

High plasma concentrations of autoantibodies against native peptide 210 of apoB-100 are related to less coronary atherosclerosis and lower risk of myocardial infarction

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Aims

We examined whether antibodies against peptides 45 and 210 of apoB-100 are related to myocardial infarction (MI) and severity of coronary atherosclerosis.

Methods and results

Three hundred and eighty-seven survivors of a first MI (aged <60 years) and 387 sex- and age-matched controls were characterized in detail. IgG and IgM autoantibodies against native and malondialdehyde (MDA)-modified peptides 45 and 210 of apoB-100 (amino acids 661–680 and 3136–3155) were quantified in plasma and quantitative coronary angiography was performed in 243 patients. Post-infarction patients had significantly lower IgG against the native peptide 210 (IgG-p210_{nat}) and higher IgM against the MDA-modified peptide 210 (IgM-p210_{MDA}) compared with controls, whereas no differences were found for other antibodies. Plasma concentrations of IgG-p210_{nat}, but not IgM-p210_{MDA}, were independently and inversely related to the degree of coronary atherosclerosis in patients. In multiple logistic regression analysis (including established risk indicators), MI risk was 0.55 (95%CI: 0.37–0.81) for individuals in the IgG-p210_{nat} upper quartile compared with the remaining individuals.

Conclusion

Circulating IgG antibodies against the native peptide 210 of apoB-100 are inversely related to the severity of coronary atherosclerosis and associated with lower risk of MI. Epitope 210 of apoB-100 emerges as a target for immunization against atherosclerosis in humans.

Keywords

Myocardial infarction • Coronary atherosclerosis • Angiography • Oxidized LDL • Autoantibodies • apoB-100

Introduction

Today, adaptive immunity is recognized to play an important role in atherosclerosis. Oxidized low-density lipoprotein (oxLDL) is immunogenic and the immune response against oxLDL is considered as a crucial and modifiable event in atherogenesis.¹ The concept of oxLDL as an important autoantigen in atherosclerosis is supported by the findings showing that (i) T-cells cloned from human lesions respond to oxLDL in an MHC class II-dependent

manner, (ii) both plasma and lesions contain high levels of antibodies recognizing various epitopes of oxLDL, and (iii) hypercholesterolaemic animals with genetic deficiencies in the adaptive immune system develop less atherosclerotic disease.^{1–5}

Although adaptive immunity is believed to have a net atherogenic effect (promoting inflammation and plaque growth), anti-atherogenic effects of immune responses against oxLDL have been described. Experimental animals immunized with oxLDL particles are partially protected from atherosclerosis^{6–10} and

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immunization targeted to induce immune responses against specific components of oxLDL (such as modified phospholipids or fragments of apoB) have also resulted in atheroprotection.^{11,12} Indeed, immune reactions against certain epitopes of apoB might have a particular impact on atherosclerosis. To investigate this, we used a library of native and aldehyde-modified polypeptides (each 20 amino acids long) covering the complete apoB-100 sequence to capture circulating IgG and IgM antibodies from human plasma. Relationships were found between the immune responses against certain aldehyde-modified peptides of apoB (such as peptides 45 and 210) and human atherosclerosis.⁵ Subsequent immunization with some of these native and aldehyde-modified peptides, or the corresponding antibodies, in apoE knockout mice resulted in altered circulating immunoglobulin composition, accompanied by up to 70% reduction in atherosclerosis.¹³ Collectively, these studies imply that activation of adaptive immunity against certain epitopes of apoB-100 may be atheroprotective. Clinical associations of antibodies against native and aldehyde-modified peptides 45 and 210 with carotid atherosclerosis were recently described,^{14,15} whereas corresponding relationships to the severity of coronary atherosclerosis and risk of myocardial infarction (MI) remain to be shown.

Against this background, we investigated the relationships between plasma levels of IgG and IgM antibodies directed against the native and malondialdehyde (MDA)-modified peptides 45 and 210 of apoB-100, severity of coronary artery disease assessed by quantitative coronary angiography (QCA), and the risk of MI in representative survivors under the age of 60 years and in population-based control subjects.

Methods

Study population

The Biobank and data set of the Stockholm Coronary Atherosclerosis Risk Factor (SCARF) study¹⁶ was used for the present study. The SCARF study comprises 387 survivors of a first MI (age <60 years) and 387 sex- and age-matched control subjects recruited from the general population of the same county. Details of recruitment procedures and representativeness have been described.¹⁶ In brief, the inclusion rate of eligible patients was 76%. Controls, the characteristics of whom are shown in Table 1 and have been reported earlier,¹⁶ were selected at random from the population register among individuals born on the same date as the corresponding patients. With the exception of subjects with symptom suggestive of coronary artery disease, all the controls were included in the study, irrespective of metabolic disturbances or clinical risk indicators discovered during the investigation. A total of five patients failed to complete the study after the matched controls had been enrolled. These controls were kept in the study and re-assigned to patients entering the study at a later stage. All the participants were interviewed, underwent a brief medical examination and donated fasting blood samples; this took place in the patients at 3 months after the cardiac event. In both the groups, 82% of the subjects were male and 99% were considered Caucasian. Data from coronary angiography (executed during the initial admission or 3 months after the event) was available in a representative subset of 243 patients. Two of the three hospitals performed angiography routinely in all patients, whereas the third only during clinical indications (and the latter patients were excluded from the angiographic analyses to avoid a selection bias).

