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Welsh, P., Murray, H.M. , Ford, I. , Trompet, S., de Craen, A.J.M., Jukema, J.W., Stott, D.J. ,McInnes, I.B. , Packard, C.J. , Westendorp, R.G.J., and Sattar, N. (2011) *Circulating interleukin-10 and risk of cardiovascular events: a prospective study in the elderly at risk. Arteriosclerosis, Thrombosis, and Vascular Biology*, 31 (10). pp. 2338-2344. ISSN 1079-5642

<http://eprints.gla.ac.uk/57424/>

Deposited on: 18 January 2012

Arteriosclerosis, Thrombosis, and Vascular Biology

JOURNAL OF THE AMERICAN HEART ASSOCIATION

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Arterioscler Thromb Vasc Biol 2011, 31:2338-2344: originally published online July 14, 2011

doi: 10.1161/ATVBAHA.111.231795

Arteriosclerosis, Thrombosis, and Vascular Biology is published by the American Heart Association, 7272 Greenville Avenue, Dallas, TX 75214

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<http://atvb.ahajournals.org/content/31/10/2338>

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Circulating Interleukin-10 and Risk of Cardiovascular Events

A Prospective Study in the Elderly at Risk

Paul Welsh, Heather M. Murray, Ian Ford, Stella Trompet, Anton J.M. de Craen, J. Wouter Jukema, David J. Stott, Iain B. McInnes, Chris J. Packard, Rudi G.J. Westendorp, Naveed Sattar, for the PROSPER Study Group

Objective—The goal of this study was to examine the association of the antiinflammatory interleukin-10 (IL-10) with risk of cardiovascular disease (CVD).

Methods and Results—In the PROSPER (PROspective Study of Pravastatin in the Elderly at Risk) cohort, we related baseline concentrations of circulating IL-10 to risk of CVD events in a nested case (n=819)-control (n=1618) study of 3.2 years of follow-up. Circulating IL-10 showed few strong associations with classical risk factors but was positively correlated with IL-6 and C-reactive protein. IL-10 was positively associated with risk of CVD events (odds ratio [OR] 1.17, 95% CI 1.05 to 1.31 per unit increase in log IL-10) after adjusting for classical risk factors and C-reactive protein. Furthermore, IL-10 was associated more strongly with CVD risk among those with no previous history of CVD (OR 1.42, 95% CI 1.18 to 1.70), compared with those with previous CVD (OR 1.04, 95% CI 0.90 to 1.19; $P=0.018$). Overall, IL-10 showed a modest ability to add discrimination to classical risk factors (C-statistic +0.005, $P=0.002$).

Conclusion—Baseline circulating levels of the antiinflammatory IL-10 are *positively* associated with risk of CVD among the elderly without prior CVD events, although the association is less evident in those with a history of CVD. Additional epidemiological and mechanistic studies investigating the role of IL-10 in CVD are warranted. (*Arterioscler Thromb Vasc Biol.* 2011;31:2338-2344.)

Key Words: cardiovascular disease prevention ■ cytokines ■ epidemiology ■ risk factors ■ inflammation

Interleukin-10 (IL-10) is a pleiotropic cytokine that is produced by a variety of immunologic cells, including Th2-type T cells, some T-regulatory cells, B cells, and macrophages, and is most widely recognized as an antiinflammatory cytokine.^{1,2} It mediates this antiinflammatory action primarily by inhibiting Th1-type T-cell cytokine production, antigen presentation, and proliferation,³ and it plays a role in limiting macrophage inflammatory responses.⁴

In keeping with an antiinflammatory role, IL-10 may be an antiatherogenic cytokine. Overexpression of IL-10 locally, systemically, or by activated T-cells reduces atherosclerosis development in mouse models,^{5,6} and IL-10 deficient atherosclerosis-prone mice more rapidly develop plaques with a phenotype conducive to rupture compared with control mice.^{7,8} This effect of IL-10 is in direct contrast to classically proinflammatory cytokines, such as IL-6 and interferon γ , which may exacerbate atherogenesis in similar mouse models.⁹ In humans, unstable atherosclerotic plaques have appar-

ently greater localized IL-10 expression in immunostained atherectomy specimens than stable plaques.¹⁰

