Review

www.advmolmed.com
doi:10.2399/amm.07.165

Advances in
Molecular Medicine
International Journal of Molecular Biology, Biochemistry and Gene Technology

Mannose-binding lectin in obstetrics

Hakan Ozan*

Department of Obstetrics and Gynecology, Uludag University Medical Faculty, Bursa, Turkey

Pregnancy is a unique situation in which a fetus with paternal alloantigens can survive in a maternal environment in a state of chronic inflammation due to placentation with trophoblastic invasion and remodeling of spiral arteries. Mannose-binding lectin (MBL) plays a role in complement activation through the lectin pathway, complement-independent opsonophagocytosis, modulation of inflammation, recognition of altered self structures, removal of immune complexes, and apoptotic cell clearance. There are insufficient and conflicting data about the association between MBL and gestational complications such as miscarriage, preterm delivery, and preeclampsia. Though most reports specify the genetic expression or concentration of MBL during pregnancy, there are speculations rather than proofs regarding its role in immunopathogenesis.

Key words: Mannose-binding lectin, pregnancy, miscarriage, preterm delivery, preeclampsia

Adv Mol Med 2007; 3(4): 165-170

MBL is one of the important factors involved in the innate immune system, and it was initially isolated from and is mainly produced by the liver.[1] Experimental data showed that MBL is also synthesized by human monocytes and monocyte-derived dendritic cells in vitro.[2] At birth, the MBL level is approximately 2/3 of the adult MBL level and it increases to the adult level in a month; it remains stable thereafter, with a minor decline in elderly.[3] MBL recognizes and binds specific structures such as sugar groups, phospholipids, nucleic acids, and non-glycosylated proteins.[4] It plays a role in complement activation through the lectin pathway, complement-independent opsonophagocytosis, modulation of inflammation, recognition of altered self-structures, removal of immune complexes, and apoptotic cell clearance.[5-8] As a host defense mechanism, MBL activates not only the classical complement pathway, but also the antibody- and C1-independent MBL pathway. MBL circulates along with MBL-associated serine protease-2 (MASP-2), and upon binding to microorganisms, autoactivation of MASP-2 promotes the cleavage of C4 and C2 to generate C3 convertase.[5,9] On the other hand, the MBL pathway requires the activation of ficolins.[5,10]

Structurally, the MBL protein is a member of the collectin family and contains both collagenous regions and lectin domains.[5,11,12] There are 2 human MBL genes, namely, a pseudogene MBL-1 and a protein encoding gene MBL-2.[5] The functional MBL-2

*Correspondence to: Hakan Ozan, MD, Assoc. Prof
İbrahimpaşa Mah, İnanç Sok,
Özpirinç Apt, A Blok, No: 20/5,
Bursa 16010 Turkey
Phone: + 90 224 224 38 15
Fax: + 90 224 224 13 94
e-mail: hozan@superonline.com
Accepted: December 24, 2007, Published online: June 7, 2008

gene is located on chromosome 10 and consists of 4 exons. In 1991, Sumiya et al, identified the complete nucleotide sequence of these 4 exons.[13] Exon 1 harbors the following 3 point mutations: Gly54 Asp, termed as variant B; Gly57Glu, variant C; and Arg52Cys, variant D.[13–15] The wild type has been named variant A. Variant B has been identified in 25% of Eurasian people and is inherited as an autosomal dominant mutation.[5] Variant C is rare in Eurasians but is seen in 50–60% of the sub-Saharan people. The polymorphisms of the regulatory elements on the promoter region of the gene also cause variations in MBL levels up to 1000 fold, among healthy individuals. Since it is an acute-phase reactant, MBL levels exhibit a 1.5–3 fold increase during acute inflammation despite inter-individual variations.[16]

Pregnancy is a unique situation in which a fetus with paternal alloantigens can survive in a maternal environment. Trophoblast invasion and remodeling of the maternal spiral arteries are necessary in the construction of the feto-maternal interface. Extending chorionic villi coated with syncitiotrophoblasts, float freely within sinuses filled with maternal blood to maintain exchange transport of nutrients to the fetus.[17] This brave exposure of paternal antigens to the maternal immune system activates the complement system, and complement components physiologically accumulate in the placenta.[18,19] This immune process is restrained by the development of immune tolerance.

