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Interleukin-10 Concentration and Coronary Heart Disease (CHD) Event Risk in the Estrogen Replacement and Atherosclerosis (ERA) Study

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Abstract

Interleukin-10 (IL-10) is a cytokine with pleiotropic properties. Limited biochemical and clinical evidence suggest a link between IL-10 and coronary heart disease (CHD). However, more data are needed to clarify the relationship between IL-10 and risk for CHD events.

METHODS—The present study was a secondary analysis of the Estrogen Replacement and Atherosclerosis (ERA) trial, a randomized clinical trial that examined the effects of hormone replacement therapy on post-menopausal women with known coronary atherosclerosis. IL-10 concentration, measured at baseline, was treated as both a continuous and categorical variable. Cox proportional hazards models were used to compute hazard ratios as estimates of relative risk for CHD events.

RESULTS—There were 71 events over an average 3.2 year follow-up. Incident rates were higher for individuals with IL-10 concentrations equal to or greater than the median level (1.04 pg/mL) compared to those individuals below the median level (30% vs. 18.5 %, $p=0.02$). The cumulative incidence of CHD events was significantly greater in individuals with IL-10 concentrations ≥ 1.04 pg/mL ($p=0.01$). A one standard deviation increase in baseline IL-10 concentration was associated with a 34% greater risk of a CHD event (HR 1.34 [1.06–1.68], $p=0.01$). This elevated risk was not altered by interleukin-6, C-reactive protein, or additional cardiovascular risk factors. IL-10 concentration and risk for CHD events was most pronounced in diabetics (HR 2.4 [1.46–3.83], $p=0.0005$).

CONCLUSION—In the ERA trial, elevated IL-10 concentration was associated with an increased risk for future cardiovascular events in post-menopausal women with established coronary atherosclerosis. Further study of the relationship between IL-10 and the pathogenesis and progression of atherosclerosis and cardiovascular events is warranted.

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Keywords

Interleukin-10; Coronary Heart Disease; Inflammation

INTRODUCTION

Interleukin-10 (IL-10) is a pleiotropic cytokine produced by CD4⁺ Th2 cells, CD4⁺ Th1 cells, CD8⁺ T cells, B cells, and macrophages in response to antigens recognized by the immune system(1). IL-10 inhibits antigen-presenting capacity of monocytes(2) and antigen-induced T cell proliferation(3). IL-10 directly inhibits release of inflammatory cytokines by monocytes and neutrophils(4) and can dampen the inflammatory response by inhibiting Th1 cell cytokine production. On the other hand, IL-10 can stimulate numerous cell types, specifically B-cells (5) and activated CD8⁺ T cells(6), exemplifying the complexity of IL-10 in immunoregulation.

Several lines of evidence suggest that IL-10 may be important in atherosclerosis. The anti-inflammatory and anti-atherogenic properties of IL-10 have been demonstrated using several models of atherosclerosis in mice(7;8). In humans, the expression of IL-10 has been demonstrated in both coronary arteries(9) and atherosclerotic plaque(10). Furthermore, serum levels of IL-10 have been shown to be greater in individuals with atherosclerosis compared to controls(11;12), suggesting IL-10, as an anti-inflammatory molecule, may be elevated in response to the pro-inflammatory environment of atherosclerosis.

Taken together, these data suggest IL-10 is a potentially relevant molecule in atherosclerosis. However, serum concentrations of IL-10 and risk for CHD events have not been previously examined in individuals with known atherosclerosis. Accordingly, we sought to determine in a group of asymptomatic women with atherosclerosis, whether there was an association between serum IL-10 concentration and risk for future CHD events in the Estrogen Replacement and Atherosclerosis (ERA) Trial.

METHODS

The ERA trial was a randomized, double-blind, placebo-controlled clinical trial that examined the effects of hormone replacement therapy on progression of coronary atherosclerosis in women. The design and main results have been previously published(13;14). A total of 309 postmenopausal women with angiographically verified coronary artery disease at baseline were randomly assigned to conjugated equine estrogen (0.625mg/d), conjugated equine estrogen (0.625mg/d) plus medroxyprogesterone acetate (2.5mg/d), or placebo and scheduled for coronary angiography an average of 3.2 years after randomization.

Women were recruited between January 1995 and December 1996 and were eligible if they were postmenopausal, not currently receiving estrogen replacement treatment, and had one or more epicardial coronary stenoses of at least 30 percent of the luminal diameter, as measured by quantitative coronary angiography before the start of the trial. Of the 815 women screened, 309 women were subsequently enrolled.

Smoking history was obtained from questionnaires and current smoking was defined as having a cigarette in the last 30 days. Use of cholesterol-lowering medications was obtained from medication lists. Diabetes was defined as a history of diabetes, a fasting glucose ≥ 126 mg/dL, a 2hr glucose ≥ 200 mg/dL, or use of hypoglycemic medications. Blood pressure was measured in the seated position after a 5-minute rest period, and measurements were based on the average of the second and third readings obtained at each visit. Body mass index (BMI) was obtained

from anthropometric measurements at baseline and calculated from the equation weight (kg)/height (m²).

