Circulating levels of interleukin-17 and cardiovascular outcomes in patients with acute myocardial infarction

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Received 18 February 2012; revised 25 June 2012; accepted 28 July 2012; online publish-ahead-of-print 6 September 2012

See page 556 for the editorial comment on this article (doi:10.1093/eurheartj/ehs399)

Aim

Interleukin (IL)-17 pathway is being clinically targeted in immune-mediated diseases, most of which are associated with a significant cardiovascular risk. We investigated the relationship between serum levels of IL-17 and the risk of cardiovascular events in patients with acute myocardial infarction.

Methods and results

We used data from 981 patients enrolled in the prospective, multicentre French registry of Acute ST elevation, or non-ST-elevation Myocardial Infarction (Fast-MI, NCT00673036). Serum levels of IL-17 were associated with the risk of all-cause death and recurrent MI at 2 years, with levels of IL-17 below the median indicative of a worse outcome. The impact of IL-17 remained significant after adjustment for known cardiovascular risk factors, C-reactive protein, and treatments including statins: hazard ratio (HR) = 1.40 (1.03–1.91); P = 0.03. IL-17 inhibited mononuclear cell adhesion to endothelium and reduced endothelial vascular cell adhesion molecule (VCAM-1) expression. Patients with low (below the median) IL-17 levels and high (above the median) soluble VCAM-1 (sVCAM-1) levels were at particularly increased risk of death and MI: adjusted HR = 2.22 (1.32–3.75) compared with the high IL-17/low sVCAM-1 group (P = 0.002).

Conclusions

Low serum levels of IL-17 are associated with a higher risk of major cardiovascular events in Caucasian patients with acute MI. Our results raise possible concern about the use of inhibitors of the IL-17 pathway in clinical settings associated with a high cardiovascular risk.

Clinical trials registration: NCT00673036.

Keywords

Myocardial infarction • Inflammation • IL17 pathway • Immune-mediated diseases • Pharmacovigilance • Prognosis

Introduction

Atherosclerosis is a complex disease of the arterial wall initiated in response to a variety of pro-atherogenic stimuli, among which modified lipids play an important role.1,2 The latter induce innate and adaptive immune responses with both deleterious and protective components. A breakdown in this balance leads to uncontrolled disease progression and precipitates severe complications.3,4 The current understanding is that the deleterious component of the adaptive immune response is driven by T helper type 1 (Th1)-related mediators, mainly interferon (IFN)-γ, which play central roles in promoting vascular inflammation, artery wall remodelling, and plaque progression.3,4

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The protective component is still poorly understood. However, several studies have shown that three cytokines, interleukin (IL)-5, IL-10, and transforming growth factor (TGF)-β, play critical counter-regulatory roles in atherosclerosis.13,15–11

Recently, a new lineage of CD4+ T cells, called Th17, has been identified and characterized.12 The Th17 subset is driven by specific transcription factors and produces IL-17A (hereafter referred to as IL-17), IL-17F, IL-21, IL-6, and IL-22.12 The discovery of Th17 lineage has revived a great interest in the potential roles of IL-17 in health and disease.13 Several studies have shown a non-redundant role for IL-17 in the clearance of specific pathogens that are not adequately controlled by Th1 or Th2 immunity, particularly extracellular bacteria and fungi.12 Beyond this role in host defence, several researchers have implicated Th17 cells and cytokines in the pathophysiology of immune-mediated diseases, such as rheumatoid arthritis, psoriasis, colitis, or asthma,12,13 even though definite proof of a pathogenic role is still lacking in humans.

On the basis of these data, several investigators hypothesized a deleterious role for IL-17 in atherosclerosis. Their results showed that IL-17 was expressed in human coronary and symptomatic carotid atherosclerotic lesions14–17 and reported enhanced production of pro-inflammatory mediators by vascular smooth muscle cells in response to IL-17, with or without help from IFN-γ.15,18 Other experimental data showed elevated expression of IL-17 at the early stages of lesion development compared with non-atherosclerotic animals,19 and reported reduction in the atherosclerosis burden20,21–23 or inflammation24 or reduction of ischaemia/reperfusion injury25 after inhibition of IL-17 signalling.

