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*J Immunol* 2007; 179:4307-4312; doi: 10.4049/jimmunol.179.7.4307

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A New Class of Reverse Signaling Costimulators Belongs to the TNF Family

Mingyi Sun and Pamela J. Fink

Recent evidence shows that many molecules of the TNF family serve as counter-receptors, inducing costimulation through reverse signals in addition to delivering signals through their respective TNF receptors. In this review, we will discuss this new class of costimulators with a focus on the mechanisms of costimulation transduced by reverse signaling through Fas ligand. The Journal of Immunology, 2007, 179: 4307–4312.

The TNF family thus far includes 19 identified members, all of which, with the exception of the secreted lymphotoxin (LT)α and a proliferation-inducing ligand (APRIL), are type II transmembrane proteins containing a C-terminal TNF homology domain that binds to cysteine repeat domains of one or more molecules belonging to the TNF receptor (TNFR) family (1). Although TNFR family members are expressed by a wide variety of cells, almost all of the TNF proteins are expressed by cells of the immune system, including B cells, T cells, NK cells, dendritic cells (DCs), and monocytes (2). TNF-TNFR interactions initiate multiple signaling pathways promoting cell survival, death, differentiation, or inflammation in TNFR-expressing cells, depending on the activation state of the cells and the expression levels of the TNFR family molecules.

Although many TNF family proteins can be expressed in soluble form or released from the cell surface following cleavage by specific proteases (2, 3), most act as membrane-bound factors and require direct cell-to-cell contact. The possibility therefore exists for the bidirectional transfer of information upon TNF-TNFR ligation. Indeed, data from many groups published over the past 17 years are consistent with the notion that many molecules of the TNF family receive as well as deliver signals through their respective receptors (Fig. 1). In this review, we use the term “reverse signal” to designate the signal delivered through the TNF family member and the term “counter-receptors” to denote the TNF family members that play such a double role (4–6). The reverse signals we describe in this review are still remains unclear (7). The N-terminal cytoplasmic domains of most TNF family members are conserved across species but not between family members, suggesting that the intracellular domains of TNF family members play a key role in their biological functions. Of interest in the search of a focal point for reverse signaling induced by these members, six (CD70, Fas ligand (FasL), CD30L, CD40L, 4-1BBL, and TNF-α) of 10 molecules with bidirectional capacity contain at least one putative casein kinase I (CKI) binding site, suggesting that reverse signaling may depend on this motif (8, 9).

CD40L (CD154)

Although CD40 signaling initiated by the interaction of CD40-bearing APC and CD40L-bearing CD4+ T cells is essential for APC activation and humoral immune responses, CD40L has been recognized for more than a decade to costimulate via CD40L-mediated reverse signaling (10). Evidence suggests that CD40L is preformed and stored in naive CD4+ T cells (11). Its expression is rapidly but transiently induced on T cells upon activation through the TCR, but coexpression with CD28 signaling is required for prolonged high-level CD40L expression (12–14). CD40L costimulation is critical for the development of CD4+ T cell-dependent effector functions (15–17), because mice lacking CD40L-transduced costimulation due to CD40 deficiency are unable to make Ab responses or generate germinal centers. The administration of soluble CD40 to CD40 knockout mice restores the missing signal delivered through CD40L (18). However, the requirement of CD40L-mediated costimulation for CD8+ T cell responses is unclear, as experiments have blocked the delivery of both forward signals through CD40 and reverse signals through CD40L (19, 20). The observed close homology between human and mouse CD40L cytoplasmic tails implies the functional importance of this domain. The molecular mechanism of CD40L costimulation remains uncertain, given that CD40L has a very short cytoplasmic tail of only 22 aa, lacking any known enzymatic activity. There is evidence indicating that the activation of T cells via CD40L stimulation alone induces tyrosine phosphorylation of cellular proteins including Lck and phospholipase Cγ (PLCγ), which further activates protein kinase C (PKC),
LIGHT-mediated reverse signaling. The molecular basis of (30), it is unknown whether this interaction influences lymphocyte attenuator to negatively regulate T cell responses (see below). Although HVEM can also deliver reverse signals initiated by anti-TNF mediated reverse signaling, regulating cell proliferation, cytokine secretion, oxidative burst, class switch, and T cell maturation in cells expressing molecules belonging to the TNF family.

