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Dominant Effects of Mutations in the Collagenous Domain of Mannose-Binding Protein

Russell Wallis

Individuals heterozygous for mutant alleles encoding serum mannose-binding protein (MBP, also known as mannose-binding lectin) show increased susceptibility to infections caused by a wide range of pathogenic microorganisms. To investigate the molecular defects associated with heterozygosity, wild-type rat serum MBP polypeptides (MBP-A: 56% identical in sequence to human MBP) and rat MBP polypeptides containing mutations associated with human immunodeficiency have been coexpressed using a well-characterized mammalian expression system. The resulting proteins are secreted almost exclusively as heterooligomers that are defective in activating the complement cascade. Functional defects are caused by structural changes to the N-terminal collagenous and cysteine-rich domains of MBP, disrupting interactions with associated serine proteases. The dominant effects of the mutations demonstrate how the presence of a single mutant allele gives rise to the molecular defects that lead to the disease phenotype in heterozygous individuals. The Journal of Immunology, 2002, 168: 4553–4558.

Mannose-binding protein (MBP, also known as mannose-binding lectin) is the first component of the lectin pathway of complement activation (1, 2). It binds directly to carbohydrate structures on the surfaces of bacterial, fungal, and parasitic cells and initiates complement fixation by activating associated serine proteases designated MBP-associated serine proteases (MASPs). MBPs are members of the collectin family of animal lectins. These proteins all contain an N-terminal serine protease domain. MASPs are homodimers stabilized through interactions involving the N-terminal CUB domain (9). The N-terminal CUB and epidermal growth factor-like domains are necessary and sufficient to reproduce the MBP-binding properties of the full-size proteins. MBP dimers bind single MASP dimers, whereas MBP trimers and tetramers bind up to two MASP dimers.

MBP-associated immunodeficiency is a common disorder that is linked to increased susceptibility to a wide range of infections (2). Individuals are particularly vulnerable in the first few years of life, before an effective adaptive immune response is established. The deficiency also manifests itself when adaptive immunity is compromised, for example, during HIV infection or following chemotherapy (10, 11). MBP-associated immunodeficiency can be caused by any one of three separate point mutations in the MBP gene. Each mutation leads to a single amino acid substitution within the first part of the collagenous domain. In both homozygous and heterozygous individuals, the disorder is characterized by reduced levels of serum MBP and structural changes to the N-terminal domains.

A model system in which the naturally occurring human variants have been recreated in rat serum MBP has proved to be very useful for characterizing the structural defects associated with homozygous genotypes (9, 12). The mutations decrease significantly the complement-fixing activities of rat MBP as a result of structural changes that disrupt interactions with MASPs. In homozygous individuals, immunodeficiency is caused by inefficient complement activation that compounds the effects of reduced serum MBP levels. However, the molecular basis of immunodeficiency in heterozygous individuals is poorly understood. Serum MBP levels are considerably higher than in homozygous subjects, implying that diminished ability to fix complement rather than decreased serum levels may be the major factor leading to immunodeficiency.

To determine whether MBPs of the type that would be produced by heterozygotes are functionally defective, wild-type and mutant rat MBPs have been coexpressed. The resulting heterooligomeric MBPs have significantly reduced complement-fixing activities as a result of structural changes that resemble the defects in homooligomeric mutant MBPs. Defective complement fixation resulting from the dominant effects of the mutations is likely to be a major cause of immunodeficiency in individuals with mutant MBP alleles.
Materials and Methods

Materials

Restriction enzymes were purchased from New England Biolabs (Beverly, MA). All tissue culture media were obtained from Life Technologies (Gaithersburg, MD). Promix cell-labeling mix (70% [35]S-methionine and 30% [35]S-labeled cysteine) was purchased from Amersham Pharmacia Biotech (Little Chalfont, U.K.). Nitrilotriacetic acid-agarose, Sepharose 6B, and protein molecular mass markers were obtained from Sigma-Aldrich (St. Louis, MO). Immun 4 microtiter wells were purchased from Dynex Technologies (Chantilly, VA). Nitrocellulose membranes were purchased from Schleicher & Schuell (Keene, NH). Anti-hemagglutinin (HA) and anti-c-myc mAbs were purchased from Roche Molecular Biochemicals (Indianapolis, IN).

