recently cloned cDNAs encoding Mac-2 and showed that the protein is secreted and has the characteristics of a galactose-specific lectin (2). A search of the computer data bases revealed that it had been identified independently by two other groups on the basis of its carbohydrate-binding property. Wang and colleagues (3, 4) identified the protein, which they called carbohydrate-binding protein 35 (CBP 35), in 3T3 mouse fibroblasts, and on the basis of immunolocalization studies and sequence homologies proposed that it might be a component of nuclear ribonucleoprotein complexes. Raz *et al.* (5) identified the same protein (under the name L-34) as a tumor cell surface lectin and suggested that it enhanced tumor metastasis by promoting the formation of multicellular emboli. A rat cytosolic protein highly similar to Mac-2/CBP 35/L-34 has been described and has been shown to bind IgE (6). We have shown that Mac-2 also has the ability to bind murine IgE (2). Recently, yet another function has been revealed by the work of Woo *et al.* (7), who showed that Mac-2 is the major nonintegrin laminin-binding protein synthesized by murine inflammatory macrophages, indicating a potential role in macrophage–extracellular matrix interactions.

Given the number of functions and subcellular locations proposed for Mac-2, further studies are clearly warranted. The high expression of this protein in inflammatory macrophages suggests that it has an important function in inflammation. In view of the potential involvement of this protein in processes relevant to human disease, we were interested in determining whether a human homolog of Mac-2 existed. In the present report we describe the cloning of the human homolog of Mac-2 (which we have designated hMac-2, for human Mac-2 antigen)* and show that the primary structure of the two proteins is highly conserved, especially in the lectin domain. The hMac-2 synthesized *in vitro* is recognized by the M3/38 monoclonal antibody to Mac-2 (1) and behaves like a galactose-specific lectin in its binding to the desialylated glycoprotein asialofetuin. It also binds to purified laminin, thus confirming and extending the results of Woo *et al.* (7).

MATERIALS AND METHODS

A cDNA library made from *Staphylococcus albus*-activated human monocytes in the vector λgt11 (kindly provided by Deborah Galson, Massachusetts Institute of Technology) was screened with the radiolabeled 970-base-pair (bp) *Eco*RI insert from the clone Mac 2.16 (2) under the following conditions: hybridization at 65° in 6× SSC (1× SSC is 0.15 M sodium chloride/0.015 M sodium citrate, pH 7)/0.2% SDS/1× Denhardt's solution (0.02% Ficoll/0.02% polyvinylpyrrolidone/0.02% bovine serum albumin)/1 mM EDTA and salmon sperm DNA at 100 μg/ml for 18 hr; wash with 3×SSC/0.1% SDS at 37°C. Positive plaques were purified, DNA was prepared, and the inserts were subcloned in the vector pBSK (Stratagene) as previously described (2).

To obtain 5′-extended hMac-2 cDNAs, the 660-bp *Eco*RI fragment from the clone hMac-2.2 was radiolabeled and used to screen, under high-stringency conditions (8), a cDNA library made from the human colonic carcinoma cell line HT29 in the vector CDM8 (9) (kindly provided by Ivan Stamenkovic, Massachusetts General Hospital). Positive colonies were purified, and plasmid DNA was prepared (8).

Double-stranded DNA sequencing was carried out with the T7 sequencing kit (Pharmacia), using appropriate restriction fragments cloned in pBSK. Oligonucleotide primers based on available sequence were used when the restriction fragment was too large to be sequenced by using the vector-based primers. Sequence comparisons were carried out with the computer program GAP (10).

In vitro transcripts of cloned cDNAs were generated by using the T7 promoter in CDM8 and the transcripts were translated *in vitro* as previously described (2). Northern analysis, Western analysis, metabolic labeling of cells, immunoprecipitations, precipitations with asialofetuin coupled to Sepharose, and SDS/PAGE were carried out as detailed earlier (2).

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Abbreviations: hMac-2, human Mac-2 antigen; PMA, phorbol 12-myristate 13-acetate.

*The sequence reported in this paper has been deposited in the GenBank data base (accession number M35368).

