J. Cell Sci. Suppl. 9, 121–133 (1988)
Printed in Great Britain © The Company of Biologists Limited 1988

The structure and function of vertebrate mannose lectin-like proteins

R. A. B. EZEKOWITZ[1] AND P. D. STAHL[2]

[1]*Children's Hospital Medical Center, Enders 7, 320 Longwood Avenue, Boston, MA 02115, USA*
[2]*Department of Cell Biology and Physiology, Washington University School of Medicine, 660 S. Euclid Avenue, St Louis, MO 63110, USA*

Summary

Sugar-specific recognition is now well established as an important determinant of cell–cell interactions and host defence mechanisms. Macrophages, in particular, are known to express a variety of lectin-like proteins that are specific for oligosaccharides terminating in mannose, fucose, galactose and sialic acid. This review focuses on mannose recognition systems. Terminal mannose is rarely found on mammalian cell surfaces whereas it is ubiquitous on the surfaces of lower organisms. Mammals have evolved at least two mechanisms to recognize terminal mannose residues. These are, first, a mannose receptor located on the surface of macrophages and second, a mannose-binding protein found in blood plasma and secreted by hepatocytes. The mannose receptor is a 175K ($K = 10^3 M_r$) membrane glycoprotein. The receptor binds glycoproteins bearing high-mannose chains avidly at neutral pH but poorly at pH 5–6. The receptor recycles rapidly between the cell surface, where ligand binding occurs, and various acid intracellular compartments, where the ligand is discharged. The pH dependency of binding and the rapid recycling of receptor allow cells to accumulate many ligand molecules over an extended time. Endocytosis via this pathway appears to be regulated since mannose receptor expression is closely regulated. For example, the receptor is absent from monocytes but strongly expressed on 3- to 4-day-old monocyte-derived macrophages. Receptor expression can be up- and down-regulated by anti-inflammatory steroids and γ interferon, respectively. The mechanisms are poorly understood as is the physiological basis for modulation.

The mannose-binding protein is a large oligomeric plasma protein secreted by liver and present in significant amounts in serum. The basic subunit of the mannose-binding protein is a 32K protein, which shows homology with other lectin-like proteins, notably certain surfactant proteins of the lung. The structure of the mannose-binding protein has been deduced from sequence information and DNA cloning experiments. The elaboration of the mannose-binding protein by the liver is regulated; it appears to be an acute phase reactant. Recent evidence suggests that the mannose-binding protein may play some opsonic role in the recognition and killing of organisms. In this review, it is suggested that the mannose receptor and the mannose-binding protein operate 'hand in glove' to deal with infectious agents that express mannose on their surfaces.

Introduction

Mannose is a ubiquitous component of bacterial pathogens, parasites, yeasts and the envelope glycoprotein of certain viruses. While most mammalian cell surface and secreted glycoproteins have complex oligosaccharide chains terminating in sialic acid, an occasional secretory protein and most lysosomal hydrolases do contain high-mannose chains. This stems from the fact that intracellular targeting of lysosomal hydrolases involves mannose phosphate residues (Kornfeld & Kornfeld, 1985). Several years ago, it became clear that mammals possess a macrophage-specific cell

surface receptor that recognizes oligosaccharides terminating in mannose or fucose (Stahl *et al.* 1976, 1978; Schlesinger *et al.* 1978). Parallel work indicated that rabbit liver (Kawasaki *et al.* 1978; Townsend & Stahl, 1981; Maynard & Baenziger, 1982) and plasma (Kawasaki *et al.* 1983) contain a soluble protein that binds mannose oligosaccharides and whose specificity is similar to the mannose receptor. Hepatocyte-derived mannose-binding proteins have now been isolated from the serum of man and rodents (Maynard & Baenziger, 1982; Summerfield & Taylor, 1986). In this review we will discuss two major areas. First, the macrophage mannose receptor, its role in endocytosis, its characterization and purification from macrophage membranes and the regulation of its surface expression. Second, the hepatocyte-derived human serum mannose-binding protein will be reviewed including its structure–function relationships, its homology to other vertebrate lectins and its up-regulation as an acute phase reactant. Finally we will discuss the relationship between these two mannose-specific lectin-like proteins and propose a model for their physiological function in host defence.

The mannose receptor: structure and function

Plasma clearance experiments (Stahl *et al.* 1976) pointed to the existence of a receptor that bound glycoproteins bearing high-mannose chains. Receptor activity was especially rich in liver and spleen, but other tissues were positive as well. Most eukaryotic glycoproteins bear complex chains terminating in sialic acid but there are several notable exceptions, in particular most lysosomal hydrolases. Others include thyroglobulin, the third component of complement and immunoglobulin M (IgM) (Kornfeld & Kornfeld, 1985). It is now known that the mannose receptor is expressed by a wide variety of macrophages and that it mediates the uptake into macrophages of an assortment of mannose glycoproteins and artificial glyconconjugates. Glycoconjugates (Lee *et al.* 1976), prepared by derivatizing bovine serum albumin with mannose, have proven to be invaluable as high-affinity ligands for a variety of receptors, including the mannose receptor, particularly when the molar ratio of sugar to protein is >20:1 (Hoppe & Lee, 1983). L-Fucose was as effective as mannose when bound to proteins and this has given rise to the term mannose/fucose receptor (Shepherd *et al.* 1981). L-Fucose, when appropriately viewed in three dimensions, has an orientation about C2, C3 and C4 that gives it properties similar to D-mannose. The only naturally occurring fucosylated glycoprotein ligand identified thus far is salivary amylase (Niesen *et al.* 1984). Glycoconjugates have been used to quantify receptor distribution. Binding studies indicate that alveolar macrophages possess about 100 000 cell surface receptors ($K_d = 20$ nM) (Stahl *et al.* 1980). However, cells whose membranes have been permeabilized with detergents contain 500 000 receptor sites (Wileman *et al.* 1984), consistent with the conclusion that macrophages contain a large intracellular pool of receptors. The existence of an intracellular pool of receptors is supported by proteolysis experiments. About 20% of the total cellular receptor pool is available to proteases when cells are incubated at