Serum samples from 30 individuals at random (out of 222 enrolled) presenting with ST-elevation MI (STEMI) at one of the participating hospitals, treated with thrombolysis and participating in a longitudinal cohort study of long-term outcome after thrombolysis in STEMI,¹⁷ were used to examine the circulating IgG and IgM antibodies against apoB peptides at three time-points after the onset of MI (at <4 h, 24 h, and 3 months).

The studies were approved by the Ethics Committee of the Karolinska University Hospital and all the subjects gave their informed consent to participation.

Biochemical analysis

Fasting concentrations of cholesterol and triacylglycerol (TG) in the very low-density lipoprotein (VLDL), low-density lipoprotein (LDL), and high-density lipoprotein (HDL) fractions were determined by a combination of preparative ultracentrifugation, precipitation of apoB-containing lipoproteins, and lipid analysis.¹⁸ Commercially available kits were used for the analysis of insulin and proinsulin (ELISAs from DAKO Ltd, Cambridgeshire, UK), fibrinogen (clotting assay IL-Test Fibrinogen C from Instrumentation Laboratory Co., Milan, Italy), and plasminogen activator inhibitor-1 (PAI-1) activity (Cromolize PAI-1 from Biopool International, Umeå, Sweden). High-sensitivity C-reactive protein was measured by particle-enhanced immunonephelometry in the BN system (Dade Behring, Liederbach, Germany).

Circulating antibodies against peptides 45 (p45) and 210 (p210) of human apoB-100 (amino acids 661–680 and 3136–3155, respectively) were quantified in plasma or serum by ELISA and detected at 405 nm as described.^{5,19} In brief, p45 and p210 were produced (KJ Ross Petersen AS, Horsholm, Denmark) and modified by treatment with 0.5 mol/L MDA. The thiobarbituric acid reactive substance assay was used to assess MDA modification of the peptides⁵ and the aldehyde content of p45 and p210 was 0.022 and 0.021 nmol/μg peptide, respectively. To remove the unbound MDA, the peptide was dialyzed against 0.15 mol/L phosphate-buffered saline (PBS) containing 1 mmol/L ethylene diamine tetraacetic acid of pH 7.4, using dialysis tubing with 1000 MW cut-off. Native and MDA-modified peptides were diluted in PBS pH 7.4 (20 μg/mL) and absorbed to microtitre plate wells and used to capture IgG and IgM antibodies directed against native or MDA-modified peptides 45 and 210 of apoB-100. The respective native and MDA-modified peptides in this study are designated p45_{natv}, p45_{MDA}, p210_{natv}, and p210_{MDA}, respectively.

Coronary angiography

Coronary angiography was performed as described, and QCA was used for the subsequent analyses.¹⁶ Minimum lumen diameter (MLD), reference diameter, percentage diameter stenosis, mean segment diameter (MSD), segment length, plaque area, segment area, and number of significant (>50%) stenosis were registered in each of the 15 coronary segments.

Statistical analyses

Statistical analyses were performed using Statview (SAS Institute Inc., USA) and STATA (Stata Corporation, USA) softwares and the level of significance was set to $P < 0.05$. Skewed data were transformed prior to the regression analysis. Owing to missing values in 25 patients and 12 controls, 362 patients and 375 controls were available for subsequent statistical analysis of SCARF participants. For group comparisons, percentages or medians (interquartile range) are presented and P -values were calculated by Mann–Whitney U test.

The upper quartiles of the concentrations of circulating antibodies against p210_{natv} and p210_{MDA} were identified and subjects with upper

Table 1 Basic characteristics of patients and control subjects

	Patients	Controls	P-value
<i>n</i>	387	387	
Age	54 (49–57)	54 (49–57)	
Male (%)	82	82	
Smokers (%)	50	25	<0.0001
Lipid-lowering medication (%)	35	0	<0.0001
BMI (kg/m ²)	26.8 (24.7–29.7)	25.6 (23.8–27.8)	<0.0001
SBP (mmHg)	130 (118–140)	128 (118–140)	0.60
DBP (mmHg)	80 (75–88)	80 (78–88)	0.19
Triacylglycerols (mmol/L)	1.6 (1.2–2.2)	1.2 (0.8–1.6)	<0.0001
LDL cholesterol (mmol/L)	3.2 (2.5–3.9)	3.4 (2.9–4.2)	<0.0001
HDL cholesterol (mmol/L)			
Men	1.0 (0.8–1.3)	1.3 (1.1–1.5)	<0.0001
Women	1.3 (1.0–1.5)	1.6 (1.4–1.9)	<0.0001
Glucose (mmol/L)	5.3 (5.0–5.9)	4.8 (4.6–5.2)	<0.0001
Insulin (pmol/L)	47.0 (32.0–69.0)	36.0 (27.5–50.5)	<0.0001
Proinsulin (pmol/L)	5.1 (3.4–7.5)	3.5 (2.6–5.4)	<0.0001
C-reactive protein (mg/L)	1.5 (0.7–3.4)	1.0 (0.5–1.8)	<0.0001
IL-6 (ng/L)	0.8 (0.6–1.4)	0.6 (0.5–1.0)	<0.0001
Fibrinogen (g/L)	3.8 (3.3–4.4)	3.5 (3.1–4.0)	<0.0001
PAI-1 (IU/mL)	12.6 (4.8–22.8)	7.5 (3.0–17.6)	<0.0001

Values are expressed as median (interquartile range) or percentage. CHD, coronary heart disease; BMI, body mass index; SBP, systolic blood pressure; DBP, diastolic blood pressure; LDL, low-density lipoprotein; HDL, high-density lipoprotein; IL-6, interleukin-6; PAI-1, plasminogen activator inhibitor-1.

quartile antibody levels were compared with the remaining individuals to test for (i) the risk of MI in all the individuals and (ii) differences in coronary angiography parameters in the subset of patients having undergone QCA.

