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IgM antibodies against malondialdehyde and phosphorylcholine are together strong protection markers for atherosclerosis in systemic lupus erythematosus: Regulation and underlying mechanisms

Mizanur Rahman [a], Sudhir Sing [a], Zahra Golabkesh [a], Roland Fiskesund [a], Thomas Gustafsson [b], Tomas Jogestrand [b], Anna G. Frostegård [a], Ingiäld Hafström [c], Anquan Liu [a], Johan Frostegård [a,*]

[a] IMM, Unit of Immunology and Chronic Disease, Karolinska Institutet, 171 65 Stockholm, Sweden
[b] Department of Laboratory Medicine, Division of Clinical Physiology, Karolinska Institutet at Karolinska University Hospital, Huddinge, 141 86 Stockholm, Sweden
[c] Department of Rheumatology, Karolinska Institutet at Karolinska University Hospital, Huddinge, 141 86 Stockholm, Sweden

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ABSTRACT

Objectives: Phosphorylcholine (PC) and malondialdehyde (MDA) are generated during lipid peroxidation and form adducts with proteins as albumin as studied herein. Atherosclerosis and cardiovascular disease (CVD) are increased in systemic lupus erythematosus (SLE). We here investigate the role and regulation of IgM antibodies against PC (anti-PC) and MDA (anti-MDA).
Methods: IgM anti-PC and anti-MDA in SLE patients (n = 114) were compared with age- and sex-matched population-based controls (n = 108). Common carotid intima-media thickness (IMT) and plaque occurrence were determined by B-mode ultrasound. Plaques were graded according to echogenicity (potentially vulnerability). Production of IgM anti-PC and anti-MDA by B cells was determined by ELISA and ELISPOT. The effect of anti-PC and anti-MDA on macrophage uptake of apoptotic cells and oxidative stress was studied by flow cytometry.
Results: Above 66rd percentile together, IgM anti-PC and anti-MDA were striking protection markers for plaque prevalence and echolucency in SLE (OR: 0.08, CI: 0.01–0.46 and OR: 0.10, CI: 0.01–0.82), respectively, and risk markers for plaque prevalence when below 33rd percentile: OR: 3.79, CI: (1.10–13.00).
In vitro, IgM anti-PC and anti-MDA were much higher when B cells were co-cultured with CD3 T cells. Anti-HLA-, anti-CD40 antibody or CD40 silencing abolished these effects. Uptake of apoptotic cells was increased by IgM anti-PC and anti-MDA. MDA induced increased oxidative stress, which was inhibited by IgM anti-MDA.
Conclusions: Unexpectedly, both IgM anti-MDA and IgM anti-PC are T-cell dependent and especially together, are strong protection markers for atherosclerosis in SLE. Underlying mechanisms include increased phagocytosis of apoptotic cells and decrease of oxidative stress.

1. Introduction

Atherosclerosis is the major cause of cardiovascular disease (CVD), and is now recognized as a chronic inflammatory condition. Activated immunocompetent cells, such as T-cells and monocytes/macrophages producing proinflammatory cytokines are abundant in atherosclerotic plaques. According to a leading hypothesis, enzymatically modified and oxidized forms of low density lipoproteins (OxLDL) play an important role in atherogenesis. Presence of OxLDL and dead cells/debris in a necrotic core of plaques represent major features of atherosclerosis [1,2].

In SLE the risk of CVD and atherosclerosis (especially the prevalence of plaques) is very high. In addition to being an important clinical problem, studies of CVD in SLE could give important insights into the role of immunity in atherosclerosis and CVD, thus SLE functioning as a "human model" of immunity and atherosclerosis [3].

When phospholipid-containing cell membranes and lipoproteins are exposed to reactive oxygen species, lipid peroxidation ensues with an array of compounds generated, both lipid- and protein-related (as apoB-100 in LDL). From an immunological point of view, two lipid-related epitopes are of special interest in atherosclerosis, phosphorylcholine (PC) and malondialdehyde (MDA). Both epitopes become immunogenic when bound to carriers, and their exposure to the immune system generates antibodies against these compounds [2].

OxLDL immunity is a complex phenomenon, where an array of antigens is generated including apoB100-related compounds, and antibody determinations against OxLDL give conflicting clinical data, sometimes being risk markers and sometimes protection markers [2].

Abbreviations: SLE, systemic lupus erythematosus; CVD, cardiovascular disease; MDA, malondialdehyde; PC, phosphorylcholine; HSA, human serum albumin; BSA, bovine serum albumin; SFM, serum free medium; OR, Odds Ratio.

* Corresponding author.
E-mail address: johan.frostegard@ki.se (J. Frostegård).

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A dominant compound in MDA-protein adducts is acetaldehyde which forms stable dihydropyridine (4-methyl-1, 4-dihydropyridine-3,5-dicarbaldehyde) which modifies the lysine into a stable product which is antigenic [4]. When LDL is modified by MDA (MDALDL), it has been known since long that this compound has interesting properties in atherosclerosis. Immunization with MDALDL (oxidation of LDL by cupper, as a model, also leads to MDA-modification) in a pivotal study from the mid-90s led to amelioration of atherosclerosis development though the exact immunological mechanisms were not elucidated [5]. LDL modified by MDA is taken up in monocytes/macrophages and could play a role in atherogenesis [6]. However, little is known about the clinical role of anti-MDA where MDA is bound to a carrier not related to oxLDL, as herein human albumin, and properties and regulation of IgM anti-PC and IgM anti-MDA.

