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Oxidized Low-Density Lipoprotein Autoantibodies, Chronic Infections, and Carotid Atherosclerosis in a Population-Based Study

Manuel Mayr, MD, PHD,*‡ Stefan Kiechl, MD,§ Sotirios Tsimikas, MD, FACC,‖ Elizabeth Miller, BS,‖ Joanna Sheldon, PHD,† Johann Willeit, MD,§ Joseph L. Witztum, MD,‖ Qingbo Xu, MD, PHD*‡

London, United Kingdom; Innsbruck, Austria; and La Jolla, California

OBJECTIVES We investigated whether associations exist between immune reactions to oxidized low-density lipoproteins (OxLDLs), chronic infections, and carotid atherosclerosis as quantified by ultrasound.

BACKGROUND Atherosclerosis is a chronic immuno-inflammatory disease wherein both oxidized lipids and infectious agents are incriminated as possible contributors.

METHODS We measured immunoglobulin (Ig)G and IgM autoantibody titers to copper-oxidized-LDL and malondialdehyde-LDL (OxLDL-AB), IgG and IgM apolipoprotein B-100-immune complexes (ApoB-IC), and titers of antibodies to *Escherichia coli* and chlamydial lipopolysaccharide (LPS), mycobacterial heat shock protein 65 (mHSP65), *Chlamydia pneumoniae*, *Helicobacter pylori*, and cytomegalovirus and evaluated their relationship to cardiovascular risk factors, chronic infections, and incident/progressive carotid atherosclerosis in the Bruneck study.

RESULTS The OxLDL-AB and ApoB-IC levels remained stable over time as indicated by strong correlations between 1995 and 2000 measurements ($p < 0.001$ each). Significant associations existed between all OxLDL markers and antibody titers to pathogens, especially to *E. coli*-LPS and mHSP65. Both OxLDL-AB and ApoB-IC levels showed a rise with increasing pathogen burden. Notably, OxLDL-ABs were also elevated in subjects with chronic infection as defined by clinical criteria. Titers of IgG, but not IgM, OxLDL-AB, or ApoB-IC inversely correlated with total cholesterol, LDL cholesterol, and apoB concentrations. The IgG OxLDL markers were positively and IgM markers were inversely associated with incident and progressive carotid atherosclerosis in univariate analyses but were not independent predictors in multivariate analyses.

CONCLUSIONS Our study provides evidence for an association between human oxLDL markers and chronic infections. Moreover, in this population-based study, neither IgG nor IgM OxLDL autoantibodies were independently predictive of atherosclerosis progression in the carotid arteries. (J Am Coll Cardiol 2006;47:2436–43) © 2006 by the American College of Cardiology Foundation

It has been demonstrated that immunoglobulin (Ig)G and IgM autoantibodies to oxidized low-density lipoprotein (OxLDL-AB) are present in the plasma of animals and humans and form immune complexes with OxLDL in atherosclerotic lesions and plasma (1,2). Increased OxLDL-AB titers after immunization with OxLDL (3–6) or *Streptococcus pneumoniae* (7), which contains an identical phosphocholine epitope on its cell wall as OxLDL, have been associated with reduced atherosclerosis progression in both murine (4,6,7) and rabbit (3,5) models of atherosclerosis. In murine models, OxLDL-AB correlate closely with atherosclerosis progression and regression (8,9). Immunization with malondialdehyde (MDA)-LDL, as a model oxidation-specific epitope of OxLDL, also provides atheroprotection (3,6) at least in part through induction of the same E06/T15 IgM antiphosphocholine antibodies mediated by interleukin-5 (10). The natural murine IgM autoantibody E06 and similar E0 autoantibodies were found to have 100% homology in the variable region to classic "natural" murine T15 anti-phosphocholine antibodies (11) that provide optimal protection against lethal infection by *S. pneumoniae* and other pathogens (12). These observations raise the intriguing possibility that infectious agents could increase the titers of this type of idiotypic human autoantibodies, which in turn influence atherogenesis.

