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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 assayed 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.

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Abbreviations and Acronyms

ACS = acute coronary syndrome
CRP = C-reactive protein
hsCRP = high-sensitivity C-reactive protein
LPC = lysophosphatidylcholine
oxLDL = oxidized low-density lipoprotein
mRNA = messenger ribonucleic acid
NCA = normal coronary arteries
PBMCs = peripheral blood mononuclear cells
RT-PCR = reverse-transcription polymerase chain reaction
TNF = tumor necrosis factor
TRAIL = tumor necrosis factor-related apoptosis-inducing ligand

METHODS

Materials, reagents, and antibodies. Oxidized low-density lipoprotein was prepared as previously described (14). Lysophosphatidylcholine (LPC), an active derivative of oxLDL, 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 applied on a 10% acrylamide sodium dodecyl sulfate gel under reduced conditions, transferred to nitrocellulose paper, and probed with anti-TRAIL antibodies (Santa Cruz Biotechnology). The same procedure was applied to atherosclerotic aortas of 10-month-old ApoE knockout mice and those of age-matched C57BL/6 mice (used as control nonatherosclerotic aortas).

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% H_2O_2, 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