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Mannan-binding protein—levels in plasma and upper-airways secretions and frequency of genotypes in children with recurrence of otitis media

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SUMMARY

We have investigated a possible association between recurrence of otitis media and low concentrations of mannan-binding protein (MBP) in plasma and upper-airway secretions. The protein concentration was measured in plasma ($n=76$), nasopharyngeal secretions ($n=83$) and middle ear effusions ($n=73$) from otitis-prone children, children with less recurrence of acute otitis media, children with no previous history of acute otitis media, but suffering from secretory otitis media, and healthy children. Moreover, genetic polymorphisms associated with low MBP plasma levels were investigated in DNA from nasopharyngeal tonsils of 89 children with recurrence of otitis media. A wide range of MBP plasma concentrations was found. No statistically significant differences in MBP plasma concentration were observed between patients and controls. Nor was there any increased frequency of the genotypes associated with low MBP plasma concentrations. Thus, our results do not support the assumption that low concentration and/or MBP deficiency alone predispose to recurrence of otitis media in Caucasian children. MBP was detected in both nasopharyngeal secretions (1/175 of plasma level) and middle ear effusions (1/4 of plasma level), suggesting a role for the protein in the local mucosal immune defence system at these locations. In contrast, MBP was undetectable in 53 samples of mixed-saliva.

Keywords mannose-binding protein mannose mannan immunodeficiency complement genotypes external secretions immunoglobulins otitis media lectins

INTRODUCTION

Recurrence of upper-airway infections is very common in small children, often leading to secretory otitis media (SOM) and occasionally to acute purulent otitis media (AOM) [1,2]. Although the typical clinical appearances of SOM and AOM are quite different, almost identical risk markers have been found for the two diseases, i.e. recurrent upper-airway infections and dysfunction of the Eustachian tube [1–4]. It is still only partly understood why some children suffer from recurrent episodes of AOM (rAOM) [5]. Increased colonization with bacteria such as *Streptococcus pneumoniae*, non-encapsulated *Haemophilus influenzae* and *Moraxella catarrhalis* is often found in the nasopharynx and occasionally in the middle ear cavity during episodes of SOM and AOM [6,7].

Various aberrations have been demonstrated in both the humoral and cellular immune apparatus of children with rAOM [5,8]. However, most of the anomalies described could to some extent be the result rather than the cause of recurrent infections. A common complement-dependent opsonic defect has been shown to occur with increased frequency in children with recurrent upper-airway infections and diarrhoea during infancy [9]. Recently, it was shown that this opsonic defect was caused by a low concentration of mannan-binding protein, synonymous with mannose-binding protein (MBP) [10]. MBP is an acute phase plasma protein synthesized in the liver [11,12]. It is a C-type lectin with a broad carbohydrate specificity, binding especially well to carbohydrate structures containing mannose or N-acetyl-glucosamine [13]. Such structures are present in the cell wall of a variety of microorganisms. MBP consists of oligomers composed of trimeric subunits formed by single peptide chains which contain a carbohydrate recognition and a collagen-like domain [14]. MBP is involved in infectious pathogen recognition and neutralization by C1q and immunoglobulin-independent activation of the classical complement pathway [15–17]. Moreover, it has an intrinsic ability to mediate opsonization through the collectin receptor (C1q receptor) [18].

Recently, an allelic variant of MBP, which is closely associated with low concentrations of MBP, has been described

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[19]. The variant allele causes an amino acid substitution in exon 1 at codon 54 in the collagen-like domain in the MBP gene (GGC (glycine) to GAC (aspartic acid)). This mutation is remarkably frequent in both Caucasians and Eskimos, with an estimated allele frequency of 0·13 in both populations [20,21]. However, this mutation alone cannot account for all cases of MBP deficiency, because MBP deficiency was also seen in some individuals found with the normal genotype (GGC). Another mutation associated with low MBP plasma concentrations at codon 57 in exon 1 in the MBP gene has been found in both West and East Africa, with allele frequencies of 0·26 and 0·23, respectively ([22], Madsen & Garred, manuscript in preparation). This allele causes an exchange of GGA (glycine) with GAA (glutamic acid).

