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ALBARBAR, Balid, DUNNILL, Christopher and GEORGOPOULOS, Nik  
<<http://orcid.org/0000-0002-6330-4947>>

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# **Regulation of cell fate by Lymphotoxin (LT) receptor signalling: functional differences and similarities of the LT system to other TNF superfamily (TNFSF) members**

Balid Albarbar, Christopher Dunnill, Nikolaos T. Georgopoulos

Department of Biological Sciences, School of Applied Sciences, University of Huddersfield, Huddersfield, U.K.

## **Correspondence:**

Nik T Georgopoulos, PhD

Department of Biological Sciences, School of Applied Sciences, University of Huddersfield, Huddersfield, HD1 3DH, UK.

E-mail: [N.Georgopoulos@hud.ac.uk](mailto:N.Georgopoulos@hud.ac.uk), Tel: +44 (0)1484 47 2721, Fax: +44 (0)1484 47 2182

## Abstract

The role of TNFR family members in regulating cell fate both in the immune system and in non-lymphoid tissues has been under extensive research for decades. Moreover, the ability of several family members (death receptors) to induce death (mainly via apoptosis) represents a promising target for cancer therapy. Many studies have focused mostly on death receptors such as TNFRI, Fas and TRAIL-R due to their strong pro-apoptotic potential. Yet, cell death can be triggered via non-classical death receptors, and the Lymphotoxin (LT) system represents a very good example of such a TNFR subfamily. Here we provide a comprehensive review of intracellular signalling pathways and cellular responses to LT-specific signalling, and compare for the first time the LT system to other TNFRs, such as CD40. Our aim is to highlight that non-classical TNFR-TNFL dyads such as the LT system demonstrate more complex, cell-type and context-specific capabilities. Understanding these complexities will permit a better understanding of the biological mechanisms via which non-death domain-containing TNFRs induce cell death, but may also allow the design of better therapeutic strategies.

## Abbreviations

AP-1: activator protein-1

APRIL: a proliferation-inducing ligand

BAFF: B-cell activating factor belonging to the TNF family

CLL: chronic lymphocytic leukaemia

DD: death domain

DR: death receptor

DcR: decoy receptor

DIF: differentiation-inducing factor

Fas: Fibroblast-associated cell-surface

LT: Lymphotoxin

LIGHT: Lymphotoxin-like exhibits Inducible expression and competes with herpes simplex virus Glycoprotein D for HVEM, HVEM being a receptor expressed on T lymphocytes

NF- $\kappa$ B: nuclear factor kappa B

NGF: nerve growth factor

HVEM: herpes-virus entry mediator

IFN- $\gamma$ : Interferon-gamma

JNK: c-Jun N-terminal kinase

RANK: receptor activator of nuclear factor-kB

TF: Transcription factor

TNF: tumour necrosis factor

TNF $\alpha$ : tumour necrosis factor-alpha

TNFSF: TNF superfamily

TNFL: TNF ligand

TNFR: TNF receptor

TRAF: TNFR-associated factor

TRAIL: TNF-related apoptosis-inducing ligand

TRAIL-R: TRAIL receptor

TL1A: TNF-like molecule 1A

## Keywords

Lymphotoxin, CD40, apoptosis, receptor cross-linking, cell signalling

## 1. The discovery of the Tumour Necrosis Factor (TNF) and Lymphotoxin (LT) proteins

The first indirect evidence for the existence of the tumour necrosis factor (TNF) family originates from experiments involving Coley's mixed toxins, which were discovered in the late 1800s. Coley's toxins consisted of a cocktail of dead bacteria and this toxic mixture was found to cause the regression of human sarcomas. The hypothesis was that the immune system needs a local trigger, such as that achieved by pathogens, which mediates the recruitment of immunocytes in order to attack tumour cells. Decades later, and in line with Coley's discovery, Gratia and colleagues found that administration of bacterial culture filtrates (consisting of *E.coli* and other bacterial types) led to liposarcoma regression in guinea pigs. In 1943, Shear and colleagues demonstrated that isolated endotoxins from Gram-ve bacterial lipopolysaccharide (LPS) were able to cause haemorrhagic necrosis in transplanted murine sarcomas. It was later suggested that the endotoxin-induced haemorrhagic necrosis in transplanted tumours was not due to direct cytotoxicity to tumours, but occurred by indirect mechanisms that resulted in hypotension, leading to vascular collapse and ischemia, thus causing tumour cell anoxia and ultimately cell death. A decade later, O'Malley and colleagues reported that administration of this endotoxin mixture could induce necrosis to tumour-bearing mice and this factor was formally named Tumour Necrosis Serum (TNS). The effectiveness of TNS was further confirmed *in vivo* and Carswell renamed this as Tumour Necrosis Factor (TNF), a ligand that appeared toxic towards malignant cells (1), now referred to as TNF $\alpha$ . A series of initial investigations carried out both *in vitro* and *in vivo* demonstrated the pro-apoptotic potential of TNF $\alpha$  and as the administration of recombinant TNF $\alpha$  in tumour-bearing mice resulted in anti-tumour responses, these observations rendered TNF a promising anticancer agent.

Soon after the identification of TNF $\alpha$ , a similar TNF-like factor was discovered and named TNF $\beta$  which was later renamed Lymphotoxin-alpha (LT $\alpha$ ), following its discovery as a protein secreted by T lymphocytes after recognition of host virus infection and of tumour antigens. Officially the two ligands were characterised when the coding sequences for both TNF $\alpha$  and LT $\alpha$  were isolated in the 1980s. The TNF $\alpha$  and LT $\alpha$  proteins are structurally and functionally closely related molecules but they demonstrate differential functional outcomes on several human cell lines (discussed in detail in subsequent sections). TNF $\alpha$  and LT $\alpha$  are now classified into a group of cytokines which have critical functional importance in immunity, inflammation, cytodifferentiation and apoptosis and represent the archetypal

members of the group of proteins that now constitutes the tumour necrosis factor superfamily (TNFSF).

## 2. The TNF ligands (TNFLs) and TNF Receptors (TNFRs)

The TNFSF consists of a large and complex network of ligands (TNFLs) and receptors (TNFRs) and each subgroup of the family may function in distinct ways, based on their signalling capacity and their ability to regulate specific gene expression and subsequently cell fate. By means of signalling triggered via interactions of these receptors with their cognate ligand(s), TNFRs play a critical role in cellular homeostasis and have multifunctional roles ranging from promotion of cell growth or induction of differentiation, to cytotoxicity by activation of cell death (mainly apoptosis). The members of TNFLs and TNFRs are summarised in Table 1 with additional information on their cellular origins and recruited intracellular proteins (see also subsequent sections). Whilst, to date, 18 ligands and 29 receptors have been identified, in this review we will mainly focus on receptors and ligands of the Lymphotoxin (LT) system, and compare them with other closely related TNFSF systems that share structural and particularly functional similarities. As described above, the discovery of LT $\alpha$  came shortly after the discovery of TNF $\alpha$ . Both ligands are able to interact with both TNFR –I and –II receptors, albeit with different affinities. Unlike other TNFRs such as Fas, TRAIL-R or CD40 which almost exclusively have a single cognate ligand, the LT system is far more complex with ligands LT $\alpha$ , LT $\beta$ , LT $\alpha$ 1 $\beta$ 2, LT $\alpha$ 2 $\beta$ 1, and LIGHT being able to interact with the two main transmembrane receptors LT $\beta$ R and HVEM, the TNFRs –I and –II as well as the soluble receptor DcR3 (however the DcR3 receptor will not be discussed in this review).

### 2.1 TNFLs

TNFLs are type II transmembrane proteins that contain an intracellular N-terminus and extracellular C-terminus with the C-terminus region characterised by a conserved TNF homology domain (THD) (2, 3). TNF $\alpha$  is expressed in full-length on the cell surface as a 26kDa membrane ligand (mTNF $\alpha$ ) and as a 17kDa soluble cytokine (sTNF $\alpha$ ) after shedding. By contrast, LT $\alpha$  is always shed as a soluble cytokine, yet LT $\beta$  is expressed only in a membrane-bound form, as the latter does not contain a cleavage site. LT $\alpha$  and LT $\beta$  can assemble together and form two membrane bound complexes of LT $\alpha$  $\beta$ ; the LT $\alpha$ 1 $\beta$ 2 complex

consists of a single  $\beta\beta$  and two unique  $\alpha\beta$  sites and exhibits high affinity to LT $\beta$ R; LT $\alpha$ 2 $\beta$ 1 on the other hand binds to TNFR –I and TNFR –II, but with less affinity to LT $\beta$ R. LIGHT, also known as TNFSF14 or TL4, exists also in either membrane form (29kDa) or a soluble form following cleavage by a yet undefined furin-like proteinase and can bind both LT $\beta$ R and the HVEM receptor, but not TNFR –I and TNFR –II (Figure 1).

## 2.2 TNFRs

The majority of the TNFRs are type I transmembrane glycosylated proteins with an extracellular N-terminus and an intracellular C-terminus, although some TNFRs are type III transmembrane proteins (BCMA, TACI, BAFFR and XEDAR (3)). Structurally, TNFRs can be divided into three regions; an extracellular domain (ECD), a transmembrane domain (TMD) and an intracellular domain (ICD). All TNFRs share a four cysteine-rich domain (CRD) in the ECD region, which is responsible for the specificity and affinity of these receptors for their cognate ligands.

In order to initiate TNFR-mediated cell signalling, TNFLs (either in soluble form or in membrane-bound form on the surface of an effector cell) induce TNFR clustering, aggregation and oligomerisation (cross-linking) on target cells. Signals are generally accepted to require trimeric ligands to achieve receptor trimerisation (as shown in Figure 1). However, TNFRs may not exist as monomers that trimerise only upon ligand binding; there is in fact evidence for the existence of a pre-ligand assembly domain (PLAD) residing within the CRD of the receptors, which appears to be critical for the function of the receptor (4). Typically, once the receptor is activated, signal transduction is triggered via recruitment of adaptor proteins in order to ultimately activate transcription factors such as NF- $\kappa$ B or c-Jun N-terminal kinase /activator protein-1 (JNK/AP-1) for induction of either proliferation, differentiation or, more typically for a large proportion of the TNFSF, cell death (Figure 1). The precise nature, timing and extent of adaptor protein recruitment and overall TNFR activation are dependent on several factors, such as cell type, cellular context/state, and quality of the signal i.e. the strength of ligand-receptor interaction (discussed further in subsequent sections). These factors are critical for adaptor protein recruitment and intracellular signalling and thus functional outcome.

## 2.3 TNFR sub-groups

TNFRs can be divided into three sub-groups based on the specific structural features that they contain within their ICD (5). An important characteristic defining the first and more classical TNFR group is that the ICD contains a death domain (DD) and this includes TNFRI, Fas and TRAIL-Rs (Table 1). The DD permits signalling initiation via adaptor protein recruitment; these adaptor proteins are modular, as they contain a DD (that allows interaction with the receptor) as well as a death effector domain (DED). Examples of such proteins include the TNFR-associated DD (TRADD) for TNFR-I and Fas-associated DD (FADD) for Fas and TRAIL-Rs. The existence of the DED permits induction of apoptotic signalling via recruitment of DED-comprising initiator pro-caspases, in particular caspase-8 and in some cases caspase-10 (5-7).

The second TNFR group is characterised by the presence of another type of domain known as the TRAF-interacting motif (TIM). Receptor activation leads to recruitment of TNFR-associated factors (TRAFs), which are zinc RING finger proteins with a C-terminal region responsible for receptor binding, and these adaptors mediate recruitment and/or activation of downstream signalling pathways, mainly Mitogen Activated Protein Kinases (MAPKs) such as p38 and JNK resulting in activation of transcription factors (TFs) such as NF- $\kappa$ B and AP-1. To date, seven TRAF proteins have been identified and different TNFRs rely on distinct signalling pathways mediated by different TRAFs following receptor activation (8).

NF- $\kappa$ B is the best studied TF and is a critical transcriptional activator of many genes involved in innate and adaptive immunity, inflammatory responses, as well as development and maintenance of the immune system. The NF- $\kappa$ B family includes Rel (c-Rel), RelA (p65), RelB, NF- $\kappa$ B1 (p50) and NF- $\kappa$ B2 (p52) and activates two pathways; the classical (canonical) and alternative (noncanonical) (detailed in Figure 1). In the context of the TNFSF, the canonical pathway often mediates inflammatory responses while the noncanonical pathway is involved in immune cell proliferation, maturation and is responsible for secondary lymphoid organogenesis (reviewed in (9)).

The third group of TNFRs is characterised by the lack of intracellular signalling function, due to the lack of an ICD in their cytoplasmic region. Yet, by maintaining the capacity to bind to TNFLs, these receptors act as decoy receptors and attenuate TNFR signalling. The best characterised such receptors are the TRAIL decoy receptors TRAIL-R3 (DcR1) and -R4 (DcR2) (10, 11) (Table 1).



## 2.4 Regulation of TNFL and TNFR function by shedding

Decoy receptors are not the only factor negatively modulating TNFR activation, because regulation of receptor signalling involves shedding of both ligands and receptors into soluble forms (Figure 2) by the action of a family of metalloprotease known as sheddases, and often and this phenomenon is associated with attenuation of ligand-mediated receptor activation. Such enzymes include disintegrin and metalloproteinase (ADAM-17), and the latter is also referred to as TNF-alpha-converting enzyme (TACE) and was originally identified for its ability to shed membrane (mTNF $\alpha$ ) to soluble ligand (sTNF $\alpha$ ) (12). Importantly, cleavage of both mTNFRs and mTNFLs into soluble forms limits the bioavailability and thus concentrations of TNFLs and their respective TNFRs and this may have a direct impact on functional outcome.

## 3. The TNF $\alpha$ system: ligand-receptor interactions and intracellular signalling

TNF $\alpha$  is either secreted or maintained as a membrane-bound ligand by various immune and non-immune cell types including natural killer cells (NK), neutrophils, macrophages, monocytes, T cells, mast cells and granulocytes, as well as neurons, keratinocytes, smooth muscle cells, fibroblasts, endothelial cells and some malignant non-lymphoid cell lines (13), where it can activate multiple signalling transduction pathways. TNF $\alpha$ , the main ligand for the TNFR –I and –II receptors, is the archetypal pro-inflammatory cytokine and is a highly pleiotropic factor that plays critical roles in a variety of physiological mechanisms (13, 14). An intriguing feature of the TNF $\alpha$ /TNFR-I/II system is that differential receptor expression and/or receptor activation by TNF $\alpha$  can regulate the balance between cell survival and apoptosis (15).