Microbial pathogens have repeatedly been implicated in atherogenesis (13,14). Evidence in favor of this hypothesis derives from in-vitro experimental and seroepidemiologic studies, which primarily focused on *Chlamydia pneumoniae*, *Helicobacter pylori*, and cytomegalovirus (CMV) (13,15). But a causal role of infections in the initiation and progression of atherosclerosis remains controversial (13,15). Notably, immune reactions triggered by infections may be a crucial determinant of their proatherogenic potencies:

From the *Department of Cardiac and Vascular Sciences and the †Protein Reference Unit, St. George's, University of London, London, United Kingdom; ‡Institute for Biomedical Aging Research, Austrian Academy of Sciences, Innsbruck, Austria; §Department of Neurology, Medical University Innsbruck, Innsbruck, Austria; and the ‖Department of Medicine, University of California San Diego, La Jolla, California. This work was supported by grants from the Oak Foundation (OCY-99-010) and British Heart Foundation, and by NHLBI grant HL56989 (La Jolla Specialized Center of Research in Molecular Medicine and Atherosclerosis) and the Donald W. Reynolds Foundation. J. Brent Muhlestein, MD, acted as guest editor for this paper.

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Abbreviations and Acronyms

ApoB	= apolipoprotein B
CMV	= cytomegalovirus
Cu-OxLDL	= copper-oxidized low-density lipoprotein
ELISA	= enzyme-linked immunosorbent assay
HSP	= heat shock protein
Ig	= immunoglobulin
IMT	= intima-media thickness
LDL	= low-density lipoprotein
LPS	= lipopolysaccharide
MDA	= malondialdehyde
mHSP65	= mycobacterial heat shock protein 65
OxLDL-AB	= autoantibodies to oxidized low-density lipoprotein
OxLDL	= oxidized low-density lipoprotein

smaller studies implicated acute infections in children (16) and the oral flora as a source of antigen for OxLDL-AB (17). However, no data exist concerning the relationship between OxLDL-AB and chronic infections or antipathogen antibodies in humans. In this population-based survey, we investigated the associations of OxLDL-AB with immune reactions to *E. coli* and chlamydial lipopolysaccharide (LPS), mycobacterial heat shock protein 65 (mHSP65), *C. pneumoniae*, *H. pylori*, and CMV as well as with carotid atherosclerosis as quantified by ultrasound.

METHODS

Subjects. Human plasma was collected as part of the Bruneck study (Northern Italy, Bolzano Province), a large population-based survey on the epidemiology and etiology of atherosclerosis approved by our ethics committee board (18,19). All participants gave their informed written consent before entering the study. The study population was recruited between July and November 1990 as an age- and gender-stratified random sample of all inhabitants of Bruneck 40 to 79 years old (125 men and 125 women in the fifth to eighth decade each, n = 1,000). A total of 93.6% participated, with data assessment completed in 919 subjects. During follow-up (1990 to the re-evaluations in 1995 and 2000), subgroups of 63 and 97 individuals died, respectively. Among survivors, follow-up was 96.5% (1995, n = 826) and 93.8% (2000, n = 684) complete. The current evaluation focused on the follow-up period between 1995 and 2000. Plasma samples for the assessment of OxLDL-AB were available in 765 (1995) and 656 subjects (2000). Population characteristics and levels of risk factors did not differ between participants of the 1995/2000 evaluations and those not participating, and also not between subjects with and without measurements of OxLDL-ABs.

Clinical examination and laboratory methods. All participants underwent a clinical examination and completed standardized questionnaires on current and past exposure to candidate vascular risk factors as extensively described previously (20). Blood samples were taken from the antecubital vein after subjects had fasted and abstained from smoking for at least 12 h. All laboratory parameters were examined by standard methods (21). Subjects with chronic infections or conditions known to be associated with recurrent episodes of infectious exacerbation, such as chronic obstructive pulmonary disease (n = 251 of the 765 subjects in the 1995 evaluation), were identified by an extensive screening consisting of two consecutive phases (19). The first step involved a detailed self-reported medical and medication history, thorough clinical examination, spirometry, extensive laboratory evaluations including urinary analysis, and a review of the Bruneck Hospital databases and other medical records. If the data were inconclusive, in a second step individuals were referred for further examinations. The diagnosis of common chronic infections was established according to standard diagnostic criteria by an expert committee including specialists from various medical fields. The diagnosis of chronic obstructive pulmonary disease required documentation of airway obstruction by spirometry (FEV_1/FVC ratio <0.70) and the presence of typical symptoms, such as dyspnea, cough, expectoration, or wheeze. Bronchitis was defined as chronic when cough with expectoration lasted $\geq$3 months in $>$2 consecutive years. Periodontitis was defined by self-report. Urinary tract infections were regarded as recurrent in the case of $\geq$3 documented episodes.

