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

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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-κB: nuclear factor kappa B
NGF: nerve growth factor
HVEM: herpes-virus entry mediator
IFN-γ: Interferon-gamma
JNK: c-Jun N-terminal kinase
RANK: receptor activator of nuclear factor-kB
TF: Transcription factor
TNF: tumour necrosis factor
TNFα: 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 TNFα. A series of initial investigations carried out both *in vitro* and *in vivo* demonstrated the pro-apoptotic potential of TNFα and as the administration of recombinant TNFα in tumour-bearing mice resulted in anti-tumour responses, these observations rendered TNF a promising anticancer agent.

Soon after the identification of TNFα, a similar TNF-like factor was discovered and named TNFβ which was later renamed Lymphotoxin-alpha (LTα), 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 TNFα and LTα were isolated in the 1980s. The TNFα and LTα 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). TNFα and LTα 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 LTα came shortly after the discovery of TNFα. 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 LTα, LTβ, LTα1β2, LTα2β1, and LIGHT being able to interact with the two main transmembrane receptors LTβR 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). TNFα is expressed in full-length on the cell surface as a 26kDa membrane ligand (mTNFα) and as a 17kDa soluble cytokine (sTNFα) after shedding. By contrast, LTα is always shed as a soluble cytokine, yet LTβ is expressed only in a membrane-bound form, as the latter does not contain a cleavage site. LTα and LTβ can assemble together and form two membrane bound complexes of LTαβ; the LTα1β2 complex

consists of a single ββ and two unique αβ sites and exhibits high affinity to LTβR; LTα2β1 on the other hand binds to TNFR –I and TNFR –II, but with less affinity to LTβR. 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 LTβR 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-κB 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-κB 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-κB 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-κB family includes Rel (c-Rel), RelA (p65), RelB, NF-κB1 (p50) and NF-κB2 (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 (mTNFα) to soluble ligand (sTNFα) (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 TNFα system: ligand-receptor interactions and intracellular signalling

TNFα 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. TNFα, 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 TNFα/TNFR-I/II system is that differential receptor expression and/or receptor activation by TNFα can regulate the balance between cell survival and apoptosis (15).

TNFα was found to act as an inducer of apoptotic cell death during the maturation of mice thymocytes (16, 17). *In vitro*, TNFα 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 TNFα only when the protein synthesis was inhibited using cycloheximide (CHX) (18). TNFα can induce both necrotic and apoptotic cell death in mouse fibroblasts *in vitro* and *in vivo* (19). In particular, recombinant TNFα 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 TNFα by apoptosis. In fact, normal skin (Detroit 551) and lung fibroblasts (WI-38) were resistant and their growth was actually stimulated by TNFα (20-22), which is also observed in some carcinoma cells types such as those of the bladder, where TNFα can be growth-promoting (23). Of note are studies demonstrating that membrane-bound TNFα 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 TNFα exhibits both cell type- and context-specificity and TNFα-mediated signalling can have highly pleiotropic effects.

TNFRs –I and –II are mainly activated by TNFα although receptor ligation can be induced by soluble LTα3 (due to its highly homologous structure to TNFα) and by LTα2β1 (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 TNFα 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 TNFα also induces the activation NF-κB (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-κB after TNFα treatment or the specific activation of TNFRII (32). Pham and colleagues further demonstrated soluble TNFα-induced apoptosis in NF-κB-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-κB, 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 mTNFα with high affinity, thus it has been suggested that there must be cell-cell contact in order for TNFα to activate TNFRII 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-κB 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-κB and AP-1 and caspases, and thus many TNF-induced responses supported by findings that ROS regulate TNF-α-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 mTNFα 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 mTNFα-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 TNFRI activation, thus demonstrating that ROS are important regulatory molecules in TNFα-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 LTβR

LTβR is activated by three ligands which are the two heterotrimeric LTαβ 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 LTβR by stromal cells in the intestine is important for normal production of IgA

after antigen recognition. Constitutive LTβR-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 (LTβR-Ig) caused inhibition of splenic germinal centre formation and defective humoral responses (55). Thus, such studies using genetically modified mice indicate that LTβR is a key molecule involved in lymphoid organogenesis and in adaptive humoral immunity. Two studies using a fusion protein to inhibit LTβR signalling through LTα1β2 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 LTβR 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 LTβR receptor *in vitro* by either LIGHT mutein (LIGHT-R228E – which is mutated form of LIGHT that preferentially interacts with LTβR over HVEM) or agonistic anti-LTβR 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-κB (59-62). Unlike TNFRI which activates the canonical pathway of NF-κB, LTβR can activate both NF-κB pathways (5, 11). Lukashev et al have previously demonstrated that agonistic multivalent pentameric anti-LTβR antibody CBE11 can reduce the growth of colon and cervical tumours *in vivo* (63). More recently, Hu et al demonstrated that LTβR activation using LTβR agonistic antibody BS-1 was found to induce growth inhibition (as well as NF-κB 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 LTβR 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 LTβR by using a different monoclonal anti-LTβR 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-LTβR monoclonal antibody alone caused cell death *in vitro* (64). Therefore, signalling through

