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Rapid communication

# Autoantibody against the amino acid sequence 661–680 in apo B-100 is associated with decreased carotid stenosis and cardiovascular events

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**Abstract**

Immunization with malondialdehyde (MDA)-modified peptides corresponding to the amino acid sequence between 661 and 680 in apo B-100 (p45) inhibits atherosclerosis in apo E knockout mice. The same effect can be obtained by treating the mice with recombinant anti-MDA-p45 IgG, suggesting that these antibodies have atheroprotective effects. In the present study we analyzed if autoantibodies against p45 and MDA-p45 are related to carotid atherosclerosis and acute cardiovascular events in humans. Using a nested case control design we determined plasma levels of IgG recognizing native and MDA-modified p45 in baseline samples from 75 subjects with acute myocardial infarction or sudden cardiac death and 148 matched controls. The control group was found to have significantly higher levels of p45 IgG than the cases. Moreover, an independent association was found between high levels of MDA-p45 IgG and a low degree of carotid stenosis ($P = 0.006$). There was a high degree of co-variation between IgG binding to native p45 and MDA-p45 ($r = 0.68$, $P < 0.0001$). The associations between lower levels of autoantibodies against the apo B-100 p45 sequence and cardiovascular disease are in agreement with previous experimental studies demonstrating that these antibodies have atheroprotective effects. Our findings support the notion that the p45 sequence of apo B-100 is a potential target for immunomodulatory treatment of atherosclerosis in humans.


*Keywords:* Apolipoproteins; Antibodies; Carotid stenosis; Peptide; Ultrasound

Activation of adaptive immunity plays an important role in the development of atherosclerosis [1,2]. T cells in human atherosclerotic plaques recognize epitopes in oxidized LDL when presented by macrophage MHC class II molecules [3] and autoantibodies against oxidized LDL are commonly expressed in man, suggesting that oxidized LDL is an important antigen in atherosclerosis [4,5]. Several lines of evidence favour the concept that adaptive immune responses are activated as part of the disease process and promote inflammation and plaque growth [1,2]. However, immunization with oxidized LDL has also been shown to reduce atherosclerosis, demonstrating the existence of an atheroprotective immune reaction to oxidized LDL [5–11]. Oxidized phospholipids [12] and aldehyde-modified peptide sequences in apo B-100 [13] are the major targets for the immune system in oxidized LDL. We have previously demonstrated that high IgM levels against a number of different aldehyde-modified peptide sequences in apo B-100 are associated with increased severity of carotid disease and risk for development of acute myocardial infarction [13]. Immunization of apo E knockout (KO) mice with some of these native and aldehyde-modified apo B-100 peptide sequences induces an immunoglobulin switch from IgM to IgG that is accompanied by an inhibition of atherosclerosis [14–16]. To study the possible atheroprotective effects of this IgG we produced recombinant human IgG1 specific for a

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malondialdehyde (MDA)-modified peptide corresponding to the sequence between amino acids 661 and 680 in apo B-100 (p45) [17]. Subcutaneous immunization with MDA-p45 peptide has previously been shown to inhibit atherosclerosis by about 50% in apo E KO mice [15]. A similar inhibition of atherosclerosis was observed in apo E KO mice following three weekly injections of recombinant anti-MDA-p45 IgG. Taken together these results suggest that IgG recognizing the MDA-modified peptide sequence between amino acids 661 and 680 in apo B-100 may protect against atherosclerosis.

The aim of the present study was to investigate plasma levels of p45 IgG autoantibodies in subjects with and without acute cardiac events as well as the association between these autoantibodies and the severity of atherosclerosis in the carotid artery as determined by B-mode ultrasound in humans.