Determination of OxLDL-AB, apolipoprotein B (ApoB)-immune complexes (IC), and OxLDL-E06. Oxidized LDL-ABs were measured in plasma, which was immediately frozen after sampling and stored in aliquots at −70°C without thawing. Measurements were performed in 2001 after one and six years of storage, respectively. Titers of IgG and IgM OxLDL autoantibodies, determined at 1:500 dilutions in human plasma, were tested for binding to MDA-LDL and copper-oxidized LDL (Cu-OxLDL) as previously described (2,22,23). Immunoglobulin G and IgM IC with ApoB-100 particles (ApoB-IC) and circulating OxLDL-E06, a measure of the oxidized phospholipid content per ApoB particle (OxPL/ApoB) assessed by means of the antibody E06, were measured as previously described (2,22,23).

Serological tests for infectious agents. Immunoglobulins (IgG, IgA, IgM, kappa, lambda) to *E. coli*-LPS and mHSP65 were measured using enzyme-linked immunosorbent assays (ELISA) following established protocols (24,25). Antibodies against chlamydial LPS, *C. pneumoniae*, *H. pylori*, and CMV were determined by commercial test systems as part of the 1995 and 2000 evaluations: chlamydial IgG recombinant LPS ELISA (Medac, Hamburg, Germany), SeroCP-IgA (Savyon Diagnostics Ltd., Ashdod, Israel), Helicobacter-IgG (Medac), and CMV-IgG ELISA (Medac). The analyses were performed and calculated according to manufacturers' instructions.

Scanning protocol and definition of ultrasound end points. The ultrasound protocol involves scanning of the internal (bulbous and distal segments) and common carotid arteries (proximal and distal segments) of either side with a

10-MHz imaging probe. Scanning was performed every five years by the same experienced sonographer, who was unaware of the subjects' clinical and laboratory characteristics. Atherosclerotic lesions were defined by two ultrasound criteria: 1) wall surface (protrusion into the lumen or roughness of the arterial boundary); and 2) wall texture (echogenicity). The maximum axial diameter of plaques was assessed in each of 16 vessel segments and a sensitive and reproducible atherosclerosis score calculated by addition of all diameters. Accuracy of this procedure was established previously (26). Incident atherosclerosis was defined by the occurrence of new plaques in previously normal vessel segments. Finally, intima-media thickness (IMT) was measured at the far wall of the left and right common carotid arteries as the distance between the lumen-intima interface and the leading edge of the media-adventitia interface. The mean maximum IMT was calculated and used in the analyses.

Statistical analyses. The correlation between OxLDL-AB, ApoB-IC, and antipathogen antibodies was estimated by Spearman rank correlation coefficients. Differences in the levels of antipathogen antibodies and various vascular risk attributes according to quartiles of OxLDL-AB were tested with the general linear model procedure of the SPSS statistical software (SPSS Inc., Chicago, Illinois). These analyses were adjusted for age and gender. The OxLDL-AB and ApoB-IC quartiles were modeled as a set of categories or as a trend. Only p values from the primary approach are presented because the results of both models were quite similar. Levels of OxLDL-AB and ApoB-IC in subjects with and without chronic infections were compared again using the general linear model procedure. The associations of OxLDL-AB and ApoB-IC with incident carotid atherosclerosis were assessed by logistic regression analysis. Base models were controlled for age, gender, and baseline atherosclerosis while multivariate equations further included apolipoprotein A_1 and B, hypertension, smoking, diabetes, ferritin, C-reactive protein, fibrinogen, and antichlamydial IgG antibody titers. Logistic regression models were supplemented by linear regression analyses that used five-year changes in the carotid atherosclerosis score as a continuous outcome variable. Odds ratios and regression coefficients presented were calculated for a one standard deviation unit change in given variables. All analyses were performed twice using either native OxLDL-AB and ApoB-IC levels or $\log_e$-transformed values. Because the findings of both procedures were very similar, only data from analyses using native values are presented for ease of presentation and interpretation.