Assay Methods

Blood samples obtained at baseline were kept frozen at -80°C until assayed. C-reactive protein (CRP) was measured using ELISA and a high-sensitivity kit (American Laboratory Products Co, Windham, NH). The assay sensitivity was 0.002 mg/L. The intra-assay coefficient of variation (CV) was 6.7%, and the interassay CV was 12%. Interleukin-6 (IL-6) was measured by ELISA according to manufacturers' specifications (Biosource International, Camarillo, CA). The assay sensitivity was 0.16 pg/mL. Intra-assay precision was 9.3% CV and the interassay precision was 9.9% CV. IL-10 was measured by ELISA using reagents from BioSource International (Camarillo, CA). The assay sensitivity was 0.4 pg/mL. Intraassay precision was 4.8% CV and interassay precision was 5.5% CV.

Clinical Cardiovascular Events

A CHD event was defined as hospitalization for unstable angina, fatal or nonfatal myocardial infarction, coronary angioplasty, or coronary artery bypass grafting (CABG) over the course of the trial (13). The mean follow-up time was 3.2 years. At each clinic visit, participants were questioned about interval hospitalizations. The hospital discharge summary was obtained for each hospitalization. A similar form was completed if the ERA clinic was informed of a participant's death. Collection of the required information to document the event (including discharge diagnosis, EKGs, cardiac enzymes, procedure notes, and, in the event of out-of-hospital death, a standardized interview with the woman's physician, family members, and witnesses) was obtained as soon as the preliminary event form was filled out.

Statistical Analysis

IL-10, IL-6, and CRP were treated as continuous variables and log-transformed to more closely approximate a normal distribution. Participants were also stratified on the median value for IL-10 (1.04 pg/mL) as having high or low IL-10. Differences in baseline characteristics by CHD event were determined by chi-square test for categorical variables, and t-test and Wilcoxon scores for parametric and nonparametric variables, respectively. Kaplan Meier curves were constructed to illustrate the cumulative incidence of CHD events by high and low IL-10 concentration, and the corresponding p-value was calculated from a log-rank test. Cox proportional-hazards models were used to compute hazard ratios as estimates of relative risk of CHD for one standard deviation difference in IL-10 concentration in univariable analysis, and after adjusting for age, race, diabetes, treatment arm, statin use, smoking, aspirin use, systolic and diastolic blood pressure, BMI, HDL cholesterol, LDL cholesterol, IL-6, and CRP concentrations. Similar univariable and multivariable models were constructed to examine the association of baseline IL-6 and CRP concentration and risk for events. Statistical software SAS 9.1 (Cary, NC) was utilized.

RESULTS

The baseline characteristics of the ERA cohort were stratified by whether participants had a documented CHD event (Table 1). Women did not significantly differ by traditional cardiovascular risk factors such as age, race, smoking, or blood pressure by CHD event category. However, a greater percentage of diabetics had CHD events compared to non-diabetics. In addition, lower HDL concentration was significantly associated with a greater likelihood of a CHD event.

With an average 3.2 years of follow-up, there were 71 CHD events with a calculated incident rate of 23% (71/309). Incident rates were higher for individuals with IL-10 concentrations

equal to or greater than the median level (1.04 pg/mL) compared to those individuals below the median level (30% vs. 18.5 %, $p=0.02$). The cumulative incidence of CHD events was significantly greater in individuals with high IL-10 concentration (Figure 1: log-rank test $p=0.01$).

A one standard deviation increase in baseline IL-10 concentration was associated with a 34% greater risk of a CHD event (HR 1.34 [1.06–1.68], $p=0.01$) (Table 2). This elevated risk was not significantly altered by IL-6, CRP or additional covariates including: age, race, diabetes, treatment arm, statin use, smoking, aspirin use, systolic and diastolic blood pressure, BMI, HDL cholesterol, and LDL cholesterol. There was no significant increase in CHD risk when separate models were constructed for IL-6 or CRP, though the risk associated with high CRP (+20%) was in the expected direction.

When stratifying on presence of diabetes, one standard deviation increase in IL-10 conferred nearly a 2.5 times greater risk for a CHD event in diabetics (HR 2.4 [1.46–3.83], $p=0.0005$) (Figure 2). This interaction was significant with a p -value of 0.03. In addition, individuals with CRP concentrations greater than 3 mg/L and higher IL-10 concentrations had a 40% greater risk for CHD events compared to only a 10% increase in risk when CRP concentrations were <3 mg/L (p -value for interaction = n.s.).

DISCUSSION

In the ERA trial, elevated levels of IL-10 were associated with an increased risk for future cardiovascular events in post-menopausal women with known atherosclerosis. This association was independent of other measured inflammatory markers or additional cardiovascular risk factors. High IL-10 concentration and risk for CHD events was most pronounced in individuals with diabetes.

Previous studies support the role of IL-10 in atherosclerosis, most apparent in atherosclerosis models in mice demonstrating the anti-inflammatory and anti-atherogenic properties of IL-10 (7;8). In humans, the expression of IL-10 has been demonstrated in both coronary arteries(9) and atherosclerotic plaque(10), and higher serum levels of IL-10 are evident in atherosclerosis patients compared to controls(11;12). These results suggest IL-10, as an anti-inflammatory molecule, may be elevated in response to the pro-inflammatory environment of atherosclerosis.