## 1. Material and methods

### 1.1. Study population

The study subjects, born between 1926 and 1945, were recruited from the "Malmö Diet and Cancer (MDC)" study cohort as previously described [13]. Participants who had a history of myocardial infarction or stroke prior to enrolment were not eligible for the present study. The study population consisted of 223 subjects, 75 cases that developed acute cardiac events, i.e. fatal or non-fatal myocardial infarction or deaths due to coronary heart disease during follow-up and 148 controls matched for age, sex, smoking habits, presence of hypertension, month of participation in the screening examination and duration of follow-up. For two of the cases it was only possible to find one instead of two matching controls. The ethical committee of Lund University, Sweden approved the study.

### 1.2. Laboratory analyses

After overnight fasting blood samples were drawn for the determination of serum values of total cholesterol, triglycerides, HDL cholesterol, LDL cholesterol and whole blood glucose. LDL cholesterol in mM was calculated according to the Friedewald formula. Oxidized LDL was measured using ELISA (Mercodia, Uppsala, Sweden) in EDTA plasma supplemented with the antioxidants DTPA and BHT.

### 1.3. B-mode ultrasound vasculography

An Acuson 128 Computed Tomography System (Acuson, Mountain View, CA) with a 7 MHz transducer was used for the assessment of percent carotid stenosis as described previously [18].

### 1.4. Determination of p45 and MDA-p45 IgG

A 20 amino acid long peptide corresponding to the sequence between amino acids 661 and 680 in human apo B-100 (p45; IEIGLEGKGFEPTLEALFGK) was produced (KJ Ross Petersen AS, Horsholm, Denmark) and used in ELISA. The peptide was modified by treatment with 0.5 M MDA [19] for 3 h at 37 °C. The MDA-modified peptide was dialyzed against PBS containing 1 mM EDTA with several changes for 18 h at 4 °C. The MDA modification of peptide was assessed using the thiobarbituric acid reactive substances (TBARS) assay as described [13]. The aldehyde content of the modified peptide was 0.022 nmol/μg peptide. The native and MDA-modified peptides were diluted in PBS pH 7.4 (20 μg/ml) and absorbed to microtiter plate wells (Nunc MaxiSorp, Nunc, Roskilde, Denmark) in an overnight incubation at 4 °C. After washing with PBS containing 0.1% Tween-20 (PBS-T) the coated plates were blocked with SuperBlock in TBS (Pierce, Rockford, IL) for 5 min at room temperature followed by an incubation test plasma, diluted 1/100 in TBS-0.1% Tween-20 containing 10% SuperBlock (TBS-T) for 2 h at RT and overnight at 4 °C. After rinsing, deposition of autoantibodies directed to the peptides was detected using mouse anti-human IgG antibodies (Sigma, St. Louis, MO) appropriately diluted in TBS-T. After incubation for 3 h at room temperature the plates were washed and the bound antibodies were detected by alkaline phosphatase conjugated goat anti-mouse IgG (Sigma), incubated for 2 h at room temperature. The colour reaction was developed by using phosphatase substrate kit (Pierce) and the absorbance at 405 nm was measured after 2 h of incubation at room temperature.

### 1.5. Statistics

Statistical analysis was done using SPSS. The results are presented as median and range and as proportions when appropriate. Spearman rank correlation coefficients were calculated to evaluate associations between p45 IgG, risk factors and carotid stenosis. Mann–Whitney test was used to assess differences between groups. $\chi^2$-test was used for comparing proportions. Multiple regression analysis was used to study independent correlations between carotid stenosis and other variables.

## 2. Results

Using a nested case control design we selected 75 subjects with coronary events (acute myocardial infarction or death due to coronary heart disease) and 148 controls matched for age, sex, smoking and hypertension from the Malmö Diet Cancer Study. The median time from inclusion to the acute coronary event was 2.8 years (range 0.1–5.9 years) among cases. The baseline characteristics of the study groups are shown in Table 1. The cases were characterized by having higher plasma triglycerides ($P < 0.05$) and lower levels of IgG against native p45 ($P < 0.05$). There was no association between plasma levels of native or MDA-p45 IgG and time from inclusion to the acute event among the cases. There were no significant differences in lipoprotein lipids, oxidized LDL or glucose between cases and controls.