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Autoantibodies against modified apolipoprotein B-100 in relation to low-density lipoprotein size and the metabolic syndrome in otherwise healthy men

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Abstract

The role of inflammation in atherosclerotic disease is well established, but the role of autoantibodies against modified apolipoprotein (apo) B-100 remains unclear. The metabolic syndrome is associated with a proinflammatory state, a predominance of small dense low-density lipoprotein (LDL) particles, and an increased risk for atherosclerotic diseases. Previous studies have shown specific autoantibodies against modified apo B-100 (within LDL) to be related to human atherosclerotic disease. The objective of the present study was to investigate whether autoantibodies against modified apo B-100 are related to parameters of the metabolic syndrome, such as small dense LDL. Two hundred ninety-one healthy men were investigated for different metabolic, anthropometric, and inflammatory variables; LDL peak particle size; and distribution of LDL in 4 subfractions. Subjects were grouped according to LDL peak size ≥23.5 nm (pattern A, n = 230) or <23.5 nm (pattern B, n = 61). Immunoglobulin (Ig) G and IgM antibodies against 2 aldehyde-modified peptide sequences, denoted as 45 and 210, within apo B-100 were quantified. Levels of IgG_{45}, but not the other autoantibodies, were significantly higher in pattern B individuals (with a predominance of small dense LDL particles) compared with pattern A ($P < .01$). Relationships for both IgG_{45} and IgG_{210} with parameters typically associated with the metabolic syndrome were found. Only IgG_{45} tended to be higher in individuals with the metabolic syndrome compared with those without ($P = .07$). We conclude that subjects with a predominance of small dense LDL particles have elevated concentrations of IgG_{45} in the circulation, which reflect an activated immune response to a specific epitope of modified apo B-100.

1. Introduction

The central role of inflammation in atherosclerotic disease is today well recognized, and attention is now focused on the immune system as a possible target in the prevention and treatment of cardiovascular disease. Atherosclerotic plaques contain several antigens, some of them endogenously derived, for example, modified low-density lipoprotein (LDL), which can trigger both innate and adaptive immune responses [1]. Low-density lipoprotein can be modified in the vascular wall, for example, by oxidation; and oxidized LDL (oxLDL) is now accepted as a probable initiator of atherosclerotic disease. Autoantibodies against oxLDL are common in man, and these antibodies are of both immunoglobulin (Ig) G and IgM nature [1]. Moreover, relationships between circulating levels of autoantibodies directed against modified sequences of apolipoprotein (apo) B-100 (the main protein within LDL) and human atherosclerotic disease have been found [2,3]. In these studies, a library of aldehyde-modified polypeptides (covering the complete sequence of apo B-100) was used to detect both IgG and IgM autoantibodies. Antibodies against certain

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peptides (such as peptides 45 and 210) showed strong relationships to human atherosclerosis [2]. Although the biological role of these autoantibodies in the atherosclerotic process remains to be clarified, promising immunization studies in mice (using peptides of modified apo B-100) may pave the way for similar studies in man [3,4].

The metabolic syndrome, with its characteristic dyslipidemia (high triacylglycerol, low high-density lipoprotein [HDL] and small dense LDL), promotes the development of atherosclerotic disease [5]. Individuals with this syndrome exhibit high concentrations of inflammatory markers, such as C-reactive protein (CRP), indicating a proinflammatory state that contributes to the development of atherosclerosis [5]. The small dense LDL particles associated with the metabolic syndrome are believed to be especially atherogenic, partly because of their propensity to become oxidized [5]. Therefore, a relationship between the occurrence of small dense LDL and circulating levels of autoantibodies directed against modified apo B-100 might be expected. Furthermore, because the small dense LDL phenotype is characteristic of the metabolic syndrome, these autoantibodies could also be expected to be related to the metabolic syndrome and its components. Against this background, we quantified IgG and IgM autoantibodies directed against specific modified sequences of apo B-100 in a sample of 63-year-old healthy men in whom all components of the metabolic syndrome had been characterized in detail.

