Objectives
We hypothesized that the expansion of unusual T lymphocytes, CD4⁺CD28null T cells, might represent a key pathogenetic mechanism of recurrent instability.

Background
Clinical presentation of acute coronary syndromes (ACS) is variable. Some patients have recurrent episodes of instability, despite optimal treatment, whereas others have a single acute event in their life. The CD4⁺CD28null T cells, with a functional profile that favors vascular injury, have recently been found both in peripheral blood and in unstable coronary plaques of patients with ACS.

Methods
Peripheral blood T cells from 120 consecutive unstable angina (UA) patients were analyzed for the distribution of T-cell subsets by flow cytometry. Patients were subgrouped according to the occurrence of prior (during the 24 months before the study enrollment) and subsequent (during the 24 months of follow-up) acute coronary events. For 51 patients, the index event was the first ever (G1); 30 patients had prior events (G2); and 39 patients had further events at follow-up (death, myocardial infarction, or UA) or both before and after the index event (G3).

Results
The CD4⁺CD28null T-cell frequency was higher in G3 than in G2 and G1 (median 9.5% [range 2.4% to 48.0%] vs. 5.1% [range 0.4% to 27.8%] and 2.3% [range 0.2% to 22.8%], respectively; p < 0.001). The expansion of these unusual T lymphocytes was higher in patients with elevated C-reactive protein levels, and it was reduced by statin therapy. On multivariate logistic regression analysis, CD4⁺CD28null T-cell frequency was an independent predictor of future acute coronary events (odds ratio 3.01, 95% confidence interval 1.1 to 8.25; p = 0.023).

Conclusions
A perturbation of T-cell repertoire is strongly associated with the recurrence of acute coronary events, conceivably playing a key pathogenetic role. (J Am Coll Cardiol 2007;50:1450–8) © 2007 by the American College of Cardiology Foundation

The spectrum of clinical presentation of acute coronary syndromes (ACS) is extremely varied. At one extreme end of the spectrum, some patients have several acute coronary events (either myocardial infarction or unstable angina [UA]) over a period of years, despite the use of the more advanced and costly pharmaceutical strategies and invasive procedures. At the other end of the spectrum, some patients have a single acute event, such as an acute myocardial infarction not preceded by anginal symptoms and, after this event, remain totally asymptomatic for years (1). The mechanisms of the occasional transition from stable to unstable atherosclerosis may not be the same in these 2 extreme groups (2). We have recently demonstrated that patients with recurrent acute coronary events have persistently high levels of C-reactive protein (CRP) and enhanced in vivo and in vitro monocyte response to proinflammatory stimuli (3–6).

Activated inflammatory cells have been found in the coronary plaques as well as in the peripheral blood of patients with ACS (7–14). In particular, patients with UA have an increased frequency of CD4⁺ T lymphocytes characterized by defective cell surface expression of CD28, a major costimulatory molecule critically involved in determining the outcome of antigen recognition by T lymphocytes. CD4⁺CD28null T cells are expanded in the
peripheral blood of patients with UA and infiltrate unstable coronary plaques, where they undergo clonal expansion, probably triggered by specific antigens (12,13). These cells are capable of releasing large amounts of interferon (IFN)-γ and they are the dominant population of IFN-γ producing cells in the peripheral blood of patients with UA (12). Because of the increased IFN-γ production, one of their functions is the activation of monocytes and macrophages; indeed, monocytes from patients with UA display a molecular fingerprint of ongoing IFN-γ stimulation (14). Therefore, CD4⁺CD28⁻ T cells might be involved in the control of plaque-infiltrating macrophages.

CD4⁺CD28⁻ T cells are distinct from classic helper T cells in several additional aspects (15). In particular, they express killer immunoglobulin-like receptors, a characteristic of natural killer cells, and have killer cell functions (16,17). Endothelial cells are susceptible to this T-cell–mediated injury (16). Furthermore, in the presence of CRP at concentrations frequently found in patients at risk for coronary events, susceptibility of endothelial cells to T-cell–mediated cytotoxicity is increased (16).

Therefore, alongside other proinflammatory mechanisms, we hypothesized that the expansion of T lymphocytes with the functional profile of CD4⁺CD28⁻ T cells might represent a key pathogenetic mechanism of recurrent instability.