In epidemiological studies, many proinflammatory cytokines and inflammatory biomarkers (such as IL-6) are positively associated with risk of cardiovascular disease (CVD).¹¹ Consequently, one may hypothesize that circulating IL-10 is inversely associated with CVD (in line with an antiatherogenic role). However, in small clinical studies, IL-10 levels have been reported to be unchanged,^{12,13} increased,^{14,15} or decreased^{16,17} in stable and unstable angina patients relative to controls. Additionally complicating matters, higher circulating IL-10 has been reported to be associated with improved^{18,19} and worsened²⁰ prognosis in patients with acute coronary syndromes. Large prospective studies examining the association of circulating IL-10 with CVD risk are hence required. In a recent prospective study (the Estrogen and Replacement and Atherosclerosis study of middle-aged women with prevalent atherosclerosis) there was a *positive*

Received on: January 13, 2011; final version accepted on: July 1, 2011.

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Arterioscler Thromb Vasc Biol is available at <http://atvb.ahajournals.org>

DOI: 10.1161/ATVBAHA.111.231795

association of circulating levels IL-10 with risk of coronary heart disease (CHD), such that a 1 standard deviation increase in IL-10 was associated with a hazard ratio of 1.34 (95% CI 1.06 to 1.68) for CHD, although this study was based on only 71 CHD events, an outcome that included angina.²¹ There is hence a need for more rigorous epidemiological assessment of the link between IL-10 and CVD risk to more clearly understand the etiologic relevance of the circulating IL-10 biomarker signal. Furthermore, it would be useful to test for the first time whether IL-10 measurements have any clinical potential in risk prediction.

In PROSPER (PROspective Study of Pravastatin in the Elderly at Risk),²² we have substantial power in terms of end points, and hence the potential to explore for differential associations of IL-10 with subsequent prespecified vascular events (including fatal versus nonfatal events) and its associations in primary versus secondary prevention groups. Moreover, we were also able to examine whether such associations were independent of traditional risk factors, as well as C-reactive protein (CRP).

Methods

Participants

The use of the PROSPER trial as a post hoc cohort study for the prospective investigation of inflammatory factors as CVD risk markers has been previously described.²³ Men and women aged 70 to 82 years were recruited between December 1997 and May 1999 if they had either preexisting vascular disease (coronary, cerebral, or peripheral) or raised risk of such disease because of smoking, hypertension, or diabetes. Further inclusion criteria included total cholesterol between 4.0 and 9.0 mmol/L (135 to 350 mg/dL) and fasting triglyceride concentrations less than 6.0 mmol/L (534 mg/dL). Participants were randomly assigned to receive either 40 mg of pravastatin daily or matching placebo. All subjects were clinically examined at baseline before randomization, which included drawing of venous blood samples and measurement of body mass index (BMI) (weight/height²) and blood pressure, among other clinical parameters, by health professionals.²² The institutional ethics review boards of all centers approved the protocol, and all participants gave written informed consent. The protocol was consistent with the Declaration of Helsinki. All data were processed and analyzed at the Robertson Centre for Biostatistics, University of Glasgow (Glasgow, United Kingdom). In the original PROSPER trial, there were 881 primary end points (definite or suspected death from CHD, nonfatal myocardial infarction, and fatal or nonfatal stroke) over 3.2 years of follow-up of 5804 elderly participants at elevated CVD risk. A blinded end points committee reviewed and adjudicated all incident vascular end points, including all possible strokes.²² In the present study, to maximize efficiency, we matched all available primary end point cases to 2 controls selected at random from those in the cohort who did not experience a primary end point during the follow-up, and we analyzed IL-10 levels in available plasma samples with sufficient residual volume.

Laboratory Analysis

Baseline classical CVD risk factors, BMI, and inflammatory markers were measured as previously reported.²²⁻²⁴ IL-10 was assayed on a saved biobank of baseline plasma samples on a commercially available ELISA (R&D Systems, Oxford, United Kingdom), with a reported interassay coefficient of variation of 4.5%. All samples were processed by technicians blinded to the identity of samples, and results were entered into a master database of which the PROSPER statistics division in Scotland (Robertson Centre, University of Glasgow) is custodian.

