

Role of Apo2L/TRAIL in immunity: Applications to rheumatoid arthritis

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Author contributions: Martinez-Lostao L and Anel A solely contributed to this paper.

Supported by the Ministerio de Ciencia e Innovación No. SAF2007-16674 and No. SAF2010-15341 to Anel A; and a Sara Borrell Postdoctoral Contract No. CD05/00082 from the Instituto de Salud Carlos III, Spain to Martinez-Lostao L.

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Received: June 2, 2011 Revised: April 18, 2012

Accepted: July 4, 2012

Published online: August 3, 2012

Abstract

Rheumatoid arthritis (RA) is the most common inflammatory disease of the musculoskeletal system primarily affecting the joints. It is characterized by massive synovial hyperplasia and subsequent destruction of articular cartilage and bone. Although various aspects in the pathogenesis of RA remain unclear, genetic, environmental and of course immunological factors have been involved. Defects in apoptosis seem to play a role in both initiation and perpetuation of RA. Apo2 ligand/tumor necrosis factor (TNF) related apoptosis-inducing ligand (Apo2L/TRAIL) is a cytokine that belongs to the TNF superfamily capable of inducing apoptosis on tumor cells through activation of the extrinsic pathway. Besides this function, like other members of the TNF superfamily, Apo2L/TRAIL has been shown to exert important functions in the regulation of the immune system. Concerning pathological conditions, the Apo2L/TRAIL signaling pathway plays an important role in the response to infections, in immune surveillance against tumors and in autoimmune diseases such as RA. Furthermore, its implication in suppression of autoimmu-

nity suggests that Apo2L/TRAIL has potential as therapeutic agent not only in cancer but also in autoimmune diseases. In fact, Apo2L/TRAIL-based therapies have been shown effective in various animal models of RA. This review summarizes the current knowledge on the biology of Apo2L/TRAIL and its role in RA.

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Key words: Apo2 ligand; Tumor necrosis factor related apoptosis-inducing ligand; Apoptosis; Rheumatoid arthritis; Autoimmunity; Immune response

Peer reviewers: Chang-Hee Suh, Professor, Department of Rheumatology, Ajou University School of Medicine, San 5, Wonchon-dong, Yeongtong-gu, Suwon 443-721, South Korea; Hazem M Youssef, MD, FRCP, Rheumatology Unit, Aberdeen Royal Infirmary, Aberdeen, AB25 2ZN, United Kingdom

Martinez-Lostao L, Anel A. Role of Apo2L/TRAIL in immunity: Applications to rheumatoid arthritis. *World J Rheumatol* 2012; 2(1): 1-11 Available from: URL: <http://www.wjgnet.com/2220-3214/full/v2/i1/1.htm> DOI: <http://dx.doi.org/10.5499/wjr.v2.i1.1>

INTRODUCTION

Multicellular organisms use apoptosis, the major mechanism of programmed cell death, to eliminate cells that are superfluous or that are irreparably damaged^[1,2]. Apoptosis plays a pivotal role during development and controls homeostasis of tissues throughout adult life^[3]. A wide variety of stimuli can trigger apoptosis such as severe damage caused by heat shock, cytotoxic drugs, radiation infection, tumor transformation, and cellular stress. Moreover, an aberrant regulation of apoptosis is implicated in the pathogenesis of a variety of major human diseases. Excessive apoptosis is implicated in neurodegenerative diseases, such as Alzheimer's and Huntington's diseases^[4],

acquired immune deficiency syndrome^[5], ischemic heart disease^[6], stroke^[7], and infertility^[8]. In contrast, deficiency in apoptosis plays a key role in the pathogenesis of cancer^[9] and is also involved in certain autoimmune disorders^[10].

There are two major apoptotic pathways: the intrinsic or mitochondrial and the extrinsic or death receptor-mediated. The intrinsic pathway is activated by intracellular events and depends on proteins of the Bcl-2 family that control the release of apoptogenic factors from the mitochondria^[11]. In contrast, the extrinsic pathway is triggered by signals received through extracellular protein ligands that bind to proapoptotic death receptors (DR) thereby initiating an intracellular signaling cascade leading to apoptosis^[12].

Mitochondrial outer-membrane permeabilization is involved in the intrinsic pathway allowing the release of proapoptotic factors such as cytochrome c, Second mitochondria-derived activator of caspase/direct inhibitor of apoptosis-binding protein with low pI (Smac/DIABLO) and apoptosis inducing factor from the mitochondria to the cytosol^[13-15], a process controlled by the Bcl-2 protein family^[16]. Once released from mitochondria to the cytosol, cytochrome c induces the formation of a multimeric complex called the apoptosome, containing the adaptor protein Apaf-1 and the initiator caspase-9. Caspase-9 is activated into the apoptosome and in turn cleaves effector caspases ultimately leading to apoptosis^[17].

