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Mini-Review

Molecules of the Tumor Necrosis Factor (TNF) Receptor and Ligand Superfamilies: Endless Stories

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Tumor necrosis factor (TNF) receptor members have unique structures composed of 2–4 cysteine — rich pseudorepeats in the extracellular domain. On ligation by trimeric ligand molecules, oligomerization of three receptor molecules occurs, which in turn activates the receptor and recruits intracellular signaling molecules to the cytoplasmic tail to initiate biological events. Recently, the numbers of tumor necrosis factor receptor and ligand family members have been rapidly expanding. Functional characterization of the new members has indicated redundant roles with other known members as well as provided insights into novel functions. In particular, identification of soluble decoy receptors which have the ability to bind multiple ligands highlights a complex control mechanism of immune responses by these molecules. Studies of the new members have also revealed that the TNF receptor and ligand family members play an important role in other than the immune system.

Keywords: Costimulation, Decoy receptor, Signaling, Tumor necrosis factor ligand, Tumor necrosis factor receptor.

Introduction

The immune system has fine mechanisms of regulating two important biological processes, cell proliferation and apoptosis, to maintain homeostasis and to properly manage immune responses to foreign antigens. If the balance between these two is disturbed, it has an unwanted effect on the body. Molecules of the TNF receptor and ligand superfamilies play a central role in regulating the two phenomena in the immune system. However, their functions are by far more diverse, extending even beyond the immune system, including embryonic development, organogenesis, and metabolism.

The numbers of the receptor and ligand family members have been rapidly expanding, mainly with the help of massive DNA sequencing and bioinformatic technology. Thus far, twenty-five members of the receptor family and twenty members of the ligand family have been described. With the emergence of new members, they bring up more functional and regulatory complexity to the superfamilies. Here, we will review recent investigations of newly identified members of the TNF receptor and ligand superfamilies.

Structural Characteristics of the TNF Receptor and Ligand Superfamilies

Molecules of the TNF receptor superfamily are Type I membrane proteins where the N-terminal region is extracellular and the C-terminal region is intracellular with a short transmembrane domain flanking between them. They are characterized by cysteine-rich pseudorepeats (CRPs) of two to six copies in the extracellular domain. The canonical CRP typically contains 30–40 amino acid residues with six disulfide-bridged cysteines (C1–C2, C3–C5, and C4–C6). Generally, the sequence homology in the extracellular domain among the human TNF receptor family members is in the range of 20–40%. However, each CRP is thought to adopt generally similar tertiary structures. Structural modeling studies suggest that the basic building block of the receptor structure is not the 6-cysteine, 3-disulfide unit, but rather a pair of smaller modules (Naismith and Sprang, 1998).

TNF ligand superfamily members generally exist as Type II membrane proteins. However, most members also

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exist as soluble proteins that are proteolytically cleaved from the membrane surface. Like TNF receptor family members, members of the ligand superfamily show a low level of sequence homology within the C-terminal extracellular domain ranging from 15–35%. However, the three-dimensional structure is similar between them: the extracellular domain of the monomer consists of a distinct fold of eight anti-parallel β-strands forming two β-sheets folded into the characteristic 'Jellyroll' topology (Banchereau *et al.*, 1994). An active form of the ligand members is a trimer. The trimeric molecule is broader at the bottom and narrower at the top (Eck and Sprang, 1989; Eck *et al.*, 1992).

The structure of the TNFR1 and LT α complex has been solved (Banner *et al.*, 1993). The TNFR1-LT α complex in the crystal shows three elongated TNFR1-LT α molecules, each binding along the surface groove between two adjacent subunits. Each receptor molecule binds in three grooves of the ligand trimer formed by the subunit interfaces. The long axes of the three receptor molecules are approximately parallel to each other and to the 3-fold axis of the ligand. The N- and C-termini of the receptor molecules protrude beyond the bottom and top of the ligand trimer, respectively. Thus, the trimer of TNF ligand molecules cross-links the three receptor molecules together into a cluster, which is thought to be the activated TNF receptor state. Consistently, receptor clustering is a general mechanism for signal transduction for growth factor receptors, and cross-linking of TNF receptor family molecules by agonistic antibodies can easily replace that by trimeric TNF ligand molecules in activating the receptor molecules.

