

A role of the TRAIL-TRAIL receptor system in the pathogenesis of diabetes

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Abstract. The TNF- α super-family of cytokines comprises structurally related proteins that play pivotal roles in regulating cell death, immune response and inflammation. A new member of the family namely Tumor necrosis factor alpha-Related Apoptosis-Inducing Ligand (TRAIL) is involved not only in apoptosis and immune regulation, but also it has a provocative role in vascular biology as reported recently. In this report we provide evidence that this new function of TRAIL may have a significance in the pathogenesis of diabetes and in particular in the vascular alterations that occur late during the natural history of the illness. Noteworthy, depending on the type of diabetes and on the disease stage, TRAIL can have a dual role, either as immune modulator as well as a regulatory molecule of the vascular wall fitness. (www.actabiomedica.it)

Key words: TRAIL, diabetes, HUVEC, streptozotocin, NO

Introduction

Over the last decade, major changes in the quality, quantity and source of food consumed in many countries, combined with a decrease in levels of physical activity among the population, have led to an increase in the prevalence of diabetes and its complications. Overweight and obesity, the main drivers of type 2 diabetes, affect worldwide around 1 billion people (1). Considering only diabetes with overt clinical presentation, over 200000 people are newly affected in USA every year and the percentage of the world population affected is 5% (1, 2). Previews on the future incidence of the disease underline a major boost of glucose intolerance and diabetes in Western countries as well as in recently developed or developing Third World countries (1). This alarming picture notwithstanding, recent progress has improved our knowledge of pathophysiology of this complex disease. This new set of data could be useful to design new therapeutic avenues. In this review we will focus on a potential

new player in the pathogenesis of diabetes, namely Tumor necrosis factor alpha-Related Apoptosis-Inducing Ligand (TRAIL), a member of the Tumor Necrosis Factor (TNF)-alpha (α) family of cytokines (3).

Links between TNF- α and diabetes

The TNF- α super-family of cytokines, which comprises structurally related proteins that play important roles in regulating cell death, immune response and inflammation, is represented by the first cytokine of the family discovered: TNF- α . A role of TNF- α in the pathogenesis of diabetes is well established (4-7). In this regard, in type I diabetes, TNF- α acts as a pro-apoptotic and pro-necrotic factor produced by T cells and macrophages in the immune response against the beta cell (4-6). Regarding the role of TNF- α in type II diabetes and more generally in the late complications of the disease, several lines of evidence demonstrate an important signalling pathway

by TNF- α that increases insulin resistance in several tissues, including muscle and adipocytes (6, 7).

TRAIL, a new member of the TNF super-family of cytokines

A role for the molecule TRAIL in diabetes is still not well delineated but it is coming of age. TRAIL (3, 8), also known as APO-2L (9) like other members of the TNF family, is a type II membrane protein, having an intracellular amino-terminal portion, an internal transmembrane domain, and a carboxyl terminus external to the cell. Among the TNF family members, TRAIL shares the highest amino-acid identity with CD95L. In fact, both TRAIL and CD95L exist as full-length membrane-bound molecules and as shorter soluble forms (9). The biological effects induced by TRAIL are mediated by interactions with cell surface TRAIL receptors.

Several studies have demonstrated an extreme complexity of the expression and function of TRAIL receptors. At least five TRAIL receptors belonging to the apoptosis-inducing TNF-receptor (R) family have been described so far in humans. TRAIL-R1 (DR4) and TRAIL-R2 (DR5) transduce apoptotic signals upon binding of TRAIL, while TRAIL-R3 (DcR1) and TRAIL-R4 (DcR2), as well as osteoprotegerin (OPG), are homologous to DR4 and DR5 in their cysteine-rich extracellular domain but lack intracellular death domain and apoptosis inducing capability (9). OPG exists apparently only in a soluble form as decoy receptor for its legends (TRAIL itself and RANKL, another member of the family principally involved in bone resumption, ref. 10).

