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Tumor Necrosis Factor Related Apoptosis Inducing Ligand (Trail) in Endothelial Response to Biomechanical and Biochemical Stresses in Arteries

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ABSTRACT

Shear stress is determined by three physical components described in a famous triad: blood flow, blood viscosity and vessel geometry. Through the direct action on endothelium, shear stress is able to radically interfere with endothelial properties and the physiology of the vascular wall. Endothelial cells (ECs) have also to sustain biochemical stresses represented by chemokines, growth factors, cytokines, complement, hormones, nitric oxide (NO), oxygen and reactive oxygen species (ROS). Many growth factors, cytokines, chemokines, hormones, and chemical substances, like NO, act and regulate endothelium functions and homeostasis. Among these cytokines Tumor Necrosis Factor Related Apoptosis Inducing Ligand (TRAIL) has been assigned a regulatory role in ECs physiology and physiopathology. Thus, the aim of this review is to provide a general overview of the endothelial response pathways after different types of biomechanical and biochemical stress in in vitro models and to analyze the crucial role of TRAIL under pathological conditions of the cardiocirculatory system like atherosclerosis, coronary artery disease, and diabetes. J. Cell. Biochem. 116: 2427–2434, 2015.

KEY WORDS: SHEAR STRESS; ENDOTHELIAL CELLS; VASCULAR SMOOTH MUSCLE CELLS; TRAIL; CARDIOVASCULAR SYSTEM

A closed-loop circulatory system connected to a pump must be formed very early in human development to distribute oxygen, nutrients, paracrine factors and to clean toxic metabolites to the far corners of the body. The arterial circulatory system supports a pulsatile blood flow and high pressure in order to ensure smooth and thorough movement of substances to deliver to all body tissues. The continuous blood return to the right section of the cardiac pump, however, must be kept at low pressure, with a low shear stress, and this is allowed by the high capacitance of the venous system. The various functions of the circulatory system continuously require considerable specialization of its components and, as noted, the heterogeneity of endothelial cells (ECs) lining the lumen of arteries and veins has a key role towards this specialization [Aitsebaomo et al., 2008; Atkins et al., 2011]. Until the late 1990s, the initial driving force in creation of heterogeneous ECs phenotypes was thought to be the exposure of these cells to flowing blood [Conway et al., 2010]. Several data demonstrate that ECs heterogeneity, while retaining the plasticity to change under a changing environment, has a genetic basis that comes into play perhaps even before hemangioblasts differentiate into ECs and hematopoietic cells. A lot of elegant experiments in zebrafish, mouse embryos and human umbilical vein ECs (HUVECs) have provided the majority of the molecular data that drive the present knowledge of ECs heterogeneity. Unfortunately, human studies are largely limited to ex vivo experiments with human-derived cells/tissues or to correlations with human vascular diseases [Aitsebaomo et al., 2008].

ECs must sustain shear stress that is the most likely cause of the discrete and focal nature of the atherosclerotic lesions. Shear stress is determined by three physical components described in a famous triad: blood flow, blood viscosity, and vessel geometry. These three elements are related to hemodynamics and are measured in dynes per square centimeter. Through the direct action on the endothelium, shear stress inherently affects the properties and functions of the endothelium and, consequently, the whole structure of the vessel

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wall. ECs have also to sustain biochemical stresses represented by chemokines, complement, cytokines, growth factors, hormones, nitric oxide (NO), oxygen (O_2), and reactive oxygen species (ROS). Many growth factors, cytokines, chemokines, hormones, and chemical substances, like NO, act and regulate endothelial functions. One of the important cytokines that shows these functions is Tumor Necrosis Factor Related Apoptosis Inducing Ligand (TRAIL). TRAIL, also known as Apo2 ligand, is a member of the TNF family expressed as either a type II trans membrane protein, similarly to other membrane bound ligands of the TNF superfamily, or as a soluble protein, detectable in the serum under physiological conditions [Di Pietro and Zauli, 2004]. In literature, a number of studies have shown that soluble TRAIL is able to induce the activation of some signal transduction pathways, such as ERK/MAPK, Akt and NF-κB pathway [Zauli et al., 2005]. Many studies demonstrated that these pathways promote the survival and/or proliferation of vascular smooth muscle cells (VSMCs) and vascular ECs [Di Pietro and Zauli, 2004; Secchiero et al., 2004]. TRAIL also exerts a protective action on the endothelium due both to its anti-inflammatory activity and to the production of NO by ECs themselves [Zauli et al., 2003; Di Pietro et al., 2006].

Therefore, the aim of this review is to provide a general overview of the endothelial response pathways after different types of biomechanical and biochemical stress in in vitro models and to analyze the crucial role of TRAIL under pathological conditions of the cardiocirculatory system like atherosclerosis, coronary artery disease, and diabetes.

BIOMECHANICAL AND BIOCHEMICAL STRESSES AND ECS HETEROGENEITY

The vascular multipotent progenitor cells, named angioblasts, originate in the lateral plate mesoderm and migrate to a midline position just ventral to the notochord, giving rice to both hematopoietic cells and ECs. Many cell-tracing experiments in vertebrate embryos have shown that arterial-venous cell fate decision of angioblasts takes place before their migration and location. The major embryonic vessels arise from the coalescence of angioblasts to form the dorsal aorta (DA), which lies ventral to the notochord, and the posterior cardinal vein located just below and parallel to DA. The response to vascular endothelial growth factor (VEGF) gradient, in addition to angioblasts genetic predestination, initiates a cascade of events, driven by a hierarchy of signaling molecules, that culminates in arterial versus venous ECs differentiation [Atkins et al., 2011].

