Plasma IL-5 concentration and subclinical carotid atherosclerosis

CrossMark

Angela Silveira [a, *], Olga McLeod [a], Rona J. Strawbridge [a], Karl Gertow [a],
Bengt Sennblad [a, b], Damiano Baldassarre [c, d], Fabrizio Veglia [d], Anna Deleskog [a],
Jonas Persson [e], Karin Leander [f], Bruna Gigante [f], Jussi Kauhanen [g], Rainer Rauramaa [h],
Andries J. Smit [i], Elmo Mannarino [j], Philippe Giral [k], Sven Gustafsson [l], Stefan Söderberg [m],
John Öhrvik [a], Steve E. Humphries [n], Elena Tremoli [c, d], Ulf de Faire [f], Anders Hamsten [a]

[a] Atherosclerosis Research Unit, Department of Medicine Solna, Karolinska Institutet, Stockholm, Sweden
[b] Science for Life Laboratory, Karolinska Institutet, Stockholm, Sweden
[c] Dipartimento di Scienze Farmacologiche e Biomolecolari, Università di Milano, Italy
[d] Centro Cardiologico Monzino, IRCCS, Milan, Italy
[e] Division of Cardiovascular Medicine, Department of Clinical Sciences, Karolinska Institutet, Danderyd University Hospital, Stockholm, Sweden
[f] Division of Cardiovascular Epidemiology, Institute of Environmental Medicine, Karolinska Institutet, Stockholm, Sweden
[g] Institute of Public Health and Clinical Nutrition, University of Eastern Finland, Kuopio, Finland
[h] Foundation for Research in Health, Exercise and Nutrition, Kuopio Research Institute of Exercise Medicine, Kuopio, Finland
[i] Department of Medicine, University Medical Center Groningen and University of Groningen, The Netherlands
[j] Internal Medicine, Angiology and Arteriosclerosis Diseases, Department of Clinical and Experimental Medicine, University of Perugia, Perugia, Italy
[k] Assistance Publique — Hopitaux de Paris, Service Endocrinologie-Metabolisme, Groupe Hôpitalier Pitie-Salpetriere, Unités de Prévention Cardiovasculaire, Paris, France
[l] Department of Molecular Medicine and Surgery, Karolinska Institutet, Stockholm, Sweden
[m] Division of Medicine, Department of Public Health and Clinical Medicine, University of Umeå, Sweden
[n] Centre for Cardiovascular Genetics, University College London, United Kingdom

A R T I C L E   I N F O

A B S T R A C T

Objective: Genetic variants robustly associated with coronary artery disease were reported in the vicinity of the interleukin (IL)-5 locus, and animal studies suggested a protective role for IL-5 in atherosclerosis. Therefore, we set this work to explore IL-5 as a plasma biomarker for early subclinical atherosclerosis, as determined by measures of baseline severity and change over time of carotid intima-media thickness (cIMT).
Methods: We used biobank and databases of IMPROVE, a large European prospective cohort study of high-risk individuals (n = 3534) free of clinically overt cardiovascular disease at enrollment, in whom composite and segment-specific measures of cIMT were recorded at baseline and after 15 and 30 months. IL-5 was measured with an immunoassay in plasma samples taken at baseline.
Results: IL-5 levels were lower in women than in men, lower in the South than in North of Europe, and showed positive correlations with most established risk factors. IL-5 showed significant inverse relationships with cIMT change over time in the common carotid segment in women, but no significant relationships to baseline cIMT in either men or women.
Conclusions: Our results suggest that IL-5 may be part of protective mechanisms operating in early atherosclerosis, at least in women. However, the relationships are weak and whereas IL-5 has been proposed as a potential molecular target to treat allergies, it is difficult to envisage such a scenario in coronary artery disease.

---

1. Introduction

Inflammation plays an important role in atherosclerosis and clinical cardiovascular disease (CVD) [1–3]. Epidemiological studies have shown that patients with chronic inflammatory diseases, such as rheumatoid arthritis, lupus erythematosus and psoriasis, run a significantly greater risk of contracting coronary artery disease (CAD) than the general population, a fact that is not fully explained by presence of traditional cardiovascular risk factors [3,4]. Instead, it appears that inflammation and an increased proportion of unstable plaques contribute substantially to the increased cardiovascular mortality in rheumatoid arthritis [4]. In addition, the absence of an increase in occurrence of clinical CAD *prior to* the onset of rheumatoid arthritis [5], and the reduced risk of CAD achieved by blocking tumor necrosis factor early in the course of rheumatoid arthritis [6], suggest that CAD develops *after* the onset of rheumatoid arthritis. Genetic studies have also supported the hypothesis that pro-inflammatory pathways play a causal role in CAD [7]. Interleukins (ILs) in particular can be considered as strong candidates because they are known to influence the cardiovascular system in either a harmful, pro-inflammatory way (IL-1, IL-2, IL-6, IL-7, IL-8, IL-15, IL-17, and IL-18) or in a protective, anti-inflammatory way (IL-4, IL-10, IL-11, IL-12, and IL-13) [8]. Accordingly, meta-analyses of genetic studies have provided strong evidence for IL-6 and the IL-6 receptor in CAD [9,10].