In 2007, van de Geijn et al, studied the MBL levels during pregnancy in a longitudinal study.[20] Blood was taken at 12, 20, and 30 weeks of gestation and at 6 and 12 weeks and between 6–9 months postpartum from 32 healthy pregnant women without adverse obstetric history. The authors assumed that the women completely recovered from the pregnancy-induced changes at 6–9 months postpartum, and the MBL level at this time point was considered to be the baseline value. They found that the serum MBL level increased to 140% compared to the baseline. The increase in MBL level was apparent at 12 weeks of gestation, and this level did not increase significantly thereafter during pregnancy. Its concentration dropped sharply to 57% of the baseline value at 6 weeks postpartum. MBL levels showed major interindividual variations, but all exhibited the same pattern during the study. When genotyping was performed, baseline serum MBL levels in women with the wild-type allele were found to be significantly higher compared to the levels in those with variant alleles. The increase in serum MBL levels during pregnancy was also significantly associated with specific genotypes as follows: 177% with AA-genotype, 126% with AO-genotype, and 117% with OO-genotype. The magnitudes of the postpartum drops in MBL levels were significant; a 55% drop occurred in the AA-genotype, 60% in the AO-genotype, and 65% in the OO-genotype. When MBL-MASP complex activity was examined, a significant increase in MBL-MASP complex activity of up to 172% was noted during pregnancy compared to that at the baseline; further, a sharp 70% postpartum drop was observed. The MBL pathway activity significantly increased during pregnancy to 160% in case of the AA-genotype and 171% in case of the AO-genotype compared to baseline. However, the MBL pathway activity did not show significant early postpartum decline in any of the genotypes. Moreover, a significant correlation was observed between the MBL serum level, MBL-MASP complex activity, and MBL pathway activity.

Increased MBL levels were hypothesized to be a compensatory response for the prevention of infections during pregnancy because the adaptive immune system has already been suppressed to enable the mother to tolerate the fetus as a semi-allograft. MBL was also considered to be necessary in the removal of apoptotic cells appearing during placentation. It was hypothesized that the placental growth hormone could stimulate MBL production because such an induction was reported in case of the human growth hormone in vitro and in non-pregnant women.[21,22]

Recurrent miscarriage (RM) is defined as 3 consecutive pregnancy losses occurring before 24 weeks of gestation and it affects 0.5–1.0% of couples.[23,24] Detection of MBL on the endothelium of the spiral arteries aroused suspicion regarding its

potential role in miscarriages.[25] In 1995, Kilpatrick et al, evaluated serum MBL levels in blood donors and found significantly low levels in 16% (21 out of 135) of female partners and 14% (15 out of 108) of male partners in couples with RM compared to <5% in case of obstetrically normal controls.[26] Subsequently, they reported their findings from 218 females and 179 male partners who had experienced recurrent miscarriage, and 376 blood donors were used as controls.[27] They evaluated 3 cut-off levels. The lower the cut-off value, the stronger was the correlation between MBL deficiency and RM. In case of female patients with MBL <0.01, <0.05 and <0.06 μg/mL the proportions were 9.2, 12.8, and 33.5%, respectively. The corresponding proportions in case of their male partners were comparable, ie, 8.4, 12.8, and 35.8%. They concluded that, low levels of MBL are over-represented in both male and female partners of couples experiencing RM and MBL value ≤0.1 mg/mL should be considered a clinically significant risk factor for spontaneous abortion. In 1999, Christiansen et al, confirmed the results of the previous study. MBL deficiency was evaluated in 146 Danish women with RM and 41 of their husbands along with 49 Scottish RM women and 41 of their husbands.[28] The frequency of MBL deficiency was higher in both Danish and Scottish females compared to the controls, but the difference was only statistically significant when the groups were combined; this might indicate the different levels of genomic expression in different populations. However, the frequency of MBL deficiency in women with exactly 3 miscarriages was 9% in the combined group, and the difference from the corresponding frequency in the controls was not significant. In the Danish cohort, there was a significant increase in the frequency of low MBL (26%) in those with at least 5 miscarriages and even a higher increase (56%) among those with at least 7 miscarriages. The authors concluded that the highly significant correlation between the frequency of MBL deficiency and the number of the miscarriages was compatible with the proposed causal relationship between these 2 factors. Two years later, Baxter et al, reported their data on RM, and did not ascertain any MBL-2 genotype differences between groups with or without RM.[29] They studied both the structural and promoter MBL variants in 76 couples with RM and 69 couples without RM. Contrary to previous studies, there was no significant difference between the groups in mean number of structural variant genes or the frequency of low, medium, or high MBL level haplotypes in both partners. The frequency of very low MBL level haplotypes, codon 52 and 54 mutations, the proportion of patients homozygous for structural variant alleles, and couples with >2 structural variant alleles were comparable in the study and control groups. Kruse et al, was the first to investigate a different aspect of MBL deficiency in RM patients.[30] They observed that the prevalence of low MBL levels was significantly increased in women with RM irrespective of a cut-off level of 50 ng/mL or 100 ng/mL. The prevalence of low MBL levels increased significantly with the number of miscarriages. The median birth weight tended to be lower in women with MBL levels of <100 ng/mL compared to women with normal MBL levels (p=0.06); however, the difference between the groups was significant when infants born at >37 weeks of gestation were considered alone (p=0.04). Authors, who established the association between MBL and RM, implicated sub-clinical infections in MBL deficient fetus and mother, hypothetically. In addition to the role of infections, Kruse et al.,[30] hypothesized that impaired immune complex elimination interferes with placental functions, thereby resulting in decreased birth weight in case of MBL deficiency.