Atherosclerosis has many epidemiological causes that are correlated with the coexistence of specific risk factors [De Caterina and Madonna, 2009]. Major risk factors for cardiovascular diseases (CVD) (hypertension, diabetes, hypercholesterolaemia, obesity, tobacco smoking, inflammatory processes, physical inactivity, and metabolic syndrome) share a common mechanism of action, since they act on the inner surface of the arterial walls [Porreca et al., 2002]. The cellular and molecular effects of such systemic risk factors, acting through bio-humoral mediators, can account for the greater or lesser propensity of different individuals to atherosclerotic vascular disease. The atherosclerotic process is localized preferentially in specific regions of the arteries such as at the areas of bifurcation and along the convex edge of curved arteries, where the endothelium is subjected to a greater shear stress. It was also observed that some arteries are more predisposed to atherosclerotic lesions than other arterial vessels, i.e. epicardial coronary arteries *versus* intra myocardial arteries or carotid arteries versus subclavian arteries [De Caterina and Madonna, 2009].

The specific site location of the atherosclerotic process is an important aspect in translational research. It is usually explained by the action of blood flow, blood viscosity and vessel geometry that cause specific modes of shear stress imposed on the arterial wall by the flow of the bloodstream [Conway et al., 2010; Atkins et al., 2011]. In the last years, much progress has been achieved in understanding how shear stress is transduced within the arterial wall to promote or protect from atherogenesis [De Caterina and Madonna, 2009; Conway et al., 2009]. Each EC is comparable to a little model of input/output system [Conway et al., 2009]. Inputs derive from the extracellular environment and are represented by biomechanical (i.e. shear stress and cyclical strain) and biochemical forces (i.e. chemokines, complement, cytokines, growth factors, hormones, NO, O_2, and ROS). The cellular phenotype represents the output and is manifested by protein and mRNA expression, cell shape, calcium flux, release of inflammatory mediators, leukocyte adhesion and/or transmigration, cell migration, proliferation, survival and/or apoptosis, vasomotor tone, and hemostatic balance. The input is linked to the output by several signaling pathways that originate at cell membrane level and lead to transcription or post-transcriptional modifications.

Biomechanical and biochemical network of signals varies across the vasculature at each time point. For instance, ECs in the nervous system interact with a number of paracrine factors released by astroglial cells that are fundamental for the blood brain barrier; instead, cardiac ECs are exposed to local forces derived from the contracting heart and paracrine factors generated from neighboring cardiomyocytes. Every single site of the vasculature net signal input changes from one moment to another. As an example, liver sinusoidal ECs are exposed to portal venous blood that is greatly different in pre- and post-prandial composition. ECs are able to sense and respond to the microenvironment by changing their phenotypes. Although environmental differences are enough to explain the morphological and functional differences of different ECs, there is also evidence that certain site-specific properties of endothelium are epigenetically programmed [Atkins et al., 2011]. In this respect, many studies based on DNA microarrays of cultured human ECs obtained from different sites of the vascular tree showed remarkable differences in the transcription profiles between ECs from arteries and veins and between ECs of major and minor vessels [Aird, 2005]. Indeed, the stimulation of ECs derived from human coronary artery with oxidized LDL produced important changes in the expression of genes involved in adhesion, proliferation and apoptosis in comparison with ECs derived from human saphenous vein, showing a specific susceptibility to atherosclerosis of arterial ECs compared to venous ECs. In turn, ECs derived from pulmonary artery and microcirculation display site-specific barrier properties that are maintained in cultured cells [Kelly et al., 1998]. This is not true for ECs derived from high endothelial venules (HEVs). In an important study carried out on human ECs from HEVs of tonsils and umbilical

cord, Lacorre et al. [2004] demonstrated changes in gene expression profiles in freshly isolated cells and after 2 days of cultivation. Altogether, these results highlight the double role of microenvironment and epigenetics in committing vascular specific phenotypes, although the data are not yet validated in vivo [Atkins et al., 2011].

A lot of mechanisms inducing epigenetic changes have been identified including DNA methylation, histone proteins methylation and hyper-activation. DNA and histones methylation is under control of methylases and de-methylases enzymes, whereas histones acetylation is under control of histones acetyl-transferases and de-acetylases enzymes. Recently, many in vitro studies have highlighted the role of DNA methylation and/or histones acetylation and/or de-methylation of specific genes in ECs [Lacorre et al., 2004]. However, the impact of epigenetics changes on ECs phenotypes in the different kind of the vessels is hitherto unknown and thus appears as a challenging field of scientific interest. Indeed, it is fascinating to hypothesise that ageing and/or disease are related with an increase in epigenetic mutations, resulting in a lower plasticity of the endothelium. For instance, human ECs isolated from intestinal sites stricken by inflammatory bowel disease (IBD) show hyper adhesiveness to leukocytes compared to cells isolated from healthy areas or from patients unaffected by IBD [Aird, 2007]. Surprisingly, these various properties, which have been attributed to differences in inducible NO synthase activity, were maintained during sequential passaging in culture. It has been demonstrated that an heterogeneous endothelial phenotype provides at least two significant advantages: first, it ensures the endothelial adaptation to different mechanical and elastic properties of the tissues in the various body districts and, in the second instance, it allows the endothelial adaptation to different microenvironments, from the highly oxygenated microenvironment of the lung alveoli to the highly hypoxic and hyperosmolar inner medullary area of the kidney [Aird, 2007].