In 2011, genetic variants in the vicinity of the IL-5 locus were found to be robustly associated with CAD in a large case–control study including a total of 32,717 cases and 75,465 control subjects [11]. IL-5 was originally defined as a "T-cell-replacing factor" that drives activated B cells for terminal differentiation into antibody-secreting plasma cells in mice [12]. In humans, IL-5 is best characterized as a major maturation and differentiation factor for eosinophils [13]. Because of the importance of eosinophils for allergy and other associated disorders, IL-5 has been proposed as a potential molecular target for the treatment of these diseases, with a couple of IL-5 antagonist therapies currently under development [13,14]. Prior to the recent gene-centric study in CAD [11], the role of IL-5 in CVD had been barely touched upon, with one publication linking plasma IL-5 levels inversely to subclinical atherosclerosis [15] and two studies implicating raised IL-5 levels in unstable angina and myocardial infarction [16] and risk of recurrent CAD events [17].

Against this background, we examined the role of IL-5 as a plasma biomarker for early, subclinical carotid atherosclerosis, as determined by measures of baseline severity and change over time in carotid intima-media thickness (cIMT), in a large prospective cohort study of high-risk individuals who were free of clinically overt CVD at enrollment [18].

2. Materials and methods

2.1. Study population

The present study was performed using the biobank and databases of a multicentre, European, longitudinal cohort study (acronym: IMPROVE (Carotid **I**ntima **M**edia Thickness (cIMT) and IMT-**PRO**gression as Predictors of **V**ascular **E**vents in a High-Risk European Population) [18]). In brief, inclusion criteria were age from 55 to 79 years, presence of at least three cardiovascular risk factors and absence of symptoms of CVD, as well as of conditions that might limit longevity or cIMT visualization. Participants were recruited in seven centers in five European countries: Italy (centers in Perugia and Milan), France (Paris), the Netherlands (Groningen), Sweden (Stockholm) and Finland (two centers in

Kuopio). These centers are located from South to North of Europe at latitudes 43, 45, 48, 53, 59 and 62, respectively. Between March 2004 and April 2005, 3711 participants were enrolled, of whom 3534 (1701 men and 1833 women) were included in the present report. Participants were excluded because of inaccuracy of data at recruitment, technical difficulty to acquire cIMT or incomplete follow-up, as detailed [19]. Ethics committee approvals for the study were obtained in each of the 7 recruiting centres and written informed consents were obtained from all participants.

Baseline characteristics of the entire IMPROVE cohort and methods for determination of established cardiovascular risk factors have been published [18].

2.2. Quantification of IL-5 in plasma

The concentration of IL-5 was measured in duplicate in EDTA-plasma samples by a sandwich immunoassay with electro-chemiluminescense detection using the ultra-sensitive kit for human IL-5 assay from MesoScale Discovery, Gaithersburg, MD, USA (Catalog #: K1151AJC-4, Lot: K0022251). All samples were incubated overnight at 4 °C with the antibody on the wells of plates, on a shaker at 600 rpm. Otherwise the protocol followed the manufacturer's instructions. The mean intra-assay coefficient of variation (CV) for all samples was 8%, and the inter-assay CV for the control plasma was 13% (n = 94 plates).

2.3. Carotid ultrasonography

Details of the protocol and validation of the carotid ultrasound measurements have been published [18]. In the present report, the following cIMT variables were studied in relation to IL-5: the mean and maximum IMT of the whole carotid tree (IMTmean and IMTmax), and the average of maximum IMT values of the whole carotid tree (IMTmean-max); the mean and maximum IMT of the common carotid arteries (CC-IMTmean and CC-IMTmax), excluding the first centimetre closest to the bifurcation; the mean and maximum IMT of the internal carotid artery (ICA-IMTmean and ICA-IMTmax); and the mean and maximum IMT of the bifurcations (Bif-IMTmean and Bif-IMTmax). Participants underwent cIMT measurements at 3 time-points: baseline and after 15 and 30 months. Changes in cIMT over 30 months, expressed in mm/year, were calculated by linear regression of IMT versus time using data from the 3 time-points.

2.4. Variable definitions

Hypertension was defined as a diagnosis of hypertension and/or treatment with antihypertensive drugs. Diabetes was defined as a diagnosis of diabetes and/or treatment with insulin or other hypoglycemic drug, and/or fasting glucose ≥ 7 mmol/L at the baseline examination. Smoking habits, identified from a structured questionnaire performed at baseline, included variables reflecting current smoking status, duration/cessation of smoking and the average number of cigarettes being consumed. As a measure of cumulative smoking, a 'pack-years' variable was calculated by multiplying the average number of cigarettes smoked per day by the number of years of smoking divided by 20. The 'pack-years' was used as a 5-level categorical variable where first group included never-smoker status and four other included quartiles of 'pack-years'. Results were presented as percent of population in smoking groups 0/1/2/3/4: 0: non-smokers; 1 to 4 correspond respectively to 0–7.99; 8–17.99; 18–29.99; 30-250 pack-years.

