Interleukin-18 and the Risk of Coronary Heart Disease in European Men

The Prospective Epidemiological Study of Myocardial Infarction (PRIME)

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Background—Interleukin (IL)-18 promotes atherosclerotic plaque growth and vulnerability. It is unknown, however, whether elevations of circulating IL-18 precede the onset of coronary events in apparently healthy individuals.

Methods and Results—We evaluated the relationship between baseline plasma levels of IL-18 and the subsequent incidence of coronary events over a 5-year follow-up in the Prospective Epidemiological Study of Myocardial Infarction (PRIME), which included 10 600 healthy European men aged 50 to 59 years at baseline. Analysis was performed in a nested case-control manner comparing 335 cases with a coronary event to 670 age-matched controls. Baseline levels of IL-18 were significantly higher in men who developed a coronary event than in controls (225.1 versus 203.9 pg/mL, \( P = 0.005 \)). After adjustment for most potential confounders, including C-reactive protein, IL-6, and fibrinogen, the relative risk of future coronary events associated with increasing tertiles of IL-18 was 1.65 (95% CI 1.14 to 2.40, \( P = 0.008 \)) in Northern Ireland, 1.29 (95% CI 0.96 to 1.73, \( P = 0.09 \)) in France, and 1.42 (95% CI 1.13 to 1.79, \( P = 0.003 \)) in both populations combined (\( P = 0.31 \) for the test of homogeneity between populations). In all models, IL-18 made an independent contribution to the prediction of risk over lipids or other inflammatory markers such as C-reactive protein, IL-6, or fibrinogen.

Conclusions—Plasma IL-18 level was identified as an independent predictor of coronary events in healthy, middle-aged European men. Determination of circulating IL-18 might improve the prediction of coronary events. (Circulation. 2003;108:2453-2459.)

Key Words: inflammation cardiovascular diseases myocardial infarction prognosis interleukins

Recently, evidence from experimental studies has emerged that expression of IL-18 is intimately related to atherosclerotic plaque progression and vulnerability.\(^{11-14}\) These results could be translated into the clinical setting, as shown in the AtheroGene Study, which suggested that the concentration of circulating IL-18 was one of the strongest predictors of future cardiovascular events in patients with stable and unstable angina.\(^{15}\) To extend these results toward a primary prevention setting, we aimed to evaluate the ability of circulating levels of IL-18 to predict future coronary events in a large, population-based cohort of initially healthy European men.

Methods

Study Population
The design of the Prospective Epidemiological Study of Myocardial Infarction (PRIME) is described elsewhere.\(^{16}\) PRIME is a population-based cohort of initially healthy European men.
population-based prospective study of coronary events. During the 1991 to 1994 recruitment period, 10,600 men aged 50 to 59 years living in the areas of Lille, Strasbourg, and Toulouse in France and Belfast, Northern Ireland, were examined. The entry examination included standardized questionnaires relating to medical history, drug intake, presence of coronary heart disease (CHD), and various habits, including tobacco and alcohol consumption. Approval from the appropriate local research ethics committee was obtained, and all subjects gave written informed consent.

During the 5-year follow-up, subjects were contacted annually by letter and, if necessary, by telephone, and a clinical event questionnaire was completed. For all possible events, clinical information was sought directly from hospital or general practitioners’ notes. All details of ECG, hospital admissions, enzymes, surgical operations, angioplasty, and treatment were collected and classified according to MONICA (Monitoring Trends and Determinants in Cardiovascular Disease) criteria.17 Death certificates were used for supporting information on cause of death. After 5 years, follow-up was achieved in >98% of the cohort.

Outcome was defined by a combined end point that included hard coronary events (nonfatal myocardial infarction [MI] and coronary deaths) and angina pectoris. Angina pectoris was defined by the presence of chest pain at rest or on exertion and 1 of the following criteria: (1) angiographic stenosis greater than 50%; (2) a positive exercise stress test (if no angiographic or scintigraphic data); or (4) ECG changes at rest but without any set of conditions for MI and no evidence of a noncoronary cause in the clinical history. Unstable angina was defined according to the Braunwald criteria. In the absence of ECG or enzyme data, the diagnosis was rejected. Full details on the determination of these end points have been described previously.18 All events were checked by a medical committee to provide independent validation of the event.

