High Rate of Early Restenosis After Carotid Eversion Endarterectomy in Homozygous Carriers of the Normal Mannose-Binding Lectin Genotype

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Background and Purpose—Mannose-binding lectin (MBL) is thought to influence the pathophysiology of cardiovascular disease by decreasing the risk of advanced atherosclerosis and by contributing to enhanced ischemia reperfusion injury. Thus, we investigated the role of MBL in restenosis after eversion endarterectomy in patients with severe carotid atherosclerosis.

Methods—In a prospective study, 123 patients who underwent carotid endarterectomy were followed-up by carotid duplex scan (CDS) sonography for 14 months. In a retrospective study, we examined 17 patients and 29 patients, respectively, who had or had not at least 50% restenosis 29 months after carotid eversion endarterectomy. MBL genotypes were analyzed by a polymerase chain reaction-based method, and MBL serum concentrations were measured.

Results—In the prospective study in the patients homozygous for the normal MBL genotype, CDS values were significantly higher after 14 months of follow-up compared with the values measured 6 weeks after surgery (P<0.001). In contrast, only a slight increase was registered in patients carrying MBL variant alleles. The differences were much more pronounced in female than in male patients. Similar differences were observed when patients with high and low MBL serum concentrations were compared. In the retrospective study, a significant increase in the frequency of MBL variant genotypes was observed in patients not experiencing restenosis compared with the patients with restenosis (P=0.007).

Conclusions—These results indicate that reoccurrence of stenosis after carotid endarterectomy is partially genetically determined and imply that MBL contributes significantly to the pathophysiology of this condition. (Stroke. 2005;36:944-948.)

Key Words: atherosclerosis ■ cardiovascular disease ■ carotid endarterectomy ■ carotid stenosis ■ complement ■ genetics ■ ischemia ■ mannose-binding lectin ■ MBL2 ■ reperfusion injury

Microsurgical carotid eversion endarterectomy is a procedure in which plaque material is removed from inside of the carotid artery to avoid a primary or recurrent cerebral attack. The beneficial effect of carotid eversion endarterectomy for carotid stenosis is well-documented. However, restenosis is common after carotid eversion endarterectomy, and it may often appear quite rapidly after the operation. According to a meta-analysis, the risk of potentially clinically significant restenosis was 10% in the first year, 3% in the second year, 2% in the third, and 1% thereafter. When a carotid stenosis reaches a maximum of >50% of the vascular diameter, it typically progresses and re-operation may become necessary.

Early restenosis, defined as disease presenting within 24 months of the initial operation, is thought to be secondary to the proliferation of medial smooth muscle cells, leading to myointimal hyperplastic lesions, whereas late disease, presenting after 24 months of the initial operation, is frequently secondary to renewed atherosclerosis. Mannose-binding lectin (MBL) is a liver-derived serum protein of importance for innate immunity. On binding to a ligand, MBL may activate the lectin pathway of complement via the MBL-associated serine protease 2. Human MBL is encoded from a single gene (MBL2) on chromosome 10. Three single-base substitutions in exon 1 of the MBL2 gene independently cause low serum levels of MBL: at codon 54.
(glycine to aspartic acid, allele B), at codon 57 (glycine to glutamic acid, allele C), and finally at codon 52 (arginine to cysteine, allele D). The common designation for these variant alleles is O, whereas the normal allele has been named A. Each of the 3 variants reduces the amount of functional MBL subunits in heterozygous individuals 5- to 10-fold.

Recently, a dual role of MBL in cardiovascular disease has been suggested because it has been shown that lack of functional alleles is associated with severe coronary artery disease and/or increased carotid plaque formation. Moreover, it has been shown that MBL deficiency may amplify the putative effect *Chlamydia pneumoniae* may have on coronary artery disease. However, experimental data and clinical observations suggest that MBL and the lectin pathway of complement may initiate the inflammatory reaction seen in relation to ischemia reperfusion injury.

Based on these findings that indicate probable involvement of the MBL system in pathological processes leading to restenosis, we investigated a possible association of MBL alleles with restenosis in patients undergoing carotid eversion endarterectomy.

### Materials and Methods

#### Subjects

Group 1 included patients examined in a prospective study. A total of 123 patients (80 men, 43 women, 63.0 ± 9.4 years old) with severe (mean: 80 ± 14%) stenosis of carotid artery who underwent eversion type carotid endarterectomy between October 2000 and March 2003 were included and followed-up.