The extrinsic pathway transmits apoptotic signals from extracellular ligands through DRs to the intracellular apoptotic machinery. Six different DRs are known: Fas, tumor necrosis factor (TNF)R1, DR3, TNF-related apoptosis-inducing ligand (TRAIL)-R1 or DR4, and TRAIL-R2 or DR5, and DR6^[18]. These DRs interact with their corresponding ligands, which belong to TNF superfamily (FasL, TNF, TL1A and TRAIL, respectively). This interaction induces receptor oligomerization and activation of caspase cascade, ultimately leading to apoptosis^[12].

Apo2L/TRAIL, a member of the TNF protein superfamily, was initially described as a death ligand capable of inducing apoptosis in transformed cells while sparing normal cells^[19,20]. Subsequently, a variety of studies, including those with knockout mice for TRAIL and TRAIL-R have been conducted to unravel the physiological role of this cytokine^[21-23]. These studies revealed that Apo2L/TRAIL-TRAIL-R signaling is implicated in the regulation of the homeostasis of the immune system. Thus Apo2L/TRAIL can be considered as an additional mechanism necessary to prevent the development of autoimmunity^[24,25].

Rheumatoid arthritis (RA) is the most common inflammatory disease of the musculoskeletal system^[26,27]. Although RA frequently shows systemic involvement, it primarily affects the joints, where chronic synovial inflammation and subsequent destruction of the articular cartilage and bone are the hallmarks of the disease. This synovial hyperplasia is caused by a massive invasion of inflammatory cells and by extensive increase of resident

synovial cells also called fibroblast-like synoviocytes (FLS), which generates a heterogeneous tissue known as pannus. RA-FLS play a pivotal role in both initiation and perpetuation of RA^[28,29]. A body of evidence has demonstrated that FLS undergo substantial changes referred to as tumor-like transformation, being active drivers of joint destruction in RA^[30,31]. Among the cellular characteristics that distinguish FLS are production of cytokines, chemokines and growth factors as well as alterations in growth and apoptosis. The later is of particular interest, because the resistance of RA-FLS to apoptotic signals provides one explanation to the development of pannus and joint destruction. Concerning inflammatory cells, T lymphocytes, mainly CD4+ T cells with a memory phenotype, but also CD8+ T cells, macrophages and B cells are present in the sublining tissue. It has also been described alterations in apoptosis in infiltrating T lymphocytes that together with alterations in RA-FLS may lead to the creation of a proinflammatory microenvironment into the joint that contributes to chronic disease maintenance.

The major aim of this review is to provide a summary of the current data on the role of the death ligand Apo2L/TRAIL in the pathogenesis as well as its use as therapeutic agent in RA.

APO2L/TRAIL SIGNALING

Apo2L/TRAIL was independently identified by two different groups as the third member of the TNF superfamily that induces apoptosis^[19,20]. Apo2L/TRAIL is capable of binding to a complex system of receptors with different affinities and possibly distinct signaling outcomes. Five receptors for Apo2L/TRAIL are known in humans called TRAIL-R1/DR4, TRAIL-R2/DR5, TRAIL-R3/DcR1 and TRAIL-R4/DcR2^[12]. Apo2L/TRAIL can bind a soluble receptor termed osteoprotegerin (OPG). Only DR4 and DR5 possess a death domain (DD) in their intracellular portion and are capable of transmitting the proapoptotic signal^[32,33] by inducing the formation of the death-inducing signaling complex (DISC)^[34,35]. DcR1 and DcR2 are two non-apoptotic cell-bound receptors for Apo2L/TRAIL^[36,37]. Apo2L/TRAIL can also bind, with rather low affinity, to a soluble receptor called OPG^[38]. OPG binds with high affinity and inhibits the action of another TNF superfamily member termed receptor activator of nuclear factor kappa B (NFκB) ligand (RANKL) involved in bone metabolism. Nevertheless, it is rather unlikely that Apo2L/TRAIL-OPG interaction may play a physiological role, at least *in vivo*, since Apo2L/TRAIL and DR5 knockout mice do not display a phenotype with alteration in bone metabolism^[39,40].

The initial step of the Apo2L/TRAIL-induced apoptosis is the binding of the trimeric ligand to DR4 or DR5. This interaction induces clustering of the receptors that recruits the adaptor protein Fas-associated DD (FADD) which in turn promotes the assembly of the DISC^[34,41]. DD of FADD binds to the homologue domain of the DRs thereby exposing the death-effector domain of

procaspase-8 or -10. Recruitment of procaspase-8 to the DISC induces its activation by autocleavage, release into the cytosol, where active caspase 8 activates the effector caspases-3, -6 and -7, the ultimate executors of the apoptotic program of cell death (Figure 1).