Binding of [^{35}S]methionine-labeled *in-vitro*-synthesized hMac-2 protein to laminin was carried out as follows. One milligram of laminin purified from the Engelbreth–Holm–Swarm sarcoma (GIBCO) was coupled (2) to 1 ml of Sepharose CL-4B (Pharmacia) and stored as an approximately 50% slurry in 0.5% Triton X-100 in 10 mM Tris·HCl, pH 7.4, with 3 mM $MgCl_2$ and 10 mM NaCl (binding buffer). hMac-2 RNA (1–2 μg) transcribed *in vitro* was translated in reticulocyte lysate (Promega) in a 50-μl reaction mixture containing 40 μCi (1 μCi = 37 kBq) of [^{35}S]methionine. At the end of the translation 20 μl of the reaction mixture was diluted in 1 ml of binding buffer and incubated with 0.2 ml of the laminin-Sepharose slurry overnight at 4°C with gentle agitation, in either the presence or the absence of competing sugars. After the beads had been washed with binding buffer, the bound proteins were eluted by boiling in sample buffer and then analyzed by SDS/PAGE.

The human cell lines THP-1 (monocytic leukemia) and HL60 (promyelocytic leukemia) were obtained from the American Type Culture Collection (Rockville, MD). The promyelocytic cell line HL60 was induced to differentiate toward the macrophage phenotype by treatment with 10^{-7}M phorbol 12-myristate 13-acetate (PMA) for 48 hr (11) and toward the myelocyte phenotype by treatment with 1.25% (vol/vol) dimethyl sulfoxide for 5 days (12).

RESULTS

Molecular Cloning of hMac-2 cDNA. Screening of a human activated monocyte cDNA library with the mouse Mac-2 probe under reduced stringency resulted in the isolation of several clones. The restriction map of the clone with the longest insert, hMac2.2, is shown in Fig. 1. Preliminary sequencing of this insert indicated that though it was highly similar to Mac-2, it did not extend sufficiently 5′ to include the translational start site. To obtain a clone with further 5′ sequence, the 660-bp *Eco*RI fragment of hMac2.2 was used to screen a cDNA library from the human colon carcinoma cell line HT-29 (which expresses hMac-2; data not shown) under high-stringency conditions. Of the clones thus obtained one, hMac2.3, was found to include the translational start site. The inserts from these clones were subcloned in the vector pBSK and used for subsequent experiments.

Sequence of hMac-2 cDNA. The nucleotide sequence and predicted amino acid sequence of the hMac-2 cDNA is shown in Fig. 2. We have obtained a total of 935 bp of cDNA sequence. Given that the size of the corresponding mRNA is approximately 2 kilobases (kb) (see below), the sequence lacks about 1 kb of the 5′ untranslated region. The single long

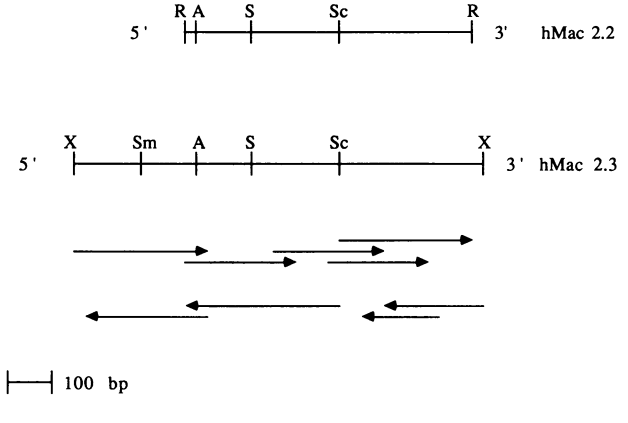

FIG. 1. Restriction map of hMac2 cDNA clones. Vertical lines indicate restriction sites: R, *Eco*RI; A, *Acc* I; S, *Sph* I; Sc, *Sca* I; Sm, *Sma* I; X, *Xho* I. Horizontal arrows indicate direction and extent of sequencing.