Statistical Analysis

Baseline variables were summarized as mean (SD) for continuous variables and as number (percentage) for categorical variables. Where necessary (eg, for IL-10), continuous variables were transformed logarithmically to give a near-normal distribution of data for parametric analysis. Statistical analyses included calculation of the univariate conditional logistic regression odds ratio (OR) for baseline characteristics, for primary end point with controls, and in subsequent subgroup analysis (primary versus secondary prevention, fatal versus nonfatal CVD events, and for analysis of subgroups by median baseline IL-10 and IL-6). The OR for continuous variables was calculated for a 1-unit increase in log IL-10. C-statistics were calculated for the Cox proportional hazards survival models²⁵ containing classical risk factors with and without addition of log IL-10 and then log CRP and are reported along with resultant probability values testing whether the inclusion of log IL-10 and log CRP leads to predictions that are more concordant with observed events. Data were analyzed using the SAS version 9.1 (SAS Institute Inc., Cary, NC) and TIBCO Spotfire S+ version 8.1 (TIBCO Software Inc) software packages.

Results

Sample Availability

Because of sample attrition from previous studies, there were 819 primary end point (case) samples and 1618 control samples that had sufficient plasma for IL-10 measurement (92.2% of the total), with no evidence of a higher degree of missingness among cases versus controls ($P=0.16$) or in primary versus secondary prevention ($P=0.15$). Missing samples were therefore missing at random.

Baseline Risk Factors

For conventional risk factors, the case and control populations had differences similar to those expected based on results from the whole cohort²³ (Table 1); cases were slightly older than controls, were more likely to be male, had lower total cholesterol (which was apparently driven by a lower high-density lipoprotein cholesterol), were more likely to be smokers, had greater prevalence of history of vascular disease and diabetes at baseline, and were less likely to be randomly assigned to pravastatin at baseline. Circulating IL-10 concentrations were higher among cases (geometric mean 3.06 pg/mL [standard deviation ± 2.27]) than controls (2.75 pg/mL [± 2.20 pg/mL]; $P=0.0025$) on univariable comparison (Table 1).

Correlations of IL-10 With CVD Risk Factors

IL-10 had limited associations with classical CVD risk factors; there was no evidence of an association with age, blood pressure, low-density lipoprotein cholesterol, BMI, smoking, diabetes, or history of CHD or stroke (Table 2). There was a weak inverse association of IL-10 with HDL-cholesterol ($r=-0.067$; $P=0.0072$) and log CRP ($r=0.097$; $P=0.0001$) and a moderate association with log IL-6 ($r=0.161$; $P<0.0001$). Males had slightly higher levels of IL-10 than females ($P=0.002$), and IL-10 levels were higher in the Netherlands than in Scotland or Ireland ($P<0.0001$).

IL-10 Associations With CVD Risk

IL-10 was positively associated with risk of a primary end point among all participants (OR 1.18 [95% confidence interval, 1.06 to 1.32]) (Table 3). This OR was virtually