Apo2L/TRAIL apoptotic signaling pathway is regulated at different levels to prevent unwanted caspase activation. In fact, not all proteins present in the DISC are proapoptotic. Cellular FLICE inhibitory protein (cFLIP), which shares high sequence homology with caspase-8 and -10, inhibits caspase activation at the DISC by competing for binding to FADD. There are two splice variants of cFLIP, a longer (cFLIP_L) and a shorter version (cFLIP_S)^[42]. However, the role of cFLIP_L is controversial. Some studies have reported that cFLIP_L is an antiapoptotic protein that works in a similar manner to cFLIP_S^[43]. In contrast, other studies describe cFLIP_L as a proapoptotic molecule^[44].

Other mechanisms of distinct nature have been described that may modulate Apo2L/TRAIL signaling. Post-translational modifications of DRs by O-glycosylation seem to be important to promote clustering of DR4 and DR5, after ligand binding which mediate recruitment and activation of the apical caspase-8^[45]. Recently, ubiquitylation of caspase-8 after death receptor ligation by Apo2L/TRAIL has been showed as a crucial mechanism which promotes the full activation of caspase-8^[46].

Finally, it has been described that Apo2L/TRAIL has more diverse effects than apoptosis. Among the non-apoptotic effects of Apo2L/TRAIL, it has been reported induction of proliferation, migration and survival signals, in distinct cell types. Receptor interacting protein (RIP), which is able to activate the inhibitor of NFκB kinase-complex (IKK complex) in TNF signaling, has been described to be present in the Apo2L/TRAIL DISC^[47]. RIP promotes phosphorylation of IKK, which phosphorylates IκB leading to its degradation. Degradation of inhibitor of NFκB (IκB) promotes phosphorylation of NFκB thereby activating this transcription factor^[48]. Furthermore, Apo2L/TRAIL can activate other proinflammatory intracellular signaling pathways such as mitogen-activated kinase (MAPK), phosphatidylinositol 3-kinase (PI3K) and c-Jun N-terminal kinase^[49]. This pro-survival effects induced by Apo2L/TRAIL are crucial in the resistance of distinct tumor cells to Apo2L/TRAIL-induced apoptosis^[50] and also seem to be important in the pathogenesis of some autoimmune diseases such as RA^[51].

BIOLOGICAL ROLE OF APO2L/TRAIL IN THE IMMUNE SYSTEM

Both Apo2L/TRAIL and TRAIL-R deficient mice do not display any overt developmental defects^[39,52,53] revealing that, Apo2L/TRAIL signaling is not essential for normal embryonic development.

The major roles of Apo2L/TRAIL have been found in the immune system playing a role in shaping and regu-

lating the immune response. This is not surprising as it was already suggested by the inducible expression of Apo2L/TRAIL in immune cells such as monocytes, dendritic cells (DCs) and natural killer (NK) cells^[54-56].

In case of T cells, Apo2L/TRAIL expression is absent in naive T cells, whereas expression of Apo2L/TRAIL protein was increased on both CD4+ and CD8+ after T-cell receptor or phytohaemagglutinin stimulation^[57,58]. Surface Apo2L/TRAIL in activated T cells seems to be stabilized by type I interferons^[57].

In human T cell blasts Apo2L/TRAIL and FasL are stored into intracytoplasmic pre-lysosomal compartments with the structure of multivesicular bodies^[59]. Apo2L/TRAIL, and FasL, are rapidly released to the supernatant of activated human T cells associated with microvesicles/exosomes of 100 nm of diameter with the death ligands on their surface^[25,60].

Although non-activated CD4+ and CD8+ T cells express DR4 and DR5, they are resistant to Apo2L/TRAIL-mediated apoptosis^[61,62]. However, activation of T cells with interleukin (IL)-2 resulted in Apo2L/TRAIL susceptibility. In fact, Apo2L/TRAIL is implicated in the homeostasis of the immune response by induction of activation-induced cell death (AICD) of human T cells^[63]. This process is dependent on the action of death ligands, especially on FasL^[64,65], but Apo2L/TRAIL also plays a role in AICD^[66]. The effect of Apo2L/TRAIL was more pronounced on the CD8+ T cell population^[67]. Inhibition of IL-2-dependent T cell blast growth, mainly in the CD8+ T cell population, by Apo2L/TRAIL does not require re-stimulation and would suggest an additional immune-regulatory role of this death ligand^[25,66,67].

There is a CD8+ T cell population which is primed in the absence of CD4 T cells, the so-called "helpless" CD8+ T cells. These cells are unable to undergo the second round of clonal expansion^[68]. The memory CD8+ T cells generated in this manner die by Apo2L/TRAIL-mediated AICD upon re-stimulation^[69].