In this study we specifically tested this hypothesis by measuring CD4⁺CD28⁻ T-cell frequencies and CRP levels in a consecutive series of unstable and stable angina patients admitted to our institution. The UA patients were carefully selected on the basis of the presence or absence of recurrent episodes of instability (and/or infarction) in the 24 months before the study enrollment, and they were followed for a further period of 24 months to assess the occurrence of further acute coronary events. Moreover, because we have recently observed a relation between statin use and frequency of CD4⁺CD28⁻ T cells (18), we also analyzed the influence of statin therapy in this cohort of UA patients.

Methods

Population. The UA patient selection and study design are presented in Figure 1. We prospectively evaluated 218 consecutive patients admitted to our coronary care unit (CCU) with a diagnosis of Braunwald class IIIIB UA between September 2000 and September 2002. Patients with UA were considered to have recurrent acute coronary events if they had at least 2 CCU admissions with diagnosis of Braunwald class IIIIB UA other than the index event and/or myocardial infarction during the 24 months before the study enrollment. Patients without any previous episode...
of UA and/or myocardial infarction were considered to be patients without recurrent acute coronary events. The UA patients who did not fulfill the criteria to be considered with or without recurrent acute coronary events (n = 42) were excluded from the study. Other exclusion criteria were the following: 1) evidence of inflammatory or infectious diseases, malignancies, or immunologic or hematologic disorders (n = 9); 2) treatment with antiinflammatory drugs other than low-dose aspirin (n = 6); 3) ejection fraction <40% (n = 13); and 4) age >75 years (n = 17). The UA patients were followed for a further period of 24 months after enrollment to assess the clinical outcome. Follow-up visits, consisting of physical examination, a standard 12-lead electrocardiogram, and a treadmill stress test were performed every 6 months. Recurrence of new acute coronary events (cardiac death, myocardial infarction, and CCU admission for UA) was recorded. For 11 patients, follow-up data were not available, and they were excluded from the study.

As a control population, we studied 67 consecutive patients (54 male, mean age 64 ± 9 years) with chronic stable effort angina (CSA) as first manifestation of ischemic heart disease lasting >2 years, admitted to our institution to undergo elective coronary angiography.

Demographic data, classic risk factors, previous revascularization procedures (percutaneous coronary intervention [PCI] or coronary bypass graft surgery), angiographic findings, left ventricular function, and medical treatments at the time of blood sampling were carefully recorded for all patients. In UA patients, history of previous acute coronary events was obtained by patient medical records.

All patients gave their written informed consent. The Ethics Committee of the Catholic University of Rome approved the study.

**Study design.** We compared extreme groups of patients with UA (Fig. 1). The UA patients were subgrouped according to the occurrence of prior and further events during the 48 months of study observation. For 51 patients, the index event was the first ever (group 1); 30 patients had recurrent instability and/or myocardial infarction during the previous 24 months, but no further events after the index one (group 2); and 39 patients had further events (25 patients were readmitted to the CCU because of UA, 11 patients had an acute myocardial infarction [8 of whom were also readmitted because of UA], and 3 patients died) during the 24 months of follow-up, and 33 of these 39 patients also had prior events (group 3). Thus, patients in group 3 exhibited a strikingly high recurrence of acute coronary events. Group 4 consisted of 67 patients with CSA (see the preceding section).

**Blood sampling.** Venous blood samples were taken at the time of the index event. Total and differential white blood cell counts and T-cell subset distributions were analyzed on fresh blood samples. Coded plasma and serum samples were stored at −70°C and analyzed for CRP in a single batch at the end of the study by laboratory staff unaware of the clinical data. In patients with UA, plasma cardiac troponin T (cTnT) was determined at the time of hospital admission as a routine measurement by the Department of Clinical Chemistry. All categorization and management of patients were independent of these results.

**T-cell analysis.** Total and differential white blood cell counts were obtained with a Bayer H-3 hematology analyzer (Bayer Diagnostic Division, Tarrytown, New York) using automated cytochemistry in flow.

Heparinized (10 U/ml) whole blood samples were stained with fluorescein isothiocyanate-conjugated anti-CD4 (Becton Dickinson, San Jose, California) and phycoerythrin-conjugated anti-CD28 (Pharmingen, San Diego, California) monoclonal antibodies (mAb) and analyzed by 2-color flow cytometry on the Coulter Epics XL (Beckman Coulter, Fullerton, California). Nonspecific staining with isotype-matched control mAb was <1%; the intra- and interassay variability was <10%.

