

Where ends the TRAIL in arthritis?

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Abstract

A hallmark of rheumatoid arthritis (RA) is the pseudo-tumoral expansion of fibroblast-like synoviocytes (FLS), as these cells invade and finally destroy the joint structure. RA FLS have been proposed therefore as a therapeutic target. The TNF-related apoptosis-inducing ligand (TRAIL) has gained much attention as a possible therapeutic reagent for the treatment of tumors, as TRAIL was described originally to induce apoptosis specifically in cancer cells but not in normal cells. The fact that FLS in RA patients exhibit tumor-like features led to investigations on the effect of TRAIL on *ex vivo* RA FLS. In this review we aim to summarize what is presently known on the role of TRAIL in RA.

Introduction

Rheumatoid arthritis (RA) is a systemic inflammatory disease affecting the joint lining tissue called synovium. The synovium is normally a relatively acellular structure with a delicate intimal lining that is one or two cell layers thick. The rheumatoid synovial tissue is characterized by hyperproliferation of fibroblast-like synoviocytes (FLS) in the intimal lining layer and infiltration of the sublining by macrophages, and T- and B-cells, which promote inflammation and destruction of bone and cartilage. The hyperplasia of synovial fibroblasts in RA contributes to joint destruction, directly through enhanced production of matrix-degrading enzymes, and indirectly through excessive release of proinflammatory cytokines. The proliferation of RA FLS is considered to be pseudo-tumoral, as RA FLS proliferate in an anchorage-independent manner, lack contact inhibition, and express oncogenes and cell cycle proteins indicative of transformation.¹ Besides an increased proliferation, insufficient apoptosis might contribute to the

increased numbers of synovial fibroblasts in RA joints, as apoptosis plays a crucial role in controlling cell numbers by eliminating old cells, unnecessary cells, and unhealthy cells.² Therefore one strategy to treat rheumatoid arthritis is the design of drugs that can restore the normal apoptotic pathways in synovial fibroblasts.²

Ligands of the tumor necrosis factor (TNF) family are trimeric cytokines that have an important role in inducing various biological responses such as cell proliferation, differentiation, survival, and apoptosis.³ TNF-like ligands capable of inducing apoptosis by binding to their cognate receptors, so-called death receptors, include TNF itself, Fas (CD95) ligand, and the TNF-related apoptosis-inducing ligand (TRAIL). The death receptors of the TNF family are potential targets for inducing apoptosis in malignant cells. In recent years, considerable attention has been focused on the potential benefits of TRAIL in cancer therapy, as a broad range of cancer cells are sensitive to TRAIL-induced apoptosis.⁴ In addition, the use of TRAIL in combination with chemotherapeutic agents or irradiation strengthens its apoptotic effects and frequently sensitizes otherwise TRAIL-resistant tumor cells. Importantly, TRAIL-exposure shows no toxic side effects of therapeutically relevant doses in primates⁵ or in humans.⁶ Likewise agonistic antibodies targeting either DR4 or DR5 are well tolerated in patients.⁷⁻¹¹ The fact that FLS in RA patients exhibit tumor-like features led to investigations on the effect of TRAIL in RA. In this review we aim to summarize what is presently known about the role of TRAIL in RA.

TRAIL in general

TRAIL is a type II membrane ligand that belongs to the TNF super-family, and is mainly known to its ability to trigger cell death in a somewhat tumor selective manner.¹² Like most TNF-related ligands however, TRAIL has been demonstrated to exert pleiotropic functions including abilities to trigger cell proliferation or differentiation.^{13,14} Apoptosis triggering by TRAIL involves principally two receptors, DR4 and DR5 also known as TRAIL-R1 and TRAIL-R2, which upon binding to their cognate ligand engage the recruitment of the adaptor protein FADD and initiator caspases (caspase-8 and/or -10) via homotypic interactions through the death-domain and the death-effector-domains, respectively, leading to the formation of the so-called DISC (death inducing signaling complex).¹⁵ Notably, the TRAIL ligand/receptor system differs between mouse and humans.^{16,17} For example, only one TRAIL receptor containing a death-domain has been identified in the mouse.