2. Subjects and methods

2.1. Subjects

In the present study, 291 individuals aged 62 to 64 years were enrolled from a previously recruited population-based cohort of 2039 Swedish men [6]. The 2039 men were divided into tertiles of fasting plasma insulin concentrations; and approximately 100 subjects from each tertile were randomly recruited to this study, resulting in a study population with a range of insulin sensitivities. Exclusion criteria were non-Swedish descent; manifest diabetes, cardiovascular disease, cancer, or chronic degenerative disease; treatment with antihypertensive or lipid-lowering agents; and body mass index (BMI) outside 19 to 35 kg/m^2. Recruitment details and clinical procedures have been described [7]. The Ethics Committee of Karolinska Institutet approved the study, and all subjects gave informed consent.

2.2. Biochemical analysis

In light of the strong relationships found previously, IgG and IgM autoantibodies directed against aldehyde-modified sequences (peptides 45 and 210; amino acids 661-680 and 3136-3155, respectively) of human apo B-100 were quantified in plasma by enzyme-linked immunosorbent assay as described [2] and designated IgG_{45}, IgG_{210}, IgM_{45}, and IgM_{210}.

The LDL particle size distribution was determined (as described by Sjogren et al [7]) by 3% to 7.5% polyacrylamide gradient gel electrophoresis, staining, and subsequent scanning, giving peak particle size of LDL and relative distribution of LDL in predefined subfractions with cutoffs: LDL-I (27.0-25.0 nm), LDL-II (25.0-23.5 nm), LDL-III (23.5-22.5 nm), and LDL-IV (22.5-21.0 nm), corresponding to densities of 1.006 to 1.030, 1.030 to 1.040, 1.040 to 1.050, and 1.050 to 1.063 kg/L, respectively. Individuals were grouped according to a predominance of either large buoyant LDL (pattern A) or small dense LDL (pattern B) based on their LDL peak particle size (pattern A, ≥23.5 nm; pattern B, <23.5 nm, corresponding to the classic boundary of 1.040 kg/L) [8]. Oxidized LDL was captured with the monoclonal antibody 4E6 (Mercodia, Uppsala, Sweden) as described [9]. Commercially available enzyme-linked immunosorbent assays were used to quantify insulin and proinsulin (DAKO, Glostrup, Denmark), CRP (Haemochrom Diagnostica, Mölndal, Sweden), tumor necrosis factor α, and interleukin-6 (R&D Systems, Abingdon, UK). The homeostasis model assessment (HOMA) of insulin sensitivity was derived as described [10].

2.3. Defining the metabolic syndrome

The metabolic syndrome was defined as present when a subject exceeded the cutoffs for at least 3 of 5 components: triacylglycerol (>1.7 mmol/L), glucose (≥5.6 mmol/L), HDL (<1.0 mmol/L), waist circumference (>102 cm), and blood pressure (BP) (systolic BP ≥130 or diastolic BP ≥85 mm Hg) according to the National Cholesterol Education Program (Adult Treatment Panel III) [11].

2.4. Statistical analysis

For statistical analyses, Statview (SAS Institute, Cary, NC) software was used. Unpaired *t* tests were performed to compare biochemical data between pattern A and pattern B individuals. If skewed, variables were log transformed before analysis; but arithmetic means ± SD are presented for ease of understanding. Nonparametric statistics (Spearman correlation and Mann-Whitney) were used with significance level set to $P < .05$. Multiple regression analysis was used to establish whether relationships were independent of small dense LDL.

3. Results

3.1. Characteristics

Basic characteristics of the 291 men included in the present study are presented in Table 1. No subject had manifest diabetes mellitus; and metabolic and anthropometric values were in the reference ranges for a group of 63-year-old healthy men, although the degree of dyslipidemia was fairly modest. Grouping the subjects according to LDL pattern A or pattern B identified 230 individuals with pattern A and 61 individuals with pattern B. Compared with

Table 1
Characteristics of the whole cohort and individuals divided into pattern A and pattern B based on their LDL peak particle size