The frequencies of total CD4+, CD4+CD28null, and CD4+CD28null T cells were determined using WinMDI software (Joseph Trotter, Scripps Research Institute, La Jolla, California). A cutoff of 4% CD4+CD28null T cells was chosen to define patients with low or high frequencies of these cells, because 4% represented the 90th percentile of distribution in 100 age-matched healthy subjects (median 0.73%, range 0.05% to 4.94%) (15). Moreover, patients having >10% CD4+CD28null T cells were considered to have very high frequencies of these cells, because this value was more than 10-fold higher than the median value in the normal population.

**Measurements of CRP and cTnT.** C-reactive protein was measured using a high-sensitivity latex-enhanced immunonephelometric assay (Latex/BN II, Dade Behring, Marburg, Germany). The working range of the assay was 0.175 to 1,100 mg/l, and the coefficient of variation was <5%. The median normal value for CRP is 0.8 mg/l, with 90% of normal values <3 mg/l. Troponin T was measured by the third-generation cTnT assay on an Elecsys 2010 (Roche Diagnostics, Mannheim, Germany). The minimum detectable concentration is 0.01 ng/ml, and the 99th percentile in healthy individuals is <0.01 ng/ml.

**Statistical analysis.** Because CRP values and CD4+CD28null T-cell frequencies do not follow a normal distribution, nonparametric tests were used: the Kruskal-Wallis test and the Dunn multiple pairwise comparisons for comparisons between groups, and the Spearman rank test for correlation. The remaining continuous variables were compared using the 1-way repeated measures analysis of variance with the Bonferroni correction for multiple pairwise comparisons as appropriate. Proportions were compared using the chi-square test. The CRP values and CD4+CD28null T-cell frequencies are expressed as median and range; the remaining variables are expressed as mean ± SD. Univariate logistic regression analysis was used to identify predictors of long-term outcome in patients with UA after the index events. The following clinical and laboratory variables were tested: age, gender, classic risk factors, previous PCI.
or bypass surgery, history of recurrent acute coronary events, left ventricular ejection fraction, multivessel disease, cTnT levels, statin treatment, CRP levels, and the percentage of CD4⁺CD28null T cells. Multivariate logistic regression analysis was then applied to individuate the variables independently associated with the outcome. Only variables with a value of $p \leq 0.1$ on univariate analysis were included in the multivariate model. Event-free survival was analyzed by the Kaplan-Meier method, and the log rank test was used for comparison among curves. The interaction between statin treatment and CD4⁺CD28null T-cell frequency on outcome was tested using the analysis of covariance. As end points we considered the recurrence of new acute coronary events (cardiac death, myocardial infarction, and CCU admission for UA) during the 24 months of prospective follow-up. Statistical analysis was performed using SigmaStat software (SPSS, Chicago, Illinois). A $p$ value of $<0.05$ (2-tailed) was considered to be statistically significant.

**Results**

**CD4⁺CD28null T-cell frequencies in different groups.** Demographic and clinical data of the study populations and the biologic parameters measured in the study are reported in Table 1.