Delivery that occurs before 37 completed weeks of gestation is defined as a preterm delivery and occurs at the rate of 5–13%.[31,32] This is one of the major problems in obstetrics and is responsible for 75% of perinatal mortality and >50% of long-term morbidity.[33] The hypothesis that inflammation might be important in the ethiopathogenesis of preterm delivery led to the evaluation of inflammatory mediators in such patients.[34] In 2006, Bodamer et al, investigated the association between MBL-2 gene polymorphisms and preterm birth.[35] A study of 204 randomly selected archived dried blood filter

cards of infants born prematurely and at term was conducted. Heterozygous and homozygous carriers of the codon 52 variant (D-allele) genotype were significantly more frequent in the preterm group compared to the term group, but the frequency in case of codons 54 and 57 was not significant. Moreover, they found a significant underrepresentation of the LYA/LYA genotype (promoter-550 C/C) carriers in the preterm group. Recently, van de Geijn et al, studied 157 uncomplicated singleton gestations of nulliparous women and did not confirm the results of the previous study.[36] Based on the genotypes, individuals were categorized into the following 3 groups: the high MBL genotype group A associated with high MBL serum levels ((H/L)YA/(H/L)YA and (H/L)YA/LXA), the intermediate MBL genotype group B associated with intermediate MBL serum levels (LXA/LXA and (H/L)YA/O), and the low MBL genotype group C associated with MBL deficiency and hence the lowest MBL serum levels (LXA/O and O/O). The mean gestational ages of the groups at delivery were 274 days, 283 days, and 284 days, respectively. Twelve of the 14 preterm births (86%) were within the high MBL genotype group A, ie, 13.6% of the deliveries in MBL genotype group A versus 2.9% of those in MBL genotype groups B and C together were preterm. MBL genotype groups were significantly associated with the length of gestational age at delivery and high MBL genotype group had a significantly shorter gestational age than the other two MBL groups, which were comparable in between. Birth weights were 3163, 3423, and 3454 g in groups A, B, and C, respectively. Conversely, no significant difference in gestational age was observed between the groups when the analysis was restricted to women that delivered at term. All neonates (n=5) that were born before 34 weeks of gestation were delivered by women with MBL genotype group A. Bodamer et al, hypothesized that codon 52 variant carrier fetuses with MBL deficiency are prone to infections and born preterm. On the contrary, van de Geijn et al., implicated high MBL levels as causing preterm delivery by aggravating the existing local or systemic inflammatory medical conditions.

