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Plasma Interleukin-5 Levels Are Related to Antibodies Binding to Oxidized Low-Density Lipoprotein and to Decreased Subclinical Atherosclerosis

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Objectives This study's aim was to assess the role of interleukin (IL)-5 in modulating the levels of antibodies binding to oxidized low-density lipoprotein (OxLDL) in human atherosclerosis.

Background Various pro- and anti-inflammatory cytokines have been implicated in atherogenesis, and recent findings in mice indicate that the cytokine IL-5 plays a protective role in atherosclerosis in part via the induction of antibodies binding to OxLDL.

Methods Plasma IL-5 levels and antibody titers to 2 most commonly used models of OxLDL (copper OxLDL and malondialdehyde-modified LDL) were measured in 1,011 Finnish middle-aged subjects with chemiluminescent enzyme-linked immunosorbent assay. Intima-media thickness (IMT) was assessed ultrasonographically from the internal carotid artery, the bifurcation, and the common carotid artery.

Results There was a significant positive association between plasma IL-5 levels and antibody titers to copper OxLDL ($p = 0.010$ and $p = 0.044$, immunoglobin [Ig] M and G, respectively) and IgM to malondialdehyde-modified LDL ($p < 0.001$) in the association analysis performed between different IL-5 quartiles. Furthermore, plasma IL-5 levels were found to be inversely associated with bifurcational IMT, and even after adjustments for traditional risk factors of atherosclerosis (age, gender, smoking, systolic blood pressure, LDL, and body mass index), IL-5 remained an independent determinant of the mean bifurcational IMT ($p = 0.010$).

Conclusions Our data demonstrate that plasma IL-5 levels are related to the plasma levels of antibodies binding to OxLDL and to decreased subclinical atherosclerosis in humans. These results are in line with earlier findings in murine atherosclerosis and indicate for the first time that IL-5 may play a role in human atherosclerosis. (J Am Coll Cardiol 2008;52:1370–8) © 2008 by the American College of Cardiology Foundation

Atherosclerosis is a complex, multifactorial vascular disease with both chronic and acute manifestations (1). One important factor modulating atherogenesis is the immune system, with both innate and adaptive immunity affecting the atherosclerotic disease processes in the artery wall (1–3). There are several candidate pathogens or pathogenic processes that can elicit localized inflammatory responses and subsequent atherosclerotic lesion formation (1–3). Importantly, several pro- and anti-inflammatory cytokines have been found to regulate this inflammatory process (4,5).

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One well-defined set of potential pathogens is generated by the oxidation of low-density lipoprotein (LDL) leading to the formation of OxLDL, which is recognized by specific innate and adaptive immune responses (6). These responses include the generation of both immunoglobin (Ig) G and M antibodies against OxLDL (6), which can be found in humans and animal models of atherosclerosis. Although the

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exact role of these autoantibodies in atherosclerosis is still elusive, there is accumulating evidence that IgM antibodies against OxLDL in particular seem to inversely correlate with surrogate markers of cardiovascular disease, suggesting a protective role of this class of antibodies (7–10). In murine models of atherosclerosis, IgM antibodies dominate the humoral response to OxLDL, of which many seem to be naturally occurring antibodies (6,11,12). These IgM antibodies have been shown to recognize their cognate epitopes/antigens in atherosclerotic lesions and have been suggested to actively modulate atherogenic processes by their ability to block the uptake of OxLDL and apoptotic cells by macrophages through scavenger receptors, thereby limiting the formation of foam cells (11,13–15). In particular, the prototypic natural germline IgM antibody T15/EO6 is expanded during atherogenesis in mice (15). T15/EO6 antibodies bind phosphocholine (PC), which is present in oxidized phospholipids of OxLDL, as well as the capsular polysaccharide of *Streptococcus pneumoniae* (15). We previously showed that immunization of LDL receptor$^{-/-}$ (LDLR$^{-/-}$) mice with pneumococcal extracts led to a near monoclonal expansion of T15/EO6 IgM and significantly decreased atherosclerotic lesion formation (16). In contrast, only very low IgG titers against PC were induced consistent with the T-cell independent nature of this response. IgM antibodies with similar reactivity seem to exist in humans as well, as we found that IgM titers to copper OxLDL (CuOx-LDL) and capsular polysaccharide correlate significantly in patients with pneumococcal pneumonia (16). Moreover, immunization of atherosclerosis-prone mice or rabbits with model oxidation epitopes, such as malondialdehyde-modified LDL (MDA-LDL) has been shown in a number of studies to induce specific antibodies and to provide atheroprotection (17–22). In a recent study, we further characterized this response and demonstrated that immunization of LDLR$^{-/-}$ mice with homologous MDA-LDL led to a dominant induction of the Th2 cytokine IL-5 and to a noncognate expansion of natural T-cell independent T15/EO6 antibodies (19). We further showed that this expansion of T15/EO6 IgM was dependent on the presence of IL-5, as IL-5 deficient mice did not exhibit this response. Importantly, reconstitution of LDLR$^{-/-}$ mice with bone marrow from IL-5 deficient donors resulted in significantly more atherosclerosis when compared with recipients of wild-type bone marrow. Thus, IL-5 has an atheroprotective role in vivo and is critically involved in the production of beneficial natural IgM Abs T15/EO6 (19). This hypothesis was recently strengthened by Miller et al. (23), who demonstrated that induction of IL-5 and antibodies to OxLDL with cytokine IL-33 resulted in atheroprotection in apolipoprotein E$^{-/-}$ mice (23). Furthermore, co-administration of the anti–IL-5 antibody with IL-33 reduced the levels of anti-OxLDL antibodies and prevented the reduction of atherosclerotic plaque size (23).

