Prediction of Myocardial Infarction in Dyslipidemic Men by Elevated Levels of Immunoglobulin Classes A, E, and G, but Not M

Petri T. Kovanen, MD; Matti Mänttäri, MD; Timo Palosuo, MD; Vesa Manninen, MD; Kimmo Aho, MD

Background: Immune mechanisms have been suggested to play an important role in the development of coronary atherosclerosis and its thrombotic complications. We evaluated the predictive value of the levels of various serum immunoglobulin classes in middle-aged men at increased risk of myocardial infarction.

Methods: Using nested case-control design and logistic regression analysis, we estimated the association between serum immunoglobulins and the risk of coronary end points (nonfatal or fatal myocardial infarction or sudden cardiac death) in dyslipidemic men (levels of non–high-density lipoprotein cholesterol ≥5.2 mmol/L, [≥201 mg/dL]) participating in the Helsinki Heart Study. The cases consisted of 135 subjects in whom a coronary end point occurred during the 5-year observation period of the study, and the controls were 135 subjects who did not suffer coronary end points during this period. Levels of IgA, IgE, IgG, and IgM were determined in serum samples collected at study entry.

Results: Levels of IgA, IgE, and IgG, but not IgM, were significantly higher in cases than in controls. After adjustment for other risk factors, such as age, smoking, and blood pressure, the risk of coronary disease showed a significant relation to the levels of IgA, IgE, and IgG. The risk in the highest quartile of each distribution as compared with the lowest quartile was 2.2-fold for IgA (95% confidence interval, 1.0-4.5), 2.8-fold for IgE (1.3-5.9), and 2.8-fold for IgG (1.3-5.9). Hypertriglyceridemia and a low level of high-density lipoprotein cholesterol were associated with increased risk of a coronary end point only if the levels of IgA, IgE, or IgG were also elevated.

Conclusion: Elevated levels of IgA, IgE, and IgG are associated with myocardial infarction and cardiac death in men with dyslipidemia. The present data suggest that, for dyslipidemia to cause coronary atherothrombosis, an immune response reflected by elevated levels of these immunoglobulin classes is an important determinant.

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The role of immunologic mechanisms in the development of atherosclerosis and its thrombotic complications (collectively named “atherothrombosis”) has attracted considerable interest during the last 2 decades.1-8 Recent evidence indicates that both cellular and humoral immune mechanisms are involved in the development of the atherosclerotic lesions. Thus, activated macrophages,9,10 T lymphocytes,11 and mast cells12 have all been found in the atherosclerotic arterial wall, and antibodies against oxidized low-density lipoproteins (LDLs)13 and circulating immune complexes composed of LDLs and antibodies against them14 have been found in the circulation of patients with atherosclerosis. The thrombotic component of the disease is also connected with immunologic processes of the cellular and humoral type. Macrophages13 and mast cells16 in unstable coronary plaques, for example, secrete proteinases that induce degradation of the extracellular matrix and may cause the plaque to rupture. The macrophages of the lesions also secrete the tissue factor, a key protein in the activation of the coagulation cascade and, hence, of thrombus formation.17 In addition, the serum of patients suffering from myocardial infarction has been found to contain antibodies against phospholipids or phospholipid-binding proteins, which have been considered to play a role in arterial thrombosis.18 The observed cross-reaction between antibodies against oxidized LDL and those against cardiolipin, an anionic phospholipid, also suggests an immunologic link between atherosclerosis and thrombosis.19 Finally, elevated antibody titers