A logistic regression model adjusted for traditional risk factors (except for LDL-C, owing to statin treatment in 134 patients) was applied to determine the association between antibodies against p210_{nat} and p210_{MDA} and the development of MI. Variables allowed to enter the model had a *P*-value <0.20 in univariable analysis. To test the predictive accuracy of the model, 10-fold cross-validation was applied that generated a mean odds ratio (with standard error) from 10 consecutive logistic regression analyses that each comprised 90% of the study population. The homeostasis model assessment (HOMA) index of insulin sensitivity was derived as described.²⁰ Smoking habits were identified from the structured interview conducted at 3 months after the acute event and smokers were defined as current smokers or former smokers and compared with never smokers. To identify individuals with dyslipidaemia, cut-offs for fasting plasma concentrations of TG >1.7 mmol/L were used for hypertriacylglycerolaemia (HyperTG) and HDL-C <1.0 mmol/L (in men) and <1.3 mmol/L (in women) for low HDL-C (HypoHDL).

Stepwise forward multiple regression analyses were used to identify the impact of antibodies on MSD, mean MLD, mean percentage stenosis, and plaque area/millimetre. These analyses were carried out in the subset of patients examined with QCA, in whom antibody data were available (*n* = 227) and adjusted for those traditional risk factors that each exhibited a univariable relationship to the respective QCA measurement with a *P*-value of less than 0.20. Stepwise multiple regression analyses were repeated with a backward approach and the accuracy of the multivariable model was tested by 10-fold

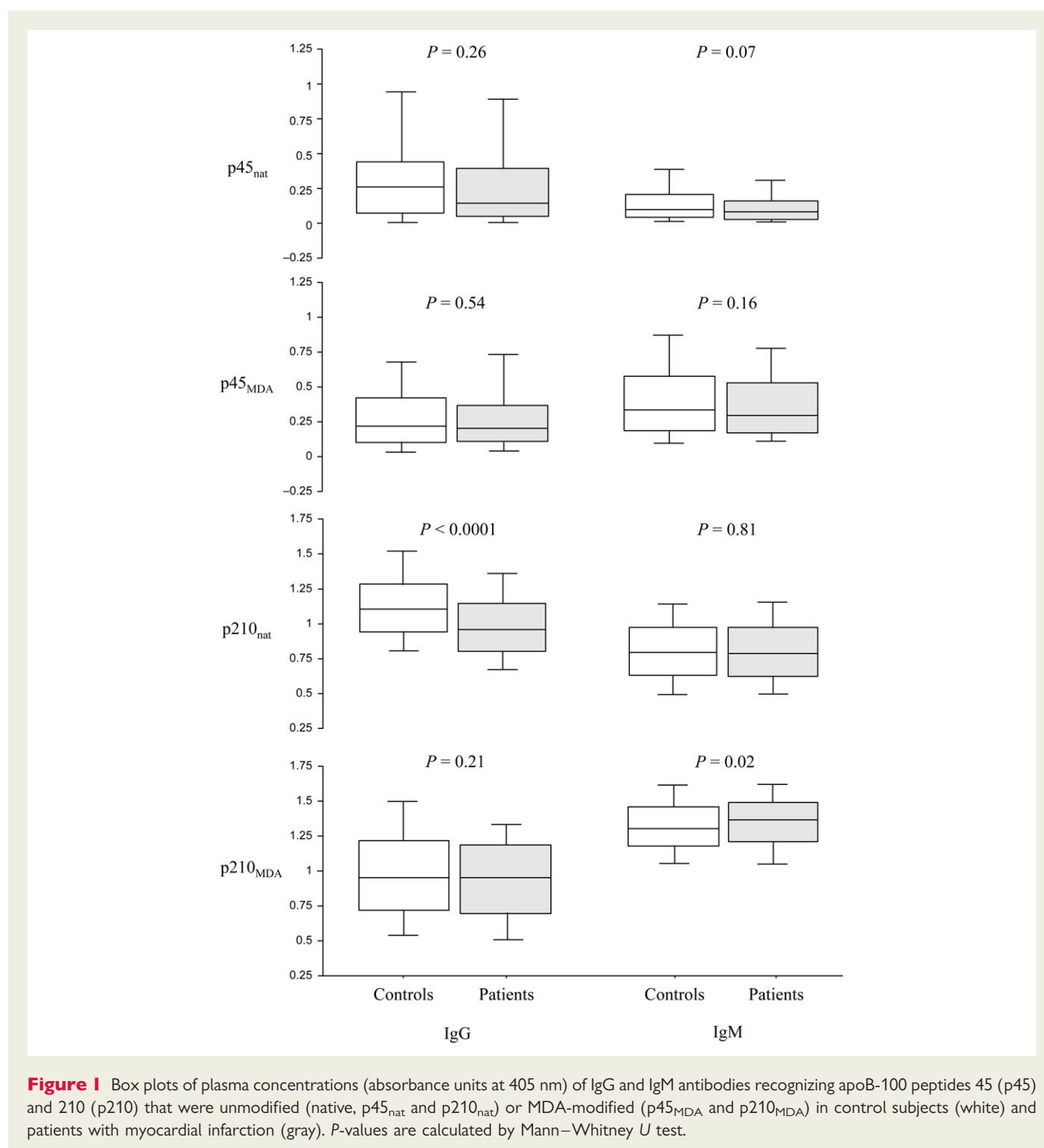
cross-validation. A mean standardized coefficient (with SE) from 10 consecutive analyses was generated. Of note, antibody determinations were forced into the models that failed to identify the antibody in question as an independent predictor (i.e. two of 10 models for MSD and three of 10 for mean MLD).