In contrast to the extracellular domain, the cytoplasmic domain has little similarity among TNF receptor family members, with one exception. Members of the TNF receptor superfamily that can induce cell death have a homologous sequence of 65–80 amino acids, termed the 'death domain' (DD). This subgroup of molecules include TNFR1, Fas, TRAILR-1, TRAILR-2, DR3, and DR6. In either case, the cytoplasmic domain does not have any enzymatic activity, but provides binding sites for adaptor molecules needed for signal transduction as discussed below.

TNF ligand superfamily members show a short cytoplasmic domain and its length is variable between members. Evidence is accumulating that the cytoplasmic domain of TNF ligand superfamily members is involved in 'reverse signaling' (Watts *et al.*, 1999). Further research will be needed to clarify this.

Signaling Aspects of the TNF Receptor Superfamily

The signaling pathways that lead to cell survival or programmed cell death (apoptosis) are regulated by molecules of the TNF receptor superfamily. Generally, DD-containing molecules regulate apoptosis, while DD-lacking molecules regulate cell survival or cell proliferation. In reality, however, this dichotomy is an oversimplification. In many cases, one molecule can exert the two seemingly opposite functions depending on the metabolic state of the cell. Nevertheless, the two distinct signaling pathways serve for cell survival or apoptosis, respectively. Here, we will restrict our discussion to the signaling pathways that promote cell survival. The signaling pathways for death receptors have been discussed in detail in a recent review by Ashkenazi and Dixit (1998).

Receptor trimerization by trimeric ligand molecules initiates to recruit intracellular signaling components to the receptor cytoplasmic tail. A family of molecules directly associate with the cytoplasmic domain of DD-lacking TNF receptor family molecules. Thus far, six members, namely TNF receptor-associated factor (TRAF) 1 through 6, have been identified and characterized. A mechanism of molecular interactions between the cytoplasmic tail of receptor family members and TRAFs has just begun to be defined. The C-terminal TRAF domain of all TRAF molecules (except TRAF4 which is localized in the nucleus) is required for binding to their associated receptors (Cheng *et al.*, 1995; Hsu *et al.*, 1995; Takeuchi *et al.*, 1996). A couple of binding motifs for TRAFs have been identified on the primary sequence of the tail region of receptors. CD40, CD30, and CD27 contain a PXQX(T/S) motif that has been demonstrated to mediate the interactions with TRAFs (Gedrich *et al.*, 1996; Aizawa *et al.*, 1997; Boucher *et al.*, 1997; Akiba *et al.*, 1998). In other receptors, TRAF binding sites are localized to stretches of 5–6 acidic amino acids (Gedrich *et al.*, 1996; Arch and Thompson, 1998). TNFR2 has an SXXE binding motif for TRAF2, which is also found in CD30 and AITR/GITR (Park *et al.*, 1999). TRAF6 interacts with only CD40 and RANK (Ishida *et al.*, 1996; Darney *et al.*, 1999). In these molecules, the TRAF6 binding site is distinct from the binding site of other TRAF molecules. However, TRAFs bind to overlapping subsets of receptors, apparently by competition for the same binding sites.

TRAFs relay a signal further downstream by interacting with other signaling molecules. Well characterized is the signaling pathway leading to the nuclear factor (NF), κB. Among the TRAFs, TRAF2, TRAF5, and TRAF6 are essential for induction of NF-κB activation. In contrast, TRAF1 and TRAF3 appear to function as a negative regulator for NF-κB activation. Activation of TRAFs may be induced by receptor-induced TRAF oligomerization or conformational change to initiate downstream events. This is supported by evidence that oligomerization of TRAF2 and TRAF6 is sufficient for induction of downstream events (Baud *et al.*, 1999). The mitogen-activated protein kinase (MAPK), NIK (NF-κB-inducing kinase) is a part of a signaling complex that is assembled by the

multimerization of TRAF proteins (Malinin *et al.*, 1997). NIK phosphorylates the IKK [IkB (inhibitor of NF-κB) kinase] complex of which the IKKβ subunit is responsible for IKK activation. IKK, in turn, phosphorylates inhibitory Ikβ proteins which form a complex with an inactive heterodimeric NF-κB, causing their degradation. The dissociation of Ikβ from the inactive NF-κB leads to activation of NF-κB, which translocates into the nucleus and binds specific NF-κB binding sites. TRAF2 also interacts with MAPK kinase kinase, MEKK1, which activates JNK (c-June-N-terminal kinase) via a MAP kinase and ultimately activates the transcription factor AP-1.