IL-5 is a pleiotropic cytokine that is known to stimulate B cells and eosinophils (24). In particular, human IL-5 was shown to enhance the responsiveness of certain pathogen-stimulated B-cells and to be an essential factor for terminal eosinophil differentiation as well as eosinophil activation (24), but its potential effects in human atherosclerosis are unknown. The aim of the present study was to investigate the potential connection between IL-5 and antibodies binding to OxLDL in a large randomly selected population-based cohort representative of a middle-aged Finnish population, and to assess how IL-5 levels are related to early subclinical atherosclerosis measured by carotid intima-media thickness (IMT).

Abbreviations and Acronyms

BMI = body mass index

CuOx-LDL = copper oxidized low-density lipoprotein

Ig = immunoglobulin

IL = interleukin

IMT = intima-media thickness

LDL = low-density lipoprotein

LDLR = low-density lipoprotein receptor

MDA-LDL = malondialdehyde-modified low-density lipoprotein

PC = phosphocholine

OxLDL = oxidized low-density lipoprotein

Methods

Subjects. Plasma IL-5 levels, antibodies binding to OxLDL and IMT were measured from a study group of 1,011 participants of the OPERA (Oulu Project Elucidating Risk of Atherosclerosis) study cohort, which is a population-based epidemiological study addressing the risk factors and disease end points of atherosclerotic cardiovascular diseases. The study design and the characteristics of the subjects have been previously described in detail (25–27). Briefly, the OPERA cohort consists of randomly selected subjects from the Finnish Social Insurance Institute register living in Oulu region in Northern Finland. The overall participation rate was 87.1%, and the initial population consisted of 1,200 subjects ages 40 to 59 years at the time of recruitment. Of the total population, 86 subjects had coronary heart disease, 93 subjects were diabetic, 542 subjects used blood pressure-lowering medication, 30 subjects used lipid-lowering medication, 58 subjects used aspirin, 23 subjects used oral diabetes medication, 13 subjects used insulin, and 112 subjects were undergoing hormone replacement therapy. A total of 189 of the original subjects were excluded from the present study due to missing samples or data. Clinical characteristics of the study subjects are expressed in Table 1. An informed consent was obtained from each participant and the study was approved by the Ethical Committee of the Faculty of Medicine, University of Oulu and followed the principles of the Declaration of Helsinki.

Carotid ultrasonography. The carotid ultrasound measurements have been previously described in detail in Päivänsalo et al. (25). Intima-media thickness was measured by a trained radiologist blinded to the clinical data. A duplex ultrasound system with 7.5-MHz scanning frequency in B-mode, pulsed Doppler mode, and color mode was used (Toshiba SSA-270A, Toshiba Corp., Tokyo, Japan). IMT, defined as the distance between the media-adventitia inter-

Table 1 Characteristics of the Study Subjects

Variable	Mean (SD)	Median (IQR)
Count (N)	1,011	—
Male, % (n)	49.1 (496)	—
Female, % (n)	50.9 (515)	—
Number of current smokers, % (n)	28.5 (288)	—
Age (yrs)	51.3 (6.0)	51.3 (46.0–56.5)
BMI (kg/m^2)	27.7 (4.6)	26.9 (24.4–30.2)
Waist-to-hip ratio	0.87 (0.09)	0.87 (0.79–0.93)
Plasma lipids (mmol/l)		
Total cholesterol	5.69 (1.05)	5.67 (4.97–6.31)
LDL-cholesterol	3.52 (0.94)	3.49 (2.91–4.12)
HDL-cholesterol	1.35 (0.38)	1.29 (1.08–1.57)
Triglycerides	1.57 (1.00)	1.31 (0.97–1.85)
Blood pressure (mm Hg)		
Systolic	148 (22)	147 (133–162)
Diastolic	89 (12)	90 (81–97)
Fasting blood glucose (mmol/l)	4.73 (1.47)	4.40 (4.10–4.80)
Plasma IL-5 (pg/ml)	1,511 (9,924)	139 (66–365)
hsCRP (mg/l)	3.75 (7.43)	1.53 (0.71–3.73)
IMT_{mean} (mm)		
ICA	0.78 (0.17)	0.75 (0.68–0.85)
BIF	0.94 (0.21)	0.90 (0.78–1.05)
CCA	0.91 (0.19)	0.88 (0.80–0.98)