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   1 GGAGCCAGCCAACGAGCGGAAAATGGCAGACAATTTTTCGCTCCATGATGCGTTATCTGG  60
   1                   M  A  D  N  F  S  L  H  D  A  L  S  G      13

  61 GTCTGGAAACCCAAACCCTCAAGGATGGCCTGGCGCATGGGGGAACCAGCCTGCTGGGCA 120
  14  S  G  N  P  N  P  Q  G  W  P  G  A  W  G  N  Q  P  A  G  Q   33

 121 GGGGCTACCCAGGGGCTTCCTATCCTGGGGCCTACCCCGGGCAGGCACCCCCAGGGCTTA 180
  34  G  L  P  R  G  F  L  S  W  G  L  P  R  A  G  T  P  R  A  Y   53

 181 TCCTGGACAGGCACCTCCAGGCGCCTACCCTGGAGCACCTGGAGCTTATCCCGGAGCACC 240
  54  P  G  Q  A  P  P  G  A  Y  P  G  A  P  G  A  Y  P  G  A  P   73

 241 TGCACCTGGAGTCTACCCAGGGCCACCCAGCGGCCCTGGCTACCCATCTTCTGGACAGCC 300
  74  A  P  G  V  Y  P  G  P  P  S  G  P  G  Y  P  S  S  G  Q  P   93

 301 AAGTGCCCCCGGAGCCTACCCTGCCACTGGCCCCTATGGCGCCCCTGCTGGGCCACTGAT 360
  94  S  A  P  G  A  Y  P  A  T  G  P  Y  G  A  P  A  G  P  L  I  113

 361 TGTGCCTTATAACCTGCCTTTGCCTGGGGGAGTGGTGCCTCGCATGCTGATAACAATTCT 420
 114  V  P  Y  N  L  P  L  P  G  G  V  V  P  R  M  L  I  T  I  L  133

 421 GGGCACGGTGAAGCCCAATGCAAACAGAATTGCTTTAGATTTCCAAAGAGGGAATGATGT 480
 134  G  T  V  K  P  N  A  N  R  I  A  L  D  F  Q  R  G  N  D  V  153

 481 TGCCTTCCACTTTAACCCACGCTTCAATGAGAACAACAGGAGAGTCATTGTTTGCAATAC 540
 154  A  F  H  F  N  P  R  F  N  E  N  N  R  R  V  I  V  C  N  T  173

 541 AAAGCTGGATAATAACTGGGGAAGGGAAGAAAGACAGTCGGTTTTCCCATTTGAAAGTGG 600
 174  K  L  D  N  N  W  G  R  E  E  R  Q  S  V  F  P  F  E  S  G  193

 601 GAAACCATTCAAAATACAAGTACTGGTTGAACCTGACCACTTCAAGGTTGCAGTGAATGA 660
 194  K  P  F  K  I  Q  V  L  V  E  P  D  H  F  K  V  A  V  N  D  213

 661 TGCTCACTTGTTGCAGTACAATCATCGGGTTAAAAAACTCAATGAAATCAGCAAACTGGG 720
 214  A  H  L  L  Q  Y  N  H  R  V  K  K  L  N  E  I  S  K  L  G  233

 721 AATTTCTGGTGACATAGACCTCACCAGTGCTTCATATACCATGATATAATCTGAAAGGGG 780
 234  I  S  G  D  I  D  L  T  S  A  S  Y  T  M  I                 248

 781 CAGATTAAAAAAAAAAAAAAAAGAATCTAAACCTTACATGTGTAAAGGTTTCATGTTCACT 840

 841 GTGAGTGAAAATTTTTACATTCATCAATATCCCTCTTGTAAGTCATCTACTTAATAAATA 900

 901 TTACAGAGAAAAAAAAAAAAAAAAAAAAAAAAAAA 935
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FIG. 2. Nucleotide sequence and predicted amino acid sequence of the hMac-2 cDNA. The repeated hexapeptide as well as the motif found in S-type lectins is underlined.