Table 1. Comparison of Baseline Characteristics Between Cases and Controls

	Cases (n=819)	Controls (n=1618)	P Value
Continuous variables, mean (SD)			
Age, y	75.7 (3.5)	75.4 (3.3)	0.011
Body mass index, kg/m ²	26.9 (4.1)	26.8 (4.1)	0.96
Systolic blood pressure, mm Hg	154.9 (22.7)	154.0 (21.4)	0.33
Diastolic blood pressure, mm Hg	82.9 (11.8)	83.6 (11.4)	0.14
Total cholesterol, mmol/L	5.61 (0.86)	5.68 (0.92)	0.046
HDL cholesterol, mmol/L	1.24 (0.34)	1.28 (0.33)	0.0067
LDL cholesterol, mmol/L	3.74 (0.76)	3.79 (0.81)	0.15
Triglyceride, mmol/L*	1.43 (1.54)	1.42 (1.52)	0.37
IL-10, pg/mL*	3.06 (2.27)	2.75 (2.20)	0.0025
Categorical variables, n (%)			
Pravastatin	377 (46.0)	813 (50.2)	0.049
Male	473 (57.8)	761 (47.0)	<0.0001
Smoking			0.036
Never	254 (31.0)	573 (25.4)	
Current smoker	222 (27.1)	448 (27.7)	
Ex smoker	254 (31.0)	597 (36.9)	
Country			<0.0001
Scotland	333 (40.7)	685 (42.3)	
Ireland	369 (45.0)	606 (37.4)	
Netherlands	117 (14.3)	327 (20.2)	
History of:			
Diabetes	123 (15.0)	177 (10.9)	0.0038
Hypertension	494 (60.3)	1003 (62.0)	0.42
Coronary disease	357 (43.6)	470 (29.0)	<0.0001
Peripheral arterial disease	131 (16.0)	164 (10.1)	<0.0001
Any vascular disease	459 (56.0)	662 (40.9)	<0.0001

P values for continuous variables are from two-sample *t* test, and P values for categorical variables are from the χ^2 test. n is the maximum number. All available data were used. Please note that because of the design structure of the trial—recruiting more subjects with hypertension/smokers and subjects with diabetes (and women) into the low-risk primary prevention group—the significance or nonsignificance of univariate comparisons in this table could be potentially misleading. HDL indicates high-density lipoprotein; LDL, low-density lipoprotein; IL, interleukin.

*Values are geometric means (SD) calculated from the log-transformed distribution.

unchanged after adjusting for conventional risk factors or for conventional risk factors plus CRP (1.17 [1.05 to 1.31]). When the population was split by treatment randomization (as was performed in previous PROSPER analysis, with no identifiable interaction for inflammatory markers²³), the positive association of baseline IL-10 with risk of primary end point was still evident in placebo recipients but not in pravastatin recipients. This finding was consistent both in the unadjusted and in the fully adjusted analysis (OR 1.31 [1.10 to 1.58] in placebo recipients versus 1.06 [0.91 to 1.24] in statin recipients the later model; *P* for interaction=0.031). The link between IL-10 and outcomes was similar for CHD death versus nonfatal myocardial infarction (Table 3).

Because of a potential for reverse causality, we examined the association of IL-10 with risk of CVD in those with and

Table 2. Associations of Log IL-10 With CVD Risk Factors Among Controls

Continuous Variables (Univariable Pearson Correlation)	n	R	P Value
Age, y	1618	0.0004	0.99
Systolic BP, mm Hg	1618	0.009	0.71
Diastolic BP, mm Hg	1618	−0.009	0.71
Total cholesterol, mmol/L	1618	−0.002	0.93
HDL cholesterol, mmol/L	1618	−0.067	0.0072
LDL cholesterol, mmol/L	1618	0.009	0.73
Log Triglycerides, mmol/L	1618	0.032	0.20
BMI, kg/m ²	1618	−0.002	0.94
Log CRP, mg/dL	1618	0.097	0.0001
Log IL6, pg/mL	1618	0.161	<0.0001
Categorical Variables*	n	Mean (SD)	P Value
Sex			0.0020
Male	761	2.94 (2.25)	
Female	857	2.60 (2.16)	
Country			
Scotland	685	2.65 (2.43)	Referent
Ireland	606	2.62 (2.10)	0.85
Netherlands	327	3.27 (1.87)	<0.0001
Diabetes			0.97
Yes	177	2.75 (2.30)	
No	1441	2.75 (2.20)	
Smoking			
Nonsmoker	573	2.71 (2.01)	Referent
Current smoker	448	2.75 (2.13)	0.74
Ex-smoker	597	2.79 (2.45)	0.51
History of CHD			0.45
Yes	470	2.82 (2.62)	
No	1148	2.73 (2.04)	
History of stroke/TIA			0.25
Yes	162	2.95 (2.06)	
No	1456	2.73 (2.22)	

IL indicates interleukin; CVD, cardiovascular disease; BP, blood pressure; HDL, high-density lipoprotein; LDL, low-density lipoprotein; CRP, C-reactive protein; CHD, coronary heart disease; TIA, transient ischemic attack.