2.5. Statistical analysis

Variables are reported as number of subjects in group (proportion) or median (interquartile range). Variables with skewed distribution were logarithmically transformed before they were used in analysis.

Differences in IL-5 levels across recruitment centers were analyzed by the Jonckheere–Terpstra test for ordered alternatives to assess trends across centers defined by their latitude (the two Finnish centers were analyzed together). Comparisons of IL-5 and other variables between men and women were calculated by Mann–Whitney U test. Associations between IL-5 levels and cardiovascular risk factors were assessed by calculation of Spearman rank correlation coefficients. Conventional risk factors and risk factors which were significantly correlated with IL-5 were used in the selection of covariates for the regression models.

Multivariable analysis of factors associated with IL-5 levels was performed by multiple linear regression. Multiple robust regression was used to assess associations between IL-5 levels and cIMT measurements [20]. Age was always forced into the models, which also included latitude, and a range of relevant clinical and biochemical variables. Because the cIMT variables were strongly correlated with each other correction for multiple testing was not performed.

Table 1
Characteristics of the participants.

	All subjects	Males	Females	P-value[a]
n	3534	1701	1833	
Age, years	64.4 (59.6–67.2)	64.3 (59.3–67.1)	64.6 (60.0–67.3)	0.055
BMI, kg/m^2	26.8 (24.3–29.5)	27.1 (25.0–29.3)	26.4 (23.6–29.7)	<0.001
Waist/hip ratio	0.92 (0.86–0.97)	0.96 (0.93–1.01)	0.87 (0.82–0.91)	<0.001
SBP, mmHg	140 (130–153)	141 (130–154)	140 (130–152)	0.148
DBP, mmHg	81 (75–88)	83 (77–90)	80 (75–88)	<0.001
Hypertension	2436 (68.9)	1151 (67.7)	1285 (70.1)	0.118
LDL-cholesterol, mmol/L	3.50 (2.82–4.22)	3.37 (2.75–4.03)	3.64 (2.93–4.38)	<0.001
HDL-cholesterol, mmol/L	1.20 (1.01–1.46)	1.10 (0.93–1.30)	1.32 (1.11–1.60)	<0.001
Triglycerides, mmol/L	1.31 (0.94–1.90)	1.38 (0.97–2.03)	1.26 (0.91–1.78)	<0.001
Glucose, mmol/L	5.5 (5–6.3)	5.7 (5.2–6.6)	5.3 (4.8–6.0)	<0.001
Diabetes mellitus	929 (26.3)	533 (31.9)	396 (21.9)	<0.001
CRP, mg/L	1.86 (0.77–3.58)	1.64 (0.67–3.24)	2.09 (0.92–3.95)	<0.001
Current smokers	512 (14.9)	274 (16.6)	238 (13.4)	0.009
Pack-years, %	49.1/12.6/12.5/12.6/13.1	32.2/15.1/16.6/15.7/20.4	64.3/10.4/8.8/9.8/6.6	<0.001
Creatinine, μmol/L	79.1 (68.5–90.7)	88.6 (79.7–98.6)	70.6 (63.0–79.3)	<0.001
Vitamin D, nmol/L	48 (34–64)	50 (37–65)	47 (30.5–61)	<0.001
Adiponectin, μg/mL	10.6 (6.2–17.2)	8.1 (4.8–12.1)	14.0 (8.6–21.9)	<0.001
IL-5, pg/mL	0.43 (0.27–0.71)	0.48 (0.30–0.77)	0.39 (0.25–0.64)	<0.001

Values are expressed as median (interquartile range) or number of subjects in group (%).
BMI, body mass index; SBP, systolic blood pressure; DBP, diastolic blood pressure; LDL, low-density lipoprotein; HDL, high-density lipoprotein; CRP, C-reactive protein; IL-5, Interleukin-5. Hypertension: diagnosis of hypertension and/or treatment with antihypertensive drugs; Diabetes: diagnosis of diabetes and/or treatment with insulin or other hypoglycemic drug, and/or fasting glucose ≥7 mmol/L at the baseline examination.
[a] P-values for comparisons between men and women were calculated by Mann–Whitney U test.

A two-sided *p*-value <0.05 was considered significant for all analyses. STATA 11.1 (StataCorp LP) and SAS 9.2 for Windows were used for the statistical analyses.

3. Results

3.1. Characteristics of the participants

Baseline anthropometric, biochemical and environmental characteristics showed significant differences between male and female participants of the present substudy for the majority of variables (Table 1). In general, the levels of the risk factors analyzed showed a more beneficial profile in women, with exception of LDL-cholesterol and Vitamin D, which were better in man.