4 °C whereas all of the receptors can be inactivated with protease at 37 °C (Stahl *et al.* 1980). Given the observation that an alveolar macrophage can take up 2×10^6 molecules h^{-1}, these data lead to the conclusion that receptors rapidly recycle and that each receptor must traverse a complete cycle every 10–15 min. Correlative morphological studies at the electron-microscope level have shown that mannosylated ligands are taken up into coated vesicles, which, after losing their coats, fuse with each other (Harding *et al.* 1985). Larger translucent vesicles become tubular within 8–10 min, a process apparently required for receptor–ligand sorting. The intracellular life-time of the receptor in cultured macrophages is likely to be several days, which indicates that a given receptor may recycle hundreds of times before it is degraded or inactivated.

The major function of the mannose receptor in macrophages is presumed to be the transfer of internalized proteins to lysosomes. However, kinetic experiments have suggested that alternative pathways may exist. For example, a small, but significant, fraction of receptor molecules traverse a pathway following internalization that does not include passage through an acid environment (Tietze *et al.* 1982). This minor pathway has been referred to as receptor–ligand cycling. Other receptor–ligand systems that follow a similar pathway in other cell types include the low-density lipoprotein (LDL) receptor (Aulinskas *et al.* 1985) and the asialoglycoprotein receptor (Townsend *et al.* 1984; Simmons & Schwartz, 1984). These processes have been variously referred to as retro-endocytosis and diacytosis, respectively. Another example of this may be illustrated by the pathway followed by internalized high-density lipoprotein (HDL) in macrophages. Recent studies suggest that HDL can enter cells by receptor-mediated endocytosis and that, within the vacuolar compartment, cholesterol can be absorbed by exchange from cytoplasmic stores (Schmitz *et al.* 1985). This is thought to be followed by the exocytotic release of HDL resulting in the net discharge of cholesterol from the cells. Whether these are independently regulated endocytic pathways in macrophages remains to be determined.

In addition to its role in receptor-mediated endocytosis, the mannose receptor also mediates phagocytosis, independently of Fc and C3 receptors (Sung *et al.* 1983). The participation of the mannose receptor in phagocytosis raises several interesting questions about receptor structure–function relationships. Receptor-mediated pinocytosis requires clustering in coated pits followed by internalization and recycling, whereas receptor-mediated phagocytosis, according to the 'zipper' hypothesis, requires mobility in the membrane (Griffin *et al.* 1975). Whether different populations of receptor molecules mediate these two physiological functions, remains to be determined.

Recent progress on the biochemistry of the mannose receptor has revealed that the receptor is a 175K ($K = 10^3 M_r$) membrane glycoprotein (Wileman *et al.* 1986). The rabbit (Lennartz *et al.* 1987*a*), human (Lennartz *et al.* 1987*b*), rat (Haltiwanger & Hill, 1986) and mouse (R. Fiani & P. D. Stahl, unpublished results) receptors have been isolated. Isolation has been achieved by affinity chromatography on mannose–Sepharose. Elution from the affinity column is achieved by mannose, EDTA or low

pH. These three conditions relate to known properties of the receptor. First, the receptor recognizes glycopeptides bearing more than one terminal mannose residue (Maynard & Baenziger, 1981). The monosaccharide, mannose, is a poor inhibitor but, as expected, it is effective at high concentrations in blocking receptor binding. Second, the receptor requires Ca^{2+} for full binding activity. Removal of Ca^{2+} with EGTA impairs binding and allows receptor elution from the column. Third, the receptor binds ligands avidly at neutral pH but poorly at pH 5 (Lennartz *et al.* 1987*a*,*b*). The pH dependency of binding accounts for the separation of receptor and ligand within acidified endosomes.

Based on digestion experiments with endoglycosidase F, the receptor is known to be a glycoprotein bearing complex oligosaccharide chains. Biosynthetic studies using anti-receptor antibodies to immunoprecipitate solubilized receptor molecules have shown that newly synthesized receptor is endoglycosidase H sensitive (M. R. Lennartz *et al.* unpublished results). An interesting question has emerged from these findings. Does the mannose receptor, which is a high-mannose chain bearing glycoprotein, bind to itself or to its neighbours in the endoplasmic reticulum immediately following its synthesis, or is the receptor synthesized as an inactive precursor? Pulse–chase studies show that exit of newly synthesized receptor from the endoplasmic reticulum is not unusually slow, suggesting that the receptor is not retained by virtue of its mannose-binding property. However, treatment of macrophages with the drug swainsonine (Chung *et al.* 1984), which blocks processing of high-mannose chains, results in a complete inactivation of mannose receptor activity within hours. This observation suggests that the environment in which the mannose receptor normally operates is deficient in high-mannose oligosaccharides.