In the present study we have investigated whether low plasma concentrations of MBP occur with increased frequency among children with rAOM and SOM. Moreover, we established the frequencies of the MBP alleles in these patient groups. Finally, we investigated the levels of MBP in mixed-saliva, nasopharyngeal secretions, and middle ear effusions.

SUBJECTS AND METHODS

Samples of EDTA-plasma, nasopharyngeal secretions, and middle ear effusions were obtained from children as previously described [23,24]. The children were classified into four groups according to their medical history and records, supplemented by otoscopy and tympanometry. None of the children in this study had acute illness, i.e. acute otitis media, rhinitis or temperature elevation at the time of sampling. Moreover, none of the children had received antibiotics during the 3 weeks before the investigation. In addition, at the time of sampling normal plasma levels of haptoglobin, orosomucoid and α1-antitrypsin were found in all children [25]. The children were Danish Caucasian, and were living in the area of Copenhagen, Denmark. They had been followed every 3–6 months by otoscopy and tympanometry during 1–2 years before they were admitted to hospital for insertion of middle-ear ventilating tubes and/or adenoidectomy. Episodes of acute purulent otitis media were in most cases diagnosed by either the general physician or an ENT specialist. Only occasionally were the middle ear effusions aspirated for culture. In those cases *Strep. pneumoniae* and *H. influenzae* were the most common pathogens cultured.

Group of otitis-prone children (OP group)
These were children having experienced more than five episodes of acute purulent otitis media during the preceding 1–2 years. Also, these children had had SOM 3–6 months before sampling.

Group of children with recurrence of acute otitis media (rAOM group)
These were children comparable to the OP group, but with less than five episodes of AOM 1–2 years before sampling.

Group of children with secretory otitis media (SOM group)
These were children who had SOM for at least 3–6 months before sampling. The children in this group were characterized by recurrence of common colds and hearing disability resulting from periodic accumulation of middle ear effusions. In contrast to the children in the OP and the rAOM groups, these children had no previous episodes of AOM according to the parents and the medical examinations performed during the 1–2 year observation period before sampling. The diagnosis of SOM was confirmed by myringotomy.

Group of healthy children
These were children hospitalized for operations to correct protruding ears, hernia, or phimosis. Additionally, samples were obtained from children who underwent adenoidectomy due to mechanical obstruction of the nasopharynx, resulting in snoring as the only symptom. None of these children had previous known episodes of AOM or SOM. All the children were examined by the same ENT specialist (C.H.S.) at the time of sampling. Normal otoscopy was verified in all cases. In some of the children the examination was supplemented by tympanometry.

Plasma samples
Plasma samples were obtained from 76 children (22 girls and 54 boys) aged 2–178 months (median 51·5 months). Of these children 24 belonged to the OP group (median age 48 months), 15 to the rAOM group (median age 63 months), 22 to the SOM group (median age 53 months) and 15 to the healthy group (median age 63 months).

Nasopharyngeal secretions
Nasopharyngeal secretions were obtained from 83 children (38 girls and 45 boys) aged 17–127 months (median 57·0 months). Of these children, 20 belonged to the OP group, 28 to the rAOM group, 29 to the SOM group, and six to the healthy group.

Middle ear effusions
Middle ear effusions were obtained from 73 children (25 girls and 48 boys) aged 8–156 months (median 54·0 months). Of these children 19 belonged to the OP group, 26 to the rAOM group, and 28 to the SOM group. Obviously, none of the children in the healthy group had middle ear effusions.

Mixed-saliva
Mixed-saliva was collected from 48 otherwise healthy 4-year-old children [26] and five healthy adults.