Apo2L/TRAIL also seems to be involved in the regulation of T helper 1 and T helper 2 responses^[70] and has been recently implicated in the induction of cell proliferation of the CD4+ CD25+ regulatory T cell population^[71,72].

Apo2L/TRAIL signaling has been implicated in intra-thymic negative selection^[23,73]. However, it is still a controversial subject. These studies suggested that negative selection was at least partially impaired in TRAIL knockout mice or in the presence of soluble blocking DR5. In contrast other studies using TRAIL knockout mice and a neutralizing anti-mouse TRAIL mAb showed that Apo2L/TRAIL signaling does not play a role in this process^[74]. Supporting this finding normal negative selection has been described in DR5 knockout mice suggesting that Apo2L/TRAIL receptor signaling is not required for negative selection^[53].

Immune effector cells involved in the fight against infections, such as NK cells and cytotoxic T cells, express Apo2L/TRAIL when they are activated and exert their

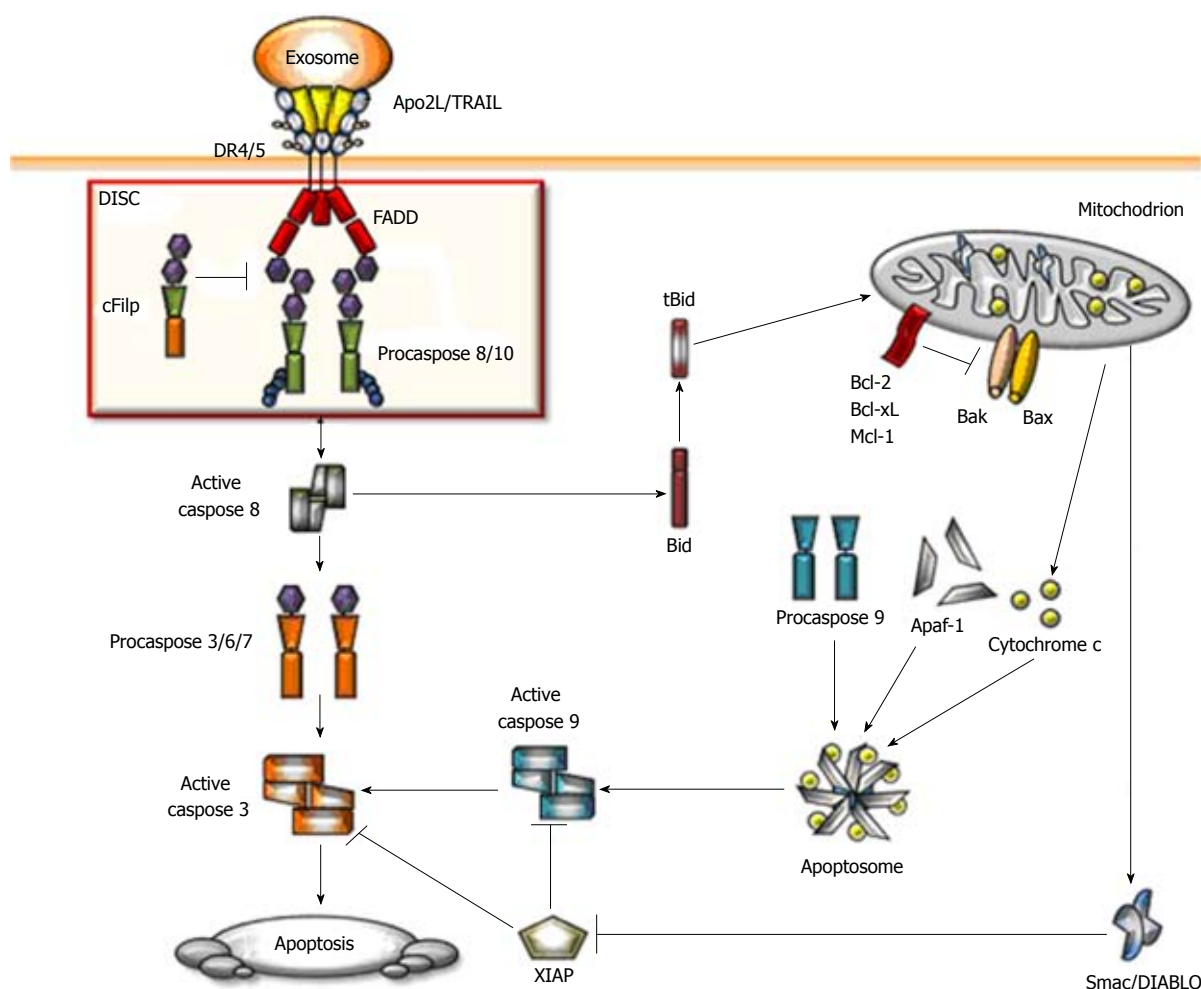


Figure 1 Schematic representation of the Apo2 ligand/ tumor necrosis factor related apoptosis-inducing ligand apoptotic signaling pathway. When Apo2 ligand/ tumor necrosis factor related apoptosis-inducing ligand (Apo2L/TRAIL) binds to their respective receptors induced their trimerisation and formation of the death-inducing signaling complex (DISC). The adaptor protein fas-associated protein with death domain (FADD) is recruited to the DISC through its death domain (DD) which interacts with DD of the receptors. Subsequently, procaspases-8 and -10 are recruited to the protein complex where they interact with FADD via the death effector domains. Cellular FLICE inhibitory protein (cFLIP) can compete with caspase-8 for the binding to FADD and high levels of cFLIP can inhibit caspase-8 activation at the DISC. DISC-activated caspases-8 and -10 trigger a caspase cascade by cleavage of caspase-3 thereby activating effector caspases. In type I cells, activation of the extrinsic pathway is sufficient to induce Apo2L/TRAIL-induced apoptosis whereas in type II cells, Bid cleavage is required for apoptosis induction by Apo2L/TRAIL. Caspase-8 cleaves Bid into tBid which initiates the mitochondrial pathway leading to release of cytochrome c and second mitochondria-derived activator of caspase/direct inhibitor of apoptosis-binding protein with low pI (Smac/DIABLO) from the mitochondria. After release from mitochondria, cytochrome c, together with Apaf-1 forms the apoptosome, which leads to activation of caspase-9. Smac/DIABLO counteracts the inhibitory function of X-linked inhibitor of apoptosis thereby allowing for full activation of caspases-3 and -9, ultimately leading to cell death.

cytotoxic function, at least in part, *via* Apo2L/TRAIL signaling^[62,75-79].