Expression of the mannose receptor appears to be closely regulated. The receptor is not expressed by circulating monocytes nor by most macrophage-like cell lines (e.g. J774, P338D1, U937). Macrophage-like cell lines treated with 5-azacytidine do express the receptor (Diment *et al.* 1987). Cells treated with the drug and cloned in soft agar continue to express the receptor after extended periods in culture. When monocytes are allowed to differentiate in culture, expression of the receptor commences (Shepherd *et al.* 1982). Expression of the receptor in cultured monocyte-derived macrophages results in an increased accumulation of uptake activity over several days. Whereas monocytes are receptor-negative, resident and thioglycollate-elicited macrophages express intermediate levels of mannose receptor (Stahl & Gordon, 1982; Ezekowitz *et al.* 1981). Alveolar macrophages express high levels. The reasons for variable expression of the receptor among tissue macrophage populations remains uncertain. Activated macrophages, either prepared from animals infected with BCG or following treatment *in vitro* with γ interferon, express low to intermediate levels of receptor (Ezekowitz *et al.* 1981, 1986). The nature of the down-regulation produced by macrophage activation (i.e. decreased receptor number, decreased ligand affinity or decreased recycling time) remains to be determined. Interestingly, the level of expression appears to be normal in macrophages infected with organisms that live and grow within intracellular vacuoles, for example, *Leishmania* (Shepherd *et al.* 1983). In fact, the evidence suggests that certain

organisms may use the mannose receptor to gain access to the vacuolar compartment of macrophages. Yet another finding, which reveals fine control of mannose receptor expression, is the modulation observed when macrophages are treated with anti-inflammatory steroids. Incubation of rat or human macrophages with dexamethasone leads to an enhancement of mannose receptor expression (Shepherd *et al.* 1985; Mokoena & Gordon, 1985). Receptor levels and uptake can be elevated by several-fold following 24 h incubation with low levels of steroid. The effect can be blocked by addition of inhibitors of protein synthesis. These data suggest that regulation of receptor levels may be achieved, in part, by regulation of receptor synthesis.

It is likely that the mannose plays a role in host defense for reasons outlined above. Whether the receptor plays some role in the transport or shuttling of lysosomal hydrolases in macrophages remains to be determined. Suggestive evidence supporting a role has been published. Shepherd *et al.* (1985) have shown that, under certain circumstances, mannan can stimulate lysosomal enzyme secretion and Diment *et al.* (1987) have shown an increased correlation between lysosomal enzyme secretion and mannose receptor expression. Lee and colleagues have shown that neoglycoproteins can elicit secretion of hydrolases when such glycoconjugates contain mannose but not galactose. Moreover, secretion was stimulated in the presence of cycloheximide, suggesting that the secretory load was derived from pre-existing lysosomes.

Mannose-binding proteins

Mannose-binding proteins (MBP) have been isolated from the livers of rats (Kawasaki & Ashwell, 1977; Mizuno *et al.* 1981; Maynard & Baenziger, 1982; Townsend & Stahl, 1981) and man (Wild *et al.* 1983; Summerfield & Taylor, 1986) and subsequently found in the serum of rats (Kawasaki & Ashwell, 1977; Mizuno *et al.* 1981), rabbits (Kawasaki *et al.* 1978) and man (Kawasaki *et al.* 1983; Summerfield & Taylor, 1986). Much more is known about the structure of mannose-binding proteins than is known about their function. Drickamer *et al.* (1986) characterized two homologous rat MBPs, the so-called rat MBP-A and rat MBP-C. They form at least trimers of a basic 32K subunit and form part of a family of lectin-like proteins, which include five membrane-bound hepatic receptors (Drickamer, 1987).

While the rat seems to possess distinct liver and serum MBPs, the presence of similar distinct forms in the human has not been clearly documented. Mannose-binding proteins isolated from human liver and serum have identical apparent molecular weights, binding characteristics and share immunological cross-reactivity (Wild *et al.* 1983; Summerfield & Taylor, 1986; Kawasaki *et al.* 1983). The isolation of cDNA clones that encode two homologous human mannose-binding proteins has extended our knowledge of the structure and function of human MBP (Ezekowitz *et al.* 1988). In this section we will review the structure of the human MBP, its relationships to other vertebrate lectins and discuss the regulation of its synthesis, which might provide insight into its role as an opsonin.

Common structural features of human MBP-C, rat MBP-C and rat MBP-A

The sequence of the human MBP is strikingly homologous to the rat MBPs, in particular rat MBP-C. As shown in Fig. 1 the human MBP and the rat MBP-C can be aligned with only three gaps. In this alignment, the sequences of these two mature proteins are 51% homologous. An alignment between human MBP and the rat MBP-A with seven gaps allows a homology of 48% between these two native proteins. The overall organization of these proteins appears identical. Human MBP-C consists of a N-terminal signal sequence followed by a short segment (21 amino acid residues) rich in cysteine residues, then a collagen-like domain of 56 residues, and finally a C-terminal non-collagen-like domain of 148 amino acids.

Signal sequences

Since MBPs are found in the circulation, the presence of a typical hydrophobic signal sequence is an expected feature of these molecules. The human MBP-C signal

