Mannose-binding lectin (MBL) codon 54 gene polymorphism protects against development of pre-eclampsia, HELLP syndrome and pre-eclampsia-associated intrauterine growth restriction

I.Sziller¹, O.Babula², P.Hupuczi¹, B.Nagy¹, B.Rigó¹, G.Szabó¹, Z.Papp¹, I.M.Linhares² and S.S.Witkin²

¹First Department of Obstetrics and Gynecology, Semmelweis University, Faculty of Medicine, Budapest, Hungary and ²Division of Immunology and Infectious Diseases, Department of Obstetrics and Gynecology, Weill Medical College of Cornell University, New York, NY, USA

Insufficient invasion of the spiral arteries by trophoblast cells is associated with the etiology of pre-eclampsia, the syndrome of hemolysis, elevated liver enzymes and low platelet counts (HELLP) and pre-eclampsia-associated intrauterine growth restriction (IUGR). Mannose-binding lectin (MBL) is a component of the innate immune system. MBL-mediated activation of the complement cascade is an important event in the destruction of invading trophoblasts. The gene coding for MBL is polymorphic, and variant alleles result in greatly reduced circulating MBL levels. The aim of this study was to test the association between an MBL polymorphism and pre-eclampsia, HELLP syndrome and IUGR. DNA was extracted from buccal swabs of 51 women with pre-eclampsia, 81 women with HELLP syndrome and 184 healthy pregnant controls. Aliquots were tested for a single nucleotide MBL gene polymorphism at codon 54 by PCR and endonuclease digestion. Homozygosity for the wild-type allele was more frequent in patients with pre-eclampsia (P = 0.04) and HELLP syndrome (P = 0.02) when compared with controls. The presence of the variant allele was more prevalent among controls than in women with pre-eclampsia (P = 0.02) or HELLP syndrome (P = 0.028). Twenty-two (55%) patients with pre-eclampsia and 43 (53%) women with HELLP syndrome delivered an IUGR neonate. MBL-54 heterozygosity was more frequent in controls (27.2%) than in pre-eclamptic women (4.5%, P = 0.025) and those with HELLP syndrome (11.7%, P = 0.05) who delivered an IUGR neonate. Genotype frequencies of neonates born to mothers in all study groups were similar. Carriage of the MBL codon 54 polymorphism protects against pre-eclampsia, HELLP syndrome and IUGR and implies that an MBL-mediated event might be involved in the pathogenesis of these disorders.

Key words: gene polymorphism/HELLP syndrome/intrauterine growth restriction/mannose-binding lectin/pre-eclampsia

Introduction

Pre-eclampsia occurs in up to 5% of all reported deliveries and is the most frequent medical complication of the second half of pregnancy, labour and the early post-partum period (Feinberg, 2006). Recent observations provided convincing evidence that pre-eclampsia is not a single disease but a spectrum of clinical presentations spanning from a mild hypertension and proteinuria with a eutrophic fetus close to term to early onset maternal systemic disturbances including hemolysis, elevated liver enzymes and low platelet counts (HELLP syndrome) (Vatten and Skjåerven, 2004; Redman and Sargent, 2005). Most cases of early onset pre-eclampsia are characterized by reduced uteroplacental blood flow, reduced fetal nutrition and intrauterine growth restriction (IUGR) (Roberts and Cooper, 2001). Pre-eclampsia and, specifically, HELLP syndrome substantially increase both maternal and neonatal morbidity and mortality (Sibai et al., 1986; Martin et al., 1991).

Despite intensive research, there is no general agreement of the mechanism(s) leading to the development of these syndromes. Uteroplacental ischaemia, endothelial cell dysfunction, excessive maternal inflammatory response to deported trophoblast and insufficient trophoblast invasion of the spiral arteries have been proposed as possible causes (Redman et al., 1999; Duckitt and Harrington, 2005; Venkatessa et al., 2006; Fekete et al., 2006).

Mannose-binding lectin (MBL) is a constituent of the innate immune system. MBL binding to carbohydrate moieties on microorganisms and altered cells leads to activation of the complement system via the lectin pathway (Neth et al., 2000). In addition to complement activation, the protein has several distinct functions including promotion of complement-independent opsono-phagocytosis, modulation of inflammation and promotion of apoptosis (Turner, 2003). Of these functions, activation of the complement has been studied the most extensively.

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The MBL gene (mbl2) is located on chromosome 10 and is polymorphic at codons 52, 54 and 57 (Hansen and Holmskov, 1998). The presence of variant alleles at codon 54 has been associated with decreased functional levels of MBL in the circulation and in the vagina (Madsen et al., 1995; Babula et al., 2004). The persistence of variant MBL alleles at relatively high frequency suggests that carriage may confer a relative advantage to the host under some environmental conditions. Whereas a genetically related MBL deficiency increases susceptibility to microbial infections (Super et al., 1989), low levels of MBL reduces the amount of complement on the surface of intracellular pathogens, which exploits complement and complement receptors to invade and replicate in host cells (Garred et al., 1992).