SHEAR STRESS AND ATHEROGENESIS

The atherosclerotic disease usually affects coronary arteries, internal and external carotid artery, abdominal aorta and superficial femoral arteries [Chien, 2008]. These arterial sites have got manifold blood flow patterns, including reversal flow (RF) during each phase of cardiac hemodynamics, which are in favor of the hypothesis that turbulent hemodynamic patterns lead to atherosclerotic lesions. These hemodynamic variations can alter the profile of gene expression and, as a consequence, ECs structure and function, influencing ECs responses to chemical factors and ECs interactions with underlying VSMCs, thus increasing the chance of atherogenesis [Chien, 2008]. Three are the types of the hemodynamic forces to which arterial endothelium is subjected. The first one is the hydrostatic pressure produced by the forces acting on the vessel wall; the second is the circumferential tension or stress resulting from traction forces applied by intercellular connections during vascular motility; the third is the shear stress which consists in biomechanical dragging frictional forces exerted on the arterial wall by the flowing blood. Shear stress is the most reasonable cause to explain the point and focal nature of atherosclerosis and is determined by three physical components measured in dynes per square centimeter: blood flow, blood viscosity, and vessel geometry. Since the shear stress acts on vascular endothelium it deeply affects endothelial function and, as a consequence, the vascular wall physiology. In linear shape arterial segments, the blood flows according to the cardiac cycle pulsatility. In these segments, ECs are subjected to a high shear stress with an average high and uniform intensity in space and time. Previous studies, comparing "anti-atherogenic" non-reversing arterial shear stress to "pro-atherogenic" reversing arterial shear stress, described the reversing shear stress as a sine wave and used high steady shear stress and static conditions as controls for the pro-atherogenic waveform [Ohura et al., 2003]. In any case, simulations of shear stress in the carotid sinus showed that the shear stress applied on the artery wall does not appear harmonic but has an articulated waveform [Yee et al., 2008]. An interesting study by Conway et al. [2010] compared the effects of different types of shear stress on gene expression, cell proliferation, and monocyte adhesiveness to human umbilical vein ECs (HUVECs), a consolidated in vitro model for arterial ECs responses, since HUVECs gene expression clusters tightly with other large vessel ECs [Yee et al., 2008]. In particular, these authors developed a physiological reversing shear stress model to compare the effects of the low steady shear stress (1 dyne/cm^2), of the reversing shear stress (time-average: 1 dyne/cm^2), and of the arterial steady shear stress (15 dyne/cm^2). They demonstrated that low steady shear stress is the greater force involved in the control of gene expression and cell proliferation, whereas reversing shear stress is responsible for the increase in monocyte adhesion. These results provided new insights into endothelial responses to mechanical forces useful to understand the mechanisms underlying atherogenesis in the vessels affected by disturbed blood flow. In this respect, in vitro studies have shown that the regions particularly prone to atherosclerosis are areas of ECs subjected to reverse flow and recirculation, and characterized by temporal and spatial gradients of shear stress compared to ECs grown in static conditions [De Caterina et al., 2007]. It appears crucial to understand the initial molecular events in atherogenesis to define ECs gene expression profiles in various sites of normal arteries. In their study Won et al. [2007] showed an increased expression and Ser^{1177} phosphorylation of endothelial nitric-oxide synthase (eNOS) in response to shear stress at the wall sites of normal mouse aorta more prone to atherosclerosis, suggesting that turbulent blood flow occurring at arterial branches and curvatures is able to interfere with ECs gene expression by modulating the nuclear transcription machinery.

TRAIL AND ATHEROGENESIS

It has been widely recognized that inflammatory processes are implicated in the pathogenesis of atherosclerosis and phenotype transition of stable atherosclerotic plaques into vulnerable ones [Libby, 2002]. Atherosclerotic plaques are composed of a lipid core, a fibrous cap, and inflammatory infiltrates, mainly containing T cells and macrophages. These cells and components together with VSMCs accumulate in the inner lining of an artery resulting in the thickening of the arterial wall. Activated T lymphocytes have been attributed a key role in the initiation and progression of atherosclerosis [Libby, 2002]. Among the numerous antigens responsible for T-cell activation, oxidized low-density lipoproteins (oxLDL) have been considered the most important ones [Stemme

et al., 1995]. Michowitz et al. [2005] demonstrated for the first time the presence of TRAIL in stable atherosclerotic lesions, an increased TRAIL expression in vulnerable plaques and the co-localization of TRAIL with plaque-infiltrating CD3 cells and oxLDL, suggesting a possible role for TRAIL in atherosclerosis. TRAIL is well known for its capability to induce apoptosis in a variety of cancer cell types, but the wide expression of TRAIL and TRAIL receptors (TRAIL-R1/DR4, TRAIL-R2/DR5, TRAIL-R3/DcR1, TRAIL-R4/DcR2, and osteoprotegerin/OPG) in many normal tissues has put in light that its physiological role is more complex than the simple activation of apoptotic pathways in cancer cells [Di Pietro and Zauli, 2004]. As a matter of fact, accumulating evidences testify a number of non-apoptotic and immune functions regulated by TRAIL and, in particular, by soluble TRAIL, which is able to activate intracellular signal transduction pathways involved in cell survival, migration and proliferation in a variety of normal cells [Zauli et al., 2003; Di Pietro and Zauli, 2004; Di Pietro, 2011]. In particular, within the vessel wall TRAIL expression has been found in VSMCs, ECs and inflammatory cells [Zauli et al., 2003] and related to the induction of apoptosis in cultured ECs [Alladina et al., 2005]. It has been demonstrated that ECs are able to exert a protective role against atherosclerosis through the release of NO, a well known vasoactive mediator with antithrombotic and anti-inflammatory activity [Sandoo et al., 2010]. The expression of TRAIL and TRAIL receptors in normal arteries, in atherosclerotic lesions [Michowitz et al., 2005] and in Monckeberg's sclerosis [Secchiero et al., 2004], a form of atherosclerosis typically occurring in diabetic patients, suggests that TRAIL may be an important factor regulating cardiovascular disease (CVD). In CVD, TRAIL$^{-/-}$ mice exposed to arterial wall injury did not display intimal thickening [Chan et al., 2010]. Moreover, in diabetic ApoE$^{-/-}$ mice TRAIL stabilized atherosclerotic plaques by increasing VSMCs content, and promoted apoptosis of inflammatory cells [Secchiero et al., 2006]. Accordingly, TRAIL has been shown to reduce the atherogenetic process and the vascular diabetic complications by regulating cholesterol and lipogenesis [Di Bartolo et al., 2011]. In this respect, a very recent paper has demonstrated that TRAIL promotes macrophage lipid uptake by modulating scavenger receptor expression [Liu et al., 2014]. Beside TRAIL, also TRAILR1/DR4 has been implicated in the development of endothelial dysfunction and atherosclerosis [Li et al., 2013]. The association of atherosclerosis with bone pathologies promoted researches of mediators and pathways common to skeletal and vascular system. Since the identification of Osteoprotegerin (OPG) as a regulator of bone metabolism, a number of discussions arose on its possible role in the atherosclerotic arterial obstructive disease, in the mechanisms of calcification of atherosclerotic plaques, and in loss of elasticity of the vascular walls. Studies carried out in vitro and in vivo demonstrate that OPG inhibits the calcification of the vascular walls. Anyhow, clinical studies prove that increased serum OPG levels are associated with vascular walls calcification, stroke, coronary artery disease, and cardiovascular accidents [Van Campenhout and Golledge, 2009].