TNF $\alpha$  was found to act as an inducer of apoptotic cell death during the maturation of mice thymocytes (16, 17). *In vitro*, TNF $\alpha$  mainly has either growth inhibitory or cytotoxic effects in some normal cell types and in human tumour cells, but in most cases tumour cell lines are sensitive to TNF $\alpha$  only when the protein synthesis was inhibited using cycloheximide (CHX) (18). TNF $\alpha$  can induce both necrotic and apoptotic cell death in mouse fibroblasts *in vitro* and *in vivo* (19). In particular, recombinant TNF $\alpha$  causes growth inhibition or cytotoxicity in a number of cell lines of human and murine origin such as human carcinoma cell lines ME-180 (cervical) and murine fibroblasts (L929). However, not all cells

respond to TNF $\alpha$  by apoptosis. In fact, normal skin (Detroit 551) and lung fibroblasts (WI-38) were resistant and their growth was actually stimulated by TNF $\alpha$  (20-22), which is also observed in some carcinoma cells types such as those of the bladder, where TNF $\alpha$  can be growth-promoting (23). Of note are studies demonstrating that membrane-bound TNF $\alpha$  induces stronger signalling via TNFR –I and –II compared with its soluble counterpart, which can fundamentally alter the functional outcome of receptor activation (24, 25). Therefore TNF $\alpha$  exhibits both cell type- and context-specificity and TNF $\alpha$ -mediated signalling can have highly pleiotropic effects.

TNFRs –I and –II are mainly activated by TNF $\alpha$  although receptor ligation can be induced by soluble LT $\alpha$ 3 (due to its highly homologous structure to TNF $\alpha$ ) and by LT $\alpha$ 2 $\beta$ 1 (see also following sections). TNFRI is constitutively expressed on most nucleated cell types but is predominantly found on cells of epithelial and fibroblast origins. Overall, TNFRI has a greater abundance than TNFRII, with TNFRII mainly expressed on monocytic, lymphocytic, myeloid, hematopoietic, endothelial and neuronal cells. During inflammation, both TNFRI and TNFRII receptors can be quickly shed into soluble receptors believed to be important in down-regulating the inflammatory effects of TNF $\alpha$  and these sTNFRs have been detected in human urine and blood serum of cancer patients (26).

Activation of TNFR –I and –II induces distinct signalling pathways; for instance, it has been demonstrated that the stimulation of individual TNFR –I or –II on mouse thymocytes and cytotoxic T cell line CT-6 by murine TNF (but not human TNF) induced differential effects in these cells (27). Agonistic antibodies specific for TNFRI caused cytotoxicity whereas antibodies for TNFRII failed to reciprocate this. Moreover, TNFRII stimulation triggered cell proliferation (27). Through its DD and TRADD, TNFRI activation can activate the caspase-mediated pathway of apoptosis in numerous tumour cell lines (28). Activation of TNFRI by TNF $\alpha$  also induces the activation NF- $\kappa$ B (29) and this is a negative regulator for apoptosis mediated by TNFRs signalling (30, 31). It has been shown that cell death could be augmented by the inhibition of NF- $\kappa$ B after TNF $\alpha$  treatment or the specific activation of TNFRII (32). Pham and colleagues further demonstrated soluble TNF $\alpha$ -induced apoptosis in NF- $\kappa$ B-deficient cells (33) which was due to JNK activation. TNFRI also contains a TIM domain which interacts with TRADD, TRAF1 and TRAF2 and this triggers the activation of receptor interacting protein kinase (RIP). RIP and TRAF2 form a complex with TRADD in order to induce either MAPKs which lead to NF- $\kappa$ B, or c-Jun N-terminal kinase (JNK)/activator protein-1 (AP-1) activation (11). RIP is a critical player that participates in

various biological processes for intracellular and extracellular stresses and is found to stimulate TNF-induced necrosis (34-37).

TNFR-II binds to mTNF $\alpha$  with high affinity, thus it has been suggested that there must be cell-cell contact in order for TNF $\alpha$  to activate TNFR-II effectively. TNFR-II also recruits TRAF1 and TRAF2, and the latter plays a critical role in activation of IKK and stress kinases JNK and p38. Activation of these is regulated by reactive oxygen species (ROS) release, which can occur either from NADPH oxidase (Nox) or following mitochondrial disruption (36, 38-46). Notably, ROS induction can trigger both pro-survival and pro-apoptotic signals; low levels of ROS activate NF- $\kappa$ B and cell survival, yet in high amounts ROS appear to activate JNK and cause apoptosis or necrosis. Reports indicate that ROS is found to also be a critical upstream component for the activation of MAPKs, NF- $\kappa$ B and AP-1 and caspases, and thus many TNF-induced responses supported by findings that ROS regulate TNF- $\alpha$ -induced apoptosis/necrosis (47). In fact, JNK can induce necrosis mediated by TNF-stimulation by ROS augmentation, thus JNK may control the balance of TNF-stimulated cell apoptosis *versus* necrosis (48). More recently mTNF $\alpha$  was shown to be highly cytotoxic to carcinoma cells due its ability to cause ROS-mediated necrosis (49). Interestingly, it was confirmed that apoptosis was driven by mTNF $\alpha$ -mediated ligation of TNFR -II and not -I (24, 49). Moreover, it has been reported that the inhibition of either ROS or JNK activity prevents the release of mitochondrial cytochrome c and caspase-3 cleavage in response to TNFR-I activation, thus demonstrating that ROS are important regulatory molecules in TNF $\alpha$ -mediated apoptosis. It should however be noted that a number of previous reports indicated that JNK activation is not always essential in TNF-induced apoptosis (47, 48, 50, 51).

## **4. The Lymphotoxin system: ligand receptor interactions and cell signalling**

### **4.1 LT receptors**

#### **4.1.1 LT $\beta$ R**

LT $\beta$ R is activated by three ligands which are the two heterotrimeric LT $\alpha\beta$  complexes and the homotrimeric LIGHT (31). This receptor is mainly expressed on stromal fibroblasts, epithelial cells, monocytes, DCs and mast cells but is absent on lymphocytes (52, 53). Expression of LT $\beta$ R by stromal cells in the intestine is important for normal production of IgA

after antigen recognition. Constitutive LT $\beta$ R-mediated signalling leads to the development of autoimmune disease, including Sjogren's disease and experimental autoimmune encephalomyelitis (EAE). Moreover, LT $\alpha^{-/-}$  and LT $\beta$ R $^{-/-}$  mice demonstrated a reduction in chemokine and adhesion molecule expression within lamina propria lymphocytes (54). Blocking of LT pathways in normal adult mice using soluble receptor-immunoglobulin fusion protein (LT $\beta$ R-Ig) caused inhibition of splenic germinal centre formation and defective humoral responses (55). Thus, such studies using genetically modified mice indicate that LT $\beta$ R is a key molecule involved in lymphoid organogenesis and in adaptive humoral immunity. Two studies using a fusion protein to inhibit LT $\beta$ R signalling through LT $\alpha$ 1 $\beta$ 2 and LIGHT attenuation, have also shown that LT receptors regulate the normal development lymph nodes of mice offspring (56, 57). More recent studies have indicated that little LT $\beta$ R expression is found in normal human colon tissue and adenomas, but receptor expression is increased on colon adenocarcinomas (58), thus indicating that LT receptor expression may increase during carcinogenesis.

Activation of the LT $\beta$ R receptor *in vitro* by either LIGHT mutein (LIGHT-R228E – which is mutated form of LIGHT that preferentially interacts with LT $\beta$ R over HVEM) or agonistic anti-LT $\beta$ R antibody, induces chemokine (IL-8) secretion in HEK293 and 375 melanoma cells and inhibits the growth of 375 melanoma cells, and this appears to be due to differential modulation of the MAPKs signalling molecules ASK1, JNK1/2, AP1, and NF- $\kappa$ B (59-62). Unlike TNFR1 which activates the canonical pathway of NF- $\kappa$ B, LT $\beta$ R can activate both NF- $\kappa$ B pathways (5, 11). Lukashev et al have previously demonstrated that agonistic multivalent pentameric anti-LT $\beta$ R antibody CBE11 can reduce the growth of colon and cervical tumours *in vivo* (63). More recently, Hu et al demonstrated that LT $\beta$ R activation using LT $\beta$ R agonistic antibody BS-1 was found to induce growth inhibition (as well as NF- $\kappa$ B activation) in colon carcinoma cell lines HT29 and CT26, mammary carcinoma 4T1 and soft-tissue sarcoma CMS4 (58). Moreover, BS-1 was able to trigger the activation of caspase -8 and -3 as well as the release of cytochrome c in tumour cells, all of which were mediated by LT $\beta$ R activation; this provides evidence that cell growth inhibition of these tumour cells could be partially driven by caspase-dependent mechanism (58). The aforementioned study also reported that the activation of LT $\beta$ R by using a different monoclonal anti-LT $\beta$ R antibody (ACH6) suppressed the colon carcinoma metastasis *in vivo* (58). These findings are in support of previous work by Browning and colleagues demonstrating that anti-LT $\beta$ R monoclonal antibody alone caused cell death *in vitro* (64). Therefore, signalling through

LT $\beta$ R either by its natural ligands or via agonistic anti-LT $\beta$ R antibodies triggers cell death for a variety of tumour cell lines (64, 65).

The growth of colon and cervical carcinoma cell lines in xenograft models was shown to be inhibited by agonistic antibody LT $\beta$ R (CBE11) and this potentiated tumour responses to chemotherapeutic treatment (63). *In vitro*, however, some contradictory findings have been reported, as the activation of LT $\beta$ R with an agonistic LT $\beta$ R antibody in melanoma cell lines (Hs294T, SKMel5, SKMel28, and WM115) leads to the activation of the NF- $\kappa$ B and enhances tumour cell proliferation (66). Mackay et al reported that activation of LT $\beta$ R with either soluble LT $\alpha$ 1 $\beta$ 2 or agonistic anti-LT $\beta$ R antibody (CBE11) induced activation of NF- $\kappa$ B in HT29 and WiDr human adenocarcinoma lines and human lung fibroblasts WI-38 (67), but Browning et al demonstrated that recombinant LT $\alpha$ 1 $\beta$ 2 together with IFN- $\gamma$  were cytotoxic to an array carcinoma cell lines, including HT29 and WiDr, breast adenocarcinoma cell line (MDA-MB-468) and cervical carcinoma (HT-3) cells (64).

Signal transduction through LT $\beta$ R involves recruitment of adaptor proteins such TRAF-2, -3, and -5 to the cytoplasmic tail of the receptor upon its ligation (65, 68, 69) and these interactions regulate TF activation. TRAF2 and TRAF5 recruitment led to NF- $\kappa$ B activation, but TRAF3 was found to be a negative regulator for NF- $\kappa$ B activation and associated with induction of cell death, as shown in the tumour cell line HT29 and in human embryonic kidney cells (HEK293T) (70), findings is an accordance with such an effect for TRAF3 in signalling triggered by other TNFSF members (71, 72). A study by Kim and colleagues also demonstrated using HeLa cells *in vitro* that TRAF2 and TRAF3 were recruited following the LIGHT/LT $\beta$ R ligation and their recruitment led to the activation of NF- $\kappa$ B and JNK (73). Bista and colleagues reported that TRAF3 functions as a pro-survival molecule during LT $\beta$ R activation, through canonical and noncanonical NF- $\kappa$ B function. In fact, LT $\beta$ R-induced signalling complexes enhanced TRAF3 recruitment, but decreased TRAF2 recruitment which attenuated the phosphorylation of I $\kappa$ B $\alpha$  and RelA genes of NF- $\kappa$ B (74). Moreover, Chen and colleagues demonstrated using human hepatoma cells (Hep3BT2), HeLa and HEK293 cells that the activation of LT $\beta$ R by using either LIGHT mutein (LIGHT-R228E) or agonistic monoclonal antibody anti-LT $\beta$ R (clone 31G4D8) led to the recruitment of TRAF3 and TRAF5 and the production of ROS, which in turn activated apoptosis signal-regulating kinase-1 (ASK1) to induce caspase-dependent and caspase-independent LT $\beta$ R-mediated death (59). Furthermore, it has been reported that a mixture of cell death features was observed following LT $\beta$ R activation and this depended on cell type; for example, apoptosis was

observed in the fibroblastoid line WEHI164, whereas a mixed type of cell death (apoptosis and necrosis) was seen in HT29 cells following LT $\beta$ R activation (75).

#### 4.1.2 HVEM

Another receptor that binds with LIGHT and LT $\alpha$  is the herpesvirus entry mediator (HVEM). This receptor is expressed by lymphoid and non-lymphoid cells, but primarily it is expressed transiently by NK cells and constitutively on naive CD4<sup>+</sup> and CD8<sup>+</sup> T cells. It is reported that most B cell malignancies express HVEM and these include those of B-chronic lymphocytic leukaemia (B-CLL), mantle cell lymphoma, acute lymphoblastic leukaemia (ALL) and Burkitt's lymphoma. HVEM is also expressed by all primary myeloma cells and in plasma cell leukaemia (31). HVEM has dual roles acting as both a receptor and as ligand and it was first described as a receptor for herpes simplex virus-1 (HSV-1) glycoprotein D (HSV-gD), the main component of the HSV envelope for entry into human and mouse cells (76, 77). HVEM functions as ligand and binds the immunoglobulin (Ig) superfamily members B and T lymphocyte attenuator (BTLA) and CD160 (glycosylphosphatidylinositol-anchored member of immunoglobulin (Ig) domain protein), and expressed by many immune cells as reviewed recently elsewhere (78).