Production of fragments of MASP-1/3 and MASP-2

The N-terminal three domains of MASP-1 and MASP-2, which reproduce wild-type CRDs and galactose-binding CRDs were purified on columns (1 ml) in 1 ml of loading buffer (50 mM Tris (pH 7.8) containing 1.25 M NaCl and 25 mM CaCl2). Columns were washed with loading buffer (3 × 1-ml fractions), and protein was eluted in 50 mM Tris (pH 7.8) containing 1.25 M NaCl and 2.5 mM EDTA (3 × 1-ml fractions). Galactose (6 mM) was included in the loading buffer for the mannose-Sepharose affinity columns to prevent weak interactions with the CRDs of WT* MBP.

Results

Heterooligomers produced by coexpression of wild-type and mutant MBPs

The first step toward understanding why MBPs produced by heterozygous individuals are functionally defective was to determine whether wild-type and mutant polypeptide chains associate with each other during biosynthesis. To address this question, the CRDs of wild-type chains, but not mutant chains, were modified to confer selectivity to galactose (Fig. 1). This strategy enabled selective purification of homoooligomeric wild-type and mutant MBPs by affinity chromatography on galactose-Sepharose or mannose-Sepharose columns while heterooligomeric MBPs bound to both columns. Because CRDs are not involved in oligomerization of subunits, the changes were not expected to disrupt assembly of the MBPs.

As expected, wild-type MBP binds to a mannose-Sepharose column and can be eluted by chelating the Ca2+ required for binding (Fig. 2). It elutes in the unbound wash fractions from a galactose-Sepharose column, indicating that it does not interact significantly with galactose. In contrast, MBP with galactose-binding specificity
To assess the ability of heterooligomeric MBPs to interact with heterooligomeric MBPs, the proteins are secreted predominantly as heterooligomers. Thus, heterozygous individuals for the human disorder are likely to produce heterooligomeric MBPs consisting of both mutant and wild-type polypeptides. Because individuals heterozygous for mutations to just some polypeptides are reduced by 10-, 7-, and over 30-fold, respectively. By comparison, the complement-fixing activities of wild-type MBP and WT/R23C protein are reduced by 1.5- to 4-fold while the activities of the WT*/G25D and WT*/G28E proteins are reduced by 5-fold and over 30-fold, respectively. By contrast, the R23C/R23C protein consists of a lower proportion of dimers, trimers, and tetramers of subunits and higher amounts of single subunits, which leads to a reduction in the overall complement-fixing activity (18). Gel filtration analysis shows that the proportion of MBP trimers and tetramers is also reduced in the WT/R23C protein, although not to the same extent as in the R23C/R23C protein (Fig. 4). The major species consist of covalent oligomers that resemble those of wild-type MBP on SDS-polyacrylamide gels, indicating that the disulfide bonds that link individual polypeptide chains together at the N terminus are not disrupted significantly (Fig. 5). Based on the relative complement-fixing activities of oligomers in wild-type MBP (4), the WT/R23C protein would be expected to have ~50% of the activity of wild-type protein if the defect were due to changes in the relative proportion of oligomers alone. This value is consistent with the 1.5- to 4-fold reduction in complement-fixing activity observed, suggesting that the primary defect is due to aberrant assembly during biosynthesis, resulting in lower amounts of trimers and tetramers of subunits.

Defective complement fixation in the R23C/R23C protein arises because the smaller MBP oligomers have lower affinities for MASP-2 (18). To compare the WT/R23C protein with its homooligomeric counterpart, the binding affinity for MASP-2 was measured using a fragment consisting of the N-terminal three domains of MASP-2 that reproduces the binding properties of the full-size protein (9). Because the affinity of the MBP-MASP-1 complex is relatively low compared to the MBP-R23C protein, the R23C/R23C protein is expected to be a major cause of immunodeficiency in such patients.