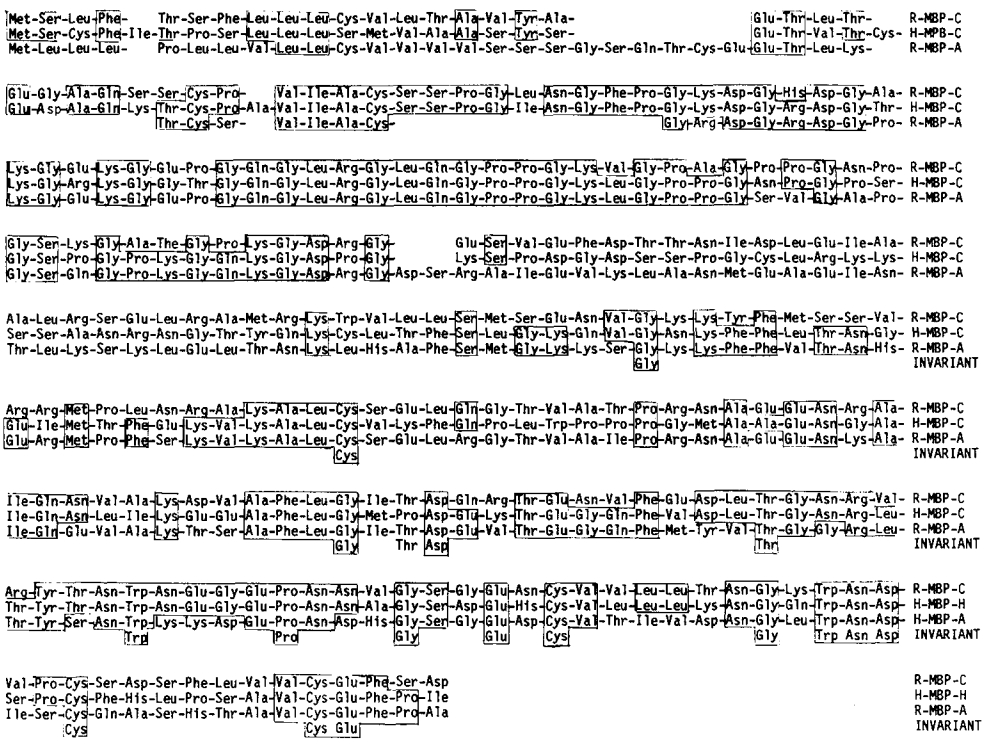

Fig. 1. Comparison of animal lectin sequences. The sequences of the two rat MBPs, R-MBP-A and -C are compared with the human MBP-C(H-MBP-C). The invariant residues represent sequences invariant in 12 lectin-like proteins including the human and rat hepatic asialoglycoprotein receptors, the avian hepatic receptor, the apoprotein of dog and human surfactant, the N-terminal portion of a galactose-specific lectin isolated from the haemolymph of *Sarcophaga perigrina*, a lectin isolated from the coelomic fluid of a sea urchin *Anthocidaris crassispina*, a chicken cartilage core proteoglycan protein and the IgE Fc receptor. Residues that are identical between the MBPs, human and rat are boxed to emphasize homology.

sequence has the features of N-terminal signal sequences found on almost all eukaryotic secretory proteins (Von Heijne, 1983). The N terminus of the mature human and rat proteins is glutamic acid. This is preceded by a serine or alanine which would conform to the general rule that the amino acid in the position preceding cleavage by the signal peptidase tends to be a residue with a small side chain like serine or alanine. The lack of specific conservation of residues within the signal or at the cleavage boundary is similar to comparisons made between signal sequences in otherwise highly homologous proteins (Von Heijne, 1983).

Interchain disulphide bonds

The presence of a number of cysteine residues in the short N-terminal non-collagen-like segment are found in both human and rat MBPs (Fig. 1). In the rat protein these interchain disulphide bonds are removed when MBPs are digested with collagenase (Drickamer *et al.* 1986). When MBP purified from human sera is analysed by polyacrylamide gel electrophoresis under non-reducing conditions it occurs as multimers, composed of an aggregate of 30K subunits. Strong reduction and alkylation are required to reduce this multimeric form to the basic subunit suggesting that the cysteines in the N-terminal domain of human MBP-C are probably involved in disulphide bond formation.

Collagen-like domains

This region is highly conserved and there are sequences of near identity between the human MBP-C and both rat MBPs. A point of note is that there is a single identical interruption in the Gly–X–Y repeat structure, the sequence Gly–Gln–Gly (residues 297–303), in a highly conserved portion of both collagenous domains. This aberration in the regular Gly–X–Y–Gly repeat is also found in some other proteins that contain collagen-like regions, like the apoproteins of pulmonary surfactant (White *et al.* 1985), and the A and C chains of C1q (Reid, 1983). It is known that the interruption is the site of an intron in human MBP (S. Herman *et al.*, unpublished results), rat MBP (Drickamer & McCleary, 1987) and in the human surfactant apoprotein gene (White *et al.* 1985). The collagen sequence is more closely related to that found in non-fibrillar collagen molecules than fibrillar collagen, in that the distortions in the triple helical structure are found in non-fibrillar collagen (for a review see Martin *et al.* 1985). All of this suggests that the collagen portion of these molecules evolved from a common ancestral gene. Other portions of the collagen-like domain resemble the triple-helix-forming segments of collagen in that a large number of the X and Y positions are occupied by prolines. The detailed analysis of the human (J. Baenziger, personal communication) and rat proteins reveals that a high proportion of the prolines in the Y position are hydroxyproline. Of some interest is the finding that the sequence Arg–Gly–Asp–Ser (RGDS), which is recognized by a family of cell surface receptors known as integrins (Hynes, 1987), is not found in the human MBP-C or the rat MBP-C whereas it is present in the rat MBP-A. Although the human protein does contain the sequence Asp–Gly–Asp–Ser, the functional significance of this motif is not known.

Comparison of human MBP with other mammalian carbohydrate-binding proteins

Several recent reports have drawn attention to striking homologies between mammalian lectin-like proteins (for a review see Drickamer, 1987). The primary structures of several animal lectins have been reported (Takahashi *et al.* 1985; Giga *et al.* 1987; Shigaku *et al.* 1986). Fig. 1 illustrates the invariant residues of 12 proteins that bear homology with the human MBP (these are listed in the legend). The homologies are greatest in the C termini of the mammalian lectin-like proteins, which are the putative carbohydrate-binding domains. Although strong sequence homology exists, each protein has a distinct pattern of carbohydrate-binding specificity; the MBPs recognize mannose, the rat and human hepatic lectins are specific for galactose and *N*-acetylgalactosamine, and the chicken hepatic lectin recognizes *N*-acetylglucosamine. The binding specificity of the sea urchin lectin is not well characterized, and the inclusion of the IgE lymphocyte Fc receptor in this family suggests that it may have lectin-like properties, which may or may not relate to IgE binding. The cartilage proteoglycans and the apoproteins, SP28-36, of pulmonary surfactant are most homologous to the galactose-binding proteins and, on the basis of these predictions, these proteins do in fact interact with galactose (Benson *et al.* 1985; Haagsman *et al.* 1987).