However, another set of data suggests a regulatory role for IL-17 in atherosclerosis. We have recently shown that mice with T cells deficient for suppressor of cytokine signalling (SOCS)3 displayed elevated levels of IL-17, associated with the reduced atherosclerotic lesion size.26 The systemic blockade of IL-17 signalling abrogated atheroprotection and promoted vascular inflammation.26 The results were related to the role of IL-17 in the regulation of endothelial vascular cell adhesion molecule (VCAM)-1 and to the control of both Th1 and Th2 responses. In addition, we found that an elevated expression of IL-17 in human carotid lesions was associated with signs of plaque stability.26 Another group recently reported accelerated progression of atherosclerosis in IL17A-deficient mice, which was prevented after supplementation with recombinant IL-17A.27

Overall, the relevance of IL-17 to human atherosclerosis remains poorly defined, and more importantly, its relevance to cardiovascular outcomes remains unexplored. This is an important issue given the current clinical testing of inhibitors of IL-17 signalling in immune-mediated diseases associated with a high cardiovascular risk. Therefore, the aim of the present study was to evaluate the relationship between circulating IL-17 levels and cardiovascular events, a composite of all-cause death and non-fatal myocardial infarction (MI), in patients who suffered from acute MI. In addition, since IL-17 has been shown to regulate VCAM-1 expression in mice,26 we examined the role of IL-17 in mononuclear cell adhesion to endothelial cells and explored the relationship between IL-17, VCAM-1 measured at acute stage and cardiovascular events at 2 years.

Methods

Study population

The study population and methods of the French registry of Acute ST-elevation and non-ST-elevation Myocardial Infarction (FAST-MI) have been described in detail in previous publications.28,29 Briefly, all patients ≥18 years of age were included in the registry if they had elevated serum markers of myocardial necrosis higher than twice the upper limit of normal for creatine kinase, creatine kinase-MB, or elevated troponins, and either symptoms compatible with acute MI and/or or electrocardiographic changes on at least two contiguous leads with pathologic Q waves (≥0.04 s) and/or persisting ST-elevation or depression >0.1 mV. The time from symptom onset to intensive care unit admission had to be <48 h. Patients were managed according to usual practice; treatment was not affected by participation in the registry. Of the 374 centres in France that treated patients with acute MI at that time, 223 (60%) participated in the registry. Among these, 100 centres recruited 1029 patients who contributed to a serum bank. Their baseline characteristics were comparable with the overall population of the registry. Written informed consent was provided by each patient. More than 99% of patients were Caucasians. The follow-up was collected through contacts with the patients’ physicians, the patients themselves, or their family, and registry offices of their birthplace. The two-year follow-up was >98% complete. The outcome events were assessed blinded to the results of IL17, IL6, and sVCAM1 measurements. The study was reviewed by the Committee for the Protection of Human Subjects in Biomedical Research of Saint Antoine University Hospital and the data file was declared to the Commission Nationale Informatique et Liberté.

Blood sampling and measurements

Blood samples used for this study were recovered at the time of admission to the intensive care unit (<48 h from symptom onset). Blood samples were stored at −80°C at the Department of Clinical Pharmacology, University of Pierre et Marie Curie. All samples were identified by number only and were analysed in random order. Serum concentrations of IL-17 were measured using the flow cytometry assay (Bender Med Systems) with a detection limit at 2.5 pg/mL. Soluble VCAM-1 was measured using an ELISA assay (Bender Med Systems). The measurement of C-reactive protein and IL6 levels was also centralized. The limit of detection for sVCAM-1 was 0.6 ng/mL. Among the 1029 patients who contributed to a serum bank, results for IL17, sVCAM-1, and sVCAM1 measurements. The study was reviewed by the Committee for the Protection of Human Subjects in Biomedical Research of Saint Antoine University Hospital and the data file was declared to the Commission Nationale Informatique et Liberté.