It has been demonstrated that engagement of soluble LIGHT with HVEM-expressing U937 cells induced a weak increase in NF- $\kappa$ B activity (79). Overexpression of HVEM in 293 cells enhances the recruitment of adaptor proteins, TRAF -1, -2, -3, and -5, which resulted in activation of NF- $\kappa$ B and AP-1 activation. By contrast, other studies demonstrated that HVEM activation more likely recruits TRAF2 and TRAF5, which are key mediators for the activation of NF- $\kappa$ B as well as AP-1 (68, 73, 80, 81). Pasero and colleagues demonstrated that LIGHT-mediated HVEM signalling is able to induce cell death in freshly isolated B-CLL tumour cells, while LT $\beta$ R was not expressed or expressed at low levels. The mechanisms responsible for cell death in the B-CLL tumour cells related to expression of FasL, p53, Bax, Bid, Bcl-Xs and mitochondrial cytochrome C release (82). Interestingly, it was found that broad caspase inhibition via z-VAD-FMK did not prevent apoptosis, suggesting that both intrinsic and extrinsic pathways of apoptosis were active (82-84).

This data suggests that despite the co-stimulatory and co-inhibitory role for HVEM during immune regulation, HVEM could in fact function as a tumour suppressor if utilised in the correct context. However, there is evidence to suggest that the way HVEM modulates

cell fate might be indirect and more complex than the aforementioned studies suggested (discussed in more detail in the following sections).

## 4.2 LT ligands

### 4.2.1 LT $\alpha$

LT $\alpha$  is often used as a term to describe the biologically active trimer LT $\alpha$ 3 (31, 85), but can exist in three different forms; soluble homotrimeric LT $\alpha$ 3, or as two transmembrane heterotrimeric complexes termed LT $\alpha$ 1 $\beta$ 2 and LT $\alpha$ 2 $\beta$ 1. LT $\alpha$ 1 $\beta$ 2 and LT $\alpha$ 2 $\beta$ 1 complex formation occurs when soluble LT $\alpha$  binds to LT $\beta$  bound on the cell membrane, thus signalling via LT $\alpha$  $\beta$ -LT $\beta$ R interaction requires target-effector cell contact. In addition to binding to TNFR (Figure 1), LT $\alpha$ 3 may bind HVEM, although this binding has been reported to be with low affinity (86). LT $\alpha$  is secreted by activated lymphocytes, resting B cells, non-hematopoietic and myeloid lineage cells. Like TNF $\alpha$ , LT $\alpha$  secretion has been found in some immortalised T cell lines including Jurkat and Hut78 (87). It is also found to be secreted following stimulation of Raji B lymphocytes with phorbol ester (88). It has been shown that when LT $\alpha$  is mutated at either D50N or Y108F, it will only remain as a homotrimer which is not able to bind TNFR1 or TNFR2 and is not able to induce HT29 cell apoptosis. The modified LT $\alpha$  ligand, however, co-assembled with LT $\beta$  and formed a stable ligand heterotrimer complex named LT $\alpha$  $\beta$ , which was functionally active and able to trigger cell death in the adenocarcinoma cell line due to a capability to bind LT $\beta$ R (89). In addition, Browning and colleagues reported that the LT $\alpha$ 1 $\beta$ 2 with mutated LT $\alpha$  was functionally active on HT29 and WiDr cells (64).

### 4.2.2 LT $\beta$ and LT $\alpha$ $\beta$ complexes

The non-cleavable membrane LT $\beta$  ligand is active when homotrimeric and it ligates with LT $\beta$ R (89). LT $\beta$  is known to be expressed in splenic naive B cells in the adult spleen, CD4<sup>+</sup> T cells, and mature DCs. Evidence suggests that the expression of LT $\beta$  on these lymphocytes enhances the immune response, and is also responsible for antiviral immunity on non-lymphocytes by facilitating antigen presentation by APCs (90). LT $\beta$  remains largely under-researched, not only perhaps due to its lack of malignant cell toxicity, but because it mainly assembles with LT $\alpha$  in order to form membrane stable complexes of LT $\alpha$  $\beta$  (89).

As in the case of LT $\beta$ , the effects of LT $\alpha\beta$  complexes (via LT $\beta$ R activation) remain relatively under investigated, despite the ability of LT $\alpha\beta$  ligands to induce cytotoxic effects *in vitro* and *in vivo* (64, 89). The expression of both LT $\alpha$ 1 $\beta$ 2 and LT $\alpha$ 2 $\beta$ 1 complexes is regulated by IL-2, which leads to their induction on human peripheral blood T cells (88). Interestingly, LT $\alpha\beta$  ligands exhibit differential receptor binding specificities due to the differences in their stoichiometry (31). In murine studies, LT $\alpha\beta$  expression shows induction on splenic T cells in response to the cytokines IL-4 and IL-7 and the chemokines CCL19 and CCL21 (91, 92), but it is still not yet reported how this relates to humans. The interaction of LT $\alpha\beta$  with its receptor LT $\beta$ R is also important for the maintenance of the gut-associated lymphoid tissues (GALT), including, lymph nodes and Peyer's patches and also for the formation of germinal centres (31, 87). This suggests that the system is important in normal development and immune regulation following adulthood. Some studies have looked the importance of the LT $\alpha\beta$  complexes in signal transduction (31, 93-96). Of the two types of LT $\alpha\beta$  membrane complexes, recombinant LT $\alpha$ 1 $\beta$ 2 was able to induce cell death in a range of carcinoma cell lines in the presence of IFN- $\gamma$  (64).

### 4.2.3 LIGHT

Lymphotoxin-like exhibits Inducible expression and competes with herpes simplex virus Glycoprotein D for HVEM, HVEM being a receptor expressed on T lymphocytes (LIGHT) was identified and classified as a TNFL member when it showed sequence homology with TNF $\alpha$  (27%), LT $\alpha$  (27%), LT $\beta$  (34%), FasL (31%) and CD40L (26%) (86). LIGHT is constitutively expressed on myeloid cells, primary immature DCs and its expression can be induced on the surface of activated T cells and macrophages (79, 86, 97). LIGHT can ligate both LT $\beta$ R and HVEM receptors (and can bind to soluble receptor DcR3) to regulate cell proliferation, differentiation and growth inhibition (see below). The interaction of LIGHT with LT $\beta$ R and HVEM plays an important role in the induction of positive co-stimulatory signals between immune cells as reviewed in detail elsewhere (98, 99). LIGHT also plays a crucial role in regulating gene expression in innate and adaptive immune system against pathogens but also conversely may be linked to disease (autoimmunity and cancer) (reviewed in (87)).

Work in transgenic mice showed that LIGHT is important for T cell proliferation and in regulation of T cell homeostasis (100). Two studies *in vitro* showed that LIGHT induces T cell proliferation, IFN- $\gamma$  secretion and NF- $\kappa$ B activation (101, 102). LIGHT also cooperates with CD40 ligand (CD154) contributing to DC maturation (103-105). LIGHT induces the



expression of chemotactic molecules CCL21 and adhesion molecule (MAdCAM-1) and also MIG/CXCL9 and IP-10/CXCL10 most likely via LT $\beta$ R signalling (106-109). The release of MIG and IP-10 possibly reduces tumour angiogenesis and enhances the infiltration of activated tumour antigen-specific T cells, which may lead to tumour regression (102, 107). A study by Petreaca and colleagues demonstrated in a cutaneous wound-healing model that LIGHT promotes apoptosis in local macrophages via LT $\beta$ R in order to reduce inflammation (110). Conversely, however, LIGHT may enhance severe inflammation in non-lymphoid tissues (31). *In vivo*, tumours expressing LIGHT have been reported to undergo autocrine LIGHT mediated apoptosis thus LIGHT overall has a tumour suppressive effect (111). Other studies in mice have also shown that the expression of LIGHT caused activation of localised NK cells and the infiltration of cytotoxic CD8 T (CTL) which assisted tumour eradication (107, 112). Transfected murine fibrosarcoma with stable membrane LIGHT resulted in tumour rejection and eradication *in vivo* and LIGHT expression enhanced the anti-tumour response mainly by priming T cells (101, 107).

Soluble LIGHT can trigger apoptosis of human tumour cells *in vitro* but this appears to require the presence of IFN- $\gamma$  (111). The combination of LIGHT/IFN- $\gamma$  in fact has the capacity to cause apoptosis of p53-normal and p53-deficient HT29 adenocarcinoma cells (113), MDA-MB-231 breast cancer cells (114), caspase-3 deficient MCF-7 breast cancer cells and human hepatoma cells (59, 101). It was recently reported that LIGHT treatment triggers the activation of caspase-3 with concomitant down-regulation of anti-apoptotic protein Bcl-2 in HCT116 colorectal carcinoma cells (115). This is in support of previous studies suggesting that the LIGHT/IFN- $\gamma$  combination induces apoptosis via down-regulation of anti-apoptotic Bcl-2 family members, where the contribution of the Bcl-2 families (pro-apoptotic and anti-apoptotic) appears to be cell type-dependent (101, 114). Some studies also demonstrated that LIGHT/IFN- $\gamma$  induced apoptosis may be caspase-independent, as caspase inhibition had little effect on cell death (75). Chen and colleagues also showed that LIGHT treatment combined with IFN- $\gamma$  drives ROS-dependent apoptosis in the human hepatoma line Hep3BT2 (101). There is now more evidence for a role of ROS in LT-related and particularly TNF $\alpha$ -induced apoptosis and ROS-induced death overall is increasingly attracting attention in the context of the TNFSF (116-118).

Interestingly, a number of studies have suggested that the functional outcome of LT $\beta$ R and HVEM receptor activation by LIGHT is dependent on the presence or relative expression levels of these receptors on the target cells. Some elegantly performed studies by Ware and colleagues using an HVEM-selective LIGHT mutant that cannot bind LT $\beta$ R

showed that IFN- $\gamma$  assisted activation of LT $\beta$ R alone is sufficient and necessary for LIGHT-induced apoptosis in HT29 cells, and apoptosis was TRAF3-dependent (65). Interestingly, however, LIGHT-induced growth inhibition occurs in carcinoma cells MDA-MB-231 and HT29 cells which express both receptors LT $\beta$ R and HVEM, and also LIGHT was not cytotoxic to cells that expressed only one of these receptors (111). In that study, LIGHT induced growth inhibition in the prostate cancer cell line PC-3 which only expresses LT $\beta$ R but not HVEM. This evidence highlighted two important points: a) LIGHT may not cause cell death for target cells expressing one of the receptors LT $\beta$ R or HVEM, b) LIGHT engagement with LT $\beta$ R or HVEM may trigger different biological mechanism in target cells (111). A recent *in vitro* study using HeLa and HT29 cells demonstrated that signal transduction (TRAF2 levels and activation of NF- $\kappa$ B) and functional outcome (apoptosis) rely on the relative expression of HVEM and LT $\beta$ R on the target cells (72). This is in line with studies showing that HVEM activation by agonistic antibodies rather than LIGHT acts to promote cell survival (119), whereas LT $\beta$ R signalling by agonists drives cell death or cell survival in a cell type-restricted manner (61, 64, 67). However, such findings contrast previous studies by Pasero et al in cells from patients with chronic lymphocytic leukaemia, which suggested that when HVEM is the primary available receptor, soluble and mainly membrane-presented LIGHT promoted cell death. Interestingly this was found to occur via cross-talk of LIGHT-mediated signalling with other TNFSF members, in particular by induction of endogenous TNF $\alpha$ , which enhanced HVEM mediated cell-death (82).

Therefore, the effect of LIGHT-induced signalling on cell fate appears complex and studies like those discussed above have not only suggested receptor level-related effects, but also, as soluble LIGHT does not bind to other TNFRs, e.g. Fas, DR4, or DR5 shown by *in vitro* binding assays (59), it is possible that indirect signalling (via cross-talk) may be important, too. It may therefore be possible that the HVEM and LT $\beta$ R receptors can signal independently, cooperatively or in an antagonistic fashion in determining cell fate in the context of LIGHT signalling.

## **5. The LT and CD40 ligand-receptor systems**

### **5.1 CD40 and its signalling and functional similarities to LT in regulating cell fate**

In addition to its similarities to the archetypal ligand-death receptor dyads (and particularly TNF $\alpha$ /TNFR-I/II), the LT system demonstrates strong and striking signalling and

functional similarities to the CD40 system. CD40 was first functionally characterised in B cells (although originally identified as an antigen expressed in bladder carcinomas) and shares homology with NGFR (120). CD40 is a type I transmembrane protein with a MW 40-45 kDa and is constitutively expressed on activated T cells, B cells, DCs, APCs, but also at low level on monocytes, platelets as well as fibroblasts, epithelial, endothelial, neuronal cells, and is also found to be expressed by a variety of carcinomas. The ligand of CD40 is CD154 (CD40L), a type II transmembrane protein with MW between 31-39 kDa (121). CD40L is predominantly expressed on activated CD4<sup>+</sup> T cells and B cells, activated APCs (such as DCs) as well as activated platelets (122-124). The CD40/CD40L dyad is critical in cellular and humoral immune responses, and is essential for lymphocyte proliferation as well as differentiation and maturation. CD40/CD40L engagement mediates DC activation and the activated DCs promote the upregulation of other co-stimulatory molecules, for instance B7 family members, resulting in potent production of proinflammatory cytokine in order to enhance productive immune responses.

One interesting characteristic of the CD40 system is that receptor expression is not restricted only to normal cells but it is also expressed in both mouse and human in many tumour cells, including hematological malignancies, such as lymphocytic leukaemia, lymphoma, multiple myeloma, acute myeloid leukaemia (AML) as well as in both non-Hodgkin's lymphomas (NHLs) and Hodgkin's lymphomas. Moreover CD40 is expressed on non-lymphoid cells where its engagement by CD40L contributes to cytokine and chemokine secretion and can also lead to fibroblast and endothelial cell proliferation. Although CD40 expression is relatively low on normal epithelial cells, it is often highly expressed on solid tumours such as melanoma and lung cancers as well as in carcinomas of the nasopharynx, bladder, cervix and in ovarian cancer, although it appears to be absent from most prostate carcinomas (125).