Finally, Apo2L/TRAIL has been implicated in immunosurveillance against cancer^[35,52,80]. Although Apo2L/TRAIL has a suppression role in the growth of grafted tumor and experimental metastasis, the importance of endogenous ligand in the immunosurveillance against primary tumors is still a matter of debate. Mice deficient in Apo2L/TRAIL and TRAIL-R do not spontaneously develop tumors in early age^[39,53]. However, TRAIL-deficient mice develop more lymphomas than wild-type mice when they are aged^[81]. Neutralization of Apo2L/TRAIL signaling enhanced fibrosarcoma development in methylcholanthrene-induced tumors in mice^[39]. In the absence of Apo2L/TRAIL or TRAIL-R, mice develop more lymphomas, carcinogen-induced tumors, skin carcinoma

and lymphoma metastasis^[21,22,81]. In any case, a definitive role of endogenous Apo2L/TRAIL in tumor suppression of primary tumors has not been yet well established and further studies in autochthonous tumor development models will be needed^[82].

APOPTOSIS IN RA-FLS

The resistance of RA-FLS to apoptotic signals has been associated with the phenotype of these cells and it may provide an explanation to the development of pannus and consequently the joint destruction^[83]. Resistance of FLS to apoptosis occurs at different levels. For example, Bcl-2 expression is induced by TNF α and IL-1 β in cultured FLS. Moreover, there is a direct correlation between Bcl-2-expression and the extent of the synovial

lining thickening and inflammation^[84]. IL-15, a cytokine with pleiotropic effects, increases Bcl-2 and Bcl-xL levels in FLS^[85]. Mcl-1 is also induced after cytokine stimulation and is found in RA synovium correlating with synovial inflammation^[86]. The apoptotic protein Bcl-2/adenovirus E1B 19-kd protein-interacting protein 3 is induced in RA synovium in response to hypoxia but is pro-apoptotic action is inhibited by TNF α and IL-1 β providing a link between inflammation and resistance to apoptosis in RA^[87].

Although FLS express a variety of death receptors such as Fas/CD95, TRAILR1 and TRAILR2 and also TNFR1^[88-90], various data indicate that FLS are relatively resistant to receptor-mediated apoptosis. TNF α is able to induce soluble Fas thus decreasing the susceptibility of FLS to Fas/CD95-induced apoptosis^[89]. Dcr3 is expressed in FLS in a TNF α -dependent manner and is able to prevent Fas/CD95-induced apoptosis^[91]. LIGHT, another member of the TNF superfamily, is found in RA and also prevents FLS from Fas/CD95-induced apoptosis^[92]. The expression of FLIP is high in RA mainly at sites of cartilage destruction^[93]. It has been suggested that the expression of FLIP depends on the stage of disease^[94]. While in RA patients with short duration of the disease showed decreased levels of apoptosis accompanied by high expression of FLIP, in patients with a long-term RA, increased levels of apoptosis were associated with low levels of FLIP. Again a connection between inflammation and resistance to apoptosis is achieved because TNF α can induce the expression of FLIP^[95]. Post-translational modifications also play a role in FLS apoptosis. Small ubiquitin-like modifier 1 (SUMO-1) is highly expressed in FLS and SUMO-1-mediated modification protects cells from Fas- and TNFR1-induced apoptosis^[96].

