CD4+CD28– T Lymphocytes Contribute to Early Atherosclerotic Damage in Rheumatoid Arthritis Patients

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Background—Peripheral blood expansion of an unusual CD4+ T-cell subset lacking surface CD28 has been suggested to predispose rheumatoid arthritis (RA) patients to develop more aggressive disease. However, the potential association between CD4+CD28null T cells and early atherosclerotic changes in RA has never been investigated.

Methods and Results—The number of circulating CD4+CD28null cells was evaluated in 87 RA and 33 control subjects who also underwent evaluation of carotid artery intima-media thickness (IMT) and endothelial function via flow-mediated vasodilation (FMV). Patients had higher IMT and lower FMV compared with control subjects. The frequency of CD4+CD28null cells was significantly higher in patients than in control subjects. Twenty patients with persistent expansion of circulating CD4+CD28null cells had more marked increase of carotid artery IMT and stronger decrease of brachial artery FMV. Blockade of tumor necrosis factor-α led to a partial reappearance of the CD28 molecule on the CD4+ cell surface.

Conclusions—Circulating CD4+CD28null lymphocytes are increased in RA. Patients with persistent CD4+CD28null cell expansion show preclinical atherosclerotic changes, including arterial endothelial dysfunction and carotid artery wall thickening, more significantly than patients without expansion. These findings suggest a contribution of this cell subset in atheroma development in RA. Moreover, the demonstration that tumor necrosis factor-α blockade is able to reverse, at least in part, the CD28 deficiency on the CD4+ cell surface may be of interest for possible innovative therapeutic strategies in cardiovascular diseases. (Circulation. 2004;109:2744-2748.)

Key Words: arthritis, rheumatoid ■ cells ■ endothelium ■ vasodilation

An increasing body of evidence suggests that rheumatoid arthritis (RA) is associated with excess cardiovascular (CV) mortality, which appears to be linked to premature atherosclerosis. Although some traditional CV risk factors may play a role, it is thought that systemic chronic inflammation may play an additional pivotal role in accelerating atherosclerotic processes in RA. Conversely, there is evidence that inflammation may be equally important in the development of “typical” atherosclerosis. Chronic inflammation, therefore, may act independently of or synergistically with traditional atherosclerotic risk factors in accelerating the atherosclerotic process in RA. How RA might contribute to the initiation and progression of the atheromatous plaque still remains a matter of debate. As a chronic inflammatory disorder, atherosclerosis shares many similarities with RA. Interestingly, a stable expansion of a CD4+ T-cell subset that completely lacks expression of the CD28 molecule has been described in the peripheral blood of some patients with RA and patients with unstable angina. Because the CD4+CD28null cell population represents a subset with high proinflammatory and tissue-damaging potential, a role of this unusual T-cell subset in favoring the atherosclerotic process and CV events in RA has been hypothesized. However, there has been no direct evidence to date that patients with peripheral blood CD4+CD28null cell expansion are more exposed to atherosclerotic risk than RA subjects without such cell expansion. In this study, the number of circulating CD4+CD28null cells has been analyzed in a group of RA patients free of overt CV disease who were simultaneously evaluated for preclinical atherosclerotic damage.

Methods

Population

Eighty-seven white Italian outpatients followed up at the Rheumatology Clinic of the University of Perugia who fulfilled the American College of Rheumatology classification criteria for RA were...
Flow-mediated vasodilation (FMV) was assessed on the brachial artery by ultrasound device (HDI 3500, Advanced Technology Laboratories) equipped with a linear multifrequency 5- to 12-MHz transducer, as described. Erythrocyte sedimentation rate, rheumatoid factor, C-reactive protein, and C-reactive protein, mg/L were evaluated in each subject. Disease duration, y was estimated by independent-samples t test and Mann-Whitney U test, respectively. Bivariate correlations between study variables were calculated by Spearman’s rank correlation coefficients. When vascular parameters in the groups with or without CD4+CD28null cell expansion were compared, both IMT and endothelial dysfunction data were adjusted by sex in an ANCOVA because of the evidence of a small, albeit nonsignificant, difference in sex distribution in the 2 groups. Differences between baseline and treatment in patients undergoing treatment with anti-tumor necrosis factor (TNF) monoclonal antibodies were estimated by 1-way ANOVA for repeated measurements with Tukey’s post hoc test. Significant differences were assumed to be at P<0.05 for 2-tailed tests.