Regulation of MBP synthesis

Variability of MBP levels isolated from different sera (from 100 ng ml^{-1} to 50 μg ml^{-1} as determined by a radioimmunoassay (R. A. B. Ezekowitz, unpublished results)) led us to investigate whether the hepatic synthesis of MBP may be regulated. Northern analyses were carried out on RNA isolated from normal liver derived from a liver biopsy sample for a staging laporotomy for Hodgkins disease and RNA isolated from a patient who had suffered major trauma 48 h prior to death. The latter RNA was greatly enriched for acute phase reactants. Radiolabelled human MBP cDNA hybridized only to the acute phase RNA. A major species of RNA of about 3·5 kb represents the human MBP-C. A larger mRNA of about 5·5 kb is a consistent finding. This larger species may represent (1) additional 5′ or 3′ untranslated sequence, (2) a larger mRNA that codes for a homologous mannose-binding protein or (3) a splicing intermediate. Further analysis revealed that the expression of MBP was restricted to liver and certain liver cell lines. Unlike some other acute phase proteins, the synthesis of MBP is not up-regulated *in vitro* by interleukin-1 and/or tumour necrosis factor (R. A. B. Ezekowitz, unpublished results). However, mRNA levels are greatly induced by γ interferon. This appears to be a rapid event, which is observed after 30 min of exposure to the lymphokine. The presence of a heat-shock element (described by Pelham, 1982) in the 5′ region of the human MBP gene (G. Herman *et al.*, unpublished results), led us to investigate whether MBP mRNA was induced by heat shock. Preliminary studies show that raising the temperature of HepG2 cells to 45 °C for 60 min greatly induces MBP RNA (R. A. B.

Ezekowitz *et al.*, unpublished results). This study supports the idea that this element is active and that the MBP is indeed a heat-shock protein.

Function of human MBP

The circumstances under which MBP synthesis is induced as part of the acute phase response, its up-regulation in response to heat shock and γ interferon suggest a role for MBP in natural immunity. Human MBP could have a primary role in the engagement of mannose-rich pathogens in the circulation. For this to occur MBP would be expected to distinguish mannose on pathogens from cell surface glycoproteins. Specificity at this level is feasible when one considers that most glycoproteins contain less than six oligosaccharide chains. Organisms coated with high-mannose chains, on the other hand, would be highly multivalent. Thus, it would be reasonable to expect the mannose-containing surface of an infectious agent to become coated with MBP, even if many of the sugar–lectin interactions were of a low affinity type.

MBP opsonizes *Salmonella*

Human MBP is able to enhance uptake of mannose-rich *Salmonella* by polymorphonuclear leukocytes in the absence of serum. In the absence of MBP or serum no specific uptake of these strains of bacteria is observed. All the MBP-dependent and 50% of the serum-related uptake can be specifically abrogated by yeast mannan, a mannose-rich glycoprotein (M. Kulhman & R. A. B. Ezekowitz, unpublished results).

MBP inhibits *in vitro* infectivity of HIV

The presence of high-mannose oligosaccharide chains on the external domain of the envelope glycoprotein of HIV, the human immunodeficiency virus, (Montagnier *et al.* 1985; T. Gregory & M. Spellman, personal communication) suggested to us that this MBP may interfere with the life cycle of this pathogenic retrovirus. The importance of glycosylation in the biology of the virus has been inferred from studies that showed that certain plant lectins, which recognize particular configurations of high-mannose, inhibit HIV infection *in vitro* (Lifson *et al.* 1986). These observations are supported by studies that show that deglycosylated forms of gp 120 fail to interact with CD4 (Matthews *et al.* 1987). We have shown that MBP was able to inhibit *in vitro* infection of susceptible cells by the human immunodeficiency virus (R. A. B. Ezekowitz *et al.*, unpublished results). The precise molecular basis for MBP-mediated inhibition of HIV infection *in vitro* is not clear. It is possible that, through its interaction with the exposed mannose chains on the envelope glycoprotein, MBP interferes with the topology of the virus, either by masking those epitopes required for adhesion to a cell surface receptor, or perhaps, less likely, by inducing some conformational change in the virus.

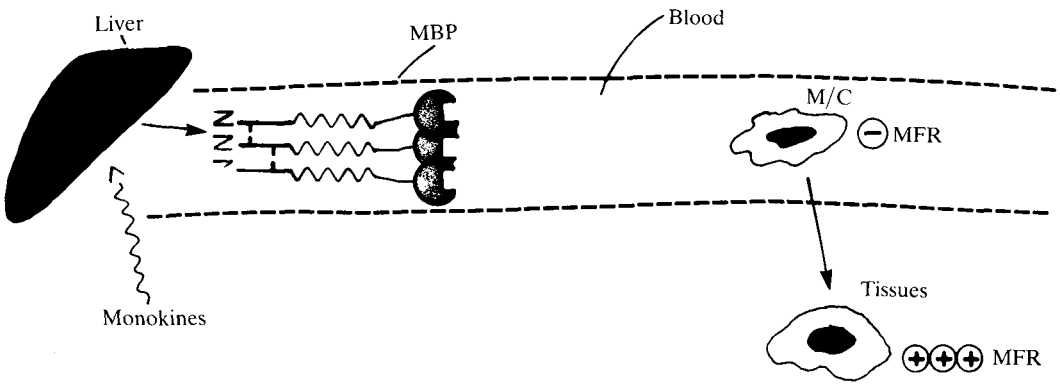

Fig. 2. A model of mannose lectin-like recognition. Monokines released in response to infection cause augmented release of MBP from the liver. These MBPs serve to recognize mannose-containing pathogens in the blood. In tissue, macrophages, which express high levels of MFR, serve to recognize mannose-rich pathogens in the absence of serum and hence circulating MBP.