RESULTS

Correlations between OxLDL-AB and ApoB-IC in 1995 and 2000. The IgG and IgM MDA-LDL, Cu-OxLDL, and ApoB-IC levels were strongly correlated and stable from 1995 to 2000 with the individual correlations exceeding $r = 0.6$ (Appendix Table 1). Levels of OxLDL-AB and ApoB-IC across age groups were also highly consistent in the 1995 to 2000 evaluations. The MDA-LDL autoantibody levels tended to be higher than Cu-OxLDL autoantibody levels. At both time points, IgM ApoB-IC levels were inversely associated with age ($p < 0.001$ for 1995 and 2000), whereas IgG Cu-OxLDL levels showed a positive association ($p < 0.001$ for 1995 and 2000), and a similar trend was observed for IgG MDA-LDL ($p < 0.1$ for 1995 and 2000). Interestingly, at each time point, IgM MDA-LDL, Cu-OxLDL, and ApoB-IC were all significantly higher in female subjects than in male subjects ($p < 0.001$ for all), whereas IgG ApoB IC were higher in men ($p < 0.001$) while no gender differences were found for IgG MDA-LDL and Cu-OxLDL. In addition, strong correlations were noted among all IgG OxLDL-AB and among IgG ApoB-IC levels and among all IgM OxLDL-AB and IgM ApoB-IC levels (Appendix Table 1) consistent with previous data in patients with acute coronary syndromes and coronary artery disease (23).

Association of OxLDL-AB and ApoB-IC with antipathogen antibodies, chronic infections, and vascular risk factors. The IgG and IgM OxLDL-AB and apoB-IC levels correlated with antibody titers to *E coli* and chlamydial LPS, mHSP65, *C. pneumoniae*, *H. pylori*, and CMV measured in 1995 and 2000 (Table 1). Correlations were slightly weakened, but remained mostly significant when adjusting for total IgG, IgA, or IgM levels (data not shown). To further clarify the scale and strength of relation between OxLDL-AB and ApoB-IC and infectious agents, inflammatory markers, lipoproteins, and other risk factors, levels of all these variables were compared across quartiles of OxLDL-AB and ApoB-IC titers in the 1995 assessment. These analyses were adjusted for age and gender. Data are shown in Table 2 for IgG and IgM Cu-OxLDL autoantibodies. Associations for other variables, including smoking, fasting glucose, blood pressure, body mass index, waist-hip ratio, alcohol and energy intake, sport index, microalbuminuria, uric acid, ferritin, thyroid-stimulating hormone, erythrocyte sedimentation rate, leucocyte count, and $alpha_1$-antitrypsin are presented in Appendix Table 2. Appendix Tables 3 and 4 summarize the data for IgG and IgM MDA-LDL autoantibodies and ApoB-IC. In brief, all IgG and IgM OxLDL-AB and ApoB-IC were significantly correlated with anti-*E. coli* LPS antibodies ($p \leq 0.001$ for most associations). Associations with antibody titers to chlamydial LPS, *C. pneumoniae*, *H. pylori*, and CMV were somewhat less consistent and more pronounced for IgG OxLDL-AB. A positive association with anti-mHSP65 antibodies was confined to IgG OxLDL-AB and IgG ApoB-IC. Very similar results were obtained once focusing on the 2000 measurements and when the analyses were additionally adjusted for hypertension, apoB, and $apoA^1$, smoking, diabetes, ferritin, C-reactive protein, and fibrinogen levels (data not presented). Notably, age- and gender-adjusted levels of OxLDL-AB and ApoB-IC showed a rise

Table 1. Correlation Between Levels of Anti-OxLDL Autoantibodies and Antipathogen Antibodies in 1995 and 2000

	MDA-LDL IgG	MDA-LDL IgM	Cu-OxLDL IgG	Cu-OxLDL IgM	ApoB-IC IgG	ApoB-IC IgM
E. coli LPS						
1995	0.125*	0.142*	0.131*	0.128*	0.197*	0.178*
2000	0.116†	0.212*	0.074	0.257*	0.213*	0.216*
Chlamydial LPS IgG						
1995	0.104†	0.094†	0.138*	0.085†	0.107†	0.028
2000	0.172*	0.094†	0.160*	0.066	0.125†	0.054
mHSP65						
1995	0.230*	0.055	0.203*	0.121†	0.168*	0.080†
2000	0.162*	0.099†	0.133*	0.111†	0.109†	0.145*
C. pneumoniae IgA						
1995	0.089†	0.086†	0.144*	0.079†	0.089†	0.030
2000	0.102†	0.054	0.116†	−0.023	0.105†	−0.020
H. pylori IgG						
1995	0.141*	−0.032	0.104†	−0.040	0.089†	0.023
2000	0.143*	−0.038	0.044	−0.002	0.057	−0.010
CMV IgG						
1995	0.169*	0.105†	0.110†	0.064	0.040	0.088†
2000	0.156*	0.134*	0.061	0.083†	0.028	0.072