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In type I cells, DISC formation and caspase-8 activation generally is sufficient to promote caspase-3 processing and apoptosis triggering. In type II cells, DISC formation and caspase-8 activation are weaker compared to type I cells, and full caspase-3 activation occurs through the mitochondrial amplification loop via Bid cleavage.¹⁸ Efficient DISC engagement is the limiting factor that defines caspase-8 activation and thus cellular dependency to type I or II signaling pathways. Accordingly, it has been demonstrated that the mitochondrial requirement in some type II cells could be overcome by increasing the concentrations of TRAIL.¹⁹ Likewise, negative regulation of TRAIL DISC-induced formation occurs in cells that express DcR1 or DcR2, also coined TRAIL-R3 and TRAIL-R4.²⁰ DcR1 and DcR2 are devoid of a functional death-domain and impair TRAIL-induced cell death either directly by competing with their cognate ligand, or through regulatory inhibitory activity leading to reduced caspase-8 processing within the TRAIL DISC.^{20,21} Similar to DcR1, the soluble receptor OPG, albeit exhibiting the lowest affinity for TRAIL among the TRAIL receptors, is able to compete for TRAIL binding and thus impair TRAIL-induced signaling.^{22,23}

The TRAIL system is probably one of the most complex members of the TNF family, owing to the large number of receptors to which TRAIL can bind and to the signaling

pathways that are engaged. DR4 and DR5 are both capable of triggering apoptosis and are the receptors that exhibit the highest affinity for TRAIL.²⁴ The existence of two apparently redundant receptors may have physiological meaning and it is thought that they could play distinct roles with respect to the control of tissue homeostasis or activation of secondary signaling pathways. Accordingly, while most cancer cell lines engage either DR4 or DR5 to trigger TRAIL-induced apoptosis,²⁵ chronic B-cell leukemia cells engage DR4 preferentially despite co-expressing DR5,²⁶ and some colon carcinoma cell lines preferentially engage DR5.²⁷ The reasons for these specific engagements are not clear. Recently DR4- and DR5-mediated apoptosis were shown to be controlled by O-glycosylation,²⁸ a finding that could help explain the specific engagement or the importance of each agonistic receptor with respect to TRAIL-induced signaling pathways. Indeed, some DR5 "specific" TRAIL mutants revealed that the JNK pathway is preferentially activated by DR5.²⁹

Besides the TRAIL membrane-bound complex I or DISC, TRAIL was recently demonstrated to initiate the formation of a secondary complex, which contrarily to TNF,³⁰ is thought to be required to trigger nonapoptotic signaling activities including the activation of the NF- κ B, JNK, or p38 pathways.³¹ TRAIL has been described to induce cell proliferation or cell survival in many cell types including vascular smooth muscle cells, synovial fibroblasts, vascular endothelial cells, or cancer cells,^{32,38} as well as to induce intestinal, skeletal myoblast, or osteoclast differentiation.^{13,14,39} Animal models in which TRAIL expression has been inactivated demonstrated that TRAIL plays a crucial role in immune tumor surveillance,⁴⁰ and that it could contribute to the control of some autoimmune diseases^{41,42} including rheumatoid arthritis.

TRAIL in rheumatoid arthritis

The first report linking TRAIL with arthritis came from a mouse study using the collagen-induced arthritis (CIA) model.^{41,43} In this model susceptible mouse strains with type II collagen (CII) from heterologous species lead to a pathology that resembles human RA called collagen-induced arthritis (CIA)⁴⁴ including foot-pad swelling, synovitis, pannus formation, and bone and cartilage destruction of paw joints. Both CII antibodies and CD4⁺ T-cells are required for the development of CIA, and the classical mouse strain used for the CIA model is DBA/1 mice. Using the CIA model Song and co-workers observed that blockade of TRAIL in mice using a soluble recombinant receptor exacerbated autoimmune arthritis, whereas