These data have stimulated an animated discussion on the prognostic value of OPG as a biomarker of vascular disease although the specific action and mechanisms through which OPG acts in cardiovascular pathophysiology are still unclear. Currently, OPG appears not only as an indicator of calcification, but also as a mediator that modulates vascular calcification, inflammatory response, and apoptosis. The role played by OPG in the vascular system is manifold and requires the interaction between its ligands, RANKL and TRAIL, and a bidirectional control involving osteogenic, inflammatory, and apoptotic events.

The OPG/RANKL/RANK axis, responsible for ossification and bone mineralization, seems to play a major role in vasculature and atherosclerosis [Papadopouli et al., 2008]. In the vasculature, OPG is normally produced by ECs and VSMCs, and RANKL is only present at calcified vessels, at high concentrations. OPG$^{-/-}$ mice present calcification of the tunica media of the arteries and osteoporosis, while transgenic administration of OPG to them reverses this process [Papadopouli et al., 2008]. In addition, administration of OPG prevents experimentally induced vascular calcification. Numerous chronic CVD show an increase in serum concentration of detectable OPG [Bucay et al., 1998]. These data indicate that serum OPG levels could be a new and an additional biomarker of cardiovascular risk that could be used in clinical practice in both the prevention of cardiovascular risk and in the estimation of cardiovascular prognosis.

TRAIL SIGNALING AT THE ATHEROSCLEROTIC PLAQUE

Up to date, the comprehension of TRAIL signaling pathways involved in atherosclerosis appears controversial. In fact, it has been reported that TRAIL is able to induce apoptosis of VSMCs and ECs in vivo whereas it can induce proliferation and migration of these cells in vitro. On the other hand, an anti-atherogenic role of TRAIL is outlined by investigations carried out in diabetic ApoE-null mice demonstrating TRAIL-induced reduction of atherosclerotic plaques and increased macrophage death and VSMCs content. Consistently, soluble TRAIL serum levels appear lower in patients prone to coronary artery disease (CAD). Based on these evidences, we can infer that TRAIL could be a relevant prognostic marker for CVD.

Acute coronary syndrome (ACS) often results by the rupture of the atherosclerotic lesion, following the infiltration of T cells and macrophages in the sub-endothelium. Sato et al. [2006] showed that plaque-infiltrating CD4$^+$ T cells effectively kill VSMCs. VSMCs sensitive to T cell–mediated killing express TRAIL-R2/DR5, and anti-TRAIL and anti-DR5 antibodies block T cell–mediated apoptosis. CD4$^+$ T cells that express TRAIL upon stimulation are augmented in patients with ACS and are able to induce VSMCs apoptosis with greater effectiveness. VSMCs apoptosis triggered by TRAIL-producing CD4$^+$ T cells, may lead, in turn, to plaque destabilization and rupture.

It has been reported that TRAIL can exert pro-survival and pro-proliferative effects on VSMCs both in vitro and in vivo. Kavurma et al. [2008] demonstrated that TRAIL co-localized with PCNA (proliferating cell nuclear antigen) and with IGF1R (insulin-like growth factor-1 receptor) inwardly the intima of saphenous vein bypass grafts sections. Moreover, the administration of 1 ng/ml of TRAIL to VSMCs cultured in vitro was capable to induce cell growth. It has been hypothesized that TRAIL binding at DR4 and DcR1 is required to exert pro-survival and pro-proliferative effects; in fact, TRAIL-induced proliferation of VSMCs is inhibited when using neutralizing antibodies to these receptors (Fig. 1). It has been also described that TRAIL is capable of increasing IGF1R expression by

