The Involvement of Tumor Necrosis Factor-Related Apoptosis-Inducing Ligand (TRAIL) in Atherosclerosis

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OBJECTIVES
Herein, we determined the significance of tumor necrosis factor (TNF)-related apoptosis-inducing ligand (TRAIL) in atherosclerotic vascular disease.

BACKGROUND
Inflammation is associated with the pathogenesis of atherosclerosis. The TNF-related apoptosis-inducing ligand/APO-2L, a member of the TNF superfamily, has a role in apoptosis induction and is recognized for its immunomodulatory properties.

METHODS
Stable and vulnerable atherosclerotic human plaques and aortas from atherosclerotic mice were assayed for the presence of TRAIL, and its inducibility was assessed by immunoblot and real-time polymerase chain reaction on peripheral mononuclear cells incubated with oxidized low-density lipoprotein (oxLDL). Enzyme-linked immunosorbent assay was used for the determination of soluble TRAIL levels in atherosclerotic patients.

RESULTS
Tumor necrosis factor-related apoptosis-inducing ligand is present in stable atherosclerotic lesions, is increased in vulnerable plaques, and is found to colocalize with CD3 cells and oxLDL. The TNF-related apoptosis-inducing ligand messenger ribonucleic acid (mRNA) and protein expression was up-regulated in peripheral blood mononuclear cells after incubation with oxLDL. Serum levels of soluble TRAIL but not TNF-alpha or Fas-ligand were reduced significantly in patients with unstable angina as compared with patients with stable atherosclerotic disease and healthy subjects. A negative correlation was demonstrated between soluble TRAIL and C-reactive protein levels but not with levels of mRNA of TRAIL in peripheral blood mononuclear cells.

CONCLUSIONS
Tumor necrosis factor-related apoptosis-inducing ligand is expressed in plaque-infiltrating CD3 cells and induced by oxLDL, whereas levels of soluble TRAIL are reduced in patients with acute coronary syndromes and negatively correlate with C-reactive protein levels. These results support a possible role for TRAIL in atherosclerosis. (J Am Coll Cardiol 2005;45:1018–24) © 2005 by the American College of Cardiology Foundation

In recent years, accumulating data implicate inflammatory processes in the pathogenesis of atherosclerosis and phenotype transition of the plaque from stable to a vulnerable one (1–3). Atherosclerotic plaques are composed of a lipid core, fibrous cap, and inflammatory infiltrates containing principally T cells and macrophages. Activated T lymphocytes play an important role in the initiation and progression of atherosclerosis (1–3). Several antigens are implicated in T-cell activation, the principal one of which is oxidized low-density lipoprotein (oxLDL) (3,4).

Acute coronary syndromes (ACS), including unstable angina and acute myocardial infarction, are caused predominantly by the rupture of the fibrous cap overlying a vulnerable coronary atherosclerotic plaque, with subsequent platelet aggregation and thrombus formation. Inflammation appears to play a key factor in these events (5,6). Inflammatory markers such as C-reactive protein (CRP) and interleukin-6 were shown to correlate with coronary adverse events (1–3), whereas interleukin-10, which exhibits anti-inflammatory properties, may have a protective role in atherosclerosis (7).

Tumor necrosis factor (TNF)-related apoptosis-inducing ligand (TRAIL)/APO-2L is a member of the TNF ligand superfamily (8,9). Its primarily recognized biologic activity is induction of apoptosis in cancer cells through its interaction with death receptors (DR4 and/or DR5) on transformed or infected cells. Although TRAIL is not constitutively expressed on the surface of inactivated cells of the immune system, its expression is up-regulated in response to stimulation with cytokines (10). Moreover, TRAIL has been demonstrated to inhibit autoantigen-specific T cells, suggesting it may suppress autoimmune responses (11).