The outcome of CD40/CD40L signalling ranges from proliferation and differentiation to growth inhibition and cell death in a cell type- and context-dependent manner. With regards to its function in tumour cells, CD40 ligation has growth inhibitory effects in ovarian, breast, bladder (urothelial) and colorectal tumour cells *in vitro* when such cells were treated with a soluble form of CD40L or agonistic anti-CD40 antibodies (23, 126, 127). Combination of soluble CD40 agonist with pharmacological protein synthesis inhibitor (cycloheximide) or chemotherapeutic agents (e.g. cisplatin) dramatically enhanced the anti-proliferative properties of CD40 ligation by rendering it pro-apoptotic in various types of carcinomas (23, 128-132).

As in the case of the LT system, due to the lack of intrinsic kinase activity, CD40 signalling starts with the recruitment of adaptor proteins, in particular TRAF1, TRAF2, TRAF3, TRAF5 and TRAF6. It has been shown that TRAF2, TRAF3 and TRAF6 bind directly to cytoplasmic tail of CD40 whereas TRAF1 and TRAF5 are recruited indirectly via interactions with TRAF2 and TRAF3, respectively. Following TRAF recruitment, signalling cascades triggered include the p38 MAPK, AKT, JNK/AP-1, signal transducer and activator of transcription 5 (STAT5) pathways and the activation of canonical and noncanonical pathways of NF- $\kappa$ B. The activation of such cascades is dependent and attributed to the precise TRAF protein recruitment pattern. In B cells, for instance TRAF2 and MEKK1 recruitment activates the JNK, p38 mitogen activated protein kinase (MAPK) and AKT pathways (133-135), whereas TRAF5 and TRAF3, are found to be required for canonical and noncanonical pathways of NF- $\kappa$ B activation (71, 136, 137). Ishida and colleagues demonstrated that recruitment of TRAF5 via CD40 mediates the activation of NF- $\kappa$ B (138). Studies in epithelial cells, where CD40 signalling is less well characterised, demonstrated the importance of the presence of TRAF6 in activation of canonical NF- $\kappa$ B, p38, JNK and AKT following CD40 ligation and its role in anti-apoptotic signalling (139, 140). By contrast, other TRAFs such as TRAF3 have been implicated as mediators of CD40-induced apoptosis (127, 141). Overall, a number of signalling and functional properties appear to be shared by CD40 and LT $\beta$ R. It has been reported that these receptors are able to induce the maturation and immunogenic activity for DCs, and this is because they share similarities in activating adaptor proteins as part of their intracellular signalling, e.g. TRAF3 induction activates the noncanonical NF- $\kappa$ B pathway.

Activation of LT $\beta$ R following binding of ligands LT $\alpha\beta$  and LIGHT, or using other soluble agonists, induced rapid LT $\beta$ R signalling via recruitment of TRAF2, TRAF3 and TRAF5 (69, 73). It has been suggested that the role of TRAF2 is important for the activation of NF- $\kappa$ B pathways (73, 81), whereas JNK activation and cell death induction through LT $\beta$ R signalling was mediated by TRAF3. Studies using HeLa cells showed that recruitment of TRAF2 and TRAF3 and activation of NF- $\kappa$ B are triggered after LT $\beta$ R activation by LIGHT. Recruitment of TRAF2 and TRAF3 through LT $\beta$ R signalling was found to be important with TRAF3 being critical in apoptosis (65, 70), but JNK activation in this context has not been reported. Moreover, two studies demonstrated that TRAF3 knockdown leads to the increase of protein expression of noncanonical pathway NF- $\kappa$ B members, e.g. p100/RelB, RelB, and stimulation-independent activation of NIK. NIK is a kinase required for the noncanonical pathway activation, and the phosphorylation-mediated processing of p100/RelB into active

form p52/RelB (74, 142, 143). NIK may act as either a pro-survival mediator by activation of NF- $\kappa$ B or as a negative regulator for TRAF2 and cellular inhibitors of apoptosis 1 and 2 (cIAP1 and cIAP2) and mediate anti-apoptotic functions (144). These observations indicate a similarity of LT $\beta$ R signalling to other TNFRs (mainly CD40 and BAFF-R), where TRAF2 functions as an inducer but TRAF3 acts as inhibitor of NF- $\kappa$ B (92, 133); yet, the precise functional roles of TRAFs in LT $\beta$ R signalling remain relatively unexplored. Moreover, HVEM can signal through binding to TRAF1, 2, 3 and 5 in epithelial cells (80). It was reported that the overexpression of HVEM contributes to the activation of JNK but signal transduction via HVEM activation is not fully understood (80, 145).

It is therefore clear that the LT system shares functional similarities to other TRAF-recruiting receptors and particularly CD40, as it can recruit one or more similar adaptor proteins with differential functional effects. Although more work is required to fully characterise such functional similarities, it is clear that understanding the precise molecular signature of LT and CD40 triggered signalling cascades and comparing their precise nature may decipher the underpinning mechanisms of the observed differential functional outcomes.

## **5.2 The importance of LT receptor and CD40 cross-linking in determining functional outcome**

One fundamental property of the TNFSF that despite its clear importance is very rarely highlighted relates to how “signal quality” (i.e. the degree of receptor activation or cross-linking) affects or determines the outcome of receptor ligation. There is a plethora of reports in the literature clearly indicating that highly cross-linked agonistic antibodies, cross-linked soluble recombinant ligands and particularly membrane-presented ligand (achieved by co-culture of target cells with growth-arrested, ligand-expressing third-party cells) induce a greater extent of carcinoma cell death *in vitro* in comparison to non-cross-linked agonists.

Studies in the CD40 system have demonstrated how ligand valency, and consequently the extent of receptor cross-linking, can dictate cell death against survival signals (23). Specifically in carcinoma cell lines, membrane-presented CD40 ligand (mCD40L), but not soluble agonists (e.g. sCD40L), induces high level of pro-inflammatory cytokine secretion and causes extensive cell apoptosis (7, 23, 146), whilst remaining a tumour-cell specific death signal (23, 147). This is not a unique property of CD40, for

instance it has been reported that mTNF $\alpha$  generates higher cytotoxicity than soluble ligand in certain tumour cell lines leading even to necrotic cell death (24).

The importance of degree of TNFR cross-linking in determining functional outcome (23, 148) is also clear in LT-specific signalling. Triggering cell signalling through the activation of LT $\beta$ R and HVEM has been demonstrated using various agonist formats: agonistic antibodies, soluble recombinant ligands (LIGHT or LT $\alpha\beta$ ) or ligation by membrane-presented ligand LIGHT. The majority of previous studies have focused on the activation of LT $\beta$ R, and to a lesser extent on HVEM, by soluble agonists and although activation of LT receptors by membrane-bound ligand (and particularly LIGHT) remains under-investigated, there are some studies that have utilised membrane-presented LT ligands.

It is well established in carcinoma cell lines that cross-linking of LT $\beta$ R in Hep3BT2, HeLa and HEK293 cells by soluble mutein LIGHT (LIGHT-R228E), which activates LT $\beta$ R, and agonistic anti-LT $\beta$ R monoclonal antibody (clone 31G4D8) were able to induce cell death in these cells (59, 65). Degli-Esposti and colleagues reported that cross-linking of LT $\beta$ R with immobilized agonistic anti-LT $\beta$ R monoclonal antibody (M12) induced secretion of IL-8 and RANTES in A375 cells, but not cell death, and similar observations were made with membrane-bound LT $\beta$  and LT $\alpha\beta$  (ligands for LT $\beta$ R) (61). On the other hand, Browning and colleagues demonstrated that immobilised agonistic monoclonal antibody anti-LT $\beta$ R (CBE11) induced cell death efficiently for HT29, WiDr MDA-MB-468 and HT-3 better than when the agonist was added to cultures in non-cross-linked form (64). Importantly, the activation of LT $\beta$ R was more enhanced when the same agonistic antibody was engineered and converted into an IgM-like oligomer and thus delivered in pentameric form (CBE11p). The pentameric agonistic antibody was shown to inhibit cell proliferation and induced cell death for HT29 in the presence or absence of IFN- $\gamma$  to a greater extent than did the monomeric mAb form CBE11 (63).

Interestingly, soluble recombinant LT $\alpha$ 1 $\beta$ 2 (another ligand for LT $\beta$ R) was toxic when combined with IFN- $\gamma$  in adenocarcinoma cell lines (64) and these studies by Browning and colleagues using different cross-linked forms of LT ligands (e.g. LT $\alpha$ 1 $\beta$ 2) and antibodies for LT $\beta$ R activation have provided some evidence for the importance of the degree of receptor cross-linking in functional outcome for a number of cell lines *in vitro*. Of note also, there is evidence that cross-linking of HVEM receptor in CLL-derived cells (showing weak or no expression of LT $\beta$ R) with agonistic antibody could induce downstream signalling involving pro- and anti-apoptotic proteins, which was more enhanced when LIGHT was presented in a

membrane-bound form (82). Moreover, recent studies by Bechill et al have demonstrated that LT $\beta$ R and HVEM in HeLa and HT29 cells activated by membrane-bound LIGHT (via target cell co-culture with CHO cells expressing LIGHT ligand) or mutant LIGHT (LIGHT-R228E) in the presence of IFN- $\gamma$  induced high levels of secretion of the CXCL10 chemokine (72).

An interesting, yet related, aspect of the LT system is the clear requirement for synergy with IFN- $\gamma$  for the induction of apoptosis. There is evidence that when LT receptor activation by LIGHT is combined with IFN- $\gamma$  this enhanced LIGHT cytotoxicity (111), in accordance with studies that cross-linking LT $\beta$ R alone with soluble LIGHT in presence of IFN- $\gamma$  is sufficient to induce cell death (65). The studies by Bechill et al showing that in HeLa and HT29 cells LIGHT/IFN- $\gamma$  induced a higher level of cytokine secretion compared with LIGHT treated cell alone further support this notion (72). Moreover, we now have evidence that cross-linking LT $\beta$ R and HVEM with wild type membrane-presented LIGHT induces extensive cell death in carcinoma cells of various tissue origins in the absence IFN- $\gamma$  compared with soluble agonists (Albarbar and Georgopoulos, unpublished observations).

Therefore, there is an emerging picture that although the activation of LT receptors (LT $\beta$ R and/or HVEM) requires the synergistic action of IFN- $\gamma$  to induce adequate cytotoxicity, membrane-presented agonist may engage the apoptotic pathway more effectively thus negating the need for IFN- $\gamma$  synergy. These observations on the importance of the quality of the signal in determining functional outcome following LT system-triggered signalling demonstrate intriguingly clear parallels with the mode of operation of the CD40/CD40L dyad, where membrane-bound agonist provides a stronger pro-apoptotic signal that overrides anti-apoptotic mechanisms (23, 127).

## 6. Concluding remarks and future perspectives

The role of the TNFLs and TNFRs in regulating cell fate in the immune system as well as in non-lymphoid tissues has been under extensive research over the past three decades. Moreover, the ability of several family members to induce death (mainly via apoptosis) represents a promising target for cancer therapy. Such efforts have focused mostly on death receptors such as TNFRI, Fas (CD95), and TRAIL-R due to their strong pro-apoptotic potential; however lack of tumour cell-specificity represents an obstacle in such therapeutic strategies. The ability of tumour-specific death induction might instead be a feature of the non-classical death receptors, and the LT and CD40 systems might represent better such

targets. However, as shown in this review, it appears that such non-classical TNFR-TNFL dyads demonstrate more complex, cell-type and clearly context-specific capabilities, so it is essential that more studies delve further into the complexities of these systems.

This complexity is evident at the signal transduction level as well as the level of receptor activation. For instance, there is clear difference between the ability of TNF agonists to trigger cell signalling when presented in a soluble versus membrane-bound form. Often soluble agonists lack cytotoxic potency when administered as a single treatment (without synergism by co-treatment with cytokines), yet membrane-presented ligands appear to be superior. And although such studies have been informative to a great extent, the use of a variety of ligand/agonist format (soluble or membrane) between different studies *per se* often makes it difficult to form a collective and unequivocal understanding of the function of these ligands. Moreover, there is often a lack of consistency when it comes to the cell models used to study the functional role of receptor-ligand interactions. This may perhaps explain some of the inconsistencies evident in the literature, some of which we mentioned above. It is thus essential that well-characterised *in vitro* models (e.g. cell lines representative of tissues of origin) are employed to study the role of TNFRs. Equally, it is important that the effect of TNFR signalling is also tested in the normal counterparts of such cells to examine tumour cell specificity.

In conclusion, the LT and CD40 systems display several signalling similarities as well as differences, but as our understanding of a) the signalling requirements, b) the cell-type specificity and c) the role of the quality (strength) of ligand-receptor interactions in determining functional outcome gradually increases, this will not only permit a better understanding of the biological mechanisms via which non DD domain-containing TNFRs induce cell death, but may also allow the design of better, i.e. more efficient, and also ideally tumour-specific therapeutic strategies.