At first, TRAIL-R3 and TRAIL-R4 have been proposed to function as decoy receptors, protecting normal cells from apoptosis. Hence, it has been shown that expression of TRAIL-R1 and/or TRAIL-R2 are necessary but not always sufficient to mediate TRAIL-induced apoptosis, while expression of TRAIL-R3 and/or TRAIL-R4 often does not correlate with the resistance or sensitivity of tumour target cells to the effects of TRAIL (11). TRAIL, show the remarkable ability to induce growth arrest and apoptosis independently of p53 wild-type function, Bcl-2

and Bcl-xL and MDR gene expression (11,12). With respect to other members of the TNF super-family, such as CD95L and TNF- α , the unique feature of TRAIL is its ability to exert anticancer activity with absent or low toxicity toward most normal tissues. Indeed, severe toxic side effects preclude both TNF- α and CD95L from use in systemic anticancer therapy (12). On the other hand, TRAIL is effective in selectively killing both in vitro and in vivo a vast array of tumour cells from lung, breast, kidney, colon, prostate, thyroid, and skin cancers (11, 12), while causing no or minimal organ toxicity and inflammation in vivo.

To dissect in more detail TRAIL function TRAIL knock-out (TRAIL^{-/-}) mice and TRAIL R-knock out (TRAIL-R^{-/-}) mice were created. Despite the limitations of the model (mice express only one receptor of TRAIL, the counterpart of the human R1 and R2), the most striking findings include a general negative regulation of innate as well adaptive immune response, increased autoimmune and tumour susceptibility and an organ-specific resistance to irradiation induced DNA-damage (13-17). TRAIL acts downstream on different effector molecules, among them nuclear factor-kappaB (NF-kB) is of particular interest. The NF-kB/Rel family of proteins are composed of a group of dimeric transcription factors that have an outstanding role in the regulation of inflammation and immunity. Control of transcription by NF-kB proteins can be of relevance to the function of TRAIL in three ways. First, induction of anti-apoptotic NF-kB dependent genes critically determines cellular susceptibility toward apoptosis induction by TRAIL-R1, TRAIL-R2, and other death receptors. Each of the multiple of known NF-kB inducers therefore has the potential to interfere with TRAIL-induced cell death. Second, TRAIL and some of its receptors are inducible by NF-kB, disclosing the possibility of autoamplifying TRAIL signalling loops. Third, the TRAIL death receptors can activate the NF-kB pathway (3, 18).

Moreover, we recently proposed a provocative, non-apoptotic role for TRAIL (19-21). Of note, human umbilical venous endothelial cells (HUVEC) cultured with recombinant TRAIL are not killed by apoptosis, but instead they display a survival and growth advantage versus normally plated HUVEC (19, 20). HUVEC express TRAIL receptors (TRAIL-R1 and

TRAIL-R2 as well as decoy receptors TRAIL-R3 e TRAIL-R4); TRAIL exerts its positive action on survival and growth by the Akt and ERK1/2 pathways (19). The same group shows that TRAIL acts in a similar way on cultured vascular smooth muscle cells (VSMC); in particular rat VSMC express TRAIL-R1 and TRAIL-R2 (as their human counterparts) and TRAIL activates in culture mainly the ERK1/2 pathway to promote an anti-apoptotic signal as well as a growth signal (22). Of note, TRAIL induces a weak Akt signalling in these cells and no induction of the p38/MAPK pathway was observed (22).

TRAIL and type I diabetes

The above TRAIL presentation is of obvious importance to understand the potential role of TRAIL and its receptors on physiopathology of diabetes. Type I diabetes is essentially an autoimmune disease, and TRAIL and its receptors are expressed on cell the immune system. As mentioned above (13-17), TRAIL *-/-* mice demonstrated a sustained increase of autoimmunity, uncovering a role of TRAIL as negative regulator of the immune response. Recently, two seminal papers (14, 15) have convincingly demonstrated the involvement of TRAIL in the pathogenesis of type 1 diabetes, exactly as a regulator of the immune response (not as apoptotic effector). Despite increased expression of TRAIL in pancreatic islets during the development of autoimmune type 1 diabetes in non-obese diabetic (NOD) mice, (and increased expression of TRAIL *in vitro* on islet beta cells plated with TNF- α and interferon-gamma), stimulation of TRAIL did not induced apoptosis; diabetogenic T cells were in this model blocked in their cell cycle progression and became anergic.