open reading frame commences with the ATG at nucleotide 23, which is surrounded by a consensus translational initiation sequence (13) and terminates with the TAA codon at nucleotide 767. It encodes a 248-amino acid protein with a predicted molecular weight of 26,294 which has two domains: an amino-terminal portion (extending up to amino acid 137) which is proline- and glycine-rich and contains the motif Pro-Gly-Ala-Tyr-Pro-Gly (PGAYPG) repeated (as such or with minor modifications) five times and a carboxyl-terminal portion containing a consensus S-type lectin motif (14). It is worth noting that hMac-2, like its murine homolog, lacks an amino-terminal signal peptide or any other region of significant hydrophobicity. The 3′ untranslated region of the hMac-2 cDNA is distinctly A+T rich and there is a long poly(A) stretch between positions 787 and 801, well upstream of the poly(A) tail at the 3′ end. The former is unlikely to be a cloning artifact since it is present in clones isolated from both the monocyte and HT29 libraries.

A comparison of the predicted amino acid sequences of the mouse and human Mac-2 proteins is shown in Fig. 3. There is an overall amino acid sequence identity of 77%. If allowance is made for conservative amino acid changes, the similarity of the two sequences is 85%. The maximum conservation of primary structure is seen in the lectin domain. The repeated motif PGAYPG in the amino-terminal domain is seen in both proteins, but hMac-2 has fewer repeats, accounting for its smaller size.

Characterization of hMac-2 Synthesized *in Vitro*. The hMac-2 protein was synthesized *in vitro* by transcription off the T7 promoter on CDM8 and translation of the mRNA in a reticulocyte lysate. The protein so produced was immunoprecipitated specifically by the monoclonal antibody M3/38 directed against the murine Mac-2 antigen (1) and not by an isotype-identical control antibody (Fig. 4, lanes 3 and 4) and comigrated with the protein immunoprecipitated by this

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  1 MADNFSLHDALSGSGNPNPQGWPGAWGNQPAGQGLPRGFLSWGLPRAGTP 50
    |||.|||:|||.|||||||||:||||||||::.|.| : .. :.| .:.|
  1 MADSFSLNDALAGSGNPNPQGYPGAWGNQPGAGGYPGAAYPGAYPGQAPP 50

 51 RAYPGQAPPGAYPG..APGAYPGAPAPGVYPGPPSGPGYPSSGQ..... 92
    |||||||||||||  :|:||||:.|||.||||....:||:|..
 51 GAYPGQAPPGAYPGQAPPSAYPGPTAPGAYPGPTAPGAYPGSTAPGAFPG 100

 93 .........PSAPGAYPATGPYGAPAGPLIVPYNLPLPGGVVPRMLITILG 134
           |||||:|||.||||.|||||.|||:|||||||:|||||||:|
101 QPGAPGAYPSAPGGYPAAGPYGVPAGPLTVPYDLPLPGGVMPRMLITIMG 150

135 TVKPNANRIALDFQRGNDVAFHFNPRFNENNRRVIVCNTKLDNNWGREER 184
    |||||||||.|||.|||||||||||||||||||||||| |||||:|||
151 TVKPNANRIVLDFRRGNDVAFHFNPRFNENNRRVIVCNTKQDNNWGKEER 200

185 QSVFPFESGKPFKIQVLVEPDHFKVAVNDAHLLQYNHRVKKLNEISKLGI 234
    ||.|||||||||||||:|||||||||||||||||:|.|.|||.|||
201 QSAFPFESGKPFKIQVLVEADHFKVAVNDAHLLQYNHRMKNLREISQLGI 250

235 SGDIDLTSASYTMI 248
    ||||.||||...||
251 SGDITLTSANHAMI 264
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FIG. 3. Comparison of the predicted amino acid sequence of hMac-2 (upper line) with that of Mac-2 (lower line). Between sequences, vertical lines indicate amino acid identity and dots indicate similarity (:, high identity score; ., moderate identity score).

antibody from a metabolically labeled lysate of the human monocyte cell line THP-1 (Fig. 4, lanes 1 and 2). The *in-vitro*-synthesized protein was also precipitated by the desialylated glycoprotein asialofetuin coupled to Sepharose (Fig. 4, lane 7) but not by Sepharose coupled to the nonglycosylated protein myoglobin (not shown). This binding was unaffected by 100 mM glucose but was inhibited by an equivalent concentration of galactose (Fig. 4, lanes 5 and 6).

hMac-2 Synthesized *in Vitro* Binds Laminin. As shown in Fig. 5, lane 1, purified laminin coupled to Sepharose bound to and precipitated hMac-2 protein synthesized *in vitro*. This binding was not inhibited by the presence of 100 mM glucose but was significantly reduced by galactose at the same concentration (Fig. 5, lanes 2 and 3). Similar results were obtained when murine Mac-2 protein synthesized *in vitro* was used (unpublished data).