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METHODS

STUDY POPULATION

The present case-control study was nested within the Helsinki Heart Study, a 5-year double-blind coronary primary prevention trial to test the hypothesis that lowering serum LDL cholesterol and triglyceride levels, and elevating serum high-density lipoprotein (HDL) cholesterol levels with gemfibrozil, would reduce nonfatal myocardial infarction and cardiac death in dyslipidemic men. The design, methods, and primary results have been described elsewhere in detail.32-34 Briefly, the participants were selected from 23,531 men aged 40 to 55 years, employed by 2 government agencies and 5 industrial companies in Finland. To be eligible, subjects had to fulfill the lipid acceptance criterion of a non-HDL cholesterol level greater than 5.2 mmol/L (201 mg/dL) at 2 successive screening visits and have no evidence of coronary heart disease or any other major disease (eg, bronchial asthma, rheumatoid arthritis). The lipid acceptance criterion selected subjects from the upper end of the total cholesterol distribution (and hence LDL cholesterol distribution), while the HDL cholesterol distribution in the study participants was close to that of the population screened. A total of 4081 men were randomly allocated to receive either gemfibrozil (n=2046) or placebo (n=2035). The cases in the present study consisted of 135 (of the 140) subjects with a definite coronary end point (nonfatal or fatal myocardial infarction or sudden cardiac death during the study period)32 from whom a sufficient volume of frozen baseline serum was available for analyses. The controls were subjects matched for drug treatment (gemfibrozil or placebo) and geographical area (clinic in the study organization), but who did not suffer coronary end points or any other major illness during the study period.32

LABORATORY METHODS

Frozen baseline serum, collected initially from all entrants to the study in 1980 to 1982 and preserved at −20°C, was used in the analyses. Levels of IgA, IgG, and IgM were quantified by standard immunoturbidimetry, by means of an automated clinical chemistry analyzer (Kone Specific R, Kone Instruments, Espoo, Finland). Calibrators (Labquality Inc, Helsinki, Finland) and antisera (Dako, Glostrup, Denmark) were obtained commercially. Total IgE levels were determined with a commercial enzyme-linked immunosorbent assay kit (Immunotech SA, Marseille, France) according to the manufacturer's instructions. Interassay and intra-assay variations, respectively, were 4.5% and 2.4% for IgA, 8.0% and 5.0% for IgE, 5.7% and 2.9% for IgG, and 5.6% and 3.7% for IgM.

STATISTICAL METHODS

For continuous variables, the significances of differences in mean level between the cases and controls were estimated by means of either the t test or analysis of variance. Because of the strongly skewed IgE distribution, logarithmic transformations of the values (log IgE) were used in the analyses, and the results are given as medians. For IgA, IgG, and IgM, with relatively normal distributions, mean levels with SDs are given. All subjects who smoked were categorized as smokers, irrespective of the quantity of tobacco consumed. All ex-smokers in this study cohort (less than 5%) had stopped smoking more than 3 months before the start of the trial and were included with the nonsmokers. The significance of immunoglobulins as a coronary risk factor was estimated with unconditional logistic regression analyses in the EGRET (Epidemiology and Statistical Corp, Seattle, Wash). For the analyses, the levels were divided into quartiles and the lowest was used as a reference (odds ratio [OR], 1). For the study of joint effects of immunoglobulins and other risk factors (eg, smoking), a new variable was added to the model. The variable consisted of all combinations of, for example, IgA (high/normal) and smoking (smoker/nonsmoker), with subjects in the normal IgA–nonsmoker group serving as a reference (OR, 1). This approach is similar to a stratified analysis, except that all combinations are held in the model simultaneously and so can be compared directly with each other.

RESULTS

There were differences between cases and controls in the baseline levels of several coronary risk factors (Table 1). This was to be expected, since the matching variables used were treatment group and geographical area. The classic risk factors (age, blood pressure, and smoking) were different in the 2 groups. However, the baseline lipid levels were similar because of the selection criteria of the Helsinki Heart Study.
With the exception of IgM, immunoglobulin levels at the study baseline were significantly higher in the cases than in the controls (IgA, 2.74±1.13 vs 2.45±0.95 g/L, P<.03; IgE, 0.012 vs 0.0091 mg/L (1 mg/L=417 [kilo–international units per liter]), P=.002; IgG, 12.3±3.6 vs 11.5±2.9 g/L, P<.05; and IgM, 1.32±0.60 vs 1.24±0.65 g/L, P=.3). The frequency distributions are given in the Figure, and in every instance the cases clearly showed a higher range of values. It should be noted that the mean values for IgA, IgG, and IgM, and the median values for IgE, were all within the normal range for the general population in Finland (normal range, 0.9-4.8 g/L for IgA, 0.24 mg/L for IgE, 6.8-15 g/L for IgG, and 0.4-2.6 g/L for IgM).

