

Ontogeny of human mannan-binding protein, a lectin of the innate immune system

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Mannan-binding protein (MBP) is a member of the collectins. These lectins are composed of polypeptide chains which contains a collagen-like region and a calcium dependent carbohydrate recognition domain (named a C-type CRD). MBP binds via the CRD to carbohydrate structures on microorganisms. MBP can activate the complement system when bound to carbohydrate. MBP is thus thought to play a role in the defence against microorganisms. The present report describes the ontogeny of MBP. The level of MBP increase during the first 3 months of life, at which time a stable level is reached, comparable to that seen in adults. On average, the level of MBP at term was 37% of the post 3-month level. Measurements on infants born prematurely showed a 3-fold increase in MBP concentration from the time of birth at around 30 weeks from conception to the 10th week of life.

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Mannan-binding protein (MBP) is a plasma protein which is thought to play an important part in the innate antimicrobial defence system of the body. MBP is a so-called collectin, i.e. a mosaic protein containing collagen-like regions and C-type lectin domains (review 1). The name of the protein is derived from the initial description based on its affinity for carbohydrate structures presented by mannan, a preparation of polysaccharides and glycoproteins from the yeast *Saccharomyces cerevisiae*. Further studies of the binding specificities of MBP have revealed a rather broad carbohydrate reactivity, with mannose and N-acetyl-D-glucosamine (GlcNAc) being particularly good ligands, which provide the molecule with the potential to bind to the carbohydrate structures found on many microorganisms. When bound to carbohydrate structures MBP activates the complement system in a manner mimicking complement component C1q, i.e. via activation of a protease complex (2, 3). The activation of the complement system causes the deposition of complement factors onto the microorganism. The bound complement factors are opsonizing (i.e. mediate phagocytosis) and also induce lysis of the microorganism. MBP thus has the potential of killing invasive microorganisms in cooperation with other components of the innate immune system.

A reduced ability to opsonize *Saccharomyces cerevisiae* was observed in about a quarter of sera from infants with frequent, unexplained infections (4). MBP was measured in serum samples from 10 children with the opsonization defect, and all were found to have low levels of MBP (5). All other immunological parameters measured were normal. Purified MBP could correct the defect *in vitro*. It is, however, noteworthy that the infectious agents causing the observed frequent infections in infants lacking MBP are as yet ill defined. The opsonic potential of sera towards *Candida albicans*, *Staphylococcus aureus* and *Escherichia coli*, correlates with that seen against yeast. This reflects the widespread occurrence of mannose and GlcNAc on microorganisms and suggest that the defect is relevant in relation to many microorganisms.

A wide range of MBP concentrations in blood is found (from < 10 ng/ml to 10 µg/ml). In each individual the concentration is stable except for increases during acute phase reactions (6). Sequence analysis of the gene for MBP has revealed certain point mutations in the exon encoding the collagenous region. The mutations are associated with low levels of MBP (7). The gene frequency of the Caucasian mutation is 13% in a sample of the Danish population and the African mutation is found in 25%

of the individuals of a sample of black Africans (8, 9). The levels of MBP in plasma are strongly associated with the mutations, but other factors must also play a role, since some individuals homozygous for the normal gene show absence of MBP (8, 10).

In infants being 6–18 months of age passively transferred maternal antibodies have disappeared and the acquired specific immune system is not yet fully developed. MBP appears to be a potent mediator of innate immunity and it thus appears of obvious interest to investigate the ontogeny of MBP during the first year of life.

Material and methods

Study population

In the present report we followed the concentration of MBP in blood samples withdrawn from 40 mature infants at the day of birth as umbilical cord blood, and as venous blood at 3 months, 6 months, 9 months and 12 months of age. Cord blood samples were also obtained from 10 infants born prematurely (ranging from 26 to 33 week of gestation) and capillary blood was taken 10 weeks later. At the time of blood withdrawal all children were without signs of infection. Blood was collected into tubes containing EDTA, centrifuged within 1 h of sampling, and the plasma was stored at -70°C until analysis. The investigation was carried out in accordance with the Helsinki declaration with informed consent from the parents.

Antibodies

Two rabbits were immunized with 25 μg mannan-binding protein in complete Freud's adjuvant, and boosted twice with 20 μg mannan-binding protein in incomplete Freud's adjuvant. The serum was stored at 4°C with 7.5 mM NaN_3 . The antibody used for coating was made from one of the rabbits whereas the second, biotinylated antibody was made from the other rabbit. Both immunoglobulin preparations were purified on a protein-A-Sepharose column (Pharmacia, Uppsala, Sweden) following the procedures described by the manufactures. The two antibody preparations were on Western blots of a crude mannan-binding protein preparation found to react only with a 32 kDa band representing mannan-binding protein. The biotinylation of the secondary antibody was performed as described by Guesdon et al. (11).

