Signal transduction by tumour necrosis factor and tumour necrosis factor related ligands and their receptors

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Many biological functions are regulated through interactions of extracellular molecules with their cognate cell surface receptors. The transduction of these signals by their receptors at the plasma membrane to the intracellular machinery results in such cellular activities as gene activation, protein phosphorylation, cell proliferation, and cell destruction. Though the kinetics of these activities may differ, their interactions are coordinated by selective interplay between the receptors' intracellular domains and a select set of intracellular receptor binding proteins. One such family of extracellular molecules and their cell-surface receptors are the tumour necrosis factor (TNF) family of related cytokines and receptors, which is the topic of this review.

Rheumatoid arthritis, a disease of joint inflammation and destruction, is the result of inappropriate activation of resident and inflammatory cells within the synovial tissue. The consequence of an initiating and as yet unknown stimulus, the cascade of inflammatory processes are chronic and self perpetuating. The inflammation in the joints characteristic of arthritis is believed to be attributable largely to misregulation of cytokine production, abnormal expression of receptors, or the absence of counter-regulatory pathways. Two proinflammatory cytokines, TNF and interleukin 1 (IL1), are believed to be the major cytokines cooperating in the pathology of this disease. Therapeutic approaches that inhibit the interaction of these ligands with their receptors has been a successful avenue in the treatment of rheumatoid arthritis.

One characteristic common to both TNF and IL1 is their ability to activate the transcription factor nuclear factor-kappa B (NF-kB), which is responsible for regulation of a number of genes necessary for the inflammation process. More recently, the elucidation of the TNF and IL1 signalling pathways has provided novel candidate molecules from which to develop therapeutic inhibitors that would block NF-kB activation. Furthermore, additional members of the TNF family have been discovered and are also capable of activating NF-kB. To date, 21 members of the TNF receptor superfamily and 17 members of the TNF ligand superfamily have been identified. Most of these ligand/receptor pairs participate in modulating various physiological processes, including the immune response, anti-tumour activity, cellular proliferation and differentiation, and apoptosis. Many of these physiological processes are controlled through a network of these and other cytokines produced by various types of cells and their aberrant regulation may result in inflammatory diseases. In this review, we would like to focus on the recent developments in the characterisation of the TNF signalling pathway learned from multiple approaches including gene disruption in mice and on reports of recently discovered members of the TNF ligand and receptor superfamilies. It is likely that these novel cytokines also cooperate in regulating the immune system, and thus may be involved in inflammatory diseases.

**TNF signal transduction**

Although produced primarily by activated macrophages, small amounts of TNF are produced by several other cell types. TNF is expressed as a 26 kDa transmembrane protein, which is processed to a soluble 17 kDa protein released via specific proteolytic cleavage. Some of the well known activities ascribed to TNF include septic shock, cytotoxicity, inflammation, and viral replication. Clearly, TNF is a pleiotropic cytokine perhaps because virtually all cells express at least one of the two types of TNF receptors. The signalling pathways initiated by TNF binding to its receptor have been extensively investigated, clarifying the signalling components linking receptor activation to biological activities. The advent of the yeast two hybrid system for identifying protein-protein interactions and the availability of expressed sequence tag (EST) databases have assisted in the identification of a unique and novel set of signalling machinery used by TNF and other related family members. These novel adaptor proteins seem to be promiscuous and thus are used by more than one TNF receptor family member for signal transduction. Although specific functions have been assigned to these adaptors in relation to the cellular responses activated by TNF receptor engagement, the physiological relevance of each adaptor protein in the context of ligand stimulation must await its targeted disruption in mice. Where these experiments have been done, however, some unexpected findings have emerged.

Signalling cascades initiated by various members of the TNF receptor family include those that activate transcription factors (that is, NF-kB and AP1), protein kinases (that is, MAPK, JNK, p38), and proteases. Over the past few years, a number of novel adaptor proteins have been identified that initiate these signalling cascades. One family, the death-
domain proteins, link death receptors to downstream proteases of the caspase family necessary for activation of apoptosis. The death domain is a protein-protein interaction motif, which is a conserved stretch of approximately 90 residues. The homophillic or heterophillic interaction between death domain containing proteins is most probably through electrostatic interactions, as revealed by the structure of the death domain of Fas, which consists of a series of antiparallel amphipathic α-helices with many exposed charged residues. For example, ligand binding to the cell surface receptor causes a rearrangement of the intracellular domain to oligomerise with adaptors and in turn initiate signal transduction cascades. Consistent with this model, forced overexpression of death receptors in cultured cells causes a ligand independent apoptotic effect indistinguishable from ligand stimulation. Thus, it seems that oligomerisation caused by ligand binding to the receptor initiates the signalling cascades.