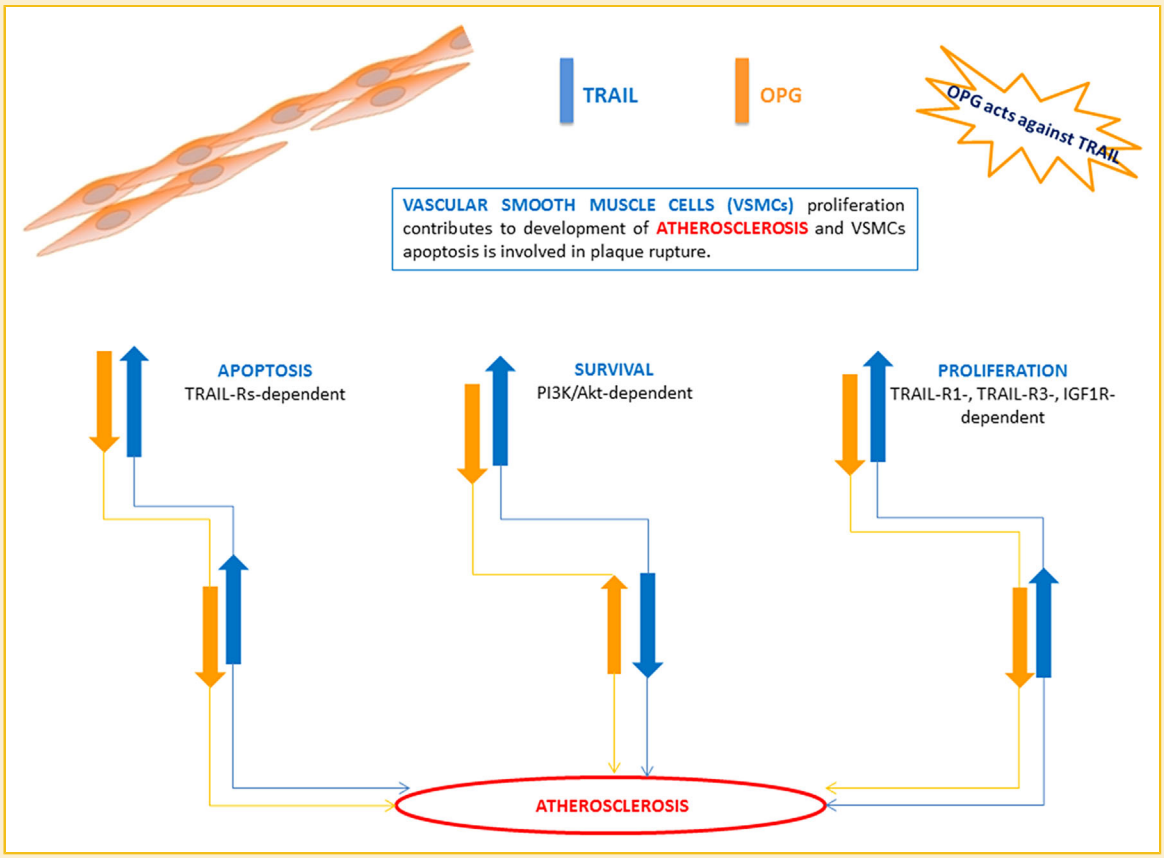

Fig. 1. TRAIL/TRAIL receptor system in VSMCs homeostasis and morbid atherosclerosis. Endothelial cells (ECs) and vascular smooth muscle cells (VSMCs) are the main cellular components of the normal arterial wall. Since TRAIL and its receptors are expressed in both normal and pathological arterial walls, it is hypothesized that TRAIL signaling could regulate vascular wall homeostasis and morbid atherosclerosis. The figure shows in vitro findings obtained in VSMCs: in the first line the blue arrows indicate the TRAIL-mediated effects on VSMCs in contrast to the orange arrows representing OPG-mediated effects. In the second line, the blue arrows indicate the reduction or increase in atherosclerosis as a consequence of the action of TRAIL on VSMCs whereas the orange arrows indicate the effects of OPG on atherosclerosis.

recruiting NF-κB pathway. Due to its intrinsic tyrosine kinase activity and downstream mediators, like Akt and MAPK, IGF1R once activated can lead to cell survival and differentiation, or proliferation, and transformation. Thus, by positively regulating IGF1R expression and consequent VSMCs proliferation, TRAIL could have a key role in promoting vascular disorders. To verify these in vitro findings, Chan et al. [2010] explored whether TRAIL could induce VSMCs proliferation in vivo. They induced the femoral artery lesion in TRAIL-deficient mice and demonstrated the absence of intimal hyperplasia or proliferation in these mice compared to wild-type mice. The proof of VSMCs proliferation was obtained when the administration of human TRAIL in wounded arteries of TRAIL-deficient mice was able to rescue neo intima proliferation. In particular, they observed that fibroblast growth factor-2 (FGF-2), released after intimal damage, could induce up regulation of TRAIL expression by stimulating Sp1 phosphorylation and interaction of phosphorylated Sp1 with NF-κB at the human TRAIL promoter. These findings are in contrast with the commonly accepted view of TRAIL as a pro-apoptotic molecule and add new insight in the comprehension of the signaling pathways recruited by TRAIL to induce vascular cell death or survival in CVD and other vascular disorders.

TRAIL AND CORONARY ARTERY DISEASE

TRAIL in its soluble form is detected at concentrations of 10–100 pg/ml in the serum/plasma. The soluble form of TRAIL has regulatory activity on ECs and, more generally, on the vascular system; moreover, it has been shown that receptors for the soluble form of TRAIL are also expressed in human placenta. Interestingly, it has been reported that a significant postpartum decrease in circulating TRAIL concentration [Zauli et al., 2011] is similar to the reduction in concentration observed in coronary artery disease [Secchiero et al., 2010]. Of note, low TRAIL levels at the patient discharge after Acute Myocardial Infarction (AMI) were related to an increased incidence of heart failure or cardiac death in the 12-month follow-up [Secchiero et al., 2006]. Furthermore, the reduction in serum TRAIL levels detectable within 24 hours after AMI were inversely related with prognostic biomarkers of cardiovascular accidents usually measured in serum after acute coronary syndrome, like circulating levels of Creatinine Kinase (CK), Creatinine Kinase – Muscle Brain

(CK-MB) and Brain Natriuretic Peptide (BNP) [Secchiero et al., 2009]. However, the very small sample size analyzed was a limitation in this study and did not allow the authors to support the prognostic meaning of TRAIL as a further biomarker in AMI prognosis. The reduction of serum or plasma levels of TRAIL could result in a worse outcome of AMI through a number of mechanisms. For instance, in vitro studies have shown that soluble recombinant TRAIL (rTRAIL) can cause apoptotic cell death of neutrophils [Lum et al., 2005]. Because neutrophils, releasing proteases in tissues damaged by ischemic events, have been demonstrated to have a fundamental role in the necrosis after AMI [Takahashi et al., 2007], it has been supposed that the dramatic decrease of TRAIL serum levels after AMI could cause a temporary alteration of neutrophil clearance activity.

Our research group has demonstrated that the anti-inflammatory activity exerted by TRAIL on endothelium is related to nitric oxide production by ECs [Di Pietro et al., 2006]. Other authors have shown that serum TRAIL has an anti-inflammatory role in CVD and that the increase in serum TRAIL levels has a protective role against graft versus host disease (GVHD), after bone marrow transplantation, in endothelial cell damage, in micro vascular inflammation, and in precocious obstruction of capillary bed [Secchiero et al., 2009]. In opposite way to what happens for the markers of inflammation and tissue damage that have been usually measured in serum of patients with AMI, the down regulation of TRAIL can be seen as the bearer of further prognostic information independent of markers already known, and can be used in current clinical practice like an outcome predictor index.