In vivo blockade of TRAIL induced signalling in NOD mice, or diabetic induction with multiple low dose streptozotocin (STZ) with TRAIL *-/-* mice significantly enhanced specific diabetogenic responses, accelerated diabetes, enhanced islet inflammation. Animal models of autoimmune diabetes thus define without any doubt a potent immune-regulatory and protective role of TRAIL toward overt disease. Nevertheless the action of TRAIL on isolated healthy

islets (23) is weakly apoptotic; further studies (even on human tissues) are needed to better understand the role of TRAIL and its receptors in the pathophysiology of type 1 diabetes.

TRAIL and type II diabetes

Endothelial cell dysfunction is an important risk factor for diabetic atherosclerosis, whose prevalence is markedly increased among diabetic individuals (24). It has been proposed a primary role of an alteration of nitric oxide (NO) synthesis and endothelium-induced vasodilatation, which play a key role in the regulation of the vascular tone (25). In this regard we have previously demonstrated that TRAIL induces the *in vitro* release of NO by vascular endothelial cells (20). Starting from our previous data on the TRAIL-NO connection and the intriguing action of TRAIL and its receptors on HUVEC and VSMC (19-22), as well as from the evidence of TRAIL expression in the vessel wall (26), we tested whether the TRAIL system has a pathophysiologic significance in the vascular complications due to diabetes on a STZ-induced, rat model (Zauli G. et al., submitted for publication).

We analyzed not only TRAIL and TRAIL receptor, but also OPG, another member of the TNF- α family of cytokines, because it acts as a decoy receptor of TRAIL. Moreover, it has been shown that OPG is produced by a wide range of tissues, including the cardiovascular system, and that OPG levels are particularly high in aortic and renal arteries (27-29). Both endothelial cells (ECs) and vascular smooth muscle cells (VSMCs) constitutively express OPG, whose release in the culture supernatants is significantly increased *in vitro* by inflammatory cytokines, such as TNF- α (29). Other studies have reported that serum OPG is significantly increased in diabetic patients (30-31), and, in particular, in patients affected by coronary artery disease, myocardial infarction and abdominal aortic aneurysm (32-34).

In our experimental approach, while STZ-treated rats did not show any significant difference in the steady-state mRNA levels of TRAIL with respect to untreated animals, a significant decrease in the mRNA levels of TRAIL was noticed in the aortas of

SZT-treated diabetic mice, which received insulin for 10 days with respect with both vehicle-treated control and SZT-treated animals. The decrease of TRAIL expression evaluated by quantitative RT-PCR was confirmed at the protein level by Western blot and immuno-histochemical analyses (Zauli G. et al., submitted for publication).

In the aortic samples obtained from the three groups of animals the OPG mRNA steady-state levels were significantly increased in SZT-treated diabetic rats with respect to both controls and diabetic animals treated with insulin. On the other hand, no statistically significant differences were noticed among control animals and diabetic animals treated with insulin. Analysis of OPG at the protein level confirmed the mRNA data. Thus, while SZT-induced diabetes significantly up-regulated OPG but not TRAIL expression in the aortic wall, treatment of rats with insulin down-regulated simultaneously TRAIL and OPG expression. However, it is important to point out that the OPG/TRAIL ratio was markedly increased in diabetic animals with respect to control animals. On the other hand, the OPG/TRAIL ratio was similar in diabetic animals treated or not with insulin.

Starting from the observation that TRAIL and OPG are predominantly expressed in the tunica media of both human and rat vessels we also probed insulin *in vitro* with VSMC to monitor TRAIL expression. Our data on trans-membrane TRAIL showed its clear expression at the surface level in VSMCs and the addition in culture of insulin induced a clear-cut decrease of surface TRAIL expression. These effects were specific for TRAIL, since no significant modulations of TRAIL-R1 and TRAIL-R2 were noticed in VSMCs treated with insulin. On the other hand, in our experiments, high glucose concentrations did not show any significant effect on TRAIL surface expression (Zauli G. et al., submitted for publication).

Our results on vascular reactivity by meaning of experiments on isolated rat thoracic aortic rings showed that increasing concentrations of TRAIL induce a significant vasorelaxation. L-NAME pre-exposure of aortic rings completely abolished TRAIL-induced relaxation, clearly indicating that the NO synthase pathway played a key role. Aortic rings from STZ-induced diabetic rats were insensitive to the

myo-relaxing action of TRAIL, irrespective of the treatment *in vivo* with insulin.