On the other hand, FLS also contribute to the accumulation of infiltrating cells by regulating their response to apoptosis through cellular interaction and soluble factors. FLS produce large amounts of stromal cell-derived factor 1 α which is able to inhibit T cell apoptosis through activation of PI3-kinase and MAPK pathways^[97]. B cells co-cultured with FLS are protected from apoptosis through a Vascular cell adhesion protein 1 and α 4 β 1 integrin-dependent mechanism^[98]. Reduced apoptosis has been associated with increased expression of Bcl-xL^[99]. The B cell-activating factor of the TNF family, that is involved in pro-survival B cell signaling, is also produced by FLS after engagement of α 5 β 1 integrins of the cell surface^[100].

In summary, FLS resistance to apoptosis contributes significantly to the pathogenesis of RA. The tumor-like transformation of FLS not only leads to profound changes in the responsiveness of these cells to apoptotic stimuli. In addition, it increases the persistence of inflammatory cells by modulating its resistance to cell death.

ROLE OF APO2L/TRAIL IN RA

Autoimmune diseases result from the inappropriate recognition of self-antigens due to defects in the regulation

of the immune system. Apo2L/TRAIL signaling seems to be able to modulate the autoimmune disease and to be implicated in a variety of autoimmune diseases. An increased number of studies have consistently shown that Apo2L/TRAIL is capable of inhibiting autoimmune diseases in a variety of animal models. In these studies, Apo2L/TRAIL seems to play distinct roles ranging from inhibiting inflammation, to inhibiting cell cycle progression, proliferation of auto-reactive T cells as well as cytokine and antibody production.

Although TRAIL- and TRAIL-deficient mice do not display spontaneous autoimmune diseases, many studies have identified profound effects when autoimmunity is induced in these mice or in the presence of Apo2L/TRAIL signaling-blocking agents. In these studies, it has been shown that mice were more susceptible to induced-autoimmune diabetes^[123,101-103]. It is noteworthy that double FasL mutant (gld) and TRAIL knockout mice developed an extreme and fatal lymphoproliferative disease which was more severe than that due to mutation in FasL alone^[104]. Apo2L/TRAIL is also implicated in experimental autoimmune thyroiditis^[71,105,106]. The most widely used mouse model which mimics multiple sclerosis is experimental autoimmune encephalomyelitis (EAE). Blockade of Apo2L/TRAIL signaling led to a high degree of inflammation in the central nervous system^[107,108]. However, a reduction of the clinical severity of EAE is observed when TRAIL-R2-Fc, an Apo2L/TRAIL signaling blocking agent, was injected into central nervous system in mice in which EAE was previously induced^[109].

A variety of studies have implicated Apo2L/TRAIL in the pathogenesis of RA. In a mice model of RA [collagen-induced arthritis (CIA)], the chronic blockade of Apo2L/TRAIL exacerbated autoimmune arthritis, leading to profound hyperproliferation of synovial cells and arthritogenic lymphocytes and increasing the production of autoantibodies and proinflammatory cytokines^[110]. In this study, Apo2L/TRAIL inhibited autoimmune inflammation by blocking cell cycle progression rather than by inducing apoptosis of inflammatory cells. TRAIL-deficient mice were also more susceptible to CIA. In line with this, TRAIL-deficient C57BL/6 mice developed the typical symptoms when immunized with collagen whereas C56BL/6 wild-type mice were not susceptible to CIA^[123].

Although numerous studies have examined the role of Apo2L/TRAIL in autoimmune diseases in experimental animal models, less is known of the role of Apo2L/TRAIL in human autoimmune diseases. Most of the studies have shown expression of DR4 and/or DR5 in FLS from RA patients^[51,90,111,112]. However, in one study neither Apo2L/TRAIL nor its receptors were detectable on lymphocytes or synovial fibroblasts obtained from synovial fluid (SF) from RA patients^[113]. Nevertheless, RA SF macrophages expressed the decoy receptor DcR1. On the other hand, it has been demonstrated that T lymphocytes from RA synovial fluids were activated and expressed a similar pattern of Apo2L/TRAIL than human

T cell blasts or T cells in the SF of traumatic patients^[114]. RA T cells were insensitive to Fas-mediated regulation, as previously reported^[115] but remarkably, they were more sensitive than *in vitro* activated T cells to regulation by Apo2L/TRAIL. Nevertheless, it was detected very low amounts of bioactive FasL and Apo2L/TRAIL associated with exosomes in SF from RA patients as compared with SF from traumatic arthritis patients^[114].