We reported that IgM anti-PC (where PC was conjugated with carriers unrelated to LDL) is a protection marker in human disease as CVD and atherosclerosis development [2,7] but also SLE where this was reported in two different SLE-cohort [8,9], one of them the same as herein [9].

Little is known about properties and regulation of IgM anti-PC and IgM anti-MDA in humans and the role of T-cells in their generation though such antibodies are often described as being T-cell independent [10]. We here report that these antibodies are, surprisingly, T-cell dependent, increase phagocytosis of apoptotic cells and are striking protection markers for atherosclerosis and potentially vulnerable plaques in SLE. The implications of these findings are discussed.

2. Materials and methods

2.1. The SLEVIC study cohort

The study - SLE Vascular Impact Cohort (SLEVIC) - has been described elsewhere [9]. Briefly, 114 SLE patients from Karolinska University Hospital, Huddinge, Sweden and 122 sex- and age-matched population based controls were included. Anti-MDA measurements were available in 114 SLE patients and 108 controls.

All patients fulfilled the 1982 revised criteria of the American College of Rheumatology (ACR) for SLE. The study is approved by the Karolinska Institutet Research Ethics.

Committee and was performed in accordance with the Declaration of Helsinki. All subjects gave informed consent before entering the study.

2.2. Study protocol

The investigation included a written questionnaire, an interview, a physical examination by a rheumatologist, laboratory determinations and an ultrasound examination of carotid arteries in all but three patients.

2.3. Carotid B-mode ultrasonography

The right and left carotid arteries were examined with a duplex scanner (Sequoia, Siemens Acuson, Mountain View, CA, USA) using a 6 MHz linear array transducer as previously described [9].

Briefly, the far wall of the common carotid artery (CCA), 0.5 to 1.0 cm proximal to the beginning of the carotid bulb, was used for measurements of the intima-media thickness (IMT) which was defined as the distance between the leading edge of the lumen-intima echo and the leading edge of the media-adventitia echo. The CCA lumen diameter was defined as the distance between the leading edge of the intima-lumen echo of the near wall and the leading edge of the lumen-intima echo of the far wall. The examinations were digitally stored for subsequent computer analyses [11]. The mean values of the IMT and lumen diameter within the 10 mm long section were calculated. When a plaque was observed in the region of the CCA measurements, the IMT was not measured.

Carotid plaque was defined as a localized intima-media thickening above 1 mm and at least a 100% increase in thickness compared with adjacent wall segments. Plaque was screened for in the common, internal and external carotid arteries. Plaque occurrence was scored as the absence of plaque, the presence of unilateral plaque, and the presence of bilateral plaque. Plaque morphology in terms of echogenicity was assessed in a modified version of the classification proposed by Gray-Weale et al. [12] and graded from 1 to 4 as echolucent, predominantly echolucent, predominantly echogenic and echogenic. Grade 1 was defined as echolucent in this study. Echolucency was defined with the arterial lumen as reference and echogenicity with the far wall adventitia as reference.

The ultrasonographic methods used have been described in detail previously [13,14].

2.4. ELISA determination of IgM antibodies against MDA and PC

Levels of IgM antibodies to MDA and PC were determined by enzyme-linked immunosorbent assay (ELISA), for anti-PC as described earlier [8]. MDA was conjugated with human serum albumin (Sigma-Aldrich AB, Stockholm, Sweden) as described [15,16]. For anti-MDA-determinations, serum from a donor with anti-MDA levels above median were used as internal standard and tested on each plate. The plateau of IgM antibody binding was reached with the antigen concentration of 10 μg/ml. 96-well Immuno plates (Thermo Scientific, Denmark) were coated with 100 μl of MDA-HSA. Coated plates were incubated overnight at 4 °C. After five washings with PBS/Tween 20 (Sigma Aldrich, MO, USA), the plates were blocked with 2% BSA in PBS for 1 h at room temperature and washed. Serum samples were diluted (1:100) in 0.2% BSA in PBS and added at 100 μl/well. Plates were then incubated 2 h at room temperature and washed.

Alkaline phosphatase conjugated goat anti-human IgM (Sigma Aldrich, MO, USA) diluted 1:7000 in the sample buffer was added at 100 μl/well and incubated for 2 h at room temperature. After five washings, color was developed by adding the alkaline phosphatase substrate (PNPP) at 100 μl/well and incubating the plates for 30 min at room temperature in the dark. The plates were read in an ELISA Multiscan Plus (Spectra Max 250, Molecular Devices, CA, USA) at 405 nm. All samples were measured in duplicates and the coefficient of variation was below 15%.

In order to investigate the specificity of anti-MDA, competition assays were performed. At a dilution giving 50% of maximal binding of anti-MDA, sera were pre-incubated with different concentrations of MDA overnight in glass tubes. After vortexing, the tubes were incubated overnight at 4 °C and centrifuged at 13,000 r.p.m. for 30 min (4 °C). The supernatants were tested for IgM antibody binding to MDA and gave above 60% inhibition (data not shown) which is thus similar to the method we described for anti-PC previously [17].

The percentage of inhibition was calculated as following:

$$\text{Percent inhibition} = (\text{OD without competitor} - \text{OD with competitor})/\text{OD without competitor} \times 100.$$

2.5. Cell separation

Buffy coats from healthy individuals were collected at Karolinska University Hospital, Stockholm, Sweden. Buffy coats-derived B, CD3 T and CD14 cells were isolated using cell enrichment cocktail or antibodies (STEMCELL Technologies Inc., France) and following Ficoll-Paque Plus protocol (GE Healthcare, Sweden). Peripheral blood mononuclear cells (PBMC) were isolated following Ficoll-Paque Plus protocol.