**Laboratory Analysis**

Anti-CD4 monoclonal antibody was from the American Type Cell Culture Collection. Anti-CD28 monoclonal antibodies were purchased from Becton Dickinson. The simultaneous presence of CD4 and CD28 was evaluated on peripheral blood mononuclear cells by flow cytometry (FACScan, Becton Dickinson) using a 2-color immunofluorescence staining technique, as previously described. Erythrocyte sedimentation rate, rheumatoid factor, C-reactive protein (laser nephelometry), total cholesterol, triglycerides (enzymatic colorimetric method) and high-density lipoprotein cholesterol (enzymatic colorimetric method after precipitation to polyethylene glycol), and low-density lipoprotein cholesterol (Friedewald formula) were also evaluated in low-density lipoprotein cholesterol (Friedewald formula).

**Flow-Mediated Vasodilation**

Flow-mediated vasodilation (FMV) was assessed on the brachial artery by ultrasound device as reported elsewhere. Any drug known to affect endothelial function was withdrawn ≥1 week before the examination. The average of 3 measurements of basal and post-hyperemia diameter was used for the analysis. FMV was expressed as the relative increase in brachial artery diameter during hyperemia, and defined as 100×[(post-hyperemia diameter−basal diameter)/basal diameter]. Blood flow was measured as arterial cross sectional area (π×r²) times mean Doppler velocity corrected for angle.

**Carotid Intima-Media Thickness**

Carotid artery intima-media thickness (IMT) was evaluated by an ultrasound device (HDI 3500, Advanced Technology Laboratories) equipped with a linear multifrequency 5- to 12-MHz transducer, as described. Each subject was characterized by mean common carotid IMT, defined as the average of 12 IMT readings: Common carotid arteries, right and left side, far and near wall, 3 sampling points per segment. The intraobserver coefficient of variation was 3.9% (mean±SD of the difference, 0.018±0.031 mm), and interobserver values were 5.6% (0.028±0.032 mm).
The percentage of patients receiving methotrexate was higher in the patient group with cell expansion, but the remaining treatment was similar in the 2 groups of RA patients (Table 2). To rule out the possibility that the observed IMT and FMV differences between groups with and without CD4+CD28null cell expansion could be caused by differences in systemic manifestations of the disease, the RA subset with extra-articular manifestations were compared with the group of patients without evidence of systemic disease (Table 3). Although patients with nodular RA and/or rheumatoid organ disease had longer duration of disease and higher C-reactive protein serum values, they did not show any differences in age and sex distribution, percentage of patients with rheumatoid factor+ and bony erosions, or more importantly, in values of IMT and FMV.

A longitudinal evaluation of CD4+CD28null cells in each patient after 30 and 60 days, without changes of treatment and stable disease activity, did not show the emergence of circulating T cells with this unusual phenotype in patients without expansion at baseline, whereas persistent cell expansion was demonstrated in patients with more than 15% of CD4+CD28null cells at baseline, with only slight variations (<30% of basal value) over time (data not shown). Seven RA patients carrying persistent CD4+CD28null cell expansion and active disease (DAS28 index >3.2), despite treatment with methotrexate (15 mg/wk), were evaluated anew 15 and 45 days after starting intravenous infusion (at days 0 and 15) of anti-TNF-α monoclonal antibody (3 mg/kg infliximab, Remicade, Schering-Plough). The additional treatment led to a significant reduction of circulating CD4+CD28null cells (from 32.3%±5.9% at baseline to 20.9±7% and 21.6±6% after 15 and 45 weeks, P<0.04 and P<0.05, respectively).