Conversely, a dual role of Apo2L/TRAIL has been suggested in RA which is characterized by expansion of FLS. It has been reported that Apo2L/TRAIL induced RA FLS proliferation in a dose-dependent manner through a mechanism involving MAPK and PI3K/Akt signaling^[51]. Previous studies have demonstrated a relative *in vitro* sensitivity of RA FLS to Apo2L/TRAIL^[90] which is increased upon treatment with actinomycin D^[116]. However, more recent studies indicated that only a fraction of FLS are sensitive to Apo2L/TRAIL-induced apoptosis^[117], depending on their proliferative state^[118], while proliferation is induced in another fraction after rApo2L/TRAIL treatment^[51,117]. More recently, it has been reported that Apo2L/TRAIL-induced apoptosis varied in FLS from different RA patients and that susceptibility of FLS to apoptosis induced by Apo2L/TRAIL inversely correlated with disease severity of RA patients^[119].

APO2/TRAIL AS TREATMENT OF RA

Although Apo2L/TRAIL-based therapies have been mostly used in cancer, its therapeutic value in autoimmune diseases has been also proposed. In this line, a number of therapeutic strategies involving Apo2L/TRAIL have been currently used to treat various experimental autoimmune diseases such as experimental autoimmune thyroiditis^[71,105] and experimental autoimmune encephalomyelitis^[10,72,120,121].

Concerning RA, distinct Apo2L/TRAIL-based therapeutic approaches have been used for treatment of arthritic joints. CIA was induced in DBA/1 mice and then animals received an intra-articular injection of an adenovirus carrying the mouse TRAIL gene^[110]. This local treatment reduced disease score. Interestingly, in this study TRAIL had no effect on apoptosis of inflammatory cells either *in vivo* or *in vitro* but inhibited DNA synthesis and prevented cell cycle progression of lymphocytes *in vitro*. A similar therapeutic strategy had been used in a rabbit model of RA. In IL-1 β -induced arthritis in rabbits, intra-articular gene transfer using an adenoviral vector carrying human Apo2L/TRAIL gene ameliorated disease in treated arthritic joints. Apo2L/TRAIL gene transfer was able to induce apoptosis in cells within the synovial cell lining, to reduce leukocyte infiltration and to stimulate matrix synthesis^[122]. Gene transfer-based therapeutic strategy which modulates Apo2L/TRAIL receptor expression may sensitize RA synoviocytes to Apo2L/TRAIL. Primary cultures established from RA synovial cells showed an increase of Dcr2 correlating with Apo2L/TRAIL

resistance of these cells. A combined treatment with a Dcr2 silencing RNA approach and gene transfer using an adenoviral vector carrying human Apo2L/TRAIL eliminated apoptosis-resistant RA synovial fibroblasts^[123].

Other therapy strategy for treatment of RA has been the use of rApo2L/TRAIL. Using the previously described rabbit model of IL-1 β -induced arthritis, intra-articular injection of human rApo2L/TRAIL into arthritic joints induced apoptosis of the synovial cells and reduced leukocyte infiltration. Furthermore, treatment with rApo2L/TRAIL had not adverse effects neither locally on cartilage metabolism nor systemic on hepatic function^[124]. Treatment with human rApo2L/TRAIL was also reported in a CIA mouse model. Soluble rApo2L/TRAIL was capable of significantly reducing the severity and incidence of CIA, joint swelling, erythema, and edema. Inflammatory cell infiltration, cartilage destruction, and bone erosion were also significantly reduced in joints of TRAIL-treated mice in a dose-dependent manner. Treatment with rApo2L/TRAIL was also effective systemically decreasing the levels of proinflammatory cytokines and anti-collagen-specific antibodies in the sera of CIA mice^[125].

Other Apo2L/TRAIL-based therapeutic strategy has been the use of genetically modified DCs in mouse models. In a CIA model on DBA/1j mice, *in vivo* administration of genetically modified DC infected with an adenovirus expressing inducible TRAIL and pulsed with collagen II significantly decreased the incidence of arthritis and infiltration of T cells in joints^[126]. Interestingly, adenoviral vector carrying Apo2L/TRAIL was not toxic to DCs or mice but could induce activated T cells to undergo apoptosis in the spleen. Anti-human DR5 mAb (TRA-8) has been also used as treatment in adjuvant arthritis in rats, a rat model of RA^[127]. Hind paw inflammation was ameliorated after treatment with TRA-8 decreasing synovial hyperplasia due to induction of apoptosis in synovial cells and infiltration of inflammatory cells.