The signaling pathway leading to cell survival must be controlled by an elaborate regulatory machinery and have a cross-talk with the signaling pathway leading to apoptosis (Arch *et al.*, 1998). The regulatory machinery may operate mostly at the proximal step. To take some examples, since TNF receptor family molecules associate with more than one TRAF molecule with a different regulatory activity, the differential recruitment of activating or inhibitory TRAF molecules seems to be one level of regulation of receptor-induced signal transduction. In addition, the possibility of homo- and/or heterodimerization of TRAF molecules that leads to the formation of higher order complexes could add another level of regulation. TRAFs have the ability to associate with other adaptor proteins such as A20, TRIP, and I-TRAP/TANK (Rothe *et al.*, 1995; 1996; Cheng and Baltimore, 1996; Song *et al.*, 1996; Lee *et al.*, 1997; Roy *et al.*, 1997). The function of these molecules is not well defined but they seem to modulate the activity of TRAFs. TRAFs also interact with signaling molecules involving the death pathway, such as TRADD, cIAPs, and RIP (Rothe *et al.*, 1995; Hsu *et al.*, 1996; Uren *et al.*, 1996). This has an implication that TRAFs are an important connection point between the death signal pathway and the cell survival pathway.

Biological Functions of Newly Identified Members of the TNF Receptor and Ligand Superfamilies

RANK, OPG/TR1/OCIF and TRANCE/OPGL/ODF

Osteoprotegerin (OPG) was originally identified as a soluble member of the TNF receptor superfamily (Simonet *et al.*, 1997). Unlike other members, it was first characterized in non-lymphoid tissue. OPG in normal mice is localized within cartilage rudiments of developing bones, as well as in the small intestine and the muscular wall of several major arteries. Transgenic mice overexpressing OPG in the liver exhibit a marked increase in bone density (osteopetrosis). In fact, it turned out that it is identical to the osteoclastogenesis inhibitory factor (OCIF), i.e., a negative regulator to inhibit osteoclast differentiation and thus to inhibit bone resorption (Simonet *et al.*, 1997; Tsuda *et al.*, 1997; Yasuda *et al.*, 1998a). This is further confirmed in OPG-deficient mice which develop early onset of osteoporosis (Bucay *et al.*, 1998). On the contrary, the ligand for OPG (OPGL) has proved to be the long-sought osteoclast differentiation factor (ODF) (Lacey *et al.*, 1998; Yasuda *et al.*, 1998b), which is identical to TRANCE (TNF-related activation-induced cytokine) encoded by an 'immediate early' gene up-regulated by TCR stimulation (Wong *et al.*, 1997a). As expected, mice with a disrupted *OPGL* gene show severe osteopetrosis and complete lack of osteoclasts as a result of an inability of osteoblasts to support osteoclastogenesis (Kong *et al.*, 1999). Taken together, OPG acts as a naturally occurring decoy receptor for OPGL expressed on osteoblasts, which initiates a signal cascade via a membrane-bound receptor for osteoclast progenitors to mature into osteoclasts. In addition, OPGL has a capacity to directly activate mature osteoclasts (Fuller *et al.*, 1998; Kwon *et al.*, 1998; Lacey *et al.*, 1998; Burgess *et al.*, 1999).

OPGL binds to a membrane-bound TNF receptor superfamily member, RANK (receptor activator of NF-κB), which was originally identified by direct sequencing of a dendritic cell (DC) cDNA library (Anderson *et al.*, 1997). Since its finding, it had been suspected that a differentiation and activation signal for osteoclasts is mediated by RANK (reviewed by Filvaroff and Derynck, 1998). Recently, Hsu *et al.* (1999) and Nakagawa *et al.* (1998) provided direct evidence that OPGL exerts its activity on osteoclast progenitors via its receptor RANK. However, the molecular mechanism by which OPGL-RANK interactions lead to the differentiation and activation of osteoclasts remains largely to be elucidated. It appears that TRAF6 is required for RANK-mediated osteoclastogenesis, even though RANK has the unprecedented ability to bind all the TRAFs that are known to be localized in the cytoplasm — TRAF1, TRAF2, TRAF3, TRAF5, and TRAF6 [This may reflect an unusually long cytoplasmic domain (383 amino acids) among the TNF superfamily (Darney *et al.*, 1998; Galibert *et al.*, 1998; Wong *et al.*, 1998).], since TRAF6-deficient mice show an osteopetrotic phenotype due to impaired osteoclast function (Lomaga *et al.*, 1999). Consistently, NF-κB knockout mice exhibit a similar phenotype (Iotsovo *et al.*, 1997). In summary, TRAF6-mediated NF-κB activation is required for osteoclast activation and differentiation. However, the possibility cannot be excluded that JNK activation is sufficient for osteoclast differentiation in the absence of NF-κB activation (Hsu *et al.*, 1999).