Time-courses for the circulating antibodies against certain epitopes of apoB were calculated on log-transformed data from participants in the longitudinal cohort study, using repeated measures of analysis of variance and Bonferroni/Dunn *post hoc* analysis. The Huynh-Feldt correction factor was used to adjust for covariance in repeated measures.²¹

Results

Basic characteristics of patients and controls are presented in Table 1 (most of which have been published previously).¹⁶ Compared with the controls, patients were more frequently smokers, were more obese, and had higher plasma concentrations of TG, glucose, insulin, proinsulin, C-reactive protein, IL-6, fibrinogen, and PAI-1, but lower HDL-C. LDL-C was significantly lower in patients, but this difference was abolished when patients on lipid-lowering drugs were excluded from the analysis. In addition, medication influencing blood pressure was taken by nearly all of the patients and no differences in blood pressure between patients and controls were found.

Data on circulating antibodies (Figure 1) revealed that patients had significantly lower titres of IgG against p210_{nat} (mean ± SD in absorbance units; 0.99 ± 0.27 vs. 1.13 ± 0.29) but higher titres of IgM against p210_{MDA} (1.35 ± 0.22 vs. 1.31 ± 0.23)



compared with controls. These differences were unrelated to sex or age of the subjects (data not shown). No significant differences in IgG and IgM antibodies against p45_{nat} or p45_{MDA} were found between the groups. Exclusion of patients on lipid-lowering treatment did neither influence the case–control differences in p210_{nat} and p210_{MDA}, nor did it affect the group comparisons for the other antibodies studied. However, it is notable that patients on lipid-lowering medication had significantly lower antibody titres than those without (e.g. IgG-p210_{nat}, 0.92 ± 0.30

vs. 1.02 ± 0.24 , $P = 0.0001$; and IgM-p210_{MDA}, 1.29 ± 0.25 vs. 1.37 ± 0.20 , $P = 0.0008$). Similarly, lipid-lowering medication (yes/no) was significantly related to p210_{nat} and p210_{MDA} titres in regression analysis (data not shown). In contrast, we found no indications of antibody-lowering effects of other compounds prescribed to the patients (such as aspirin, ACE-inhibitors, and calcium-channel inhibitors).

Multiple logistic regression analysis with MI as the outcome variable was performed in all the individuals in whom the risk

Table 2 Logistic regression analysis of the risk of myocardial infarction (MI)^a

	Univariable analysis		Multivariable analysis ^b	
	OR (95% CI)	P	OR (95% CI)	P
HyperTG	3.26 (2.37–4.48)	<0.0001	2.00 (1.33–2.99)	0.0008
HypoHDL	4.36 (3.11–6.11)	<0.0001	2.77 (1.89–4.05)	<0.0001
Smoking (yes/no)	2.03 (1.49–2.76)	<0.0001	1.67 (1.15–2.44)	0.008
HOMA index	9.49 (5.17–17.4)	<0.0001	3.27 (1.16–9.22)	0.02
Proinsulin	8.78 (4.80–16.1)	<0.0001	3.09 (1.21–7.87)	0.02
BMI	1.12 (1.08–1.17)	<0.0001	0.98 (0.92–1.04)	0.54
C-reactive protein	2.75 (1.99–3.81)	<0.0001	1.30 (0.84–2.00)	0.24
Fibrinogen	1.60 (1.33–1.91)	<0.0001	1.24 (0.97–1.58)	0.08
PAI-1	1.19 (1.10–1.29)	<0.0001	0.91 (0.81–1.02)	0.13
Upper quartile of IgG-p210 _{nat}	0.52 (0.37–0.73)	0.0002	0.55 (0.37–0.81)	0.003
Upper quartile of IgM-p210 _{MDA}	1.19 (0.86–1.67)	0.30	NE	

^aApplying a reverse statistical approach, i.e. using antibody levels instead of MI as the dependent variable in the model, resulted in corresponding relationships between the antibodies investigated and MI.