Expression of hMac-2 in Various Cell Lines. The hMac-2 cDNA was used to probe a Northern blot of total cellular RNAs from various human cell lines. The results are shown in Fig. 6 and indicate that the hMac-2 probe recognizes an approximately 2-kb mRNA which is expressed at a low level in the monocytic cell line THP-1 and at a higher level in the promyelocytic cell line HL60. Expression increases in HL60 when it is induced to differentiate, especially by PMA. hMac-2 expression can also be detected in the fibroblast cell line SL68 and in various epithelial cell lines, including HeLa, SCC, HT-29, and CaCo 2, while it is not detectable in the lymphoid cell line BJAB (data not shown).

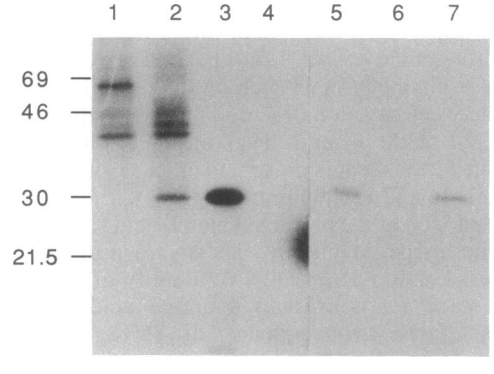

FIG. 4. Characterization of *in-vitro*-synthesized hMac-2 protein by immunoprecipitation of metabolically labeled cell lysates and SDS/PAGE. Lanes 1 and 2 show immunoprecipitates of a lysate of THP-1. In lane 2 M3/38, the monoclonal antibody to Mac-2, was used; in lane 1 an isotype-identical control antibody was used. Lanes 3 and 4 show immunoprecipitates of *in-vitro*-synthesized hMac-2 protein, with M3/38 in lane 3 and control antibody in lane 4. Lanes 5–7 show precipitation of *in-vitro*-synthesized hMac-2 protein by asialofetuin coupled to Sepharose. The precipitations were carried out in the absence of competing sugar (lane 7), in the presence of 100 mM galactose (lane 6), or in the presence of 100 mM glucose (lane 5). The numbers to the left indicate molecular weights $\times 10^{-3}$.

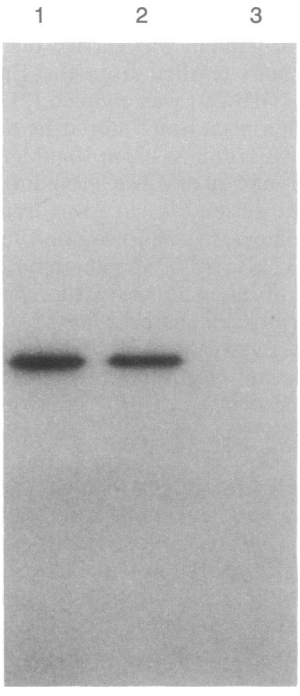

FIG. 5. Binding of [^{35}S]methionine-labeled *in-vitro*-translated hMac-2 protein to laminin-Sepharose: SDS-PAGE analysis. Lane 1, binding carried out in the absence of competing sugar; lane 2, binding in the presence of 100 mM glucose; lane 3, binding in the presence of 100 mM galactose.