**TABLE 3. Clinical Characteristics of RA Patients Subdivided According to the Presence or Absence of Extra-Articular Manifestations**

<table>
<thead>
<tr>
<th>Data</th>
<th>All (n=87)</th>
<th>Without EMs (n=70)</th>
<th>With EMs (n=17)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age, y</td>
<td>63.0 (10)</td>
<td>63.0 (10)</td>
<td>65.0 (11)</td>
</tr>
<tr>
<td>Men, %</td>
<td>29</td>
<td>27</td>
<td>35</td>
</tr>
<tr>
<td>Disease duration, y</td>
<td>8 (5 to 13)</td>
<td>7 (4 to 11)</td>
<td>12 (9 to 27)*</td>
</tr>
<tr>
<td>Rheumatoid factor +, %</td>
<td>61</td>
<td>59</td>
<td>65</td>
</tr>
<tr>
<td>Bony erosions, %</td>
<td>59</td>
<td>59</td>
<td>65</td>
</tr>
<tr>
<td>ESR, mm/h</td>
<td>27 (18)</td>
<td>25 (18)</td>
<td>35 (19)</td>
</tr>
<tr>
<td>C-reactive protein, mg/L</td>
<td>18.8 (20)</td>
<td>15.5 (10)</td>
<td>31.7 (33)*</td>
</tr>
<tr>
<td>Common carotid IMT, mm</td>
<td>0.84 (0.2)</td>
<td>0.84 (0.2)</td>
<td>0.85 (0.2)</td>
</tr>
<tr>
<td>Flow-mediated vasodilation, %</td>
<td>3.7 (1.4)</td>
<td>3.8 (1.5)</td>
<td>3.6 (1.4)</td>
</tr>
</tbody>
</table>

EMs indicates extra-articular manifestations. Data are expressed as mean (SD) or median (interquartile range).

P<0.05 vs patients without EM.
Discussion

This study confirms that a subset of RA patients is characterized by peripheral blood expansion of CD4+ cells that lack the CD28 molecule on their surface.5,6 In addition, it supports recent findings showing that RA patients have lower FMV and higher carotid IMT, both recognized markers of early atherosclerotic disease and CV risk, than control subjects.16–19 Interestingly, however, the group of RA patients with blood expansion of CD4+CD28null cells had FMV impairment and IMT increases more marked than RA subjects without evidence of circulating CD4+CD28null cells. This observation strongly suggests that this unusual T-cell subpopulation is involved, at least in part, in the development of early atherosclerotic vessel damage.

Different pathogenic mechanisms may explain this observation. It has been postulated that emergence of CD28-deficient CD4+ T cells characterizes RA patients with aggressive disease and extra-articular manifestations.6 Also, in the present study, the RA patients with CD4+CD28null cell expansion had a higher incidence of extra-articular manifestations. This may suggest that vasculitic events, characterizing systemic disease, are primary and direct triggers of vessel damage, whereas CD4+CD28null cell expansion is only secondary to vessel inflammation in this RA subset. This hypothesis, however, is not supported by our observation: Although a longer disease duration and higher C-reactive protein levels characterized patients with systemic disease, IMT and FMV values of the RA subset with extra-articular manifestations were strictly overlapping with those of patients without evidence of systemic disease.

Phenotypic and functional properties of CD28-lacking CD4+ cells may provide alternative explanations for a possible pathogenic role of this cell subset in favoring CV disease in RA. The expression of CD28 is regulated by a complex cytokine network. T-cell activation in the presence of interleukin-12, a proinflammatory cytokine highly expressed in the inflammatory environment of a rheumatoid joint,20,21 results in the restoration of CD28 gene transcription and cell surface appearance of a functional CD28 molecule on CD4+CD28null cells.22 This may account for the finding that CD4+CD28null cells partially regain expression of CD28 in rheumatoid synovium.9 More interestingly, the upregulatory action exerted by interleukin-12 on CD28 expression is contrasted by downmodulation induced by another proinflammatory cytokine, TNF-α, which plays a fundamental role in several chronic inflammatory conditions.23 Thus, the description of CD4+CD28null T cells in vivo not only during normal aging,24,25 in which elevated levels of TNF-α have been described,26 but also among patients with inflammatory diseases, including RA,9 Wegener’s granulomatosis,27 and multiple sclerosis,28 appears to be more than coincidental. In these disorders, CD4+CD28null cells have been postulated to represent a pool of prematurely senescent T cells resulting from chronic immune activation.22 Interestingly, they have been implicated in autoimmune phenomena and have a proinflammatory activity. In particular, they produce large amounts of interferon-γ,27,29,30 a typical Th1-cytokine that is involved not only in rheumatoid synovitis but also in atherosclerosis development.31,32 It remains unclear, however, whether the higher percentage of CD4+CD28null cells in RA are the result of the inflammatory process, ie, prematurely senescent CD4+ cells that are unable to die but still secrete cytokines, or represent a subset of RA subjects whose pathogenetic process includes generation of this subset at an early stage.