Assays
MBP concentrations were measured in a double enzyme immuno-assay (EIA) as described elsewhere [21]. Briefly, microtitre plates (Maxisorp, Nunc, Roskilde, Denmark) were coated with a mouse anti-MBP MoAb (clone 131-1, IgG1, kappa) kindly provided by Dr Claus Koch (Division of Immunology, State Serum Institute, Copenhagen, Denmark). Plasma samples were diluted in a Tris/HCl buffer containing EDTA and 0·05% Tween 20. A pool of EDTA plasma with a known concentration of MBP was used as a standard. Biotinylated clone 131-1 anti-MBP MoAb was used as the detector antibody. This is possible since MBP comprises up to 18 identical subunits. Finally, horseradish peroxidase-labelled streptavidin (Amersham, Aylesbury, UK) was added. The substrate was *o*-phenylene diamine hydrochloride in citrate-phosphate buffer, pH 5·0. The colour reaction was stopped by the addition of H_2SO_4. Optical density (OD) was read at 492 nm. Values are given as μg MBP/*l*. Parallel plates were coated with equivalent amounts of mouse IgG1 and processed as above. This was done to reveal the

binding of rheumatoid factors, anti-mouse immunoglobulins, and non-specific binding of MBP interfering in the system.

The albumin, IgA, IgD, IgE, IgG, IgM, secretory IgA, and secretory IgM results have previously been reported [7,24,27,28].

MBP genotyping by DNA analysis was performed on biopsies of nasopharyngeal tonsils collected from another group of 89 children, who, according to the above criteria belonged to one of the OP, rAOM, and SOM groups, respectively. Immediately after removal of the tissue, specimens were placed in cold PBS for 24 h before ethanol fixation and paraffin embedding [28]. The paraffin blocks were stored at 4°C until use. Paraffin-embedded tonsils were deparaffinized by repeated extractions with octane, and residual octane was removed by the addition of 100% ethanol followed by evaporation [29]. DNA was extracted from the tonsils according to a standard procedure including non-ionic detergent lysis of the cells and harvest of the nuclei [30]. The MBP exon 1 sequences were amplified utilizing the polymerase chain reaction (PCR). As primers a pair of oligonucleotides 5′-GTA GGA CAG AGG GCA TGC TC-3′ (upper strand) and 5′-CAG GCA GTT TCC TCT GGA AGG-3′ (lower strand) were used as previously described [19,20]. PCR was performed in the presence of 1·5 mM $MgCl_2$ in the following 40 cycles: 30 s at 94°C, 1 min at 60°C and 2 min at 72°C. This was preceded by a 4-min denaturation step at 94°C and followed by 5 min extension at 72°C. The PCR product was digested with the restriction enzymes *Ban*I and *Mbo*II (Promega, Madison, WI), recognizing 5′-G↓GP$_y$P$_u$CC-3′ and 5′-GAAGA(N)$_8$↓-3′, respectively. Subsequently restriction fragment length polymorphism analysis (RFLP) was performed [20]. As a molecular weight marker pBR322 (Boehringer, Mannheim, Germany) digested with *Hae*III (Amersham) was used. The *Ban*I restriction enzyme cleaves the normal MBP GGC-type PCR product into two fragments, but leaves the variant MBP GAC-type PCR product undigested. In contrast, the *Mbo*II restriction enzyme cleaves the African variant MBP GAA-type PCR product into two fragments and leaves the normal MBP GGA-type PCR product undigested. Alternatively, the PCR product was hybridized with allele sequence-specific oligonucleotide probes recognizing GGC (5′-GG CGT GAT GGC ACC AAG G-3′) and GAC (5′-GG CGT GAT GAC ACC AAG G-3′) in the presence of tetramethylammonium chloride [20].

Statistical analysis

Kruskal–Wallis, Mann–Whitney, Fisher's exact test and Spearman rank correlation were used for statistical analyses. Probability $P < 0.05$ was chosen as the level of significance (two-tailed).

RESULTS

Plasma

Levels of MBP in plasma samples from the four groups of children investigated are shown in Fig. 1. A wide range of MBP concentrations was found in the children tested ($n = 76$), ranging from 29 to 9658 μg/*l*. The median concentration was 1232 μg/*l* and the interquartile range was 420–3120 μg/*l*. No statistically significant difference was observed in MBP plasma concentrations between the study groups ($P > 0.30$). Neither was there any statistically significant difference in the number of the patients with low levels of MBP (< 250 μg/*l*) compared with the controls ($P = 1.0$). No significant correlation was observed between the levels of plasma MBP and the other plasma parameters investigated (albumin, IgA, IgE, IgD, IgG and IgM) ($P > 0.30$).