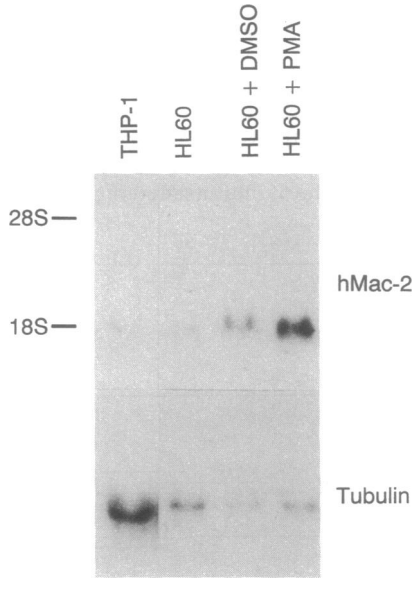

FIG. 6. Northern blot of total cellular RNA from two cell lines hybridized to hMac2 and tubulin probes. HL60 cells were grown in the presence of dimethyl sulfoxide (DMSO) or PMA as indicated. In the lane marked THP-1 30 μg of RNA was loaded; all other lanes were loaded with 5 μg of RNA. On the left, 28S and 18S indicate the positions of the corresponding ribosomal RNAs.

DISCUSSION

The properties of the murine Mac-2 antigen which we previously described indicated clearly that it is a galactose-specific lectin (2). Its marked up-regulation in inflammatory macrophages in comparison to resident tissue macrophages suggested that it might be involved in the inflammatory process. Its close homology to a rat IgE-binding protein (6) raised the possibility that it may have a role in the regulation of IgE biosynthesis or function. Several other proteins with sizes and galactose-binding properties similar to Mac-2 have been described. cDNAs encoding the lectins described by Jia and Wang (3) and Raz *et al.* (4) have been cloned and their sequences are identical to the sequence of Mac-2 cDNA. Various functions have been attributed to the individual members of this group of proteins, some of which have clear relevance to human disease. To obtain further clues to the function of Mac-2 and its relatives and because of their potential clinical importance, we have cloned the human homolog of these various rodent proteins and we have designated it hMac-2.

The primary structures of Mac-2 and hMac-2 are highly conserved, with 85% of the amino acids being similar. Like the murine protein, hMac-2 demonstrates a clear two-domain organization and the conservation of primary structure is especially marked in the carboxyl-terminal lectin domain in which there are long, uninterrupted stretches of amino-acid sequence identity. The amino-terminal half of the protein contains the motif PGAYPG seen in Mac-2 but the number of repeats of this sequence is smaller in the human protein. While the exact function of this repetitive sequence is not clear, its evolutionary conservation suggests that it has some important role.

As was observed in the case of Mac-2, the predicted amino acid sequence of hMac-2 does not contain a signal peptide or transmembrane domain, raising questions regarding the mechanism by which these proteins are localized to the cell surface. We have previously demonstrated the existence of two distinct Mac-2 cDNAs which are alternatively spliced at their 5′ ends upstream of the initiation codon, and we suggested that one of the alternatively spliced forms encoded a signal peptide (2). The limited sequence that we have obtained for the 5′ untranslated region of the hMac-2 cDNA does not bear similarity to the corresponding region of either of the murine cDNAs, suggesting that this region is not of sufficient functional significance to be evolutionarily conserved. In keeping with this observation we have now found that neither of the alternatively spliced Mac-2 cDNAs encodes a protein with a signal peptide. Secretion appears to occur by a signal-peptide-independent mechanism, and some of the secreted protein is retained on the cell surface by binding to galactose-containing glycoproteins or glycolipids (B.J.C. and S.P., unpublished results). We assume that hMac-2 utilizes a similar mechanism for export and surface localization. Mac-2 and hMac-2, being secreted proteins with the ability to bind to carbohydrates on the cell surface and on extracellular molecules, are well suited to serving a "bridging" function in cell–cell and cell–substratum interactions.

Given the extent of conservation of the primary structure of Mac-2 and hMac-2, it is not surprising that the two proteins have similar antigenic and ligand-binding properties. hMac-2 is specifically recognized in immunoprecipitations and Western analyses by the monoclonal antibody M3/38, which was raised against the murine protein. This finding indicates that M3/38 could be used for the detection of hMac-2 in human cells and tissues and paves the way for the use of this reagent in investigations of the involvement of hMac-2 in human disease states. Like the murine protein, hMac-2 has the properties of a galactose-specific lectin, as demonstrated by its galactose-inhibitable binding to the desialylated glycoprotein asialofetuin.