A second family of adaptor proteins identified as signalling components of the TNF receptor family is the TNF receptor associated factor (TRAFs) family, which appears to function primarily in the activation of transcription factors and protein kinases. The TRAF family consists of six distinct proteins, each containing a ring and zinc finger motif in their N-terminal and C-terminal domains that appear to be responsible for self association and protein interaction (fig 1). All, except for TRAF4, were identified through yeast two-hybrid screening using a cytoplasmic domain of various members of the TNF receptor family. To date, TRAF4 has no known function. The interaction of TRAF1, TRAF2, and TRAF5 with various cytoplasmic domains of TNF receptor

**Figure 1** The TRAF family of proteins. Top, each of the TRAF molecules is depicted with the indicated motifs. Bottom, members of the TNF receptor family are listed with the TRAF molecules that directly interact with the receptor. LMP-1 and IL1 receptors are not members of this family, but have been shown to bind to TRAF molecules.
family members requires a specific motif in the receptor (that is, PXQXT). Unlike these TRAF molecules, TRAF6 uses a distinct motif (that is, QXPXE), which has been identified in CD40 and RANK. However, of the known TRAF molecules, only TRAF2, TRAF5, and TRAF6 have been demonstrated to mediate NF-κB and JNK activation.

Over the past few years, the signalling machinery linking the TNF receptor to three downstream targets (that is, apoptosis, NF-κB, and JNK activation) has been elucidated (fig 2). To understand the complexity of the identification of the signalling components of a pathway, one must first understand how they are identified. For example, after the adaptor is identified, it is examined for its ability to either activate or inhibit downstream signalling pathways by transfection of its cDNA into cultured cells. Furthermore, a mutant version of the potential adaptor molecule is introduced into cells and examined for its ability to inhibit a specific ligand dependent end point. If the mutant version blocks this pathway, then one concludes this adaptor molecule participates in signalling by the tested ligand. Although this is not a foolproof scheme, it has become a very powerful tool in the study of the signalling events affected by TNF and other members of this family. There are potential pitfalls to arriving at general conclusions when performing these types of experiments. However, the physiological role of these adaptor molecules in TNF signalling and development has been recently revealed by targeted disruption of their genes in mice, most notably TRAF2, RIP, FADD, caspase 8, and FAN.

One of the first molecules to be identified and required for NF-κB and JNK activation by TNF was TRAF2. Initially, when it was discovered, this protein was shown to activate NF-κB, and was later found to activate JNK when overexpressed in cultured cells. Furthermore, a mutant version of TRAF2 could inhibit TNF induced NF-κB and JNK. Thus, from the early reports it appeared that TRAF2 was essential for TNF dependent NF-κB activation. However, from the TRAF2 knockout mouse model, TNF could surprisingly still activate NF-κB in embryonic fibroblasts, but not JNK. Furthermore, TRAF2 -/- mice appeared normal at birth but became progressively runted and died prematurely. Defects in B cell precursors and atrophy of the thymus were also observed. Moreover, these mice exhibited increased serum concentrations of TNF, and thymocytes and haematopoietic cells were highly sensitive to TNF induced apoptosis. These observations suggest that TRAF2 is required for TNF induced JNK activation and also important in the regulation of lymphocyte function and growth.

Figure 2 Schematic diagram of TNF signal transduction molecules and the biological activities activated by the adaptor proteins.
TNF related ligands and their receptors

RIP, or receptor interacting protein, which was initially identified as a Fas associated death domain kinase, seems not to play a part in Fas mediated apoptosis but rather in TNF mediated NF-κB activation. In vitro RIP activates apoptosis, NF-κB and JNK; however, the physiological role of RIP was determined by targeted disruption of its gene in mice. RIP deficient mice appear normal at birth but begin to deteriorate by extensive apoptosis in both the lymphoid and adipose tissues and die at 1–3 days of age. Although TNF and Fas are able to activate apoptosis in RIP −/− cells, TNF fails to activate NF-κB. Thus, it appears that RIP, but not TRAF2, is required for TNF induced NF-κB activation.

The Fas associated death domain, or FADD (Mort1), was originally identified by its ability to associate with the Fas death domain. Subsequently, a mutant version of FADD inhibited TNF, Fas, and DR3 induced apoptosis, but not activation of NF-κB, suggesting that the activation of NF-κB and apoptosis are separable. The physiological role of FADD was elucidated in mice lacking FADD. The FADD −/− mice did not survive past day 11.5 of embryogenesis because of extensive abdominal haemorrhage and cardiac failure. Furthermore, FADD −/− mice are not susceptible to TNF, Fas, and DR3 induced apoptosis, but the apoptosis pathway induced by DR4 remains intact. Thus, not only is FADD required to initiate apoptosis by some death receptors, but also FADD appears to be required for embryonic development.