LTβR either by its natural ligands or via agonistic anti-LTβR 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 LTβR (CBE11) and this potentiated tumour responses to chemotherapeutic treatment (63). *In vitro*, however, some contradictory findings have been reported, as the activation of LTβR with an agonistic LTβR antibody in melanoma cell lines (Hs294T, SKMel5, SKMel28, and WM115) leads to the activation of the NF-κB and enhances tumour cell proliferation (66). Mackay et al reported that activation of LTβR with either soluble LTα1β2 or agonistic anti-LTβR antibody (CBE11) induced activation of NF-κB in HT29 and WiDr human adenocarcinoma lines and human lung fibroblasts WI-38 (67), but Browning at al demonstrated that recombinant LTα1β2 together with IFN-γ 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 LTβR 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-κB activation, but TRAF3 was found to be a negative regulator for NF-κB 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/LTβR ligation and their recruitment led to the activation of NF-κB and JNK (73). Bista and colleagues reported that TRAF3 functions as a pro-survival molecule during LTβR activation, through canonical and noncanonical NF-κB function. In fact, LTβR-induced signalling complexes enhanced TRAF3 recruitment, but decreased TRAF2 recruitment which attenuated the phosphorylation of Ikβα and RelA genes of NF-κB (74). Moreover, Chen and colleagues demonstrated using human hepatoma cells (Hep3BT2), HeLa and HEK293 cells that the activation of LTβR by using either LIGHT mutein (LIGHT-R228E) or agonistic monoclonal antibody anti-LTβR (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 LTβR-mediated death (59). Furthermore, it has been reported that a mixture of cell death features was observed following LTβR 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 LTβR activation (75).

4.1.2 HVEM

Another receptor that binds with LIGHT and LTα 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 $CD4^+$ and $CD8^+$ 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-κB 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-κB 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-κB 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 LTβR 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 LTα

LTα is often used as a term to describe the biologically active trimer LTα3 (31, 85), but can exist in three different forms; soluble homotrimeric LTα3, or as two transmembrane heterotrimeric complexes termed LTα1β2 and LTα2β1. LTα1β2 and LTα2β1 complex formation occurs when soluble LTα binds to LTβ bound on the cell membrane, thus signalling via LTαβ-LTβR interaction requires target-effector cell contact. In addition to binding to TNFR (Figure 1), LTα3 may bind HVEM, although this binding has been reported to be with low affinity (86). LTα is secreted by activated lymphocytes, resting B cells, non-hematopoietic and myeloid lineage cells. Like TNFα, LTα 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 LTα is mutated at either D50N or Y108F, it will only remain as a homotrimer which is not able to bind TNFRI or TNFRII and is not able to induce HT29 cell apoptosis. The modified LTα ligand, however, co-assembled with LTβ and formed a stable ligand heterotrimer complex named LTαβ, which was functionally active and able to trigger cell death in the adenocarcinoma cell line due to a capability to bind LTβR (89). In addition, Browning and colleagues reported that the LTα1β2 with mutated LTα was functionally active on HT29 and WiDr cells (64).

4.2.2 LTβ and LTαβ complexes

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

As in the case of LTβ, the effects of LTαβ complexes (via LTβR activation) remain relatively under investigated, despite the ability of LTαβ ligands to induce cytotoxic effects *in vitro* and *in vivo* (64, 89). The expression of both LTα1β2 and LTα2β1 complexes is regulated by IL-2, which leads to their induction on human peripheral blood T cells (88). Interestingly, LTαβ ligands exhibit differential receptor binding specificities due to the differences in their stoichiometry (31). In murine studies, LTαβ 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 LTαβ with its receptor LTβR 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 LTαβ complexes in signal transduction (31, 93-96). Of the two types of LTαβ membrane complexes, recombinant LTα1β2 was able to induce cell death in a range of carcinoma cell lines in the presence of IFN-γ (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 TNFα (27%), LTα (27%), LTβ (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 LTβR 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 LTβR 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-γ secretion and NF-κB 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 LTβR 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 LTβR 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-γ (111). The combination of LIGHT/IFN-γ 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-γ 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-γ 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-γ 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 TNFα-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 LTβR 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 LTβR

showed that IFN-γ assisted activation of LTβR 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 LTβR 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 LTβR but not HVEM. This evidence highlighted two important points: a) LIGHT may not cause cell death for target cells expressing one of the receptors LTβR or HVEM, b) LIGHT engagement with LTβR 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-κB) and functional outcome (apoptosis) rely on the relative expression of HVEM and LTβR 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 LTβR 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 TNFα, 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 LTβR 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 TNFα/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 $CD4^+$ 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-κB. 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-κB activation (71, 136, 137). Ishida and colleagues demonstrated that recruitment of TRAF5 via CD40 mediates the activation of NF-κB (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-κB, 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 LTβR. 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-κB pathway.