	All (n = 291)	Pattern A (n = 230)	Pattern B (n = 61)
LDL peak size (nm)	23.9 ± 4.5	24.1 ± 0.3	23.2 ± 0.2***
LDL-II (%)	53 ± 9	57 ± 6	41 ± 7***
LDL-III (%)	19 ± 9	15 ± 5	33 ± 6***
oxLDL (U/L)	62 ± 19	60 ± 17	72 ± 20***
oxLDL/LDL-C	17 ± 4	17 ± 4	19 ± 5***
BMI (kg/m^2)	25.8 ± 3.1	25.5 ± 3.0	27.0 ± 2.9**
Waist circumference (cm)	96.2 ± 8.9	95.3 ± 8.9	99.8 ± 8.0**
Systolic BP (mm Hg)	135 ± 17	135 ± 17	138 ± 17
Diastolic BP (mm Hg)	81 ± 9	81 ± 8.5	82 ± 8.9
Smoking (n/d)	2 ± 6	2 ± 6	2 ± 7
Insulin (pmol/L)	41 ± 24	38 ± 22	53 ± 27***
Glucose (mmol/L)	5.1 ± 1.0	5.0 ± 0.9	5.4 ± 1.4**
HOMA	1.6 ± 1.2	1.4 ± 1.0	2.2 ± 1.6***
Triacylglycerol (mmol/L)	1.2 ± 0.6	1.0 ± 0.4	1.9 ± 0.8***
HDL-C (mmol/L)	1.7 ± 0.4	1.7 ± 0.4	1.4 ± 0.2***
LDL-C (mmol/L)	3.7 ± 0.9	3.6 ± 0.9	3.8 ± 0.9
CRP (mg/L)	2.0 ± 2.5	2.0 ± 2.7	2.1 ± 1.6*
Interleukin-6 (μg/L)	1.5 ± 1.4	1.4 ± 1.2	1.8 ± 2.2*
Tumor necrosis factor α (μg/L)	2.3 ± 1.1	2.3 ± 1.2	2.2 ± 1.1

Values are mean ± SD. *P* values are calculated from log-transformed data when skewed.
* $P < .05$ compared with pattern A individuals.
** $P < .01$ compared with pattern A individuals.
*** $P < .0001$ compared with pattern A individuals.

pattern A, pattern B individuals had significantly higher triacylglycerol, insulin, BMI, oxLDL, and inflammatory markers, lower HDL cholesterol (HDL-C), but similar LDL cholesterol (LDL-C) concentrations (Table 1).

3.2. IgG and IgM autoantibodies

Quantification of IgG and IgM autoantibodies revealed peptide 45 of apo B-100 to capture more IgG antibodies than IgM (approximately 50% more), whereas the contrary was true for peptide 210.

Group comparisons showed only IgG_{45} to be significantly higher in pattern B compared with pattern A individuals (Fig. 1). Correlation analyses showed IgG_{45} to be the antibody most consistently related to parameters of the metabolic syndrome (Table 2). IgG_{45} was negatively related to HDL-C and positively related to BMI, waist circumference, plasma triacylglycerol, CRP, and different measures of small dense LDL (the latter not always statistically significant; LDL-II, $P = .06$; LDL-III, $P = .08$). In turn, IgG_{210} was negatively related to HDL-C, LDL-C, large buoyant LDL (LDL-II), and oxLDL ($P = .08$) and positively related to diastolic BP. To investigate whether the statistically significant correlations between IgG-antibodies and metabolic parameters identified in Table 2 were mediated via small dense LDL, the correlations were adjusted for measurements of LDL particle size (LDL peak size for IgG_{45} and LDL-II for IgG_{210} because these measures were correlated with the respective autoantibodies). Significant relationships remained between IgG_{210} and diastolic BP ($r = 0.15$, $P = .013$) and LDL-C ($r = -0.13$, $P = .023$) when adjusted for LDL-II, whereas only a borderline significant relationship remained between IgG_{45} and BMI ($r = 0.12$, $P = .051$) when adjusted for LDL peak size. Controlling for LDL particle size abolished the relationships between IgG antibodies and the other metabolic parameters (waist, triacylglycerol, HDL-C, and CRP). Finally, 31 individuals were identified as having the metabolic syndrome; and these subjects had higher levels of IgG_{45} and IgG_{210} (although not statistically significant) compared with those without the syndrome (mean ± SD, absorbance at 405 nm): 0.43 ± 0.35 vs 0.36 ± 0.37, $P = .07$ for IgG_{45} and 0.75 ± 0.27 vs 0.67 ± 0.22, $P = .08$ for IgG_{210}.

No significant correlations were found between IgM_{45} or IgM_{210} and the parameters included in Table 2 (data not shown) except that both correlated positively with the oxLDL/LDL-C ratio ($r = 0.14$, $P < .05$ and $r = 0.12$, $P = .05$, respectively). Furthermore, IgM_{210} was significantly correlated with LDL peak size ($r = -0.12$, $P < .05$) and the amount of large buoyant LDL (LDL-II, $r = -0.19$, $P < .01$), but not with LDL-III.