RANK has the highest homology with CD40 within the extracellular domain. The receptor appears to be expressed specifically on DCs among antigen-presenting cells (Anderson *et al.*, 1997; Wong *et al.*, 1997b). RANKL (OPGL) expression is more restricted than RANK

expression, and strictly limited to activated T lymphocytes (Wong *et al.*, 1997a). Like CD40L, OPGL acts as a DC survival factor and enhances DC cluster formation (Anderson *et al.*, 1997; Wong *et al.*, 1997b). Unlike CD40L, however, OPGL does not elevate the expression levels of MHC class I, cell adhesion molecules, or co-stimulatory molecules such as CD80 and CD86 on DCs. When stimulated with either CD40L or OPGL, DCs, in turn, induce T-cell proliferation. Thus, it appears that stimulation of RANK augments the co-stimulatory capacity of DCs by an undefined CD80/CD86-independent mechanism. Since signals through RANK or CD40 can mutually up-regulate each other on DCs, it is also possible that CD40L-CD40 and OPGL-RANK interactions on DCs-T cells regulate T-cell priming in a cooperative way (Kwon *et al.*, 1999a). The third possibility is that functions of OPGL and RANK can substitute for those of CD40L and CD40 in communicating between DCs and T cells. This hypothesis is supported by evidence that T-cell activation is not impaired in CD40-deficient or CD40L-deficient mice (Bachmann *et al.*, 1998). Furthermore, OPGL-RANK interaction provides the co-stimulation required for efficient $CD4^+$ T-cell priming during viral infection in the absence of CD40L or CD40 (Bachmann *et al.*, 1999). Likewise, DCs of OPGL-deficient mice do not have any defects in priming T cells, even though OPGL-deficient mice have developmental defects in T cells (Kong *et al.*, 1999).

Surprisingly, OPGL-deficient mice exhibit many defects other than osteopetrosis (Kong *et al.*, 1999). For example, there are defects in early differentiation of T and B lymphocytes in OPGL-deficient mice. This appears to be due to intrinsic defects in bone-marrow-derived cells. Perhaps the most intriguing finding in the OPGL-deficient mice is that they lack lymph nodes, yet show normal Peyer's patches and normal structure of spleen. This feature of OPGL-deficient mice differs from that of mice deficient in TNF-α, LT-α, LT-β, TNFR1, or LTβR. Thus, OPGL is an important regulator of lymphocyte development and, specifically, lymph node organogenesis.

The biological consequences of OPG action is not definitely demonstrated in the immune system. By analogy with bone metabolism, OPG could function as a natural decoy receptor for RANK, i.e., it could attenuate OPGL-mediated signals provided to/by T cells by interfering with the interaction between OPGL and RANK (Chun *et al.*, 1998). Given that OPGL is a survival factor for DCs, it is likely that OPG may function as an important regulator in counter-balancing the activity of DCs between cell death and survival (Kwon *et al.*, 1999a). In relation to this, one interesting fact is that OPG binds TRAIL, a strong inducer of apoptosis (Emery *et al.*, 1998). *In vitro* results indicate that OPG can negatively regulate apoptosis. Thus, OPG may have dual blades which can regulate two opposite processes — apotosis and cell survival.