Group 2 included patients examined in a cross-sectional study. Seventeen patients (10 men, 7 women, 66.7 ± 2.1 years old) who underwent operation by eversion-type carotid endarterectomy 29 months (range, 14 to 85 months) before and had an at least 50% restenosis measured by carotid color duplex scan (CDS) sonography measurements. Twenty-nine patients were controls (22 men, 7 women, 67.5 ± 2.3 years old) who underwent the same operation without detectable restenosis matched with the patients experiencing restenosis by the length of follow-up time, age, gender, and serum lipid levels.

#### Surgery and Follow-up of the Patients During the Prospective Study

The study protocol was approved by the Institutional Review Committee at Semmelweis University, and the subjects gave informed consent. After detailed medical examination, careful medical history was taken. All consecutive patients who underwent eversion endarterectomy at the Department of Vascular and Cardiac Surgery were included in the study. Indication for carotid eversion endarterectomy was in accordance with American Heart Association guidelines. The operation and the clinical follow-up were performed as described previously in accordance with American Heart Association guidelines.

#### Measurement of MBL Serum Concentration

MBL serum concentration was measured in a double-sandwich enzyme-linked immunosorbent assay as previously described in samples obtained preoperatively. This assay preferentially detects higher-order oligomerized MBL (detection limit, 20 μg/L) and is closely associated with the function of the MBL activation pathway of complement.

#### Statistical Analyses

Statistical analyses were made using GraphPad Prism V 3.0 for Windows software package (GraphPad Software). Several group comparisons were performed with the Kruskall–Wallis 2-way ANOVA or nonparametric repeated measures ANOVA (Friedman) tests. Comparison of categorical variables was performed with the χ² test for trend test.

### Results

#### MBL Genotypes and Serum Concentrations

We found that the MBL genotypes correlated highly significantly with the MBL serum concentrations (Kruskall–Wallis *P* < 0.0001) (Figure 1). Median MBL serum concentration at baseline was 2016 μg/L (interquartile range, 1192 to 3560) in patients with the A/A genotype (n = 76), 336 (range, 106–800) for patients with the A/O (n = 41) genotype and below...
Evidence for a Role of MBL in Restenosis After Carotid Endarterectomy

After removal of plaques by endarterectomy, the carotid CDS values decreased to 0% in 100 of 123 patients (controlled ±6 weeks after the operation). After that period, a fraction of the patients experienced restenosis. During the 14-month follow-up period, 10% to 19% restenosis was observed in 6 patients, 20 to 39% in 13 patients, 40% to 49% restenosis in 10 patients, whereas restenosis equal to or exceeding 50% was recorded in 16 patients. There was no significant difference (P=0.532) in the restenosis rate between patients operated by different surgeons (n=7).

According to an analysis by nonparametric ANOVA followed by a post hoc test, in A/A homozygous carriers an increase in the CDS values could be detected already 7 months after endarterectomy (P<0.05), and it progressed until the end of the follow-up period (P<0.001). By contrast, the increase in the CDS values became weakly significant (P<0.05) only at the end of the follow-up period in the group of the patients who were heterozygotes (A/O) or homozygous for MBL variant genotypes (O/O) (Figure 2). Testing for MBL regulatory variants did not provide additional information.

Gender has been shown to be a significant parameter in reoccurrence of stenosis after carotid eversion endarterectomy, and when gender-related and MBL genotype-related effects were considered together, significant (P=0.0038) differences between male and female patients in the CDS sonography values were found only in patients with the normal (A/A) genotype (Figure 3A). The data were evaluated by 2-way ANOVA test. The analysis showed that females carrying the normal MBL A/A genotype increased significantly in CDS values already at 7 months (P<0.01) and further at 14 months (P<0.001), whereas in male patients the increase in the CDS values became significant (P<0.05) only at 14 months after surgery. By contrast, no significant CDS increase was seen either in male or in female patients carrying MBL variant genotypes (A/O + O/O) (Figure 3B).

MBL variant alleles have been shown to modulate the effect C pneumoniae may have on coronary artery disease. In this cohort, no correlation between the corrected CDS values measured either at 7 months or at 14 months after endarterectomy and seropositivity for C pneumoniae was observed (data not shown), nor did the presence of MBL variant alleles influence this observation.