MBL is present on the endothelium of the spiral arteries and particularly localized in vessels containing endovascular trophoblast (Bulla et al., 2006). We hypothesize that a decreased capacity for MBL-mediated complement activation may also be advantageous in limiting complement-mediated destruction of semi-allogeneic fetal cells during pregnancy and, thereby, altering the likelihood of developing pre-eclampsia and HELLP syndrome. The aim of the present study was to test whether an MBL-dependent mechanism plays a role in the etiology of pre-eclampsia and HELLP syndrome.

Material and methods
Between September 2003 and January 2006, consecutive Hungarian pregnant women with pre-eclampsia (n = 51) and HELLP syndrome (n = 35) who delivered at Semmelweis University and their neonates were enrolled in the study. An additional 46 currently non-pregnant women, who had delivered at the same department between January 1998 and August 2003 and had been diagnosed as having HELLP syndrome during their pregnancy by the same criteria as the consecutive pregnant women with HELLP syndrome, were also enrolled and tested. These non-pregnant women (n = 46) were recruited from among attendants of the ‘HELLP syndrome clinic’ of the department that had been organized to offer medical follow-up for those women who had delivered as HELLP patients at the department from January 1995. The total number of patients with HELLP syndrome in this study was 81.

The pregnant control group consisted of healthy full-term pregnant women who delivered a full-term eutrophic neonate at the same department on the same day as a pregnant woman either with pre-eclampsia or HELLP syndrome. Enrollment into the study of a pregnant woman with pre-eclampsia or HELLP syndrome and her neonate was followed by the enrollment of at least one subsequent full-term pregnant woman and her neonate who delivered in the same hospital on the same day. Enrollment of the 46 non-pregnant outpatients with a history of HELLP syndrome who attended for their annual medical follow-up was also followed by the enrollment of one full-term pregnant woman and her neonate without pre-eclampsia or HELLP syndrome who delivered in the same hospital on the same day. The total number of the pregnant control group was 184.

Since the frequency of the MBL gene polymorphisms might vary with ethnicity, only white ethnic Hungarian natives were enrolled. Each participant gave informed consent, and the study was approved by the Ethical Committee of the Semmelweis University.

The diagnosis of pre-eclampsia was made by the attending physician on the basis of a blood pressure of 140/90 mmHg or more after the 20th week of gestation and proteinuria of at least 300 mg 24 h\(^{-1}\) in a collected urine sample or ++ on dipstick urine analysis.

HELLP syndrome was diagnosed by the presence of thrombocytopenia (\(<150\,000\,\text{cells}\,\mu\text{l}^{-1}\)), evidence of hepatic dysfunction (increased aspartate aminotransferase level of \(>70\,\text{IU}\,\text{l}^{-1}\), increased alanine aminotransferase level of \(>70\,\text{IU}\,\text{l}^{-1}\), or both, with increased lactate dehydrogenase level of \(>600\,\text{IU}\,\text{l}^{-1}\)) and evidence of hemolysis (increased serum-bilirubin level of \(>1.2\,\text{mg}\,\text{dl}^{-1}\) and increased lactate dehydrogenase level of \(>600\,\text{IU}\,\text{l}^{-1}\)).

The diagnosis of IUGR was made if the birth weight of the neonate was below the 10th percentile value for his/her given gestational age compared with a growth chart for Hungarian male and female neonates (Papp et al., 1991).

Cells from the buccal mucosa of mothers and neonates were obtained by rotating a cotton swab against the inside of the cheek. Specimens were collected within 1 h after delivery and stored at 4 °C until DNA isolation. In 46 non-pregnant women with a history of HELLP syndrome, buccal sampling was performed by the same method, but only maternal samples were available for testing. Those performing the gene polymorphism testing were blinded to all clinical data.

DNA was extracted by suspending the cells in a 1% solution of the non-ionic detergent, Brij 35, in Tris buffer containing 5 mg ml\(^{-1}\) proteinase K, followed by incubation at 56°C for 60 min. Proteinase K was subsequently inactivated by incubation at 95°C for 10 min. The extracts were diluted 1:5 in 10 mmol l\(^{-1}\) Tris–HCl that contained 1.5 mmol l\(^{-1}\) MgCl\(_2\), 50 mmol l\(^{-1}\) KCl, 0.2 mmol l\(^{-1}\) each of dATP, dCTP, dGTP and TTP, 1.25 units of Taq DNA polymerase and 30 pmol of oligonucleotide primers that amplified the codon 54 polymorphic region of the MBL gene (Madsen et al., 1994; Babula et al., 2004). The final volume was 50 μl. Samples were incubated in a thermal cycler (PTC-100 Programmable Thermal Cycler, MJ Research Inc., Waltham, MA, USA) for 2 min at 94°C, followed by 35 cycles of 94°C for 50 s, 58°C for 1.5 min and 72°C for 15 s, and then by a final incubation at 72°C for 5 min.