Smoking had no effect on IgA level, whereas IgE level was higher (0.013 vs 0.0096 mg/L, P<.001) and IgG level was lower (11.3±3.2 vs 12.5±3.3 g/L, P<.003) in smokers than in nonsmokers. No associations were found between the immunoglobulins and blood pressure or lipid levels.

There was a significant positive correlation between the baseline immunoglobulin level and the risk of coronary heart disease in the study cohort, which remained significant after adjustment for age and smoking (Table 2). Further adjustment for additional risk factors (eg, blood pressure) did not change this correlation. The results also remained essentially unchanged (data not presented) when the analyses were stratified in nonsmokers, in the placebo group, or in a subset excluding the 7 subjects (4 cases and 3 controls) with elevated IgE levels (<0.24 mg/L; reference values, 0.00-0.24 mg/L), suggesting atopy.

A study of the joint effects of immunoglobulins and other risk factors for coronary heart disease (smoking, low HDL cholesterol level, or elevated triglyceride level) disclosed the following effects. The joint effect of elevated IgG level and smoking was almost multiplicative (OR, 8.3), while elevated IgE level increased the risk by 70% in nonsmokers (from an OR of 1 to 1.7) and only by 35% in smokers (from an OR of 2.8 to 3.8), even if the absolute increase in risk was greater in smokers (Table 3). The effect of low HDL cholesterol level as a risk factor was dependent on immunoglobulin (A, E, and G) levels, there being no increment in risk when the immunoglobulin levels were low (Table 4). When elevated triglyceride levels were used in the analyses instead of low HDL cholesterol levels, the risk increased only by 20% (from an OR of 1.0 to 1.2) in the low IgG category but by 125% (from an OR of 2.0 to 4.5) in the high IgG category (data not presented).

Finally, studies of the joint effects of individual immunoglobulin classes disclosed the following associations. Table 5 shows that a high IgE level increased the risk by 60% when the IgA level was low but only by about 30% (from an OR of 1.8 to 2.4) when the IgA level was high. Similarly, a high IgE level increased the risk by 70% when the IgG level was low, but did not increase the risk any further (OR of 2.8 vs 2.7) when the level of IgG was also elevated. The study of the joint effects of IgG and IgE on the risk of coronary heart disease is presented in Table 5.
In the subjects participating in the Helsinki Heart Study, we previously found that those who developed myocardial infarction had significantly higher levels of 2 specific autoantibodies, those against oxidized LDL and against cardiolipin. Moreover, there was a significant correlation between the levels of these antibodies and the total IgG level (P.T.K., M.M., and T.P.; unpublished data; 1995). In the Helsinki Heart Study, an increased risk of myocardial infarction has also been found among subjects with elevated levels of IgG and IgA antibodies against C pneumoniae. Since each specific antibody usually constitutes only a tiny fraction of the immunoglobulins synthesized, the observed increases in the above 3 specific antibodies are not likely to explain the association between total IgG (or IgA) and risk of myocardial infarction.

In this prospective study, we found that in initially healthy hyperlipidemic men who suffered myocardial infarction, the levels of IgA, IgE, and IgG were significantly higher than in their matched controls. In each of these immunoglobulin classes, the risk of myocardial infarction increased with the increase in immunoglobulin level and was not influenced by adjustment for confounding factors, such as age, smoking, or blood pressure.

Both autoantigens and several exogenous antigens have been implicated in the pathogenesis of myocardial infarction. In the subjects participating in the Helsinki