*P values are from 2-sample *t* tests.

without history of prior CVD (Table 4). Among those with no history of CVD, circulating IL-10 levels were associated with the primary end point (regardless of treatment of group) in the fully adjusted model (OR 1.42 [1.18 to 1.70]) in all participants. However, IL-10 was not associated with risk of primary end point in those with a history of CVD events (1.04 [0.90 to 1.19]). These 2 associations showed significant interaction (*P*=0.018).

To further investigate whether IL-10 is positively or negatively associated with CVD risk in the context of ongoing inflammation, we devised a model splitting the cohort into high and low IL-10 and IL-6 categories by the median observed value of each marker (2.97 and 2.51 pg/mL, respectively) (Supplemental Data, available online at

Table 3. Unadjusted and Adjusted Conditional Logistic Regression Analysis for Baseline IL-10 and the Risk of a Coronary or Stroke Event

CVD Event Type	Events	Unadjusted		Adjusted*		Adjusted†	
		Odds Ratio (95% CI)	P Value	Odds Ratio (95% CI)	P Value	Odds Ratio (95% CI)	P Value
Primary end point							
All‡	819	1.18 (1.06,1.32)	0.0027	1.19 (1.06,1.32)	0.0023	1.17 (1.05,1.31)	0.0049
Placebo	442	1.34 (1.14,1.58)	0.0003	1.33 (1.13,1.57)	0.0008	1.31 (1.10,1.54)	0.0018
Pravastatin	377	1.05 (0.91,1.22)	0.51	1.07 (0.92,1.24)	0.39	1.06 (0.91,1.24)	0.44
CHD death or nonfatal MI							
All‡	588	1.17 (1.03,1.32)	0.014	1.17 (1.03,1.32)	0.017	1.15 (1.01,1.31)	0.030
Placebo	324	1.36 (1.13,1.62)	0.0009	1.35 (1.21,1.63)	0.0016	1.32 (1.10,1.60)	0.0033
Pravastatin	264	1.00 (0.84,1.20)	0.98	1.00 (0.84,1.20)	0.99	0.99 (0.83,1.19)	0.93
Fatal or nonfatal stroke							
All‡	231	1.23 (1.04,1.47)	0.019	1.22 (1.02,1.46)	0.031	1.20 (1.00,1.43)	0.047
Placebo	118	1.32 (1.00,1.71)	0.040	1.25 (0.96,1.63)	0.099	1.23 (0.94,1.61)	0.13
Pravastatin	113	1.17 (0.92,1.48)	0.20	1.20 (0.94,1.53)	0.15	1.19 (0.93,1.52)	0.17
Fatal or nonfatal events							
CHD death							
All‡	153	1.29 (1.04,1.61)	0.022	1.22 (0.98,1.52)	0.077	1.17 (0.94,1.46)	0.16
Placebo	92	1.36 (1.02,1.83)	0.039	1.27 (0.95,1.70)	0.11	1.23 (0.92,1.65)	0.17
Pravastatin	61	1.22 (0.88,1.71)	0.23	1.19 (0.85,1.68)	0.31	1.14 (0.80,1.62)	0.49
Nonfatal MI							
All‡	435	1.13 (0.98,1.29)	0.093	1.14 (0.99,1.31)	0.071	1.13 (0.98,1.30)	0.087
Placebo	232	1.35 (1.10,1.65)	0.0039	1.36 (1.10,1.67)	0.0041	1.34 (1.09,1.66)	0.0062
Pravastatin	203	0.95 (0.78,1.15)	0.58	0.94 (0.78,1.15)	0.56	0.94 (0.77,1.15)	0.55
Fatal stroke							
All‡	27	1.11 (0.68,1.82)	0.67	0.95 (0.58,1.56)	0.83	0.90 (0.54,1.50)	0.69
Placebo	11	0.81 (0.42,1.56)	0.53	0.76 (0.36,1.57)	0.46	0.77 (0.37,1.62)	0.49
Pravastatin	16	1.38 (0.76,2.54)	0.29	1.00 (0.54,1.89)	0.99	0.91 (0.46,1.78)	0.78
Nonfatal stroke							
All‡	204	1.25 (1.04,1.51)	0.0017	1.24 (1.03,1.50)	0.026	1.23 (1.02,1.48)	0.035
Placebo	107	1.40 (1.06,1.83)	0.017	1.31 (0.99,1.74)	0.061	1.29 (0.97,1.71)	0.082
Pravastatin	97	1.14 (0.89,1.48)	0.30	1.19 (0.92,1.54)	0.19	1.19 (0.92,1.54)	0.20