**Molecular defects in the WT/R23C protein**

Previous studies have revealed that the overall complement-fixing activity of MBP is a function of the distribution of oligomers and the specific activities of each oligomer (4). Wild-type MBP comprises covalent oligomers ranging from monomers to tetramers of subunits, of which trimers and tetramers have the highest complement-fixing activities. By contrast, the R23C/R23C protein consists of a lower proportion of dimers, trimers, and tetramers of subunits and higher amounts of single subunits, which leads to a reduction in the overall complement-fixing activity (18). Gel filtration analysis shows that the proportion of MBP trimers and tetramers is also reduced in the WT/R23C protein, although not to the same extent as in the R23C/R23C protein (Fig. 4). The major species consist of covalent oligomers that resemble those of wild-type MBP on SDS-polyacrylamide gels, indicating that the disulfide bonds that link individual polypeptide chains together at the N terminus are not disrupted significantly (Fig. 5). Based on the relative complement-fixing activities of oligomers in wild-type MBP (4), the WT/R23C protein would be expected to have ~50% of the activity of wild-type protein if the defect were due to changes in the relative proportion of oligomers alone. This value is consistent with the 1.5- to 4-fold reduction in complement-fixing activity observed, suggesting that the primary defect is due to aberrant assembly during biosynthesis, resulting in lower amounts of trimers and tetramers of subunits.
The apparent molecular mass of the smallest MBP species was determined covalent structures in MBP oligomers have been described previously (18). These oligomers are assembled primarily from smaller covalent structures of 110, 45, and 26 kDa that comprise four and two polypeptide species, implying that they are assembled from trimeric subunits. However, these oligomers are assembled primarily from smaller covalent structures of 110, 45, and 26 kDa that comprise four and two polypeptide species, along with some single polypeptide chains. Possible arrangements of such covalent structures in MBP oligomers have been described previously (18). The apparent molecular mass of the smallest MBP species was determined from the migration position on 17.5% gels using molecular mass markers of 6.5–66 kDa as standards. Also sensitive to structural defects in the collagenous domain of MBP (13), interactions with the corresponding MASP-1/3 fragment were quantified as well. Solid-phase competition assays reveal that the WT/R23C protein binds to the N-terminal three domains of either MASP-1/3 or MASP-2 more weakly than wild-type MBP binds (Fig. 6). However, MASP binding by WT/R23C is less perturbed than MASP binding by the R23C/R23C protein (Table I), an effect that parallels the degree of disruption to oligomerization in homooligomeric and heterooligomeric MBPs (Fig. 4). Thus, defective binding to MASP-2 by the WT/R23C protein can also be accounted for by changes in the distribution of oligomers and probably leads directly to aberrant complement fixation.

The molecular defects in the R23C/R23C protein result from the presence of cysteine residues within the collagen-like domain (18). Adventitious disulfide bond formation between cysteine 23 residues is believed to disrupt association of the subunits during biosynthesis. The WT/R23C protein contains more of the larger oligomers than the R23C/R23C protein. Thus, incorporation of a cysteine residue into just some polypeptides of the collagen triple helix is less disruptive to oligomer formation than the presence of a cysteine residue in all three polypeptide chains.

Structural defects in the WT/G25D and WT/G28E proteins

Previous studies have shown that reductions in the proportion of trimers and tetramers of MBP subunits account for an ~3-fold decrease in the complement-fixing activities of the G25D/G25D and G28E/G28E proteins (18). Gel filtration analysis reveals that the distribution of oligomers in the WT/G25D and WT/G28E proteins is also perturbed compared with wild-type MBP (Fig. 4), although percentages of trimers and tetramers of subunits are somewhat higher than for the G25D/G25D and G28E/G28E proteins. Changes in the distribution of oligomers alone could only account for a reduction of ~25% in the complement-fixing activities, which is much less than the 5-fold and ~30-fold reductions observed. Therefore, individual heterooligomers of the WT/G25D and WT/G28E proteins must also have lower specific complement-fixing activities than corresponding homooligomers of their wild-type counterparts.

Reduced specific complement-fixing activities of individual oligomers of the G25D/G25D and G28E/G28E proteins reflect perturbation of the MASP-2 binding site caused by local disruption to the collagen-like domain and the adjacent cysteine-rich domain (18). These changes lead to a 4-fold decrease in complement-fixing activity. Comparable structural defects in the cysteine-rich domains are detected in the WT/G25D and WT/G28E proteins, as evidenced by the pattern of bands observed on SDS-polyacrylamide gels, in which the predominant covalent species comprise two and four polypeptide chains (Fig. 5). The covalent structures are also comparable to proteins isolated from the serum of immunodeficient individuals that consist of low molecular mass covalent forms.