Cell adhesion assay

Human peripheral blood mononuclear cells (PBMCs) were isolated on a PANCOLL gradient (Biotech GmbH). After a washout with PBS, cells were stained with the use of a fluorescent probe (0.5 μM; CellTracker Orange CMTMR; Molecular Probes). Briefly, the cells were incubated with the fluorescent probe during 30 min in RPMI and resuspended in culture medium after a 30 min washout for an adhesion assay. Human umbilical cord endothelial cells (HUVECs) (Pomocell) were plated in 48-well plates and stimulated for 24 h with 10 ng/mL tumour necrosis factor (TNF)-α (R&D Systems) in the absence or in the presence of recombinant IL-17 (R&D Systems) at 10 ng/mL or 100 ng/mL, prior to a cell adhesion assay. After washout, fluorescent PBMC cells were made to adhere to HUVECs for 1 h. After two more washouts, adherent cells were fixed in 4% paraformaldehyde and counted in five
ELISA assay
Human umbilical cord endothelial cells were stimulated with 100 ng/mL of TNF-α in the presence or not of either 10 or 100 ng/mL of recombinant IL-17 during 48 h. Then supernatants were collected for the ELISA assay of sVCAM-1 (Bender Med Systems) and IL-6 (BD Biosciences). The limit of detection for VCAM-1 was 0.6 ng/mL, and the lowest concentration of the standard sample for IL-6 was 4.7 pg/mL. The coefficients of variation of IL-17 and sVCAM-1 were 3.4 and 3.1% for the mean intra-assay and 4.9 and 5.2% for the mean inter-assay, respectively.

Statistical analysis
An outcome event was defined as all-cause death or non-fatal MI during the 2-year follow-up period. The primary endpoint, a composite of all-cause death and non-fatal MI defined as the episode index at inclusion, and was adjudicated by a committee whose members were unaware of patients’ medications, and blood measurements. Continuous variables are described as mean ± SD and categorical variables as frequencies and percentages. Serum levels of IL-17, C-reactive protein, IL-6, and sVCAM were log-transformed to remove positive skewness, before being used as continuous variables. Baseline demographic and clinical characteristics, treatment factors, and therapeutic management during hospitalization were compared as pre-specified among the median range of IL-17 levels using χ² or Fisher’s exact tests for discrete variables, and by unpaired t-tests, Wilcoxon sign-rank tests for continuous variables. Median level IL-17 and sVCAM1 were based on the distribution of their baseline levels among the entire population. Survival curves according to the median IL17 level and sVCAM1 are estimated using the Kaplan–Meier estimator. We used a multivariable Cox proportional hazards model to assess the independent prognostic value of variables with the primary endpoint during the 2-year follow-up period. The multivariable model comprised sex, age, previous or current smoking, body mass index, family history of coronary disease, history of hypertension, acute MI, heart failure, renal failure, diabetes, heart rate at admission, Killip class, left ventricular ejection fraction, hospital management (including reperfusion therapy, statins, beta-blockers, clopidogrel, diuretics, digitalis, heparin), and log C-reactive protein levels. The statistical interaction between IL-17 and sVCAM-1 was tested by adding an interaction term with both variables in the Cox model. The results are expressed as hazard ratios for Cox models with 95% confidence intervals (CIs). All statistical tests were two-sided and performed using the SAS software version 9.1. For analysis of the cell adhesion and in vitro cytokines assays, we performed multiple comparisons using ANOVA and the Bonferroni/Dunn test.

Results
Baseline demographics and clinical presentation
Of the 981 patients enrolled, 176 patients (18%) died or had an MI during the 2-year follow-up period. Patients who died or had an MI during the follow-up were older (75 ± 12 vs. 64 ± 13 years) with a higher proportion of females (43 vs. 27%), than those without an outcome event (see Supplementary material online, Table S1). They also had a higher rate of hypertension (80 vs. 58%), diabetes (51 vs. 28%), prior heart failure (15 vs. 3%), prior MI (30 vs. 15%), prior stroke or transient ischaemic event (14 vs. 6%), and chronic renal failure (14 vs. 3%) (all P < 0.001). They were less likely to be on statin therapy (68 vs. 81%), beta-blockers (51 vs. 76%), clopidogrel, heparin, but more likely to be on diuretics, or digoxin (all P < 0.004) compared with patients without an outcome event during the follow-up (Supplementary material online, Table S1).

