Natural Mannose-Binding Lectin (MBL) Down-regulates Phagocytosis of Helicobacter pylori

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Abstract

Considering the role of lectin-carbohydrate interactions between *Helicobacter pylori* bacteria and the host cells we addressed the question on how mannose binding lectin – MBL, present in human plasma, may influence the phagocytosis of *H. pylori* by peripheral blood granulocytes. For phagocytosis assay the granulocytes separated from peripheral blood of healthy *H. pylori*-seronegative donors were used. Phagocytosis was estimated by fluorescence assay using FITC-labelled *H. pylori* cells. The MBL level in the serum samples as well as MBL-binding to *H. pylori* bacteria were estimated by ELISA. In this study all *H. pylori* isolates bound recombinant mannose binding lectin-MBL as shown by ELISA. The ingestion of *H. pylori* bacteria in the medium with human serum depleted in natural MBL (nMBL) was more intensive than in the medium with complete serum containing nMBL. Moreover, the ingestion of *H. pylori* bacteria in the medium with complete serum was increased by an addition of anti-rMBL IgG. The results indicate that interaction of bacterial and host lectins may regulate the phagocytosis of *H. pylori* bacteria and in this way influence an outcome of the infection caused by these microbes.

Key words: *Helicobacter pylori*, mannose binding lectin (MBL), phagocytosis

Introduction

*Helicobacter pylori* related gastroduodenal infections are associated with strong infiltration of the gastric mucosa by neutrophils, macrophages, lymphocytes and plasma cells (Rudnicka and Andersen, 1999). Despite mobilization of phagocytes to inflammatory foci, the bacteria are not eliminated. It has been suggested that they may evade destruction by phagocytes due to a temporary persistence in the cytosol of epithelial cells (Petersen and Krogfeld, 2003). Many *H. pylori* strains express adhesin proteins that bind to specific host cell macromolecule receptors. The best defined *H. pylori* adhesin-receptor interaction, described by Ilver et al. (1998), is that between the Lewis b (Le b) blood group antigen binding adhesin, BabA, a member of a family of *H. pylori* outer membrane proteins.

Mahdavi et al. (2002), identified sialyl-dimeric Lewis X glycosphingolipid as a receptor for *H. pylori*. The corresponding sialic-acid-binding adhesin (SabA) was isolated and the *sabA* gene was identified (Mahdavi et al., 2002). It has also been established that *H. pylori* strains express heparan sulphate binding proteins (Hirmo et al., 1995).

Two molecular mechanisms of microbial recognition by phagocytes are distinguished: direct – opsonin independent, and indirect – opsonin dependent (Ofek et al., 1995) In our previous study we found that antibodies specific to various *H. pylori* antigens may have opposite effects on the course of phagocytosis of these bacteria. We showed that opsonization of *H. pylori* with anti-Lewis X monoclonal antibody (IgM)
made Lewis X-positive but not Lewis X-negative \textit{H. pylori} bacteria more susceptible to phagocytosis (Chmiela et al., 1997, 1998, Rudnicka et al., 2001). However, sera from dyspeptic patients with IgG against \textit{H. pylori} surface antigens reduced the susceptibility of these bacteria to phagocytosis (Rudnicka et al., 1998). The importance of opsonic activity of the complement in the ingestion of \textit{H. pylori} bacteria by neutrophils was also shown (Mc Kinlay et al., 1993).

On the other hand, Rautelin et al. (1994), showed that about one third of \textit{H. pylori} strains isolated from human gastric biopsy specimens, induced strong chemiluminescence in neutrophils, even without serum opsonins.

Lectinophagocytosis is a known example of opsonin independent phagocytosis. It includes the reaction between surface lectins and carbohydrates on microbial or phagocytic cells (Ofek et al., 1995). Previously we showed that interaction between bacterial surface structures such as sialic acid specific haemagglutinins, heparin binding proteins and corresponding phagocyte receptors was necessary for the ingestion of \textit{H. pylori} (Chmiela et al., 1998). On the other hand, our results suggested that \textit{H. pylori} can use widely distributed host compounds: sialic acid or heparin/heparan sulfate glycosaminoglycans, hyaluronic acid or vitronectin (in the presence of complement) to avoid phagocytosis (Chmiela et al., 1998).