Values presented are Spearman rank correlation coefficients between antibody levels assessed in 1995 (n = 765) and 2000 (n = 656), antibody measurements were compared within the same year: *$p < 0.001$; †$p < 0.05$.

ApoB = apolipoprotein B; CMV = cytomegalovirus; *C. pneumoniae* = *Chlamydia pneumoniae*; CuOxLDL = copper oxidized low-density lipoprotein; *H. pylori* = *Helicobacter pylori*; IC = immune complexes; Ig = immunoglobulin; LDL = low-density lipoprotein; LPS = lipopolysaccharide; MDA = malondialdehyde; mHSP65 = mycobacterial heat-shock protein 65; OxLDL = oxidized low-density lipoprotein.

with increasing pathogen burden. Moreover, OxLDL-ABs were elevated in subjects with chronic infections as assessed by standard clinical criteria (19) (Table 3).

In contrast, levels of IgG and IgM OxLDL-AB and ApoB-IC titers were not associated with OxLDL-E06 (OxPL/apoB) concentration, whereas IgG OxLDL-AB showed a significant inverse relation with concentrations of plasma total cholesterol, LDL-C, and apoB, as shown previously (2,27–29). Finally, all OxLDL-AB and apoB-IC did not correlate with other vascular risk factors or with markers of inflammation, including C-reactive protein, indicating that they are independent of all these parameters. Analogous findings were obtained once focusing on 2000 OxLDL-AB and ApoB-IC data (not shown).

Association of OxLDL-AB and ApoB-IC with carotid atherosclerosis. Results for associations with incident and progressive carotid atherosclerosis (1995 to 2000) are summarized in Table 4. In unadjusted analyses, IgG Cu-OxLDL, MDA-LDL, and ApoB-IC were positively correlated with the 1995 common carotid artery IMT

Table 2. Association Between Serum and Inflammatory Parameters, Vascular Risk Factors, and Cu-OxLDL IgG and IgM Autoantibodies

	IgG Quartile Groups					IgM Quartile Groups				
Variables (Units)	Q1	Q2	Q3	Q4	p Value	Q1	Q2	Q3	Q4	p Value
Antibodies (titer)										
E. coli LPS	3.40	3.84	3.76	3.90	<0.001	3.54	3.60	3.84	3.92	0.015
Chlamydial LPS IgG	2.59	2.91	3.09	3.16	0.005	2.76	2.98	2.96	3.05	0.092
mHSP65	2.95	3.40	3.45	3.60	<0.001	3.18	3.33	3.38	3.51	0.100
C. pneumoniae IgA	1.95	2.32	2.44	2.40	0.005	2.09	2.33	2.28	2.41	0.068
H. pylori IgG	3.30	3.72	3.76	3.65	0.001	3.67	3.75	3.39	3.63	0.091
CMV IgG	2.87	2.92	3.18	3.32	0.033	3.00	3.02	2.95	3.32	0.349
Lipids/lipoproteins										
LDL (mg/dl)	149	145	141	144	0.082	147	140	143	151	0.098
HDL (mg/dl)	57.8	58.6	58.8	60.6	0.377	59.7	58.4	58.9	58.7	0.608
Total cholesterol (mg/dl)	237	230	223	229	0.008	232	225	228	234	0.385
Triglycerides (mg/dl)	145	131	125	127	0.078	131	138	132	127	0.725
Apolipoprotein A_1 (mg/dl)	166	165	164	170	0.153	169	166	165	164	0.145
Apolipoprotein B (mg/dl)	121	117	111	114	0.005	117	115	114	116	0.861
Lipoprotein(a) (mg/dl)	26.6	26.3	26.6	24.9	0.940	24.1	23.5	27.6	29.2	0.316
OxLDL (RLU)	9,814	10,720	10,284	9,654	0.753	9,600	8,951	10,719	11,210	0.064
Vascular risk factors										
Fibrinogen (mg/dl)	289	275	297	292	0.116	286	286	288	292	0.808
HbA_1C (%)	5.51	5.46	5.59	5.47	0.278	5.57	5.56	5.43	5.47	0.258
C-reactive protein (mg/l)	2.72	1.48	4.02	3.15	0.021	2.63	2.65	2.95	3.14	0.704

Values presented are means of given variables. The p values are from analyses adjusted by age and gender.