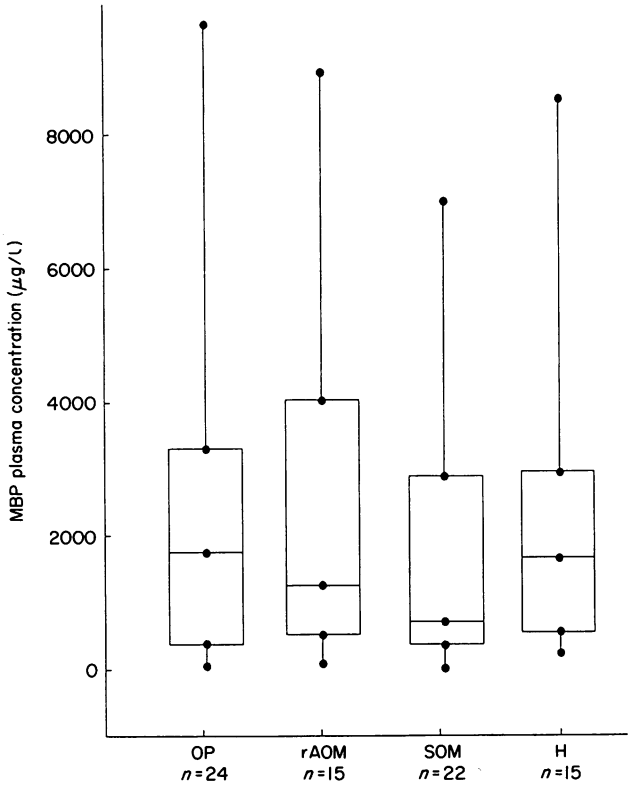

Fig. 1. Plasma concentrations of mannan-binding protein (MBP) in otitis-prone chldren (OP), children with less recurrence of acute otitis media (rAOM), children with secretory otitis media (SOM) and healthy children (H). Range, interquartile ranges (boxes) and medians are indicated.

Nasopharyngeal secretion

The concentration of MBP in nasopharyngeal secretions ($n = 83$) ranged from 0 to 414 μg/*l* (median, 10 μg/*l*; interquartile range, 0–18 μg/*l*). No statistically significant difference in the MBP concentrations was observed between the groups of children investigated ($P = 0.67$). A statistically significant positive correlation was observed between the levels of MBP and albumin ($n = 76$; $r_s = +0.22$; $P = 0.047$), between MBP and IgG ($n = 59$; $r_s = +0.26$; $P = 0.042$) and between levels of MBP and total IgM ($n = 81$; $r_s = +0.34$; $P = 0.002$). Furthermore, there was a statistically significant negative correlation between levels of MBP and the ratio of secretory IgA to total IgA ($n = 81$; $r_s = -0.23$; $P = 0.041$) and the ratio of secretory IgM to total IgM ($n = 81$; $r_s = -0.29$; $P = 0.007$). No statistically significant difference was observed with the other parameters investigated in nasopharyngeal secretions (secretory IgA and IgM, total IgA and IgD) ($P > 0.3$).

Middle ear effusions

The concentration of MBP in middle ear effusions ($n = 73$) ranged from 0 to 2150 μg/*l* (median, 250 μg/*l*; interquartile range, 50–650 μg/*l*). No statistically significant difference in the MBP concentration was observed between the groups of children investigated ($P = 0.87$). There were no statistically significant correlations between levels of MBP and those of