It has been shown that activated $CD4^+$ T cells expressing TRAIL are augmented in the blood of patients with Acute Coronary Artery Syndrome (ACAS) and induced endothelial damage, which could determine the atherosclerotic plaque rupture. Precocious treatment with rosuvastatin, fluvastatin or pitavastatin could suppress T cell-mediated ECs inflammatory lesions in atherosclerotic lesions and, accordingly, could preserve from cardiovascular ischemic disease, like Chronic Obstructive Coronary Artery Disease (COCAD) [Sato et al., 2010]. It is widely known that the TRAIL receptor DR5 is up regulated in ECs. Anti TRAIL specific antibodies block endothelial apoptosis mediated by $CD4^+$ T cells, suggesting that the TRAIL pathway is involved in T cell-mediated ECs apoptosis. Moreover, both the activating antigen CD69 and TRAIL have been found increased in T cells in ACAS and COCAD. It is worth noting, however, that most of the available studies, performed in standard culture conditions, have shown that soluble rTRAIL causes the activation of intracellular signaling pathways, i.e. ERK/MAPK, Akt and NF-κB, which induce and lead to the survival and/or proliferation of ECs and VSMCs [Secchiero et al., 2004; Secchiero et al., 2009].

It has also been shown how metalloproteinase inhibitors can improve biological activity of soluble TRAIL [Zauli et al., 2003], so it would be plausible and very interesting for further researches to investigate whether soluble TRAIL can be also cleaved by metalloproteases. Soluble TRAIL was also inversely related with ACAS and COCAD and was ascribed a protective effect with a markedly reduced mortality in patients with high levels of this biomarker [Niessner et al., 2003]. On the other hand, high levels of circulating TRAIL have been found in severe chronic Heart Failure (HF) patients [Levine et al., 1990].

It has been demonstrated that TRAIL increases endothelial nitric oxide synthase (eNOS) phosphorylation, NOS activity and NO synthesis in cultured HUVECs without inducing apoptotic cell death. Although an important factor that regulates eNOS activity is its localization within the cells, little information has been gained about the role of TRAIL in the regulation of eNOS trafficking among cellular compartments and the cytoskeleton involvement in this mechanism. Interestingly, our own research group has demonstrated that TRAIL is able to affect NO production by regulating eNOS sub-cellular distribution through modifications of the cytoskeleton and the Golgi complex in cultured HUVECs [Di Pietro et al., 2006].

TRAIL AND DIABETES

Recent studies have suggested that the anti-inflammatory and immunomodulatory activity of endogenous TRAIL might counteract the severity of type I diabetes. In fact, the administration of rTRAIL in streptozotocin (STZ)-treated animals developing type I diabetes is able to preserve, at least in part, the morphology and function of pancreatic islets [Zauli et al., 2010]. Furthermore, it appears that the blood concentration of TRAIL is lower in patients affected by CVD [Schoppet et al., 2006], and particularly in those who are also affected by diabetes [Corallini et al., 2007]. Consistently, TRAIL deficiency has been correlated with autoimmune diabetes in mice models and the antagonization of TRAIL signaling in non-obese diabetic (NOD) mice or STZ-treated $TRAIL^{-/-}$ mice increased the onset of diabetes compared with the control group [Lamhamedi-Cherradi et al., 2003]. On the other hand, TRAIL seems to be involved in the vascular alterations that occur in the advanced state of the illness [Vaccarezza et al., 2007]. With respect to cultured human coronary arteries endothelial cells (HCAECs) a differential regulation of death receptors has been demonstrated under high glucose conditions [Allison et al., 2005]. In particular, gene and surface protein expression levels of TNF-R1 and Fas were highly up regulated in HCAECs whereas no significant up regulation of DR4 and DR5 was found [Allison et al., 2005]. Therefore, TRAIL could have a key role as a protective factor in atherosclerosis and diabetes although the full regulatory mechanisms are still to be clarified.

CONCLUDING REMARKS

Further clinical studies are needed to evaluate the role of TRAIL in the diagnosis of obstructive coronary artery disease, in osteoclast differentiation and, consequently, in the ability to determine modifications in metabolism of calcium and, therefore, in the progression of the process of calcification of the great vessels and of epicardial coronary arteries related to both normal shear stress, which these vases are daily subjected to, and in pathological conditions such as in the case of high blood levels of glucose. There are missing data on the response of TRAIL endothelium subjected to biomechanical stress inversion of flow in both healthy subjects and in subjects with high blood levels of circulating glucose. Moreover, further investigations are needed to define the mechanism of aortic valve calcification in order to prevent and/or to delay the onset of this disease. We hope that in the near future

we will give our contribution to gain new insight into this interesting matter.

REFERENCES

Aird WC. 2005. Spatial and temporal dynamics of the endothelium. J Thromb Haemost 3:1392–1406.

Aird WC. 2007. Phenotypic heterogeneity of the endothelium: structure, function, and mechanisms. Circ Res 100:158–173.

Aitsebaomo J, Portbury AL, Schisler JC, Patterson C. 2008. Brothers and sisters: molecular insights into arterial-venous heterogeneity. Circ Res 103:929–939.

Alladina SJ, Song JH, Davidge ST, Hao C, Easton AS. 2005. TRAIL-induced apoptosis in human vascular endothelium is regulated by phosphatidylinositol 3-kinase/Akt through the short form of cellular FLIP and Bcl-2. J Vasc Res 42(4):337–347.

Allison J, Thomas HE, Catterall T, Kay TW, Strasser A. 2005. Transgenic expression of dominant-negative Fas-associated death domain protein in β cells protects against fas ligand-induced Apoptosis and reduces spontaneous diabetes in nonobese diabetic mice. J Immunol 175:293–301.