Events) risk score (185 ± 34 vs. 158 ± 36) and fewer patients had undergone coronary angioplasty PCI (44 vs. 72%) or thrombolyis (10 vs. 18%) during hospitalization (all P < 0.009). The median of IL17 and sVCAM1 for the entire sample was 6.26 pg/mL (0–22.8) and 695.47 ng/mL (528–977), respectively. Patients who had an event had a significantly lower median of IL17 and higher sVCAM1 and IL6 at baseline (Supplementary material online, Table S1). However as shown in Table 1, the baseline characteristics of patients according to the median of IL-17 levels did not differ with the exception of a higher rate of previous or current smokers in those with baseline IL-17 levels <6.26 pg/mL (58 vs. 51% in patients with baseline IL-17 ≥6.26 pg/mL, P = 0.03). No significant correlation was observed between IL-17 and C-reactive protein (Pearson coefficient = −0.2%, P = 0.47). A weak positive correlation of IL-17 was observed with VCAM-1, Pearson coefficient = 0.13, P < 0.001).

Interleukin-17 and clinical outcomes at 2 years
The probability of outcome events as a function of the baseline IL-17 level is presented in Figure 1. At 2 years, event rates for death and MI was higher in patients with IL-17 levels <6.26 pg/mL (21%) compared with those with levels above the median (15%). The corresponding hazard ratio for event rates was 1.44 (95% CI = 1.07–1.95) (P = 0.02). After adjustment for known cardiovascular risk factors, C-reactive protein, and treatments including statins, low IL-17 levels remained an independent correlate of the risk of death or MI, HR 1.40 (1.03–1.91) (P = 0.03).

The adjusted HRs of death and recurrent MI associated with an increase of 1 pg/mL of IL-17 were 0.88 (0.79–0.99; P = 0.03), and 1.20 (1.05 to 1.37) for an increase of 1 pg/mL of IL-6, P = 0.007. We also tested for trend over tertiles of IL-17 and IL-6 to examine the association over a wider range of IL-17 and IL-6 levels. Compared with tertile 1, chosen as a reference, adjusted HRs were 0.66 (0.45–0.96) and 0.68 (0.47–0.99) for tertile 2 and tertile 3, respectively, for IL-17 (P = 0.03). The Cochran-Armitage trend test was significant (P = 0.021). Similarly, the adjusted HRs for tertiles 2 and 3 of IL6 were 1.09 (0.68–1.75) and 1.60 (1.04–2.45), respectively, compared with tertile 1 (P = 0.04).

The results were consistent with similar trends when examining separately the risk of death or MI, for IL17 and IL6, although statistical significance was not achieved due to fewer events.

Soluble vascular cell adhesion molecule-1 and clinical outcomes at 2 years
At 2 years, 25.7% of patients among those with sVCAM-1 levels above the median (≥695.47 ng/mL) died or had a non-fatal MI compared with 9.5% in those with levels below the median

different fields per condition under fluorescence microscope (Zeiss microscope).