<b>TNFR</b>	<b>Cellular origin</b>	<b>Signal initiation</b>	<b>TNFL</b>	<b>Reference</b>
TNFR1, TNFRSF1A, p55-60, and CD120a, TNFR60 and TNFRSF1A	Nucleated cells and all tissues	DD plus TRAF2,5	TNF $\alpha$ , cachectin, DIF, LT $\alpha$ 3	(149-153)
TNFR2, CD120b, p75-80 and TNFRSF1B	Inducible on immune cells and hematopoietic	TRAF1,2,3	LT $\alpha$ 3, TNF $\alpha$ , LT $\alpha$ 2 $\beta$ 1	(15, 150, 154, 155)
LT $\beta$ R, TNFRSF3, CD18, TNFCR, TNFR3	Fibroblast, epithelial, myeloid cells and most tumour cells	TRAF2,3,5	LT $\beta$ , LT $\alpha$ 2 $\beta$ 1, LT $\alpha$ 1 $\beta$ 2, LIGHT	(68-70, 74, 81, 156, 157)
CD95, APO-1, Fas, TNFRSF6 APT1 and DR2	T and B cells and epithelial cells	DD plus TRAF2	FasL, APT1LG1 CD278, and TNFSF6	(158-161)
DR3, WSL-LR, TRAMP, TR3, LARD, APO-3, DDR3, TNFRSF12	Activated T cells and tissues of thymus, spleen and fetal kidney	DD plus TRAF2	APO-3L, TWEAK, DR3LG, TL1A, TNFSF12	(159, 162-165)
DR4, Apo2, TRAILR1 and TNFRSF10A	Most cells and cell lines	DD	TRAIL, Apo2L, TL2, TNFSF10	(166-170)
DR5, TRAILR2, KILLER, TRICK2 and TNFRSF10B	Most cells and cell lines	DD	TRAIL, Apo2L, TL2, TNFSF10	(167-171)
DR6, TR-7, TNFRSF21	Lymphoid organs, tissues lymphoid cells and tumours	DD plus TRAF2	N.D	(153, 172-174).
DcR1, TRAILR3, TRID, Apo2, LIT and TNFSF10C	Various human tissues	Absent	TRAIL, TL2, TNFSF10	(166, 171, 175-177)
DcR2, TRAILR4, TRUNDD	Various human tissues	Absent	TRAIL, TL2, TNFSF10	(175, 178, 179)
DcR3, TR6, M68, TNFRSF6B	Monocytes, dendritic cells, lung tissues, adenocarcinomas	Absent	FasL, LIGHT, TL1A	(158, 180)
CD27, TNFRSF7, S152 and Tp55	T, B cell and some tumours	TRAF2,3,5	CD27L, TNFSF7 and CD70	(181)
CD30, TNFSF8	Lymphoid cells and some tumours	TRAF1,2,3,5	CD30L	(150)

CD40, GP39, HIGM1, IMD3, TNFRSF5, TRAP	T, B cells and some tumours	TRAF2,3,5,6	CD40L, CD154, CD140, HIGM1, TNFSF5	(136, 138, 150, 182)
OX40, gp34, TNFRSF4, TXGP1L, CD134, ACT35	T cells and some tumours	TRAF1,2,3,5,6	OX40L, TNFSF4, TXGP1	(8, 183-187)
NGFR, P75, P75NGFR, P75NTR, CD271, TNFRSF16	Nervous system, kidney, lung, hair follicles and some tumours	DD plus TRAF1,2,3,4,5,6	NGF, TNFSF16	(188)
AITR, GITR and TNFRSF18	T cells and some tumours	TRAF1,2,3,4,5	AITRL, TL6, hGITRL and TNFSF18	(143, 189-193)
HVEM, HveA, TL1, CD270, TNFRSF14, ATAR, TR2	T cells, lymphoid and non-lymphoid cells, and some tumours	TRAF1,2,3,5	LIGHT, LTα3, CD258, HVEM-L TL4, TNFSF14	(80, 145)
4-1BB, TNFRSF9, CD137 and ILA	T cells and thymocytes	TRAF1,2,3	4-1BBL and TNFSF9	(8, 194-196)
RANK, TRANCE-R, TNFRSF11A	Activated T cells, dendritic cells, lymph nodes	TRAF1,2,3,5,6	RANKL, OPGL, ODF TRANCE, TNFRSF11A, TNFRSF11B	(195, 197-199)

## Table 1

### TNFL and TNFR members

The table summarises all known TNFRs and their cognate ligands, the cell types in which TNFRs are expressed and the adaptor proteins involved in signalling triggered by the receptor in each case. N.D, not determined.

## Figure captions

### Figure 1 : Interactions of TNFLs and TNFRs and associated intracellular signalling pathways

Schematic representation of TNFL and TNFR interactions and associated signalling, with the upper portion showing TNFL expression by an effector cell and lower portion showing TNFR expression by target cell. TNF $\alpha$  can be both either membrane-bound or secreted and binds to and activates TNFRI and TNFRII, whereas LT $\alpha$ 3 exists in soluble homotrimeric form. LT $\beta$  is not shed into soluble form and can bind with LT $\alpha$  to form LT $\alpha\beta$  complexes. Heterotrimeric LT $\alpha$ 1 $\beta$ 2 binds LT $\beta$ R and LT $\alpha$ 2 $\beta$ 1 binds with TNFRI, TNFRII as well as LT $\beta$ R. LIGHT binds LT $\beta$ R and HVEM as well as soluble receptor DcR3. Arrows indicate high affinity interactions, the dotted red arrow indicates possible binding and the dashed lines indicate binding at low affinity. TNFR-mediated signalling is triggered via intracellular proteins associating with either the death domain (as for TNFRI) or a TRAF binding motif (as for CD40, LT $\beta$ R). Members of the TRAF family are indicated: TRAF1 (purple), TRAF2 (black), TRAF3 (green), TRAF5 (red), TRAF6 (blue). The two main signalling axes are JNK and NF- $\kappa$ B (for precise explanations see text). Activation of ROS triggers ASK1 and subsequently cell death which can be either caspase-dependent or independent. Activation of NF- $\kappa$ B may involve canonical (classical) and noncanonical (alternative) pathways. The canonical pathway depends on NIK and activation of trimeric complex of IKK $\alpha\beta\gamma$  and phosphorylation of IKB $\alpha$  to p50/RelA; the noncanonical pathway of NF- $\kappa$ B is dependent on NIK and IKK $\alpha$  and followed by activation of p100/RelB to p52/RelB. The activity of p50/RelA and p52/RelB in the nucleus leads to activation of specific gene transcription.

### Figure 2 : Mechanism of TNFLs and TNFRs shedding

TNFLs (top) and TNFRs (bottom) are expressed in two forms, membrane-bound (via a transmembrane domain anchoring the protein within the cell membrane) or soluble trimeric.

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Signalling via membrane-bound forms of TNFLs requires cell-cell contact to achieve activation of membrane-bound forms of TNFRs to induce receptor trimerisation and trigger intracellular signalling. Soluble TNFLs or TNFRs are membrane-bound forms that had been cleaved into soluble forms by metalloproteinase.

## References

1. Carswell EA, Old LJ, Kassel RL, Green S, Fiore N, Williamson B. Endotoxin-induced serum factor that causes necrosis of tumors. *Proc Natl Acad Sci U S A*. 1975 1975;72(9):3666-70.
2. Idriss HT, Naismith JH. TNF alpha and the TNF receptor superfamily: Structure-function relationship(s). *Microscopy Research and Technique*. 2000 Aug 1;50(3):184-95.
3. Bodmer J-L, Schneider P, Tschopp J. The molecular architecture of the TNF superfamily. *Trends in biochemical sciences*. 2002;27(1):19-26.
4. Chan FK-M. Three is better than one: Pre-ligand receptor assembly in the regulation of TNF receptor signaling. *Cytokine*. 2007 Feb;37(2):101-7.
5. Dempsey PW, Doyle SE, He JQ, Cheng G. The signaling adaptors and pathways activated by TNF superfamily. *Cytokine & growth factor reviews*. 2003 Jun-Aug;14(3-4):193-209.
6. Micheau O, Tschopp J. Induction of TNF receptor I-mediated apoptosis via two sequential signaling complexes. *Cell*. 2003 Jul 25;114(2):181-90.
7. Engels IH, Totzke G, Fischer U, Schulze-Osthoff K, Janicke RU. Caspase-10 sensitizes breast carcinoma cells to TRAIL-induced but not tumor necrosis factor-induced apoptosis in a caspase-3-dependent manner. *Mol Cell Biol*. 2005 Apr;25(7):2808-18.
8. Wajant H, Henkler F, Scheurich P. The TNF-receptor-associated factor family: scaffold molecules for cytokine receptors, kinases and their regulators. *Cellular signalling*. 2001;13(6):389-400.
9. Vallabhapurapu S, Karin M. Regulation and Function of NF-kappa B Transcription Factors in the Immune System. *Annual Review of Immunology* 2009. p. 693-733.
10. Ashkenazi A. Targeting death and decoy receptors of the tumour-necrosis factor superfamily. *Nature Reviews Cancer*. 2002;2(6):420-30.
11. Hehlhans T, Pfeffer K. The intriguing biology of the tumour necrosis factor/tumour necrosis factor receptor superfamily: players, rules and the games. *Immunology*. 2005 May;115(1):1-20.
12. Moss ML, Jin SLC, Milla ME, Burkhart W, Carter HL, Chen WJ, et al. Cloning of a disintegrin metalloproteinase that processes precursor tumour-necrosis factor-alpha. *Nature*. 1997 Feb 20;385(6618):733-6.
13. Aggarwal BB. Tumour necrosis factors receptor associated signalling molecules and their role in activation of apoptosis, JNK and NF-kappa B. *Annals of the Rheumatic Diseases*. 2000 Nov;59:6-16.
14. Beutler B, Cerami A. The biology of cachectin/TNF--a primary mediator of the host response. *Annual review of immunology*. 1989;7(1):625-55.
15. Cabal-Hierro L, Lazo PS. Signal transduction by tumor necrosis factor receptors. *Cellular signalling*. 2012;24(6):1297-305.
16. Hernandez-Caselles T, Stutman O. Immune functions of tumor necrosis factor. I. Tumor necrosis factor induces apoptosis of mouse thymocytes and can also stimulate or inhibit IL-6-induced proliferation depending on the concentration of mitogenic costimulation. *The Journal of Immunology*. 1993;151(8):3999-4012.
17. Giroir BP, Brown T, Beutler B. Constitutive synthesis of tumor necrosis factor in the thymus. *Proceedings of the National Academy of Sciences*. 1992;89(11):4864-8.
18. Ruggiero V, Latham K, Baglioni C. Cytostatic and cytotoxic activity of tumor necrosis factor on human cancer cells. *The Journal of Immunology*. 1987;138(8):2711-7.
19. Kamata H, Honda S-i, Maeda S, Chang L, Hirata H, Karin M. Reactive oxygen species promote TNF $\alpha$ -induced death and sustained JNK activation by inhibiting MAP kinase phosphatases. *Cell*. 2005;120(5):649-61.
20. Sugarman BJ, Aggarwal BB, Hass PE, Figari IS, Palladino MA, Shepard HM. Recombinant human-tumor necrosis factor-alpha - effects on proliferation of normal and transformed-cells invitro. *Science*. 1985 1985;230(4728):943-5.

21. Fransen L, Ruyschaert MR, Vanderheyden J, Fiers W. Recombinant tumor-necrosis-factor - species specificity for a variety of human and murine transformed-cell lines. *Cell Immunol.* 1986 Jun;100(1):260-7.
22. Haranaka K, Satomi N. Cytotoxic activity of tumor necrosis factor (TNF) on human cancer cells in vitro. *The Japanese journal of experimental medicine.* 1981;51(3):191-4.
23. Bugajska U, Georgopoulos NT, Southgate J, Johnson PW, Graber P, Gordon J, et al. The effects of malignant transformation on susceptibility of human urothelial cells to CD40-mediated apoptosis. *Journal of the National Cancer Institute.* 2002;94(18):1381-95.
24. Ardestani S, Li B, Deskins DL, Wu H, Massion PP, Young PP. Membrane versus soluble isoforms of TNF- $\alpha$  exert opposing effects on tumor growth and survival of tumor-associated myeloid cells. *Cancer research.* 2013;73(13):3938-50.
25. Grell M, Douni E, Wajant H, Lohden M, Clauss M, Maxeiner B, et al. The transmembrane form of tumor necrosis factor is the prime activating ligand of the 80 kDa tumor necrosis factor receptor. *Cell.* 1995 Dec 1;83(5):793-802.
26. Engelmann H, Novick D, Wallach D. Two tumor necrosis factor-binding proteins purified from human urine. Evidence for immunological cross-reactivity with cell surface tumor necrosis factor receptors. *Journal of Biological Chemistry.* 1990;265(3):1531-6.
27. Tartaglia LA, Weber RF, Figari IS, Reynolds C, Palladino MA, Goeddel DV. The two different receptors for tumor necrosis factor mediate distinct cellular responses. *Proceedings of the National Academy of Sciences.* 1991;88(20):9292-6.
28. Ashkenazi A, Dixit VM. Death receptors: Signaling and modulation. *Science.* 1998 Aug 28;281(5381):1305-8.
29. Legler DF, Micheau O, Doucey MA, Tschopp J, Bron C. Recruitment of TNF receptor 1 to lipid rafts is essential for TNF $\alpha$ -mediated NF- $\kappa$ B activation. *Immunity.* 2003 May;18(5):655-64.
30. Karin M, Lin A. NF- $\kappa$ B at the crossroads of life and death. *Nat Immunol.* [Review]. 2002 Mar;3(3):221-7.
31. Ware CF. Network communications: lymphotoxins, LIGHT, and TNF. *Annu Rev Immunol.* 2005;23:787-819.
32. Wajant H, Pfizenmaier K, Scheurich P. Tumor necrosis factor signaling. *Cell Death Differ.* [Review]. 2003 Jan;10(1):45-65.
33. Pham CG, Bubici C, Zazzeroni F, Papa S, Jones J, Alvarez K, et al. Ferritin heavy chain upregulation by NF- $\kappa$ B inhibits TNF $\alpha$ -induced apoptosis by suppressing reactive oxygen species. *Cell.* 2004;119(4):529-42.
34. Zhang D, Lin J, Han J. Receptor-interacting protein (RIP) kinase family. *Cellular & molecular immunology.* 2010;7(4):243-9.
35. Kelliher MA, Grimm S, Ishida Y, Kuo F, Stanger BZ, Leder P. The death domain kinase RIP mediates the TNF-induced NF- $\kappa$ B signal. *Immunity.* 1998;8(3):297-303.
36. Hsu HL, Huang JN, Shu HB, Baichwal V, Goeddel DV. TNF-Dependent recruitment of the protein kinase RIP to the TNF receptor-1 signaling complex. *Immunity.* 1996 Apr;4(4):387-96.
37. Lin Y, Choksi S, Shen H-M, Yang Q-F, Hur GM, Kim YS, et al. Tumor necrosis factor-induced nonapoptotic cell death requires receptor-interacting protein-mediated cellular reactive oxygen species accumulation. *Journal of Biological Chemistry.* 2004;279(11):10822-8.
38. Hsu HL, Xiong J, Goeddel DV. The TNF receptor 1-associated protein TRADD signals cell-death and NF- $\kappa$ B activation. *Cell.* 1995 May 19;81(4):495-504.
39. Devin A, Lin Y, Liu Zg. The role of the death-domain kinase RIP in tumour-necrosis-factor-induced activation of mitogen-activated protein kinases. *EMBO reports.* 2003;4(6):623-7.
40. Hsu H, Shu HB, Pan MG, Goeddel DV. TRADD-TRAF2 and TRADD-FADD interactions define two distinct TNF receptor 1 signal transduction pathways. *Cell.* 1996 Jan 26;84(2):299-308.