Recent studies have reported that TRAIL confers endothelial cell protection from apoptosis and proliferation by the Akt and extracellular signal-regulated kinase pathways (12,13). These results are complemented by observations showing that the addition of TRAIL to primary human endothelial cells increased the phosphorylation of endothelial nitric oxide synthase activity, with subsequent nitric oxide synthesis (13). In view of the key role of T cells and endothelial cells in the pathogenesis of atheroma and plaque destabilization, we reasoned that TRAIL could be expressed within atherosclerotic plaques and may be associated with immune-modulating properties that could prove protective.
**Methods**

**Materials, reagents, and antibodies.** Oxidized low-density lipoprotein was prepared as previously described (14). Ly-sophosphatidylcholine (LPC), an active derivative of ox-LDL, was purchased from Sigma (St. Louis, Missouri). Antibodies used for immunohistochemistry and Western blotting consisted of rabbit polyclonal anti-human TRAIL antibodies that are reactive with the amino acids 25-281 at the carboxy terminus of TRAIL (Santa Cruz Biotechnology, Santa Cruz, California) and are cross-reactive with murine TRAIL. Anti-CD3 antibodies were from DAKO (Carpinteria, California). Anti-oxLDL antibodies were either polyclonal, prepared as previously described (14), or mouse anti-Apo B100 IgG (ICN Pharmaceuticals Inc., Aurora, Ohio).

**Immunoblotting for detection of TRAIL in human and murine plaques.** Human atherosclerotic plaques from carotid endarterectomy samples (n = 8) and sections of left internal mammary arteries (n = 7) obtained during coronary artery bypass graft operations were kept frozen at −80°C after washing them from surrounding the tissue and blood clots. For comparison of stable and unstable (vulnerable) lesions with regard to TRAIL expression, we obtained samples of plaques removed electively from patients with peripheral vascular disease (stable plaques; n = 5) and from patients with ACS that underwent rheolytic therapy with the Angiojet system (n = 4; Possis Medical, Minneapolis, Minnesota). This technique allows for a suction force that disintegrates and removes thrombus and plaque content from culprit arteries in patients with ACS. The generated fluid was centrifuged, red cells were lysed, and plaque content was homogenized and frozen at −80°C until blotting was performed. These plaques are considered to be vulnerable because of their association with the thrombus and the respective clinical picture. On the day of immunoblotting, samples were defrosted at room temperature, washed with phosphate-buffered saline, and homogenized in a lysis buffer. After centrifugation, the supernatant were washed with phosphate-buffered saline, and homogenized in a lysis buffer. After centrifugation, the supernatant were prepared as previously described (14). Ly-sophosphatidylcholine (LPC) and the respective clinical picture. On the day of immunoblotting, samples were defrosted at room temperature, washed with phosphate-buffered saline, and homogenized in a lysis buffer. After centrifugation, the supernatant were prepared as previously described (14). Ly-sophosphatidylcholine (LPC), an active derivative of ox-LDL, was purchased from Sigma (St. Louis, Missouri). Antibodies used for immunohistochemistry and Western blotting consisted of rabbit polyclonal anti-human TRAIL antibodies that are reactive with the amino acids 25-281 at the carboxy terminus of TRAIL (Santa Cruz Biotechnology, Santa Cruz, California) and are cross-reactive with murine TRAIL. Anti-CD3 antibodies were from DAKO (Carpinteria, California). Anti-oxLDL antibodies were either polyclonal, prepared as previously described (14), or mouse anti-Apo B100 IgG (ICN Pharmaceuticals Inc., Aurora, Ohio).

**Immunohistochemical study of human atherosclerotic lesions.** Five-micrometer thick frozen sections of human carotid plaques were sectioned. After fixation and blocking with nonimmune serum, we added the primary antibodies (anti-TRAIL antibodies, anti-CD3 antibodies, and anti-oxLDL antibodies) for 1 h at room temperature. After washing, biotinylated affinity-purified secondary antibodies were added. The slides were then incubated with 0.3% H2O2, followed by additional rinses and incubation streptavidin-peroxidase conjugate (Jackson Laboratories, Bar Harbor, Maine) for 30 min. The slides were developed with 3-amino-9 ethylcarbazole substrate (DAKO) for 15 min and counterstained with hematoxylin.

**Patients.** Three group of subjects were selected. Group 1 comprised patients with ACS that were admitted in the intensive coronary care unit (n = 40), group 2 comprised patients with stable angina pectoris with angiographically documented atherosclerosis (n = 28), and group 3 comprised subjects with normal coronary arteries (NCA) as determined by angiography (n = 20). Acute coronary syndrome in all patients was defined as chest pain accompanied by definite ischemic electrocardiographic changes (ST-segment changes and/or T-wave inversions). Myocardial infarction was diagnosed if either: 1) elevation of troponin I (>0.8 ng/ml) or creatine kinase-myocardial band (>), or 2) definite (>2 mm) ST-segment elevations in at least two consecutive leads also was present.