Supplementary material online, Table S1

<table>
<thead>
<tr>
<th>IL17A (pg/mL)</th>
<th>&lt;6.26 (n = 490)</th>
<th>≥6.26 (n = 491)</th>
<th>P-value&lt;sup&gt;b&lt;/sup&gt;</th>
</tr>
</thead>
<tbody>
<tr>
<td>Demographic and risk factors</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Male sex (%)</td>
<td>341 (70)</td>
<td>343 (70)</td>
<td>0.93</td>
</tr>
<tr>
<td>Age, year</td>
<td>66 ± 14</td>
<td>66 ± 14</td>
<td>0.97</td>
</tr>
<tr>
<td>Hypertension (%)</td>
<td>300 (61)</td>
<td>306 (62)</td>
<td>0.72</td>
</tr>
<tr>
<td>Hypercholesterolaemia (%)</td>
<td>247 (50)</td>
<td>268 (55)</td>
<td>0.19</td>
</tr>
<tr>
<td>Diabetes mellitus (%)</td>
<td>165 (34)</td>
<td>149 (30)</td>
<td>0.26</td>
</tr>
<tr>
<td>Family history of CAD (%)</td>
<td>109 (22)</td>
<td>127 (27)</td>
<td>0.18</td>
</tr>
<tr>
<td>Previous or current smokers (%)</td>
<td>285 (58)</td>
<td>252 (51)</td>
<td>0.03</td>
</tr>
<tr>
<td>Prior myocardial infarction (%)</td>
<td>77 (16)</td>
<td>92 (19)</td>
<td>0.21</td>
</tr>
<tr>
<td>Prior PCI or CABG (%)</td>
<td>85 (17)</td>
<td>85 (17)</td>
<td>0.99</td>
</tr>
<tr>
<td>Prior stroke or TIA (%)</td>
<td>43 (9)</td>
<td>32 (7)</td>
<td>0.18</td>
</tr>
<tr>
<td>Prior heart failure (%)</td>
<td>25 (5)</td>
<td>23 (5)</td>
<td>0.76</td>
</tr>
<tr>
<td>Chronic renal failure (%)</td>
<td>26 (5)</td>
<td>22 (4)</td>
<td>0.55</td>
</tr>
<tr>
<td>Clinical presentation</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Body mass index (kg/m²)</td>
<td>27 ± 5</td>
<td>27 ± 4</td>
<td>0.57</td>
</tr>
<tr>
<td>Systolic blood pressure at admission</td>
<td>140 ± 30</td>
<td>140 ± 27</td>
<td>0.79</td>
</tr>
<tr>
<td>Diastolic blood pressure at admission</td>
<td>80 ± 17</td>
<td>80 ± 16</td>
<td>0.63</td>
</tr>
<tr>
<td>Heart rate at admission</td>
<td>80 ± 21</td>
<td>79 ± 19</td>
<td>0.89</td>
</tr>
<tr>
<td>STEMI, n (%)</td>
<td>260 (53)</td>
<td>250 (51)</td>
<td>0.5</td>
</tr>
<tr>
<td>Killip entree class ≥ 2 or more (%)</td>
<td>138 (29)</td>
<td>119 (25)</td>
<td>0.17</td>
</tr>
<tr>
<td>GRACE score</td>
<td>163 ± 38</td>
<td>162 ± 36</td>
<td>0.99</td>
</tr>
<tr>
<td>Left ventricular ejection fraction</td>
<td>53 ± 12</td>
<td>53 ± 13</td>
<td>0.63</td>
</tr>
<tr>
<td>Baseline biological exams</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>C-reactive protein (mg/L)&lt;sup&gt;a&lt;/sup&gt;</td>
<td>5.2 (1.86–18.36)</td>
<td>4.92 (1.89–15.43)</td>
<td>0.36</td>
</tr>
<tr>
<td>VCAM-1 (ng/mL)&lt;sup&gt;a&lt;/sup&gt;</td>
<td>675.8 (509–940.5)</td>
<td>727.2 (550.8–1023.8)</td>
<td>0.01</td>
</tr>
<tr>
<td>IL6 (pg/mL)&lt;sup&gt;a&lt;/sup&gt;</td>
<td>4.1 (2.3–9.6)</td>
<td>3.8 (2.1–8.3)</td>
<td>0.21</td>
</tr>
<tr>
<td>Medication previous admission</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Statins (%)</td>
<td>142 (29)</td>
<td>142 (29)</td>
<td>0.98</td>
</tr>
<tr>
<td>Aspirin (%)</td>
<td>168 (34)</td>
<td>163 (33)</td>
<td>0.72</td>
</tr>
<tr>
<td>In-hospital management</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>PCI, n (%)</td>
<td>320 (65)</td>
<td>340 (69)</td>
<td>0.19</td>
</tr>
<tr>
<td>Thrombolysis, n (%)</td>
<td>77 (16)</td>
<td>83 (17)</td>
<td>0.7</td>
</tr>
<tr>
<td>Coronary artery bypass surgery, n (%)</td>
<td>21 (4)</td>
<td>14 (3)</td>
<td>0.23</td>
</tr>
<tr>
<td>Statins (%)</td>
<td>389 (79)</td>
<td>383 (78)</td>
<td>0.59</td>
</tr>
<tr>
<td>Beta-blockers</td>
<td>350 (71)</td>
<td>347 (71)</td>
<td>0.79</td>
</tr>
<tr>
<td>Calcium channel blockers (%)</td>
<td>109 (22)</td>
<td>93 (19)</td>
<td>0.2</td>
</tr>
<tr>
<td>ACE inhibitors or ARB (%)</td>
<td>251 (51)</td>
<td>275 (56)</td>
<td>0.13</td>
</tr>
<tr>
<td>Nitrate derivatives (%)</td>
<td>261 (53)</td>
<td>250 (51)</td>
<td>0.46</td>
</tr>
<tr>
<td>Aspirin (%)</td>
<td>454 (93)</td>
<td>442 (90)</td>
<td>0.14</td>
</tr>
<tr>
<td>Clopidogrel (%)</td>
<td>435 (89)</td>
<td>431 (88)</td>
<td>0.63</td>
</tr>
<tr>
<td>Heparin (%)</td>
<td>405 (83)</td>
<td>405 (82)</td>
<td>0.94</td>
</tr>
<tr>
<td>Low molecular weight heparin (%)</td>
<td>323 (66)</td>
<td>290 (59)</td>
<td>0.03</td>
</tr>
<tr>
<td>Diuretics (%)</td>
<td>169 (34)</td>
<td>160 (33)</td>
<td>0.53</td>
</tr>
<tr>
<td>Glycoprotein IIb/IIIa inhibitor (%)</td>
<td>189 (39)</td>
<td>198 (40)</td>
<td>0.57</td>
</tr>
<tr>
<td>Digitals glycosides (%)</td>
<td>10 (2)</td>
<td>9 (2)</td>
<td>0.81</td>
</tr>
</tbody>
</table>