41. Stanger BZ, Leder P, Lee T-H, Kim E, Seed B. RIP: a novel protein containing a death domain that interacts with Fas/APO-1 (CD95) in yeast and causes cell death. *Cell*. 1995;81(4):513-23.
42. Rothe M, Pan M-G, Henzel WJ, Ayres TM, V Goeddel D. The TNFR2-TRAF signaling complex contains two novel proteins related to baculoviral inhibitor of apoptosis proteins. *Cell*. 1995;83(7):1243-52.
43. Rothe M, Sarma V, Dixit VM, Goeddel DV. TRAF2-mediated activation of NF-kappa B by TNF receptor 2 and CD40. *Science*. 1995 Sep 8;269(5229):1424-7.
44. Park YC, Ye H, Hsia C, Segal D, Rich RL, Liou H-C, et al. A novel mechanism of TRAF signaling revealed by structural and functional analyses of the TRADD-TRAF2 interaction. *Cell*. 2000;101(7):777-87.
45. Westwick JK, Weitzel C, Minden A, Karin M, Brenner DA. Tumor necrosis factor alpha stimulates AP-1 activity through prolonged activation of the c-Jun kinase. *J Biol Chem*. 1994 Oct 21;269(42):26396-401.
46. Fiers W, Beyaert R, Declercq W, Vandenabeele P. More than one way to die: apoptosis, necrosis and reactive oxygen damage. *Oncogene*. 1999 Dec 16;18(54):7719-30.
47. Sakon S, Xue X, Takekawa M, Sasazuki T, Okazaki T, Kojima Y, et al. NF-kB inhibits TNF-induced accumulation of ROS that mediate prolonged MAPK activation and necrotic cell death. *The EMBO journal*. 2003;22(15):3898-909.
48. Lamb JA, Ventura JJ, Hess P, Flavell RA, Davis RJ. JunD mediates survival signaling by the JNK signal transduction pathway. *Mol Cell*. 2003 Jun;11(6):1479-89.
49. Ardestani S, Deskins DL, Young PP. Membrane TNF-alpha-activated programmed necrosis is mediated by Ceramide-induced reactive oxygen species. *Journal of molecular signaling*. 2013;8(1):12.
50. Liu ZG, Hsu H, Goeddel DV, Karin M. Dissection of TNF receptor 1 effector functions: JNK activation is not linked to apoptosis while NF-kappaB activation prevents cell death. *Cell*. 1996 Nov 1;87(3):565-76.
51. Natoli G, Costanzo A, Ianni A, Templeton DJ, Woodgett JR, Balsano C, et al. Activation of SAPK/JNK by TNF receptor 1 through a noncytotoxic TRAF2-dependent pathway. *Science*. 1997;275(5297):200-3.
52. Browning JL, Sizing ID, Lawton P, Bourdon PR, Rennert PD, Majeau GR, et al. Characterization of lymphotoxin-alpha beta complexes on the surface of mouse lymphocytes. *Journal of Immunology*. 1997 Oct 1;159(7):3288-98.
53. Stopfer P, Mannel DN, Hehlhans T. Lymphotoxin-beta receptor activation by activated T cells induces cytokine release from mouse bone marrow-derived mast cells. *Journal of Immunology*. 2004 Jun 15;172(12):7459-65.
54. Kang H-S, Chin RK, Wang Y, Yu P, Wang J, Newell KA, et al. Signaling via LTβR on the lamina propria stromal cells of the gut is required for IgA production. *Nature immunology*. 2002;3(6):576-82.
55. Mackay F, Majeau GR, Lawton P, Hochman PS, Browning JL. Lymphotoxin but not tumor necrosis factor functions to maintain splenic architecture and humoral responsiveness in adult mice. *European journal of immunology*. 1997;27(8):2033-42.
56. Fava RA, Notidis E, Hunt J, Szanya V, Ratcliffe N, Ngam-ek A, et al. A role for the lymphotoxin/LIGHT axis in the pathogenesis of murine collagen-induced arthritis. *The Journal of Immunology*. 2003;171(1):115-26.
57. Rennert PD, Browning JL, Mebius R, Mackay F, Hochman PS. Surface lymphotoxin alpha/beta complex is required for the development of peripheral lymphoid organs. *The Journal of experimental medicine*. 1996;184(5):1999-2006.
58. Hu X, Zimmerman MA, Bardhan K, Yang D, Waller JL, Liles GB, et al. Lymphotoxin β receptor mediates caspase-dependent tumor cell apoptosis in vitro and tumor suppression in vivo despite induction of NF-kB activation. *Carcinogenesis*. 2013;34(5):1105-14.

59. Chen MC, Hwang MJ, Chou YC, Chen WH, Cheng G, Nakano H, et al. The role of apoptosis signal-regulating kinase 1 in lymphotoxin-beta receptor-mediated cell death. *J Biol Chem*. 2003 May 2;278(18):16073-81.
60. Chang Y-H, Hsieh S-L, Chen M-C, Lin W-W. Lymphotoxin  $\beta$  receptor induces interleukin 8 gene expression via NF- $\kappa$ B and AP-1 activation. *Experimental cell research*. 2002;278(2):166-74.
61. Degli-Esposti MA, Davis-Smith T, Din WS, Smolak PJ, Goodwin RG, Smith CA. Activation of the lymphotoxin beta receptor by cross-linking induces chemokine production and growth arrest in A375 melanoma cells. *The Journal of Immunology*. 1997;158(4):1756-62.
62. Hehlhans T, Männel DN. Recombinant, soluble LIGHT (HVEM ligand) induces increased IL-8 secretion and growth arrest in A375 melanoma cells. *Journal of Interferon & Cytokine Research*. 2001;21(5):333-8.
63. Lukashev M, LePage D, Wilson C, Bailly V, Garber E, Lukashin A, et al. Targeting the lymphotoxin-beta receptor with agonist antibodies as a potential cancer therapy. *Cancer research*. 2006 Oct 1;66(19):9617-24.
64. Browning JL, Miatkowski K, Sizing I, Griffiths D, Zafari M, Benjamin CD, et al. Signaling through the lymphotoxin beta receptor induces the death of some adenocarcinoma tumor lines. *Journal of Experimental Medicine*. 1996 Mar 1;183(3):867-78.
65. Rooney IA, Butrovich KD, Glass AA, Borboroglu S, Benedict CA, Whitbeck JC, et al. The lymphotoxin- $\beta$  receptor is necessary and sufficient for LIGHT-mediated apoptosis of tumor cells. *Journal of Biological Chemistry*. 2000;275(19):14307-15.
66. Dhawan P, Su Y, Thu YM, Yu Y, Baugher P, Ellis DL, et al. The lymphotoxin- $\beta$  receptor is an upstream activator of NF- $\kappa$ B-mediated transcription in melanoma cells. *Journal of biological chemistry*. 2008;283(22):15399-408.
67. Mackay F, Majeau GR, Hochman PS, Browning JL. Lymphotoxin beta receptor triggering induces activation of the nuclear factor kappaB transcription factor in some cell types. *J Biol Chem*. 1996 Oct 4;271(40):24934-8.
68. Nakano H, Oshima H, Chung W, Williams-Abbott L, Ware CF, Yagita H, et al. TRAF5, an activator of NF- $\kappa$ B and putative signal transducer for the lymphotoxin- $\beta$  receptor. *Journal of Biological Chemistry*. 1996;271(25):14661-4.
69. VanArsdale TL, VanArsdale SL, Force WR, Walter BN, Mosialos G, Kieff E, et al. Lymphotoxin-beta receptor signaling complex: role of tumor necrosis factor receptor-associated factor 3 recruitment in cell death and activation of nuclear factor kappa B. *Proceedings of the National Academy of Sciences*. 1997;94(6):2460-5.
70. Force WR, Cheung TC, Ware CF. Dominant negative mutants of TRAF3 reveal an important role for the coiled coil domains in cell death signaling by the lymphotoxin-beta receptor. *The Journal of biological chemistry*. 1997 Dec 5;272(49):30835-40.
71. Hauer J, Püschner S, Ramakrishnan P, Simon U, Bongers M, Federle C, et al. TNF receptor (TNFR)-associated factor (TRAF) 3 serves as an inhibitor of TRAF2/5-mediated activation of the noncanonical NF- $\kappa$ B pathway by TRAF-binding TNFRs. *Proceedings of the National Academy of Sciences of the United States of America*. 2005;102(8):2874-9.
72. Bechill J, Muller WJ. Herpesvirus entry mediator (HVEM) attenuates signals mediated by the lymphotoxin  $\beta$  receptor (LT $\beta$ R) in human cells stimulated by the shared ligand LIGHT. *Molecular immunology*. 2014;62(1):96-103.
73. Kim Y-S, Nedospasov, S. A. & Liu, Z.-G. TRAF2 plays a key, nonredundant role in LIGHT-lymphotoxin  $\beta$  receptor signaling. *Molecular and cellular biology*. 2005;25(6):2130-7.
74. Bista P, Zeng W, Ryan S, Bailly V, Browning JL, Lukashev ME. TRAF3 controls activation of the canonical and alternative NF $\kappa$ B by the lymphotoxin beta receptor. *Journal of Biological Chemistry*. 2010;285(17):12971-8.



75. Wilson CA, Browning JL. Death of HT29 adenocarcinoma cells induced by TNF family receptor activation is caspase-independent and displays features of both apoptosis and necrosis. *Cell Death Differ.* 2002 Dec;9(12):1321-33.
76. Montgomery RI, Warner MS, Lum BJ, Spear PG. Herpes simplex virus-1 entry into cells mediated by a novel member of the TNF/NGF receptor family. *Cell.* 1996 Nov 1;87(3):427-36.
77. Spear PG, Manoj S, Yoon M, Jogger CR, Zago A, Myscofski D. Different receptors binding to distinct interfaces on herpes simplex virus gD can trigger events leading to cell fusion and viral entry. *Virology.* 2006 Jan 5;344(1):17-24.
78. Šedý J, Bekiaris V, Ware CF. Tumor Necrosis Factor Superfamily in Innate Immunity and Inflammation. *Cold Spring Harbor perspectives in biology.* 2014;a016279.
79. Harrop JA, McDonnell PC, Brigham-Burke M, Lyn SD, Minton J, Tan KB, et al. Herpesvirus entry mediator ligand (HVEM-L), a novel ligand for HVEM/TR2, stimulates proliferation of T cells and inhibits HT29 cell growth. *J Biol Chem.* 1998 Oct 16;273(42):27548-56.
80. Marsters SA, Ayres TM, Skubatch M, Gray CL, Rothe M, Ashkenazi A. Herpesvirus entry mediator, a member of the tumor necrosis factor receptor (TNFR) family, interacts with members of the TNFR-associated factor family and activates the transcription factors NF- $\kappa$ B and AP-1. *Journal of Biological Chemistry.* 1997 May 30;272(22):14029-32.
81. Kuai J, Nickbarg E, Wooters J, Qiu Y, Wang J, Lin L-L. Endogenous association of TRAF2, TRAF3, cIAP1, and Smac with lymphotoxin  $\beta$  receptor reveals a novel mechanism of apoptosis. *Journal of Biological Chemistry.* 2003;278(16):14363-9.
82. Pasero C, Barbarat B, Just-Landi S, Bernard A, Aurran-Schleinitz T, Rey J, et al. A role for HVEM, but not lymphotoxin-beta receptor, in LIGHT-induced tumor cell death and chemokine production. *European journal of immunology.* 2009 Sep;39(9):2502-14.
83. Korsmeyer SJ, Shutter JR, Veis DJ, Merry DE, Oltvai Z. Bcl-2/Bax: A rheostat that regulates an anti-oxidant pathway and cell death. *Proceedings of the American Association for Cancer Research Annual Meeting.* 1994 1994;35(0):671-2.
84. Gross A, McDonnell JM, Korsmeyer SJ. Bcl-2 family members and the mitochondria in apoptosis. *Genes Dev.* 1999 Aug 1;13(15):1899-911.
85. Ware CF, VanArsdale S, VanArsdale TL. Apoptosis mediated by the TNF-related cytokine and receptor families. *J Cell Biochem.* 1996 Jan;60(1):47-55.
86. Mauri DN, Ebner R, Montgomery RI, Kochel KD, Cheung TC, Yu GL, et al. LIGHT, a new member of the TNF superfamily, and lymphotoxin alpha are ligands for herpesvirus entry mediator. *Immunity.* 1998 Jan;8(1):21-30.
87. Gommerman JL, Browning JL. Lymphotoxin/light, lymphoid microenvironments and autoimmune disease. *Nature Reviews Immunology.* 2003 Aug;3(8):642-55.
88. Ware CF, Crowe PD, Grayson MH, Androlewicz MJ, Browning JL. Expression of surface lymphotoxin and tumor-necrosis-factor on activated t-cell, b-cell, and natural-killer-cells. *Journal of Immunology.* 1992 Dec 15;149(12):3881-8.
89. Williams-Abbott L, Walter BN, Cheung TC, Goh CR, Porter AG, Ware CF. The lymphotoxin- $\alpha$  (LT $\alpha$ ) subunit is essential for the assembly, but not for the receptor specificity, of the membrane-anchored LT $\alpha$ 1 $\beta$ 2 heterotrimeric ligand. *Journal of Biological Chemistry.* 1997;272(31):19451-6.
90. Junt T, Tumanov AV, Harris N, Heikenwalder M, Zeller N, Kuprash DV, et al. Expression of lymphotoxin beta governs immunity at two distinct levels. *European journal of immunology.* 2006;36(8):2061-75.
91. Browning JL, Ngamek A, Lawton P, Demarinis J, Tizard R, Chow EPC, et al. Lymphotoxin-beta, a novel member of the tn timer family that forms a heteromeric complex with lymphotoxin on the cell-surface. *Cell.* 1993 Mar 26;72(6):847-56.