4. Discussion

Cross-sectional data from this well-characterized cohort have been reported previously [7,9,12-14]. In the present study, we have demonstrated that healthy 63-year-old men with a predominance of small dense LDL particles have increased concentrations of circulating IgG_{45}, an antibody directed against a modified epitope (amino acids 661-680) of apo B-100. This particular antibody was also weakly related to parameters typical of the metabolic syndrome (obesity, dyslipidemia, and CRP), which was reflected in a tendency

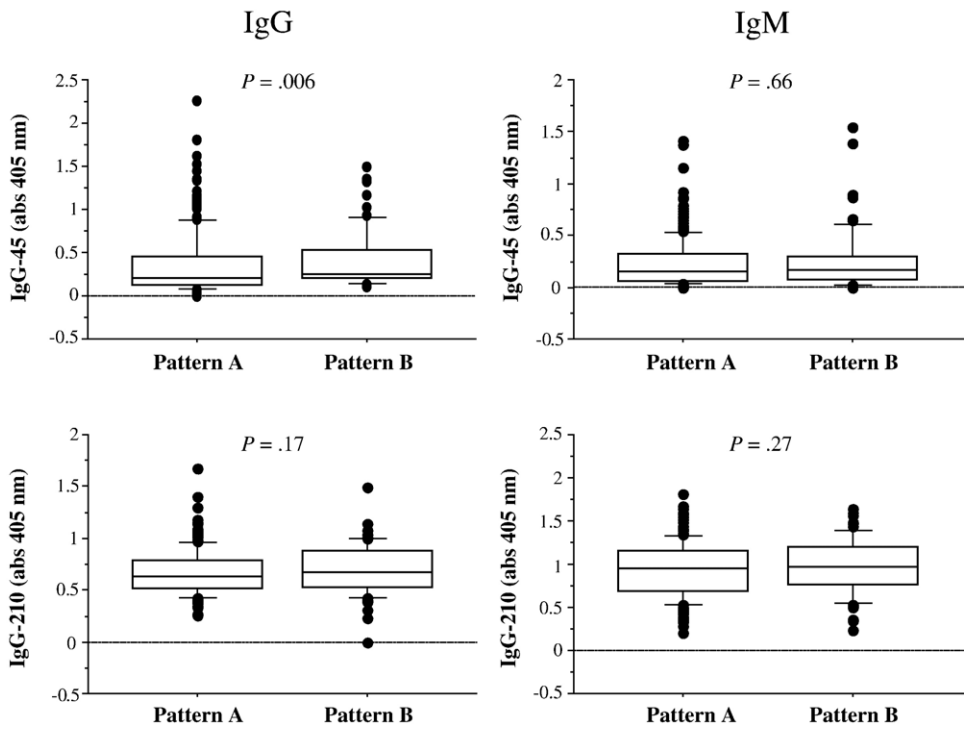

Fig. 1. Specific IgG and IgM autoantibodies in men grouped according to LDL peak particle size ≥23.5 nm (pattern A, n = 230) or <23.5 nm (pattern B, n = 61). Immunoglobulin G_{45} and IgG_{210} in the left panel; IgM_{45} and IgM_{210} in right panel. *P* values calculated from Mann-Whitney test.

toward increased IgG_{45} levels in subjects with the metabolic syndrome. These relationships might have an impact on atherosclerotic risk in subjects with a predominance of small dense LDL and/or the metabolic syndrome because patients with coronary heart disease demonstrated increased levels of IgG_{45} [2]. However, it is uncertain if this antibody should be considered proatherogenic. Immunization studies with peptide 45 have shown beneficial effects on atherosclerosis in mice (discussed below). Furthermore, treating mice with recombinant antibodies specific for aldehyde-modified peptide 45 significantly reduced plasma concentrations of oxLDL [15], suggesting that IgG_{45} could enhance clearance of oxLDL as a response to activated immunity.