A 4-fold decrease in complement-fixing activity due to structural perturbations in the N-terminal domains that disrupt the interaction with MASP-2 combined with the small reduction in complement-fixing activity due to the changes in the distribution of oligomers would account for the overall 5-fold decrease in complement-fixing activity of the WT/G25D protein. However, the WT/G28E protein is a further 8- to 10-fold less active at fixing complement than the WT/G25D protein. This reduction mirrors the 10-fold difference in complement-fixing activities between the G25D/G25D and G28E/G28E proteins. Thus, the additional defect caused by the G28E mutation is also a dominant effect.

**Table 1. Distribution of MBP oligomers in WT/mutant MBPs determined by gel filtration chromatography**

<table>
<thead>
<tr>
<th>MBP</th>
<th>Monomer (%)</th>
<th>Dimer (%)</th>
<th>Trimer (%)</th>
<th>Tetramer (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>WT</td>
<td>13 ± 2</td>
<td>36 ± 10</td>
<td>22 ± 2</td>
<td>29 ± 5</td>
</tr>
<tr>
<td>WT/R23C</td>
<td>31 ± 1</td>
<td>44 ± 2</td>
<td>14 ± 6</td>
<td>11 ± 6</td>
</tr>
<tr>
<td>WT/G25D</td>
<td>21 ± 3</td>
<td>50 ± 6</td>
<td>14 ± 4</td>
<td>10 ± 5</td>
</tr>
<tr>
<td>WT/G28E</td>
<td>23 ± 3</td>
<td>41 ± 2</td>
<td>35 ± 6</td>
<td>1 ± 1</td>
</tr>
</tbody>
</table>

**FIGURE 5.** SDS-PAGE of homooligomeric and heterooligomeric MBPs. Proteins were separated under nonreducing conditions on 10% polyacrylamide gels and were detected with Coomassie blue. The migration positions of molecular mass markers and of covalent oligomeric forms of wild-type MBP are shown on the left and right, respectively. The covalent structure of WT* MBP resembles the structure of wild-type protein, demonstrating that changes to the CRDs do not affect oligomerization of subunits. As shown in Fig. 4., oligomers of the glycine mutants elute from a gel filtration column at comparable positions to oligomers of wild-type MBP, implying that they are assembled from trimeric subunits. However, these oligomers are assembled primarily from smaller covalent structures of 110, 45, and 26 kDa that comprise four and two polypeptide species, along with some single polypeptide chains. Possible arrangements of such covalent structures in MBP oligomers have been described previously (18). The apparent molecular mass of the smallest MBP species was determined from the migration position on 17.5% gels using molecular mass markers of 6.5–66 kDa as standards.

**FIGURE 6.** Binding of wild-type and heterooligomeric MBPs to MASP fragments analyzed using a solid-phase competition assay. Increasing concentrations of MBP were incubated with immobilized fragments, comprising the N-terminal three domains of either MASP-1/3 or MASP-2 using 35S-labeled MBP as the reporter ligand.
As expected, the structural changes in the WT/G25D and WT/G28E proteins lead to defective interactions with MASPs. Indeed, solid-phase competition assays reveal that the affinities of the complexes are broadly comparable to those of complexes between the corresponding homooligomeric mutant MBPs and MASPs. Thus, the dominant nature of the phenotypes implies that replacing a glycine residue at the 25 or 28 position by an acidic residue in just one or two polypeptides of the collagen triple helix is almost as disruptive as when all three polypeptide chains contain the mutation. The mutation at the 28 position affects binding to either MASP-1 or MASP-2 more than the mutation at the 25 position.

The simplest explanation to account for differences in affinities and complement-fixing activities between the two glycine mutants is that the MASPs bind closer to the 28 position, near the point at which individual subunits diverge from the central core in the bouquet-like structures.

Discussion

The results reported here suggest that inefficient complement fixation resulting from defective interactions with MASPs is likely to be a major cause of immunodeficiency in individuals heterozygous for mutant MBP alleles. On coproduction of wild-type and mutant polypeptides, MBPs are secreted predominantly as heterooligomers that have the same structural and functional defects as homooligomeric mutant proteins.