BIF = bifurcation enlargement; BMI = body mass index; CCA = common carotid artery; HDL = high-density lipoprotein; hsCRP = highly sensitive C-reactive protein; ICA = internal carotid artery; IL = interleukin; IMT = intima-media thickness; IQR = interquartile range; LDL = low-density lipoprotein.

face and the lumen-intima interface, was measured on the following 5 points at each side: internal carotid artery, bifurcation, and common carotid artery (at 3 different locations). The intrareader reproducibility of the IMT measurements was assessed from the videotapes 1.5 years after the subject examinations. The IMT measurements were done for 31 randomly selected subjects (10 men age >57 years, 11 women age <43 years, and 10 women age >57 years) by 2 radiologists blind to the original results. The intrareader variability and correlation coefficient for the mean IMT (common carotid artery/bifurcation/internal carotid artery) were 3% and 0.97 (Pearson's coefficient).The respective interreader variability and correlation values were 7.2% and 0.93.

Clinical measurements. Routine clinical laboratory tests were carried out in the Central Laboratory of the Oulu University Hospital. All the laboratory blood test samples were obtained after a 12-h request fast. Venous blood was drawn into ethylenediaminetetraacetic acid tubes. Plasma was separated by centrifugation at 2,000 rpm for 10 min and kept at 4°C until further analysis. Plasma total cholesterol and triglyceride levels were determined by enzymatic colorimetric methods, and lipoprotein fractions were separated by ultracentrifugation. The glucose concentrations were measured using the glucose-oxidase method (Diagnostica, Merck, Whitehouse Station, New Jersey). C-reactive protein was measured using commercially available enzyme-linked immunosorbent assay kit (Diagnostic Systems Laboratories, Webster, Texas). Body mass index (BMI) was calculated as weight (kilograms) divided by height (meters) squared.

Measurements of plasma IL-5. Plasma IL-5 concentration was determined in duplicates by a chemiluminescence-based enzyme-linked immunosorbent assay using commercially available antibodies and recombinant IL-5 (R&D Systems, Inc., Minneapolis, Minnesota). The interassay coefficient of variation for the measurements was 15%.

Autoantibody measurements to OxLDL. The levels of IgM, IgG, and IgG2 autoantibodies binding to MDA-LDL and CuOx-LDL were determined by chemiluminescence-based enzyme-linked immunosorbent assay as described (8). Antigens, prepared as previously described, were coated at 10 μg/ml in phosphate-buffered saline with 0.27 mmol/l ethylenediaminetetraacetic acid and incubated overnight at 4°C in white MicroFluor plates (Dynatech Laboratories, Chantilly, Virginia). Plasma samples were diluted 1:1,000 for IgM, 1:500 for total IgG, and 1:50 for IgG2 and incubated 1 h at room temperature. Plates for IgM and IgG were incubated with an alkaline phosphatase-labeled goat antihuman-IgM or -IgG (Sigma-Aldrich, St. Louis, Missouri) for 1 h at room temperature. Luminescence was measured 90 min after addition of LumiPhos 530 (Lumigen, Southfield, Michigan) using a Victor^2 Luminometer (Wallac, Perkin-Elmer, Waltham, Massachusetts). For IgG2 binding, plates were first incubated with biotin-labeled mouse antihuman-IgG2 (Pharmingen, BD Bioscience, San Jose, California), followed by alkaline phosphatase-labeled NeutrAvidin (Pierce, Thermo-Scientific, Rockford, Illinois) and LumiPhos 530. Triplicate determinations were performed for each plasma sample. A standard curve of human IgM or IgG and a control plasma sample was added to each plate to correct potential variations between the assays, and the data are expressed as relative units equivalent to control IgM or IgG. The interassay coefficients of variation were as follows: IgM, 13.6% for CuOx-LDL and 10.0% for MDA-LDL; IgG, 11.5% for CuOx-LDL and 9.1% for MDA-LDL; and IgG2, 11.9% for CuOx-LDL and 10.3% for MDA-LDL.

