



Autoantibodies against basement membrane collagen type IV are associated with myocardial infarction

Olga McLeod ^{a,*}, Pontus Dunér ^{b,1,2}, Ann Samnegård ^{c,2}, Per Tornvall ^{d,2}, Jan Nilsson ^{b,2}, Anders Hamsten ^{a,2}, Eva Bengtsson ^{b,2}

^a Department of Medicine, Atherosclerosis Research Unit, Karolinska Institutet, Stockholm, Sweden

^b Department of Clinical Sciences, Skåne University Hospital, Malmö, Sweden

^c Department of Clinical Sciences, Cardiology Unit, Danderyd Hospital, Karolinska Institutet, Stockholm, Sweden

^d Department of Clinical Sciences, Cardiology Unit, Södersjukhuset, Karolinska Institutet, Stockholm, Sweden

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ABSTRACT

Background: Collagen type IV is the major constituent of basement membranes underlying endothelial cells and is important for endothelial cell attachment and function. Autoantibodies against native collagen type IV have been found in various autoimmune diseases. Oxidation of LDL in the vascular wall results in the formation of reactive aldehydes, which could modify surrounding matrix proteins. Like oxidized LDL, these modified matrix proteins are likely to induce immune responses. We examined whether autoantibodies against native or aldehyde-modified collagen type IV are associated with myocardial infarction.

Methods: IgM and IgG against native and aldehyde-modified collagen type IV were measured by ELISA in serum from 387 survivors of a first myocardial infarction and 387 age- and sex-matched controls.

Results: Post-infarction patients had significantly increased levels of IgM against native collagen type IV, and IgG against native collagen type IV was present at detectable level in 17% of patients as opposed to 7% of controls ($p < 0.001$). Controlling for major cardiovascular risk factors demonstrated that the presence of IgG against native collagen type IV was associated with myocardial infarction (OR 2.9 (1.6–5.4), $p = 0.001$). Similarly, subjects in the highest quartile of IgM against native collagen type IV had increased risk of having suffered myocardial infarction (OR 3.11 (1.8–5.4), $p < 0.001$) after adjusting for cardiovascular risk factors. In contrast, IgG against aldehyde-modified collagen type IV was decreased in myocardial infarction patients, but this association was not independent of established cardiovascular risk factors.

Conclusion: Autoantibodies against collagen type IV are associated with myocardial infarction independently of traditional cardiovascular risk factors.

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1. Introduction

Acute coronary events mainly arise from plaque rupture or plaque erosion [1]. Plaques prone to rupture are characterized by a large lipid-rich necrotic core with an overlying thin fibrous cap [1], which is believed to rupture due to increased inflammation resulting in the degradation of extracellular matrix components. The mechanisms for plaque erosion are not known, but lesions are characterized by the absence of endothelium [1]. The endothelium regulates the vascular

tone, controls blood coagulation and regulates inflammatory processes [2]. Endothelial dysfunction predicts clinical events caused by atherothrombosis, and case-control studies indicate an association between endothelial dysfunction and acute coronary syndromes [2,3]. Although endothelial dysfunction clinically is measured by parameters of vasodilation, it appears that this condition is equated with a loss of atheroprotection and promotion of atherothrombosis.

Endothelial cells are lying on and adhere to the basement membrane, a thin sheet of extracellular matrix. The main component of the basement membrane is the network forming collagen type IV, which comprises 50% of the basement membrane. Collagen type IV binds to cells via integrins on the cell surface or via discoidin domain receptors [4–7]. As expected, the collagen type IV interaction with endothelial cells is important for maintaining endothelial cell function [8]. Interestingly, autoantibodies against collagen type IV are present in various inflammatory and autoimmune diseases [9–13].

* Corresponding author at: Department of Medicine, Atherosclerosis Research Unit, CMM, L8:03, Karolinska University Hospital, Solna, S-171 76 Sweden. Tel.: +46 8 51773257, +46 76 8600802 (mobile); fax: +46 8 311298.

¹ Both authors contributed equally.

² This author takes responsibility for all aspects of the reliability and freedom from bias of the data presented and their discussed interpretation.

Increased levels of autoantibodies against collagen type IV have been associated with diabetes and development of microangiopathy [14] and have also been reported in patients with systemic vasculitis and Crohn's disease [13,15]. Moreover, autoantibodies against collagen type IV have been detected in children with type 1 diabetes and hypertension [16,17].

Accumulation and oxidation of LDL in the vessel wall are considered as key events in the development of atherosclerotic lesions [18]. Thus, efforts so far have almost exclusively been focused on characterizing modifications of the LDL particles. Oxidation of fatty acids in LDL leads to the formation of reactive aldehydes, such as malondialdehyde (MDA) [19]. These aldehydes react with the LDL protein apoB100, and MDA-modified apoB100 becomes a target for the immune system. Accordingly, autoantibodies against oxidized LDL are associated with cardiovascular disease [19]. We have previously shown that during oxidation of LDL, reactive aldehydes leak out of the LDL particle and modify surrounding extracellular matrix proteins [20]. These modifications on extracellular matrix may impair structural and cell-binding properties of the proteins, resulting in decreased plaque stability. It is also possible that modification of basement membrane proteins enhances inflammation, as shown by increased monocyte attachment and intracellular adhesion molecule (ICAM)-1 expression on endothelial cells attached to oxidized laminin [21]. Similar to oxidized LDL, modified matrix proteins are likely to result in immune responses against the extracellular matrix in the plaque. Indeed, we have identified autoantibodies against several aldehyde-modified matrix proteins, including MDA-modified laminin present in basement membranes. Interestingly, these autoantibodies were associated with less cardiovascular disease in a prospective cohort, which may imply a protective effect in humans [22].