^b*n* = 696. NE, not entering. For other abbreviations, see Table 1. Variables with *P* < 0.20 in univariable regression analysis were considered for inclusion in the multivariable model.

associated with high antibody titres against p210 was evaluated (Table 2). Low HDL-C, smoking as well as high values of plasma TG, HOMA, and proinsulin were all independently associated with increased risk of MI. Conversely, high plasma IgG against p210_{nat} was associated with a substantially reduced risk (OR: 0.55, 95% CI 0.37–0.81) of MI, whereas high plasma IgM against p210_{MDA} was found not to be predictive of MI. Obesity and plasma concentrations of C-reactive protein, fibrinogen, and PAI-1 were not associated with MI, and inclusion of LDL-C in the model did not result in notable alterations of the risk estimates (data not shown). Exclusion of the patients on lipid-lowering medication did not materially influence the independent relationship of plasma IgG against p210_{nat} to MI (OR: 0.54, 95% CI 0.34–0.88). Of note, 10-fold cross-validation revealed that being in the upper quartile of IgG against p210_{nat} predicted MI in all the 10 models with a mean OR of 0.55 (\pm 0.004).

To evaluate the representativeness for antibody determinations of samples drawn at 3 months after the acute event, serial measurements of antibody concentrations starting during the acute stage of MI (blood samples drawn <4 h, 24 h, and 3 months after the onset of MI) were categorized as a separate group of 30 MI patients presenting with STEMI (Figure 2). Significantly higher IgG-p210_{nat} concentrations were found during the first 4 h compared with 24 h and 3 months after the onset of MI, while no alterations over time were noted for IgM-p210_{MDA}.

Antibodies against apoB-100 peptides were further analysed in relation to QCA findings in 227 patients belonging to the first sample of post-infarction patients, in whom all the measurements were available (Table 3). Patients with IgG against p45_{nat} in the upper quartile had significantly higher mean stenosis and plaque area/millimetre and those with IgG against p210_{nat} in the upper quartile had significantly higher MSD and mean MLD compared with the remaining patients. No relationships were found between any of the IgM autoantibodies and angiographic

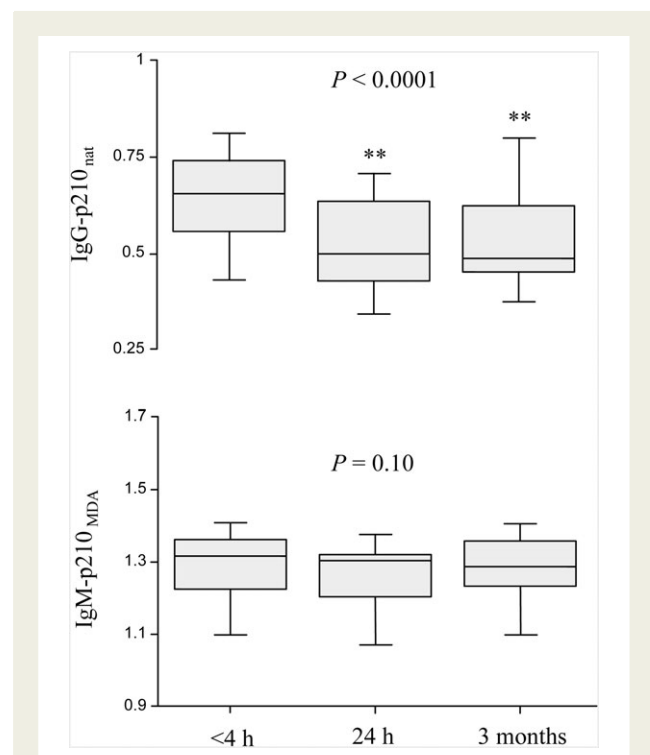


Figure 2 Serial measurements of antibody titres for IgG-p210_{nat} and IgM-p210_{MDA} after an ST-elevation myocardial infarction (*n* = 30). *P*-values are calculated from log-transformed data using repeated measures of analysis of variance with subsequent Bonferroni/Dunn *post hoc* analysis. ***P* < 0.0001 vs. <4 h.

measurements. Since only titres of IgG-p45_{nat} and IgG-p210_{nat} were related to the measures of coronary atherosclerosis, univariable correlations between these antibodies and established clinical

Table 3 Quantitative coronary angiography (QCA) data in patients divided according to the upper quartile of circulating IgG and IgM antibodies recognizing apoB-100 peptides 45 (p45) and 210 (p210) that were unmodified (native, p45_{nat} and p210_{nat}) or MDA-modified (p45_{MDA} and p210_{MDA})