One of the most important factors causing preterm delivery is preeclampsia, which is characterized by hypertension (>140/90 mmHg) and proteinuria (>0.3 g/day) in the second half of pregnancy. It is seen in 5–7% of pregnancies and leads to complications such as preterm birth, intrauterine growth retardation (IUGR) and even death.[37–39] Maternal complications are renal and hepatic failure, cerebral edema and seizures, HELLP (Hemolysis, Elevated Liver enzymes, and Low Platelet count) syndrome; and rarely death. Roberts and Gammill proposed that preeclampsia is a two-stage disorder;[40] the first step is defective trophoblast invasion and spiral artery remodelling that causes decreased placental perfusion ending with hypoxia, and the second step is the multisystemic maternal involvement. Local oxygen tension and immune-mediated interactions are the main determinants of pathogenesis with marked deposition of early and late complement components.[41–43] Disequilibrium between oxidants and antioxidant capacity in collaboration with inappropriate immune responses, increases placental apoptosis in preeclamptic patients.[44] Since an abnormal immune response is suspected to be the most important factor in preeclampsia ethiopathogenesis, MBL has been the subject of interest in 2 studies in 2007. Sziller et al, evaluated the MB codon 54 gene polymorphism in 51 preeclampsia and 35 HELLP syndrome patients.[45] The frequency of the homozygosity and carriage of the wild type A allele among women with preeclampsia (86.3% and 92.2%, respectively) and HELLP syndrome (84.0% and 91.4%, respectively) were significantly higher compared to non-preeclamptic full-term controls (71.2% and 84.8%, respectively). Moreover, heterozygous carriage of the variant B allele was significantly more prevalent among healthy controls (27.2%) compared to patients with preeclampsia (11.8%) and HELLP syndrome (14.8%). It was also significantly more prevalent when compared to IUGR cases of mothers with preeclampsia (4.5%) or HELLP syndrome (11.7%). MBL codon 54 genotyping of the neonates of the 3 groups were comparable in terms of genotype distribution and allelic frequencies. They concluded that maternal but not

fetal heterozygosity at codon 54 of the MBL gene had a protective function against development of preeclampsia and HELLP syndrome. Carriage of the variant B allele was also protective against the development of IUGR in women with both preeclampsia and HELLP syndrome. The authors hypothesized that high MBL expression activates the complement system and causes the destruction of trophoblast cells and leads to insufficient invasion of the spiral arteries at the feto-maternal interface. The subsequent hypoxia initiates the sequence of events progressing to preeclampsia. Maternal carriage of the variant-B allele with decreased MBL levels would prevent this clinical occurance. On the other hand, van de Geijn et al,, genotyped 157 preeclampsia patients and 157 uncomplicated pregnancies for the presence of wild type A allele; variants B, C, and D; and 2 haplotypes [-550 (H/L) and -221 (X/Y)] of the MBL-2 gene.[46] They did not confirm the previous results and found that the MBL genotypes not associated with preeclampsia.

Pregnancy can be considered as a state of chronic inflammation.[47] Placentation with trophoblastic invasion and remodeling of spiral arteries should be accompanied by inflammatory reactions to some extent. Destruction of existing architecture and formation of new structures within the uterine tissue last throughout the whole gestation. MBL expression or its concentration may be important because suboptimal or supraoptimal levels may either suppress or aggravate inflammatory reactions and hence cause complications during pregnancy. The description of the accurate role of MBL in pregnancy is rather important because it can be replaced in MBL deficient women or suppressed in patients with high levels of MBL.[48] Further studies on larger populations with different ethnicities might contribute to the clarification of the subject.