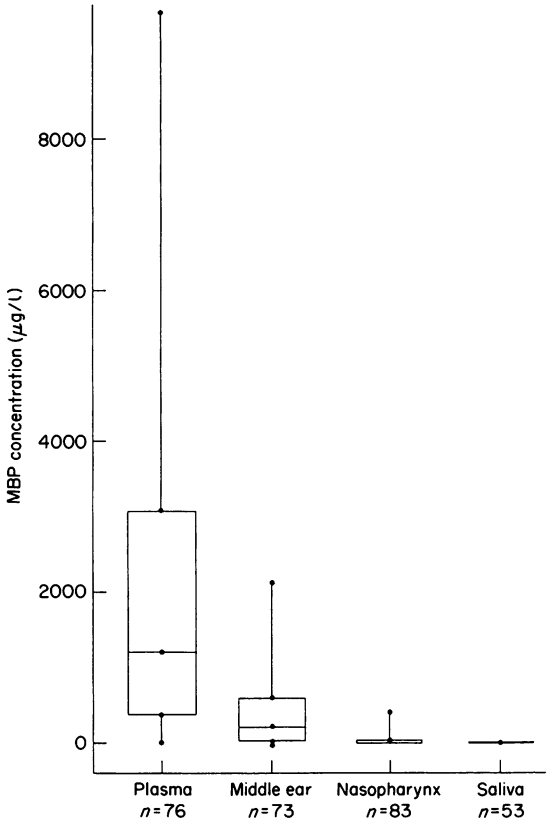

Fig. 2. The concentration of mannan-binding protein (MBP) in plasma, nasopharyngeal secretions, middle ear effusions and mixed-saliva. The MBP levels in the different compartments represent pooled data from the various groups examined. Range, interquartile ranges (boxes) and medians are indicated.

Table 1. Frequencies of mannan-binding protein genotypes in children with recurrent otitis media

	No. of individuals		
	Observed	Expected	χ^2
GGC/GGC	64 (71·9)	62·64	0·04
GGC/GAC	21 (23·5)	24·28	0·44
GAC/GAC	4 (4·5)	1·64	1·14
Total	89	89	1·62

Figures in parentheses are percentages. Expected numbers were calculated assuming Hardy–Weinberg equilibrium and using frequencies of 0·84 and 0·16 for the GGC and GAC alleles, respectively. The low χ^2 value of 1·62 with 1 degree of freedom agrees with the expected value.

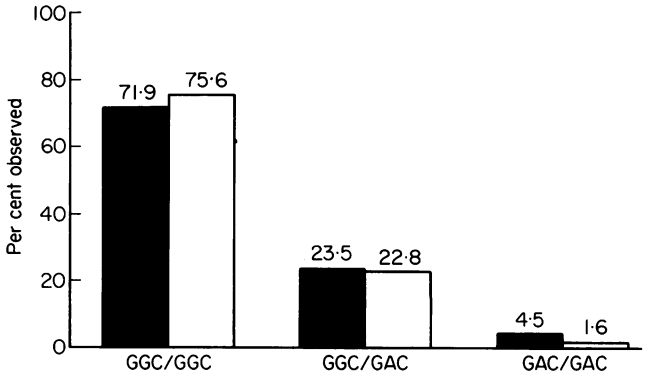

Fig. 3. Frequencies of mannan-binding protein genotypes in Danish children ($n=89$) compared with healthy Danish blood donors ($n=123$). The frequencies in the blood donors have previously been published [20]. ■, Otitis media; □, controls.

various immunoglobulins investigated in middle ear effusions (total IgA and IgM, secretory IgA and secretory IgM) ($P>0·30$).

Correlation and comparison of plasma, nasopharyngeal secretions and middle ear effusions

No statistically significant correlation was observed between plasma MBP and nasopharyngeal MBP concentrations ($n=13$; $r_s=+0·26$; $P=0·38$) or between plasma MBP and middle ear MBP ($n=19$; $r_s=+0·23$; $P=0·32$). A statistically significant positive correlation was observed between levels of MBP in nasopharyngeal secretions and MBP levels in middle ear effusions ($n=42$; $r_s=+0·47$; $P=0·0017$).

The concentration of MBP in the three compartments differed highly significantly ($P<0·0001$). The median MBP level in plasma was 3·9 times higher than in middle ear effusions and 175 times higher than in nasopharyngeal secretions. MBP was undetectable in samples of mixed-saliva (Fig. 2).