<table>
<thead>
<tr>
<th>Table 1</th>
<th>Clinical Characteristics of the Study Populations and Biological Parameters</th>
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</thead>
<tbody>
<tr>
<td></td>
<td>Unstable Angina</td>
</tr>
<tr>
<td></td>
<td>Group 1: First-Ever Event</td>
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<tr>
<td>Patients, n</td>
<td>51</td>
</tr>
<tr>
<td>Age (yrs, mean ± SD)</td>
<td>61 ± 11</td>
</tr>
<tr>
<td>Gender (M/F)</td>
<td>42/9</td>
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<tr>
<td>Risk factors</td>
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<tr>
<td>Family history of IHD</td>
<td>14 (27)</td>
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<tr>
<td>Hypercholesterolemia</td>
<td>27 (53)</td>
</tr>
<tr>
<td>Diabetes</td>
<td>4 (8)</td>
</tr>
<tr>
<td>Overweight/obesity</td>
<td>4 (8)</td>
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<tr>
<td>Hypertension</td>
<td>19 (37)</td>
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<tr>
<td>Smoking</td>
<td>30 (59)</td>
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<tr>
<td>Medications</td>
<td></td>
</tr>
<tr>
<td>Beta-blockers</td>
<td>32 (63)</td>
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<tr>
<td>ACE inhibitors</td>
<td>17 (33)</td>
</tr>
<tr>
<td>Aspirin</td>
<td>43 (84)</td>
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<tr>
<td>Clopidogrel</td>
<td>42 (82)</td>
</tr>
<tr>
<td>Statins</td>
<td>27 (53)</td>
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<td>Previous history</td>
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<tr>
<td>Unstable angina</td>
<td>NA</td>
</tr>
<tr>
<td>Myocardial infarction</td>
<td>NA</td>
</tr>
<tr>
<td>Previous PCI/CABG</td>
<td>NA</td>
</tr>
<tr>
<td>Multivessel disease</td>
<td>21 (41)</td>
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<tr>
<td>PCI/CABG for the index event</td>
<td>28/14 (55/27)</td>
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<td>Follow-up events</td>
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<tr>
<td>Unstable angina</td>
<td>NA</td>
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<tr>
<td>Myocardial infarction</td>
<td>NA</td>
</tr>
<tr>
<td>Death</td>
<td>NA</td>
</tr>
<tr>
<td>Biologic parameters, median (range)</td>
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<tr>
<td>Troponin T (ng/ml)</td>
<td>0.01 (&lt;0.01–0.12)</td>
</tr>
<tr>
<td>C-reactive protein (mg/l)†</td>
<td>3.9 (0.6–27.7)</td>
</tr>
<tr>
<td>Lymphocyte count (10⁹/l)</td>
<td>1.6 (1.1–2.4)</td>
</tr>
<tr>
<td>Total CD4⁺ T-cell frequency (%)</td>
<td>48.8 (12.9–70.2)</td>
</tr>
<tr>
<td>CD4⁺CD28null T-cell frequency (%)¶</td>
<td>2.3 (0.2–22.8)</td>
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</table>

Values are expressed as n (%) unless otherwise indicated. Group 1: 51 patients with the first-ever event; group 2: 30 patients with myocardial infarction and/or unstable angina during the 24 months before the study enrollment; group 3: 39 patients with further events during the 24 months of follow-up (death, myocardial infarction, unstable angina); 33 of them also with prior events; group 4: 67 patients with chronic stable angina. Because some patients in group 2 and group 3 had both recurrent episodes of unstable angina and myocardial infarction during the 24 months before study enrollment, the percentage of patients having prior unstable angina and prior myocardial infarction in these 2 groups is higher than 100%. Similarly, because 8 patients in group 3 who had an acute myocardial infarction during the 24 months of follow-up were also readmitted because of unstable angina, the percentage of patients having further episodes of unstable angina and a new myocardial infarction during the follow-up period is higher than 100%. The percentage of patients having further episodes of unstable angina and prior myocardial infarction in these 2 groups is higher than 100%. Therefore, patients with unstable angina and prior myocardial infarction during the follow-up period are included in the analysis of recurrence of new acute coronary events. 

ACE = angiotensin-converting enzyme; CABG = coronary artery bypass graft surgery; CSA = chronic stable angina; IHD = ischemic heart disease; NA = not applicable; PCI = percutaneous coronary intervention.
in Table 1. No difference was found in the clinical variables, in lymphocyte count, and in total CD4+ T-cell frequency among the different groups.

CD4⁺CD28null T-cell frequency was significantly higher in group 3 than in the other groups. The median frequencies (ranges) of CD4⁺CD28null T cells were 2.3% (0.2% to 22.8%) in group 1 patients, 5.1% (0.4% to 27.8%) in group 2 patients, 9.5% (2.4% to 48.0%) in group 3 patients, and 0.5% (0.02% to 21.5%) in group 4 patients (p < 0.001) (Fig. 2A, Table 1). A CD4⁺CD28null T-cell frequency of ≥4% was found in 18 of the 51 group 1 patients (35%), 17 of 30 group 2 patients (57%), 30 of 39 group 3 patients (77%), and 8 of 67 group 4 patients (12%; p < 0.001). Intriguingly, the difference among groups was almost exclusively due to the percentage of patients having very high frequencies (>10%) of CD4⁺CD28null T cells: 5 of the 51 group 1 patients (10%), 9 of 30 group 2 patients (30%), 20 of 39 group 3 patients (51%), and 6 of 67 group 4 patients (9%; p < 0.001).