**Isolation of peripheral blood mononuclear cells (PBMCs).** Peripheral blood mononuclear cells were isolated from 30 ml of freshly drawn heparinized blood using Isopaque-Ficoll (Amersham Biosciences, Buckinghamshire, United Kingdom) gradient centrifugation. To eliminate contamination by monocyte/macrophage cells were seeded on plastic plates for 4 h and nonadherent cells were collected and subjected for further assays.

**Detection of TRAIL in oxLDL/LPC-primed PBMCs by Western blot.** Peripheral blood mononuclear cells from healthy volunteers were suspended in RPMI 1640 plus antibiotics (50 mg/ml of penicillin and streptomycin), L-glutamine, and 10% fetal calf serum. The cells were cultured in six-well plates and incubated for 24 h at 37°C with oxLDL (50 μg/ml), LPC (50 and 100 μmol/l), or medium. The cells were harvested, washed with phosphate-buffered saline, and lysed with a lysis buffer. We then analyzed them by western blot using anti-TRAIL antibodies (Santa Cruz Biotechnology) as described previously.

**Real-time quantitative reverse-transcription polymerase chain reaction (RT-PCR) for the detection of TRAIL mRNA.** Total ribonucleic acid from the PBMCs of random patients from the three groups (nine from group 1, six from group 2, and five from group 3) was isolated using...
RNeasy kit (Qiagen, Hilden, Germany). Reverse transcription to complementary deoxyribonucleic acid was performed with random hexamers using the TaqMan reverse transcription reagents according to manufacturer's instructions. Real-time quantitative RT-PCR was performed on an ABI Prism 7700 sequence detection system (Applied Biosystems, Cheshire, United Kingdom). The sequence of the primers used was as follows: TRAIL forward, 5'-CAGAGGAAGAAGCAACACATTCTCT-3'; TRAIL reverse, 5'-TGATGATTCCCGAGGATTTTGTG-3'; beta-actin forward, 5'-TGACGCGGGCTACAGCTT-3'; beta-actin reverse, 5'-TCTTAAATGTACGCACGATT-3'.

**Enzyme-linked immunosorbent assay (ELISA) for the detection of soluble TRAIL, Fas-ligand (sFasL), and TNF-alpha levels.** Blood samples were taken within 48 h of hospital admission in patients with ACS. After separation according to standard procedures, serum samples were kept at −80°C until further testing. Blood samples from patients in groups 2 and 3 were obtained in the ambulatory clinic. Quantitative determination of soluble TRAIL was performed using a sandwich ELISA kit with a detection limit of 20 pg/ml according to the manufacturer's protocol (BioSource International Inc., Camarillo, California).

Serum concentrations of sFasL were determined in duplicate with commercially available ELISA kits (Diaclone Research, Besancon, France). The sensitivity for detection of sFasL was 12 pg/ml. Serum TNF-alpha levels were determined using ELISA according to the manufacturer's recommendations (R&D Systems, Minneapolis, Minnesota). The sensitivity of the ELISA was 80 pg/ml for TNF-alpha.

**High-sensitivity CRP (hsCRP) concentrations.** The assay for hsCRP was conducted according to manufacturer's instructions (Dade Behring Inc., Deerfield, Illinois). Briefly, the principle of the method includes using polystyrene particles coated with monoclonal antibodies to CRP. These particles agglutinate with CRP. The CRP level was determined according to the intensity of the scattered light in the nephelometer compared with standards of a known concentration.

**Statistical analysis.** A comparison among the three different groups of patients was performed by one-way analysis of variance for continuous variables and chi-square test for categorical variables. The Fisher exact test was used for categorical variables when the number of observations per cell was expected to be ≤5. The Dunnett multiple comparison procedure and logistic regression models were used for pair-wise comparisons between patients with ACS and the two other groups.

Because of the small number of patients tested for the amount of mRNA of TRAIL in PBMCs, the comparison among the three different groups regarding this variable was performed with the Kruskal-Wallis nonparametric analysis of variance test.