It has been suggested that immunodeficiency in heterozygous individuals arises because only a small fraction of the MBP oligomers will be normal (19). Thus, only 1 in 8 (1 in 2 × 2 × 2) single subunits, 1 in 64 dimers, 1 in 512 trimers, and 1 in 4096 tetramers will contain exclusively wild-type polypeptides, assuming random association of chains. This hypothesis is entirely consistent with data for the WT/G25D and WT/G28E proteins, because the specific complement-fixing activities of these heterooligomeric MBPs are equivalent to the activities of their homooligomeric mutant counterparts. The complement-fixing activity of the WT/R23C protein is less impaired than would be predicted from the model, implying that the defect results from the presence of more than one mutant polypeptide in an MBP oligomer.

Trimerization of MBP polypeptides is initiated by interactions between the C-terminal CRDs and the α helices that form the neck regions and proceed in a C- to N-terminal direction (20, 21). Because WT/mutant MBPs form trimeric subunits and contain both wild-type and mutant polypeptides in comparable proportions, the mutations do not prevent subunit formation. All three mutations are near the N terminus of MBP and so it is likely that only the N-terminal part of the collagenous domain and the cysteine-rich domain are misfolded.

Reduced complement-fixing activities of the WT/R23C and R23C/R23C proteins can be accounted for by changes in the population of oligomers, resulting in a reduction in the amounts of the larger species, although oligomerization of the WT/R23C protein is less impaired. Previous studies have shown that at least some cysteine 23 residues are disulfide bonded to corresponding residues within individual subunits of the homooligomeric mutant MBPs (18). The disulfide bonds probably disrupt interactions between subunits by distorting the collagen triple helix, thus preventing oligomerization of subunits. In heterooligomeric MBPs, in which just half of the polypeptides contain cysteine 23 residues, only a fraction of subunits are likely to contain aberrant interchain disulfide bonds. This distinction probably accounts for the differences in the distribution of oligomers between the WT/R23C and R23C/R23C proteins.

Each protomer of a MASP dimer must bind to a separate subunit of a MBP oligomer to form a stable complex capable of fixing complement (9). Therefore, disruption of even a single MBP subunit due to the presence of a polypeptide containing a mutation is likely to destabilize a MBP-MASP complex. The dominant phenotype resulting from the G25D and G28E mutations in heterozygous individuals probably arises because almost all of MBPs will be heterooligomers that bind defectively to MASP-2.

Reduction in the affinities of MBP/MASP-2 complexes alone cannot account for the lower complement-fixing activities of the homooligomeric and heterooligomeric MBPs (Table I). Binding of MASP-1/3 is also affected by the mutations and the possibility that MASP-1, MASP-2, and MASP-3 function cooperatively cannot be completely ruled out. Because MASP-2 in complex with MBP autoactivates and can trigger complement activation, it is more probable that MASP-2 activation itself is defective in complexes with mutant MBPs. Defective interactions between MBP and MASP-2 probably prevent efficient zymogen activation, thus precluding complement fixation.

There is a complex relationship between disruption of the structure of the collagenous domain of MBP, formation of larger oligomers, MASP-2 binding, and complement activation. For example, the WT/G25D and WT/R23C proteins bind to MASP-2 with broadly comparable affinities but the complement-fixing activity of the WT/G25D protein is ~2.5-fold lower than the activity of the WT/R23C protein (Fig. 3). The discrepancy between affinities and complement-fixing activities probably arises because the principal molecular defect is different in these two MBPs. In the WT/R23C protein, reduced complement-fixing activity is due largely to defective oligomerization of subunits, whereas in the WT/G25D protein it is caused by structural changes in the collagenous domain. Although both changes affect MASP-2 binding affinity to comparable extents, the effects on MASP-2 activation must be different.

Serum MBP concentrations are considerably higher in heterozygous individuals than in homozygotes. For example, British Caucasoïds heterozygous for the mutation equivalent to G25D in rat MBP have only 4- to 5-fold lower serum MBP levels than wild-type individuals, while trace amounts of MBP could be detected in the serum of homozygotes (22). Data reported here indicate that complement-fixation activities of WT/mutant MBPs are all significantly reduced. Thus, defective complement activation may make a more important contribution to the immunodeficient phenotype of heterozygotes than of homozygotes.

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References