We also performed subgroup analysis between groups 2 and 3 according to the feature of coronary events (UA, myocardial infarction, or death) experienced earlier or after the index admission. Within group 2, no difference in CD4⁺CD28null T-cell frequency were observed between patients (n = 9) with a history of UA (7.3%, 0.2% to 48%), and patients (n = 21) with a history of both UA and myocardial infarction (6.7%, 0.6% to 36%; p = 0.30). Within group 3, the percentage of CD4⁺CD28null T cells was significantly higher in patients with acute myocardial infarction or death during the follow-up (n = 14) compared with patients readmitted to the CCU because of UA (n = 25): 18.2% (4.7% to 48%) versus 5.0% (0.4% to 35%; p = 0.027).

In UA patients, we also compared CD4⁺CD28null T-cell frequencies according to the presence or absence of coronary instability before the index event independently of the prospective outcome. The median frequencies (range) of CD4⁺CD28null T cells were 2.5% (0.2% to 26.8%) in 57 patients without previous coronary instability and 5.4% (0.4% to 48.0%) in 63 patients with previous coronary instability (p = 0.006) (Fig. 2B). Frequencies ≥4% were found in 22 (39%) and 43 (68%) patients, respectively (p = 0.001). Very high frequencies (>10%) were found in 7 (12%) and 27 (43%) patients, respectively (p < 0.001). Of note, the percentage of patients on statins was similar in the 2 groups: 31 of 57 (54%) in patients without previous coronary instability versus 28 of 63 (44%) in patients with previous coronary instability (p = 0.27).

Moreover, CD4⁺CD28null T-cell frequency significantly increased according to the number of acute coronary events occurring during the 24 months before the study enrollment, from 2.5% (0.2% to 26.8%) in patients with a single event to 14.2% (0.6% to 48.0%) in patients with 4 to 5 events (p < 0.001) (Fig. 3).

CD4⁺CD28null T-cell frequencies according to CRP, cTnT, and statin therapy status in patients with UA.

As shown in Figure 4A, CD4⁺CD28null T cells were preferentially expanded in patients with UA and elevated

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**Figure 2**

**CD4⁺CD28null T-Cell Frequencies in Different Groups**

Frequencies of CD4⁺CD28null T cells were determined by 2-color flow-cytometry. (A) The unstable angina (UA) patients were subgrouped according to the occurrence of both prior and further events during the 48 months of study observation. Group 1: 51 patients with the first-ever event; group 2: 30 patients with myocardial infarction and/or UA during the 24 months before study enrollment; group 3: 39 patients with further events during the 24 months of follow-up (death, myocardial infarction, UA), the majority of whom (85%) also had prior events; group 4: 67 patients with chronic stable effort angina (CSA). The CD4⁺CD28null T-cell frequency was significantly higher in group 3 than in the other groups (p < 0.001; Kruskal-Wallis test). (B) The UA patients were subgrouped according to the occurrence of events (none versus 2 or more) during the 24 months before the study enrollment independently of the prospective outcome. CD4⁺CD28null T-cell frequency was significantly higher in 63 patients with previous events than in 57 patients without previous events (p = 0.006). Data are presented as single data points. The dashed lines indicate CD4⁺CD28null T-cell frequencies ≥4% (90th percentile of distribution in 100 age-matched healthy subjects) and >10% (10-fold higher than the median value in healthy subjects). Blue, green, and red dots indicate patients with CD4⁺CD28null T-cell frequencies <4%, 4% to 10%, and >10%, respectively.
levels of CRP (median frequency 8.5%, range 0.2% to 48%). In contrast, CD4^+CD28null T cells were infrequent in patients with UA and low levels of CRP (median frequency 2.7%, range 0.2% to 21.9%; p < 0.001 vs. UA high CRP). A high frequency (>4%) was found in 67% of UA patients with high CRP levels and in 32% of UA patients with low CRP levels (p < 0.001). A very high frequency (>10%) was found in 38% of UA with high CRP levels and in 15% of UA with low CRP levels (p = 0.011).

In the overall population including UA and CSA patients, a statistically significant correlation was found between CD4^+CD28null T-cell frequencies and CRP levels (r = 0.39; p < 0.001) (Fig. 4B).

Although 48 (66%) out of 73 UA patients with high CRP levels had a high level of cTnT (>0.01 ng/ml) during the first 24 h after CCU admission, compared with 11 (23%) out of 47 UA patients with low CRP levels (p < 0.001), no difference was found in CD4^+CD28null T-cell frequency according to cTnT seropositivity. Thus, the median CD4^+CD28null T-cell frequency was 5.1% (range 0.6% to 48%) in UA patients with positive cTnT and 3.9% (range 0.2% to 38.5%) in UA patients with negative cTnT (p = 0.40).