Statistical analyses. Statistical analyses were performed using the SPSS (version 10.1, SPSS, Inc., Chicago, Illinois) statistical package. The association of IL-5 to other variables determined from the OPERA cohort was studied by correlation analysis, 1-way analysis of variance and linear regression analysis. The correlation between IL-5 and the other variables was assessed using partial Pearson correlation analysis. After that, the subjects were divided into quartiles of IL-5 and the means of identified variables were compared using 1-way analysis of variance. To control the effect of confounding factors on IMT, results were adjusted for the known risk factors of atherosclerosis (age, gender, systolic blood pressure, smoking as pack-years, LDL-cholesterol, and BMI) (28) using analysis of covariance. In further analysis, the role of IL-5 as a possible independent determinant on IMT was assessed using multiple linear regression analysis. The same risk factors for atherosclerosis as

above were entered into the regression model together with IL-5. The mean wall value of the IMT of the carotid bifurcation (indicated as BIF_{mean}) was used as the independent variable in all regression analysis. In all analyses, log-transformed values were used whenever needed to normalize the skewed distributions. Statistical significance was defined as $p < 0.05$.

Results

Antibodies binding to oxidized LDL and IL-5 levels. Our studies in mice have demonstrated that the induction of the oxidized phospholipid-specific natural IgM antibody EO6 triggered by immunization with MDA-LDL is dependent on the cytokine IL-5. Therefore, we first evaluated plasma IL-5 levels as well as antibody titers to the 2 most widely used models of oxidized-LDL (i.e., CuOx-LDL and MDA-LDL). The median for plasma IL-5 concentration of the whole study group was 139 (interquartile range: 66 to 365 pg/ml). The mean plasma IL-5 concentration of the whole study group (n = 1,011) was 1,511 (9,924) pg/ml (mean ± SD), and it was significantly higher in men (1,740 ± 11,730 pg/ml, p = 0.002) than in women (1,289 ± 7,805 pg/ml). For further analyses, the entire study group was divided up into quartiles according to the measured plasma IL-5 levels. Figure 1 demonstrates IgM, total IgG, and IgG2 antibody titers to CuOx-LDL and MDA-LDL within the various plasma IL-5 quartiles, after adjusting for gender. The levels of IgM antibodies binding to both models of OxLDL were lowest in the lowest IL-5 quartile and highest in the highest IL-5 quartile (p = 0.010 and p < 0.001, CuOx-LDL and MDA-LDL, respectively) (Figs. 1A and 1B), demonstrating a clear correlation between oxidation-epitope–specific IgM and IL-5 in the plasma. In contrast, only IgG antibodies to CuOx-LDL, but not to