In keeping with its homology to the rat IgE binding protein, we have demonstrated that Mac-2 is able to bind specifically to murine IgE and that this binding is inhibited by galactose (2). These findings were also obtained independently by Laing *et al.* (15) for carbohydrate-binding protein 35. The data from these studies did not indicate whether this binding occurred through a galactose-containing sugar side chain on IgE or whether a distinct IgE-binding site on Mac-2 was involved. Preliminary experiments from our laboratory indicate that hMac-2 binds to both human and murine IgE at a low but detectable level. The mechanism and significance of this binding are not yet known.

Recently, Woo *et al.* (7) have identified Mac-2 as being the major nonintegrin laminin-binding protein expressed by murine thioglycollate-elicited peritoneal macrophages. This finding has important implications for the role of Mac-2 and hMac-2 in the inflammatory process. One of the early responses in inflammation is the induction of cell adhesion molecules such as ICAM-1 (16), INCAM-110 (17), VCAM-1 (18), and ELAM-1 (19) on the surface of vascular endothelial cells at the site of tissue injury. These molecules allow circulating monocytes, neutrophils, and lymphocytes to adhere to and subsequently make their way between the endothelial cells, through the basement membrane, and into the tissue space. During this process the leukocytes must be capable of transiently interacting with the basement membrane. One of the major components of the basement membrane is laminin, a large multisubunit protein which has sites for binding to cells and to other constituents of the basement membrane (20). While the $\alpha_6\beta_1$ integrin, a member of the integrin family of transmembrane receptors (21), is likely to play a central role in the adhesion of macrophages to basement membrane laminin (22), a secreted protein such as Mac-2, which has the ability to bind to laminin and to the cell surface, may be of importance in directing cell movement towards the basement membrane. Mac-2 may also facilitate the actual adhesion process. The results of Woo *et al.* (7) and the experiments reported here indicated that both Mac-2 and hMac-2 bind to laminin through the numerous terminal galactose residues which have been demonstrated on the sugar side chains of the latter protein (23). Such a protein–carbohydrate interaction could stabilize the protein–protein interaction involved in the integrin-mediated binding of cells to laminin.

In addition to potentially having a role in inflammation, Mac-2 may be involved in noninflammatory processes. Raz and colleagues showed that the level of expression of L-34 (Mac-2) on melanoma and fibrosarcoma cells correlated with their metastatic ability (24) and that a monoclonal antibody to L-34 inhibited the formation of metastases by these cells (25). Since the process of metastasis is analogous to inflammatory exudation in that it involves the crossing of basement membranes by circulating tumor cells, the laminin-binding property of Mac-2 provides a plausible explanation for these results.

The pattern of tissue-specific expression of hMac-2 is very similar to that of Mac-2; namely, it is expressed in cells of the monocyte-macrophage series, in various epithelial cells, and in fibroblasts, while expression is not detectable in lymphoid cells. It is interesting that induction of macrophage differentiation in the promyelocytic line HL60 results in a significant increase in hMac-2 expression. A similar increase in Mac-2 expression has been correlated with the state of differentiation of various murine macrophage cell lines (26). Mac-2 expression reaches its highest level in thioglycollate-elicited peritoneal inflammatory macrophages (1, 2). We have not yet examined the expression of hMac-2 in human macrophages activated by inflammatory stimuli, but the PMA-induced

upregulation of hMac-2 in HL60 suggests that a similar increase in expression may occur during the inflammatory activation of macrophages, since stimulation of protein kinase C is known to be involved in this process (27). It is an intriguing possibility that interaction between the adhesion molecules induced on vascular endothelium and cognate receptors on the surface of circulating monocytes may be responsible for the massive upregulation of Mac-2 in inflammatory macrophages. With the availability of the cloned Mac-2 and hMac-2 cDNAs and the antibody to the proteins, we are in a position to examine such possibilities more closely. Involvement of hMac-2 in various disease states will also now be amenable to investigation.

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