Strong Correlation Between MBL Serum Concentration and Restenosis

Changes in CDS values according to MBL serum concentration are shown in Figure 3C and 3D. The data were evaluated by 2-way ANOVA test. Analysis revealed that female carriers...
Distribution of Carriers of Normal and Variant MBL Genotypes in 17 and 29 Patients, Respectively, Who Had or Had Not at Least 50% Restenosis After Carotid Eversion Endarterectomy Operations

<table>
<thead>
<tr>
<th>Variable: MBL Genotypes</th>
<th>Patients With Restenosis, n=17 (%)</th>
<th>Patients With No Restenosis, n=29 (%)</th>
<th>P, χ² for Trend</th>
</tr>
</thead>
<tbody>
<tr>
<td>A/A carriers</td>
<td>10 (59)</td>
<td>7 (24)</td>
<td>0.007</td>
</tr>
<tr>
<td>A/O carriers</td>
<td>7 (41)</td>
<td>16 (55)</td>
<td></td>
</tr>
<tr>
<td>O/O carriers</td>
<td>0 (0)</td>
<td>6 (21)</td>
<td></td>
</tr>
</tbody>
</table>

A/A vs A/O+O/O, χ², P=0.024.

with high MBL concentration (>500 μg/L) increased significantly in CDS values already at 7 months (P<0.001), whereas no significant increase in CDS values was observed in males (Figure 3C). By contrast, CDS values did not significantly change in the male and female patients with low (<500 μg/L) MBL concentration (Figure 3D).

Confirmatory evidence for a role of MBL in restenosis after carotid endarterectomy, normal MBL genotype, dominates in patients who had restenosis in a retrospective matched case-control study.

MBL genotypes were determined in 17 and 29 patients, respectively, who underwent carotid endarterectomy and did or did not have at least 50% restenosis. The frequency of patients carrying MBL variant genotypes (A/O+O/O) was significantly lower in the restenosis than in the control group (P=0.024) (Table). Moreover, a χ² for trend analysis clearly indicated that the effect was gene dose dependent, showing that homozygous (O/O) carriers of MBL variant alleles were more protected than the heterozygous carriers of MBL variant alleles, which again were more protected than the patients homozygous for the normal A/A genotype (P=0.007). In fact, none of the 6 homozygous carriers belonged to the restenosis group (Table).

Discussion

This prospective study shows that patients undergoing eversion endarterectomy for carotid stenosis are at higher risk for experiencing restenosis provided they are homozygous for the normal MBL A/A genotype than those carrying 1 or 2 variant MBL alleles (A/O or O/O). As has been shown in other studies a higher rate of restenosis after carotid eversion endarterectomy was seen in females than in males in the prospective study. However, significant differences between male and female patients in the restenosis rate were seen only in those who carried the A/A genotype (Figure 3). The findings were corroborated by the analysis of the relationship between MBL serum concentration and restenosis. The observation in the prospective study was substantiated in a retrospective study performed >2.5 years after surgery. In the latter matched case-control study, the gender effect could not be tested. The mechanisms behind the gender effect are at present unknown but suggest a complicated interplay between different genetic and hormonal factors. We cannot rule out conclusively that our findings are caused by linkage disequilibrium to another gene than MBL2 on chromosome 10. However, the results were independently borderline significant at 5% level when we examined the B and D alleles separately, which points at the MBL2 variant genotypes as protective loci (data not shown).

The pathophysiological mechanisms behind the association between the MBL genotype and restenosis are unknown. However, it is pertinent to suggest that it involves activation of the complement system, because MBL-associated serine protease 2 initiates activation of the lectin pathway of complement. Complement has been implicated in the pathogenesis of ischemia-reperfusion injury in experimental models and an involvement of MBL mediated activation of complement has been suggested. It is highly probable that early restenosis—defined as disease presenting within 24 months of initial operation—is secondary to the proliferation of medial smooth muscle cells leading to myointimal hyperplastic lesions, whereas late restenosis is caused by atherosclerotic processes. Fiande et al found substantial MBL-dependent complement activation and cytokine production in patients undergoing thoracoabdominal aortic aneurysm repair with thoracoabdominal cross-clamping, a human in vivo model of ischemia-reperfusion injury. Thus, it may be suggested that MBL is deposited after carotid eversion endarterectomy and that complement activation products including the highly active C5a may activate endothelial cells. Activated endothelial cells produce cytokines and growth factors and other molecules, which have been shown to be essential for smooth muscle proliferation, migration, and matrix formation, and for triggering neointimal hyperplasia, giving rise to a viscous circle resulting in restenosis.

It has been shown that MBL deficiency may be associated with accelerated atherosclerosis and also cardiovascular occlusion. Thus, it is likely that the MBL genetic system may promote and protect against inflammation depending on the pathophysiological scenario within the vessel wall and that it is a fine-tuned balance that determines whether complement is an advantage or disadvantage in cardiovascular disease settings.

In conclusion, it is shown that the reoccurrence rate of early restenosis after evasion carotid endarterectomy is partially genetically determined because female patients homozygous for the MBL A/A genotype have a significantly higher risk for restenosis than patients with variant MBL genotypes. The effect was found in 2 independent studies and suggests that MBL is a central player in the pathophysiology of this condition.

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