FADD-homologous ICE/CED-3-like protease, or FLICE (MACH1), was originally discovered in a stimulated Fas complex and by a yeast two-hybrid screen using FADD as the bait. Based on its homology to other caspases, FLICE was later designated caspase 8. Upon ligation, the TNF receptor recruits the death domain protein TRADD (TNF receptor associated death domain), which interacts with FADD and engages caspase 8 to initiate the apoptotic pathway. This signalling pathway was verified in mice lacking caspase 8. Similar to the FADD −/− mice, targeted disruption of caspase 8 in mice was lethal because of impaired heart muscle development and congested accumulation of erythrocytes. Although the ability of TNF to activate NF-κB and JNK was not impaired, caspase 8 −/− mice exhibited a defect in activation of apoptosis by TNF, Fas, and DR3. Thus, of these known death receptors, all appear to require caspase 8 as the initiating caspase leading to apoptosis.

Besides its apoptotic and inflammatory responses, TNF also generates other signalling molecules including ceramide, which is a lipid second messenger. Ceramide is generated from the lipid sphingosine by the activation of neutral sphingomyelinase (N-SMase). To link TNF receptor activation to sphingomyelinase activity, another protein was identified by a yeast two-hybrid screen and designated FAN, or factor associated with N-SMase activation. TNFR1 interacts with FAN through a small region N-terminal to the death domain. To analyse the physiological role of FAN in TNF activation of N-SMase, FAN deficient mice were generated. FAN −/− mice are born healthy and exhibit no overt phenotypic abnormalities, but the ability of TNF to activate N-SMase was impaired in FAN −/− mice. Signalling through TNFR1, TNF promotes skin permeability barrier repair involving sphingomyelinase. As this repair process of the cutaneous barrier leads to the proliferation of the epidermis, FAN −/− mice have a reduced ability to cause this repair process. Although the lack of FAN does not appear to inhibit other TNF signalling pathways, FAN does appear to be involved in the activation of N-SMase by TNF.

New members of the TNF receptor family

The TNF receptor family consists of 21 known members, which are characterised by two to four homologous cysteine rich repeats in their extracellular domain. Members of this receptor superfamily contain no significant homology within their intracellular domains, except for those that possess a death domain. Despite not having intrinsic enzymatic activity, the TNF receptor family recruits novel adaptor proteins, primarily death domain containing proteins and proteins of the TRAF family. Some members of the TNF ligand superfamily bind more than one receptor, as is the case for TRAIL, which binds five distinct receptors (that is, TRAIL R1-R4 and OPG) and LIGHT, which binds two receptors (that is, HVEM and LTβR). However, which receptor-ligand pairs are physiologically relevant remains to be determined. The previously described TNF receptor family members (TNFRI, TNFRII, LTβR, Fas, NGFR, CD27, CD30, CD40, OX40, and 41BB) have been reviewed elsewhere. In this review we will introduce the recently discovered members of this receptor family (table 1). The TNF receptor family can be divided into three groups: (1) those that contain a death domain, (2) those that do not contain a death domain, and (3) those that lack a transmembrane domain, and thus are secreted, soluble forms that may in fact inhibit cytokine signalling.

DEATH RECEPTOR 3 (DR3, LARD, WSL-1, TRAMP)

Death receptor 3 was identified by a search for TNF receptors using the extracellular domain, the death domain homologous regions, and an EST database. Others identified this receptor and named it LTαD, WSL-1, or TRAMP. DR3 encodes a protein of 417 amino acids with a death domain contained between residues 335 and 413. The mRNA expression pattern was restricted to spleen, thymus, colon, intestine, prostate, and PBLs. Upon T cell activation, a selective change in its alternative splicing results in predominantly the membrane bound form, which may have implications in lymphocyte proliferation after activation. The ligand for DR3 has now been demonstrated to be TWEAK. Signal transduction by DR3 seems to use adaptor proteins such as TRADD, TRAF2, FADD, and FLICE. When overexpressed, DR3 activates NF-κB, apoptosis, and JNK.
Table 1 New members of the TNF receptor superfamily

<table>
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<th>Abbreviation</th>
<th>Receptor name</th>
<th>Alternative names</th>
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The first receptor for TRAIL was identified through a search of an EST database for TNF receptor family members and was termed death receptor 4 (DR4). It also known as TRAIL-R1, after which various laboratories identified three more receptors for TRAIL, TRAIL-R2 (DR5, TRICK2, KILLER, Apo2) and TRAIL-R3 (DeR1, TRID) and TRAIL-R4 (DeR2, TRUNDD, LIT).