Mannose binding lectin – MBL, a C-type lectin, interacts with various microbial carbohydrates (mannose, N-acetyloglucosamine, fucose and N-acetylomannosamine) (Sastry and Ezekowitz, 1993; Turner, 1996). The bacterial capsule and especially LPS could be a major determinant for MBL binding (Devyatyarnova-Johanson et al., 2000). MBL activates complement on lectin pathway, independent of C1q and antibodies, in the presence of MBL-associated serine proteases (MASP1 and MASP2, homologues of C1q and C1s) (Gal and Ambrus, 2001; Kase et al., 1999; Matsushita and Fujita, 1992). Garred et al. (2003), proposed a dual role of MBL dependent on the lectin’s concentration. Low concentrations have been associated with recurrent or severe infections in children and adults caused by extracellular pathogens and also with autoimmune diseases. High concentrations may enhance targeting of intracellular organisms to host phagocytes. MBL also modulates disease severity, at least in part through a complex, dose dependent influence of cytokine production (Matsushita and Fujita, 1992).

In this study we addressed the question on how MBL may influence the phagocytosis of \textit{H. pylori} by human granulocytes. In order to answer this question we estimated: 1) interaction of MBL with \textit{H. pylori} clinical isolates and reference strains, 2) MBL concentration in the sera from \textit{H. pylori} infected and uninfected children/adolescents and adults, 3) the intensity of \textit{H. pylori} ingestion by human granulocytes in the presence or absence of natural (nMBL) and recombinant (rMBL) mannose binding lectin as well as anti-rMBL IgG antibodies.

**Experimental**

**Material and Methods**

**Serum samples.** A total of 224 sera were examined for MBL concentration. Sera from \textit{H. pylori} positive (69) and negative (49) children/adolescents (average age 13 years) diagnosed for \textit{H. pylori} infection in Mother Health Center Institute in Łódź, Poland, were used for the study. The serum samples from \textit{H. pylori} positive (66) or negative (40) adult dyspeptic patients (average age 53 years) were obtained from K. Jonscher Hospital in Łódź, Poland. \textit{H. pylori} status was determined by endoscopy, rapid urease test and histology. Moreover, in all subjects the titers of anti-\textit{H. pylori} IgG and IgA antibodies were estimated by immunoenzymatic test – ELISA with glycine acid extract of the reference \textit{H. pylori} strain, as previously described (Rechciński et al., 1997). The study was approved by the local Ethical Committee. All patients signed informed consent.

**ELISA for serum MBL concentration.** The microtitre plates (Nunc Immunoplate Maxisorp, Nunc, Kastrup, Denmark) were coated with \textit{S. cerevisiae} mannann (Sigma, St. Louis, Michigan, USA) at a concentration of 250 µg/ml in carbonate buffer pH 9.6 (Aittoniemi et al., 1996). The plates were washed with phosphate buffered saline (PBS), pH 7.4, containing 0.05% Tween 80 (PBS/T), and the remaining binding sites were blocked with 1% bovine serum albumin, BSA, Sigma, in PBS (PBS/BSA). Next the serum samples diluted 1:40 and 1:80 in Tris-HCl pH 8.0 with 50 mM CaCl$_2$ (Tris-HCl/CaCl$_2$) supplemented with 1% BSA were added to the wells and the plates were incubated for 2 h, at 37°C. After washing, the plates were incubated for 2 h, at 37°C with rabbit antibodies against recombinant human MBL (rMBL), and then for 1 h with swine antibodies against rabbit immunoglobulins labelled with horseradish peroxidase-HRP (Dako, Glostrup, Denmark). The colour reaction was developed in the presence of substrate solution: 1 mg o-phenylenediamine dihydrochloride-OPD (Sigma) in 1 ml citric-acid phosphate buffer, pH 5.0 supplemented with 0.5 µl/µl of 30% H$_2$O$_2$. The reaction was stopped with citric acid and the absorbance was measured at 450 nm using Victor2 reader (Wallak, Oy, Turku, Finland). The standard curve was prepared by incubation of mannann-coated wells with a known amount of rMBL (0.073–1.2 µg/ml), and then with rabbit anti-rMBL antibodies and swine-HRP antibodies to rabbit immunoglobulins. In every ELISA the control wells were used for excluding the unspecific reactions.

**Sera depleted in MBL.** For phagocytosis assay two types of serum samples were used: 1) containing nMBL; 2) depleted in nMBL by absorption with \textit{S. cerevisiae} mannann coated sepharose (Sigma, St. Louis, Michigan, USA) (Kase et al., 1991). In brief, 500 µl of mannann bound sepharose was sedimented by centrifugation for 2 min, 300×g, and stabilized for 18 h, at 4°C with
The level of MBL in \textit{H. pylori} infected and uninfected individuals. There was a high variation in MBL amount in the serum samples in each group under the study (Table I). The MBL concentration was in the range 2.0–50 µg/ml. There was no significant difference in the frequency of the MBL concentration: 0–2 mg/ml; 2–4 µg/ml; 4–10 µg/ml and >10 µg/ml, between the groups of \textit{H. pylori} infected and uninfected children/adolescents and adults or between males and females.