HDL = high-density lipoprotein; Q = quartile; other abbreviations as in Table 1.

Table 3. Association Between Levels of Anti-OxLDL Autoantibodies and Pathogen Burden and Chronic Infection

Antibody (AU/ml)	Pathogen Burden					Chronic Infection		
	0	1	2	3	Trend Test (p Value)	No	Yes	p Value
MDA-LDL IgG	11,004	13,320	14,750	16,548	<0.001	14,854	16,285	0.048
MDA-LDL IgM	11,932	14,632	15,003	15,804	0.036	14,706	16,515	0.006
Cu-OxLDL IgG	5,004	7,708	7,919	9,100	<0.001	7,959	9,274	0.001
Cu-OxLDL IgM	3,005	3,526	3,932	4,000	0.055	3,715	4,303	0.005
ApoB-IC IgG	5,470	6,027	6,003	6,614	0.007	6,254	6,311	0.82
ApoB-IC IgM	3,703	3,952	4,320	4,501	0.035	4,226	4,596	0.089

Values presented are age- and gender-adjusted geometric means. Pathogen burden was defined by the seroprevalence of antibodies to *C. pneumoniae, H. pylori,* CMV, and individuals were grouped into subjects without seroconversion (n = 10), seropositivity for 1 (n = 94), 2 (n = 306), or 3 pathogens (n = 355), seroconversion was defined in accordance with the manufacturers' instructions and with our previous publication (*C. pneumoniae* IgA titer ≥16, *H. pylori* IgG ≥8 U/ml, CMV IgG ≥5 U/ml (46), chronic infection was defined by standard clinical criteria (n = 251).

Abbreviations as in Table 1.

(Spearman rank correlations 0.083 [$p < 0.05$], 0.069 [$p = 0.05$], and 0.045 [p = NS], respectively) and the 1995 carotid atherosclerosis score (Spearman rank correlations 0.089, 0.081, 0.074 [$p < 0.05$ each]). In contrast, IgM Cu-OxLDL, MDA-LDL, and ApoB-IC were inversely correlated with IMT (Spearman rank correlations −0.079 [$p < 0.05$], −0.034 [p = NS], and −0.191 [$p < 0.001$]) and atherosclerosis score (Spearman rank correlations −0.049 [p = NS], −0.028 [p = NS], and −0.167 [$p < 0.001$]). After adjustment for age and gender (± other risk factors as listed in the footnote of Table 4), significance was lost for all these associations. The IgM MDA-LDL autoantibodies showed an inverse association with five-year progression of the carotid atherosclerosis score, but this association was only of moderate strength and did not reach a conventional level of significance in the multivariate analysis (p = 0.07, Table 4). All other associations between OxLDL-AB and ApoB-IC and both incident carotid atherosclerosis and five-year progression of the atherosclerosis score were not statistically significant in multivariate analyses.

DISCUSSION

The present study provides evidence of an association between OxLDL-AB and ApoB-IC and chronic infections as defined by seroprevalence of antipathogen antibodies and standard clinical criteria. Interestingly, these associations were independent of age, gender, and other vascular risk factors suggesting that exposure to infectious agents influences immune reactions to oxLDL.

Our observation of an association between OxLDL-AB and antipathogen antibodies in humans expands our knowledge about interactions between microbial pathogens and immune reactions implicated in atherosclerosis. Oxidized LDL-AB showed modest associations with antipathogen antibodies, which became more pronounced with increasing pathogen burden and may result from molecular mimicry or increased oxidative stress associated with inflammatory reactions. Importantly, these associations were further substantiated by clinical evidence of chronic infections. Previously, we found that anti-*E. coli* LPS antibodies were inversely associated with the development of carotid atherosclerosis (25) while immune reactions to HSP60/65 provided a possible link between infections and atherogenesis (30). Abundant bacterial HSP60/65 may evoke an anti-self-immune response in susceptible individuals due to its high sequence homology with the human homologue, as shown previously in the Bruneck study (24). These serum antibodies cross-react with bacterial and human HSP60 and mediate cytotoxicity on stressed endothelial cells (30,31). In