The TRAIL receptors have been extensively reviewed. These receptors are present in a wide variety of normal tissues and in normal and tumour cell lines. Unlike TRAIL-R1 and -R2, TRAIL-R3 does not contain an intracellular domain and TRAIL-R4 contains an incomplete death domain. These data suggest that TRAIL-R3 and -R4 could serve as decoy receptors for TRAIL on the cell surface and protect the cells from TRAIL induced apoptosis. Consistent with this idea, overexpression of either decoy receptor in TRAIL sensitive cell lines protect them from TRAIL induced apoptosis. There are reports, however, suggesting that cells expressing R3 and R4 are still susceptible to TRAIL induced apoptosis, (Griffiths and Lynch and unpublished data), though there appears to be a direct correlation of the expression of the cytoplasmic caspase inhibitor (FLIP) to the protection of TRAIL induced apoptosis. However, the results of extensive studies by various laboratories have been somewhat contradictory. Some of the adaptor proteins that may be used by the TRAIL receptors include TRADD, FADD, caspase 8, caspase 10, and FLIPs. Furthermore, there are contradictory reports on whether TRAIL or its receptors activate caspase 8, caspase 10, and FLIPs. Further, there remains to be determined.

The TRAIL receptors have been extensively reviewed. The transcript for DR6 was expressed abundantly in brain, heart, placenta, pancreas, lymph node, thymus, and prostate and minimally expressed in liver and PBLs. Among cell lines examined, non-lymphoid tumour cells (HeLa S3, SW480, A549, and G361) had the highest expression of DR6; haematopoietic cell
lines (HL-60, K562, MolT4, Raji) the lowest. As with other death receptors, overexpression of DR6 induced apoptosis of HeLa cells but this was not observed in MCF-7 cells, indicating cell type specificity. As MCF-7 cells are known to be quite sensitive to TNFR1, Fas, and DR4, it may be that the mechanism of cell killing by DR6 differs from that of other death receptors. The deletion of the death domain from DR6 abolished its ability to induce apoptosis. In co-transfection and immunoprecipitation assays DR6 interacted weakly with TRADD and not at all with other death domain proteins, including FADD, RIP, and RAI1. Thus, DR6 may activate apoptosis by associating with other novel, unknown death proteins.

Like most other death receptors, overexpression of DR6 induced NF-κB activation; this was abolished when the death domain was eliminated, suggesting there is a common adaptor molecule for NF-κB and apoptosis. Overexpression of DR6 also activated JNK; this response was not abolished on truncation of the death domain, indicating that JNK activation is mediated by a cytoplasmic region distinct from that activating NF-κB and apoptosis. As TRAF molecules are capable of activating JNK and NF-κB and as DR6 contains two potential TRAF binding motifs, it is possible that DR6 uses TRAF molecules to activate NF-κB and JNK.

Unlike other death receptors (that is, TNFR1, Fas, DR3, DR4, and DR5), which are expressed in most tissues and haematopoietic cells, DR6 is expressed only in cells of non-haematopoietic origin, suggesting that its physiological role may differ. In addition, the death domain of DR6 is located proximal to the transmembrane domain, rather than at the C-terminus of the receptor, and DR6 contains at least three more protein interaction motifs than the other death receptors (leucine zipper, SH3, and a C-terminal helical domain) located C-terminal to the death domain, which suggests that DR6 may in fact activate other signalling cascades. DR6 induces apoptosis in a cell type specific manner and is a potent activator of NF-κB and JNK. Finally, because TRADD interacts weakly with DR6, other, alternate signalling components may be used by DR6.

DECOY RECEPTOR 3 (DCR3/TR6)

A search of the EST databases for other TNF receptor related genes identified a novel member of the TNF receptor family, which was named DcR3 or TR6. DcR3 encodes a protein of 300 amino acids with a molecular mass of approximately 40 kDa. Unlike other members of this receptor family, DcR3 does not contain a transmembrane domain and thus is secreted as a soluble protein similar to OPG (see below). Its mRNA appears to be expressed in lung, brain, liver, spleen, and colon. The DcR3 transcript was detected weakly in most haematopoietic cell lines and was induced upon T cell activation. Interestingly, DcR3 was constitutively expressed in endothelial HUVEC cells. DcR3 binds to both LIGHT and FasL and was able to inhibit apoptosis by both of these cytokines. The expression of this soluble decoy receptor may contribute to immune system evasion by certain tumours.