### Table 2. Immunoglobulin Levels and Risk of Coronary Heart Disease

<table>
<thead>
<tr>
<th>Quartile</th>
<th>Odds Ratio (95% CI)*</th>
</tr>
</thead>
<tbody>
<tr>
<td>IgA†</td>
<td></td>
</tr>
<tr>
<td>1</td>
<td>1</td>
</tr>
<tr>
<td>2</td>
<td>1.2 (0.6-2.5)</td>
</tr>
<tr>
<td>3</td>
<td>1.8 (0.8-3.7)</td>
</tr>
<tr>
<td>4</td>
<td>2.2 (1.0-4.5)</td>
</tr>
<tr>
<td>IgE‡</td>
<td></td>
</tr>
<tr>
<td>1</td>
<td>1</td>
</tr>
<tr>
<td>2</td>
<td>1.9 (0.9-3.9)</td>
</tr>
<tr>
<td>3</td>
<td>1.8 (0.9-3.8)</td>
</tr>
<tr>
<td>4</td>
<td>2.8 (1.3-5.9)</td>
</tr>
<tr>
<td>IgG§</td>
<td></td>
</tr>
<tr>
<td>1</td>
<td>1</td>
</tr>
<tr>
<td>2</td>
<td>1.0 (0.5-2.1)</td>
</tr>
<tr>
<td>3</td>
<td>1.8 (0.9-3.8)</td>
</tr>
<tr>
<td>4</td>
<td>2.8 (1.3-5.9)</td>
</tr>
</tbody>
</table>

*IgA disclosed that the OR associated with a high IgG level was 2.1 (95% confidence interval, 1.0-4.5) when the IgA level was low, but 3.6 (95% confidence interval, 1.6-8.0) when the IgA level was high (data not presented).

### Table 3. Joint Effects of Immunoglobulins and Smoking on Coronary Heart Disease Risk*

<table>
<thead>
<tr>
<th>Immunoglobulin Level†</th>
<th>Nonsmoker</th>
<th>Smoker</th>
</tr>
</thead>
<tbody>
<tr>
<td>IgA Low (&lt;2.8 g/L)</td>
<td>1</td>
<td>2.9 (1.6-5.5)</td>
</tr>
<tr>
<td>High (≥2.8 g/L)</td>
<td>1.8 (0.9-3.7)</td>
<td>5.0 (2.2-11.6)</td>
</tr>
<tr>
<td>IgE Low (&lt;0.016 mg/L)</td>
<td>1</td>
<td>2.5 (1.5-5.3)</td>
</tr>
<tr>
<td>High (≥0.016 mg/L)</td>
<td>1.7 (0.8-3.8)</td>
<td>3.8 (1.9-7.7)</td>
</tr>
<tr>
<td>IgG Low (&lt;13 g/L)</td>
<td>1</td>
<td>3.5 (1.8-6.6)</td>
</tr>
<tr>
<td>High (≥13 g/L)</td>
<td>2.4 (1.2-4.9)</td>
<td>8.3 (3.0-22.7)</td>
</tr>
</tbody>
</table>

*Results of a logistic regression analysis with age as a covariate. All combinations were in the model simultaneously, with the risk in subjects with low immunoglobulin level used as a reference (odds ratio, 1). CI indicates confidence interval.

†Low includes lowest and middle tertiles; high, highest tertile.

In this prospective study, we found that in initially healthy hyperlipidemic men who suffered myocardial infarction, the levels of IgA, IgE, and IgG were significantly higher than in their matched controls. In each of these immunoglobulin classes, the risk of myocardial infarction increased with the increase in immunoglobulin level and was not influenced by adjustment for confounding factors, such as age, smoking, or blood pressure.