The PCR amplicons were digested with Ban I endonuclease (New England BioLabs, Beverly, MA, USA) by incubation at 37°C for 18 h. Fragments were analysed on 2% agarose gels and stained with ethidium bromide. Ban I digestion resulted in either formation of two 260 and 89 base pair fractions (wild type, allele A) or a single uncut 349 base pair fraction (variant, allele B). Duplicate analysis of a subset of samples always yielded identical results.

Parameters with continuous distribution were compared using Student’s t-test. Genotype and allele frequencies were determined by direct counting and then divided by the number of chromosomes to obtain allele frequency and by the number of women to obtain genotype frequency. Associations between MBL genotypes and presence of pre-eclampsia or HELLP syndrome were tested by the χ\(^2\)-test or Fisher exact test if appropriate. Goodness of fit to Hardy–Weinberg equilibrium was determined by comparing the expected genotype frequencies with the observed values, using the χ\(^2\)-test (Chakravarti, 1999). A P-value < 0.05 was considered significant. In addition, odds ratios and 95% confidence intervals were calculated.

Results
During the study period, a total of 51 pregnant women with pre-eclampsia and 81 women with HELLP syndrome (cases), as well as 184 healthy full-term pregnant women (controls) were enrolled and tested for MBL genotype at codon 54.

As seen in Table I, demographic characteristics of cases and controls were comparable with regard to their mean age and parity. In contrast, mean birth weights of neonates born to mothers with pre-eclampsia and HELLP syndrome were 1134 g and 1808 g less than those born to controls (P < 0.01). Moreover, mean gestational age of pregnant women with pre-eclampsia and HELLP syndrome was 49.5 and 6.7 weeks less than that of neonates born to controls (P < 0.01). Frequency of Cesarean delivery was significantly higher in patients with pre-eclampsia (68.6%) or HELLP syndrome (95.1%) than in the control (20.7%) group (P < 0.01).

Table II summarizes the distribution of maternal MBL codon 54 genotypes in healthy pregnant women and those with pre-eclampsia. Frequency of the carriage of the wild-type A allele among women with pre-eclampsia was significantly higher compared with non-pre eclamptic full-term controls (P = 0.04). Moreover, heterozygous carriage of the variant B allele was significantly more prevalent among healthy controls (P = 0.02). The genotype distribution of women with pre-eclampsia and controls were in Hardy–Weinberg equilibrium (P > 0.05).

Similar differences were seen in the distribution of MBL-54 genotypes between women with HELLP syndrome and healthy controls (Table III). The frequency of homozygosity for the wild type of
The MBL-54 gene was significantly higher among patients with HELLP syndrome (84%) when compared with controls (71.2%), whereas heterozygous carriage of the variant B allele was higher among healthy full-term controls (27.2%) than pregnant women with HELLP syndrome (14.8%) (P = 0.02). The presence of variant allele B was associated with healthy full-term pregnancy when compared with HELLP syndrome (P = 0.04). A similar but non-significant tendency was seen between controls and pre-eclamptic women as well.

Twenty-two (43.1%) of 51 pregnant women with pre-eclampsia and 43 (53.1%) of 81 women with HELLP syndrome delivered a neonate with IUGR. The distribution of maternal MBL-54 genotype in pre-eclamptic women and those with HELLP syndrome was summarized in Table IV. Heterozygosity for the variant B allele was 27.2% in healthy full-term pregnant women as opposed to 4.5% of pre-eclamptic women with IUGR (P = 0.025) and 11.7% of women with HELLP syndrome and IUGR (P = 0.05).

MBL-54 genotype testing was performed in 270 neonates of whom 51 were born to mothers with pre-eclampsia, 35 to women with HELLP syndrome and 184 to healthy pregnant women. Neonates born to mothers with HELLP syndrome before 2003 were not considered to be tested for MBL-54 genotype. The genotype distribution and allele frequencies in the three subgroups were similar (Table V) and in Hardy–Weinberg equilibrium.

### Discussion

In the present study, maternal but not neonatal heterozygosity at codon 54 of the MBL gene was protective against development of pre-eclampsia and HELLP syndrome, and homozygosity for the wild type allele A of the MBL-54 gene was more frequent among patients with pre-eclampsia and HELLP syndrome. Carriage of the variant B allele was also protective against development of IUGR in women with both pre-eclampsia and HELLP syndrome.