ACE, angiotensin-converting enzyme inhibitors; ARB, angiotensin receptor blockers; PCI, percutaneous coronary angioplasty; CABG, coronary artery bypass surgery.

<sup>a</sup>Based on the median of baseline values from the entire population.

<sup>b</sup>P is given by Wilcoxon rank-sum or Kruskal–Wallis (continuous variables) and an exact Pearson χ² or Fisher test (categorical variables).

<sup>c</sup>mean ± SD, <sup>d</sup>median, Q1, Q3.
Figure 1  The probability of outcome events (death or recurrent acute myocardial infarction) as a function of baseline circulating IL-17 level.

Figure 2  Adjusted hazard ratios for the risk of all cause death and recurrent MI at 1 year in Fast-MI using a combined assessment of IL-17 (below or above the median) and sVCAM-1 (below or above the median).
(<695.47 ng/mL). The adjusted HR of death and recurrent MI associated with log sVCAM was 1.85 (1.31–2.60; P = 0.0005). Soluble VCAM-1 levels above the median were associated with an increased risk of death or recurrent MI at 2 years (HR = 2.69, 95% CI = 2.11–4.16 compared with sVCAM-1 below the median, P < 0.0001). After adjustment for known cardiovascular risk factors, C-reactive protein, IL-17 levels, and treatments including statins, high sVCAM-1 levels remained an independent correlate of outcome events (HR = 1.75, 95% CI = 1.21–2.52, P = 0.003). The results remained significant with sVCAM categorized on tertiles. The results were consistent with similar trends when examining separately the risk of death or MI.

Combined assessment of Interleukin-17 and soluble vascular cell adhesion molecule-1 and clinical outcomes

At 2 years, 43% of death and MI occurred in patients with low (below the median) baseline IL-17 and high (above the median) baseline sVCAM-1 levels compared with 12% in those patients with high baseline IL-17 and low baseline sVCAM-1. The corresponding hazard ratio for event rates was 4.03 (95% CI = 2.48–6.55, P < 0.0001) and remained highly significant in the Cox multivariate analysis (adjusted HR = 2.22, 95% CI = 1.32–3.75, P = 0.002) (Figure 2). There was no statistically significant interaction between IL-17 and sVCAM-1 (P = 0.15).

Interleukin-17 reduces adherence of mononuclear cells and endothelial soluble vascular cell adhesion molecule-1 production

In the light of these results and our previous finding that the IL-17 protective role in a mouse model of atherosclerosis was associated with reduced endothelial sVCAM-1 expression, we tested the hypothesis that IL-17 modulates human mononuclear cell adhesion on endothelial cells. We observed a significant reduction in PBMC adhesion to TNFα-activated HUVECs in the presence of IL-17, which was associated with a significant reduction in sVCAM-1 expression (Supplementary material online, Figure). As a control, IL-17 enhanced IL-6 production in the same supernatants (Supplementary material online, Figure).