In the present work, we investigated the presence of autoantibodies against native and aldehyde-modified collagen type IV in patients with a recent myocardial infarction (MI) and population-based matched controls.

2. Methods

2.1. Cohort

The Stockholm Coronary Atherosclerosis Risk Factor (SCARF) study database and biobank were used for the present study. As reported elsewhere [23], 387 survivors of a first MI aged less than 60 years were recruited along with 387 age- and sex-matched controls from the general population of the same county. In both groups 82% of the subjects were male. All participants were interviewed, underwent a brief medical examination and donated fasting blood samples at an examination which took place 3 months after the cardiac event for the patients. Details of recruitment, representativeness and basic characteristics of the groups have been published earlier [23]. The study was approved by the ethics committee of the Karolinska University Hospital, and conducted in agreement with the Declaration of Helsinki. All patients and control persons gave their informed consent to participation.

2.2. Coronary angiography

Routine coronary angiography was performed in a subset of 243 patients during the initial hospital stay ($n = 35$) or 3 months after the cardiac event ($n = 208$) [23]. Angiograms were analyzed by quantitative computer-based evaluation (QCA; Medis QCA-CMS system, Leiden). Minimal lumen diameter, reference diameter, percentage diameter stenosis, mean segment diameter, segment length, plaque area, segment area and number of significant stenoses ($>50\%$) were registered in each of the 15 coronary segments.

2.3. Biochemical analysis

Analyses of C-reactive protein (CRP) [24], plasminogen activator inhibitor-1 (PAI-1) [23], matrix metalloproteinase-3 (MMP-3) [23] and MMP-9 [25] have been the subject of previous reports.

2.4. ELISA for autoantibodies against native and MDA-modified collagen type IV

Proteins were MDA-modified at 37 °C during 3 h in a solution using 0.05 mol/L MDA in PBS, pH 7.4. MDA modifications were assayed using thiobarbituric acid reactive substances assay (TBARS) and Western blot using anti MDA antibody. Antibodies in plasma against native and MDA-modified collagen IV were detected by ELISA, essentially as described [26]. Briefly, 10 µg/mL of native or MDA-modified collagen IV was coated on microtiter plates (Nunc MaxiSorp, Roskilde, Denmark) and blocked with Superblock in Tris buffered saline (Pierce, Rockford, IL, USA). Plasma (dilution 1:100) was added to the wells and detection of bound antibodies was done with biotinylated rabbit anti-human IgG (Abcam) or IgM (Dako, Stockholm, Sweden), followed by alkaline phosphatase-conjugated streptavidin (Sigma), and alkaline phosphatase substrate kit (Pierce). Absorbance from wells coated with native collagen type IV was subtracted with absorbance from PBS-coated wells. Absorbance from wells coated with MDA-collagen type IV was subtracted with absorbance from wells coated with native collagen type IV to exclude antibodies binding to native epitopes of MDA-modified collagen type IV. Absorbance values were normalized against a plasma pool present on each plate. Arbitrary units (AU) are defined as percentage of the absorbance to the plasma pool. Intra-assay ($n = 10$) and inter-assay ($n = 10$) coefficients of variation for the antibody ELISA directed against native collagen IV were 2.6 and 7.6% for the IgM and 4.5 and 6.1% for the IgG, respectively. Intra-assay ($n = 10$) and inter-assay ($n = 10$) coefficients of variation for the antibody ELISA directed against MDA-modified collagen IV were 7.0 and 5.5% for the IgM and 11.9 and 10.2% for the IgG, respectively. Absorbance for antibodies against collagen type IV was in general low, but detectable levels were present in 592 individuals for IgM against native collagen type IV, in 89 individuals for IgG to native collagen type IV, in 536 individuals for IgM against MDA-collagen type IV, and in 723 individuals for IgG to MDA-collagen type IV, out of a total of 747 individuals. The specificities of ELISAs were determined by competition assay. Binding of both IgM and IgG in plasma to native collagen type IV was completely inhibited by the addition of 100 µg/mL native collagen type IV, whereas addition of MDA-collagen type IV did not compete (IgM) or only partly competed out the interaction (IgG) (Supplementary Fig. 1A). Binding of IgM and IgG in plasma to MDA-collagen type was completely inhibited by the addition of 100 µg/mL MDA-collagen type IV, whereas native collagen type IV or MDA-modified albumin did not compete or competed to a lesser extent (Supplementary Fig. 1A).

2.5. Statistical methods

Owing to missing samples in 19 patients and 8 controls, 368 patients and 379 controls were available for subsequent statistical analysis. For all types of antibodies (except IgG against native collagen type IV) values under the detection limit were replaced with 0 values. Variables are summarized as number (percentage) or medians (interquartile range). Differences in group comparisons were assessed by using Mann-Whitney test for continuous traits, and by Pearson's chi-squared test for binary. Detectable levels of IgG against native collagen type IV for patients and control individuals are summarized as group percentages and are compared using chi-square test. In univariable analysis, skewed data were transformed prior to analysis: anti-collagen IV IgM was divided into 5 groups (values below the detection limit, and 4 quartiles of positive values), anti-MDA-collagen IV IgM and IgG underwent square-root transformation. Anti-collagen IV IgG

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