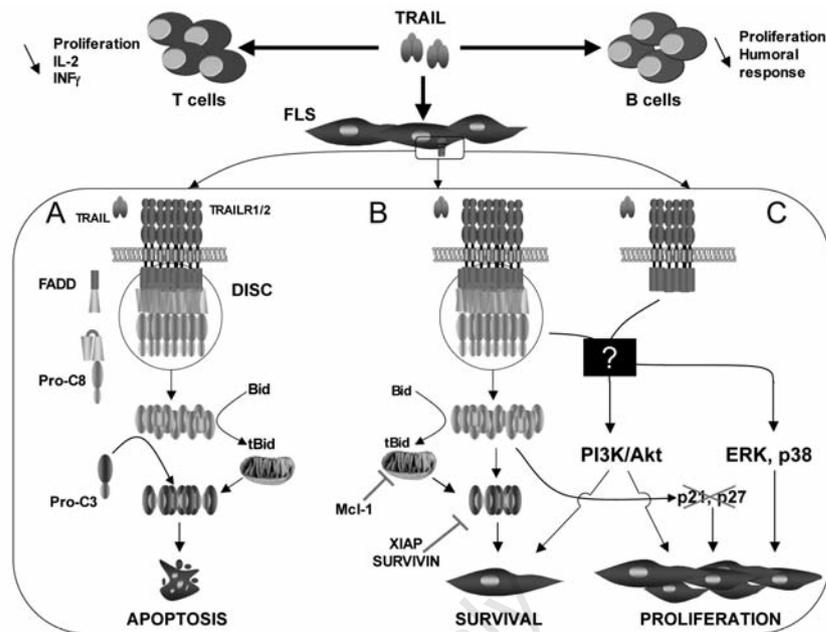


Figure 1. The complex role of TRAIL in rheumatoid arthritis. TRAIL appears to control T- and B-cell responses. On human synovial fibroblasts (FLS), TRAIL acts as a pleiotropic cytokine: (A) TRAIL can trigger apoptosis by forming the DISC in FLS cells. Activated caspase-8 initiates apoptosis by direct cleavage of downstream effector caspase-3 (Pro-C3) and Bid. (B) FLS overexpressing antiapoptotic molecules such as Mcl-1 or IAPs members, XIAP or survivin for example, are resistant toward TRAIL-induced apoptosis and survive. Activation of the PI3 kinase/Akt signaling pathway mediates FLS survival as well. (C) FLS can proliferate upon TRAIL treatment by activating PI3K and MAPK pathways. Activation of caspase-8 triggers FLS proliferation by cleavage of the cell cycle inhibitors p21 and p27.

intra-articular TRAIL adenoviral gene transfer diminished the symptoms.⁴³ Blockage of TRAIL resulted in an increased proliferation of synoviocytes and intra-articular lymphocytes. Moreover, the authors could demonstrate that TRAIL prevented cell cycle progression of lymphocytes *in vitro*. The capacity of TRAIL to control CIA was confirmed using TRAIL^{-/-} mice.⁴¹ TRAIL^{-/-} C57BL/6 mice developed severe arthritis indistinguishable from arthritis in DBA1, whereas TRAIL^{+/-} C57BL/6 mice were not susceptible to CIA owing to their genetic background. Mice deficient for TRAIL were found to have increased cellular and humoral immune responses against self-antigens. Two other studies employed adenoviral-mediated gene transfer of TRAIL in animal models of arthritis. In an Il-1 β triggered rabbit model of arthritis intra-articular adenoviral TRAIL delivery was shown to modulate inflammation.⁴⁵ In this study the attenuation of inflammation was correlated with TRAIL-induced apoptosis in cells within the synovium. The same authors obtained similar results by using recombinant TRAIL instead of TRAIL-encoding adenovirus.⁴⁶ In a preclinical study by Liu *et al.* dendritic

cells were transduced with an adenovirus-based vector able to express TRAIL and pulsed with collagen, the autoantigen responsible for disease in the CIA model. The primed dendritic cells were predicted to specifically interact and thus eliminate, only those T-lymphocytes that recognize the collagen. Indeed, treatment with TRAIL-expressing collagen-pulsed dendritic cells limited the incidence of arthritis in the CIA model by modulating T-cell responses and correlated with detection of apoptotic T-cells in the spleen. In addition, B-cell responses were affected as titers of anti-collagen II antibodies were lower. Taken together, the results obtained in animal models for arthritis support the concept that TRAIL has a therapeutic potential although the underlying mechanisms remain to be clarified.

The pseudo-tumoral proliferation of RA FLS is considered to be the major mechanism for the hyperplastic growth of the RA synovium and can be mimicked by *in vitro* culturing, since *ex vivo* RA FLS cells grow in normal medium without requiring additional stimulation. Various groups have tested how TRAIL does modulate these RA FLS cultures: a study by

Ichikawa *et al.* described TRAIL-R1 and -R2 expression on primary isolated FLS from RA patients and analyzed the effect of TRAIL on RA FLS for short culturing periods; that is, up to 24 hours. Varying levels of apoptosis were induced by TRAIL on the different RA FLS cultures tested, in which a portion of cells survived.⁴⁷ These RA FLS strongly expressed TRAIL-R2 and were highly susceptible to an agonistic anti-TRAIL-R2 antibody, identifying TRAIL-R2 as the receptor mediating TRAIL-induced apoptosis.⁴⁷ These observations are in agreement with a report by Miranda-Carus *et al.*⁴⁸ In this study, 50 fibroblasts from RA synovial fluid samples were analyzed and about half of them were found to express TRAIL-R2. These cells underwent apoptosis when treated *in vitro* with an agonistic anti-TRAIL-R2 antibody.⁴⁸ While these reports suggested the specific targeting of TRAIL-R2 on RA FLS as a potential therapeutic approach, Perlman *et al.* drew the opposite conclusion because they could not detect the expression of TRAIL-R1 or TRAIL-R2, or the susceptibility to TRAIL in RA FLS.⁴⁹ Another study by Park *et al.* concluded that cultured FLS are not sensitive to TRAIL-induced apoptosis in spite of TRAIL-R1 expression, whereas FLS became sensitive in the presence of actinomycin D or cycloheximide.⁵⁰ One report correlated resistance of RA FLS for TRAIL-induced apoptosis with expression levels of TRAIL-R4, as TRAIL-R4 siRNA could sensitize FLS.⁵¹