The interaction of \textit{H. pylori} with MBL. The \textit{H. pylori}-MBL interaction was evaluated for 31 clinical isolates and two reference strains. The results showed that all \textit{H. pylori} strains interacted with rMBL when investigated by ELISA. The specific ELISA OD450 counts for \textit{H. pylori} strains were in the range 1.0–2.0 (mean 1.5 ± 0.25) and for \textit{M. bovis} 0.2–0.6 (mean 0.4 ± 0.05) (Table II). Positive ELISA counts for rMBL coated wells were in the range 0.8–1.0.

The intensity of phagocytosis of FITC-\textit{H. pylori} bacteria by granulocytes in the presence or absence of MBL. The phagocytosis of MBL-binding \textit{H. pylori} strain CCUG 17874 by human granulocytes,
Table I
The concentration of MBL in the sera from children/adolescents and adults infected or uninfected with *H. pylori*

<table>
<thead>
<tr>
<th>Group investigated</th>
<th>Serum MBL concentration (µg/ml)</th>
<th>Prevalence (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Range (r)</td>
<td>Below 2.0</td>
</tr>
<tr>
<td>Children/adolescents</td>
<td></td>
<td></td>
</tr>
<tr>
<td><em>H. pylori (+) mean:</em></td>
<td>r = 2.0–38.0</td>
<td>12/69 (17%)</td>
</tr>
<tr>
<td>n = 69</td>
<td>10.1 ± 9.0</td>
<td></td>
</tr>
<tr>
<td><em>H. pylori (-) mean:</em></td>
<td>r = 2.0–49.6</td>
<td>5/49 (10%)</td>
</tr>
<tr>
<td>n = 49</td>
<td>12.2 ± 10.9</td>
<td></td>
</tr>
<tr>
<td>Adults</td>
<td></td>
<td></td>
</tr>
<tr>
<td><em>H. pylori (+) mean:</em></td>
<td>r = 2.0–30.5</td>
<td>10/66 (15%)</td>
</tr>
<tr>
<td>n = 66</td>
<td>9.6 ± 7.6</td>
<td></td>
</tr>
<tr>
<td><em>H. pylori (-) mean:</em></td>
<td>r = 2.0–43.2</td>
<td>7/40 (18%)</td>
</tr>
<tr>
<td>n = 40</td>
<td>10.4 ± 10.7</td>
<td></td>
</tr>
</tbody>
</table>

n – number of subjects

Table II
The interaction of recombinant mannose binding lectin (rMBL) with *H. pylori* and *M. bovis* estimated by ELISA

<table>
<thead>
<tr>
<th>Bacterial strains</th>
<th>ELISA</th>
<th></th>
<th></th>
</tr>
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<tbody>
<tr>
<td></td>
<td>Specific OD&lt;sub&gt;450&lt;/sub&gt; counts</td>
<td>Range of unspecific OD&lt;sub&gt;450&lt;/sub&gt; counts</td>
<td></td>
</tr>
<tr>
<td></td>
<td>mean</td>
<td>range</td>
<td></td>
</tr>
<tr>
<td><em>H. pylori</em></td>
<td>1.0</td>
<td>1.5 ± 0.25</td>
<td>0.1 – 0.4</td>
</tr>
<tr>
<td><em>M. bovis</em> (low binding control)</td>
<td>0.2 – 0.6</td>
<td>0.4 ± 0.05</td>
<td>0.03 – 0.04</td>
</tr>
<tr>
<td>rMBL (positive ELISA control)</td>
<td>Range 0.8–1.0</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

The interaction of rMBL with *H. pylori* and *M. bovis* was estimated by ELISA using *S. cerevisiae* mannan as coating antigen. Polyclonal rabbit anti-rMBL IgG were used for recognition of MBL bound with bacterial cells and swine antibodies against rabbit immunoglobulins labeled with horse-radish peroxidase for detection of such complex.