RANK (RECEPTOR ACTIVATOR OF NF-κB)

A recently described TNF receptor family member, RANK (for receptor activator of NF-κB), bears high similarity in its extracellular domain to CD40. It consists of a 616-amino acid transmembrane receptor, of which 383 amino acids reside in the intracellular domain, and does not appear to be homologous to any other family member. RANK mRNA is ubiquitously expressed in human tissues, but cell surface RANK is expressed only on dendritic cells, the CD4+ T cell line MP-1, foreskin fibroblasts, osteoclast progenitors, and activated B and T cells. However, its ligand RANKL (see below) appears to be restricted to activated B and T cells. RANK appears to use the TRAF family of signal transducers to activate NF-κB and JNK pathways. Furthermore, a novel TRAF6 interaction motif was identified and shown to be required for activation of NF-κB. More-over, transgenic mice expressing a soluble form of RANK have severe osteopetrosis because of a reduction in osteoclasts, similar to OPG transgenic mice (see below). The observations that RANK interacts with TRAF6 and that TRAF6 deficient mice exhibit an osteopetrotic phenotype because of a defect in bone resorption suggest a direct involvement of RANK and its ligand in osteoclastogenesis. Thus, how each of these TRAF molecules regulates RANK/RANKL signal transduction pathways resulting in osteoclast differentiation and B and T cell modulation remains to be determined.

OSTEOPROTEGERIN (OPG/OCIF/TR1/FDCR-1)

OPG was first identified by sequence homology as a possible novel TNF receptor family member during a rat intestine cDNA sequencing project. OPG was also identified by various other laboratories and named OCIF, TR1, and FDCR-1. OPG binds not only RANKL, but also TRAIL. Unlike the other TNF receptor family members, OPG, a 401 amino acid protein, does not contain a transmembrane domain and thus is secreted as a soluble receptor. Its mRNA is expressed in heart, placenta, lung, liver, bone marrow, spleen, lymph node, and kidney, and at lower levels in the thymus, prostate, testis, ovary, and small intestine. Initially expressed as a 55 kDa protein, OPG is converted to a disulphide linked dimer of approximately 110 kDa and is secreted into the medium. Others have confirmed that OPG is membrane associated, most likely through association with the extracellular matrix. In its carboxy terminus, OPG contains a homologous death domain that, when expressed as a transmembrane form, activates apoptosis. The main physiological feature of OPG appears to inhibit RANKL from binding to osteoclast progenitors, and thus inhibits osteoclastogenesis. Consistent with inhibition of osteoclastogenesis,
TGF-β1 upregulated OPG mRNA while suppressing RANKL in murine bone marrow cultures.76 Moreover, OPG deficient mice exhibit an early onset of osteoporosis.77 The unique ability of OPG to increase bone mass has resulted in a potential treatment for osteoporosis, which is entering phase I clinical trials in post-menopausal women.

### HERPES VIRUS ENTRY MEDIATOR (HVEM/TR2/ATAR)

A novel TNF receptor family was identified by searching an EST database for a TNF related receptor protein and was termed HVEM,80 ATAR81 and TR2.82 This receptor was also identified through a screen for receptors that would enable entry of herpes simplex virus-1 into cells.83 This receptor encodes a protein of 281 amino acids, whose mRNA expression is restricted to spleen, thymus, bone marrow, lung, small intestine, PBLS, and kidney. The ligand for this receptor was recently identified as LIGHT84 or HVEM-L.85 The cytoplasmic domain is much shorter than in other members of this family. It uses TRAF1, TRAF2, TRAF3, and TRAF585-86 to activate NF-κB and JNK signalling pathways. Furthermore, a TR2-Fc fusion protein inhibited a mixed lymphocyte reaction mediated proliferation, suggesting that this receptor and its ligand may participate in T cell stimulation.

### GLOUCOCORTICOID INDUCED TNF FAMILY RELATED GENE (GITR/ATR)

GITR, also known as AITR,86 was identified by searching an EST database for homologues to the TNF receptor family.87 Initially, a murine GITR was identified by comparing untreated and dexamethasone treated murine T cell hybridoma by using the differential display technique.88 GITR encodes a protein of 241 amino acids, whose mRNA expression is restricted to spleen, thymus, bone marrow, lung, small intestine, PBLS, and kidney. In murine cells, GITR may modulate T lymphocyte survival. Unlike the mouse homologue, human GITR was not induced by dexamethasone in peripheral blood T cells.85-87 The observations that this receptor activates NF-κB and protects against activation induced cell death suggests that GITR and its ligand may participate in T lymphocyte survival in peripheral tissues and perhaps during interaction with the vascular endothelium.