MBP genotypes

We investigated the frequency of the known MBP genotypes in DNA obtained from nasopharyngeal tonsils of children with recurrent otitis media (Table 1). Eighty-nine individuals were tested for polymorphism at codon 54 (GGC or GAC), encoded in exon 1 of the MBP gene; 64 were homozygous for the GGC allele (71·9%); 21 were heterozygous for the GAC allele (23·5%); and four were homozygous for the GAC allele (4·5%), respectively (Table 1). This gives a frequency of the GAC allele of 0·16, which is not significantly different from that of healthy Danish controls (0·13) ($P=0·65$) [20] (Fig. 3). None of the children expressed the GAA allele at codon 57, which has been described among black Africans (GGA replaced by GAA).

DISCUSSION

We have investigated a possible association between recurrence of otitis media and low concentrations of MBP. It has previously been shown that infection (especially otitis media) occurred significantly more frequently in 26 infants with a common opsonic defect (subsequently linked with low MBP levels) than in a matched control group [31]. In our study, however, no statistically significant differences in MBP concentrations were seen between patients and controls. Nor was any

increased frequency (0·16) found of the MBP allele associated with low plasma concentrations in Caucasians [19,20]. Moreover, the allele associated with low MBP concentrations in Africans was not found in this material. Thus, our results do not support the assumption that a low plasma concentration of MBP alone predisposes to otitis media in Caucasian children.

Several suggestions may be put forward to explain why our results differ from those of Richardson *et al.* [31]: (i) the larger sample size in our study; (ii) the studied populations may be different, i.e. the children in the present study may represent another group of patients who have sustained problems with the upper airways after infancy; (iii) the yeast opsonic deficiency may in some cases have causes other than low concentrations of MBP; (iv) different strains of the same bacteria with various MBP-binding capabilities may be involved in the pathogenesis of otitis media.

The latter argument is supported by a recent study of the binding patterns of MBP to various bacteria which suggests that encapsulated strains of *Neisseria meningitidis* serogroup B and *H. influenzae* type b only bind about 10% of the amount of MBP bound by their non-encapsulated counterparts (Emmerik *et al.*, submitted for publication). The results obtained with *N. meningitidis* are in accordance with the findings that serum levels of MBP do not differ from controls in survivors of meningococcal serogroup B and C disease [32]. Moreover, other groups of encapsulated bacteria including *Escherichia coli* K1, *Strep. suis*, *Strep. pneumoniae* and *N. meningitidis* serogroup A showed intermediate MBP binding capacity. Thus, it may be that MBP deficiency only becomes clinically relevant with a restricted number of pathogens, in spite of the presence of the relevant carbohydrates in the cell wall [33]. This should be taken into consideration when different clinical studies examining the role of MBP are compared. With respect to the pathogenesis of otitis media, several bacteria have been shown to be involved, including *Strep. pneumoniae*, non-encapsulated *H. influenzae* and *M. catarrhalis* [26]. It is, therefore, of interest to perform studies in children with recurrent upper-airways infections with defined microbial diagnosis, in whom both the binding specificity and avidity of MBP towards the relevant microorganisms have been determined.

We detected MBP in middle ear effusions (27% of plasma level) and in nasopharyngeal secretions (0·6% of plasma level). In contrast, MBP was not detected in mixed-saliva. Whether or not MBP participates in the mucosal immune system is not known. However, the presence of MBP in upper-airways secretions may indicate such a role, as has been suggested for other complement factors [34].