The role of VSMCs on this phenomenon was assessed probing VSMCs with recombinant TRAIL: an increase in NO synthase activity and in cGMP activity (a bioactive NO derivative) were registered in the culture supernatants. L-NAME completely blocked NO synthase activity on VSMCs *in vitro* in this set of experiments. Thus, TRAIL likely induce myo-relaxation through a VSMC-specific NO synthase pathway.

Our results have several important insights regarding diabetic dependent-macroangiopathy and microangiopathy. Firstly, the imbalance of OPG versus TRAIL expression in the aortic wall of diabetic rats is particularly noteworthy in light of the fact that TRAIL promotes myo-relaxation activity when added to rat aortic rings and that OPG is a potent soluble decoy receptor able to neutralize the biological activity of TRAIL. Secondly, the mio-relaxing activity is particularly noteworthy also in light of the fact that TNF- α , the prototype member of the TNF super-family of cytokines, impairs the endothelium-dependent vasodilatation *in vivo* (35). The loss of mio-relaxation activity TRAIL induced is a key feature of endothelial dysfunction, which invariably precedes permanent vascular alterations (32).

The experimental data obtained underline an important role of the TRAIL-TRAIL receptor system as an endogenous regulator of the vascular tone. The ability of insulin to down-regulate TRAIL expression in rat aortas *in vivo*, as well as in human VSMCs *in vitro* is particularly noteworthy, identifying TRAIL down-regulation as a new pathway of diabetic vasculopathy.

A possible mechanism able to explain our findings and our working hypothesis is the pivotal role of the transcription factor Egr-1, that is up-regulated by both hyperglycaemia and insulin as reported (36); in turn, over-expression of Egr-1 down regulates TRAIL expression in vascular endothelial cells (37). Egr-1 expression is additively increased by glucose and insulin, and interestingly vascular endothelial growth factor receptor-1 (flt-1) and plasminogen activator inhibitor-1, two known Egr-1 responsive genes, are increased as well in presence of glucose and insulin. Egr-1-mediated events collectively have a pronounced atherogenic phenotype, and its influence on TRAIL and

vaso-relaxation has further significance. Egr-1 up regulation, which is frequently observed in atherosclerotic lesions (38) is likely to be involved in insulin-mediated TRAIL down-regulation.

Concluding remarks

Our model (Figure 1) implies TRAIL as an important player in a more advanced diabetic state, after islet destruction or exhaustion and hyperglycaemia stabilisation. Vascular dysfunction associates with persistent hyperglycaemia followed by insulin treatment and develops over time, linking the local intravascular TRAIL pathway to the pathogenesis of the vascular damage associated with diabetes. Restoration of TRAIL expression/response could be a useful asset to improve vascular function in advanced diabetes, and it could be effective even after the process of islet cells damage. More studies on primary human cells and on human samples are needed to clarify the role of the TRAIL-TRAIL receptor system on the pathogenesis of diabetes. However, our model describes a potential role of this new protein of the TNF alpha superfamily in the pathogenic scenario of diabetes.

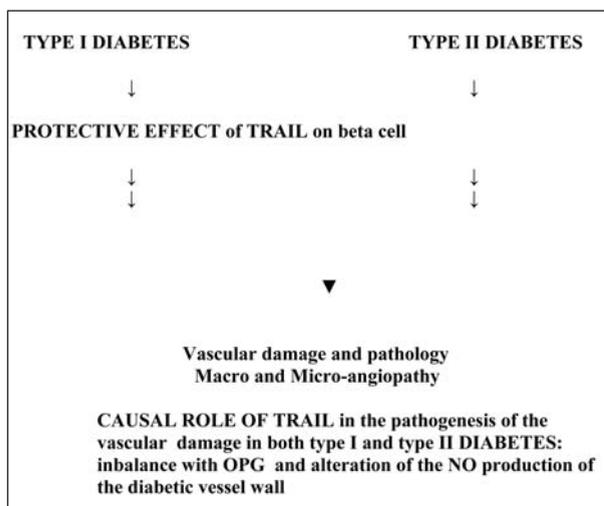


Figure 1. Diagram of the proposed role of the TRAIL-TRAIL receptor system in the pathogenesis of diabetes. Interestingly, TRAIL could have an early protective role on the onset of disease in type I diabetes, whereas it could have a modulating role of the vascular complications in both type I and type II diabetes

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