3.2. Plasma IL-5 concentration according to latitude and sex

The median plasma IL-5 concentration in all IMPROVE participants was 0.43 (0.27–0.71) pg/mL [median (interquartile range)], a figure that differed significantly across centers, defined by their latitudes, [0.38 (0.23–0.66), 0.32 (0.20–0.55), 0.41 (0.25–0.66), 0.45 (0.29–0.71), 0.49 (0.29–0.78) and 0.50 (0.33–0.77) pg/mL at latitudes 43, 45, 48, 53, 59 and 62, respectively], with a trend for lower levels in the South of Europe (p < 0.001).

Plasma IL-5 concentrations also differed by sex, with lower values in women [0.39 (0.25–0.64) versus 0.48 (0.30–0.77) pg/mL in men, p < 0.001, Table 1].

3.3. Relationships of IL-5 to risk factors for cardiovascular disease

Table 2 shows the relationships of IL-5 to cardiovascular risk factors. In both men and women, significant positive associations were found between IL-5 concentration and latitude, age, body mass index (BMI), SBP, hypertension, glucose, diabetes, CRP, creatinine and vitamin D, and a significant negative association

Table 2
Relationships of plasma IL-5 concentration to risk factors for cardiovascular disease in male and female participants.

	Males		Females	
	Rho	P-value	Rho	P-value
Latitude	0.146	<0.001	0.163	<0.001
Age, years	0.086	<0.001	0.063	0.007
BMI, kg/m^2	0.048	0.048	0.090	<0.001
Waist/hip ratio	0.039	0.105	0.023	0.318
SBP, mmHg	0.059	0.016	0.085	<0.001
DBP, mmHg	0.014	0.559	0.023	0.321
Hypertension	0.088	<0.001	0.095	<0.001
LDL-cholesterol, mmol/L	−0.019	0.450	−0.112	<0.001
HDL-cholesterol, mmol/L	0.041	0.092	−0.021	0.380
Triglycerides, mmol/L	−0.017	0.481	0.022	0.357
Glucose, mmol/L	0.067	0.006	0.057	0.014
Diabetes	0.065	0.008	0.073	0.002
Current smokers	0.007	0.763	−0.022	0.353
Pack-years, %	−0.013	0.602	−0.009	0.717
CRP, mg/L	0.053	0.030	0.081	0.001
Creatinine, μmol/L	0.080	0.001	0.093	<0.001
Vitamin D, nmol/L	0.057	0.020	0.053	0.026
Adiponectin, μg/mL	−0.058	0.017	−0.115	<0.001
Statin use	0.037	0.130	0.022	0.355

Values are Spearman rank correlation coefficients.
BMI, body mass index; SBP, systolic blood pressure; DBP, diastolic blood pressure; LDL, low-density lipoprotein; HDL, high-density lipoprotein; CRP, C-reactive protein. Hypertension: diagnosis of hypertension and/or treatment with antihypertensive drugs; Diabetes: diagnosis of diabetes and/or treatment with insulin or other hypoglycemic drug, and/or fasting glucose ≥7 mmol/L at the baseline examination.

with adiponectin. The magnitude of these associations was however rather weak; latitude was the strongest associated factor. The only sex-specific difference was a negative association of IL-5 with LDL-cholesterol, observed in women.

Multivariable analyses showed that independently related variables accounted for a total of 3.9% and 3.8% of the variability in plasma IL-5 concentration in men and women, respectively. The contributing factors in men included age, latitude, SBP, hypertension, HDL-cholesterol, CRP, creatinine and diabetes, whereas the model in women included age, latitude, LDL-cholesterol, CRP, creatinine and adiponectin.

3.4. Relationships of plasma IL-5 levels to measures of baseline cIMT and change over time in cIMT

At baseline, after adjustment by age, the plasma IL-5 concentration correlated significantly and positively with all composite

Table 3
Relationships of plasma IL-5 concentration to baseline and progression measures of carotid artery intima-media thickness.