A sustained integrity of the epithelial barrier in the nasopharynx and middle ear cavity is maintained by a well functioning secretory immune system, mucociliary clearance, and antigen non-specific antimicrobial activity such as complement factors [5,26]. Occasionally in children, increased colonization of microorganisms gives rise to nasopharyngitis and/or otitis media. Because of mucosal leakage various amounts of plasma IgA, plasma IgM, secretory IgA and secretory IgM are found in nasopharyngeal secretions and middle ear effusions [23,24]. Therefore, to some extent, the ratio of, for example, secretory IgA/total IgA gives an impression of the mucosal integrity. Previously, we have found this ratio to be close and negatively correlated with the corresponding transudation index of albumin, indicating an increased transudation to the secretions of plasma IgA and other plasma proteins such as albumin during mucosal inflammation [24]. According to present knowledge, MBP is synthesized in the liver only [12], and the occurrence of this protein in nasopharyngeal secretions and middle ear effusions therefore indicates transudation from plasma. The positive correlation between albumin, IgG and MBP and the negative correlation between secretory IgA/total IgA, secretory IgM/total IgM and MBP, as found in this study, support the idea that MBP is transudated from plasma to external secretions. However, in contrast to the 1:10 median transudation index of albumin from plasma to nasopharyngeal secretions, as previously shown [24] the corresponding figure for MBP in the present study was calculated to be only 1:175. The marked differences in the molecular sizes of albumin and MBP may in part explain the poor transudation of MBP. The positive and close correlation between IgM and MBP in nasopharyngeal secretions supports this view. Moreover, we found a very close correlation between MBP levels in nasopharyngeal secretions and middle ear effusions. At present, it is not known whether these observations indicate some kind of additional active transport of MBP into the secretions, or represent a uniform inflammatory state and thereby MBP consumption in the nasopharynx and middle ear cavity.

This study does not support the assumption that low levels of MBP are associated with recurrence of otitis media. However, it does show that MBP is present in nasopharyngeal secretions and middle ear effusions, suggesting a role in local mucosal defence.

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REFERENCES

1 Henderson FW, Collier AM, Sanyal MA *et al.* A longitudinal study of respiratory viruses and bacteria in the etiology of acute otitis media with effusion. N Engl J Med 1982; **306**:1377–83.

2 Sørensen CH, Holm-Jensen S, Tos M. Middle ear effusion and risk factors. J Otolaryngol 1982; **11**:46–51.

3 Ingvarsson L, Lundgren K, Olofsson B, Wall S. Epidemiology of acute otitis media in children. Acta Otolaryngol (Stockh) 1982; **Suppl. 388**:1–52.

4 Sipilä M, Karma P, Pukander J, Timonen M, Kataja M. The Bayesian approach to evaluation of risk factor in acute and recurrent otitis media. Acta Otolaryngol (Stockh) 1988; **106**:94–101.

5 Bernstein JM, Prellner K, Rynell-Dagöö B, Sipilä P, Palva T. Otitis media: the immunological factor. In: Sadé J, ed. Acute and secretory otitis media. Amsterdam: Kugler, 1986:203–10.

6 Kamme C, Lundgren K, Mårdh P-A. The aetiology of acute otitis media in children. Scand J Infect Dis 1971; **3**:217–23.

7 Sørensen CH, Andersen LP, Tos M, Thomsen J, Holm-Jensen S. Nasopharyngeal bacteriology and secretory otitis media in young children. Acta Otolaryngol (Stockh) 1988; **105**:126–31.

8 Prellner K, Nilsson NI. Complement aberrations in serum from children with otitis due to *S. pneumonia* or *H. influenzae*. Acta Otolaryngol (Stockh) 1982; **94**:275–82.

9 Turner MW, Super M, Levinsky RJ, Summerfield JA. The molecular basis of a common defect in opsonization. In: Chapel HM, Levinsky RJ, Webster ADB, eds. Progress in immune deficiency III. London: Royal Society of Medicine Services, 1991:177–83.