**Laboratory Methods**

Blood was drawn into EDTA tubes after a 12-hour fast. Samples were stored in liquid nitrogen until analysis. Plasma IL-18 was measured by single determination with a commercially available ELISA method (MBL Co, Ltd). The within-run coefficients of variation were determined to be 3.2% at a mean of 218.5 pg/mL (7 samples) and 8.9% at a mean of 518.9 pg/mL (7 samples); between-run coefficients of variation were 3.2% at 218.5 pg/mL (7 samples) and 8.1% at 290.1 pg/mL (7 samples). Samples for these methodological analyses were taken from the German AtheroGene study population. To directly compare the concentrations between the AtheroGene Study13 and the PRIME study, the absolute values for IL-18 in AtheroGene have to be multiplied by a factor of 5 because of different dilution procedures. Concentrations of IL-18 did not differ regardless of whether they were determined in serum or plasma (data not shown). CRP was measured by immunonephelometry (Dade Behring), IL-6 by ELISA (R&D Systems), and fibrinogen by the Clauss method. Lipids were determined by routine methods.

**Statistical Analysis**

The present study was conducted in a nested case-control sample that included all cases with a coronary event (n=335) and 2 matched controls for each case (n=670). Controls were age-matched (±3 years) participants recruited in the same center and on the same day (±3 days) as the corresponding case and were free of CHD at the time of recruitment. Details of the cases include some baseline characteristics as shown in Table 1.

**TABLE 1. Baseline Characteristics of PRIME Study Participants**

<table>
<thead>
<tr>
<th>Characteristics</th>
<th>Controls (France/N. Ireland; n=394/276)</th>
<th>Cases (France/N. Ireland; n=197/138)</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age, y</td>
<td>55.2±0.1</td>
<td>55.3±0.1</td>
<td>0.5</td>
</tr>
<tr>
<td>BMI, kg/m²</td>
<td>26.6±0.1</td>
<td>27.3±0.2</td>
<td>0.007</td>
</tr>
<tr>
<td>Alcohol, mL/d</td>
<td>34.4±1.6</td>
<td>31.9±2.3</td>
<td>0.4</td>
</tr>
<tr>
<td>SBP, mm Hg</td>
<td>133.9±0.8</td>
<td>140.1±1.0</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>DBP, mm Hg</td>
<td>83.4±0.5</td>
<td>86.4±0.7</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>History, %</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Hypertension</td>
<td>10.2</td>
<td>20.3</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Diabetes mellitus</td>
<td>2.2</td>
<td>6.9</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Family history of CHD</td>
<td>31.8</td>
<td>39.4</td>
<td>0.015</td>
</tr>
<tr>
<td>Smoking status, %</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Never</td>
<td>28.1</td>
<td>21.2</td>
<td></td>
</tr>
<tr>
<td>Former</td>
<td>42.8</td>
<td>40.3</td>
<td>0.005</td>
</tr>
<tr>
<td>Current</td>
<td>29.1</td>
<td>38.5</td>
<td></td>
</tr>
<tr>
<td>Lipid status</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Total cholesterol, g/L</td>
<td>2.23±0.02</td>
<td>2.34±0.02</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>HDL cholesterol, g/L</td>
<td>0.47±0.005</td>
<td>0.44±0.007</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Triglycerides, g/L</td>
<td>1.39 (1.34–1.45)</td>
<td>1.53 (1.44–1.62)</td>
<td>0.01</td>
</tr>
<tr>
<td>Markers of inflammation</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>CRP, mg/L⁺</td>
<td>1.38 (1.27–1.50)</td>
<td>1.96 (1.75–2.20)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Fibrinogen, g/L⁺</td>
<td>3.29 (3.23–3.36)</td>
<td>3.51 (3.42–3.61)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>IL-6, pg/mL⁺</td>
<td>1.35 (1.28–1.43)</td>
<td>1.76 (1.63–1.90)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>IL-18, pg/mL⁺</td>
<td>203.9 (195.7–212.4)</td>
<td>225.1 (212.8–238.1)</td>
<td>0.005</td>
</tr>
</tbody>
</table>

SBP indicates systolic blood pressure; DBP, diastolic blood pressure.