Discussion

The major finding of this study is that elevated levels of IL-17 are associated with a better outcome in patients with acute MI, supporting a protective regulatory role of IL-17 in coronary heart disease. Moreover, the highest risk of death and recurrent MI was observed in patients with low levels of IL-17 and high levels of sVCAM-1, suggesting an important modulatory role of IL-17 on vascular inflammation.

Only a few studies have previously reported measurements of circulating IL-17 levels in patients with coronary heart disease. The first published investigations, performed in populations with <26 Chinese patients, suggested increased levels of IL-17 and Th17 subset in patients with coronary artery disease, more particularly in patients with acute coronary syndromes. However, these results were not replicated in Caucasians. Eid et al. found no difference in IL-17 levels between patients with coronary artery disease (n = 108) and referent outpatients without a diagnosis of coronary atherosclerosis (n = 59). In addition, IL-17 levels did not differ between patients with stable and those with unstable coronary syndromes. Our present results are in agreement with those of Eid et al. in that the median levels of IL-17 that we detect in a Caucasian population are far below the levels reported in Chinese patients. This issue would merit further investigation.

Importantly, the previous clinical studies have generated assumption about the role of IL-17 in coronary artery disease but none had assessed the relationship between IL-17 levels and cardiovascular outcomes. Our study is the first to tackle this issue and shows that the detection of elevated levels of IL-17 in patients with acute MI is associated with a better cardiovascular outcome, i.e. reduced mortality and recurrent MI after 1 year of the follow-up. Thus, the currently held dogma that IL-17 promotes coronary artery disease requires reconsideration.

Some experimental studies have proposed a pro-atherogenic role of IL-17 in atherosclerosis. However, as discussed recently, the evidence for the efficient and sustained blockade of IL-17 signalling was relatively weak in some of the previous work. Our experimental studies led us to different conclusions. We found that mice with increased IL-17 expression displayed significantly smaller atherosclerotic lesions, and the protection was abrogated after IL-17 neutralization. Moreover, the administration of recombinant IL-17 to LDLr–/– mice significantly inhibited lesion development. This regulatory role of IL-17 in atherosclerosis has also been supported by a recent study showing that IL-17A deficiency enhances lesion development in the ApoE–/– model. Interestingly, IL-17 expression in human carotid lesions was associated with a lower macrophage but a higher smooth muscle cell content and a fibrous plaque phenotype, suggesting a role for IL-17 in promoting plaque stability. The atheroprotective role of IL-17 was associated with inhibition of Th1 responses, which is consistent with recent studies showing that IL-17 protects against Th1-mediated diseases like colitis, in part through the modulation of Th1 polarization.

The role of IL-17 in the modulation of endothelial cell activation is still poorly understood. In a previous study, we showed a down-regulation of mouse endothelial VCAM-1 expression in vitro and in vivo in response to IL-17, in part through the modulation of NF-κB activation. Here, we have extended these findings to human cells and showed that IL-17 inhibits mononuclear cell adherence to pre-activated HUVECs in culture. Our results are consistent with other reports showing down-regulation of VCAM-1 expression in other cell types in response to IL-17. Further studies are required to fully delineate the mechanisms by which IL-17 alters endothelial cell activation and mononuclear cell adherence, and to identify the determinants and the cell subsets that produce IL-17 in the setting of acute ischaemic injury.