Activation of LTβR following binding of ligands LTαβ and LIGHT, or using other soluble agonists, induced rapid LTβR 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-κB pathways (73, 81), whereas JNK activation and cell death induction through LTβR signalling was mediated by TRAF3. Studies using HeLa cells showed that recruitment of TRAF2 and TRAF3 and activation of NF-κB are triggered after LTβR activation by LIGHT. Recruitment of TRAF2 and TRAF3 through LTβR 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-κB 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-κB 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 LTβR signalling to other TNFRs (mainly CD40 and BAFF-R), where TRAF2 functions as an inducer but TRAF3 acts as inhibitor of NF-κB (92, 133); yet, the precise functional roles of TRAFs in LTβR 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 mTNFα 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 LTβR and HVEM has been demonstrated using various agonist formats: agonistic antibodies, soluble recombinant ligands (LIGHT or LTαβ) or ligation by membrane-presented ligand LIGHT. The majority of previous studies have focused on the activation of LTβR, 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 LTβR in Hep3BT2, HeLa and HEK293 cells by soluble mutein LIGHT (LIGHT-R228E), which activates LTβR, and agonistic anti-LTβR monoclonal antibody (clone 31G4D8) were able to induce cell death in these cells (59, 65). Degli-Esposti and colleagues reported that cross-linking of LTβR with immobilized agonistic anti-LTβR 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 LTβ and LTαβ (ligands for LTβR) (61). On the other hand, Browning and colleagues demonstrated that immobilised agonistic monoclonal antibody anti-LTβR (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 LTβR 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-γ to a greater extent than did the monomeric mAb form CBE11 (63).

Interestingly, soluble recombinant LTα1β2 (another ligand for LTβR) was toxic when combined with IFN-γ in adenocarcinoma cell lines (64) and these studies by Browning and colleagues using different cross-linked forms of LT ligands (e.g. LTα1β2) and antibodies for LTβR 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 LTβR) 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 LTβR 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-γ 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-γ for the induction of apoptosis. There is evidence that when LT receptor activation by LIGHT is combined with IFN-γ this enhanced LIGHT cytotoxicity (111), in accordance with studies that cross-linking LTβR alone with soluble LIGHT in presence of IFN-γ is sufficient to induce cell death (65). The studies by Bechill et al showing that in HeLa and HT29 cells LIGHT/IFN-γ 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 LTβR and HVEM with wild type membrane-presented LIGHT induces extensive cell death in carcinoma cells of various tissue origins in the absence IFN-γ compared with soluble agonists (Albarbar and Georgopoulos, unpublished observations).

Therefore, there is an emerging picture that although the activation of LT receptors (LTβR and/or HVEM) requires the synergistic action of IFN-γ to induce adequate cytotoxicity, membrane-presented agonist may engage the apoptotic pathway more effectively thus negating the need for IFN-γ 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 administrated 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.

TNFR	Cellular origin	Signal initiation	TNFL	Reference
TNFRI, TNFRSF1A, p55-60, and CD120a, TNFR60 and TNFRSF1A	Nucleated cells and all tissues	DD plus TRAF2,5	TNFα, cachectin, DIF, LTα3	(149-153)
TNFRII, CD120b, p75-80 and TNFRSF1B	Inducible on immune cells and hematopoietic	TRAF1,2,3	LTα3, TNFα, LTα2β1	(15, 150, 154, 155)
LTβR, TNFRSF3, CD18, TNFCR, TNFRIII	Fibroblast, epithelial, myeloid cells and most tumour cells	TRAF2,3,5	LTβ, LTα2β1, LTα1β2, 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. TNFα can be both either membrane-bound or secreted and binds to and activates TNFRI and TNFRII, whereas LTα3 exists in soluble homotrimeric form. LTβ is not shed into soluble form and can bind with LTα to form LTαβ complexes. Heterotrimeric LTα1β2 binds LTβR and LTα2β1 binds with TNFRI, TNFRII as well as LTβR. LIGHT binds LTβR 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, LTβR). 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-κB (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-κB may involve canonical (classical) and noncanonical (alternative) pathways. The canonical pathway depends on NIK and activation of trimeric complex of IKKαβγ and phosphorylation of IKBα to p50/RelA; the noncanonical pathway of NF-κB is dependent on NIK and IKKα 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.

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.

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