	Baseline measures		Progression measures	
	β ± 2 SE	p-value	β ± 2 SE	p-value
A. Adjustment for age				
MALES	N = 1701		N = 1663*	
IMTmean	0.0083 ± 0.0056	0.003	−0.0003 ± 0.0020	0.769
IMTmax	0.0112 ± 0.0102	0.028	0.0038 ± 0.0084	0.369
IMTmean-max	0.0087 ± 0.0058	0.003	0.0006 ± 0.0032	0.707
CC-IMTmean	0.0024 ± 0.0044	0.283	−0.0001 ± 0.0016	0.940
CC-IMTmax	0.0054 ± 0.0068	0.107	−0.0012 ± 0.0040	0.540
ICA-IMTmean	0.0101 ± 0.0090	0.025	0.0011 ± 0.0032	0.522
ICA-IMTmax	0.0129 ± 0.0122	0.033	0.0041 ± 0.0068	0.233
Bif-IMTmean	0.0072 ± 0.0088	0.102	−0.0020 ± 0.0040	0.326
Bif-IMTmax	0.0081 ± 0.0106	0.128	0.0023 ± 0.0078	0.560
FEMALES	N = 1833		N = 1772*	
IMTmean	0.0023 ± 0.0046	0.314	−0.0002 ± 0.0016	0.802
IMTmax	0.0041 ± 0.0094	0.379	0.0006 ± 0.0066	0.854
IMTmean-max	0.0024 ± 0.0048	0.327	0.0001 ± 0.0024	0.951
CC-IMTmean	0.0010 ± 0.0034	0.550	−0.0017 ± 0.0012	0.007
CC-IMTmax	0.0003 ± 0.0044	0.893	−0.0024 ± 0.0028	0.085
ICA-IMTmean	0.0042 ± 0.0070	0.226	0.00004 ± 0.0022	0.966
ICA-IMTmax	0.0075 ± 0.0098	0.127	0.0027 ± 0.0046	0.244
Bif-IMTmean	0.0006 ± 0.0078	0.870	0.0009 ± 0.0034	0.585
Bif-IMTmax	0.0015 ± 0.0094	0.746	0.0012 ± 0.0066	0.707
B. Adjustment for age and latitude				
MALES	N = 1701		N = 1663*	
IMTmean	0.0046 ± 0.0054	0.089	−0.0009 ± 0.0020	0.399
IMTmax	0.0057 ± 0.0102	0.259	0.0027 ± 0.0086	0.535
IMTmean-max	0.0044 ± 0.0058	0.123	0.0001 ± 0.0032	0.938
CC-IMTmean	0.0004 ± 0.0044	0.872	−0.0003 ± 0.0016	0.669
CC-IMTmax	0.0026 ± 0.0068	0.437	−0.0016 ± 0.0040	0.429
ICA-IMTmean	0.0055 ± 0.0090	0.214	0.0002 ± 0.0034	0.901
ICA-IMTmax	0.0074 ± 0.0120	0.217	0.0033 ± 0.0068	0.342
Bif-IMTmean	0.0021 ± 0.0086	0.634	−0.0030 ± 0.0042	0.150
Bif-IMTmax	0.0017 ± 0.0104	0.748	0.0009 ± 0.0080	0.822
FEMALES	N = 1833		N = 1772*	
IMTmean	−0.0007 ± 0.0044	0.749	−0.0003 ± 0.0016	0.726
IMTmax	−0.0009 ± 0.0092	0.839	0.0007 ± 0.0066	0.840
IMTmean-max	−0.0012 ± 0.0048	0.605	−0.0001 ± 0.0026	0.951
CC-IMTmean	−0.0009 ± 0.0032	0.594	−0.0019 ± 0.0012	0.003
CC-IMTmax	−0.0015 ± 0.0042	0.469	−0.0025 ± 0.0028	0.073
ICA-IMTmean	0.0003 ± 0.0068	0.910	−0.0001 ± 0.0022	0.900
ICA-IMTmax	0.0025 ± 0.0098	0.611	0.0024 ± 0.0048	0.315
Bif-IMTmean	−0.0033 ± 0.0076	0.387	0.0011 ± 0.0034	0.528
Bif-IMTmax	−0.0033 ± 0.0996	0.488	0.0013 ± 0.0066	0.707

Values are β, regression coefficient and SE, standard error. Different measurements of baseline IMT were log-transformed to achieve normal distribution. *Number of participants with complete IMT data at all time points (baseline, 15 and 30 months). IMT, intima-media thickness; IMTmean, mean IMT of the whole carotid tree; IMTmax, maximum IMT of the whole carotid tree; IMTmean-max, average of maximum IMT values of the whole carotid tree; CC, common carotid artery; ICA, internal carotid artery; Bif, bifurcation (bulb).

(IMTmean, IMTmax, IMTmean-max) and with segment-specific (ICA-IMTmean and ICA-IMTmax) IMT measurements in men but not in women (Table 3A). Because of these preliminary sex-specific relationships of IL-5 to cIMT, subsequent multivariable analyses were then performed separately in men and women. Successive adjustments were made, first by latitude followed by risk factors for cardiovascular disease. Adjustment by latitude blunted all significant associations between IL-5 and baseline cIMT variables in men (Table 3B). Further adjustments (by waist/hip ratio, triglycerides, LDL-cholesterol, HDL-cholesterol, hypertension, diabetes, smoking, CRP, statin use, heart rate, and mean arterial pressure) did not change these results (not shown).

Similar analysis for the relationships of plasma IL-5 concentration to change over time in values of cIMT produced statistically significant inverse relationships for CC-IMTmean (with a trend for CC-IMTmax) in women after adjustment for age (Table 3A) and age plus latitude (Table 3B). Further adjustments did not materially change these results. In the final model, which included adjustments for age, latitude, waist/hip ratio, triglycerides, LDL-cholesterol, HDL-cholesterol, hypertension, diabetes, smoking, CRP, statin use, heart rate and mean arterial pressure, values for [β ± 2 SE] and p were [−0.0020 ± 0.0012] and 0.002, respectively, for the relation between IL-5 and changes in CC-IMTmean in women. In men no statistically significant relationship was observed between plasma IL-5 and change over time in any cIMT measure. Inclusion of an interaction term in the multivariable analysis showed a trend for sex differences in the IL-5 effect on CC-IMTmean progression (p = 0.097) but not on CC-IMTmax (p = 0.512).