92. Luther SA, Bidgol A, Hargreaves DC, Schmidt A, Xu Y, Paniyadi J, et al. Differing activities of homeostatic chemokines CCL19, CCL21, and CXCL12 in lymphocyte and dendritic cell recruitment and lymphoid neogenesis. *Journal of Immunology*. 2002 Jul 1;169(1):424-33.
93. Browning JL, Douglas I, Ngamek A, Bourdon PR, Ehrenfels BN, Miatkowski K, et al. Characterization of surface lymphotoxin forms - use of specific monoclonal-antibodies and soluble receptors. *Journal of Immunology*. 1995 Jan 1;154(1):33-46.
94. Androlewicz MJ, Browning JL, Ware CF. Lymphotoxin is expressed as a heteromeric complex with a distinct 33-KDa glycoprotein on the surface of an activated human T-cell hybridoma. *Journal of Biological Chemistry*. 1992 Feb 5;267(4):2542-7.
95. Ware C, VanArsdale T, Crowe P, Browning J. The ligands and receptors of the lymphotoxin system. *Pathways for Cytolysis: Springer*; 1995. p. 175-218.
96. Browning JL, Androlewicz MJ, Ware CF. Lymphotoxin and an associated 33-kDa glycoprotein are expressed on the surface of an activated human T cell hybridoma. *The Journal of Immunology*. 1991;147(4):1230-7.
97. Morel Y, Schiano de Colella JM, Harrop J, Deen KC, Holmes SD, Wattam TA, et al. Reciprocal expression of the TNF family receptor herpes virus entry mediator and its ligand LIGHT on activated T cells: LIGHT down-regulates its own receptor. *J Immunol*. 2000 Oct 15;165(8):4397-404.
98. Steinberg MW, Cheung TC, Ware CF. The signaling networks of the herpesvirus entry mediator (TNFRSF14) in immune regulation. *Immunol Rev*. 2011 Nov;244:169-87.
99. Ware CF, Šedý JR. TNF Superfamily Networks: bidirectional and interference pathways of the herpesvirus entry mediator (TNFSF14). *Current opinion in immunology*. 2011;23(5):627-31.
100. Wang J, Chun T, Lo JC, Wu Q, Wang Y, Foster A, et al. The critical role of LIGHT, a TNF family member, in T cell development. *J Immunol*. 2001 Nov 1;167(9):5099-105.
101. Tamada K, Shimozaki K, Chapoval AI, Zhai Y, Su J, Chen S-F, et al. LIGHT, a TNF-like molecule, costimulates T cell proliferation and is required for dendritic cell-mediated allogeneic T cell response. *The Journal of Immunology*. 2000;164(8):4105-10.
102. Tamada K, Shimozaki K, Chapoval AI, Zhu G, Sica G, Flies D, et al. Modulation of T-cell-mediated immunity in tumor and graft-versus-host disease models through the LIGHT co-stimulatory pathway. *Nature medicine*. 2000;6(3):283-9.
103. Duhen T, Pasero C, Mallet F, Barbarat B, Olive D, Costello RT. LIGHT costimulates CD40 triggering and induces immunoglobulin secretion; a novel key partner in T cell-dependent B cell terminal differentiation. *European journal of immunology*. 2004;34(12):3534-41.
104. Pasero C, Truneh A, Olive D. Cosignaling molecules around LIGHT-HVEM-BTLA: from immune activation to therapeutic targeting. *Current molecular medicine*. 2009;9(7):911-27.
105. Morel Y, Truneh A, Sweet RW, Olive D, Costello RT. The TNF superfamily members LIGHT and CD154 (CD40 ligand) costimulate induction of dendritic cell maturation and elicit specific CTL activity. *J Immunol*. 2001 Sep 1;167(5):2479-86.
106. Ngo VN, Korner H, Gunn MD, Schmidt KN, Riminton DS, Cooper MD, et al. Lymphotoxin  $\alpha/\beta$  and tumor necrosis factor are required for stromal cell expression of homing chemokines in B and T cell areas of the spleen. *The Journal of experimental medicine*. 1999;189(2):403-12.
107. Yu P, Lee Y, Liu W, Chin RK, Wang J, Wang Y, et al. Priming of naive T cells inside tumors leads to eradication of established tumors. *Nature immunology*. 2004;5(2):141-9.
108. Farber JM. Mig and IP-10: CXC chemokines that target lymphocytes. *Journal of leukocyte biology*. 1997;61(3):246-57.
109. Sharma S, Yang S-C, Hillinger S, Zhu LX, Huang M, Batra RK, et al. SLC/CCL21-mediated anti-tumor responses require IFN $\gamma$ , MIG/CXCL9 and IP-10/CXCL10. *Mol Cancer*. 2003;2(1):22.
110. Petreaca ML, Yao M, Ware C, Martins-Green MM. YOUNG INVESTIGATOR AWARD ARTICLE: Vascular endothelial growth factor promotes macrophage apoptosis through stimulation of tumor

- necrosis factor superfamily member 14 (TNFSF14/LIGHT). *Wound Repair and Regeneration*. 2008;16(5):602-14.
111. Zhai Y, Guo R, Hsu T-L, Yu G-L, Ni J, Kwon BS, et al. LIGHT, a novel ligand for lymphotoxin beta receptor and TR2/HVEM induces apoptosis and suppresses in vivo tumor formation via gene transfer. *Journal of Clinical Investigation*. 1998;102(6):1142.
112. Fan Z, Yu P, Wang Y, Wang Y, Fu ML, Liu W, et al. NK-cell activation by LIGHT triggers tumor-specific CD8+ T-cell immunity to reject established tumors. *Blood*. 2006;107(4):1342-51.
113. Chang YH, Chao Y, Hsieh SL, Lin WW. Mechanism of LIGHT/Interferon-gamma-induced cell death in HT-29 cells. *J Cell Biochem*. 2004 Dec 15;93(6):1188-202.
114. Wu Y, Han B, Sheng H, Lin M, Moore PA, Zhang J, et al. Clinical significance of detecting elevated serum DcR3/TR6/M68 in malignant tumor patients. *International journal of cancer*. 2003;105(5):724-32.
115. Wang H, Yu Z, Liu S, Liu X, Sui A, Yao R, et al. Lentivirus-mediated LIGHT overexpression inhibits human colorectal carcinoma cell growth in vitro and in vivo. *Oncology letters*. 2013;6(4):927-32.
116. Garg AK, Aggarwal BB. Reactive oxygen intermediates in TNF signaling. *Molecular immunology*. 2002;39(9):509-17.
117. Shen H-M, Pervaiz S. TNF receptor superfamily-induced cell death: redox-dependent execution. *The FASEB Journal*. 2006;20(10):1589-98.
118. Morgan MJ, Liu Z-g. Reactive oxygen species in TNF $\alpha$ -induced signaling and cell death. *Molecules and cells*. 2010;30(1):1-12.
119. Cheung TC, Steinberg MW, Osborne LM, Macauley MG, Fukuyama S, Sanjo H, et al. Unconventional ligand activation of herpesvirus entry mediator signals cell survival. *Proceedings of the National Academy of Sciences*. 2009;106(15):6244-9.
120. Stamenkovic I, Clark E, Seed B. A B-lymphocyte activation molecule related to the nerve growth factor receptor and induced by cytokines in carcinomas. *The EMBO journal*. 1989;8(5):1403.
121. van Kooten C, Banchereau J. CD40-CD40 ligand. *Journal of leukocyte biology*. 2000;67(1):2-17.
122. Higuchi T, Aiba Y, Nomura T, Matsuda J, Mochida K, Suzuki M, et al. Cutting edge: ectopic expression of CD40 ligand on B cells induces lupus-like autoimmune disease. *The Journal of Immunology*. 2002;168(1):9-12.
123. Henn V, Slupsky JR, Gräfe M, Anagnostopoulos I, Förster R, Müller-Berghaus G, et al. CD40 ligand on activated platelets triggers an inflammatory reaction of endothelial cells. *Nature*. 1998;391(6667):591-4.
124. Danese S, de la Motte C, Sturm A, Vogel JD, West GA, Strong SA, et al. Platelets trigger a CD40-dependent inflammatory response in the microvasculature of inflammatory bowel disease patients. *Gastroenterology*. 2003;124(5):1249-64.
125. Dallman C, Johnson P, Packham G. Differential regulation of cell survival by CD40. *Apoptosis*. 2003;8(1):45-53.
126. Jiang E, He X, Chen X, Sun G, Wu H, Wei Y, et al. Expression of CD40 in ovarian cancer and adenovirus-mediated CD40 ligand therapy on ovarian cancer in vitro. *Tumori*. 2008;94(3):356.
127. Georgopoulos NT, Steele LP, Thomson M, Selby PJ, Southgate J, Trejdosiewicz LK. A novel mechanism of CD40-induced apoptosis of carcinoma cells involving TRAF3 and JNK/AP-1 activation. *Cell Death & Differentiation*. 2006;13(10):1789-801.
128. Eliopoulos AG, Davies C, Knox PG, Gallagher NJ, Afford SC, Adams DH, et al. CD40 induces apoptosis in carcinoma cells through activation of cytotoxic ligands of the tumor necrosis factor superfamily. *Mol Cell Biol*. 2000 Aug;20(15):5503-15.
129. Hess S, Engelmann H. A novel function of CD40: induction of cell death in transformed cells. *The Journal of experimental medicine*. 1996;183(1):159-67.

130. Ghamande S, Hylander BL, Oflazoglu E, Lele S, Fanslow W, Repasky EA. Recombinant CD40 ligand therapy has significant antitumor effects on CD40-positive ovarian tumor xenografts grown in SCID mice and demonstrates an augmented effect with cisplatin. *Cancer research*. 2001;61(20):7556-62.
131. Melichar B, Patenia R, Gallardo S, Melicharová K, Hu W, Freedman RS. Expression of CD40 and growth-inhibitory activity of CD40 ligand in ovarian cancer cell lines. *Gynecologic oncology*. 2007;104(3):707-13.
132. Georgopoulos NT, Merrick A, Scott N, Selby PJ, Melcher A, Trejdosiewicz LK. CD40-mediated death and cytokine secretion in colorectal cancer: A potential target for inflammatory tumour cell killing. *International journal of cancer*. 2007;121(6):1373-81.
133. Hostager BS, Haxhinasto SA, Rowland SL, Bishop GA. Tumor necrosis factor receptor-associated factor 2 (TRAF2)-deficient B lymphocytes reveal novel roles for TRAF2 in CD40 signaling. *Journal of Biological Chemistry*. 2003;278(46):45382-90.
134. Gallagher E, Enzler T, Matsuzawa A, Anzelon-Mills A, Otero D, Holzer R, et al. Kinase MEKK1 is required for CD40-dependent activation of the kinases Jnk and p38, germinal center formation, B cell proliferation and antibody production. *Nature immunology*. 2006;8(1):57-63.
135. Lee SY, Reichlin A, Santana A, Sokol KA, Nussenzweig MC, Choi Y. TRAF2 is essential for JNK but not NF- $\kappa$ B activation and regulates lymphocyte proliferation and survival. *Immunity*. 1997;7(5):703-13.
136. Bishop GA, Moore CR, Xie P, Stunz LL, Kraus ZJ. TRAF proteins in CD40 signaling. *TNF Receptor Associated Factors (TRAFs)*: Springer; 2007. p. 131-51.
137. Nakano H, Sakon S, Koseki H, Takemori T, Tada K, Matsumoto M, et al. Targeted disruption of Traf5 gene causes defects in CD40-and CD27-mediated lymphocyte activation. *Proceedings of the National Academy of Sciences*. 1999;96(17):9803-8.
138. Ishida T, ToJo T, Aoki T, Kobayashi N, Ohishi T, Watanabe T, et al. TRAF5, a novel tumor necrosis factor receptor-associated factor family protein, mediates CD40 signaling. *Proceedings of the National Academy of Sciences*. 1996;93(18):9437-42.
139. Davies CC, Mason J, Wakelam MJ, Young LS, Eliopoulos AG. Inhibition of phosphatidylinositol 3-kinase-and ERK MAPK-regulated protein synthesis reveals the pro-apoptotic properties of CD40 ligation in carcinoma cells. *Journal of Biological Chemistry*. 2004;279(2):1010-9.
140. Rowland SL, Tremblay MM, Ellison JM, Stunz LL, Bishop GA, Hostager BS. A novel mechanism for TNFR-associated factor 6-dependent CD40 signaling. *The Journal of Immunology*. 2007;179(7):4645-53.
141. Elmetwali T, Young LS, Palmer DH. CD40 ligand-induced carcinoma cell death: a balance between activation of TNFR-associated factor (TRAF) 3-dependent death signals and suppression of TRAF6-dependent survival signals. *The journal of immunology*. 2010;184(2):1111-20.
142. Dejardin E, Droin NM, Delhase M, Haas E, Cao Y, Makris C, et al. The lymphotoxin- $\beta$  receptor induces different patterns of gene expression via two NF- $\kappa$ B pathways. *Immunity*. 2002;17(4):525-35.
143. Basak S, Kim H, Kearns JD, Tergaonkar V, O'Dea E, Werner SL, et al. A fourth I $\kappa$ B protein within the NF- $\kappa$ B signaling module. *Cell*. 2007;128(2):369-81.
144. Wang C-Y, Mayo MW, Korneluk RG, Goeddel DV, Baldwin AS. NF- $\kappa$ B antiapoptosis: induction of TRAF1 and TRAF2 and c-IAP1 and c-IAP2 to suppress caspase-8 activation. *Science*. 1998;281(5383):1680-3.
145. Hsu H, Solovyyev I, Colombero A, Elliott R, Kelley M, Boyle WJ. ATAR, a novel tumor necrosis factor receptor family member, signals through TRAF2 and TRAF5. *Journal of Biological Chemistry*. 1997;272(21):13471-4.
146. Zapata JM, Pawlowski K, Haas E, Ware CF, Godzik A, Reed JC. A diverse family of proteins containing tumor necrosis factor receptor-associated factor domains. *Journal of Biological Chemistry*. 2001;276(26):24242-52.
147. Shaw NJ, Georgopoulos NT, Southgate J, Trejdosiewicz LK. Effects of loss of p53 and p16 function on life span and survival of human urothelial cells. *International journal of cancer*. 2005;116(4):634-9.