Odds ratios are for a 1-unit increase in log IL-10. IL indicates interleukin; CVD, cardiovascular disease; CHD, coronary heart disease; MI, myocardial infarction.

*Adjusted for age, sex, low-density lipoprotein cholesterol, high-density lipoprotein cholesterol, log triglyceride, systolic blood pressure, diastolic blood pressure, body mass index, smoking status, diabetes, use of antihypertensive therapy, previous CVD (CHD, peripheral arterial disease, stroke, and transient ischemic attack), and country.

†Also adjusted for log C-reactive protein.

‡Also adjusted for randomized treatment allocation.

<http://atvb.ahajournals.org>). Relative to those with low levels of both IL-6 and IL-10, those with elevated levels of either or both cytokines were at increased risk of CVD in the model of all participants. These observations of increased risk in every group where cytokine concentrations were elevated were consistent in the model restricted to those with no history of CVD (Supplemental Data). However, increases in risk were less pronounced among those with a history of CVD (consistent with the results from Table 4) (Supplemental Data). Finally, if those with high IL-10 and low IL-6 were used as the referent (assuming this reflects an “antiinflammatory” profile), those with low IL-10 and high IL-6 had relative odds of CVD close to unity: OR 0.98 (95% CI 0.76 to 1.26) among all participants, OR 0.87 (95% CI 0.60 to 1.27) among those

with no history of CVD, and OR 1.14 (95% CI 0.79 to 1.64) among those with no history of CVD.

IL-10 in CVD Risk Prediction

Among all participants, the classical risk factors (age, sex, country, low-density lipoprotein cholesterol, high-density lipoprotein cholesterol, systolic blood pressure, smoking, BMI, history of diabetes, history of hypertension, and baseline treatment allocation) yielded a C-statistic of 0.592. Addition of IL-10 to the model increased the C-statistic marginally, but statistically significantly, by +0.005 (to 0.597, *P*=0.002), and further addition of CRP again only slightly improved the model including IL-10 by 0.005 (to 0.602, *P*<0.0001 versus the basic model). Restricting the

Table 4. Unadjusted and Adjusted Conditional Logistic Regression Analysis for Baseline IL-10 and the Risk of a Coronary or Stroke Event

	Events	Unadjusted		Adjusted*		Adjusted†	
		Odds Ratio (95% CI)	P Value	Odds Ratio (95% CI)	P Value	Odds Ratio (95% CI)	P Value
No history of CVD							
All‡	360	1.38 (1.15, 1.64)	0.0004	1.44 (1.20, 1.72)	<0.0001	1.42 (1.18, 1.70)	0.0002
Placebo	188	1.47 (1.14, 1.89)	0.0025	1.45 (1.12, 1.88)	0.0046	1.44 (1.11, 1.87)	0.0059
Pravastatin	172	1.29 (1.00, 1.65)	0.046	1.41 (1.08, 1.83)	0.010	1.38 (1.06, 1.80)	0.016
History of CVD							
All‡	459	1.06 (0.93, 1.21)	0.40	1.05 (0.91, 1.21)	0.49	1.04 (0.90, 1.19)	0.60
Placebo	254	1.23 (1.00, 1.52)	0.050	1.22 (0.98, 1.52)	0.072	1.18 (0.95, 1.48)	0.13
Pravastatin	205	0.94 (0.78, 1.12)	0.48	0.91 (0.75, 1.10)	0.32	0.91 (0.75, 1.10)	0.31

The primary end point was coronary heart disease death or nonfatal myocardial infarction and fatal or nonfatal stroke. Odds ratios are for a 1-unit increase in log IL-10. IL indicates interleukin.