In unstable angina patients, there is conflicting data on serum levels of IL-10 concentration (12;15;16). There is evidence for an increase in IL-10 expression in unstable plaque(17), and CD4+ and CD8+ T cells during ACS(12). Importantly, however, in patients with elevated serum levels of C-reactive protein (CRP) during ACS, low IL-10 concentrations are associated with worse cardiovascular outcomes(18). This data is supported by biochemical studies demonstrating treatment with CRP significantly decreases LPS-induced IL-10 transcription and IL-10 secretion from human monocyte-derived macrophages(19). Interestingly, anti-inflammatory adipokine, adiponectin, upregulates IL-10 expression and protein secretion (20), illustrating the complexity of pro- and anti-inflammatory molecules during a stress response. Taken together, these studies suggest levels of IL-10 are dynamic in the atherosclerosis and ACS process, and expression of IL-10 is influenced by other pro- and anti-inflammatory molecules.

In the current study, diabetics with high IL-10 concentrations were at highest risk for CHD events. Previous studies illustrate a potential relationship between IL-10 and development of type 1 diabetes in mouse models, but the results have been contradictory(21-23). No known studies have assessed the role of IL-10 in atherogenesis among diabetic individuals.

There are limitations to the present study. The sample size and strength of association are modest, suggesting that the results should be interpreted with caution. The study consisted predominately of Caucasian women with known coronary atherosclerosis. Therefore, generalizations cannot be made about this association in men, among different ethnic groups, or in individuals without known atherosclerosis.

In conclusion, in women with known coronary atherosclerosis, elevated concentrations of IL-10 are independently predictive of CHD events. Future study is needed to understand the relationship between IL-10 and Th1 and Th2 cells, critical in the atherosclerosis milieu, and other molecules involved in the pro- and anti-inflammatory response. In addition, important questions remain concerning the potential pro- and anti-inflammatory risk markers, in aggregate, that improve global risk prediction in individuals with known atherosclerosis.

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Figure 1.
Cumulative Event Rate by IL-10 Category in ERA Solid line= IL-10 ≥ 1.04 pg/mL Dotted line= IL-10 < 1.04 pg/mL

Figure 2.
IL-10 Concentration and Risk for CHD After Stratifying by Diabetes and CRP

Table 1
Baseline Characteristics of ERA Cohort By CHD Event

<u>Baseline Characteristics</u>	<u>CHD event</u>		<u>p-value</u>
	No (n=238) mean ($\pm$ SD) or n (%)	Yes (n=71) mean ($\pm$ SD) or n (%)	
Age (years)	65 (9)	65 (11)	0.6
Race			0.4
White	195 (76)	58 (24)	
African-American	35 (81)	8 (19)	
American Indian	7 (58)	5 (42)	
Treatment Arm			0.7
Placebo	80 (76)	25 (24)	
Estrogen and Progestin	83 (80)	21 (20)	
Estrogen	75 (75)	25 (25)	
Diabetes			0.2
yes	95 (73)	35 (27)	
no	143 (79)	36 (21)	
BMI	30.3 (8.9)	29.1 (5.6)	0.7
Smoking			0.8
yes	51 (77)	15 (23)	
no	185 (76)	58 (24)	
Statin Use			0.7
yes	73 (76)	23 (24)	
no	165 (77)	48 (23)	
Aspirin Use			0.04
yes	161 (74)	57 (26)	
no	77 (85)	14 (15)	
Systolic BP (mmHg)	135 (19)	137 (18)	0.3
Diastolic BP (mmHg)	74 (10)	75 (8)	0.6
LDL (mg/dL)	136 (37)	133 (43)	0.7
HDL (mg/dL)	45 (12)	40 (10)	0.03

Table 2

Baseline IL-10, IL-6, and CRP Concentration and Risk for CHD event

	Univariable HR (CI)	p-value	Multivariable HR (CI) [*]	p-value
Interleukin-10	1.34 (1.06–1.68)	0.01	1.35 (1.04–1.77)	0.03
Interleukin-6	0.95 (0.72–1.26)	0.7	0.96 (0.66–1.40)	0.8
C-reactive protein	1.09 (0.87–1.36)	0.4	1.23 (0.87–1.74)	0.2

* Full model for IL-10 included: IL-6, CRP, age, race, diabetes, treatment arm, BMI, statin use, smoking, aspirin use, systolic and diastolic blood pressure, HDL cholesterol, and LDL cholesterol. Model for IL-6 included: CRP, IL-10, age, race, diabetes, treatment arm, BMI, statin use, smoking, aspirin use, systolic and diastolic blood pressure, HDL cholesterol, and LDL cholesterol. Model for CRP included: IL-6, IL-10, age, race, diabetes, treatment arm, BMI, statin use, smoking, aspirin use, systolic and diastolic blood pressure, HDL cholesterol, and LDL cholesterol.