Table 4. Association of Anti-OxLDL Autoantibodies (1995) With Incident and Progressive Carotid Atherosclerosis (1995–2000) (n = 656)

	Incident Atherosclerosis (1995–2000)				Five-Year Progression of Atherosclerosis Score (1995–2000)			
	Age-/Gender-Adjusted Model		Multivariate Model		Age-/Gender-Adjusted Model		Multivariate Model	
Antibody	OR (95% CI)	p Value	OR (95% CI)	p Value	Reg. Coeff. (95% CI)	p Value	Reg. Coeff. (95% CI)	p Value
MDA-LDL IgG	1.06 (0.90–1.25)	0.39	1.08 (0.91–1.29)	0.50	0.10 (−0.09–0.28)	0.31	0.10 (−0.08–0.28)	0.27
MDA-LDL IgM	0.90 (0.75–1.07)	0.39	0.92 (0.77–1.11)	0.23	−0.22 (−0.41–−0.03)	0.02	−0.17 (−0.36–0.02)	0.07
Cu-OxLDL IgG	1.04 (0.87–1.24)	0.48	1.07 (0.89–1.28)	0.69	0.02 (−0.16–0.21)	0.81	0.03 (−0.15–0.22)	0.71
Cu-OxLDL IgM	0.90 (0.75–1.07)	0.44	0.93 (0.78–1.12)	0.23	−0.07 (−0.26–0.12)	0.46	−0.02 (−0.21–0.17)	0.85
ApoB-IC IgG	0.96 (0.81–1.15)	0.74	1.03 (0.86–1.24)	0.68	−0.01 (−0.19–0.17)	0.90	0.02 (−0.15–0.20)	0.80
ApoB-IC IgM	1.00 (0.84–1.20)	0.50	1.07 (0.88–1.29)	0.98	0.04 (−0.15–0.23)	0.70	0.11 (−0.08–0.30)	0.25

Odds ratios (OR) and 95% confidence intervals (CI) were derived from logistic regression analysis of incident atherosclerosis on OxLDL-AB, age, gender, baseline atherosclerosis, and vascular risk factors and calculated for a 1-SD U change in OxLDL-AB. Regression coefficients (Reg. Coeff.) and 95% CI were derived from linear regression analysis of five-year changes in the carotid artery atherosclerosis score on OxLDL-AB, age, gender, and vascular risk factors and calculated for a 1-SD U change in OxLDL-AB. Each equation included only one type of OxLDL-AB. Multivariate models were adjusted for age, gender, baseline atherosclerosis (OR only), apolipoprotein A_1 and B, hypertension, smoking, diabetes, ferritin, C-reactive protein, fibrinogen, and anti-chlamydial IgG antibody titers.

Abbreviations as in Table 1.

addition to molecular mimicry, HSP60 activates vascular cell functions relevant to atherogenesis and lesion complications (32). We now provide evidence that immune reactions to mHSP65 are correlated with anti-OxLDL antibodies and apoB-IC in the general community.

Previous data from the Bruneck study showed that chronic infections were associated with a higher risk of carotid atherosclerosis (19). In the current study, IgG OxLDL-AB and ApoB-IC levels were positively correlated and IgM levels inversely correlated with carotid atherosclerosis in univariate analysis. However, despite the positive associations with chronic infection and antipathogen antibodies, OxLDL-ABs were not independently predictive of incident or progressive carotid atherosclerosis in this particular cohort with asymptomatic carotid atherosclerosis after adjustment for established and potential vascular risk factors. Interestingly, there was a strong trend for an inverse association of MDA-LDL IgM autoantibodies and five-year progression of carotid atherosclerosis (Table 4, $p = 0.07$). This is consistent with a recent population-based Finnish study showing that MDA-LDL IgM autoantibodies were inversely correlated with mean carotid IMT ($p = 0.026$), maximal IMT ($p = 0.067$), and number of plaques ($p = 0.081$), independent of other risk factors (33). In that study, no association was shown with IgG OxLDL-AB and carotid atherosclerosis, similar to the current study. An inverse association of OxLDL IgM ABs has also been reported in patients with hypertension (34), myocardial infarction, or coronary artery disease (23,35), and for femoral atherosclerosis in middle-aged Swedish men, although this association lost its significance after adjustment (36). However, IgG OxLDL-AB and immune complexes tended to predict an increased risk of carotid atherosclerosis (36,37), coronary artery disease, and myocardial infarction (23,38–40), but this has not been a consistent finding (34,41).