References

1. Summerfield JA, Taylor ME. 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* 1986; 883: 197-206.
2. Downing I, Koch C, Kilpatrick DC. Immature dendritic cells possess a sugar-sensitive receptor for human mannan-binding lectin. *Immunol* 2003; 109: 360-4.
3. Ip WK, To YF, Cheng SK, Lau YL. Serum mannose-binding lectin levels and mbl2 gene polymorphisms in different age and gender groups of southern Chinese adults. *Scand J Immunol* 2004; 59: 310-4.
4. Palaniyar N, Nadesalingam J, Clark H, Shih MJ, Dodds AW, Reid KB. Nucleic acid is a novel ligand for innate, immune pattern recognition collectins surfactant proteins A and D and mannose-binding lectin. *J Biol Chem* 2004; 279: 32728-36.
5. Dommett RM, Klein N, Turner MW. Mannose-binding lectin in innnate immunity: past, present and future. *Tissue Antigens* 2006; 68: 193-209.
6. Ogden CA, deCathelineau A, Hoffmann PR, et al. C1q and mannose binding lectin engagement of cell surface calreticulin and CD91 initiates macropinocytosis and uptake of apoptotic cells. *J Exp Med* 2001; 194: 781-95.
7. Neth O, Jack DL, Dodds AW, Holzel H, Klein NJ, Turner MW. Mannose-binding lectin binds to a range of clinically relevant microorganisms and promotes complement deposition. *Infect Immun* 2000; 68: 688-93.
8. Turner MW. The role of mannose-binding lectin in health and disease. *Mol Immunol* 2003; 40: 423-9.
9. Feinberg H, Uitdehaag JC, Davies JM, Wallis R, Drickamer K, Weis WI. Crystal structure of the CUB1-EGF-CUB2 region of mannose-binding protein associated serine protease-2. *EMBO J* 2003; 22: 2348-59.
10. Liu Y, Endo Y, Iwaki D, et al. Human M-ficolin is a secretory protein that activates the lectin complement pathway. *J Immunol* 2005; 175: 3150-6.
11. Thiel S, Frederiksen PD, Jensenius JC. Clinical manifestations of mannan-binding lectin deficiency. *Mol Immunol* 2006; 43: 86-96.
12. Worthley DL, Bardy PG, Mullighan CG. Mannose-binding lectin: biology and clinical implications. *Intern Med J* 2005; 35: 548-55.
13. Sumiya M, Super M, Tabona P, et al. Molecular basis of opsonic defect in immunodeficient children. *Lancet* 1991; 337: 1569-70.
14. Lipscombe RJ, Sumiya M, Hill AV, et al. High frequencies in African and non-African populations of independent mutations in the mannose binding protein gene. *Hum Mol Genet* 1992; 1: 709-15.
15. Madsen HO, Garred P, Kurtzhals JA, et al. A new frequent allele is the missing link in the structural polymorphism of the human mannan-binding protein. *Immunogenetics* 1994; 40: 37-44
16. 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: 31-5.
17. Redman CW, Sacks GP, Sargent IL. Pre-eclampsia: an excessive maternal response to pregnancy. *Am J Obstet Gynecol* 1999; 180: 499-506.