The implication of these recent studies is that MBP may provide a natural *in vivo* mechanism for preventing either HIV infection or limiting viral spread. This prospect is supported by studies that show the ability of MBP to distinguish HIV-infected H9 cells from uninfected H9 cells. This may apply to other viruses and virally infected cells, which may express high-mannose oligosaccharides as part of their viral envelope or on the external surface of infected cells. Therefore MBP may be part of an early response to infection.

Conclusion

It now appears that vertebrate lectin-like proteins, which recognize certain configurations of mannose oligosaccharides, have a key role in host defence (Fig. 2). The macrophage mannose receptor is expressed at high levels on tissue macrophages. These cells are positioned along the portals of maximum antigen entry forming a lattice beneath epithelial surfaces in the skin, gut, kidney and lung. At these sites, they are able to bind and ingest mannose-rich pathogens directly. This results in the release of a wide range of macrophage products like reactive oxygen intermediates, neutral proteinases, arachidonic acid metabolites and monokines (for a review see Ezekowitz, 1988).

The macrophage mannose receptor is not expressed on circulating monocytes. However, plasma contains a MBP, which appears to serve as the mannose recognition protein in the circulation. The proposed functional relationship between these lectin-like proteins is shown in Fig. 2. Present studies indicate that the similarity in binding characteristics between these proteins is based on structural homology (R. A. B. Ezekowitz & P. D. Stahl, unpublished observations). It is hoped that future studies will allow better definition of the structural and functional relationship between these lectin-like proteins.

References

AULINSKAS, T. H., ORAM, J. F., BIERMAN, E. L., COETZEE, G. A., JEVERS, W. & VANDER WESTHUYZEN, D. R. (1985). Retro-endocytosis of low density lipoprotein by cultured human skin fibroblast. *Arterioscelerosis* **5**, 45–54.

BENSON, B., HAWGWOOD, S., SCHILLING, J., CLEMENTS, J., DANIM, P., CORDELL, B. & WHITE, R. T. (1985). Structure of canine pulmonary surfactant apoprotein: cDNA and complete amino acid sequence. *Proc. natn. Acad. Sci. U.S.A.* **82**, 6379–6383.

CHUNG, K. N., SHEPHERD, V. L. & STAHL, P. D. (1984). Swainsonine and castanospermine blockade of mannose glycoprotein uptake by macrophages: Apparent inhibition of receptor-mediated endocytosis by endogenous ligands. *J. biol. Chem.* **259**, 14 637–14 641.

DIMENT, S., LEECH, M. & STAHL, P. D. (1987). Generation of macrophage variants with 5-azacytidine: Selection for mannose receptor expression. *J. Leukocyte Biol.* **42**, 485–590.

DRICKAMER, K. (1987). Structure and biosynthesis of membrance receptors which mediate endocytosis of glycoproteins. *Kidney International* **32 (Suppl 23)**, 67–81.

DRICKAMER, K., DORSAL, M. S. & REYNOLDS, L. (1986). Mannose-binding proteins isolated from rat liver contain carbohydrate-recognition domains linked to collagenous tail. *J. biol. Chem.* **261**, 6578–6588.

DRICKAMER, K. & MCCREARY, V. (1987). Exon structure of a mannose-binding protein gene reflects its evolutionary relationship to the asialoglycoprotein receptor and non fibrillar collagens. *J. biol. Chem.* **262**, 2582–2593.

EZEKOWITZ, R. A. B. (1988). The macrophage. In *Biological Aspects in Natural Immunity* (ed. D. Nelson), New York: Academic Press (in press).

EZEKOWITZ, R. A. B., AUSTYN, J., STAHL, P. & GORDON, S. (1981). Surface properties of activated mouse macrophages – BCG infection reduces expression of antigen F4/80 and Fc and mannose-specific receptors for endocytosis whilst enhancing IA antigens. *J. exp. Med.* **154**, 60–76.

EZEKOWITZ, R. A. B., DAY, L. & HERMAN, G. (1988). A human mannose-binding protein in an acute phase reactant that shares sequence homology with other vertebrate lectins. *J. exp. Med.* **167**, 1034–1046.

EZEKOWITZ, R. A. B., HILL, M. & GORDON, S. (1986). Interferon alpha/beta selectively antagonizes down-regulation of mannosyl fucosyl receptors on macrophages activated by interferon gamma. *Biochem. biophys. Res. Commun.* **136**, 737–744.

GIGA, Y., ATUSUSHI, I. & TAKAHASHI, K. (1987). The complete amino acid sequence of echinoiden, a lectin from the coelomic fluid of the sea urchin *Anthocidaris crassispina*. *J. biol. Chem.* **262**, 6197–6209.

GRIFFIN, F., GRIFFIN, J., LEIDER, J. & SILVERSTEIN, S. (1975). Studies on the mechanism of phagocytosis. I. Requirements for circumferential attachment of particle bound ligands to specific receptors on the macrophage plasma membrane. *J. exp. Med.* **142**, 1263–1275.

HAAGSMAN, H. P., HAGWOOD, S., SEARGENT, T., BUCKLEY, D., WHITE, R. T., DRICKAMER, K. & BENSON, B. J. (1987). The major lung surface protein, SP 28-36 is a calcium-dependent carbohydrate-binding protein. *J. biol. Chem.* **262**, 13 877–13 886.