Atkins GB, Jain KM, Hamik A. 2011. Endothelial differentiation: molecular mechanisms of specification and heterogeneity. Arterioscler Thromb Vasc Biol 31:1476–1484.

Bucay N, Sarosi I, Dunstan CR, Morony S, Tarpley J, Capparelli C, Scully S, Tan HL, Xu W, Lacey DL, Boyle WJ, Simonet WS. 1998. Osteoprotegerin-deficient mice develop early onset osteoporosis and arterial calcification. Genes Dev 12:1260–1268.

Chan J, Prado-Lourenco L, Khachigian LM, Bennett MR, Di Bartolo BA, Kavurma MM. 2010. TRAIL promotes VSMC proliferation and neointima formation in a FGF-2-, Sp1 Phosphorylation-, and Nfkappab-dependent manner. Circ Res 106:1061–1071.

Chien S. 2008. Effects of disturbed flow on endothelial cells. Ann Biomed Eng 36:554–562.

Conway DE, Sakurai Y, Weiss D, Vega JD, Taylor WR, Jo H, Eskin SG, Marcus CB, McIntire LV. 2009. Expression of CYP1A1 and CYP1B1 in human endothelial cells: regulation by Fluid shear stress. Cardiovasc Res 81:669–677.

Conway DE, Williams MR, Eskin SG, McIntire LV. 2010. Endothelial cell responses to atheroprone flow are driven by two separate flow components: low time-average shear stress and fluid flow reversal. Am J Physiol Heart Circ Physiol 298(2):H367–H374.

Corallini F, Celeghini C, Rizzardi C, Pandolfi A, Di Silvestre S, Vaccarezza M, Zauli G. 2007. Insulin downregulates TRAIL expression in vascular smooth muscle cells both in vivo and in vitro. J Cell Physiol 212:89–95.

De Caterina R, Madonna R. 2009. Cytochromes CYP1A1 and CYP1 B1: new pieces in the puzzle to understand the biomechanical paradigm of atherosclerosis. Cardiovas Res 81:629–632.

De Caterina R, Zampolli A, Del Turco S, Libby P. 2007. Mechanisms of plaque progression and complications. In: De Caterina R, Libby P, editors. Endothelial dysfunctions and vascular disease. Singapore: Blackwell Futura. 629–632.

Di Bartolo BA, Chan J, Bennett MR, Cartland S, Bao S, Tuch BE, Kavurma MM. 2011. TNF-related apoptosis-inducing ligand (TRAIL) protects against diabetes and atherosclerosis in Apoe−/− mice. Diabetologia 54:3157–3167.

Di Pietro R 2011. Signalling pathways leading to TRAL resistance. In: Advances in cancer therapy, Book 3, ISBN 979-953-307-209-7, Eds. Hala Gali-Muhtasib. 201–226.

Di Pietro R, Mariggiò MA, Guarnieri S, Sancilio S, Giardinelli A, Di Silvestre S, Consoli A, Zauli G, Pandolfi A. 2006. Tumor necrosis factor-related apoptosis-inducing ligand (TRAIL) regulates endothelial nitric oxide synthase (Enos) Activity and its localization within the human vein endothelial cells (HUVEC) in culture. J Cell Biochem 97:782–794.

Di Pietro R, Zauli G. 2004. Emerging non-apoptotic functions of tumor necrosis factor-related apoptosis inducing ligand (TRAIL)/Apo2L. J Cell Physiol 201(3):331–340.

Kavurma MM, Schoppet M, Bobryshev YV, Khachigian LM, Bennett MR. 2008. Trail stimulates proliferation of vascular smooth muscle cells via activation of NF-Kappa B and induction of insulin-like growth factor-1 receptor. J Biol Chem 283(12):7754–7762.

Kelly JJ, Moore TM, Babal P, Diwan AH, Stevens T, Thompson WJ. 1998. PUlmonary microvascular and macrovascular endothelial cells: differential regulation of Ca^{2+} and permeability. Am J Physiol 274:L810–L819.

Lacorre DA, Baekkevold ES, Garrido I, Brandtzaeg P, Haraldsen G, Amalric F, Girard JP. 2004. Plasticity of endothelial cells: rapid dedifferentiation of freshly isolated high endothelial venule endothelial cells outside the lymphoid tissue microenvironment. Blood 103:4164–4172.

Lamhamedi-Cherradi SE, Zheng SJ, Maguschak KA, Peschon J, Chen YH. 2003. Defective thymocyte apoptosis and accelerated autoimmune diseases in TRAIL−/− mice. Nat Immunol 4:255–260.

Levine B1, Kalman J, Mayer L, Fillit HM, Packer M. 1990. Elevated circulating levels of tumor necrosis factor in severe chronic heart failure. N Engl J Med. 323(4):236–241.

Li X, Han WQ, Boini KM, Xia M, Zhang Y, Li PL. 2013. TRAIL death receptor 4 signaling via Lysosome fusion and membrane raft clustering in coronary arterial endothelial cells: evidence from asm knockout mice. J Mol Med (Berl) 91(1):25–36.

Libby P. 2002. Inflammation in atherosclerosis. Nature 420:868–874.

Liu FF, Wu X, Zhang Y, Wang Y, Jiang F. 2014. TRAIL/DR5 signaling promotes macrophage foam cell formation by modulating scavenger receptor expression. PLoS One 9(1):e87059.

Lum JJ, Bren G, McClure R, Badley AD. 2005. Elimination of senescent neutrophils by tnf-related apopotosis-inducing ligand. J Immunol 175:1232–1238.

Michowitz Y, Goldstein E, Roth A, Afek A, Abashidze A, Ben Gal Y, Keren G, George J. 2005. The involvement of tumor necrosis factor-related apoptosis-inducing ligand (TRAIL) in atherosclerosis. J Am Coll Cardiol 45(7):1018–1024.