3.5. Relationships of plasma IL-5 levels to blood eosinophil count

In men and women, from the five centers in which the data were available (no eosinophil count was recorded in either Finish centers), IL-5 was consistently, positively and strongly correlated to the eosinophil counts (n = 2494, r = 0.560, p < 0.001, Spearman rank correlation).

4. Discussion

Interleukins are key players in the chronic vascular inflammation typical of atherosclerosis, with roles that can be intrinsically pro-atherogenic or anti-atherogenic. In this context, we examined IL-5 as a potential plasma biomarker for early subclinical carotid atherosclerosis and change over time in cIMT in a large prospective European cohort study of high-risk men and women who were free of clinically overt CVD at enrollment.

The plasma concentration of IL-5 in all participants of this study was in general low, on average 0.43 (0.27–0.71) pg/mL, a level that could be accurately measured in this study by increasing the incubation time of the samples with the antibodies on the plate of the immunoassay. Using other commercially available assays, plasma IL-5 levels have been reported as low or undetectable, particularly in samples from subjects in control groups [16,17,21,22]. However, Sämpi et al. [15] reported IL-5 levels at a much higher level [139 (66–365) pg/mL)], using an assay based on the same principle as ours, but with antibodies and calibrator from another commercial source (R&D Systems). For comparison, we set up the assay as described by Sämpi et al. and re-analyzed a set of 80 random non-used aliquots of samples from the original cohort but found similar results as with our assay. The IL-5 concentration in the calibrator included in the MSD™ IL-5 kit was as reported by the manufacturers when analyzed in the assay with the R&D Systems reagents, which was also true for the reverse test (R&D systems calibrator analyzed with the MSD™ kit, Supplemental Fig. I shows similar

calibration curves for the MSD™ kit and the assay using R&D Systems materials). It is therefore not appropriate to directly compare our absolute results with those of Sämpi et al. [15]. In the absence of an internationally validated calibrator, we should limit the comparisons to interrelations between variables examined within each study.

Whereas Sämpi et al. reported low IMT in the bifurcational section of the carotid artery of subjects with high plasma levels of IL-5, we observed no significant association between these variables. Actually we found no association between IL-5 and any baseline measure of cIMT in the present study. No obvious explanations for this discrepancy are apparent, but differences related to the study populations (e.g., age difference of 10 years, differences in risk profile) or even analytes measured by the respective assays should be considered. In this context, we should note that IL-5 is considered as a key factor in the regulation of growth, differentiation, recruitment, activation and survival of eosinophils, with abundant data from *in vitro* experiments and animal models suggesting that IL-5 inhibition would be an effective approach to treat eosinophilia [14]. The plasma levels of IL-5 in both male and female participants of the present study were strongly and positively correlated with circulating eosinophils across all centers, corroborating that the protein species measured in our assay was indeed IL-5.

We have observed a south-to-north gradient for IL-5 plasma concentration in the present study, clustering IL-5 with cardiovascular risk factors that also follow a similar pattern. A previous publication based on the IMPROVE study reported a south-to-north gradient of cIMT [18]. Consequently, albeit only in men, IL-5 was positively associated with baseline measures of cIMT, associations that completely disappeared in multivariable analysis when adjustment by latitude was made, implying that from south-to-north there are factors, most likely of genetic, population nature, with which both IL-5 and cIMT are associated, without being directly associated with each other. These results suggest that the plasma IL-5 concentration is not a risk indicator for CVD, contrary to what was observed in a relatively small study in which IL-5 was elevated in plasma of patients with unstable angina or myocardial infarction compared with controls [16], but in line with another study in which an IL-5 association with (recurrent) cardiovascular events in univariable analysis disappeared after adjustments [17].

Regarding changes in cIMT during the observation period of 30-month, the plasma IL-5 concentration showed a consistent and significant inverse association with changes in IMTmean, (and also a trend for IMTmax) in the common carotid segment in women but not in men. This sex- and segment-specific finding can be added to the collection of data suggesting involvement of IL-5 in protection against atherosclerosis, a notion that has been put forward by early mouse studies of immunoresponse to oxidized (ox)-LDL [23,24]. It was observed that immunization of LDLR−/− mice with ox-phospholipid specific-LDL induced expansion of atheroprotective antibodies from B1-cells that was dependent of the presence of IL-5 because IL-5−/− mice did not exhibit this response [23]. Reconstitution of LDLR−/− mice with bone marrow from IL-5 deficient mice resulted in significantly more atherosclerosis when compared with recipients of wild-type bone marrow [23]. Induction of antibodies to ox-LDL dependent of IL-5 has also been reported in the apolipoprotein E−/- mouse model [24]. Moreover, immunization with ox-LDL in mice caused expansion of specific T-cells of the Th2 subtype that secreted IL-5, which in turn induced expansion of atheroprotective antibodies [23]. Translating data generated in experiments with genetically altered mice to the human situation represents in general a challenge, in particular when involving features of the immune system in which important differences exist between mouse and humans [25]. Nevertheless, in line with the mouse models, a recent publication from the Malmö Diet and Cancer (MDC) Study reported the first clinical evidence for a protective role of Th2 immunity in CVD, high Th2 cell counts observed to be independently associated with lower IMTmean in the common carotid segment and with reduced risk of acute myocardial infarction in women [26]. Although these segment- and sex-specificities might seem in line with our results, one should keep in mind that the assumption that Th2 cells are the source of IL-5 has been challenged both in mice [27] and humans [26]. Of note, in the MDC Study IL-5 was found not to be associated with baseline cIMT or with incident cardiovascular events during a 15 year follow-up of 700 participants, which is consistent with our results, inasmuch IL-5 in IMPROVE was not associated with baseline cIMT or with incident coronary events (n = 125) during the median follow-up period of 21.5 months (later results not shown).