Data presented are percentages for categorical variables, population-adjusted mean±SEM for normal continuous variables, and *population-adjusted geometric means (95% CI) for skewed continuous variables.
date of the coronary event of the case. Mean levels of continuous risk factors were compared between cases and controls by ANOVA adjusted for population. Variables with a skewed distribution were log transformed for analysis. Conditional logistic regression analysis for matched case-control studies was used to investigate the association between outcome and explanatory variables. In these analyses, IL-18 and other inflammatory markers were treated in tertiles derived from the distribution of control individuals from the 2 populations pooled. Three models were considered for testing IL-18 effect: (1) unadjusted; (2) adjusted for classic risk factors (body mass index [BMI], smoking status, diabetes, hypertension, total cholesterol, HDL cholesterol, and triglycerides); and (3) additionally adjusted for inflammatory markers (CRP, fibrinogen, and IL-6). The relative risk (RR) associated with increasing tertiles of IL-18 was estimated by considering the tertiles as an ordinal variable (coded 0, 1, or 2). Homogeneity of the effect of IL-18 between populations was tested for matched case-control studies was used to investigate the association between coronary risk and CRP, fibrinogen, and IL-6. This was in sharp contrast with the strong associations observed within the cluster of the 3 acute-phase reactants. There was no association between IL-18 levels and lipid-lowering drugs, ACE inhibitors, or β-blockers (data not shown).

**Results**

**Baseline Characteristics**

Baseline characteristics of cases and controls are reported in Table 1. As expected, men who developed an incident coronary event were more likely to have a higher prevalence of classic risk factors than their matched controls and had higher baseline levels of inflammatory markers (CRP, fibrinogen, and IL-6), as already reported.19

**Correlates of IL-18 Compared With Other Inflammatory Markers**

As outlined in Table 2, smoking status strongly affected concentrations of CRP, fibrinogen, and IL-6. Hypertension was also associated with elevated levels of CRP and IL-6, whereas diabetes mostly related to IL-6 levels. In contrast, the concentration of IL-18 was independent of any of these factors. Moreover, unlike the acute-phase reactants, IL-18 levels did not correlate with age or BMI (Table 3). The negative correlation of IL-18 with HDL cholesterol was of similar magnitude to that observed for the other inflammatory markers. IL-18 concentrations did not correlate with fibrinogen levels and showed only a modest correlation with CRP and IL-6. This was in sharp contrast with the strong associations observed within the cluster of the 3 acute-phase reactants. There was no association between IL-18 levels and lipid-lowering drugs, ACE inhibitors, or β-blockers (data not shown).

**IL-18 and Coronary Risk**

Baseline concentrations of IL-18 were significantly higher among patients who experienced a coronary event during follow-up than among those who did not (225.1 versus 203.9 pg/mL; P=0.005). The unadjusted RRs for the combined coronary event end point were 1.32 (95% CI 0.90 to 1.93, P=0.16) in the second tertile and 2.07 (95% CI 1.40 to 3.05, P<0.001) in the third tertile compared with the first tertile of IL-18. These unadjusted RRs were of similar magnitude to those associated with tertiles of CRP and fibrinogen and lower than those associated with IL-6 (Figure 1). However, adjustment for cardiovascular risk factors significantly attenuated the relationship between coronary risk and CRP, fibrinogen, and IL-6. Conversely, the association between IL-18 and outcome remained unaffected by adjustment on risk factors and by further adjustment on CRP taken as representative of the acute-phase reactants cluster (Figure 1).

Circulating levels of IL-18 were higher in Northern Ireland than in France (218.1 versus 188.9 pg/mL; P=0.001; 262.7 versus 196.2 pg/mL in cases, P<0.001). This observation was in accordance with similar differences observed for the other inflammatory markers (CRP, fibrinogen, and IL-6) and for lipid factors (data not shown). These differences paralleled that of the prevalence of CHD between the 2 countries.

The association between increasing IL-18 tertiles and coronary risk appeared stronger in Northern Ireland (RR=1.65, 95% CI 1.14 to 2.40, P for trend=0.008) than in France (RR=1.29, 95% CI 0.96 to 1.73, P for trend=0.09),
although the 2 RRs were not statistically different (Figure 2A). The RR associated with increasing tertiles of IL-18 was 1.42 (95% CI 1.13 to 1.79, \(P=0.003\)) in both countries combined. When we separately analyzed MI/coronary death (Figure 2B) and angina pectoris (Figure 2C), the association appeared to be stronger for angina pectoris (RR=1.79, 95% CI 1.23 to 2.61, \(P\) for trend=0.003) than for MI/coronary death (RR=1.38, 95% CI 0.98 to 1.93, \(P\) for trend=0.06), but again, the difference between the 2 RRs did not reach statistical significance (\(P=0.31\) for the test of homogeneity).