Linear regression model was applied to test the association among the three groups of patients and soluble TRAIL levels after adjusting for possible confounding variables. All statistically significant variables on a univariate analysis (p < 0.05) were candidates for the regression equation; several variable selection methods were then used (all variables, forward selection, backward elimination). Statistical significance was defined as a value of p < 0.05. The SAS system 8.01 software for Windows (SAS Institute, Cary, North Carolina) was used for all calculations.

**RESULTS**

Using Western blotting, we observed TRAIL protein expression in human atherosclerotic plaques, whereas control arteries (left internal mammary arteries) representing non-atherosclerotic vessels exhibited significantly lower TRAIL expression (Fig. 1A). We then compared the expression of TRAIL in vulnerable plaques known to reside in patients with ACS to stable plaques removed from the femoral arteries on elective surgery (Fig. 1B). We found that vulnerable plaques contain a significantly higher content of TRAIL as compared with stable plaques. Aiming to confirm the presence of TRAIL in an additional species, we explored and detected TRAIL expression in the atherosclerosis process.

**Figure 1.** (A) Shown is the expression of tumor necrosis factor-related apoptosis-inducing ligand (TRAIL) in atherosclerotic and normal vessels detected by Western blotting in sections of human atherosclerotic plaques and nonatherosclerotic arteries (left internal mammary arteries [LIMA]) and compared with the expression of the housekeeping protein beta-tubulin. (B) Shown is the expression of TRAIL in vulnerable and stable atherosclerotic plaques. Stable plaques removed from femoral arteries (a), and representative vulnerable plaques (b and c) were removed by suction after the application of a rheolytic device from culprit arteries. The expression of TRAIL was assayed by Western blotting and compared with expression of beta tubulin. Densitometric analysis is shown in the graph.
rotic aortas of 10-month-old apo-E–deficient mice as compared with control C57BL/6 littermates of similar genetic background exhibiting no atherosclerosis (data not shown).

Next, we aimed to define the nature of cells expressing TRAIL protein and to determine its possible colocalization with oxLDL. Indeed, TRAIL was expressed in carotid atherosclerotic plaques within CD3-positive cells, and its expression was abundant in areas that stained positive for oxLDL (Fig. 2). The principal regions in which TRAIL-positive cells were evident were the interface regions between the lipid core and the media and within the shoulder regions of the plaques where lymphocytes are predominantly known to reside.

Because oxLDL was found to colocalize with TRAIL, we reasoned that it could play a causative role by inducing the expression of the latter. Indeed, incubation of PBMCs with oxLDL but not with its active derivative, LPC, up-regulated expression of TRAIL messenger ribonucleic acid (mRNA) (Fig. 3A) and protein (Fig. 3B) in these cells. Induction of TRAIL mRNA was evident and peaked already 6 h after incubation with 50 μg/ml of oxLDL.

After having confirmed the presence of TRAIL and its inducibility in atherosclerotic plaques, we went on to test the functional significance of this protein. For this purpose, we assayed serum levels of soluble TRAIL in the sera of three groups of patients. Group 1 consisted of patients with ACS (n = 40), group 2 consisted of patients with stable atherosclerotic disease (n = 28), and group 3 consisted of subjects with angiographically confirmed normal coronary arteries (n = 20). To exclude a confounding effect of atherosclerotic burden on TRAIL levels, we matched the patients with ACS and those with stable angina for coronary artery affliction. This resulted in two groups with a similar number of afflicted atherosclerotic coronary arteries. Baseline clinical characteristics of the three subject groups are presented in Table 1.

We found that levels of soluble TRAIL were significantly lower in patients with ACS as compared with both other groups of patients (p < 0.0001, compared with stable atherosclerosis patients; mean difference, 344 pg/ml, 95% confidence interval 191.8 to 496.4, and when compared with subjects with NCA; mean difference, 398.5 pg/ml, 95% confidence interval 224.2 to 567.7) (Fig. 4A). This difference remained statistically significant, even after using multiple linear regression analysis and including all variables that were significant on univariate analysis. No difference was found in soluble TRAIL levels between patients with stable atherosclerotic coronary disease and patients with NCA (p = 0.56), although the mean level was slightly higher in the latter group. In group 1 there were 24 patients with acute myocardial infarction and 16 with unstable angina. No difference in soluble TRAIL levels existed between these two subgroups (p = 0.51).