HALTIWANGER, R. S. & HILL, R. C. (1986). The isolation of a rat alveolar macrophage lectin. *J. biol. Chem.* **261**, 7440–7449.

HARDING, C., LEVY, M. & STAHL, P. (1985). Morphological analysis of ligand uptake and processing: The role of multivesicular endosomes and CURL in receptor ligand processing. *Eur. J. Cell Biol.* **36**, 230–238.

HOPPE, C. & LEE, Y. C. (1983). The binding and processing of mannose-bovine serum albumin derivatives by rabbit alveolar macrophages. *J. biol. Chem.* **258**, 14 193–14 202.

HYNES, R. D. (1987). Integrins. A family of cell surface receptors. *Cell* **48**, 549–551.

KAWASAKI, T. & ASHWELL, G. (1977). Isolation and characterization of an avian hepatic binding protein specific for *N*-acetylglucosamine-terminated glycoproteins. *J. biol. Chem.* **252**, 6536–6544.

KAWASAKI, T., ETON, R. & YAMASHURA, I. (1978). Isolation and characterization of a mannose-binding protein from rabbit liver. *Biochem. biophys. Res. Commun.* **81**, 1018–1026.

KAWASAKI, N., KAWASAKI, T. & YAMASHURA, I. (1983). Isolation and characterization of a mannose-binding protein from human serum. *J. Biochem.* **94**, 937–952.

KORNFELD, R. & KORNFELD, S. (1985). Assembly of asparagine linked oligosaccharides. *A. Rev. Biochem.* **54**, 631–653.

LEE, Y. C., STOWELL, C. P. & KRANTZ, M. J. (1976). 2-Imino-2-methoxyethyl-1-thioglycosides: New reagents for attaching sugars to proteins. *Biochemistry* **15**, 3956–3965.

LENNARTZ, M. R., COLE, F. S., SHEPHERD, V., WILEMAN, T. E. & STAHL, P. D. (1987*b*). Isolation and characterization of a mannose-specific endocytosis receptor from human placenta. *J. biol. Chem.* **262**, 9942–9944.

LENNARTZ, M. R., WILEMAN, T. E. & STAHL, P. D. (1987*a*). Isolation and characterization of a mannose-specific endoytosis receptor from rabbit alveolar macrophages. *Biochem. J.* **245**, 705–711.

LIFSON, J., COUTRE, S., HAUNG, E. & ENGELMAN, E. (1986). Role of envelope glycoprotein carbohydrate in human immunodeficiency virus (HIV) infectivity and virus-induced cell fusion. *J. exp. Med.* **164**, 2101–2106.

MARTIN, G. R., TIMPL, R., MULLER, P. K. & KUHN, K. (1985). The genetically distinct collagens. *Trends Biochem. Sci.* **10**, 285–287.

MATTHEWS, T. J., WEINHOLD, K. J., LYERLY, H. K., LANGLOIS, A. J., WIGZELL, H. & BOLOGNESI, D. (1987). Interaction between the human T-cell lymphotropic virus type III_B envelope glycoprotein GP 120 and the surface antigen CD4: Role of carbohydrate in binding and cell fusion. *Proc. natn. Acad. Sci. U.S.A.* **84**, 5424–5428.

MAYNARD, Y. & BAENZIGER, J. U. (1981). Oligosaccharide specific endocytosis by isolated rat hepatic reticuloendothelial cells. *J. biol. Chem.* **256**, 8063–8072.

MAYNARD, Y. & BAENZIGER, J. U. (1982). Characterization of a mannose and *N*-acetylglucosamine-specific lectin present in rat hepatocytes. *J. biol. Chem.* **257**, 3708–3716.

MIZUNO, Y., KOZUTSUMI, Y., KAWASAKI, T. & YAMASHINA, I. (1981). Isolation and characterization of a mannose-binding protein from rat liver. *J. biol. Chem.* **256**, 4247–4256.

MOKENA, T. & GORDON, S. (1985). Human macrophage activation modulation of mannosyl, fucosyl receptor *in vitro* by lymphokines, gamma and alpha interferons and dexamethasone. *J. clin. Invest.* **75**, 624–635.

MONTAGNIER, L., CLAVEL, F., KRUST, B., CHAMMARET, S., REY, F., BARRE-SINOUSSI, F. & CHERMANN, J. C. (1985). Identification and antigenicity of the major envelope glycoprotein of lymphadenopathy-associated virus. *Virology* **114**, 283–289.

NIESEN, T. E., ALPERS, D. H., STAHL, P. D. & ROSENBLUM, J. L. (1984). Metabolism of glycosylated human salivary amylase: *in vivo* plasma clearance by rat hepatic endothelial cells and *in vitro* receptor mediated pinocytosis by rat macrophages. *J. Leukocyte Biol.* **36**, 307–320.

PELHAM, H. R. B. (1982). A regulatory upstream promoter element in the *Drosophila* Hsp 70 heat-shock gene. *Cell* **30**, 517–528.

REID, K. B. M. (1983). Proteins involved in the activation and control of the two pathways of human complement. *Biochem. Soc. Trans.* **11**, 1–11.

SCHLESINGER, P., MANDELL, B., MILLER, J., RODMAN, J., WHITE, R., DOEBBER, T. & STAHL, P. (1978). Liver Kupffer cell mediated plasma clearance of glycoproteins and lysosomal glycosidases, β-glucuronidase and *N*-acetyl-β-D-glycosaminidase. *Biochem. J.* **176**, 103–109.