	IgG			IgM		
	Q1–3	Q4	P	Q1–3	Q4	P
p45_{nat}						
MSD (mm)	2.9 (2.7–3.3)	3.0 (2.7–3.3)	0.53	3.0 (2.7–3.4)	2.9 (2.7–3.2)	0.64
Mean MLD (mm)	2.2 (2.0–2.5)	2.2 (1.9–2.5)	0.52	2.2 (1.9–2.5)	2.2 (1.9–2.5)	0.68
Mean stenosis (%)	31 (26–37)	34 (27–40)	0.03	32 (27–38)	28 (23–38)	0.08
Plaque area/mm	0.21 (0.17–0.25)	0.24 (0.20–0.27)	0.04	0.22 (0.17–0.26)	0.20 (0.16–0.24)	0.10
p45_{MDA}						
MSD (mm)	2.9 (2.7–3.3)	3.0 (2.7–3.3)	0.75	3.0 (2.7–3.3)	2.9 (2.7–3.3)	0.45
Mean MLD (mm)	2.2 (2.0–2.5)	2.2 (1.8–2.5)	0.63	2.2 (1.9–2.5)	2.2 (1.9–2.5)	0.70
Mean stenosis (%)	31 (26–38)	33 (27–38)	0.11	31 (27–38)	32 (23–38)	0.95
Plaque area/mm	0.21 (0.17–0.25)	0.23 (0.18–0.27)	0.15	0.22 (0.17–0.26)	0.20 (0.16–0.24)	0.07
p210_{nat}						
MSD (mm)	2.9 (2.7–3.3)	3.1 (2.9–3.5)	0.004	2.9 (2.7–3.3)	3.0 (2.7–3.2)	0.91
Mean MLD (mm)	2.1 (1.9–2.5)	2.4 (2.1–2.6)	0.002	2.1 (1.9–2.5)	2.3 (2.0–2.5)	0.34
Mean stenosis (%)	31 (27–38)	32 (23–37)	0.54	31 (27–38)	32 (22–37)	0.29
Plaque area/mm	0.21 (0.17–0.26)	0.22 (0.17–0.26)	0.79	0.22 (0.17–0.26)	0.22 (0.17–0.25)	0.26
p210_{MDA}						
MSD (mm)	2.9 (2.7–3.3)	3.0 (2.7–3.3)	0.33	3.0 (2.7–3.3)	3.0 (2.7–3.3)	0.97
Mean MLD (mm)	2.2 (1.9–2.5)	2.3 (2.0–2.5)	0.17	2.2 (1.9–2.5)	2.3 (1.9–2.6)	0.40
Mean stenosis (%)	32 (27–38)	30 (26–34)	0.20	31 (27–38)	32 (23–38)	0.75
Plaque area/mm	0.22 (0.17–0.26)	0.21 (0.17–0.25)	0.67	0.21 (0.17–0.26)	0.22 (0.17–0.26)	0.81

Values are median (interquartile range) in groups Q1–3 (quartiles 1–3 pooled) and Q4 (quartile 4). *P*-values calculated by the Mann–Whitney *U* test. MSD, mean segment diameter; mean MLD, mean minimal lumen diameter. Number of individuals in the various groups (Q1–3/Q4) were for IgG: p45_{nat} (170/57); p45_{MDA} (170/57); p210_{nat} (173/54); p210_{MDA} (169/58), and for IgM: p45_{nat} (170/57); p45_{MDA} (169/58); p210_{nat} (168/59); p210_{MDA} (171/56).

and biochemical risk indicators for MI are presented in Table 4. IgG-p210_{nat} was negatively related to plasma concentrations of TG, glucose, C-reactive protein, and IL-6, while positive relationships were found with LDL cholesterol as well as to titres of IgG-p45_{nat} and IgM-p210_{MDA} (the latter included because of the case–control difference observed). No corresponding relationships were found between IgG-p45_{nat} and risk indicators of MI.

Predictors of angiographic measurements were determined by multiple stepwise regression analyses, which included variables with a *P*-value < 0.20 in univariable analyses. Plasma concentrations of IgG recognizing p210_{nat} was independently and positively related to both MSD and mean MLD in multivariable analyses using both forward and backward approaches (Table 5). Of note, 10-fold cross-validation generated identical mean standard coefficients for IgG-p210_{nat} against MSD (mean $\beta = 0.17 \pm 0.08$) and mean MLD (mean $\beta = 0.15 \pm 0.07$). Although the number of patients decreased to 153 when individuals on lipid-lowering treatment were excluded, the univariable and multivariable relationships of plasma IgG recognizing p210_{nat} to MSD and mean MLD remained essentially unchanged (*F*-values: 4.4 for MSD and 3.7 for mean MLD in multiple stepwise regression analysis). The corresponding multivariable analyses did not identify any independent relationships between IgG-p45_{nat} and mean stenosis or plaque area/

millimetre (data not shown). The limited number of female patients precluded separate QCA analyses according to gender.

Discussion

The results from the present study indicate that individuals with high plasma levels of IgG recognizing the native form of p210 within apoB-100 have less coronary atherosclerosis and a 45% lower risk of developing MI. These findings are in line with the previous observations of atheroprotective effects of certain components of adaptive immunity and further support the notion that immune responses against specific epitopes of apoB may be especially important in atherogenesis. The fact that IgG antibodies against p210_{nat}, but not p210_{MDA}, demonstrated inverse relationships with coronary atherosclerosis and risk of MI is of particular interest. Immune responses to a native peptide could at first sight appear dubious and be interpreted to reflect non-specific binding of the antibody. However, it is more reasonable to assume that the antibody recognizing p210_{nat} is generated *in vivo* as a response to a mildly modified p210 that plays a more pivotal role in atherogenesis than immune responses against the more severely modified MDA variant (p210_{MDA}). Indeed, a recent publication showed atheroprotection from immunization

Table 4 Univariate correlations between the selected clinical and biochemical risk indicators and titres of IgG against the native apoB-epitopes 45 and 210

	IgG-p45 _{nat}		IgG-p210 _{nat}	
	r	P	r	P
BMI	0.05	0.15	-0.05	0.21
Plasma triacylglycerols	0.06	0.12	-0.08	0.03
Plasma cholesterol	0.06	0.08	0.07	0.06
LDL-C	0.06	0.13	0.10	0.009
HDL-C	-0.04	0.23	0.03	0.51
Insulin	0.02	0.51	-0.06	0.14
Proinsulin	-0.03	0.46	-0.03	0.38
Glucose	-0.02	0.59	-0.14	0.0002
C-reactive protein	0.06	0.13	-0.11	0.003
IL-6	0.01	0.73	-0.13	0.0006
Fibrinogen	0.03	0.44	-0.04	0.32
PAI-1	0.07	0.06	-0.01	0.79
IgG-p45 _{nat}			0.16	<0.0001
IgM-p210 _{MDA}	0.10	0.007	0.28	<0.0001

n = 737. For abbreviations, see Table 1. r-Values are Spearman rank correlation coefficients.