**Risk Prediction of IL-18 Beyond Lipid and Classic Inflammatory Variables**

We further explored to what extent IL-18 might add to the predictive value of the strongest classic lipid predictor (total cholesterol/HDL cholesterol ratio) or of the inflammatory markers (Table 4). In these analyses, subjects were classified as being below or above the median for any of the risk factors considered (medians derived from the distributions of controls). Elevation of IL-18 alone was associated with an RR that ranged from 1.86 to 2.51, which was significant in all cases (Table 4). Furthermore, IL-18 added to the predictive value of the other predictors, as demonstrated by the increase in risk when elevation of IL-18 was associated with another risk factor.

Finally, we investigated which marker, IL-18 or CRP (taken as representative of the acute-phase reactants cluster), was the most predictive of coronary events over lipid testing. IL-18, CRP, and the total cholesterol/HDL cholesterol ratio (all 3 variables dichotomized according to their median value) were introduced simultaneously in a model additionally adjusted for BMI, smoking status, diabetes, and hypertension. The adjusted RRs were 1.65 (95% CI 1.19 to 2.29, \(P=0.003\)) for the lipid ratio, 1.63 (95% CI 1.17 to 2.27, \(P=0.004\)) for CRP, and 1.82 (95% CI 1.30 to 2.55, \(P<0.001\)) for IL-18, respectively.

**Discussion**

In this prospective, population-based cohort of healthy European men, baseline plasma level of IL-18 was independently associated with the future development of coronary events.
The relationship between IL-18 and the risk of CHD appeared to be independent of classic risk factors and other inflammatory biomarkers. In the present study, subjects in the highest tertile (>235 pg/mL) had a clear elevation of risk compared with those in the lowest tertile (<160 pg/mL). It must be further evaluated from different studies and various clinical settings which cutoff value of IL-18 appears to be the most useful to identify individuals at coronary risk.

Atherothrombosis is a chronic process that implicates responses of innate and acquired immunity. Alongside increasing experimental evidence, circulating markers of inflammation have been shown to predict cardiovascular risk in initially healthy people. Among these, CRP appears to be a consistent predictor in men and women across different populations, and the clinical usefulness of CRP has been postulated, although its application to clinical practice has been recently discussed. In the PRIME study, CRP was no longer associated with the risk of coronary events when controlled for IL-6 levels, nor was fibrinogen. In addition to acute-phase reactants, other inflammatory biomarkers have been shown to provide prognostic information in a variety of clinical settings. Among them, IL-18 enjoys an important place as a regulator of innate and acquired immune responses. The present results obtained in healthy individuals extend the previous results observed in patients with established coronary artery disease. Epidemiological evidence and emerging experimental data support the hypothesis that the IL-18 receptor/ligand dyad might play a key role in the inflammatory response that contributes to atherosclerotic plaque formation and vulnerability. IL-18 has been identified in human atherosclerotic lesions, with significantly higher levels of IL-18 mRNA in unstable plaques. Administration of IL-18 leads to an increase in lesion size and promotes an elevation in the number of lesion-associated T lymphocytes in animal models. Both effects were abolished in interferon-γ-deficient mice, strongly suggesting the importance of the interferon-γ-dependent pathway. Furthermore, inhibition of IL-18 signaling by IL-18 binding protein has been shown to reduce lesion progression and to stabilize plaque composition, with a decrease in inflammatory cells.

Figure 2. RRs for coronary event according to tertiles of IL-18, separated by end point and country. Solid squares indicate RRs from both countries pooled, respective symbols RRs from France (F) and Belfast (B). All RRs are fully adjusted (see model 3, Figure 1). A, Combined end point (for trend=q<0.003 in both countries pooled, q=0.31 for homogeneity between countries). B, Coronary death/MI (for trend=q=0.06, q=0.48 for homogeneity). C, Angina pectoris (for trend=q=0.003, q=0.35 for homogeneity).
and lipid content and an increase in smooth muscle cells and collagen.\textsuperscript{12}

Given the experimental data and the conclusive epidemiological evidence in different clinical settings, IL-18 appears to be an attractive candidate biomarker for clinical use. Indeed, IL-18 adds significant prognostic information over the classic lipid and inflammatory markers. Whereas CRP, fibrinogen, and IL-6 are influenced by classic risk factors, and adjustment for these confounders attenuates the predictive power of these biomarkers, the effect of IL-18 was not attenuated by adjustment for classic risk factors.