These observations are relevant in the context of the observed increased CV risk in RA. It has been shown, indeed, that excess CV morbidity is associated with increased CV mortality in RA.33–35 Excessive CV deaths could be explained only by 2 reasons: CV disease is either more prevalent or more deadly in RA patients than in the general population. Several studies have demonstrated that the ischemic heart disease rate (IHD) is higher in RA than in control subjects.33–36 In addition, it has been shown that the prevalence of IHD, evaluated by myocardial perfusion single-photon emission computed tomography scans, is twice that in matched control subjects.37 Taken together, these observations suggest that IHD is both more common and more likely to lead to death in RA. The main cause of IHD is atherosclerotic coronary disease, but RA patients do not seem to have excessive traditional CV risk factors to explain their excess coronary risk.38 In addition, RA seems to be an independent predictor of IHD.36,37 Some inflammatory markers and prothrombotic factors produced by both chronic rheumatoid inflammation and anti-rheumatic therapies may have an important pathogenic role in early atherosclerosis development.39 In this context, methotrexate may play a bivalent role by reducing inflammation or enhancing serum levels of homocysteine, which is an important independent risk factor for atherosclerosis.1 Of note, in the present study, the percentage of patients receiving methotrexate was higher in the RA group with cell expansion. However, the overlapping values of serum homocysteine in the 2 patient subsets, probably caused by the supplementation of folic acid, seem to rule out the possibility that this difference in treatment may account for the observed differences in vessel damage.

The findings of the present study also suggest an involvement of the immune system in the early atherosclerotic injury of RA. The fact that CD4+CD28null cells have been found in the blood of patients with unstable angina and in extracts from coronary arteries containing unstable plaques7,8 seems to support the idea that the expansion of circulating CD28-lacking CD4+ cells in RA not only sustains synovium inflammation but also plays a pathogenic role in atherosclerotic plaque development and rupture, possibly via the synthesis of high levels of proinflammatory cytokines.4–10

The present study showed that although the CD4+CD28null cell phenotype is largely stable in the peripheral blood of the subset of RA patients with cell expansion, infusion of anti–TNF-α monoclonal antibodies,39–41 an accepted targeted therapy interfering with the complex cytokine interplay implicated in the inflammatory processes of RA,41,42 leads to a partial reappearance of CD28 on CD4+ T cells in these patients. This observation is not surprising if the downmodulatory properties on CD28 expression of TNF-α, chronically elevated in RA, are considered.20,21 In addition, because it has been described that anti–TNF-α treatment is able to improve endothelial function not only in patients with advanced heart failure42 but also in RA subjects,43 our phenotypic finding may suggest that the positive effect on vascular wall induced by TNF-α blockade in RA is also mediated, at least in part, by reduction of the proportion of circulating CD4+CD28null cells.
In conclusion, although traditional CV risk factors and the disease themselves could play a key role in favoring early atherosclerosis in RA, the present study supports at least a partial role for circulating CD4+ cells lacking CD28 surface molecule in inducing functional impairment of arterial endothelium that is currently considered to be the earliest stage of atheroma development and promoter of CV disease progression. We believe that our findings may contribute to enhancing our knowledge of the pathogenesis of atherosclerotic vascular damage in RA, but they also suggest the need for further studies to test their clinical relevance and their importance in introducing innovative and fascinating approaches for prevention and treatment of atherosclerosis and its complications.

References