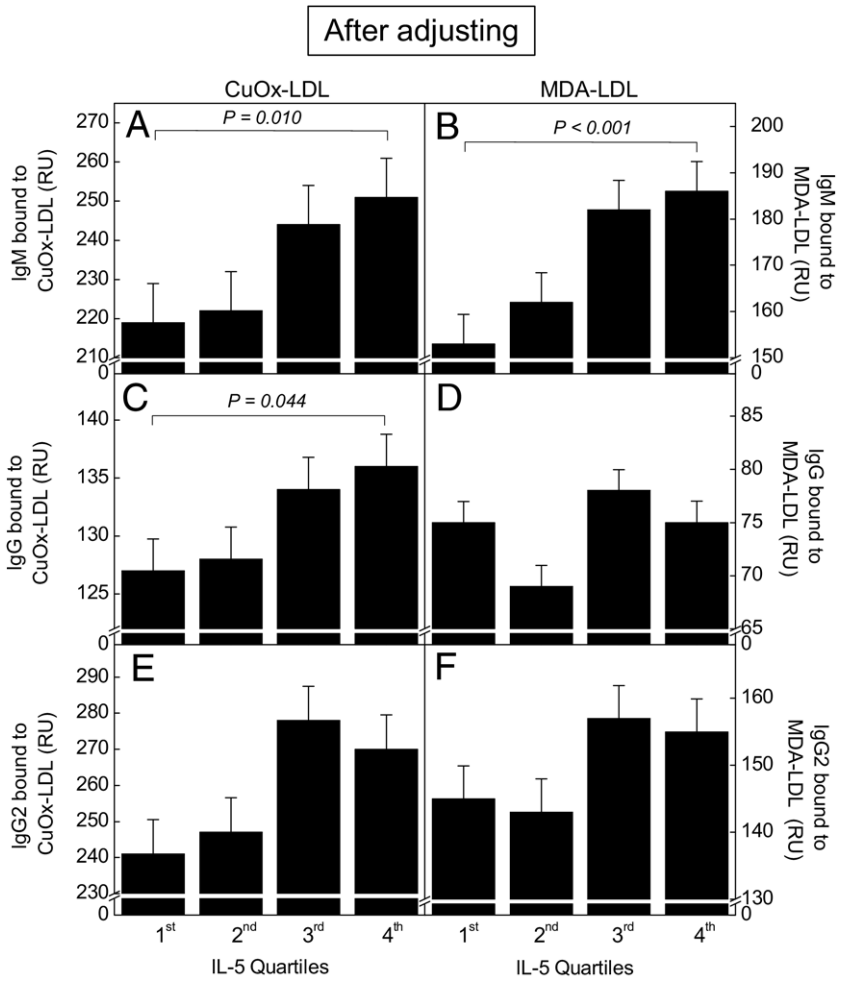

Figure 1 **Plasma Antibody Titers to OxLDL by IL-5 Quartiles**

Gender-adjusted IgM **(A, B)**, total IgG **(C, D)**, and IgG2 **(E, F)** antibodies binding to CuOx-LDL and MDA-LDL by IL-5 quartiles among the whole study group (N = 1,011). The data shown is mean (standard error of the mean). CuOx-LDL = copper oxidized low-density lipoprotein; Ig = immunoglobin; IL = interleukin; MDA-LDL = malondialdehyde-modified low-density lipoprotein; OxLDL = oxidized low-density lipoprotein; RU = relative units.

MDA-LDL showed a similar—albeit less pronounced—relationship (p = 0.044). Moreover, we also assessed IgG2 levels, as this isotype dominates the anti-PC response in humans (29). However, IgG2 titers to CuOx-LDL, which contains PC epitopes, did not show this strict relationship with IL-5 plasma levels. Because men had significantly higher IL-5 levels than women, we conducted additional analyses separately for men and women. The results were analogous: lowest levels of antibodies binding to oxidized LDL were observed in the lowest IL-5 quartile and vice versa. Thus, in humans predominantly IgM antibodies and to a lesser degree IgG antibodies to OxLDL are associated with plasma IL-5 levels, indicating a role for this cytokine in the production of anti-OxLDL antibodies.

IL-5 levels and subclinical atherosclerosis. We then assessed the possible link between plasma IL-5 concentrations and subclinical atherosclerosis measured by IMT. We have earlier demonstrated in mice that natural antibodies binding to OxLDL possess atheroprotective properties and confer atheroprotection in vivo (14,16), and that mice deficient in IL-5 have diminished levels of natural IgM to OxLDL and accelerated atherosclerosis (19). The connection between IMT and cardiovascular disease in humans has been established by others (30). Figure 2 demonstrates the IMT in the carotid bifurcation according to IL-5 quartiles, before and after adjusting for traditional risk factors of atherosclerosis (age, gender, smoking, systolic blood pressure, LDL cholesterol, and BMI) among all the study subjects. The mean carotid artery bifurcational intima media was thinnest in the