SNPs in the vicinity of the *IL-5* gene were identified as being associated with clinical disease in a gene-centric study of CAD [11], but as far as we know, replication of this locus has not been reported. Furthermore, we do not know how the *IL-5* SNPs in that study were related to the plasma concentration of IL-5. We cannot discard the possibility that the association of that locus with CAD is due to the effect(s) of other gene(s) in the vicinity of *IL-5*.

The strength of the present study is the uniquely detailed ultrasound protocol and the repeated ultrasound measurements, which for the first time allowed study of IL-5 in relation to change over time in cIMT, and also its large sample size. However there are also limitations to be considered, including the fact that carotid IMT does not solely reflect atherosclerosis although it is a widely used surrogate marker of subclinical atherosclerosis and predictor of coronary and cerebrovascular events. Also, multiple test correction was not applied even when a large number of tests was performed, which could potentially increase the possibility of spurious findings. However the cIMT variables studied were strongly correlated with each other or were composite variables of related measurements and therefore provide similar information. Of note, investigation of the effects of latitude (North-to-South gradient over Europe) was not an objective of the IMPROVE study. In the cross-sectional analysis of the IMPROVE baseline data a North-to-South gradient of carotid IMT was observed independent of differences in vascular risk factors, suggesting involvement of mechanisms of genetic and/or environmental nature [18]. Thus, latitude in this context was used as a proxy for center to gain statistical power (continuous versus nominal scale).

In conclusion, atherosclerosis and cIMT are complex traits to which many factors contribute. It is plausible that IL-5 is one of these factors, exerting a protective effect. This effect was clearly seen in animal models of atherosclerosis because they have extreme gene defects, with defined trial diets, special experimental environment and limited immunological challenges. In humans, a protective effect of IL-5 may be observed depending on the clinical context, and therefore IL-5 would have limited utility as a classical biomarker. Whereas IL-5 has been proposed as a potential molecular target to treat allergies, it is difficult to envisage such a scenario in CAD.

Funding sources

Disclosures

None.

Appendix A. Supplementary data

Supplementary data related to this article can be found at http:// dx.doi.org/10.1016/j.atherosclerosis.2014.12.046.

References

[1] G.K. Hansson, Inflammation, atherosclerosis and coronary artery disease, N. Engl. J. Med. 352 (2005) 1685—1695.

[2] P. Libby, Inflammation and cardiovascular disease mechanisms, Am. J. Clin. Nutr. 83 (Suppl. l) (2006) 456S—460S.

[3] G.K. Hansson, A. Hermansson, The immune system in atherosclerosis, Nat. Immunol. 12 (2011) 204—211.

[4] S.E. Gabriel, Cardiovascular morbidity and mortality in rheumatoid arthritis, Am. J. Med. 121 (2008) S9—S14.

[5] M.E. Holmqvist, S. Wedrén, L.T. Jacobsson, L. Klareskog, F. Nyberg, S. Rantapää-Dahlqvist, L. Alfredsson, J. Askling, No increased occurrence of ischemic heart disease prior to the onset of rheumatoid arthritis: results from two Swedish population-based rheumatoid arthritis cohorts, Arthr. Rheum. 60 (2009) 2861—2869.

[6] W.G. Dixon, K.D. Watson, M. Lunt, K.L. Hyrich, , British Society for Rheumatology Biologics Register Control Centre Consortium, A.J. Silman, D.P. Symmons, British Society for Rheumatology Biologics Register, Reduction in the incidence of myocardial infarction in patients with rheumatoid arthritis who respond to anti-tumor necrosis factor alpha therapy: results from the British society for rheumatology biologics register, Arthr. Rheum. 56 (2007) 2905—2912.

[7] R. McPherson, R.W. Davies, Inflammation and coronary artery disease: insights from genetic studies, Can. J. Cardiol. 28 (2012) 662—666.

[8] E.Z. Fisman, M. Motro, A. Tenenbaum, Cardiovascular diabetology in the core of a novel interleukins classification: the bad, the good and the aloof, Cardiovasc Diabetol. 2 (2003) 1—10.

[9] W. Niu, Y. Liu, Y. Qi, Z. Wu, D. Zhu, W. Jin, Association of interleukin-6 circulating levels with coronary artery disease: a meta-analysis implementing Mendelian randomization approach, Int. J. Cardiol. 157 (2012) 243—252.