*Adjusted for age, sex, low-density lipoprotein cholesterol, high-density lipoprotein cholesterol, log triglyceride, systolic blood pressure, diastolic blood pressure, body mass index, smoking status, diabetes, use of antihypertensive therapy, and country.

†Also adjusted for log C-reactive protein.

‡Also adjusted for randomized treatment allocation.

analysis to primary end points only yielded C-statistics that were broadly similar to the all-subjects analysis: C-statistics were 0.608, 0.619 ($P=0.0023$), and 0.622 ($P=0.0002$), respectively.

Discussion

These prospective data in the powerful PROSPER study allow several important observations regarding circulating IL-10 concentrations and risk of CVD. First, we confirmed and extended previous data based on smaller event numbers in a population at risk for CVD²¹ by demonstrating circulating IL-10 to be independently and positively associated with increased risk of CVD in the overall cohort. There was also evidence that IL-10 was a stronger marker of CVD risk in those without prior CVD. Indeed, IL-10 was associated with increased risk of CVD (regardless of treatment) in those without history of CVD, whereas it was not associated with risk in those who had previously experienced a myocardial infarction or stroke (regardless of treatment). By splitting the cohort on the basis of baseline median concentrations of IL-10 and IL-6, we showed that, generally, elevation of either or both cytokines is associated with an increased risk of CVD. Finally, we showed that IL-10 may have modest clinical utility based on incremental information to the C-statistic for conventional risk factors, although this requires further investigation.

Overall, these results suggest that the epidemiological signal for relative risk given by circulating IL-10 is complex and is likely to be highly context dependent. In many ways, this makes sense from what is known of basic IL-10 biology. IL-10 is believed to be a potentially important cytokine in terms of regulating inflammation within arterial plaques, where it appears to act in an antiinflammatory manner.^{1,2,5–8} IL-10 modulates proinflammatory cytokine production, in particular the Th-1 cytokines typical of atherogenesis²; it may modulate endothelial function and leukocyte recruitment in vivo in proinflammatory conditions²⁶; it may influence anti-

gen presentation (including oxidized lipids) from macrophages and dendritic cells²; and it may even stabilize rupture-prone plaques by suppressing apoptotic pathways in foam cells.²⁷ From this, some investigators may hypothesize an inverse association of circulating IL-10 with CVD risk. However, enhanced expression of a true “antiinflammatory” cytokine makes much more sense in the context of ongoing inflammation within the tissue, as a counterregulatory mechanism to arrest and limit further inflammation. Consistent with this, circulating IL-10 is positively associated with IL-6 and CRP. Furthermore elevation of IL-10, with or without elevation in IL-6, is associated with an increased risk of CVD. Thus, although it is an antiinflammatory cytokine, circulating IL-10 may be a surrogate marker of ongoing systemic inflammation and related pathophysiological processes, and it may be acting to buffer or counterregulate proinflammatory vascular effects. Furthermore, it must be taken into consideration that the inflammation-driven circulation of cytokines is a dynamic process and as such will not result in consistent expression of cytokines; expression of cytokines will have some fluidity, a process not fully captured by taking blood at 1 baseline time point. In addition, elevation of counterregulatory IL-10 may reflect the presence of other proinflammatory cytokines that are associated with CVD risk but that we have not measured, such as IL-18.²⁸

When the population was split by treatment randomization (as was performed in previous PROSPER analysis, with no identifiable interaction for inflammatory markers²³), the results showed that IL-10 was associated with risk in the placebo group but not in the group on treatment. It is possible that pravastatin reduces risk of CVD to a greater extent in those with elevated circulating IL-10 compared with those with lower IL-10, which would explain our observation. However, there are sparse data in the literature that may support this possibility, although Ridker et al have previously reported in a nested-case-control study from the CARE trial of pravastatin that CRP and serum amyloid A concentrations

were related to risk of CHD in those assigned to placebo (relative risk=2.11, $P=0.048$) but not among those randomly assigned to pravastatin (relative risk=1.29, $P=0.5$).²⁹ In PROSPER, this effect modification by treatment group was no longer evident after also stratifying by history of CVD. Given that we have not observed an association of other inflammatory markers with CVD risk, only in placebo recipients in PROSPER, we suggest that this may be a chance finding. Additional epidemiological studies in statin trial cohorts may be useful to expand on our findings.

