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Early complement activation follows eversion carotid endarterectomy and correlates with the time of clamping of the carotid artery

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ABSTRACT

Background: Complement activation plays an important role in ischemia/reperfusion (I/R) injury. The objective of the present study was to detect the presence and mechanism of complement activation in patients who underwent carotid endarterectomy (CEA).
Methods: Complement activation products C1rsC1-inhibitor, C4d, C3a and SC5b-9 and concentrations of C-reactive protein (CRP) were measured in samples serially taken from 16 patients with eversion CEA and 10 with carotid artery stenting (CAS) in the first 24 h post-surgery/intervention. *MBL2* genotypes were also determined.
Results: In patients with CEA an intense increase in C3a levels were observed immediately after surgery ($p < 0.001$), accompanied by a slight elevation in SC5b-9 levels ($p < 0.05$). C3a levels remained elevated until 4 h post-surgery, compared with the baseline values and with CAS patients. Peak C3a levels correlated with the time of carotid clamping ($r = 0.5921$, $p = 0.02$). No significant changes were detected in C1rsC1-inhibitor or C4d levels following CEA, and we found no association between the generation of C3a and *MBL2* genotypes or CRP levels. Complement activation was not present in patients with CAS.
Conclusions: Early complement activation follows CEA and correlates with the time of I/R injury. The lack of C4d generation suggests the role of the alternative and not the lectin pathway in the process.

The complement system is part of the innate immune system and has an essential role in host defense against pyogenic bacteria, bridging innate and adaptive immunity and in the elimination of circulating immune complexes and products of inflammatory injury (Walport, 2001a, 2001b). Uncontrolled or inappropriate complement activation plays an important role in several disease conditions, such as autoimmune diseases, sepsis, acute pancreatitis, atherosclerosis and cardiovascular diseases (Oksjoki et al., 2003, 2007). Increasing evidence shows, that complement has an essential role in ischemia/reperfusion (I/R) injury (Arumugam et al., 2004, 2006; Kilgore et al., 1994; Oksjoki et al., 2007) and targeting complement might be effective in the reduction of tissue damages caused by I/R injury (Dong et al., 1999; Weisman et al., 1990). However, most of these data comes from animal experimental models and only scarce data is available on in vivo complement activation due to I/R injury in humans (Arumugam et al., 2006; Bottiger et al., 2002; Fiane et al., 2003; Groeneveld et al., 1997; Straatsburg et al., 2000).

Carotid endarterectomy (CEA) is a well-established surgical method, which provides protection against stroke in some patients with severe carotid atherosclerosis (Anon., 1995). However, early recurrent stenosis develops in 10–15% of the cases in the first 2 years following CEA, which is thought to be secondary to an inflammatory neointimal hyperplasia of smooth muscle cells (Trisal et al., 2002). Previously, we have shown that carriers of the normal (A) allele of mannose-binding lectin gene (*MBL2*) and those with low levels of C1-inhibitor (C1-INH) of complement have higher risks to develop early restenosis after eversion CEA (Rugonfalvi-Kiss et al., 2005; Széplaki et al., 2007) and high complement C3 levels are associated with a high degree of restenosis (Széplaki et al., 2006). These observations indirectly highlighted, that activation of the complement system might play an important role in the pathomechanism of early restenosis after CEA.

During CEA, cross-clamping of the carotid artery is performed, which might trigger I/R injury and possibly complement activation. However, there are no published data on the occurrence and pos-

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sible consequences of complement activation following CEA. Thus our primary aim in the present study was to detect the presence and to investigate the mechanism of early complement activation in patients who underwent CEA. Moreover, we aimed to study the relation of complement activation to the acute-phase response, assessed by determination of C-reactive protein (CRP) levels as well.

1. Materials and methods

1.1. Study subjects and setup

We included 16 Caucasian patients (9 male and 7 female, aged 41–84 years), who underwent eversion type CEA at the Department of Cardiovascular Surgery, Semmelweis University, Budapest between January and May 2007 in the present study. Only patients without clinical signs or medical documentation of acute or severe chronic infection, autoimmune disease, sepsis or malignancy were included. Indication of CEA was in accordance with the American Heart Association guidelines (Biller et al., 1998); the details of the surgical procedure has been described previously (Entz et al., 1996). The time of clamping of the carotid artery was recorded during surgery. Ten Caucasian patients (seven males and three females, aged 50–75 years) who underwent percutaneous transluminal carotid artery balloon angioplasty and stenting (CAS) at the same department between March and May 2007 were also studied as patient controls. Indication for CAS was in accordance with the American Heart Association/American Stroke Association guidelines (Sacco et al., 2006); CAS was performed with currently accepted anticoagulant and antiplatelet prophylaxis, with distal protection. All patients gave informed consent and the study was approved by the Ethical Committee of the Semmelweis University, Budapest and conformed to the principles outlined in the declaration of Helsinki. Table 1 shows the descriptive statistics of the study population. Blood samples were serially drawn from antecubital veins prior CEA/CAS (baseline – BL) and immediately after (0 h), 1 (1 h), 4 (4 h), 8 (8 h) and 24 hours (24 h) following surgery/intervention. All clinically relevant complications during the 24 h post-surgery/intervention were recorded into the study database.