Mannan-binding protein assay

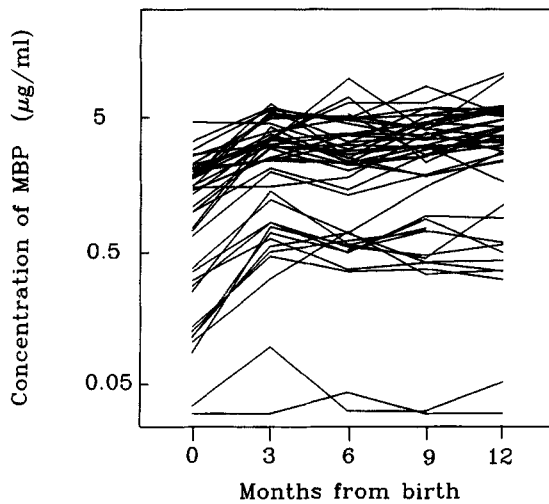
Mannan-binding protein was measured by a sandwich ELISA using polyclonal antibody (6). 1) Microtiter wells (Maxisorp, Nunc, Kamstrup, Den-

mark) were coated with immunoglobulins by incubating 0.1 μg rabbit anti-MBP antibody or 0.1 $\mu\text{g}/\text{ml}$ normal rabbit IgG (Code X903, DAKO, Glostrup, Denmark) in 100 μl coating buffer (15 mM Na_2CO_3 , 35 mM NaHCO_3 , 0.02% NaN_3 , pH 9.6) in each well overnight at room temperature in a humid chamber. The wells were washed 3 times with TBS/Tween (10 mM Tris-base, 150 mM NaCl, pH 7.4 (TBS) containing 0.05% Tween 20) and if not used immediately they were stored with 200 μl TBS in each well. 2) Samples to be tested were diluted in TBS/Tween/EDTA (TBS/Tween containing 5 mM EDTA) and 100 μl were added to each well. Serum or plasma samples were tested in duplicate at three dilutions (1/25, 1/250 and 1/800) in wells coated with rabbit anti-MBP and in wells coated with normal rabbit IgG. 3) Following a 3 h incubation at room temperature in a humid chamber the wells were washed three times with TBS/Tween. Then 100 μl of biotinylated rabbit anti-mannan-binding protein diluted in TBS/Tween was added to each well (at a 1000 dilution of the stock containing approx. 1 mg IgG/ml TBS). 4) Following an overnight incubation at 4°C in a humid chamber the wells were washed three times with TBS/Tween. Then 100 μl of alkaline-phosphatase-conjugated avidin diluted in TBS/Tween was added (at a 3000 dilution of the stock solution containing approx. 0.25 mg alkaline-phosphatase-conjugated avidin per ml (Sigma No. A-2527)). 5) Following 2 h of incubation the wells were washed and 100 μl of p-nitro-phenyl-phosphate (1 mg/ml in 10% diethanolamine, 0.1 mg/ml MgCl_2 , 0.02% NaN_3 , pH 9.8) was added to each well. 6) The optical density at 405 nm of the wells was monitored, after approx 3 h incubation at 37°C in a humid chamber, using an ELISA reader. For the simultaneous construction of a standard curve, dilutions of one normal serum with known MBP content were used in all assays. OD readings obtained in wells coated with normal rabbit IgG were subtracted from the values obtained in the anti-MBP-coated wells to compensate for the presence of rheumatoid factors and anti-rabbit IgG antibodies in the samples. The same concentration of MBP was found in serum and EDTA plasma samples withdrawn at the same time from a number of individuals. The sensitivity of this assay, defined as the concentration of purified MBP giving a signal 2 standard deviations above background levels, was 10 ng MBP/ml. The inter-assay coefficient of variation (CV) was 6%.

Statistical methods

For statistical purposes Wilcoxon matched-pairs signed-ranks test was used to compare the data from the samples taken at different times.

1 A.



1 B.

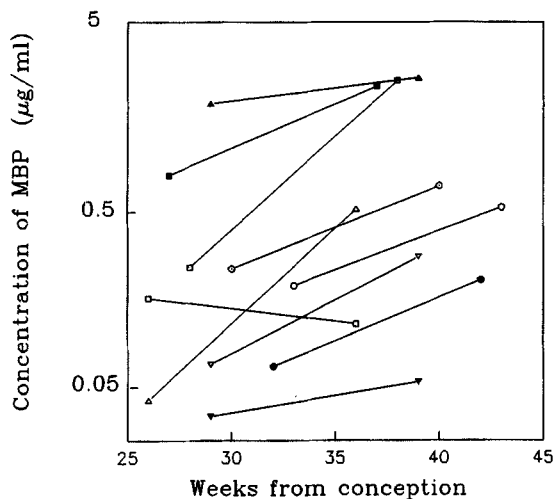


Fig. 1 A. The concentrations of MBP in the plasma from 41 mature infants. Each line indicate an individual infant. Mean values at birth: 1.3 $\mu\text{g/ml}$ (SD 0.9 $\mu\text{g/ml}$), at 3 months: 2.6 $\mu\text{g/ml}$ (SD 1.6 $\mu\text{g/ml}$), at 6 months: 2.5 $\mu\text{g/ml}$ (SD 1.9 $\mu\text{g/ml}$), at 9 months: 2.6 $\mu\text{g/ml}$ (SD 1.8 $\mu\text{g/ml}$) and at 12 months: 3.1 $\mu\text{g/ml}$ (SD 2.3 $\mu\text{g/ml}$).