A total of 59 patients were treated with statins before the study enrollment (74 ± 28 days): atorvastatin at 20 mg/day (45 patients, 76%), atorvastatin at 40 mg/day (8 patients), and pravastatin at 40 mg/day (6 patients). The 59 patients on statins before the study enrollment were compared with the 61 patients who never took statins. The percentage of CD4^+CD28null T cells was significantly lower in patients on statins (median 2.4%, range 0.2% to 38.5%) than in patients who never took statins (median 5.9%, range 0.6% to 48.0%; p < 0.001). Also, CRP levels were lower in patients on statins (median 2.4 mg/l, range 0.6 to 37.2 mg/l) than in patients not on statins (median 7.4 mg/l, range 0.8 to 86.3 mg/l; p < 0.001).

Prognostic significance of CD4^+CD28null T cells in patients with UA. Univariate logistic regression analysis was used to identify predictors of long-term outcome after the index event. A CD4^+CD28null T-cell frequency higher than 4% was the strongest predictor of future acute coronary events (odds ratio [OR] 3.73, 95% confidence interval [CI] 1.56 to 8.94; p = 0.003), followed by history of recurrent instability (OR 3.33, 95% CI 1.39 to 7.98; p = 0.006), diabetes (OR 3.13, 95% CI 1.14 to 8.56; p = 0.025), CRP levels (OR 1.78, 95% CI 1.02 to 3.98; p = 0.036), and cTnT levels (OR 1.28, 95% CI 0.96 to 2.51; p = 0.065). On multivariate logistic regression analysis, a history of recurrent instability (OR 2.73, 95% CI 1.08 to 6.90; p = 0.032) and CD4^+CD28null T-cell frequency (OR 3.01, 95% CI 1.1 to 8.25; p = 0.023) were the only variables independently associated with the clinical outcome (Table 2).
The 24-month survival free of readmission for unstable angina, myocardial infarction, and death was significantly higher in patients with a percentage of CD4<sup>+</sup>CD28<sup>−</sup> T cells ≤4% than in patients with CD4<sup>+</sup>CD28<sup>−</sup> T cells ≥4% as assessed by the log rank test (p = 0.004) (Fig. 5).

Finally, the event rate according to CD4<sup>+</sup>CD28<sup>−</sup> T-cell frequency (≤4%, 4% to 10%, >10%) and statin treatment was 15%, 30%, and 33% in patients treated with statins (p = 0.58), and 17%, 25%, and 64% in patients who never took statins (p = 0.003) (Fig. 6). However, no interaction was demonstrated between CD4<sup>+</sup>CD28<sup>−</sup> T-cell frequency and statin treatment by analysis of covariance.

**Discussion**

The spectrum of clinical presentations of ACS is extremely varied. Some patients present recurrent episodes of instability (myocardial infarction and/or UA), despite the use of the more advanced and costly therapeutic strategies, whereas others have a single acute event in their life. The pathogenetic mechanisms and/or the specific triggers of the occasional transition from stable to unstable atherosclerosis may be different in these 2 subsets of patients (1,2).

The present study supports the notion that a perturbation of the T-cell repertoire may affect the tendency of coronary instability to recur. Indeed, in a well-selected population of patients with recurrent instability, the median frequency of CD28-lacking CD4<sup>+</sup> T cells was about 4-fold higher than in patients with UA as first-ever event. A gradient of values has been observed, with an intermediate value in patients with UA and history of recurrent instability and/or myocardial infarction during the previous 24 months but no further events after the index admission.

CD4<sup>+</sup>CD28<sup>−</sup> T-cell expansion in UA was related to systemic evidence of inflammation, because significantly higher frequencies of CD4<sup>+</sup>CD28<sup>−</sup> T cells were found in UA patients with high CRP levels than in those with low CRP levels. A positive correlation was observed between CD4<sup>+</sup>CD28<sup>−</sup> T cells and CRP levels. In contrast, no difference was found in CD4<sup>+</sup>CD28<sup>−</sup> T-cell frequencies according to cTnT status, thus making unlikely the influence of myocardial cell damage per se.
Finally, as already demonstrated by our group in a different subset of patients with UA (18), the present study also suggests that statin therapy might be able to reduce CD4+CD28null T-cell frequency. However, prospective randomized studies are needed to confirm this novel anti-inflammatory property of statins and the beneficial effects on the recurrence of ACS.