HVEM/HveA/TR2/ATAR, DcR3/TR6, and LIGHT α-Herpesviruses utilize various types of cellular proteins as their co-receptor. Thus far, four cellular proteins have been identified as an entry receptor for α-herpesviruses (Montgomery *et al.*, 1997; Geraghty *et al.*, 1988; Warner *et al.*, 1998). Among these, HVEM [for herpesvirus entry mediator, later renamed HveA (herpesvirus entry protein A)] is a member of the TNF receptor superfamily, and the other three are immunoglobulin superfamily members. HveA interacts specifically with the gD of HSV-1 and HSV-2, and it is postulated that HveA is the principal receptor for entry of HSV into human lymphoid cells but not into other cell types (Montgomery *et al.*, 1997). In fact, HveA expression is most prominent in lymphoid tissue such as spleen and peripheral blood leukocytes (PBL), although it has a wide tissue distribution; HveA mRNA was detected in resting and activated $CD4^+$ and $CD8^+$ T cells, $CD19^+$ B cells, and in monocytes (Kwon *et al.*, 1997). Indeed, anti-HveA serum inhibits HSV-1 infection into activated T cells (Montgomery *et al.*, 1997). The molecular mechanism of gD-HveA interactions is not fully understood. Current data indicate that gD binds directly to HveA (Whitbeck *et al.*, 1997; Nicola *et al.*, 1998), and mediates fusion of the HSV viral envelope with the host cell membrane. Furthermore, HveA participates in HSV-1-induced cell fusion (Terry-Allison *et al.*, 1998), and enhances entry of HSV-1 and HSV-2 by cell-cell spread (Roller and Rauch, 1998). From the perspective of evolution, it will be interesting to find out how HSV viruses have evolved to use HveA as their co-receptor in competition with natural ligands of HveA, LIGHT, and LT-α (Mauri *et al.*, 1998). Although gD is able to compete with LIGHT and LT-α in binding HveA, evidence has been provided that the binding sites of gD and LT-α on the HveA protein are not identical (Sarrias *et al.*, 1999). Another interesting question is whether HSV viruses can modulate activities of T or B lymphocytes via HveA during binding, entry, or egress (Kwon *et al.*, 1999a). There are several lines of evidence that the hypothesis may be true. First, it is well known that HSV replicates in activated T cells and that gD is expressed on the surface of infected cells. Furthermore, gD-expressing T cells have a lower infectivity for HSV viruses, which is presumably because cellular gD interacts with HveA in a juxtacrine way, thus preventing interactions between viral gD and the HveA of T cells. This indicates that gD can function as a virokine that can modulate activities of lymphocytes via HveA, just like its natural ligand. Secondly, there is evidence that HSV entry triggers tyrosine phosphorylation of host cellular proteins (Qui *et al.*, 1999). Thirdly, the recombinant extracellular fragment of gD was shown to be sufficient to induce IFN-α in lymphocytes (Ankel *et al.*, 1998).

Expression of LIGHT, the ligand of HveA, is restricted in activated $CD4^+$ and $CD8^+$ T cells (Mauri *et al.*, 1998).

Antagonistic anti-HveA monoclonal antibodies inhibit $CD4^+$ T-lymphocyte proliferation, whereas a recombinant soluble LIGHT stimulates T-cell proliferation (Kwon *et al.*, 1997; Harrop *et al.*, 1998a; 1998b). Thus, LIGHT provides an HveA-specific stimulatory signal to activated T cells in an autocrine (cell-autonomous) and/or in a paracrine (homotypic cell-cell contact) way (Kwon *et al.*, 1999a). Blockage of HveA-LIGHT interaction results in various phenotypic changes in $CD4^+$ T cells followed by T-cell activation with anti-CD3 and anti-CD28 antibodies: inhibition of cytokine production, i.e., IL-2, TNF-α, IL-4, and IFN-γ; suppression of expression of the proliferation-associated marker CD71 and CD25, the early activation marker CD69, the co-stimulatory receptors CD30 and OX40, and the B cell stimulatory marker CD40L, and the adhesion molecules CD54 (Harrop *et al.*, 1997a). In addition to the co-stimulatory activity of HveA in T lymphocytes, HveA may be important for enforcing antigen-presenting cells (APC) activities by activated T cells, given that HveA expression is demonstrated in APCs such as B cells and monocytes/macrophages (Kwon *et al.*, 1997).