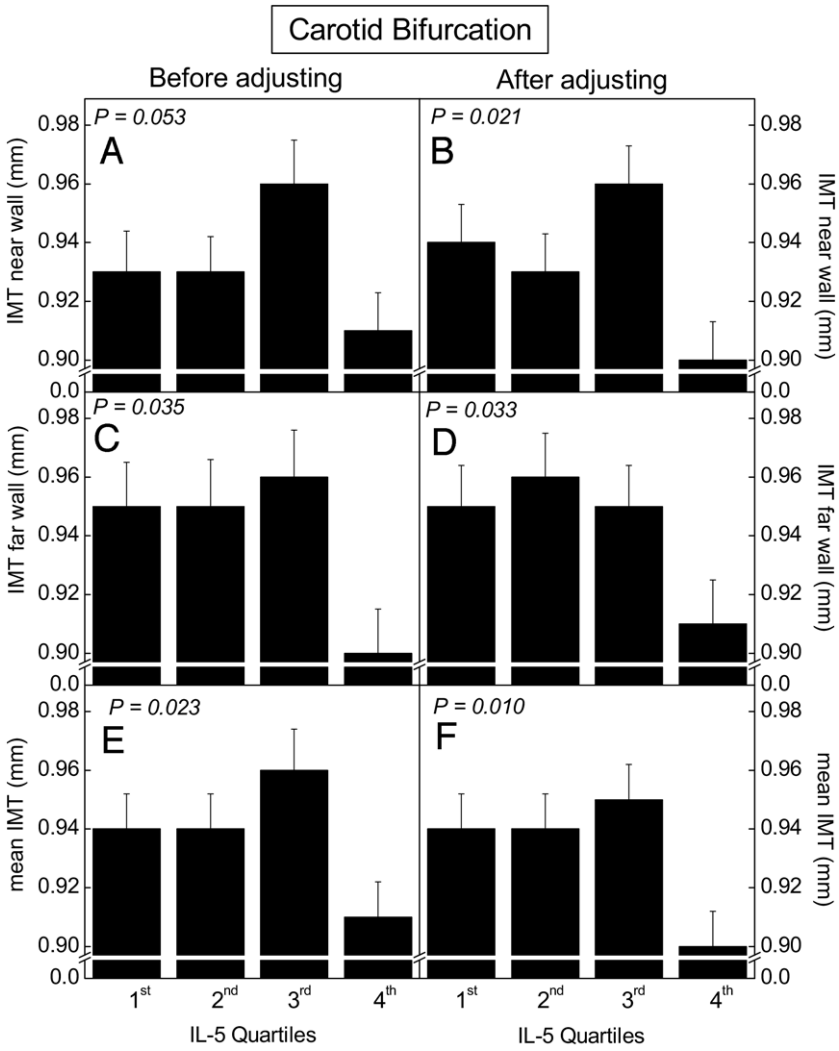

Figure 2 **Bifurcational Carotid Artery IMT by IL-5 Quartiles**

Bifurcational carotid artery IMT by IL-5 quartiles among the whole study group (N = 1,011) before and after adjustments. Analyses were performed on the near-wall measurements **(A, B)**, far-wall measurements **(C, D)**, and mean IMT **(E, F)**. Adjustments were made for age, gender, smoking, systolic blood pressure, low-density lipoprotein cholesterol, and body mass index. The data shown is mean (standard error of the mean). IMT = intima-media thickness; other abbreviations as in Figure 1.

highest IL-5 quartile among all the study subjects ($p = 0.023$ and $p = 0.010$, before and after adjustments, respectively) (Figs. 2E and 2F). Also, the bifurcational near-wall IMT (Figs. 2A and 2B) and far-wall IMT measurements (Figs. 2C and 2D) were smallest in the highest IL-5 quartile (Fig. 2). In a multiple linear regression model analysis on factors affecting the bifurcational IMT, the plasma level of IL-5 was an independent determinant of the mean bifurcational carotid artery IMT (BIF_{mean}) in the study population (Table 2). In fact, the regression model was able to explain 14.0% ($R^2 = 0.140$, $p < 0.001$) of the total variance in the mean bifurcational IMT of the study population. In this model, IL-5 alone could explain 0.6% of the variance of the mean bifurcational IMT (R^2 change for IL-5 = 0.006, $p = 0.008$). The IMT measurement was also performed in other parts of the carotid artery; yet, IL-5 levels were inversely related only to the IMT of bifurcational section of the carotid artery, but not to the internal or common carotid artery (data not shown). Collectively, these data imply that there is an epidemiological link between plasma IL-5 levels and atherosclerosis in humans and suggest that IL-5 is also an atheroprotective cytokine in humans.

The variation of several other known risk factors of atherosclerosis and the bifurcational IMTs between IL-5 quartiles is presented in Table 3. High-density lipoprotein cholesterol levels were inversely related to IL-5 levels ($p < 0.001$), and therefore the association analyses were additionally adjusted for high-density lipoprotein cholesterol. When the analyses were repeated after adjusting for age, gender, smoking, systolic blood pressure, and high-density lipoprotein cholesterol, the bifurcational IMT remained the only statistically significant carotid artery location in different IL-5 quartiles ($p = 0.013$, 0.035, and 0.008 for near-wall, far-wall, and mean IMT, respectively).

Discussion

There is clear evidence that both the adaptive and innate immune system are involved in the natural course of atherogenesis (2,3), and our recent study (19) in mice demonstrated an atheroprotective role for the Th2 cytokine IL-5 in part through the induction of natural antibodies against oxidized phospholipids. Cytokines have been implicated as major players in the atherosclerotic disease process in many ways (5). The purpose of the present study was to evaluate in humans the possible involvement of the candidate cytokine IL-5 in modulating the levels of antibodies binding to OxLDL and its role in human atherosclerosis. We here show that plasma IL-5 levels are related to the levels of antibodies binding to OxLDL in a large randomly selected population-based cohort representative of middle-aged Finnish population. Importantly, we also found that IL-5 levels are inversely related to early subclinical atherosclerosis as measured by bifurcational carotid artery IMT.

IL-5 is expressed in human carotid atherosclerotic plaques (31) and in abdominal aortic aneurysms (32), though irregularly and at low levels. Until now, only small amounts of data were available on the relationship of plasma IL-5 with human atherosclerosis. In a recent study, Inoue et al. (33) evaluated the predictive value of plasma cytokine levels, including IL-5, on cardiovascular events in 158 patients. In their study, IL-5 levels predicted cardiovascular events, though only in an univariate analysis (33). In another study by Avramakis et al. (34), only patients with myocardial infarction and unstable angina had detectable levels of IL-5, whereas IL-5 was not detected in the plasma of healthy controls. Thus, in these studies, plasma IL-5 levels seemed to be increased in patients at risk of clinical vascular events (33,34). It is noteworthy that the mean plasma IL-5 levels reported in both studies mentioned were significantly lower (<20 pg/ml) than what we measured in our healthy and much larger population (mean 1,511 pg/ml). Because methodological origins for the low IL-5 levels in these studies are not clearly evident, this could imply that patients with advanced cardiovascular disease may already have much lower plasma IL-5 levels than the healthy population does. In analogy to our data, the median serum IL-5 levels of healthy mothers and their 1-year-old babies were reported to be above 120 pg/ml (35). In light of this, the lower plasma IL-5 levels found in patients with cardiovascular disease (33,34) further support a protective role of IL-5. Furthermore, we found that high plasma IL-5 levels were associated with smaller bifurcational carotid artery IMT, and that this was statistically significant after adjusting for other well-established risk factors, such as age, gender, smoking, systolic blood pressure, LDL-cholesterol, and BMI. Interestingly, our finding on the association between