Both autoantigens and several exogenous antigens have been implicated in the pathogenesis of myocardial infarction. In the subjects participating in the Helsinki Helsinki Heart Study, we previously found that those who developed myocardial infarction had significantly higher levels of 2 specific autoantibodies, those against oxidized LDL and against cardiolipin. Moreover, there was a significant correlation between the levels of these antibodies and the total IgG level (P.T.K., M.M., and T.P.; unpublished data; 1995). In the Helsinki Heart Study, an increased risk of myocardial infarction has also been found among subjects with elevated levels of IgG and IgA antibodies against C pneumoniae. Since each specific antibody usually constitutes only a tiny fraction of the immunoglobulins synthesized, the observed increases in the above 3 specific antibodies are not likely to explain the association between total IgG (or IgA) and risk of myocardial infarction. Rather, in our selected series of subjects, it is plausible to suppose that myocardial infarction was preceded by immunologic processes probably involving a multitude of antigen-antibody systems. The results of recent prospective studies focusing on serum complement components, or plasma C-reactive protein concentrations, are in accord with our findings.
It has been suggested that both in humans and in experimental animals, a significant component of human atheromatus disease is caused by deposition of immune complexes in the arterial wall. Such immune complexes may be formed in association with incidental immune reactions or with persistent low-grade immune reactions. Experimental evidence suggests that the pathogenetic role of immune complexes is particularly significant when the levels of plasma lipids are also increased. Thus, Howard et al showed in baboons that only modest aortic lesions develop in the presence of either hyperlipidemia or immune complexes, but that extensive lesions develop if the animals are both hyperlipidemic and their plasma contains immune complexes. In the Helsinki Heart Study, the subjects were selected on the basis of elevated LDL cholesterol levels (with or without hypertriglyceridemia). Thus, the present findings apply to hyperlipidemic subjects, and it is possible that the association between increased immunoglobulin levels and an increased risk of myocardial infarction applies specifically to patients with elevated plasma lipid levels. Indeed, a low HDL cholesterol level was associated with an increased risk of myocardial infarction only if the level of IgA, IgE, or IgG was elevated. This observation strongly suggests that the underlying immunologic processes, as reflected in elevated immunoglobulin levels, operated in concert with plasma lipids. In contrast, the 2 other major risk factors for coronary heart disease, smoking and elevated blood pressure (data for blood pressure not presented), exerted their effects independently of IgA and IgG levels.

The levels of IgE are generally higher in smokers than in nonsmokers, and we observed this association in the current study also. Thus, it could be argued that part of the effect of elevated IgE level on coronary heart disease risk was mediated by smoking. However, an elevated IgE level was a risk factor for myocardial infarction in nonsmokers also, the effect of IgE being even greater in nonsmokers than in smokers. These results suggest that IgE and smoking exerted their effects, at least in part, independently. The IgE-mediated potentially atherogenic effects are likely to be restricted to cellular events triggered by antigenic stimulation of IgE-bearing cells. Both mast cells and platelets bear IgE receptors on their surfaces, and, provided the receptors are occupied by IgE, interaction of specific antigens with the receptor-bound IgE will activate these cells.

Several mechanistic links can be speculated to exist between IgE and the development of atherosclerotic lesions, especially their late thrombotic complications. First, cholesterol-containing foam cells may be formed in the arterial wall as a consequence of local antigenic stimulation of IgE-bearing mast cells. Second, IgE-mediated release of histamine from mast cells may trigger coronary artery spasms at sites where atherosclerosis has developed. Third, IgE-mediated antigenic stimulation of mast cells in coronary atheromas may trigger local degradation of extracellular matrix, as the stimulated mast cells secrete neutral proteases capable of activating matrix metalloproteinases secreted locally by other cells. Finally, by releasing tumor necrosis factor alpha, a potent proinflammatory cytokine, mast cells may play an active role in the inflammatory reactions of the rupture-prone areas of coronary atheromas. Thus, activated mast cells may contribute not only to the early events of atherogenesis, such as cholesterol deposition, but also to the late events leading to plaque destabilization, rupture, and ultimately coronary thrombosis. Support for this concept was obtained recently, as it was found that IgE predicts future nonfatal myocardial infarction in men, and that this association was independent of smoking and unrelated to allergy. In summary, the present prospective data of elevated plasma immunoglobulin levels preceding coronary end points (myocardial infarction or cardiac death) provide strong clinical support for the hypothesis that immunologic mechanisms are involved in the development of atherothrombosis. The observation of polyclonally elevated IgA, IgG, and IgE before the coronary end points suggests the existence of a multitude of atherogenic inflammatory mechanisms, yet to be discovered, all tending to elicit an immune response.

This study has strengths and limitations. The major limitation is the patient population, consisting of dyslipidemic white men. The results may thus not be applicable to other cases and women. However, a major strength of this study is the selection of the controls, a central issue in case-control studies dealing with infections and inflammation. The cases and controls in this tested case-control study come from the same workplace and had identical access to health care services.

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