Niessner A, Hohensinner PJ, Rychli K, Neuhold S, Zorn G, Richter B, Hulsmann M, Berger R, Ohura N, Yamamoto K, Ichioka S, Sokabe T, Nakatsuka H, Baba A, Shibata M, Nakatsuka T, Harii K, Wada Y, Kohro T, Kodama T, Ando J. 2003. Global analysis of shear stress-responsive genes in vascular endothelial cells. J Atheroscler Thromb 10:304–313.

Ohura N, Yamamoto K, Ichioka S, Sokabe T, Naktsuka H, Baba A, Shibata M, Nakatsuka T, Harii K, Wada Y, Kohro T, Kodama T, Ando J. 2003. Global analysis of shear stress-responsive genes in vascular endothelial cells. J Atheroscler Thromb 10:304–313.

Papadopouli AE, Klonaris CN, Theocharis SE. 2008. Role of OPG/RANKL/RANK axis on the vasculature. Histol Histopathol 23:497–506.

Porreca E, Di Febbo C, Di Castelnuovo A, Baccante G, Amore C, Angelini A, Di Nisio M, Donati MB, Cuccurullo F, Iacoviello L. 2002. Association of factor VII Levels with inflammatory parameters in hypercholesterolemic patients. Atherosclerosis 165:159–166.

Sandoo A, Veldhuijzen van Zanten JJCS, Metsios GS, Carroll D, Kitas GD. 2010. The endothelium and its role in regulating vascular tone. Open Cardiovasc Med J 4:302–312.

Sato K, Niessner A, Kopecky SL, Frye RL, Goronzy JJ, Weyand CM. 2006. TRAIL-expressing T cells induce apoptosis of vascular smooth muscle cells in the atherosclerotic plaque. J Exp Med 203:239–250.

Sato K, Nuki T, Gomita K, Weyand CM, Hagiwara N. 2010. Statins reduce endothelial cell apoptosis via inhibition of TRAIL expression on activated CD4 T cells in acute coronary syndrome. Atherosclerosis 213(1):33–39.

Secchiero P, Candido R, Corallini F, Zacchigna S. 2006. Systemic tumor necrosis factor-related apoptosis-inducing ligand delivery shows antiatherosclerotic activity in apolipoprotein E-null diabetic mice. Circulation 114:1522–1530.

Secchiero P, Corallini F, Ceconi C, Parrinello G, Volpato S, Ferrari R, Zauli G. 2009. Potential prognostic significance of decreased serum levels of TRAIL after acute myocardial infarction. PlosOne 4(2):1–6.

Secchiero P, Gonelli A, Corallini F, Ceconi C, Ferrari R. 2010. Metalloproteinase 2 cleaves in vitro recombinant TRAIL: potential implications for the decreased serum levels of TRAIL after acute myocardial infarction. Atherosclerosis 211:333–336.

Secchiero P, Zerbinati C, Rimondi E, Corallini F, Milani D. 2004. TRAIL promotes the survival, migration and proliferation of vascular smooth muscle cells. Cell Mol Life Sci 61:1965–1974.

Schoppet M, Sattler AM, Schaefer JR, Hofbauer LC. 2006. Osteoprotegerin (OPG) And Tumor Necrosis Factor-Related Apoptosis-Inducing Ligand (TRAIL) Levels In Atherosclerosis. Atherosclerosis 184:446–447.

Stemme S, Faber B, Holm J, Wiklund O, Witztum Jl, Hansson Gk. 1995. T lymphocytes from human atherosclerotic plaques recognize oxidized low-density lipoprotein. Proc Natl Acad Sci USA 92:3893–3897.

Takahashi T, Hiasa Y, Ohara Y, Miyazaki S, Ogura R. 2007. Relation between neutrophil counts on admission, microvascular injury, and left ventricular functional recovery in patients with an anterior wall first acute myocardial infarction treated with primary coronary angioplasty. Am J Cardiol 100:35–40.

Vaccarezza M, Delbello G, Zauli G. 2007. A role of the TRAIL-TRAIL receptor system in the pathogenesis of diabetes. Acta Biomed 78(Suppl.1):262–267.

Van Campenhout A, Golledge J. 2009. Osteoprotegerin, vascular calcification and atherosclerosis. Atherosclerosis 204:321–329.

Won D, Zhu S-N, Chen M, Teichert A-M, Fish JE, Matouk CC, Bonert M, Ojha M, Marsden PA, Cybulsky MI. 2007. Relative reduction of endothelial nitric-oxide synthase expression and transcription in atherosclerosis-prone regions of the mouse aorta and in an in vitro model of disturbed flow. Am J Pathol 171(5):1691–1704.

Yee A, Bosworth KA, Conway DE, Eskin SG, McIntire LV. 2008. Gene expression of endothelial cells under pulsatile non-reversing vs. steady shear stress; comparison of nitric oxide production. Ann Biomed Eng 36:571–579.

Zauli G, Monasta L, Rimondi E, Vecchi Brumatti L, Radillo O, Rofani L, Montico M, D′Ottavio G, Alberico S, Secchiero P. 2011. Circulating TRAIL shows a significant post-partum decline associated to stressful conditions. PlosOne 6(12):e27011.

Zauli G, Pandolfi A, Gonelli A, Di Pietro R, Guarnieri S, Ciabattoni G, Rana R, Vitale M, Secchiero P. 2003. Tumor necrosis factor - related apoptosis - inducing ligand (TRAIL) sequentially upregulates nitric oxide and prostanoid production in primary human endothelial cells. Circ Res 92:732–740.

Zauli G, Sancilio S, Cataldi A, Sabatini N, Bosco D, Di Pietro R. 2005. PI-3K/Akt and NF-kB/IkBa pathways are activated in Jurkat T cells in response to TRAIL treatment. J Cell Physiol 201(3):900–911

Zauli G, Toffoli B, di Iasio MG, Celeghini C, Fabris B, Secchiero P. 2010. Treatment with recombinant tumor necrosis factor-related apoptosis-inducing ligand alleviates the severity of streptozotocin-induced diabetes. Diabetes 59(5):1261–1265.