Table 2 Linear Regression Analysis Model of Factors Affecting the IMT_{mean} of BIF_{mean} of the Study Population (N = 1,011)

		Change Statistics				
Variable	R^2	R^2 Change	p Value	B (SEM)	Beta	p Value
Age	0.065	0.065	**<0.001**	0.004 (0.000)	0.244	<0.001
Gender	0.108	0.043	**<0.001**	−0.034 (0.005)	−0.189	<0.001
LDL-cholesterol	0.118	0.010	**0.001**	0.011 (0.003)	0.112	<0.001
IL-5	0.124	0.006	**0.008**	−0.011 (0.004)	−0.080	0.007
BMI	0.131	0.007	**0.005**	−0.142 (0.040)	−0.111	<0.001
Systolic BP	0.140	0.009	**0.002**	0.000 (0.000)	0.100	0.002

Model $R^2 = 0.140$; $p < 0.001$. **Bold** p values are statistically significant.
BP = blood pressure; SEM = standard error of the mean; other abbreviations as in Table 1.

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Table 3 Characteristics of the Study Subjects According to the IL-5 Quartiles (N = 1,011)

	IL-5 Quartiles (pg/ml)				
	1st 38 (24–50)	2nd 95 (80-115)	3rd 206 (170–275)	4th 809 (511–1,605)	p Value
n	256	249	253	253	0.500
Male/female, % (n)	**10.5** (106)/**14.8** (150)	**11.3** (114)/**13.4** (135)	**12.8** (129)/**12.3** (124)	**14.5** (147)/**10.5** (106)	**0.001**
Age (yrs)	**51** (51–52)	**51** (50–52)	**51** (51–52)	**51** (50–52)	0.810*
BMI (kg/m^2)	**27.7** (27.2–28.3)	**28.1** (27.5–28.7)	**27.7** (27.1–28.3)	**27.2** (26.6–27.8)	0.211*†
Smoking (pack-yrs)	**14.2** (12.6–15.8)	**13.3** (11.7–15.0)	**13.0** (11.4–14.6)	**10.6** (9.0–12.3)	**0.037***†
Systolic BP (mm Hg)	**150** (147–152)	**149** (147–152)	**147** (145–150)	**147** (144–149)	0.326‡
hsCRP (mg/l)	**1.60** (0.73–4.53)	**1.51** (0.68–4.26)	**1.54** (0.76–3.38)	**1.53** (0.66–3.26)	0.322†‡
Diabetes, % (n)	**2.2** (22)	**1.2** (12)	**1.0** (10)	**1.2** (12)	0.096
Total cholesterol (mmol/l)	**5.80** (5.67–5.92)	**5.67** (5.54–5.80)	**5.76** (5.64–5.89)	**5.54** (5.41–5.67)	**0.023**‡
LDL-cholesterol (mmol/l)	**3.53** (3.42–3.64)	**3.49** (3.38–3.61)	**3.65** (3.53–3.76)	**3.43** (3.31–3.54)	0.052‡
HDL-cholesterol (mmol/l)	**1.42** (1.38–1.46)	**1.33** (1.29–1.38)	**1.33** (1.29–1.38)	**1.32** (1.27–1.36)	**0.009**†‡
Triglycerides (mmol/l)	**1.63** (1.51–1.75)	**1.64** (1.51–1.76)	**1.53** (1.41–1.65)	**1.49** (1.37–1.61)	0.370†‡
IMT BIF near wall (mm)	**0.94** (0.92–0.97)	**0.93** (0.91–0.96)	**0.96** (0.93–0.98)	**0.90** (0.87–0.93)	**0.021**†§
IMT BIF far wall (mm)	**0.95** (0.92–0.97)	**0.96** (0.93–0.98)	**0.95** (0.93–0.98)	**0.91** (0.88–0.93)	**0.033**†§
IMT BIF mean (mm)	**0.94** (0.92–0.97)	**0.94** (0.92–0.97)	**0.95** (0.93–0.98)	**0.90** (0.88–0.93)	**0.010**†§

Data are mean (95% CI), except male/female is % (n) and IL-5 and hsCRP are median (IQR). Statistical significance was defined as $p < 0.05$. **Bold** p values are statistically significant. *Adjusted for gender. †The p value is calculated from log-transformed sample values. ‡Adjusted for age and gender. §Adjusted for age, gender, smoking, systolic BP, LDL, cholesterol, and BMI.
Abbreviations as in Tables 1 and 2.