### Novel members of the TNF family

The TNF family consists of 17 known members. All members have a similar core sequence that is predicted to contain all 10 β-sheet forming sequences characteristic of TNF. This TNF-like core domain and the EST databases have led to the identification of new TNF related ligands. The previously described TNF family members (TNF, LT, FasL, NGF, CD27L, CD30L, CD40L, OX40L, and 41BBL) have been reviewed elsewhere.81-82 In this review we will introduce the recently identified members of this family (table 2).

### TRAIL (TNF RELATED APOPTOSIS INDUCING LIGAND)

One of the first TNF related ligands that was identified was named TRAIL89 or Apo2L90 in mice. TRAIL is a ubiquitous type II transmembrane protein of 281 amino acids. It can be cleaved to a soluble protein. TRAIL specifically interacts with four membrane bound receptors known as TRAIL R1-R4 (see above) and the soluble receptor OPG, which can inhibit TRAIL induced apoptosis.89,90

TRAIL appears to cause apoptosis in a variety of cell types without affecting normal (non-transformed) cells. In T cells stimulated with PMA, ionomycin, anti-CD3, interferon α, IL2, or IL15 expression of TRAIL is upregulated.81-82 Furthermore, TRAIL is upregulated upon IFN α or γ stimulation of monocytes, which then acquire the ability to kill tumour cells.83 Others have demonstrated the ability of ‘TRAIL to induce apoptosis in human melanoma cells through caspase 8 and 3, in melanoma cells that were resistant to FasL induced cell killing,93 and in phenotypically
immature CD161+/CD56- NK cells. Most remarkably, TRAIL when administered systemically caused tumoricidal activity of the mammary adenocarcinoma cell line MDA-MB-231 in mice without causing toxic side effects. TRAIL's ability to selectively kill transformed and not normal cells and its inability to activate the NF-κB pathway suggest that TRAIL may be a powerful treatment for cancer.

APRIL (A PROLIFERATION INDUCING LIGAND)

APRIL was discovered by screening a public database using a profile search based on an optimal alignment of all the currently known TNF ligand family members. An identical molecule was identified by a similar search and named TALL2. The cDNA clone encoded a type II transmembrane protein of 250 amino acids, which contained 28 amino acids in the cytoplasmic domain, 21 amino acids in the transmembrane domain, and 201 amino acids in the extracellular domain. The sequence of APRIL showed highest similarity in its extracellular domain with FasL (21%), TNFα (20%), and LTβ (18%). Expression of its mRNA revealed that APRIL was weakly expressed and restricted to a few tissues, most notably prostate, colon, spleen, pancreas, and PBLS. Interestingly, APRIL was expressed in various tumour cell lines including HL60, HeLa S3, K562, Molt-4, Raji, SW-480, A549, and G361. Remarkably, APRIL mRNA was increased in thyroid carcinoma and in lymphoma, but in the corresponding normal tissue the expression was either weak or absent.

APRIL's expression in tumour derived tissues, but not normal tissue suggested that APRIL may serve in tumour growth proliferation. Indeed, recombinant APRIL caused proliferation in Jurkat T lymphoma cells, in some B cell lymphomas (that is, Raji, mouse A20), and in some cell lines of epithelial origin such as COS, HeLa, and some melanomas. Further NIH-3T3 cells engineered to express APRIL increased tumour growth rate in nude mice as compared with NIH-3T3 cells expressing no ligand. The mechanism by which APRIL induces cellular proliferation is not known, but it does not appear to activate NF-κB or JNK. The APRIL receptor has not yet been identified, but it does not appear to be any of the known members of the TNF receptor family. The little information we do have about APRIL and its expression in tumour cells (compared with normal tissue) suggests that APRIL may play a part in tumorigenesis. Thus, antagonistic antibodies to APRIL or its receptor may have a potential for therapeutic intervention.

TWEAK (TNF RELATEDNESS AND WEAK INDUCER OF APOPTOSIS)

TWEAK was first identified as a clone that weakly hybridised to an erythropoietin probe whose primary sequence was similar to ligands of the TNF family. An identical molecule was identified through a screen of an EST database by its homology to TNF family members and was named Apo3L. TWEAK is a 249 amino acid type II transmembrane protein whose mRNA is expressed in essentially all tissues examined. Soluble recombinant TWEAK caused IL8 secretion in HT29, A375, WI-38, and A549 cells. Additionally, TWEAK caused weak induction of apoptosis in HT29 cells when cultured with IFNγ. In contrast, others have shown that TWEAK activates apoptosis strongly in MCF-7 cells, the activation being dependent on FADD and caspase activation. TWEAK specifically interacts with the death receptor, DR3. The activation of NF-κB by TWEAK was also demonstrated to be TRAF2, TRADD, RIP, and NIK dependent. TWEAK induces proliferation in a variety of normal endothelial cells and in aortic smooth muscle cells and reduces culture requirements of serum and growth factors. TWEAK induces a strong angiogenic response when implanted in rat corneas, suggesting a physiological role for TWEAK in vasculature formation in vivo.