Antibodies against peptide 210 (both IgG and IgM) were not elevated in pattern B individuals. However, IgG_{210} correlated negatively with large buoyant LDL (LDL-II), although the absence of significant correlations with other measures of LDL size suggests that this may not be a direct relationship to LDL size distribution. Furthermore, IgG_{210} correlated negatively with LDL-C and of borderline significance with oxLDL, suggesting that also IgG_{210} could mediate the clearance of circulating LDL particles (native as well as oxidized), a concept suggested in the literature [16]. Although IgM_{210} correlated negatively with LDL peak size and large buoyant LDL (similar to IgG_{45}), overall, few relationships were found for the IgM antibodies.

The healthiness of the individuals included in this cohort is apparent from the demographic data presented in Table 1. This may well reflect our stringent exclusion criteria that were chosen to exclude conditions such as severe obesity and treatment with lipid-lowering agents, which are known to affect many of the metabolic parameters under investigation. On the other hand, the stringent selection procedure may have

Table 2
Relationships between specific IgG autoantibodies against modified peptide sequences within apo B and selected parameters

	IgG_{45} (*r*)	IgG_{210} (*r*)
LDL peak size (nm)	−0.15 *	−0.05
LDL-II (%)	−0.11	−0.20 **
LDL-III (%)	0.10	0.04
oxLDL (U/L)	0.05	−0.10
oxLDL/LDL-C	0.10	0.04
BMI (kg/m^2)	0.16 **	0.03
Waist circumference (cm)	0.14 *	0.04
Systolic BP (mm Hg)	−0.01	0.05
Diastolic BP (mm Hg)	0.05	0.17 **
Smoking (n/d)	−0.07	−0.10
Insulin (pmol/L)	0.07	0.05
Glucose (mmol/L)	0.04	0.07
HOMA	0.08	0.05
Triacylglycerol (mmol/L)	0.15 *	0.07
HDL-C (mmol/L)	−0.13 *	−0.13 *
LDL-C (mmol/L)	−0.03	−0.17 **
CRP (mg/L)	0.15 *	0.05
Interleukin-6 (μg/L)	0.01	0.08
Tumor necrosis factor α (μg/L)	0.03	0.05

n = 291. *P* values calculated from Spearman correlation analyses.
* $P < .05$.
** $P < .01$.

restricted the possibility to identify biochemical differences within the population and lead to an underestimation of the strength of any relationship. This might have contributed to the relatively weak correlations that were found between the autoantibodies quantified and the parameters investigated. Furthermore, the relationships between autoantibodies and most of the metabolic parameters were no longer statistically significant once LDL particle size was taken into account (multivariate analysis), suggesting that the associations between autoantibodies against modified apo B-100 and parameters of relevance for the metabolic syndrome were largely mediated through small dense LDL particles.

The complex role of the immune response in atherosclerosis is not yet fully understood, and the interpretation of autoantibodies in this context is not straightforward. Although the literature contains some conflicting results, most studies investigating whole classes of autoantibodies have shown elevated IgG titers to oxLDL to be related to atherosclerotic disease, whereas IgM titers show the reverse [16]. However, investigating antibodies against specific epitopes of apo B-100 (as in the present study) might cause this concept to be revised because recent findings have identified certain epitopes to be highly associated with atherosclerotic disease [3]. One such epitope is peptide 45, and increased concentrations of antibodies against this sequence of modified apo B have been related to human atherosclerosis (as discussed above) [2]. On the other hand, immunization studies with peptide 45 in apo E–deficient mice resulted in increased levels of IgG_{45} (but not IgM_{45}) and a beneficial effect on atherosclerosis (decreased by 48% compared with controls) despite increases in both plasma cholesterol and triacylglycerol [17]. Interestingly, the atheroprotective effect of this immunization was concomitant with a shift in IgG antibody expression from Th1 to Th2 response, which strengthens the idea of Th2 having an antiatherogenic effect [1,3]. Whether this immunologic response is also of relevance in man remains to be clarified. Clearly, further studies are needed to understand the complex role of immunity in atherosclerotic disease.

In summary, this cross-sectional study showed IgG_{45}, an antibody against a specific epitope of apo B-100 (and previously associated with atherosclerotic disease), to be elevated in healthy 63-year-old men with a predominance of small dense LDL particles. Furthermore, associations between IgG_{45} and small dense LDL mediated relationships of this autoantibody to parameters of the metabolic syndrome; but whether these associations are of importance for atherosclerotic risk in these individuals remains to be shown in studies of prospective design.

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