Table III
The intensity of phagocytosis of FITC-labelled *H. pylori* bacteria by human granulocytes in the presence or absence of natural (nMBL) or recombinant mannose binding lectin (rMBL) and anti-rMBL antibodies

<table>
<thead>
<tr>
<th>Phagocytosis milieu</th>
<th>Fluorescence counts</th>
<th>Phagocytosis index</th>
<th>Fluorescence counts</th>
<th>Phagocytosis index</th>
<th>Fluorescence counts</th>
<th>Phagocytosis index</th>
<th>Fluorescence counts</th>
<th>Phagocytosis index</th>
</tr>
</thead>
<tbody>
<tr>
<td>supplemented with: 20% serum containing 30 µg/ml nMBL (control culture)</td>
<td>1284</td>
<td>1.0</td>
<td>1940</td>
<td>1.5</td>
<td>3372</td>
<td>2.6</td>
<td>2848</td>
<td>2.2</td>
</tr>
<tr>
<td>supplemented with: 20% nMBL-depleted serum</td>
<td>1048</td>
<td>5707</td>
<td>5.4</td>
<td>4578</td>
<td>4.3</td>
<td>1125</td>
<td>1.0</td>
<td></td>
</tr>
<tr>
<td>supplemented with: 20% serum containing 30 µg/ml nMBL and anti-rMBL rabbit IgG</td>
<td>1486</td>
<td>4169</td>
<td>2.8</td>
<td>6180</td>
<td>4.1</td>
<td>1161</td>
<td>0.8</td>
<td></td>
</tr>
<tr>
<td>supplemented with: 20% nMBL depleted serum and 30 µg/ml rMBL</td>
<td>2560</td>
<td>6074</td>
<td>2.3</td>
<td>5759</td>
<td>2.2</td>
<td>2926</td>
<td>1.1</td>
<td></td>
</tr>
<tr>
<td>Mean: 1595 ± 668</td>
<td>Mean: 1.0</td>
<td>Mean: 4473 ± 1879</td>
<td>Mean: 3.0</td>
<td>Mean: 4972 ± 1264</td>
<td>Mean: 3.3</td>
<td>Mean: 2015 ± 1008</td>
<td>Mean: 1.3</td>
<td></td>
</tr>
</tbody>
</table>

The ingestion of *H. pylori*-FITC labeled bacteria by granulocytes was estimated fluorimetrically. The intensity of phagocytosis was expressed as mean of the fluorescence counts from four experiments, evaluated in the fluorescence reader Victor<sup>2</sup>. The phagocytosis index was calculated with relation to the intensity of phagocytosis in the medium supplemented with 20% of serum containing 30mg/ml nMBL (control culture).

▲ Difference statistically significant (p<0.05).
in the medium with 20% of complete human serum containing natural MBL-nMBL (30 µg/ml), was expressed as relative fluorescence units – RFU (1595 ± 668) and as Phagocytosis Index 1.0 (Table III). The replacement of the complete serum by the same serum depleted in nMBL (nMBL-depleted serum) on mannan coated sepharose caused a significant (p<0.05) increase of fluorescence counts from 1595 ± 668 to 4473 ± 1879 and Phagocytosis Index up to 3.0. The preservation of complement activity in MBL-depleted serum was proved by using monoclonal anti-C5-C9 complex antibodies. Data in Table III also show that addition of rabbit IgG against recombinant MBL (rMBL) to the phagocytosis samples with human complete serum containing natural MBL increased the fluorescence counts from 1595 ± 668 to 4972 ± 1264 and Phagocytosis Index from 1.0 to 3.3. The replenishment of removed human serum by the addition of rMBL did not cause the increase of phagocytosis intensity (RFU 2015 ± 1008, Phagocytosis Index 1.3) as compared with the intensity of ingestion in the medium with nMBL (RFU 1595 ± 668, Phagocytosis index 1.0).

Discussion

Previously we showed that interaction between H. pylori surface structures, namely sialic acid-specific haemagglutinin or heparin/heparan sulfate binding proteins, and corresponding macrophage receptors is required for engulfment of H. pylori bacteria. On the other hand, these microbes can use host’s sialylated compounds, heparin/heparan sulfate glycosaminoglycans, hyaluronic acid or vitronectin in the presence of complement to escape phagocytosis (Chmiela et al., 1998; Drogari-Apiranthitou et al., 1997; Rudnicka et al., 1998, 2001).