Some desirable characteristics of a cardiovascular risk predictor include standardized assays, stability of the biomarker, independence from established risk factors and improved risk prediction over and above them, a wide range of similar results in different populations, and easy application with reasonable costs. These criteria are fulfilled for CRP, although some doubts remain, especially with regard to its utility in patients at high risk or in patients already taking cardiovascular medications. In contrast, IL-18 has been shown to be predictive in initially healthy men in the PRIME study, as well as in patients with established coronary artery disease in the AtheroGene Study,\textsuperscript{15} but this marker is not yet ready for clinical application. Assessment of IL-18 is presently an expensive and time-consuming procedure, and before it enters the clinical setting, standardized reproducible assays should be available, as well as a consistent series of results from prospective studies.

A limitation of the present study is that the determination of IL-18 was only performed once on samples that were stored in liquid nitrogen. We therefore cannot exclude the possibility of protein degradation. However, this should affect both cases and controls in a similar way, and the consequence would be an attenuation of the relationship with disease.

In conclusion, in this prospective study of initially healthy men, we demonstrated a strong and independent association between plasma levels of IL-18 and future coronary events. This finding is in accordance with results obtained in high-risk patients and supports the concept, already suggested from experimental work, that inhibition of IL-18 might constitute a new therapeutic strategy in plaque stabilization.

Appendix

The PRIME Study Group

The PRIME study is organized under an agreement between INSERM and the Merck, Sharpe and Dohme-Chibret Laboratory, with the following participating laboratories: the Strasbourg MONICA Project, Strasbourg, France (D. Arveiler, B. Haas); the Toulouse MONICA Project, INSERM U558, Toulouse, France (J. Ferriere, J.B. Ruidavets); the Lille MONICA Project, INSERM U508, Lille, France (P. Amouyel, M. Montaye); the Department of Epidemiology and Public Health, Queen’s University Belfast, Northern Ireland (A. Evans, J. Yarnell); the Department of Atherosclerosis, SERLIA-INSERM U545, Lille, France (G. Luc, J.M. Bard); the Laboratory of Hematology, La Timone Hospital, Marseille, France (I. Juhan- Vague); the Laboratory of Endocrinology, INSERM U326, Toulouse, France (B. Perret); the Vitamin Research Unit, The University of Bern, Bern, Switzerland (F. Gey); the Trace Element Laboratory, Department of Medicine, Queen’s University Belfast, Northern Ireland (D. McMaster); the DNA Bank, INSERM U525/SC7, Paris, France (F. Cambien); and the Coordinating Center, INSERM U258, Villejuif, France (P. Ducimetière, P.Y. Scarabin, A. Bingham).

Acknowledgment

Dr Blankenberg was supported by a grant of the Institut National de la Santé et de la Recherche Médicale (INSERM), Paris, France. We thank the following organizations, which allowed the recruitment of the PRIME subjects: the health screening centers organized by the Social Security of Lille (Institut Pasteur), Strasbourg, Toulouse, and Tourcoing; Occupational Medicine Services of Haute-Garonne, of the PRIME subjects: the health screening centers organized by the Social Security of Lille (Institut Pasteur), Strasbourg, Toulouse, and Tourcoing; Occupational Medicine Services of Haute-Garonne, of the Urban Community of Strasbourg; the Association Inter-entreprises des Services Médicaux du Travail de Lille et environs; the Comité pour le Développement de la Médecine du Travail; the Mutuelle Générale des PTT du Bas-Rhin; the Laboratoire d’Analyses de l’Institut de Chimie Biologique de la Faculté de Médecine de Strasbourg; and the Department of Health (NI) and the Northern Ireland Chest Heart and Stroke Association. We are indebted to Susanne Prigge, Ruediger Walscheid, and Axel Thuy for determining the IL-18 measurements and to Margot Neuser for her graphical work.

References


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Circulation. 2003;108:2453-2459; originally published online October 27, 2003;
doi: 10.1161/01.CIR.0000099509.76044.A2
Circulation is published by the American Heart Association, 7272 Greenville Avenue, Dallas, TX 75231
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Print ISSN: 0009-7322. Online ISSN: 1524-4539

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