**Key features of CD4+CD28null T cells.** CD4+CD28null T cells are a population of lymphocytes rarely found in healthy individuals (15). The number of these cells increases with age and, although mechanisms leading to immunosenescence are complex, CD28null T cells are the most consistent biologic indicators of aging in the immune system (19). Despite the loss of the CD28 molecule, these cells are functionally active and possess specific immunologic properties (12,14–17). Disease-associated expansions of these cells have been reported in inflammatory disorders such as rheumatoid arthritis, Wegener’s granulomatosis, multiple sclerosis, and ankylosing spondylitis and in chronic infections (19). The severity of clinical manifestations of these diseases is correlated with the frequency of CD28null T cells. In rheumatoid arthritis, the frequency of CD4+CD28null T cells correlates with extra-articular manifestations and in particular with vasculitic complications (15). Patients with rheumatoid arthritis and persistent CD4+CD28null T-cell expansion show signs of atherosclerotic vessel damage, thus suggesting that these cells might play a pathogenetic role in the excess of cardiovascular mortality associated with rheumatoid arthritis (20,21).

**Potential mechanisms of CD4+CD28null T-cell expansion.** Environmental as well as genetic mechanisms could underlie the expansion of CD4+CD28null T cells (19). The expression of CD28 is regulated by a complex cytokine network. T-cell activation in the presence of the proinflammatory cytokine interleukin-12 results in the restoration of CD28 gene transcription and cell surface appearance of a functional CD28 molecule on CD4+CD28null cells (22), whereas tumor necrosis factor-α has a down-regulatory action (23). The defect in CD28 cell surface expression might result from chronic exposure to antigens. Therefore, the expansion of CD4+CD28null T cells in UA patients may reflect a persistent immune response to microorganisms or autoantigens contained in atherosclerotic plaques as well as more diffuse to the entire coronary tree or circulating in peripheral blood. We have previously shown that, in patients with UA, circulating and coronary plaque-infiltrating CD4+CD28null T cells are highly oligoclonal, suggesting ongoing antigenic stimulation (13). Accordingly, Zal et al. (24) have recently demonstrated that the circulating CD4+CD28null T lymphocytes in UA produce large quantities of IFN-γ and, consequently, of proinflammatory cytokines in response to very restricted antigenic stimulation.

**Study limitations.** Because the goal of this study was to investigate the role of CD4+CD28null T cells in recurrent instability, we enrolled consecutive patients admitted with UA at the two extremes of the spectrum: at one extreme patients without any history of ischemic heart disease, at the other extreme patients who had at least 2 episodes of coronary instability in the 2 years before admission. Therefore, the findings of the present study cannot be extrapolated to a general population of patients admitted to CCUs with diagnosis of Braunwald class IIIIB UA.

Furthermore, our findings on statins come from a retrospective analysis; therefore, no final conclusion can be drawn on the potential novel anti-inflammatory property of statins to reduce the frequency of CD4+CD28null T cells in UA.

Finally, we recognize the limitation of some purely “exploratory” subgroup analyses we performed in the study.

**Conclusions**

In the spectrum of clinical presentations of ACS, some patients exhibit a strikingly high recurrence of acute coronary events. These patients have a defect in T-cell homeostasis resulting in considerably high frequencies of CD4+CD28null T cells, an unusual subset of T lymphocytes with functional activities that may predispose to vascular injury. Patients with UA and frequent recurrence of acute coronary events have median frequency of CD28-lacking CD4+ cells about 4-fold higher than patients with a first-ever acute coronary event during 4 years of clinical observation, and about 9-fold higher than patients with CSA. Moreover, on multivariate logistic regression analysis, CD4+CD28null T-cell frequency was an independent predictor of future acute coronary events. Our findings might not only help to better understand the inflammatory process involved in the pathogenesis of ACS, but they also might have clinical relevance. Indeed, our group has recently demonstrated that statin treatment, as well as a specific anti-tumor necrosis factor-α treatment, is able to modulate CD4+CD28null T lymphocytes in UA (18,25).

Further studies are warranted to test the clinical relevance of our findings and their importance in introducing innovative approaches for prevention and treatment of ACS.

**REFERENCES**