Novel Apo2L/TRAIL formulations have been developed to improve its biological half-life, stability and/or bioactivity and have been used as treatment for RA in distinct animal models. Nano-sized complexes (nano-complexes) based on hyaluronic acid and polyethylene glycol (PEG)-derivatized human TRAIL (PEG-TRAIL) formed by N-terminal specific PEGylation has been used in a CIA mouse model^[128]. The therapeutic effect of this formulation injected intra-peritoneally was higher than soluble TRAIL, concerning clinical scores and histology. Additionally, sustained delivery of PEG-TRAIL resulted in significant reduction of serum inflammatory cytokines and collagen-specific antibodies that are responsible for the pathogenesis of RA. As previously discussed, infiltrating T lymphocytes in synovial fluid (SF) from RA patients, although resistant to Fas, were unexpectedly more susceptible to human rApo2L/TRAIL than were *in vitro* activated T cells. However, the amount of bioactive Apo2L/TRAIL associated with exosomes in SF from RA patients was extremely low compared with SF from

control patients with traumatic arthritis^[114]. Consequently, administration of Apo2L/TRAIL associated to the surface of liposomes resembling the natural exosomes may be a reasonable therapeutic strategy in RA. Treatment of the arthritic knee joints by intra-articular injection with human rApo2L/TRAIL associated with liposomes (LUV-Apo2L/TRAIL) in an antigen-induced arthritis rabbit model showed a higher effectiveness than soluble rApo2L/TRAIL reducing joint swelling. Histological parameters such as synovial hyperplasia, inflammatory infiltrate vascularity and formation of villi were also significantly reduced when arthritic joint were treated with LUV-Apo2L/TRAIL^[129]. In consequence, the association of Apo2L/TRAIL to liposome surface improves its bio-activity. Interestingly, treatment with this Apo2L/TRAIL novel formulation did not have adverse effects previously described for soluble form of Apo2L/TRAIL such as hepatotoxicity.

CONCLUSION

Since the first description of Apo2L/TRAIL, more than ten years ago, and the identification of its two cognate pro-apoptotic receptors, Apo2L/TRAIL signaling has provided a unique novel model system for studying the extrinsic apoptotic pathway. During the last decade, a body of evidence has accumulated illustrating that Apo2L/TRAIL is clearly implicated not only in cancer but also in immunity. Immunosuppressive and immunoregulatory functions important for immune homeostasis, immunosurveillance and autoimmunity have been demonstrated for Apo2L/TRAIL.

Biological therapies such as anti-TNF and anti-IL1 agents have been successfully used in RA. However, these therapies targeting immune system do not have a response over 60%. Therefore, other therapeutic approaches have been set up. In line with this, apart from the use of Apo2L/TRAIL as anti-tumor therapy, an increasing number of studies have shown that this molecule is a promising therapeutic agent to treat autoimmune diseases including RA. Distinct studies using *in vivo* animal models of RA have provided evidences that Apo2L/TRAIL is capable of diminishing the incidence and the severity of the autoimmune disease. A variety of experimental approaches, including gene transfer, soluble molecule, pro-apoptotic agonistic receptor antibodies and lately, novel Apo2L/TRAIL formulations based on association of the death ligand with different kind of nanoparticles have been used as treatment for arthritis in several animal models. In summary, Apo2L/TRAIL signaling is a promising molecular target for autoimmune disease immunotherapeutics.

In spite of these promising data obtained in RA, further studies are required to optimally exploit the Apo2L/TRAIL-TRAIL pathway in this disease. In this line, Apo2L/TRAIL-based nanoparticles have been shown to improve its biological half-life, stability and bioactivity compared with the soluble form and could

open new perspectives in the use of Apo2L/TRAIL as therapeutic agent in RA. With regard to the route of possible administration of Apo2L/TRAIL-based therapy, in most of studies carried out in animal models of RA, administration of Apo2L/TRAIL has been performed intra-articularly. Further studies should be performed in order to establish the viability of a systemic administration, more feasible in humans given the large number of involved joints.

Pending the outcomes of clinical trials targeting the Apo2L/TRAIL pathway in patients with cancer, clinical trials could be considered to determine the therapeutic efficacy of targeting the Apo2L/TRAIL in patients with RA. Regardless, Apo2L/TRAIL has appeared as a significant molecule in immune system regulation, with a promising future as treatment in RA.

ACKNOWLEDGMENTS

The authors thank Dr. Julian Pardo for their critical comments through preparation of this manuscript. The authors also acknowledge Dr. Avi Ashkenazi for their support through the years.

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S- Editor Wu X L- Editor Stewart G E- Editor Wu X