IL-5 levels and IMT was significant only in measurements performed at the bifurcation of the carotid artery, but not in measurements performed in other parts of the carotid. This suggests that the atheroprotective role of IL-5 is particularly prominent at sites of disturbed flow that are especially susceptible to lesion formation. Thus, an IL-5–dependent protective mechanism may be specifically important at these sites.

IL-5 has a central role in B-cell biology (24,36,37). It is particularly important in the homeostatic proliferation, survival, and Ig production of murine B-1 cells (38), which constitutively express the IL-5R and are the major source of natural IgM antibodies (39), including T15/EO6 (15,16). IL-5 transgenic mice have higher levels of serum IgM and IgA, as well as increased numbers of B-1 cells (40). In contrast, B-cells from IL-5Rec–deficient mice have defective thymus independent type 2 IgM antibody responses, such as those directed against PC (41). The role of IL-5 in human B-cell biology is less well established. Here we show that IL-5 plasma levels are associated with IgM and to lesser degree IgG antibodies to epitopes of OxLDL, thereby indicating an in vivo function of IL-5 in stimulating specific human (IgM) antibody responses. These findings are analogous to our previous data in LDLR$^{-/-}$ mice, in which IL-5 stimulated the production of B-1 cell-derived IgM Abs of the T15 idiotype, but not IgG antibodies (19). Importantly, recent epidemiological studies suggest a differential effect of IgM versus IgG antibodies against OxLDL (9). The IgM autoantibodies to OxLDL have been documented to be inversely related to measures of human atherosclerosis (8,9), suggesting a possible protective role, whereas IgG to OxLDL did not show such a relationship (9). Thus, IL-5 may mediate its protective effect by preferentially promoting anti-OxLDL IgM responses. In addition, the significant associations between IL-5 and various isotypes of antibodies binding to OxLDL presented in the current paper seem to favor the PC epitope dominant in CuOx-LDL over the malondialdehyde epitope in MDA-LDL (Fig. 1). This is also consistent with our earlier findings in mice, in which we showed that IL-5 promotes the production of natural EO6 IgM antibodies that specifically recognize PC. As discussed before, PC is a dominant structure in *Streptococcus pneumoniae* as well as in OxLDL and gives rise to atheroprotective IgM (EO6) and IgA (T15) subtypes of antibodies in mice. In humans, however, the nature, role, and subtype of anti–PC-specific antibodies in atherosclerosis has not been established, although IgM but not IgG titers to CuOx-LDL (which contains PC epitopes) were found to be inversely correlated with coronary stenosis (9). Altogether, our findings demonstrate for the first time a link between plasma IL-5 levels and specific (IgM) antibodies to neoself epitopes, thereby documenting such a functional role of IL-5 in humans.

Study limitations. In the present study, we have searched for associations and did not examine the possible underlying mechanisms linking IL-5, antibodies to oxidation epitopes, and IMT in humans. It is possible that instead of having an active role in the atherosclerotic disease, such as through induction of protective antibodies, IL-5 is merely another marker. Nevertheless, our earlier studies in IL-5–deficient LDLR$^{-/-}$ mice demonstrate a functional role for IL-5 in atherogenesis in mice. In this respect, we find it central to our findings that the IL-5 levels of our study subjects were strongly related to IgM antibody titers to OxLDL, which have been clearly demonstrated to have atheroprotective properties in mice (14,16) and have been inversely associated with human atherosclerosis (7–10). We believe that among these antibodies in humans there are those resem-

bling murine T15/EO6 type of natural antibodies that exhibit atheroprotective properties (16). Although we cannot rule out the possibility that IL-5 also mediates atheroprotective effects via other mechanisms, such as the promotion of cytotoxic T-cell responses (42) or via natural killer T-cell–mediated immune regulation (43), our findings strongly suggest a link between IL-5 production and antibody induction to oxidation epitopes in humans that is similar to that observed in mice. This link may provide further mechanistic insight into earlier studies showing a protective role of IgM antibodies to OxLDL in humans (8,10).

Conclusions

Our findings demonstrate that human plasma IL-5 levels are related to antibodies binding to OxLDL and to decreased subclinical atherosclerosis. These results are in line with the earlier findings in mice and support the concept of IL-5–activated induction of antibodies to oxidation epitopes and a protective role of IL-5 in atherosclerosis. Because strategies are currently being developed to inhibit IL-5 actions in patients with asthma and other allergic diseases (44), it is important to gain a detailed understanding of the role of IL-5 in human atherogenesis.

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