Table 5 Multiple forward stepwise regression analyses of independent determinants of mean segment diameter (MSD) and mean minimal lumen diameter (mean MLD)

	β	F-to-remove	Adjusted R ²
MSD			
HOMA index	-0.29	13.8	0.12
LDL-C	-0.20	8.9	
IgG-p210 _{nat}	0.17	6.8	
SBP	0.17	6.6	
BMI	0.19	5.8	
Mean MLD			
LDL-C	-0.21	10.4	0.13
HOMA index	-0.20	9.2	
Age	-0.15	5.6	
IgG-p210 _{nat}	0.15	5.5	

n = 227. β indicates standardized coefficient. Additional variables considered for inclusion, but not entering the models were fibrinogen for the MSD model and plasma concentrations of triacylglycerol, proinsulin, IL-6, and PAI-1 for the mean MLD model. IgG-p210_{nat}, IgG antibodies against native peptide 210. For other abbreviations, see Tables 1 and 2.

with another native apoB-peptide²² and unpublished experimental data indicate that native p210 induces stronger atheroprotective effects than its MDA-modified counterpart (Fredrikson et al., manuscript in preparation). The finding that high IgG, but not IgM titres against the apoB p210 epitope are associated with a lower risk for MI suggest the involvement of T cell-dependent

immune responses in the protective phenotype. It remains to be clarified whether this reflects a higher target specificity of p210 IgG or if the IgG titre acts as a marker for other protective T cell-mediated immune responses. However, the finding is well in line with the observation from animal studies that atheroprotection by immunization with apoB peptides is associated with increased levels of specific IgG but not IgM.^{12,23}

Previous experimental studies, based on this library of apoB-100 peptides, have shown pronounced atheroprotective effects when immunizing against p45, using both passive and active immunization strategies.^{23,24} Furthermore, in a recent prospective case-control study of 75 patients with acute MI or sudden cardiac death, cases had lower baseline circulating levels of IgG antibodies against p45_{nat}, and titres of IgG against p45_{MDA} were inversely and independently related to carotid stenosis in both cases and controls.¹⁴ However, in the present study, none of the antibodies recognizing p45 (native or MDA-modified) were related to MI or measures of coronary atherosclerosis. Instead, patients in our study exhibited lower titres of IgG against p210_{nat} and higher titres of IgM against p210_{MDA}. This is in line with a recent publication on 751 individuals with asymptomatic carotid disease, which showed a positive relationship between IgM against p210_{MDA} and progression of carotid atherosclerosis.¹⁵ Importantly, however, the IgG antibody recognizing native p210 (strongly related to MI and coronary atherosclerosis in our subjects) was not quantified in that study. Although the results from our study could indicate that higher antibody titres against p210_{MDA} in patients might reflect a more advanced stage of disease with more severely modified LDL accumulating in plaques, the lack of association between p210_{MDA} antibodies and coronary atherosclerosis argue against this explanation.

The consensus today is that both adaptive immunity and oxLDL mediate proatherogenic effects. Nonetheless, there is convincing evidence from animal studies that immunization with oxLDL is atheroprotective.¹³ It is presently unclear which mechanisms drive the anti-atherosclerotic effects of immunization, and whether the same effects would be achievable in man. So far, clinical studies in humans have identified circulating antibodies against oxLDL that are related to atherosclerotic disease.²⁵ The findings are, however, not consistent, describing both protective and detrimental relationships for anti-oxLDL antibodies, and a large proportion of this inconsistency is likely to be explained by the heterogeneity of oxLDL. In a recent study of 504 patients undergoing coronary angiography, no independent relationships were found between coronary atherosclerosis and antibodies recognizing LDL modified by different techniques.²⁶ Nevertheless, immune responses to other epitopes of oxLDL could have a pivotal role in atherosclerosis. The present study contributes novel information to this field by demonstrating that high plasma concentrations of antibodies against native p210 of apoB-100 are related to lower risk of MI and less coronary atherosclerosis. It remains uncertain, although, whether the antibodies themselves are responsible for these beneficial effects or whether the underlying immune responses alter the course of atherosclerosis. Immunization studies show that protection against the progression of atherosclerosis correlates with antibody concentrations,^{9,27} but a recent immunization study with oxLDL in B-cell-deficient mice

revealed atheroprotection even in the absence of antibodies.²⁸ This effect was paralleled by a significant increase in circulating IL-10 and T-regulatory cells, suggesting an immunization-induced modulation of T-cell activity with anti-inflammatory and anti-atherosclerotic effects. Importantly, although, T-cell-deficient mice develop less atherosclerosis after immunization,²⁹ underlining the complexity of these responses and suggesting a possible interaction between adaptive and innate immunity in immunization-induced atheroprotection. Clearly, further studies are needed to explore how the balance between protective and aggravating immune responses can be controlled in atherosclerosis.