The positive association of IL-10 with CVD risk was much stronger among those with no history of CVD events. This is perhaps not entirely surprising given that classical risk factors are better predictors of risk among those without history of CVD in this elderly at risk group. Apart from this simplistic epidemiological observation, is it possible to explain why IL-10 is apparently a poor predictor of risk among those who have a history of CVD? It has been observed that cultured peripheral blood monocytes taken from acute myocardial infarction patients and unstable angina patients have markedly reduced potential to express IL-10 in response to lipopolysaccharide or tumor necrosis factor- α stimulation relative to stable angina patients or healthy controls.³⁰ Those results are in agreement with epidemiological findings from the Leiden-85 study of incident stroke.³¹ These intriguing observations require further study.

Limitations of the data require consideration. This PROSPER analysis was based on a nested case-control study and thus did not include all control samples available. This may have reduced statistical power but likely only minimally, given that we had more than 800 cases and more than 1600 controls. Nested case-control is more efficient and cost effective than measuring novel biomarkers on an entire cohort and, if conducted well, is unlikely to introduce biases.³² Frozen sample storage at -80°C before analysis of IL-10 may have resulted in some degradation of the detectable IL-10 signal, although even if this was true, we have no reason to believe any degradation would be proportionally different between cases and controls. Moreover, there are some limited data projecting that IL-10 will be stable for many years in frozen serum.³³ Our observed association of IL-10 with proinflammatory markers also suggests that the signal we have detected is biologically relevant. We did not adjust for regression dilution because of lack of repeat measurement of IL-10, although this does not alter the primary implications of our findings. PROSPER comprises elderly at-risk patients, and we cannot exclude the possibility that this group may show some differences in association of inflammation markers with CVD events as compared with studies in middle-aged populations. However, the observed adjusted risk associations of IL-6 and CRP with primary CVD end point²³ are broadly consistent with findings from recent relevant major metaanalyses that also incorporated younger populations.^{34,35} Although PROSPER has a high-vascular-risk population, participants were otherwise clinically healthy, as per trial exclusion criteria.²² The study only had up to an average of 3.2 years of follow-up, although short follow-up may be appropriate in elderly populations. The study is more relevant to reveal novel associations than to test

predictions in general populations, and for this reason we have not given net-reclassification or integrated discrimination indexes in the present report. However, given the limited and context-dependent association of IL-10 with CVD risk and limited improvement in the C-statistic, we believe a conclusion that IL-10 is likely to have limited clinical utility to predict CVD risk is justified by the available data. Finally, although we can speculate about the mechanisms, our results in isolation cannot prove or disprove any physiological role for IL-10 in CVD risk.

In conclusion, IL-10 is positively associated with risk of CVD among the elderly without history of CVD events, but this association is weaker in patients with a history of CVD events. Additional studies on IL-10 in relation to incident CVD events in younger cohorts appear to be warranted, as well as mechanistic exploration of our findings, particularly to examine whether IL-10 is acting to counter proinflammatory vascular processes.

Sources of Funding

Funding for the IL-10 assays was provided by the Chest, Heart, & Stroke Association, Scotland. Dr Welsh is supported by British Heart Foundation Fellowship FS/10/005/28147.

Disclosures

None.

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SUPPLEMENTAL MATERIAL

Figure I: Association of IL-10 and IL-6 with risk of CVD by dichotomous cutoff at the median observed concentration of each cytokine, relative to the low IL-10 low IL-6 groups. Figures given for each observation correspond to: odds ratio (95% CI); N=controls, n= cases.