Pre-eclampsia and its related disorders, HELLP syndrome and intrauterine growth restriction, are pregnancy specific conditions with no recognized counterpart in non-pregnant women. Although pre-eclampsia presents clinically in the second half of pregnancy, in many cases it has its origins at the time of implantation. Indeed, complement components, extravillous trophoblast cells infiltrate the maternal spiral arteries supplying the placental bed. Remodelling of spiral arteries results in the formation of high-capacity sinuses that provide optimal space for the exchange of gases and nutrients between mother and fetus (Redman and Sargent, 2005; Feinberg, 2006). Chorionic villi that float into the maternal blood are covered by fetal trophoblast cells and, thus, they are directly exposed to the maternal immune system. Expression of maternal antigens on these fetal cells may induce activation of the maternal complement cascade (Faulk et al., 1980; Tedesco et al., 1990). Remodelling of the spiral arteries has also been shown to promote complement activation in first trimester placentas (Girardi et al., 2006). Indeed, complement components are present on fetal cells in the placenta and decidual spiral arteries.
during normal pregnancy and increased deposition of early and late complement components have been identified in placentas from women with pre-eclampsia (Sinha et al., 1984; Tedesco et al., 1990). We hypothesize that complement activation via the MBL pathway contributes to the destruction of trophoblast cells at the maternal–fetal interface. Excessive MBL-mediated trophoblast damage would increase the possibility of insufficient invasion of the spiral arteries at the time of implantation. The subsequent hypoxia initiates the sequence of events culminating in pre-eclampsia. Carriage of the variant MBL B allele by the mother would result in decreased MBL levels and, consequently, with a decreased capacity for complement-dependent lysis of trophoblast cells via the MBL pathway.

Carriage of the MBL-54 B variant has been reported in 22–28% of European and North American populations, which is in accordance with the rate found in healthy pregnant women in our study. The preservation of a relatively high prevalence of this variant allele in the population suggests that carriage of the variant B allele may confer a biological advantage in certain environments (Garred et al., 1992). A reduced risk for development of pre-eclampsia, HELLP syndrome and the associated IUGR may be one such situation.

The trigger(s) for an exaggerated MBL-mediated, complement-directed anti-trophoblast response leading to pre-eclampsia in some women who are homozygous for the wild-type A allele remains to be determined. However, our observations are apparently inconsistent with an infection-related triggering mechanism. The B allele at codon 54 is associated with increased susceptibility to infection (Super et al., 1989). Thus, if microorganisms were responsible for complement activation that resulted in destruction of trophoblast by a bystander mechanism, we would have expected to find the opposite results, i.e., an association between allele B carriage and pre-eclampsia. Altered carbohydrate expression on the trophoblast cell surface due to genetically determined and/or diet or lifestyle-induced atypical enzyme expression or deposition from lysed host or microbial cells may contribute to the deleterious MBL-mediated immune response. Further studies are needed to differentiate between these possibilities.

A single nucleotide polymorphism in the gene coding for an inducer of the complement system is probably not the single underlying cause for the relative protection against pre-eclampsia, HELLP syndrome or the associated IUGR. This is also evident by the 12–15% frequency of patients with pre-eclampsia and HELLP syndrome who were heterozygotes but developed these disorders. Focusing on the genetic regulation of a single protein in a complex mechanism involving multiple inducers, inhibitors and regulators for the production and deposition of complement and complement complexes might lead to incomplete results.

There were some limitations to the present study. Polymorphism in the MBL gene (mb12) in codons 52 and 57 of exon 1 were not examined, because polymorphism in the former codon is reported with low frequencies in European populations, whereas mutation in the latter is characteristic of sub-Saharan African populations (Madsen et al., 1994; Turner, 2003). Moreover, our study did not test single nucleotide polymorphisms in the promoter region of the MBL gene (−550 and −221 positions) that have also been associated with variable levels of circulating MBL (Leung et al., 2006; Bodamer et al., 2006). Finally, the role of other covariates and the interaction of these polymorphisms with other maternal risk factors were not considered.

Epidemiological studies have consistently demonstrated a familial predisposition to pre-eclampsia leading to intensive genetic research in this field (Chappel and Morgan, 2006). The endeavour to identify susceptibility genes or genome-wide associations is aimed at a better understanding of the biological processes leading to pre-eclampsia. Further studies on larger numbers and in other populations might contribute to further understanding of the association between pre-eclampsia, HELLP syndrome and genetic variations of the MBL system.

References


Submitted on December 13, 2006; resubmitted on January 9, 2007; accepted on January 12, 2007