Our group has analyzed in two studies TRAIL-responses of RA FLS.^{35,52} We detected expression of the TRAIL receptors 1 and 2 on FLS, and described that both recombinant TRAIL as well as an agonistic anti-TRAIL-R2 antibody induces apoptosis only in a subset of RA FLS that is followed by an induction of proliferation in the surviving cells.³⁵ Notably, the observed dual functionality of TRAIL on RA FLS concurs with the previously reported pleiotropic responses of TRAIL in primary human tumor cells.^{35,53} We observed a variation in TRAIL-sensitivity of RA FLS according to the patients from which they are derived, which is in agreement with the report of Ichikawa *et al.*⁴⁷

A possible explanation for the opposing observations on TRAIL-sensitivity of RA FLS might be the use of different protocols to isolate and/or culture synovial fibroblasts obtained from biopsies. For example, RA FLS were shown to produce the endogenous decoy receptor OPG that can interfere with the efficiency of recombinant TRAIL on cell cultures that have not received fresh medium.²² Another important factor appears to be the cell cycle dependency of RA FLS for TRAIL-induced apoptosis.⁵⁴ The discrepancy in reported TRAIL receptor expression might be because of the different antibodies used.

While most studies focus on TRAIL responses on synovial fibroblasts, one study analyzed

synovial T-cells.⁵⁵ Lorenzo *et al.* reported that synovial fluid T-cells from RA patients are sensitive to TRAIL- but not Fas ligand-induced apoptosis. Several reports characterized TRAIL-induced signaling in RA FLS. The group of Zhang analyzed FLS treated with the proteasome inhibitor lactacystin and described a specific role for p53 in TRAIL-R2-mediated apoptosis. Under these conditions p53 siRNA was able to reduce TRAIL-induced apoptosis.⁵⁶ Caspase-4 was found to induce cleavage of vimentin associated in a complex with p53, thus releasing p53. We could demonstrate the involvement of the ERK p38 and PI3 kinase/Akt signaling pathways in TRAIL-induced RA FLS proliferation, but that only PI3 kinase/Akt protects RA FLS from TRAIL-triggered apoptosis. In line with this is a study by Miyashita *et al.* reporting that Akt renders RA FLS resistant to TRAIL-induced apoptosis.⁵⁷ Moreover, we found that not only TRAIL-induced apoptosis, but also TRAIL-triggered proliferation in RA FLS is mediated by caspases via degradation of the cell cycle inhibitors p21 and p27. We therefore suggest that caspases act synergistically with PI3 kinase and/or MAP kinases to mediate TRAIL-induced proliferation in RA FLS. A correlation of p21 expression levels with cell viability in TRAIL-treated RA FLS has been concluded also by Juengel *et al.* These authors showed that the histone deacetylases inhibitor trichostatin sensitizes RA FLS for TRAIL-induced cell death and induced cell cycle arrest by upregulating p21 levels.⁵⁸

In summary, a pattern is emerging indicating that TRAIL acts as a pleiotropic cytokine on tumor-like human RA FLS by inducing different responses (Figure 1). This differs from previous reports describing that TRAIL has a protective role in the collagen-induced mouse model of arthritis by blocking the proliferation of synovial cells. This discrepancy could reflect the different pathogenic mechanisms between RA in the joints of patients and the respective mouse model. Moreover, the organization of TRAIL signaling between human and mouse appears to be different, as only one membrane-anchored TRAIL receptor and two soluble decoy receptors have been identified in the mouse. One recent report compared TRAIL and TRAIL receptor expression in synovial tissues of RA, osteoarthritis, and spondyloarthritis patients by immunohistochemistry and found the highest expression for TRAIL and its membrane-bound receptors in tissues of RA patients.⁵⁹ Moreover, increased levels of the apoptosis inhibitors survivin and XIAP were found in synovial tissues of patients with active RA when compared with those of inactive RA. This finding could explain not only the opposing effects of TRAIL on cultured RA FLS but also suggests that resistance for TRAIL-induced apoptosis correlates with disease severity.

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