Although the case–control design is a useful tool in epidemiological and clinical studies, the retrospective nature of the present study might have conferred a selection bias. Thus, it cannot be excluded that patients who died during the acute phase of MI, and consequently were not enrolled, had different plasma concentrations of autoantibodies directed against apoB compared with the participating survivors. Judged from the serial measurements made in patients presenting with STEMI, it appears that the circulating concentrations of IgG-p210_{nat} increase during the early stage of MI. This could be owing to the strong oxidative stress present in the ischaemic myocardium, promoting LDL oxidation, and a rapid boost of the antibody response in the already primed individuals. Secondly, the serial antibody determinations indicate that antibody measurements at 3 months after the acute event reflect a steady-state condition, rendering this time-point appropriate for antibody quantification.

Lipid-lowering medication in 35% of the patients (statin monotherapy in the vast majority of patients on treatment) could potentially result in spurious findings regarding antibody titre relationships to the severity of coronary atherosclerosis and risk of MI. Indeed, patients prescribed lipid-lowering compounds were found to have significantly lower plasma antibody concentrations than those without. The apparent antibody-lowering effect of statin treatment is most likely explained by the anti-inflammatory actions previously described for statins. However, the fact that all the associations between plasma antibody concentrations against p210_{nat} remained unaltered in the subset of patients without lipid-lowering medication speaks strongly against the possibility of spurious results.

In conclusion, this is the first study to demonstrate that circulating IgG antibodies against native peptide 210 of apoB-100 are strongly and inversely related to the severity of coronary atherosclerosis and associated with lower risk of MI. These findings provide clinical support for the previous and ongoing experimental studies showing atheroprotective effects of immune responses against certain peptides of apoB-100 and identify p210 as an interesting target for immunization and prevention against coronary atherosclerosis in humans.

Conflict of interest: none declared.

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CLINICAL VIGNETTE

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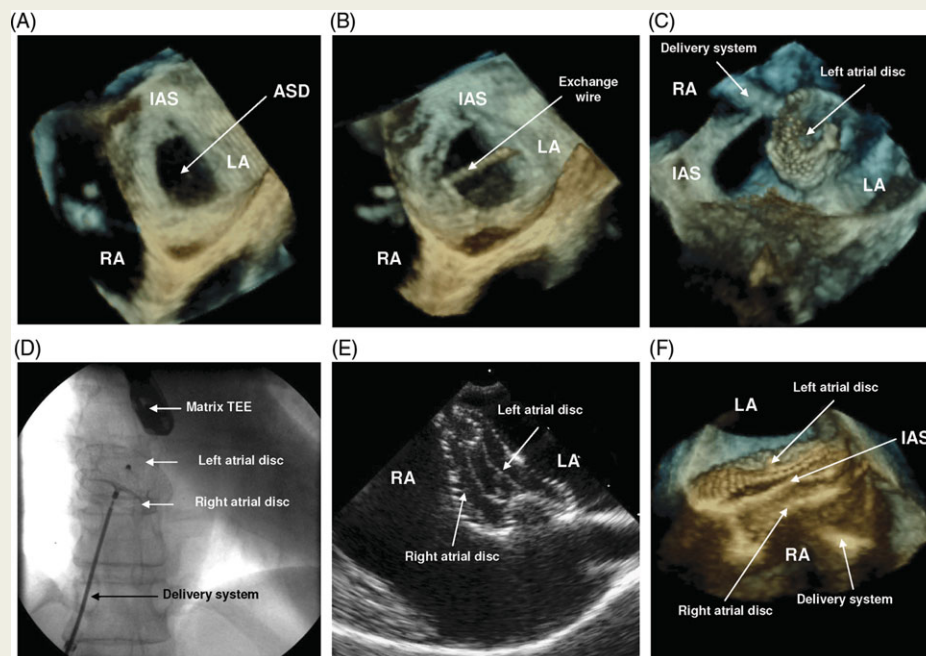
Real-time three-dimensional transoesophageal echocardiography for guidance of atrial septal defect closures

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ASD closure in a 53 year old female with significant left-to-right shunting was performed under fluoroscopic and TEE guidance. We used a novel matrix array three-dimensional (3D) TEE probe (X7-2t, Philips Medical Systems), providing exact imaging of cardiac structures in real-time. Panel A shows a 3D image of the ASD displaying an en-face view from the left atrium. The size and shape of the defect including the rim of the interatrial septum (IAS) are displayed on-line allowing assessment of the complete circumference of the ASD. Panel B demonstrates the setting of the exchange wire, centrally passing through the ASD orthogonally, which can be depicted with only a single 3D perspective.



After unfolding of the left atrial disc, spatial orientation of the device as well as distance and relation of disc size to defect size can easily be assessed (Panel C). After unfolding of the right atrial disc, exact positioning of the device has to be ensured prior to definite release. Fluoroscopy fails to delineate the relation of the device to the IAS (Panel D). Conventional 2D TEE (Panel E) demonstrates the appropriate localization of discs but only the 3D image enables to exactly visualize the correct position of the occluder and its relation to the IAS in a real-time mode (Panel F). We here show that the application of real-time 3D TEE is feasible for the guidance of percutaneous transcatheter ASD closures, highlighting advantages and the capability of the technique to increase safety, accuracy, and efficacy of the procedure