Levels of sFasL did not differ between patients with stable angina (34.2 ± 7.3 pg/ml) and unstable angina (38.6...
No detectable levels were noted in the cultured medium of PMBCs. Similarly, no significant differences were evident with regard to serum levels of TNF-alpha: 12.3 ± 3.5 pg/ml in the stable angina patients versus 13.4 ± 4.5 pg/ml in the unstable angina patients.

Because CRP is associated with high likelihood of adverse events in patients with atherosclerosis and also has been suggested to play a detrimental role in plaque formation, we evaluated the correlation between TRAIL protein levels and CRP concentrations. Interestingly, we found a negative correlation between CRP concentrations and soluble TRAIL levels ($r^2 = -0.26; p = 0.016$). The expression of TRAIL mRNA in PBMCs assayed by real-time RT-PCR demonstrated no differences among the three study groups. Moreover, no correlation was evident between mRNA level and soluble TRAIL concentrations.

**DISCUSSION**

In the current study, we demonstrate for the first time that TRAIL is expressed in atherosclerotic plaques from humans and atherosclerotic ApoE knockout mice. The control nonatherosclerotic vessels also exhibit TRAIL protein expression, although to a lesser extent, which is consistent with reports demonstrating its presence within smooth muscle cells (15). Tumor necrosis factor-related apoptosis-inducing ligand was found to colocalize with CD3 cells and with oxLDL by immunohistochemical staining. Moreover, we showed that oxLDL but not its active derivatives (LPC) can induce TRAIL mRNA and protein expression in PBMCs.

In different studies, oxLDL was shown to have proinflammatory and proatherogenic properties, that is, promoting T cells and monocyte chemotaxis and adhesion and induction of proinflammatory genes (reviewed in references 1 to 3). It also has been suggested to trigger T-cell-dependent autoimmune responses in the atherosclerotic plaques (4). These inflammatory reactions were proposed to play a critical role in atherosclerotic plaque formation, progression, and vulnerability. By demonstrating that TRAIL is colocalized with CD3 cells and oxLDL and is inducible by oxLDL priming, it may be speculated that TRAIL has a role in controlling the balance between proatherosclerotic and antiatherosclerotic programs within the atheroma.

The rational for assuming a functional role for TRAIL in the progression and destabilization of the atherosclerotic plaque stems from several observations. Tumor necrosis factor-related apoptosis-inducing ligand was found to display T-cell immunomodulatory properties (8,9,16). Studies in animal models have shown that TRAIL affects growth of T cells and effector functions. Soluble active TRAIL negatively regulates calcium influx through store-operated calcium release-activated calcium channels, which is crucial to activation of lymphocytes. Furthermore, soluble TRAIL was shown to inhibit the production of interferon-gamma and IL4 and to block cell-cycle progression from G1 to S phase and subsequent proliferation in human T cells (10,11,16). The neutralization of TRAIL receptors in animals caused exacerbation of autoimmune encephalomyelitis (17) and type I diabetes mellitus (18), whereas soluble TRAIL levels correlated with the response in patients with multiple sclerosis to interferon-beta (19). An additional beneficial property of TRAIL is its endothelial protective properties, which are related in part to nitric oxide generation (12,13). Dysfunctional endothelium and autoimmune T cells are thought to influence atherosclerosis initiation and progression (1–3), and their amelioration by TRAIL may thus bear antiatherogenic attributes in this context.

Tumor necrosis factor-related apoptosis-inducing ligand
is a transmembrane protein with an extracellular, carboxy-terminal domain. Soluble TRAIL is generated through the enzymatic cleavage of this extracellular domain. We found that soluble TRAIL levels in patients with ACS are drastically reduced as compared with patients with stable atherosclerotic disease and individuals with normal coronary disease. Interestingly, other members of the TNF superfam-

Table 1. Clinical Data of the Three Different Groups of Patients: Univariate Analysis