Of note is that HveA and LIGHT have cross-specificities with LTβ receptors (LTβR) and LTα (Mauri *et al.*, 1998). *In vivo* significance of the cross-specificity is unknown. Since HveA and LTβR have distinct tissue expression patterns, it is likely that LIGHT delivers different signals compared with LTα, being dependent on different microenvironments (Kwon *et al.*, 1999a). In the adenocarcinoma cell, HT29, which expresses both HveA and LTβR, however, it appears that LIGHT-induced apoptotic signal is delivered through both receptors (Zhai *et al.*, 1998), thus implying that cooperative signaling through both receptors is required for at least some LIGHT-triggered biological functions to be exerted (Kwon *et al.*, 1999a). To further complicate the story, LIGHT binds a recently identified decoy receptor 3 (DcR3 or TR6) (Pitti *et al.*, 1999; Yu *et al.*, 1999), which is a secreted protein with high sequence identity with OPG (31% amino acids identity in the extracellular domain). DcR3 has the ability to bind FasL and to inhibit the activation-induced cell death of Jurkat cells, peripheral blood T cells, and natural killer cells (Pitti *et al.*, 1999). The gene for DcR3 is amplified in certain tumors and DcR3 mRNA is expressed in malignant tissue. These observations strongly suggest that certain tumors may escape FasL-dependent immune-cytotoxic attack by expressing a decoy receptor that blocks FasL. The *in vivo* significance of LIGHT-DcR3 interactions is still obscure. One interesting characteristic shared by LIGHT and FasL is that they have anticancer activities, presumably by their pro-inflammatory activities (Chen *et al.*, 1998; Zhai *et al.*, 1998). Thus, it was proposed that LIGHT-DcR3 or FasL-DcR3 interactions might be an important immune evasion mechanism for cancer cells by inhibiting the pro-inflammatory activities of LIGHT and FasL (Kwon *et al.*, 1999a).

An interesting question in relation to HSV infections is whether host cells have a counteracting mechanism for viral infection by neutralizing the viral ligand gD with DcR3. If not, DcR3, on the contrary, may enhance HSV infection into host cells by blocking HveA-LIGHT interactions in some circumstances. In this case, HSV viruses adopt a smart strategy to efficiently infect host cells in DcR3-rich environments.

AITR/GITR and AITR-L/GITR-L GITR (glucocorticoid-induced TNF receptor family-related gene) or AITR (activation-inducible TNF receptor superfamily member) was initially identified by comparing untreated and dexamethasone-treated murine T cell hybridoma cells (Nocentini *et al.*, 1997). In human PBMCs (peripheral blood mononuclear cells), however, the expression of AITR/GITR is not induced by dexamethasone treatment (Gurney *et al.*, 1999; Kwon *et al.*, 1999b). Amino acids sequence analysis shows that the cytoplasmic domain of AITR/GITR has a remarkable homology with that of 4–1BB and CD27. This indicates that AITR/GITR may promote cell proliferation like 4-1BB and CD27. This possibility is further supported by evidence that AITR/GITR utilizes a similar signaling pathway to that utilized by 4–1BB; both molecules interact with TRAF1, TRAF2, and TRAF3 and induce NF-κB activation via TRAF2 (Kwon *et al.*, 1999b).

Currently, virtually nothing is known about the biological functions of AITR/GITR. However, we can infer its biological roles based on the following facts revealed so far: first, AITR/GITR expression is induced by T-cell activation and limited in activated T cells; second, the ligand of AITR/GITR (AITR-L/GITR-L) appears to be expressed specifically in endothelial cells (Gurney *et al.*, 1999; Kwon *et al.*, 1999b); third, AITR/GITR and its ligand protect T cells from activation-induced cell death (Nocentini *et al.*, 1997; Gurney *et al.*, 1999). Thus, AITR/GITR-AITR-L/GITR-L interaction may be important for interactions between activated T cells and blood vessels, which could lead to enhancement of the activation and/or transmigration capacity of T lymphocytes (Kwon *et al.*, 1999a).

TL1/VEGI Expression of TL1 (TNF ligand 1) or VEGI (vascular endothelial growth inhibitor) is specific for endothelial cells (Tan *et al.*, 1997; Zhai *et al.*, 1999). When murine MC-38 colon cancer cells were engineered to locally produce soluble TL1/VEGI, their growth was completely suppressed in syngeneic mice (Zhai *et al.*, 1999). It appears that this is largely due to TL1/VEGI's angiogenesis inhibitory effect. In this case, any elevated neutrophil infiltration was not responsible for tumor rejection. Although there are other explanations for this result, for example, enforcement of cytotoxic activities of

T cells by soluble TL1/VEGI, current *in vitro* evidence strongly indicates that soluble TL1/VEGI inhibits angiogenesis by inducing apoptosis of endothelial cells (Yue *et al.*, 1999). TL1/VEGI-mediated apoptosis in endothelial cells is accompanied by activation of JNK, p38 MAPK, and caspase-3. Thus, it seems that endothelial cells have a program to commit suicide in an autocrine way under some biological circumstances.