18. Faulk WP, Jarret R, Keane M, Johnson PM, Boackle RJ. Immunological studies of human placentae: complement components in immature and mature chorionic villi. *Clin Exp Immunol* 1980; 40: 299-305.
19. Tedesco F, Radillo O, Candussi G, Nazzaro A, Mollnes TE, Pecorari D. Immunohistochemical detection of terminal complement complex and S protein in normal and pre-eclamptic placentae. *Clin Exp Immunol* 1990; 80: 236-40.
20. van de Geijn FE, Roos A, de Man YA, et al. Mannose-binding lectin levels during pregnancy: a longitudinal study. *Hum Reprod* 2007; 22: 362-71.
21. Sorensen CM, Hansen TK, Steffensen R, Jensenius JC, Thiel S. Hormonal regulation of mannan-binding lectin synthesis in hepatocytes. *Clin Exp Immunol* 2006; 145: 173-82.
22. Hansen TK. Growth hormone and mannan-binding lectin: emerging evidence for hormonal regulation of humoral innate immunity. *Minerva Endocrinol* 2003; 28: 75-84.
23. Exalto N. Recurrent miscarriage. *Int Congress Series* 2005; 1279: 247-50.
24. Stirrat GM. Recurrent miscarriage. *Lancet* 1990; 336: 673-5.
25. Bulla R, Bossi F, Agostini C, Tedesco F. The multiple functions of the complement system in pregnancy. *Am J Reprod Immunol* 2006; 56: 24-5.
26. Kilpatrick DC, Bevan BH, Liston WA. Association between mannan binding protein deficiency and recurrent miscarriage. *Mol Hum Reprod* 1995; 1: 2501-5.
27. Kilpatrick DC, Starrs L, Moore S, Souter V, Liston WA. Mannan binding lectin concentration and risk of miscarriage. *Hum Reprod* 1999; 14: 2379-80.
28. Christiansen OB, Kilpatrick DC, Souter V, Varming K, Thiel S, Jensenius JC. Mannan-binding lectin deficiency is associated with unexplained recurrent miscarriage. *Scand J Immunol* 1999; 49: 193-6.
29. Baxter N, Sumiya M, Cheng S, et al. Recurrent miscarriage and variant alleles of mannose binding lectin, tumor necrosis factor and lymphotoxin a genes. *Clin Exp Immunol* 2001; 126: 529-34.
30. Kruse C, Rosgaard A, Steffensen R, Varming K, Jensenius JC, Christiansen OB. Low serum level of mannan-binding lectin is a determinant for pregnancy outcome in women with recurrent spontaneous abortion. *Am J Obstet Gynecol* 2002; 187: 1313-20.
31. Goldenberg RL, Culhane JF, Iams JD, Romero R. Epidemiology and causes of preterm birth. *Lancet* 2008; 371: 75-84.
32. Slattery MM, Morrison JJ. Preterm delivery. *Lancet* 2002; 360: 1489-97.
33. McCormick, MC. The contribution of low birth weight to infant mortality and childhood morbidity. *N Engl J Med* 1985; 312: 82-90.
34. Lin D, Moss K, Beck JD, Hefti A., Offenbacher S. Persistently high levels of periodontal pathogens associated with preterm pregnancy outcome. *J Periodontol* 2007; 78: 833-41.
35. Bodamer OA, Mitterer G, Maurer W, Pollak A, Mueller MW, Schmidt WM. Evidence for an association between mannose-binding lectin 2 (MBL2) gene polymorphisms and pre-term birth. *Genet Med* 2006; 8: 518-24.
36. van de Geijn FE, Dolhain RJ, van Rijs W, Willemsen SP, Hazes JM, de Groot CJ. Mannose-binding lectin genotypes are associated with shorter gestational age An evolutionary advantage of low MBL production genotypes? *Mol Immunol* 2008; 45: 1514-8.
37. Sibai BM, Caritis S, Hauth J. What we have learned about preeclampsia. *Semin Perinatol* 2003; 27: 239-46.
38. Walker JJ. Pre-eclampsia. *Lancet* 2000; 356: 1260-5.
39. Zhang J, Meikle S, Trumble A. Severe maternal morbidity associated with hypertensive disorders in pregnancy in the United States. *Hypertens Pregnancy* 2003; 22: 203-12.
40. Roberts JM, Gammill HS. Preeclampsia: recent insights. *Hypertension* 2005; 46: 1243-9.
41. Hung T-H, Skepper JN, Charnock-Jones DS, Burton GJ. Hypoxia-reoxygenation: a potent inducer of apoptotic changes in the human placenta and possible etiological factor in preeclampsia. *Circ Res* 2002; 90: 1274-81.
42. Saito S, Shiozaki A, Nakashima A, Sakai M, SasakiY. The role of the immune system in preeclampsia. *Mol Aspects Med* 2007; 28: 192-209.
43. Girardi G, Bulla R, Salmon JE, Tedesco F. The complement system in the pathophysiology of pregnancy. *Mol Immunol* 2006; 43: 68-77
44. Allaire AD, Ballenger KA, Wells SR, McMahon MJ, Lessey BA. Placental apoptosis in preeclampsia. *Obstet Gynecol* 2000; 96: 271-6.
45. Sziller I, Babula O, Hupuczi P, et al. Mannose-binding lectin (MBL) codon 54 gene polymorphism protects against development of pre-eclampsia, HELLP syndrome and pre-eclampsia-associated intrauterine growth restriction. *Mol Hum Reprod* 2007; 13: 281-5.
46. Van de Geijn FE, Dolhain RJEM, van Rijs W, Hazes JMW, de Groot CJM. Mannose-binding lectin genotypes and pre-eclampsia: A case-control study. *Hum Immunol* 2007; 68: 888-93.
47. Sargent IL, Borzychowski AM, Redman CW. NK cells and human pregnancy-an inflammatory view. *Trends Immunol* 2006; 27: 399-404
48. Valdimarsson H, Vikingsdottir T, Bang P, et al. Human plasma-derived mannose-binding lectin: a phase I safety and pharmacokinetic study. *Scand J Immunol* 2004; 59: 97-102.