<table>
<thead>
<tr>
<th></th>
<th>Acute Coronary Syndrome (n = 40)</th>
<th>Stable Angina (n = 28)</th>
<th>Normal Coronary Arteries (n = 20)</th>
<th>p Value</th>
</tr>
</thead>
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<tr>
<td>Men/women</td>
<td>34/6</td>
<td>25/3</td>
<td>15/5</td>
<td>0.48‡</td>
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<tr>
<td>Age (yrs)</td>
<td>61.2 ± 14.8</td>
<td>64.1 ± 10.7</td>
<td>55.6 ± 9.2</td>
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<td>LVEF (%)</td>
<td>49.6 ± 10.8</td>
<td>53.5 ± 8.6</td>
<td>59.4 ± 2.3</td>
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<td>NYHA-FC</td>
<td>4</td>
<td>1.46 ± 0.63</td>
<td>1.15 ± 0.48</td>
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<td>CAD extent (n × vessels)</td>
<td>1.59 ± 0.8</td>
<td>2 ± 0.9</td>
<td>0 (0)</td>
<td>0.12‡</td>
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<td>Past history</td>
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<tr>
<td>Previous MI, n (%)</td>
<td>14 (35)</td>
<td>15 (53.5)</td>
<td>0 (0)</td>
<td>0.12*</td>
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<td>Previous CABG, n (%)</td>
<td>3 (7.5)</td>
<td>8 (28.5)</td>
<td>0 (0)</td>
<td>0.08‡</td>
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<tr>
<td>Previous PTCA, n (%)</td>
<td>15 (37.5)</td>
<td>20 (71.4)</td>
<td>0 (0)</td>
<td>0.006*</td>
</tr>
<tr>
<td>Previous TIA/CVA, n(%)</td>
<td>1 (2.5)</td>
<td>2 (7)</td>
<td>1 (5)</td>
<td>†‡</td>
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<tr>
<td>CRF†</td>
<td>6 (15)</td>
<td>4 (14.2)</td>
<td>1 (5)</td>
<td>†‡</td>
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<td>Risk factors</td>
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<td>Hypertension, n (%)</td>
<td>19 (47.5)</td>
<td>16 (57.1)</td>
<td>8 (40)</td>
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<td>Diabetes, n (%)</td>
<td>8 (20)</td>
<td>12 (42.8)</td>
<td>1 (5)</td>
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<td>Smoking</td>
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<td>Current smoker, n (%)</td>
<td>21 (52.5)</td>
<td>12 (42.8)</td>
<td>5 (25)</td>
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<td>Past smoker, n (%)</td>
<td>10 (25)</td>
<td>6 (21.4)</td>
<td>4 (20)</td>
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<td>Hyperlipidemia, n (%)</td>
<td>27 (67.5)</td>
<td>24 (85.7)</td>
<td>8 (40)</td>
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<td>Medications</td>
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<td>Beta-blockers, n (%)</td>
<td>18 (45)</td>
<td>22 (78.5)</td>
<td>3 (15)</td>
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<td>ACEI, n (%)</td>
<td>10 (25)</td>
<td>14 (50)</td>
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<td>ARB, n (%)</td>
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<td>1 (3.5)</td>
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<td>Aspirin, n (%)</td>
<td>25 (62.5)</td>
<td>22 (78.5)</td>
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<td>7 (25)</td>
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<td>Statins, n (%)</td>
<td>20 (50)</td>
<td>22 (78.5)</td>
<td>4 (20)</td>
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<td>Calcium blocker, n (%)</td>
<td>4 (10)</td>
<td>11 (39.2)</td>
<td>2 (10)</td>
<td>0.006</td>
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<td>Nitrates, n (%)</td>
<td>11 (27.5)</td>
<td>11 (39.2)</td>
<td>0 (0)</td>
<td>0.007</td>
</tr>
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<td>Diuretics, n (%)</td>
<td>5 (12.5)</td>
<td>8 (28.5)</td>
<td>2 (10)</td>
<td>0.15‡</td>
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<td>Hypoglycemics, n (%)</td>
<td>2 (5)</td>
<td>5 (17.8)</td>
<td>0 (0)</td>
<td>0.112‡</td>
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<td>Biochemistry</td>
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<td>Soluble-TRAIL, pg/ml</td>
<td>238.6 ± 199.3</td>
<td>582.7 ± 349.1</td>
<td>637 ± 281.2</td>
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<td>CRP</td>
<td>12.2 ± 16.8</td>
<td>8.2 ± 16.7</td>
<td>4.9 ± 5.1</td>
<td>0.19</td>
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</table>

Data are in number (range) or mean ± SD. *Comparison only between acute coronary syndrome (ACS) and stable angina pectoris patients. †Defined as baseline creatinine above 1.5 mg/dl. ‡Categorical variables that were analyzed by the Fisher exact test.