148. Holler N, Tardivel A, Kovacsics-Bankowski M, Hertig S, Gaide O, Martinon F, et al. Two adjacent trimeric Fas ligands are required for Fas signaling and formation of a death-inducing signaling complex. *Molecular and cellular biology*. 2003;23(4):1428-40.
149. Loetscher H, Pan YCE, Lahm HW, Gentz R, Brockhaus M, Tabuchi H, et al. Molecular-cloning and expression of the human 55-kd tumor-necrosis-factor receptor. *Cell*. 1990 Apr 20;61(2):351-9.
150. Armitage RJ. Tumor necrosis factor receptor superfamily members and their ligands. *Current opinion in immunology*. 1994 Jun;6(3):407-13.
151. Vince JE, Pantaki D, Feltham R, Mace PD, Cordier SM, Schmukle AC, et al. TRAF2 must bind to cellular inhibitors of apoptosis for tumor necrosis factor (TNF) to efficiently activate NF- $\kappa$ B and to prevent TNF-induced apoptosis. *Journal of Biological Chemistry*. 2009;284(51):35906-15.
152. Terry Powers JL, Mace KE, Parfrey H, Lee S-J, Zhang G, Riches DW. TNF receptor-1 (TNF-R1) ubiquitous scaffolding and signaling protein interacts with TNF-R1 and TRAF2 via an N-terminal docking interface. *Biochemistry*. 2010;49(36):7821-9.
153. Kitson J, Raven T, Jiang Y-P, Goeddel DV, Giles KM, Pun K-T, et al. A death-domain-containing receptor that mediates apoptosis. *Nature*. 1996;384(6607):372-5.
154. Smith CA, Davis T, Anderson D, Solam L, Beckmann MP, Jerzy R, et al. A receptor for tumor-necrosis-factor defines an unusual family of cellular and viral-proteins. *Science*. 1990 May 25;248(4958):1019-23.
155. Cabal-Hierro L, Rodríguez M, Artime N, Iglesias J, Ugarte L, Prado MA, et al. TRAF-mediated modulation of NF- $\kappa$ B AND JNK Activation by TNFR2. *Cellular signalling*. 2014;26(12):2658-66.
156. Crowe PD, Vanarsdale TL, Walter BN, Ware CF, Hession C, Ehrenfels B, et al. A lymphotoxin-beta-specific receptor. *Science*. 1994 Apr 29;264(5159):707-10.
157. Sanjo H, Zajonc DM, Braden R, Norris PS, Ware CF. Allosteric regulation of the ubiquitin: NIK and ubiquitin: TRAF3 E3 ligases by the lymphotoxin- $\beta$  receptor. *Journal of Biological Chemistry*. 2010;285(22):17148-55.
158. Pitti RM, MARSTERS, S. A., LAWRENCE, D. A., ROY, M., KISCHKEL, F. C., DOWD, P., HUANG, A., DONAHUE, C. J., SHERWOOD, S. W., BALDWIN, D. T., GODOWSKI, P. J., WOOD, W. I., GURNEY, A. L., HILLAN, K. J., COHEN, R. L., GODDARD, A. D., BOTSTEIN, D. & ASHKENAZI, A. Genomic amplification of a decoy receptor for Fas ligand in lung and colon cancer. *Nature*. 1998;396(6712):699-703.
159. Chinnaiyan AM, O'Rourke K, Yu G-L, Lyons RH, Garg M, Duan DR, et al. Signal transduction by DR3, a death domain-containing receptor related to TNFR-1 and CD95. *Science*. 1996;274(5289):990-2.
160. Muntané J. Harnessing tumor necrosis factor receptors to enhance antitumor activities of drugs. *Chemical research in toxicology*. 2011;24(10):1610-6.
161. Leithäuser F, Dhein J, Mechttersheimer G, Koretz K, Brüderlein S, Henne C, et al. Constitutive and induced expression of APO-1, a new member of the nerve growth factor/tumor necrosis factor receptor superfamily, in normal and neoplastic cells. *Laboratory investigation; a journal of technical methods and pathology*. 1993;69(4):415-29.
162. Scream GR, Xu XN, Olsen AL, Cowper AE, Tan RS, McMichael AJ, et al. LARD: A new lymphoid-specific death domain containing receptor regulated by alternative pre-mRNA splicing. *Proc Natl Acad Sci U S A*. 1997 Apr 29;94(9):4615-9.
163. Bodmer J-L, Burns K, Schneider P, Hofmann K, Steiner V, Thome M, et al. TRAMP, a novel apoptosis-mediating receptor with sequence homology to tumor necrosis factor receptor 1 and Fas (Apo-1/CD95). *Immunity*. 1997;6(1):79-88.
164. Marsters SA, Sheridan JP, Donahue CJ, Pitti RM, Gray CL, Goddard AD, et al. Apo-3, a new member of the tumor necrosis factor receptor family, contains a death domain and activates apoptosis and NF- $\kappa$ B. *Current Biology*. 1996;6(12):1669-76.

165. Tan K, Harrop J, Reddy M, Young P, Terrett J, Emery J, et al. Characterization of a novel TNF-like ligand and recently described TNF ligand and TNF receptor superfamily genes and their constitutive and inducible expression in hematopoietic and non-hematopoietic cells. *Gene*. 1997;204(1-2):35-46.
166. Pan GH, Orourke K, Chinnaiyan AM, Gentz R, Ebner R, Ni J, et al. The receptor for the cytotoxic ligand TRAIL. *Science*. 1997 Apr 4;276(5309):111-3.
167. Kischkel FC, Lawrence DA, Chuntharapai A, Schow P, Kim KJ, Ashkenazi A. Apo2L/TRAIL-dependent recruitment of endogenous FADD and caspase-8 to death receptors 4 and 5. *Immunity*. 2000;12(6):611-20.
168. van Geelen CM, Pennarun B, Le PT, de Vries EG, de Jong S. Modulation of TRAIL resistance in colon carcinoma cells: different contributions of DR4 and DR5. *BMC cancer*. 2011;11(1):39.
169. Chaudhary PM, Eby M, Jasmin A, Bookwalter A, Murray J, Hood L. Death receptor 5, a new member of the TNFR family, and DR4 induce FADD-dependent apoptosis and activate the NF- $\kappa$ B pathway. *Immunity*. 1997;7(6):821-30.
170. Schneider P, Thome M, Burns K, Bodmer J-L, Hofmann K, Kataoka T, et al. TRAIL receptors 1 (DR4) and 2 (DR5) signal FADD-dependent apoptosis and activate NF- $\kappa$ B. *Immunity*. 1997;7(6):831-6.
171. MacFarlane M, Ahmad M, Srinivasula SM, FernandesAlnemri T, Cohen GM, Alnemri ES. Identification and molecular cloning of two novel receptors for the cytotoxic ligand TRAIL. *Journal of Biological Chemistry*. 1997 Oct 10;272(41):25417-20.
172. Pan GH, Bauer JH, Haridas V, Wang SX, Liu D, Yu GL, et al. Identification and functional characterization of DR6, a novel death domain-containing TNF receptor. *FEBS Lett*. 1998 Jul 24;431(3):351-6.
173. Pan G, Bauer JH, Haridas V, Wang S, Liu D, Yu G, et al. Identification and functional characterization of DR6, a novel death domain-containing TNF receptor. *FEBS letters*. 1998;431(3):351-6.
174. Kasof GM, Lu JJ, Liu D, Speer B, Mongan KN, Gomes BC, et al. Tumor necrosis factor- $\alpha$  induces the expression of DR6, a member of the TNF receptor family, through activation of NF- $\kappa$ B. *Oncogene*. 2001;20(55):7965-75.
175. Marsters S, Sheridan J, Pitti R, Huang A, Skubatch M, Baldwin D, et al. A novel receptor for Apo2L/TRAIL contains a truncated death domain. *Current Biology*. 1997;7(12):1003-6.
176. Degli-Esposti MA, Smolak PJ, Walczak H, Waugh J, Huang C-P, DuBose RF, et al. Cloning and characterization of TRAIL-R3, a novel member of the emerging TRAIL receptor family. *The Journal of experimental medicine*. 1997;186(7):1165-70.
177. Sheridan JP, Marsters SA, Pitti RM, Gurney A, Skubatch M, Baldwin D, et al. Control of TRAIL-induced apoptosis by a family of signaling and decoy receptors. *Science*. 1997;277(5327):818-21.
178. Pan GH, Ni J, Yu GL, Wei YF, Dixit VM. TRUND, a new member of the TRAIL receptor family that antagonizes TRAIL signalling. *FEBS Lett*. 1998 Mar 6;424(1-2):41-5.
179. Degli-Esposti MA, Dougall WC, Smolak PJ, Waugh JY, Smith CA, Goodwin RG. The novel receptor TRAIL-R4 induces NF- $\kappa$ B and protects against TRAIL-mediated apoptosis, yet retains an incomplete death domain. *Immunity*. 1997;7(6):813-20.
180. Yu K-Y, Kwon B, Ni J, Zhai Y, Ebner R, Kwon BS. A newly identified member of tumor necrosis factor receptor superfamily (TR6) suppresses LIGHT-mediated apoptosis. *Journal of Biological Chemistry*. 1999;274(20):13733-6.
181. Yamamoto H, Kishimoto T, Minamoto S. NF- $\kappa$ B activation in CD27 signaling: involvement of TNF receptor-associated factors in its signaling and identification of functional region of CD27. *The Journal of Immunology*. 1998;161(9):4753-9.
182. Van den Oord J, Maes A, Stas M, Nuyts J, Battocchio S, Kasran A, et al. CD40 is a prognostic marker in primary cutaneous malignant melanoma. *The American journal of pathology*. 1996;149(6):1953.

183. Mallett S, Fossum S, Barclay AN. Characterization of the MRC ox40 antigen of activated CD4 positive lymphocytes-T - a molecule related to nerve growth-factor receptor. *Embo Journal*. 1990 Apr;9(4):1063-8.
184. Croft M, So T, Duan W, Soroosh P. The significance of OX40 and OX40L to T-cell biology and immune disease. *Immunological reviews*. 2009;229(1):173-91.
185. Kawamata S, Hori T, Imura A, Takaori-Kondo A, Uchiyama T. Activation of OX40 signal transduction pathways leads to tumor necrosis factor receptor-associated factor (TRAF) 2-and TRAF5-mediated NF- $\kappa$ B activation. *Journal of Biological Chemistry*. 1998;273(10):5808-14.
186. Dürkop H, Latza U, Himmelreich P, Stein H. Expression of the human OX40 (hOX40) antigen in normal and neoplastic tissues. *British journal of haematology*. 1995;91(4):927-31.
187. Imura A, Hori T, Imada K, Kawamata S, Tanaka Y, Imamura S, et al. OX40 expressed on fresh leukemic cells from adult T-cell leukemia patients mediates cell adhesion to vascular endothelial cells: implication for the possible involvement of OX40 in leukemic cell infiltration. *Blood*. 1997;89(8):2951-8.
188. Wheeler E, Gong H, Grimes R, Benoit D, Vazquez L. p75NTR and Trk receptors are expressed in reciprocal patterns in a wide variety of non-neural tissues during rat embryonic development, indicating independent receptor functions. *Journal of Comparative Neurology*. 1998;391(4):407-28.
189. Nocentini G, Giunchi L, Ronchetti S, Krausz LT, Bartoli A, Moraca R, et al. A new member of the tumor necrosis factor nerve growth factor receptor family inhibits T cell receptor-induced apoptosis. *Proc Natl Acad Sci U S A*. 1997 Jun 10;94(12):6216-21.
190. Kim YS, Jung HW, Choi J, Kwon BS, Ham S-Y, Jung AK, et al. Expression of AITR and AITR ligand in breast cancer patients. *Oncology reports*. 2007;18(5):1189-94.
191. Esparza E, Arch R. TRAF4 functions as an intermediate of GITR-induced NF- $\kappa$ B activation. *Cellular and Molecular Life Sciences CMLS*. 2004;61(24):3087-92.
192. Esparza EM, Lindsten T, Stockhausen JM, Arch RH. Tumor necrosis factor receptor (TNFR)-associated factor 5 is a critical intermediate of costimulatory signaling pathways triggered by glucocorticoid-induced TNFR in T cells. *Journal of Biological Chemistry*. 2006;281(13):8559-64.
193. Suvas S, Kim B, Sarangi PP, Tone M, Waldmann H, Rouse BT. In vivo kinetics of GITR and GITR ligand expression and their functional significance in regulating viral immunopathology. *Journal of virology*. 2005;79(18):11935-42.
194. Vinay DS, Kwon BS. Differential expression and costimulatory effect of 4-1BB (CD137) and CD28 molecules on cytokine-induced murine CD8(+) Tc1 and Tc2 cells. *Cell Immunol*. 1999 Feb 25;192(1):63-71.
195. Inoue J-i, Ishida T, Tsukamoto N, Kobayashi N, Naito A, Azuma S, et al. Tumor necrosis factor receptor-associated factor (TRAF) family: adapter proteins that mediate cytokine signaling. *Experimental cell research*. 2000;254(1):14-24.
196. Pollok KE, Kim Y-J, Zhou Z, Hurtado J, Kim K, Pickard R, et al. Inducible T cell antigen 4-1BB. Analysis of expression and function. *The Journal of Immunology*. 1993;150(3):771-81.
197. Simonet WS, Lacey DL, Dunstan CR, Kelley M, Chang MS, Luthy R, et al. Osteoprotegerin: A novel secreted protein involved in the regulation of bone density. *Cell*. 1997 Apr 18;89(2):309-19.
198. Wong BR, Josien R, Lee SY, Vologodskaya M, Steinman RM, Choi Y. The TRAF family of signal transducers mediates NF- $\kappa$ B activation by the TRANCE receptor. *Journal of Biological Chemistry*. 1998;273(43):28355-9.
199. Kanazawa K, Azuma Y, Nakano H, Kudo A. TRAF5 Functions in Both RANKL-and TNF $\alpha$ -Induced Osteoclastogenesis. *Journal of Bone and Mineral Research*. 2003;18(3):443-50.