APRIL APRIL (for a proliferation-inducing ligand) is a new addition of TNF ligand superfamily members. The extracellular domain of APRIL shows high homology with BAFF (33% amino acid identity; Schneider *et al.*, 1999), FasL (21%), and TNF-α(20%). APRIL mRNA is expressed extensively in tumor cells (Hahne *et al.*, 1998). This unique expression pattern of APRIL prompted the authors to test whether it was associated with tumor cell growth. Indeed, cell proliferation was induced by treatment of various human tumor cell lines with recombinant soluble APRIL protein. The protein exerted a similar effect on mouse and monkey tumor cell lines as well. Furthermore, an APRIL-transfected mouse cells displayed an increased cell proliferation both *in vitro* and *in vivo*. Although the mechanism by which APRIL promotes tumor cell growth is currently not known, there are several features to point out in relation to this. APRIL-mediated tumor cell proliferation appears to be operated in an autocrine or paracrine way, like many TNF ligand superfamily members. However, it should be distinguished from co-stimulatory effects triggered by other members, since APRIL does not activate NF-κB or JNK (Hahne *et al.*, 1998). This point may be clarified if the receptor for APRIL is discovered.

BAFF/THANK Molecular identification of BAFF (for B cell activating factor belonging to the TNF family) or THANK (a TNF homologue that activates apoptosis, nuclear factor-κB, and JNK) was recently reported independently by two research groups (Mukhopadhyay *et al.*, 1999; Schneider *et al.*, 1999). BAFF/THANK has the highest sequence homology in the extracellular domain with APRIL (33% amino acid homology), as mentioned previously. The extracellular domain of BAFF/THANK is well conserved between human and mouse (86% identity). However, mouse BAFF/THANK has an insertion of 28 amino acids in the stalk region between the transmembrane and the first of several β strands which constitute the receptor binding domain in all TNF ligand superfamily members. It appears that BAFF/THANK exists as a secreted form as well as a membrane-bound form (Schneider *et al.*, 1999). The cleavage site consists of polybasic amino acids (R-N-K-R), which are also found in APRIL and TWEAK.

Expression of BAFF/THANK mRNA is restricted in lymphoid tissues, being prominent in PBL (peripheral blood leukocytes) and spleen (Mukhopadhyay *et al.*, 1999; Schneider *et al.*, 1999). Further examination of its expression pattern revealed that BAFF/THANK is expressed specifically in T cells and dendritic cells (Schneider *et al.*, 1999). When cell lines of various origins were screened, the BAFF/THANK receptor expression was detected only in B cell lines. Thus, these expression data suggest that BAFF/THANK on T cells initiates signals that lead to some biological activities in B cells via the BAFF/THANK receptor, as is seen in interactions of CD30L-CD30 and of CD40L-CD40. Now, it is clear that BAFF/THANK is able to function as a co-stimulator of B-cell proliferation. However, unlike CD40L, BAFF/THANK cannot counteract apoptotic signals in B cells after engagement of the B cell receptor (Schneider *et al.*, 1999). Furthermore, unlike CD30L and CD40L, BAFF/THANK is expressed on dendritic cells. Given the highly specific location of its receptor on B cells in contrast to the wide expression patterns of CD30 and CD40, the roles played by BAFF/THANK may be restricted in B cells. There is also a possibility that BAFF/THANK has a pleiotrophic effect: BAFF/THANK not only readily induces NF-κB and JNK activation, but also suppresses cell growth with comparable potency with TNF-α in histiocytic lymphoma U937 cells. The latter phenomenon is correlated with caspase 3 activation (Mokhopadhyay *et al.*, 1999). Taken together, it may be predicted that BAFF/THANK utilizes multiple receptors and transmits a distinct signal, depending on the receptor that it binds. Another possibility is that BAFF/THANK's functions are cell type-dependent.

Conclusions

Due mainly to the potential clinical importance, a great deal of efforts have been invested to identify new members of the TNF receptor and ligand superfamilies by searching EST (expressed sequence tag) databases. Functional characterization of the new members identified by this approach has revealed many interesting features that were unknown previously. Some TNF receptor family members and their ligands play an important role in other than the immune system, that is, in bone metabolism and in the entry process of viruses. Some TNF family members exist only as a soluble protein and they function as decoy receptors. These decoy receptors have the capacity to bind multiple ligands, implying that they are involved in a complex regulatory network in the body. It appears that many members have the ability to trigger differential signaling pathways leading to opposite biological effects — cell survival and apoptosis. This implies that there is a cross-talk between the death signaling pathway and the survival signaling pathway. Defining this connection merits future work.

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