VEGI (VASCULAR ENDOTHELIAL GROWTH INHIBITOR)

To identify an autocrine inhibitor of angiogenesis specific to endothelial cells, a cDNA library was constructed from RNA derived from various TNF homologues in this EST database showed a type II transmembrane protein of 174 amino acids with 20–30% homology to TNF family members. As the new protein was subsequently found to be able to inhibit endothelial cell growth, it was designated VEGI. Unlike other members of the TNF family, VEGI is expressed predominantly in endothelial cells. Local production of a secreted form of VEGI via gene transfer caused complete suppression of the growth of MC-38 murine colon cancers in syngeneic C57BL/6 mice. Histological examination showed marked reduction of vascularisation in MC-38 tumours that expressed soluble but not membrane bound VEGI or were transfected with control vector. The conditioned media from soluble VEGI expressing cells showed marked inhibitory effect on in vitro proliferation of adult bovine aortic endothelial cells. VEGI is a novel angiogenesis inhibitor of the TNF family and functions in part by directly inhibiting endothelial cell proliferation, suggesting that VEGI may be highly valuable in angiogenesis based cancer therapy.

RANKL (RECEPTOR ACTIVATOR OF NF-κB LIGAND)

Human RANK ligand (also known as OPGL, TRANCE, ODF) is a type II transmembrane protein with an approximate molecular mass of 45 kDa and is expressed primarily on activated T and B cells and osteoclast progenitors. A recent review is available. Like other ligands of the TNF superfamily, RANKL has been demonstrated to activate NF-κB and JNK. Furthermore, stimulation of dendritic cells with RANKL up regulates the expression of the anti-apoptotic protein Bcl-XL, suggesting a potential role for RANKL in dendritic cell survival. RANKL was also demonstrated to be cleaved from the
cell surface by the TNF converting enzyme.\textsuperscript{106} Moreover, RANKL has been demonstrated to play an essential part in osteoclast differentiation and activation.\textsuperscript{66, 70, 71, 107, 108} Targeted disruption of RANKL in mice resulted in the essential requirement for RANKL to induce osteoclastogenesis. Additionally, RANKL deficient mice had poor lymphocyte development and lymph node organogenesis.\textsuperscript{109} A similar phenotype was also observed in TRAF6 deficient mice.\textsuperscript{71} Moreover, in rheumatoid arthritis (RA) patients, IL17 in synovial fluids upregulated RANKL.\textsuperscript{110} Concentrations of IL17 in synovial fluids were significantly higher in RA patients than in osteoarthritis patients. Anti-IL17 antibody significantly inhibited osteoclast formation induced by conditioned media from RA synovial tissues. These findings suggest that IL17 first acts on osteoblasts, producing a mediator that stimulates both COX-2 dependent PGE2 synthesis and RANKL gene expression, which in turn induces differentiation of osteoclast progenitors into mature osteoclasts. They also suggest that IL17 is a crucial cytokine for osteoclastic bone resorption in RA patients.

THANK (A TNF HOMOLOGUE THAT ACTIVATES APOPTOSIS, NF-κB, AND JNK)

By using an amino acid sequence motif of TNF and searching an EST database, a novel TNF homologue encoding 285 amino acids was identified and named THANK.\textsuperscript{111} The predicted extracellular domain of THANK is 15, 16, 18, and 19% identical to LIGHT, FasL, TNF, and LTα, respectively. Northern blot analysis of its mRNA indicated expression in PBLs, spleen, thymus, lung, placenta, small intestine, and pancreas. THANK mRNA expression was highest in HL60 followed by K562, A549, and G361, but there was no expression in HeLa, Molt-4, Raji, and SW-480. Recombinant THANK protein activated NF-κB and JNK in the promyeloid cell line U937. Additionally, THANK induced activation of apoptosis in U937 cells. The receptor for THANK is at present unknown, but THANK does not bind TNFR1 or TNFR2. Identical molecules to THANK were identified and named TALL\textsuperscript{109} and BAFF.\textsuperscript{112}