ACEI = angiotensin-converting enzyme inhibitors; ARB = angiotensin receptor blocker; CABG = coronary artery bypass graft; CAD = coronary artery disease; CRF = chronic renal failure; CVA = cerebrovascular accident; LVEF = left ventricular ejection function; MI = myocardial infarction; NYHA-FC = New York Heart Association functional class; PTCA = percutaneous transluminal coronary angioplasty; TIA = transient ischemic attack.

is a transmembrane protein with an extracellular, carboxy-terminal domain. Soluble TRAIL is generated through the enzymatic cleavage of this extracellular domain. We found that soluble TRAIL levels in patients with ACS are drastically reduced as compared with patients with stable atherosclerotic disease and individuals with normal coronary disease. Interestingly, other members of the TNF superfamily that were assayed (TNF-alpha and Fas-Ligand) in the serum of the patients with stable and unstable angina did not differ between the groups, suggesting the results obtained for soluble TRAIL were not of a “class effect.” Patients with ACS are a subgroup of patients with atherosclerosis that are thought to display vulnerable plaques and thus suffer clinical sequelae due to vessel obstruction (5,6). The significantly lower TRAIL levels in this group of patients suggest a possible protective stabilizing role for this protein. Although this observation is speculative, it is somewhat supported by the finding that TRAIL expression was more robust in plaques with a vulnerable phenotype as compared with stable lesions. This finding may be related to a consumption effect of TRAIL into the lesions that are more likely to be infiltrated by immune cells.

Interestingly, we found that TRAIL mRNA expression in PBMCs was not different in the patients from the three groups and that no significant correlation to soluble TRAIL levels existed. These findings could suggest that the TRAIL protein may not be reduced in ACS because of diminished production by circulatory T cells but rather consumed into the plaque, as suggested after the results obtained with the Western blotting assays. Recently, Nakajima et al. (20) reported that the expression of TRAIL by flow cytometry on peripheral lymphocytes in patients with acute myocardial infarction was increased when compared with healthy volunteers. Unlike our study, TRAIL levels were evaluated in the PMBCs of patients with established acute ST-segment elevation myocardial infarction in which cardiomyocyte necrosis was evident, whereas in our study most patients had unstable angina. Moreover, the control patients were healthy and, therefore, their atherosclerosis burden was not similar and could have initially confounded the interpretation of the data.
associated with plaque instability and oxidative stress (22). For this purpose and to avoid the effect of plaque burden, we chose patients with ACS and stable lesions that had a similar extent of coronary affliction by atherosclerosis. We demonstrated that TRAIL is expressed in atherosclerotic plaques and is colocalized with CD3 cells and oxLDL. We also demonstrated that TRAIL is induced in PBMCs by oxLDL. In ACS, the levels of soluble TRAIL are reduced and correlate negatively with the proinflammatory acute-phase reactant CRP, whereas expression of TRAIL is significantly higher in plaques with a vulnerable as compared with a stable phenotype. If further validated, in vivo TRAIL could have a role as a protective agent in patients with ACS.

For this purpose and to avoid the effect of plaque burden, we chose patients with ACS and stable lesions that had a similar extent of coronary affliction by atherosclerosis.

C-reactive protein, a nonspecific “acute-phase” protein, is a known marker of acute vascular events (21) and is closely associated with plaque instability and oxidative stress (22). Soluble TRAIL levels were found to be negatively correlated with hsCRP concentrations, supporting a possible protective role of TRAIL in atherosclerosis and plaque instability.

Conclusions. We demonstrated that TRAIL is expressed in atherosclerotic plaques and is colocalized with CD3 cells and oxLDL. We also demonstrated that TRAIL is induced in PBMCs by oxLDL. In ACS, the levels of soluble TRAIL are reduced and correlate negatively with the proinflammatory acute-phase reactant CRP, whereas expression of TRAIL is significantly higher in plaques with a vulnerable as compared with a stable phenotype. If further validated, in vivo TRAIL could have a role as a protective agent in patients with ACS.

REFERENCES