10 Super M, Thiel S, Lu J, Levinsky RJ, Turner MW. Association of low levels of mannan-binding protein with a common defect in opsonization. Lancet 1989; **2**:1236–9.
11 Thiel S, Holmskov U, Hviid L, Laursen SB, Jensenius JC. The concentration of C-type lectin, mannan-binding protein, in human plasma increases during an acute phase response. Clin Exp Immunol 1992; **90**:31–35.
12 Ezekowitz RAB, Day LE, Herman GA. A human mannose-binding protein is an acute-phase reactant that shares sequence homology with other vertebrate lectins. J Exp Med 1988; **167**:1034–46.
13 Childs RA, Drickamer K, Kawasaki T, Thiel S, Mizuochi T, Feizi T. Neoglycolipids as probes of oligosaccharide recognition by recombinant and natural mannose-binding proteins of the rat and man. Biochem J 1989; **262:**131–8.
14 Thiel S, Reid KBM. Structures and functions associated with the group of mammalian lectins containing collagen sequences. FEBS Lett 1989; **250**:78–84.
15 Lu J, Thiel S, Wiedemann H, Timpl R, Reid KBM. Binding of the pentamer/hexamer forms of mannan-binding protein to zymosan activates the proenzyme $C1r_2C1s_2$ complex, of the classical pathway of complement, without involvement of C1q. J Immunol 1990; **144**:2287–94.
16 Matsushita M, Fujita T. Activation of the classical complement pathway by mannose-binding protein in association with a novel C1s-like serine protease. J Exp Med 1992; **176**:1497–503.
17 Kawasaki N, Kawasaki T, Yamashina I. A serum lectin (mannan-binding protein) has complement-dependent bactericidal activity. J Biochem (Tokyo) 1989; **106**:483–9.
18 Malhotra R, Thiel S, Reid KBM, Sim RB. Human leukocyte C1q receptor binds other soluble proteins with collagen domains. J Exp Med 1990; **172**:955–9.
19 Sumiya M, Super M, Tabona P *et al.* Molecular basis of opsonic defect in immunodeficient children. Lancet 1991; **337**:1569–70.
20 Garred P, Thiel S, Madsen HO, Ryder LP, Jensenius JC, Svejgaard A. Gene frequency and partial protein characterization of an allelic variant of mannan binding protein associated with low serum concentrations. Clin Exp Immunol 1992; **90**:517–21.
21 Garred P, Madsen HO, Kurtzhals JAL *et al.* Diallelic polymorphism may explain variations of blood concentration of mannan-binding protein in Eskimos, but not in black Africans. Eur J Immunogenetics 1992; **19**:403–12.
22 Lipscombe RJ, Sumiya M, Hill AVS *et al.* High frequencies in African and non-African populations of independent mutations in the mannose binding protein gene. Hum Mol Gen 1992; **1**:709–15.
23 Sørensen CH. Quantitative aspects of IgD and secretory immunoglobulins in middle ear effusions. Int J Ped Otolaryngol 1983; **6**:247–53.
24 Sørensen CH, Nielsen LK. Nasopharyngeal secretory immunoglobulins in children with recurrent acute otitis media and secretory otitis media. APMIS 1988; **96**:199–205.
25 Sørensen CH, Nielsen LK. Plasma IgG, IgG subclasses and acute-phase proteins in children with recurrent acute otitis media. APMIS 1988; **96**:676–80.
26 Sørensen CH. Quantitative aspects of the mucosal immunity and bacteriology of the nasopharynx and middle ear cavity. APMIS 1990; **98**(Suppl. 16): 1–41.
27 Sørensen CH, Brygge K. Microflora and mucosal immunology at the level of the Eustachian tube. ENT J;(in press).
28 Sørensen CH, Larsen PL. IgD in nasopharyngeal secretions and tonsils from otitis-prone children. Clin Exp Immunol 1988; **73**:149–54.
29 Wright DK, Manos MM. Sample preparation from paraffin-embedded tissues. In: Innis M, Gelfand D, Sninsky JJ, White TJ, eds. PCR protocols—a guide to methods and applications, 1st edn. San Diego: Academic Press, Inc., 1990:153–8.
30 Böhme J, Owerbach D, Denaro M, Lernmark Å, Peterson PA, Rask L. Human class II major histocompatibility antigen β-chains are derived from at least three loci. Nature 1983; **301**:82–84.
31 Richardson VF, Larcher VF, Price JF. A common congenital immunodeficiency predisposing to infection and atopy in infancy. Arch Dis Child 1983; **58**:799–802.
32 Garred P, Michaelsen TE, Bjune G, Thiel S, Svejgaard A. A low serum concentration of mannan-binding protein is not associated with serogroup B or C meningococcal disease. Scand J Immunol 1993; **37**:468–70.
33 Jantzen E, Bryn K, Bøvre K. Cellular monosaccharide patterns of Neisseriaceae. APMIS 1976; **84**:177–88.
34 Prellner K. Complement and other amplification mechanisms in otitis media. In: Bernstein J, Ogra P, eds. Immunology of the ear. New York: Raven Press, 1987:345–61.