LIGHT

An additional member of the TNF family, named LIGHT, was identified by searching an EST database for sequence similarity to TNF family members.\textsuperscript{84, 113} An identical molecule was identified by its interaction with HVEG and designated HVEG-L.\textsuperscript{85} LIGHT mRNA is highly expressed in splenocytes, activated PBLs, CD8+ tumour infiltrating lymphocytes, granulocytes, and monocytes, but it is not expressed in the thymus or in tumour cells. Additionally, LIGHT is upregulated in CD4+ and CD8+ T cells when exposed to PMA. LIGHT encodes a type II transmembrane protein of 240 amino acids. LIGHT binds not only to HVEG, but also to the LTβ receptor. A soluble, secreted form of LIGHT stimulates proliferation of T lymphocytes during allogeneic responses, inhibits HT-29 cell growth, and weakly stimulates NF-κB dependent transcription.\textsuperscript{85} The MDA-MB-231 human breast carcinoma transected with LIGHT caused complete tumour suppression in mice. Histological examination showed marked neutrophil infiltration and necrosis.\textsuperscript{64} IFNγ dramatically increases LIGHT mediated apoptosis, and LIGHT induces apoptosis of various tumour cells that express both LTβ and HVEG receptors. However, LIGHT was not cytolytic to the tumour cells that express only the LTβR or HVEG or haematopoietic cells that express only the HVEM, such as PBLs, Jurkat cells, or CD8+ TIL cells. In contrast, treatment of the activated PBLs with LIGHT resulted in release of IFNγ. Taken together, LIGHT triggers distinct biological responses based on the expression patterns of its receptors on the target cells. Thus, LIGHT may play a part in the immune modulation and have a potential value in cancer therapy.

GITRL (GLUCOCORTICOID INDUCED TNF FAMILY RELATED LIGAND)

The ligand for GITRL was identified by a yeast based signal sequence trap method from a HUVEC cDNA library.\textsuperscript{87} This ligand was also identified in a EST database search for TNF related ligands.\textsuperscript{86} GITRL encodes a 177 amino acid type II transmembrane protein with a calculated mass of 20 kDa. Analysis of its mRNA revealed highest expression in small intestine, ovary, testis, and kidney, and lower to no expression in other tissues. Expression of membrane-bound GITRL was detected on cultured HUVEC.\textsuperscript{87} Expression of either GITRL or its receptor or both the ligand and receptor in Jurkat cells inhibited activation induced cell death.\textsuperscript{87} Consistent with the inhibition of apoptosis, GITRL activated the proapoptotic transcription factor NF-κB.\textsuperscript{85, 87} Thus, GITRL may modulate peripheral T cell interaction with blood vessels in the periphery.

Conclusions and future perspectives

As new members of the TNF ligand and receptor superfamilies are being discovered, one interesting characteristic seems to be common, that most of these ligands have the ability to activate the transcription factor NF-κB. As this factor is one of the primary modulators of the inflammatory process, it would not be surprising to find more than one of these cytokines involved in RA and other types of inflammatory diseases. As most of these ligands seem to be synthesised by cells of the immune system, it will be most important to understand how each of these cytokines act under physiological conditions. For instance, RANKL and its soluble receptor OPG, whose cDNAs were just described 18 months ago, are essential for osteoclastogenesis. Uncovering the physiological role for RANKL and OPG has recently led to the initiation of phase I clinical trials for the treatment of osteoporosis. With the availability of antibodies to these new ligands and their recombinant proteins, we are poised to investigate their physiological roles in the immune
system and in various diseases such as RA. For example, a recent report demonstrated that the increased levels of IL17 in synovial fluid from RA patients caused an increase in osteoclastogenesis. This was most probably attributable to the increased expression of a novel member of the TNF family, RANKL, which is required for osteoclastogenesis, suggesting that IL17 present in synovial tissues and fluids from RA patients may be involved in the joint destruction associated with this disease. Thus, with the identification of the signalling pathways and the physiological roles associated with these new ligands, it may be possible to develop new therapeutic approaches to combat various inflammatory diseases and cancer.

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10 Darnay BG, Ni J, Moore PA, Aggarwal BB. Activation of tumor necrosis factor receptor 1 and Fas (Apo-1/CD95) results in the production of active NF-kB but not all inducers of apoptosis. Science 1998;279:512-5.


13 Karin M, Delhase M. JNK or IKK, AP-1 or NF-kB, which mediates tumor necrosis factor receptor 1 activation of NF-kB but not all inducers of apoptosis. Science 1998;279:512-5.
54 Degli-Esposti M. To die or not to die-the quest of the TRAIL receptors. J Leuk Biol 1999;65:535–42.
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