1.2. Laboratory methods

Serum specimens were allowed to clot for 2 h prior separation and were deep frozen at −80 °C. Plasma samples for the measurement of complement activation products were collected in EDTA containing vacuum tubes, were separated within 1 h and deep frozen at −80 °C immediately until laboratory use. Total genomic DNA was extracted from white blood cells using the method of Miller (Miller et al., 1988). Determination of the alleles of the *MBL2* gene were performed by polymerase chain reaction using sequence specific priming (PCR-SSP), as described previously (Garred et al., 2003a). We used commercially available enzyme-linked immunoassay (EIA) kits for the detection of complement activation products C4d, C3a and SC5b-9 (Quidel, San Diego, CA), while we used an in-house ELISA system for the detection of C1rsC1-INH complexes as described previously (Nagy et al., 2000), which is referred as arbitrary units per milliliter (AU/mL). Serum CRP concentrations were measured by particle-enhanced immunturbidimetric assay performed on a computerized laboratory analyzer (Roche Cobas Integra 400, Basel, Switzerland).

1.3. Statistical analysis

Statistical analysis was performed with SPSS for Windows 13.0.1 (SPSS Inc., Chicago, IL, http://www.spss.com) and Prism for Windows 4.02 (GraphPad Software, San Diego, CA, http://www.graphpad.com) statistical software products. As many of the variables had non-Gaussian distributions we used non-parametric tests throughout the analysis. We used the Mann–Whitney's *U*-test to compare two independent groups, the Fisher's exact test to compare categorical variables, the Friedman test for variance analysis (using Dunn's post hoc test comparisons between two repeated measures), Spearman's Rho to calculate correlations and multiple logistic regression models for multivariate analysis. All statistical analyses were performed two-tailed and $p < 0.05$ was considered as significant. Values presented in the text are medians (interquartile ranges), unless otherwise stated.

2. Results

2.1. Early complement activation follows CEA

Concentrations of the complement activation products were determined in the samples serially taken from the study subjects (Table 2). We found that alterations in C3a were significant in patients with CEA ($p < 0.0001$ for Friedman's test), but not in patients with CAS ($p = 0.1481$). C3a increased sharply immediately after surgery (0 h) and remained higher compared with the baseline values at 1 and 4 h following CEA (Table 2). Patients who underwent CEA had higher C3a levels at 0, 1 and 4 h post-surgery/intervention,

Table 1
Descriptive statistics and baseline levels of the complement activation products of the patients

	Carotid endarterectomy	Carotid artery stenting	*p*
N	16	10	–
Age, years	67.5 (53.5–71.5)	64.0 (54.0–69.5)	0.7318
Sex, male (%)	9 (56.3%)	7 (70.0%)	0.6834*
ICA stenosis (%)	90.0 (80.0–90.0)	85.0 (77.5–90.0)	0.4977
History of stroke, *n* (%)	9 (56.3%)	2 (20.0%)	0.1092*
History of CAD, *n* (%)	4 (25.0%)	6 (60.0%)	0.1087*
Hypertension, *n* (%)	14 (87.5%)	6 (60.0%)	0.1627*
Diabetes mellitus, *n* (%)	9 (56.3%)	4 (40.0%)	0.6882*
Hyperlipidaemia, *n* (%)	7 (43.8%)	2 (20.0%)	0.3989*
CRP (mg/L)	12.31 (2.50–20.00)	2.42 (1.49–13.59)	0.2357
MBL2 genotype A/A, *n* (%)	9 (56.3%)	6 (60.0%)	1.0000*
C1rsC1-INH (AU/mL)	68.11 (60.03–91.91)	72.24 (66.76–85.50)	0.4768
C4d (μg/mL)	1.50 (1.06–2.55)	0.59 (0.31–1.37)	0.0165
C3a (ng/mL)	125.3 (50.7–183.0)	76.5 (14.1–295.5)	0.7319
SC5b-9 (ng/mL)	197.2 (121.5–258.2)	163.3 (101.8–341.5)	0.8537

Values presented as medians (interquartile ranges) and absolute numbers (percentages). *p*-Values were calculated with the Mann–Whitney's non-parametric test and the *Fisher's exact test. ICA indicates internal carotid artery; CAD, coronary artery disease; *MBL2*, mannose-binding lectin gene, CRP, C-reactive protein.