Notably, divergent findings between IgM and IgG OxLDL autoantibodies in cardiovascular disease are increasingly being reported as investigators are more frequently measuring both subtypes in the same datasets (2,23,27,33,34,36,42). Although the underlying reasons for this are not apparent in humans, in mice a large percentage of IgM antibodies are germ-line encoded and are present at or near birth without antigen exposure suggesting they are a product of natural selection and therefore may function in protective housekeeping functions (43). Therefore, it is possible that such IgM OxLDL autoantibodies may be atheroprotective, as has been shown in immunization experiments with OxLDL or *S. pneumoniae* (7). In humans, it is possible that elevated basal levels of IgM OxLDL autoantibodies, which also bind to bacterial cell membranes containing phosphocholine and apoptotic cells (43), may be intrinsically protective against the pro-inflammatory consequences of OxLDL, which is consistent with the studies noted above in carotid atherosclerosis and coronary artery disease where inverse associations were noted. Further research is required to understand the mechanistic underpinnings of these observations in humans. Overall, these findings suggest that, compared with animal studies where OxLDL-AB levels correlate with atherosclerosis progression and regression (8,9) or in immunization experiments with OxLDL or *S. pneumoniae* (7) where robust antiatherogenic effects are noted, the potential effects of unstimulated levels of OxLDL-AB on human atherosclerosis and clinical cardiovascular disease seem to be less pronounced (14).

Study limitations. The analysis of immune reactions to OxLDL was not a pre-defined aim in the Bruneck study. Accordingly, the study may be considered explorative and awaits future confirmation. Furthermore, causality cannot be inferred from an epidemiologic study even if carefully designed and longitudinal in design. Previous evidence from animal experiments, however, would support a causal role for the associations obtained. Other limitations inherent to a longitudinal design include the risk of a potential selection bias due to the loss of participants between surveys and differences in the duration of sample storage. The latter, however, has been addressed in a serious of experiments demonstrating that OxLDL antibodies were stable during prolonged storage at −70°C with a mean absolute difference near zero and a rank correlation for repeated measurements >0.9. Finally, correlations between antibodies against OxLDL and other infectious antibodies might be confounded by the varying natural tendency of people to make antibodies in general, but the correlations reported remained mostly significant after adjusting for total Ig levels (data not shown) and were further substantiated by the assessment of chronic infections using clinical criteria.

Clinical implications. It is widely accepted that immune reactions contribute to atherogenesis, but the role of infections remains controversial as results from recent clinical trials using antibiotics were disappointing (44,45). We (24,25,30,46,47) and others (48–50) have demonstrated previously that infections are associated with (auto)immune reactions to HSPs. The present study suggests a possible involvement of infections and immune reactions to pathogens in the production of OxLDL autoantibodies. Thus, future clinical trials should account for immunomodulatory effects of infections on atherogenesis.

Conclusions. In summary, the present investigation provides evidence that OxLDL markers are modestly correlated with antipathogen antibodies in the general population and elevated in subjects with chronic infections, suggesting a role of infections and immune reactions to pathogens in the production of OxLDL autoantibodies. Furthermore, we demonstrated that in this random population cohort IgG OxLDL-AB and ApoB-IC were positively correlated and IgM OxLDL-AB and ApoB-IC were inversely correlated with subclinical carotid atherosclerosis, but no independent relationship existed after adjustment of a variety of clinical and laboratory variables that also may influence the development of carotid atherosclerosis. Further research is required to assess whether phosphocholine-based immunization strategies may impact human atherosclerosis (7,10,14).

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Reprint requests and correspondence: Dr. Qingbo Xu, Department of Cardiac and Vascular Science, St. George's, University of London, Cranmer Terrace, London SW17 0RE, United Kingdom. E-mail: q.xu@sgul.ac.uk.

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APPENDIX

For supplementary tables 1 to 4, please see the online version of this manuscript.