The modulation of VCAM-1 expression by IL-17 prompted us to look at the interaction between IL-17 and VCAM-1 in vivo with relation to cardiovascular outcomes. Previous studies have reported independent association between circulating levels of sVCAM-1 and future cardiovascular outcomes, particularly in patients with...
stable coronary artery disease, and in patients with diabetes. Such association could not be established in apparently healthy individuals. In acute ischaemic settings, sVCAM-1 levels show sustained elevation for up to 6 months following the index event. However, the prognostic value of elevated sVCAM-1 levels in patients with acute coronary syndromes remains relatively unexplored. Only very small studies that included a majority of patients with unstable angina have been published in this setting with contrasting conclusions. Our present study is the largest one to report on the relationship between sVCAM-1 levels and cardiovascular outcomes in patients with acute MI. The results clearly show that elevated levels of sVCAM-1 at the time of admission for acute MI are associated with a 2.6-fold increase in the risk of death and recurrent MI during the first year of the follow-up. Interestingly, IL-17 levels were associated with outcome events in these patients, the worst prognosis being for patients with both high sVCAM-1 and low IL-17 levels. These findings are consistent with the modulatory role of IL-17 on endothelial VCAM-1 expression and adhesion of mononuclear cells, and suggest an important role for IL-17 in the control of atherosclerosis-related vascular inflammation. It is important to note that in the same study sample, we found that IL-6 (known to induce IL-17 and vice versa) was associated with the worse outcome. This emphasizes the complexity of the system and the need for targeting very specific pathways to ensure both efficacy and safety.

It should be noted that blood sampling for the measurement of IL-17 and sVCAM-1 was performed at the time of admission to the intensive care unit or concomitantly with the onset of treatment, <48 h after symptom onset. Cytokine levels may fluctuate during the first 48 h after acute MI. However, the available data indicate that IL-17 levels are not increased in patients with acute coronary syndromes compared with chronic stable patients, suggesting no major fluctuations during the acute setting. In addition, we believe that fluctuations in IL-17 levels, if any, might have reduced the probability of detecting a significant association between IL-17 and cardiovascular events. Another limitation is the observational nature of this registry-type study, which does not allow a cause–effect demonstration. However, it should be noted that the study was a prospective nationwide registry that closely represents everyday up-to-date current clinical practice. The monitoring of the data by clinical research assistants, the multivariable analyses, the centralized measurement of the biomarkers, blinded to the phenotype data including outcomes, limit, although they do not preclude, the risk of bias.

In conclusion, we show that low levels of IL-17 are independently associated with 1-year mortality and recurrent MI in a Caucasian population of patients admitted for acute MI. These results need confirmation in larger studies and in different patient populations. However, we believe that they may have immediate clinical implications. Clinical trials are currently investigating the efficacy of therapeutic strategies aimed at the inhibition of IL-17 pathway in different clinical settings, including diseases associated with a high cardiovascular risk. Although our study did not assess the direct relationship inhibition of IL17 and cardiovascular events, our findings suggest that patients included in trials using IL17 inhibitors, particularly those with identifiable cardiovascular risk factors, may be at potentially higher risk of serious cardiovascular events and should therefore be closely monitored. In this regard, it is noteworthy that major adverse cardiovascular events have been reported in psoriatic patients assigned to Ustekinumab or Briakinumab, two blockers of IL-12 and IL-23 that reduce both Th1 and Th17 pathways, which was not the case for patients assigned to placebo or treated with etanercept, an inhibitor of tumour necrosis factor-α.

Supplementary material
Supplementary material is available at European Heart Journal online.

Acknowledgements
We thank the physicians who cared for the patients at the participating institutions, the International Clinical Trials Association Contract Research Organization (Fontaine-lès-Dijon, France), Elodie Drouet and the Clinical Research Assistant team of Unité de Recherche Clinique de l’Est Parisien (Assistance Publique–Hôpitaux de Paris and UPMC Paris 06), Benoît Pace, Vincent Bataille and Geneviève Mulak (French Society of Cardiology) for their assistance in designing the electronic case-record form and data management during the follow-up period. We are indebted to Carla Sebella-Arguelles and Paula Doceur (Aterovax, Paris, France) for help in IL-17 measurements.

Funding
Fast-MI is a registry if the French Society of Cardiology, supported by unrestricted grants from Pfizer and Servier and a research grant from the French Caisse Nationale d’Assurance Maladie. This work on IL-17 was also supported by Inserm, by Agence Nationale de la Recherche (ANR), by the transatlantic Leducq Network LINK, the European Union Seven Framework programme TOLERAGE. S.T. was supported by a post-doctoral fellowship from Fondation Lefoulon Delalande. Neither of these sponsors had a role in the management, analysis, and interpretation of data; drafting or approval of the manuscript.

Conflict of interest: none declared.

References