[10] IL6R Genetics Consortium Emerging Risk Factors Collaboration, N. Sarwar, A.S. Butterworth, D.F. Freitag, J. Gregson, et al., Interleukin-6 receptor pathways in coronary heart disease: a collaborative meta-analysis of 82 studies, Lancet 379 (2012) 1205—1213.

[11] The IBC 50K CAD Consortium, Large-scale gene-centric analysis identifies novel variants for coronary artery disease, PLoS Genet. 7 (2011) e1002260, http://dx.doi.org/10.1371/journal.pgen.1002260.

[12] K. Takatsu, Interleukin-5 and IL-5 receptor in health and diseases, Proc. Jpn. Acad. Ser. B Phys. Biol. Sci. 87 (2011) 463—485.

[13] T. Kouro, K. Takatsu, IL-5- and eosinophil-mediated inflammation: from discovery to therapy, Int. Immunol. 21 (2009) 1303—1309.

[14] J. Corren, Inhibition of interleukin-5 for the treatment of eosinophilic diseases, Discov. Med. 13 (2012) 305—312.

[15] M. Sämpi, O. Ukkola, M. Päivänsalo, Y.A. Kesäniemi, C.J. Binder, S. Hörkkö, Plasma interleukin-5 levels are related to antibodies binding to oxidized low-density lipoprotein and to decreased subclinical atherosclerosis, J. Am. Coll. Cardiol. 52 (2008) 1370—1378.

[16] G. Avramakis, E. Papadimitraki, D. Papakonstandinou, K. Liakou, M. Zidianakis, A. Dermitzakis, D.P. Mikhailidis, E.S. Ganotakis, Platelets and white blood cell subpopulations among patients with myocardial infarction and unstable angina, Platelets 18 (2007) 16—23.

[17] T. Inoue, H. Komoda, M. Nonaka, M. Kameda, T. Uchida, K. Node, Interleukin-8 as an independent predictor of long-term clinical outcome in patients with coronary artery disease, Int. J. Cardiol. 124 (2008) 319—325.

[18] D. Baldassarre, K. Nyyssonen, R. Rauramaa, U. de Faire, A. Hamsten, A.J. Smit, E. Mannarino, S.E. Humphries, P. Giral, E. Grossi, F. Veglia, R. Paoletti, E. Tremoli, Cross-sectional analysis of baseline data to identify the major determinants of carotid intima-media thickness in a European population: the IMPROVE study, Eur. Heart J. 31 (2010) 614—622.

[19] O. McLeod, A. Silveira, G.N. Fredrikson, et al., Plasma autoantibodies against apolipoprotein B-100 peptide 210 in subclinical atherosclerosis, Atherosclerosis 232 (2014) 242—248.

[20] O. Renaud, M.-P. Victoria-Feserb, A robust coefficient of determination for regression, J. Stat. Plan. Inference 140 (2010) 1852—1862.

[21] K. Martin, K. Viera, C. Petr, N. Marie, T. Eva, Simultaneous analysis of cytokines and co-stimulatory molecules concentrations by ELISA technique and of probabilities of measurable concentrations of interleukins IL-2, IL-4, IL-5, IL-6, CXCL8 (IL-8), IL-10, IL-13 occurring in plasma of healthy blood donors, Mediat. Inflamm. 2006 (2006) 1—7.

[22] J.M. Peake, K. Suzuki, M. Hordern, G. Wilson, K. Nosaka, J.S. Coombes, Plasma cytokine changes in relation to exercise intensity and muscle damage, Eur. J. Appl. Physiol. 95 (2005) 514—521.

[23] C.J. Binder, K. Hartvigsen, M.K. Chang, M. Miller, D. Broide, W. Palinski, L.K. Curtiss, M. Corr, J.L. Witztum, IL-5 links adaptive and natural immunity specific for epitopes of oxidized LDL and protects from atherosclerosis, J. Clin. Invest 114 (2004) 427—437.

[24] A.M. Miller, D. Xu, D.L. Asquith, L. Denby, Y. Li, N. Sattar, A.H. Baker, I.B. McInnes, F.Y. Liew, IL-33 reduces the development of atherosclerosis, J. Exp. Med. 205 (2008) 339—346. Erratum in J Exp Med. 2012;209:2515.

[25] M. Wigren, J. Nilsson, D. Kolbus, Lymphocytes in atherosclerosis, Clin. Chim. Acta 413 (2012) 1562—1568.

[26] D. Engelbertsen, L. Andersson, I. Ljungcrantz, M. Wigren, B. Hedblad, J. Nilsson, H. Björkbacka, T-helper 2 immunity is associated with reduced risk of myocardial infarction and stroke, Arterioscler. Thromb. Vasc. Biol. 33 (2013) 637—644.

[27] A. Tedgui, H. Ait-Oufella, Innate lymphoid cells. New players in atherosclerosis? Arterioscler. Thromb. Vasc. Biol. 33 (2014) 2697—2698.