Fig. 1 B. The concentration of MBP in serum from preterm infants at the day of delivery and 10 weeks later. Each symbol indicates an individual infant.

Results and discussion

The results of MBP analysis on blood samples from mature infants are shown in Fig. 1 A. The level of MBP increased during the first 3 month of life, after which there was no further statistically significant increase ($p < 0.001$ for cord blood compared with 3-month samples; $p > 0.1$ for 3-month versus 6-, 9- or 12-month). On average, the level of individual MBP concentrations at term was 37% $\pm$ 11% of the post 3-month levels.

The study of preterm infants (Fig. 1 B.) show an increase of the MBP level (starting at a lower level of MBP as compared to the mature infants) during their first 10 weeks of life. The level of MBP at Week 40 from conception (normal term) seem to be lower than in the mature infants cord blood sample, although this needs to be confirmed with a more suitable set of samples covering this time of life of preterm infants.

MBP is synthesized by hepatocytes and the ontogeny found in the present report is equivalent to what has been reported for other proteins synthesized by the liver. The complement factors with which MBP cooperate are also present at birth (12). The period after the 3rd month up to 18 months of age represent the time when the maternally transferred antibodies have been catabolized and the child's own specific immunosystem has not yet matured. Since MBP appears to be an important component of the anti-microbial defence system the presence of MBP at this time of life could play a significant role for the combat of infections. The lower levels of MBP in preterm infants, even at a gestational age of 40 weeks, adds to the list of factors potentially resulting in an immature microbial defence system in these children, findings that are concordant with preterm infants being especially susceptible to infections. An increased susceptibility to invasive microorganisms due to lack of MBP is likely to be manifest only until the maturation of the immune response.

The presence of MBP in functionally significant amounts already at birth strengthen the hypothesis that MBP plays a role in the innate immune defence. It should be realized that this supposition is still circumstantial as experiments aimed at demonstrating the *in vivo* efficacy of MBP are lacking.

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References

1. THIEL S. Mannan-binding protein, a complement activating animal lectin. *Immunopharm* 1992; 24: 91-99.
2. IKEDA K, SANNOH T, KAWASAKI N, KAWASAKI T, YAMASHINA I. Serum lectin with known structure activates complement through the classical pathway. *J Biol Chem* 1987; 262: 7451-7454.
3. LU JH, THIEL S, WIEDEMANN H, TIMPL R, REID KBM. Binding of the pentamer/hexamer forms of mannan-binding protein to zymosan activates the proenzyme C1r2C1s2 complex, of the classical pathway of complement, without involvement of C1q. *J Immunol* 1990; 144: 2287-2294.
4. SOOTHILL JF, HARVEY BA. Defective opsonization. A common immunity deficiency. *Arch Dis Child* 1976; 51: 91-99.

5. SUPER M, THIEL S, LU J, LEVINSKY RJ, TURNER MW. Association of low levels of mannan-binding protein with a common defect of opsonisation. *Lancet* 1989; 2: 1236–1239.
6. THIEL S, HOLMSKOV U, HVIID L, LAURSEN SB, JENSENIUS JC. The concentration of the C-type lectin, mannan-binding protein, in human plasma increases during an acute phase response. *Clin Exp Immunol* 1992; 90: 31–35.
7. SUMIYA M, SUPER M, TABONA P, et al. Molecular basis of opsonic defect in immunodeficient children. *Lancet* 1991; 337: 1569–1570.
8. GARRED P, THIEL S, MADSEN HO, et al. Gene frequency and partial protein characterization of an allelic variant of mannan binding protein associated with serum concentrations. *Clin Exp Immunol* 1992; 90: 517–521.
9. 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 Molec Gen* 1992; 1: 253–258.
10. MADSEN HO, GARRED P, KURTZHALS JA, LAMM LU, RYDER LP, THIEL S, SVEJGAARD A. A new Frequent allele is the missing link in the structural polymorphism of the human mannau-binding protein. *Immunogenetics* 1994; 40: 37–44.
11. GUESDON JL, TERNYNCK T, AVREAMEAS S. The use of avidin-biotin interaction in immunoenzymatic techniques. *J Histochem Cytochem* 1979; 27: 1131–1141.
12. GEELLEN SP, FLEER A, BEZEMER AC, GERARDS LJ, RIJKERS GT, VERHOEF J. Deficiencies in opsonic defence to pneumococci in the human newborn despite adequate levels of complement and specific IgG antibodies. *Pediatr Res* 1990; 27: 514–518.