SCHMITZ, G., ROBENER, H., LOHMANN, U. & ASSMANN, G. (1985). Interaction of high density lipoproteins with cholesteryl ester-laden macrophages: Biochemical and morphological characterization of cell surface receptor binding, endocytosis and resecretion of high density lipoproteins by macrophages. *EMBO J.* **4**, 613–622.

SHEPHERD, V., CAMPBELL, E., SENIOR, R. & STAHL, P. (1982). Characterization of the mannose/fucose receptor on human mononuclear phagocytes. *J. Reticuloendothelial Soc.* **32**, 423–431.

SHEPHERD, V. L., KONISH, M. & STAHL, P. (1985). Dexamethasone increases expression of mannose receptors and decreases extracellular accumulation of lysosomal enzymes in macrophages. *J. biol. Chem.* **260**, 160–164.

SHEPHERD, V., LEE, Y. C., SCHLESINGER, P. H. & STAHL, P. D. (1981). L-Fucose terminated glycoconjugates are recognized by pinocytosis receptors on macrophages. *Proc. natn. Acad. Sci. U.S.A.* **78**, 1019–1022.

SHEPHERD, V. L., STAHL, P., BERN, T. P. & RABINOVITCH, M. (1983). Receptor-mediated entry of β-glucuronidase into the parisitophorous vacuoles of macrophages infected with *Leishmania mexicana amazonensis*. *J. exp. Med.* **157**, 1471–1482.

SHIGAKU, S., TAKAAKI, T., KOSHER, R. A. & TANSIER, M. L. (1986). Cloning and sequence analysis of a partial cDNA for chicken cartilage proteoglycan core protein. *Proc. natn. Acad. Sci. U.S.A.* **83**, 5081–5090.

SIMMONS, C. F. & SCHWARTZ, A. L. (1984). Cellular pathways of galactose-terminal ligand movement in a cloned human hepatoma cell line. *Mol. Pharmacol.* **26**, 509–519.

STAHL, P. & GORDON, S. (1982). Expression of a mannosyl-fucosyl receptor for endocytosis on cultured primary macrophage and their hybrids. *J. Cell Biol.* **93**, 49–56.

STAHL, P., RODMAN, J. S., MILLER, J. & SCHLESINGER, P. (1978). Evidence for receptor-mediated binding of glycoproteins, glycoconjugates and lysosomal glycosidases by alveolar macrophages. *Proc. natn. Acad. Sci. U.S.A.* **75**, 1399–1403.

STAHL, P., RODMAN, J. S., SCHLESINGER, P. & DOEBBER, T. (1976). Clearance of lysosomal glycosidases from plasma: inhibition with agalacto-orosomucoid. *Nature, Lond.* **264**, 86–88.

STAHL, P., SCHLESINGER, P. H., SIGARDSON, E., RODMAN, J. S. & LEE, Y. C. (1980). Receptor mediated pinocytosis of mannose-glycoconjugates by macrophages: characterization and evidence for receptor recycling. *Cell* **19**, 207–215.

SUMMERFIELD, J. A. & TAYLOR, M. E. (1986). Mannose-binding proteins in human serum: identification of mannose-specific immunoglobulins and a calcium-dependent lectin of broader carbohydrate specificity, secreted by hepatocytes. *Biochim. biophys. Acta* **883**, 197–206.

SUNG, S.-S. J., NELSON, R. S. & SILVERSTEIN, S. C. (1983). Yeast mannans inhibit binding and phagocytosis of zymosan by mouse peritoneal macrophages. *J. Cell Biol.* **96**, 160–166.

TAKAHASHI, H., KOMANO, H., KAWAGUCHI, N., KITAMURA, N., NAKANISHI, S. & NATON, S. (1985). Cloning and sequencing of a cDNA of *Sarcophaga perigrina* humoral lectin induced by injury of the body wall. *J. biol. Chem.* **260**, 12228–12236.

TIETZE, C., SCHLESINGER, P. & STAHL, P. (1982). The mannose-specific endocytosis receptor of alveolar macrophages: Demonstration of two functionally distinct intracellular pools of receptors and their roles in receptor recycling. *J. Cell Biol.* **92**, 417–424.

TOWNSEND, R. & STAHL, P. (1981). Isolation and characterization of a mannose/*N*-acetylglucosamine/fucose binding protein from rat liver. *Biochem. J.* **194**, 209–214.

TOWNSEND, R. R., WALL, D. A., HUBBARD, A. L. & LEE, Y. C. (1984). Rapid release of galactose-terminated ligands after endocytosis by hepatic parenchymal cells: Evidence for a role of carbohydrate structure in the release of internalized ligand from receptor. *Proc. natn. Acad. Sci. U.S.A.* **81**, 466–470.

VON HEIJNE, G. (1983). Patterns of amino acids near signal-sequence cleavage sites. *Eur. J. Biochem.* **133**, 17–26.

WHITE, R. T., DANOM, D., MILLER, J., SPRATL, K., SCHILLING, J., HAGWOOD, S., BENSON, B. & CORDELL, B. (1985). Isolation and characterization of the human pulmonary surfactant apoprotein gene. *Nature, Lond.* **317**, 361–368.

WILD, J., ROBINSON, D. & WINCHESTER, B. (1983). Isolation of mannose-binding proteins from human liver. *Biochem. J.* **210**, 167–177.

WILEMAN, T., BOSHANS, R., SCHLESINGER, P. & STAHL, P. (1984). Monensin inhibits recycling of macrophage mannoseglycoprotein receptor and ligand delivery to lysosomes. *Biochem. J.* **220**, 665–675.

WILEMAN, T., LENNARTZ, M. & STAHL, P. (1986). Identification of the macrophage mannose receptor as a 175 KD membrane protein. *Proc. natn. Acad. Sci. U.S.A.* **83**, 2501–2505.