In this study we found that all H. pylori strains bound recombinant MBL as estimated by ELISA. These bacteria bound MBL more intensively than the cells of M. bovis. Fungi of Candida species and Aspergillus fumigatus as well as bacteria Staphylococcus aureus, exhibited strong binding of MBL, whereas Escherichia coli, Klebsiella spp., and Haemophilus influenzae type b were characterized by heterogeneous binding patterns (Neth et al., 2000). In contrast, beta-haemolytic group B streptococci, S. pneumoniae and S. epidermidis showed low levels of binding. The MBL binding by H. pylori could be mediated by mannose residues in various bacterial cell surface structures but also by fucose moieties of Lewis X or Lewis Y determinants present in the LPS of the majority of H. pylori strains (Moran et al., 1996). Jack et al. (2001) and Devyataynova-Johanson et al. (2000), showed that bacterial LPS was of major importance in determining the binding of MBL to Gram-negative organisms Salmonella spp. and Neisseria spp.

In general, it is thought that MBL mediates protection against infections due to its opsonic activity, by activating the complement system in the presence of MASp (Garred et al., 2003; Matsushita and Fujita, 1992). However, in this study the H. pylori bacteria were ingested more intensively by human granulocytes in the medium with MBL-depleted or anti-MBL sera as compared with the intensity of phagocytosis in the medium with complete fresh sera containing natural MBL (nMBL). The complement was possibly involved in this process. During phagocytosis, in the presence of complete serum, the lytic complex could be generated on lectin pathway due to the interaction of nMBL with H. pylori bacteria. In the post-phagocytosis supernatants the C5b-C9 terminal complement complex was detected. The lysis due to complement could diminish the number of ingested bacteria in the milieu of nMBL. The complement could be activated on lectin pathway both in the medium with or without nMBL, by serum ficolins which may bind mannose or GlcNAc present on the surface structures of H. pylori (Holmsovk et al., 2003; Matsushita et al., 2001). During the depletion of the sera in nMBL the activity of C5b-C9 complex was preserved. The mechanism of antibody blocking of the MBL inhibition of phagocytosis could be through blocking of nMBL binding to H. pylori or blocking of its inhibiting qualities. The interaction of anti-MBL IgG with nMBL bound to mannose residues on the surface of granulocytes or binding of nMBL-anti-nMBL IgG complexes to phagocyte Fc receptors could not be excluded. Another explanation for diminished phagocytosis of H. pylori in the medium containing nMBL as compared to the medium without nMBL is that bacteria avoid phagocytosis by intensive nMBL binding, a phenomenon which was earlier observed by us for vitronectin and sialic acid (Chmiela et al., 1998). MBL may mask the H. pylori surface adhesins important for recognition and engulfment of these bacteria by phagocytes. A weak H. pylori phagocytosis in nMBL-depleted serum with rMBL confirms this suggestion. The more extreme environment for MBL binding in the gastric mucosa, where phagocytic cells infiltrate during infections, can be neutralized by H. pylori urease. Similarly, to the results of our study, Swanson et al. (1998), showed the 50% inhibition of the interaction of Chlamydia trachomatis, C. pneumoniae and C. psittaci with the leukocytes by rMBL.
Considering the known role of phagocyte receptors for Fc fragment of IgG (FcγR) in the ingestion of bacteria it was interesting to compare the outcome of *H. pylori* phagocytosis in the medium with the sera from *H. pylori* infected individuals, seropositive for anti-*H. pylori* IgG and from uninfected, seronegative donors. In this study, we could see no difference in the ingestion of MBL-binding *H. pylori* bacteria in the medium with sera containing or free of anti-*H. pylori* IgG. Similarly, we observed no significant difference in the MBL levels in the sera from *H. pylori* infected or uninfected children and adults. Also Klabunde et al. (2000), showed no differences in serum MBL concentration in the patients infected with *Schistosoma* sp. and in healthy controls though *Schistosoma* cercariae and adult worms, like *H. pylori*, bind MBL. In contrast, MBL deficiencies were detected with a high frequency in the patients infected with HIV, hepatitis B virus or *Neisseria meningitidis* (Devyatyanova-Johanson et al., 2000; Saifuddin et al., 2000; White et al., 2000). The lack of significant correlation between MBL concentration and *H. pylori* infection in this cohort study implies that MBL is not an essential factor in the disease process. However, in some *H. pylori* infected patients, the elevated MBL concentration by blocking *H. pylori* phagocytosis may allow these bacteria permanent colonization of gastric mucosa. In the summary, our results indicate that *H. pylori* bacteria may use MBL to avoid engulfment by phagocytes. The interactions of bacterial compounds and host lectins may regulate *H. pylori* phagocytosis and on this way influence an outcome of *H. pylori* related infections.

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**Literature**


Role of MBL in H. pylori phagocytosis