CD40L cross-linking by anti-CD40L also triggers JNK and p38 activation (21, 22). These data suggest that CD40L also functions as a direct stimulatory molecule for T cells despite its short cytoplasmic tail.

**LIGHT**

LIGHT (homologous to lymphotoxin, exhibits inducible expression and competes with herpes simplex virus glycoprotein D for herpes simplex virus entry mediator [HVEM], a receptor expressed on T cells; designation is derived from underlined letters) binds to HVEM, a costimulatory receptor belonging to the TNFR family, the LTαβ receptor, and TR6/DcR3, a soluble TNFR family member that also binds to FasL and TL1A (23, 24). Induced on activated T cells and immature DCs, LIGHT has multiple functions (23, 25). LIGHT can induce apoptosis in cells expressing HVEM and LTβR (26) and can also act as a receptor to costimulate T cell proliferation, CTL activity, and cytokine production upon ligation by engineered, solid phase receptors, TNFR1 and TNFR2, and induce multiple signals (52).

Although HVEM can also deliver reverse signals through FasL-mediated reverse signaling (see below). Although HVEM can also deliver reverse signals through the Ig superfamily member B and T lymphocyte attenuator to negatively regulate T cell responses (30), it is unknown whether this interaction influences LIGHT-mediated reverse signaling. The molecular basis of LIGHT-mediated costimulation is still unclear, although recent studies show that LIGHT and TCR co-cross-linking enhances Erk1/2 activation (27).

**TRAIL**

Although TRAIL is another death-inducing TNF family member (31), it also has the capacity to costimulate T cells. It is expressed in a variety of cell types and can be up-regulated on activated NK cells and T cells (32–34). In conjunction with suboptimal anti-CD3, cross-linking TRAIL by a plate-bound TRAIL receptor 1-Fc fusion protein can selectively enhance CD4+ T cell proliferation and IFN-γ production (35, 36). Moreover, the IFN-γ augmentation can be reversed by a p38 MAPK inhibitor, suggesting that p38 MAPK is involved in TRAIL-mediated costimulation. Some studies also suggest that enhanced reactivity of T cells to autoantigens as a result of TRAIL costimulation may play a role in the development of human autoimmune diseases such as systemic lupus erythematosus (36).

**TRANCE**

TRANCE (TNF-related activation-induced cytokine), also known as receptor activator of NF-κB [RANK] ligand or osteoprotegrin ligand, can be induced on activated T cells, particularly CD4+ Th1 cells, and secreted by osteoblasts/stromal cells (37, 38). TRANCE surface expression can be further enhanced by CD28 costimulation (39). TRANCE is not only a regulator of the immune system and osteoclastogenesis via interaction with RANK and osteoprotegrin (39–41), it also mediates p38 MAPK-dependent reverse signaling and costimulates Th1 cells to enhance IFN-γ secretion by co-cross-linking TCR and TRANCE with immobilized anti-TCRβ and a RANK-Fc fusion protein (42). In addition to its role in T cell costimulation, recent studies show that reverse signaling through TRANCE also occurs in B cell chronic lymphocytic leukemia to induce IL-8 production, contributing to the pathogenesis of these cells (43).

**4-1BBL**

Although studies of 4-1BB/4-1BBL interactions have been mainly focused on 4-1BB-mediated costimulation in T cells, it has become clear that 4-1BBL can signal in both directions (44). 4-1BBL is constitutively expressed on macrophages, monocytes, DCs, and B cells; it can also be induced on activated T cells (45, 46). Reverse signaling has been demonstrated for 4-1BBL (47): cross-linking 4-1BBL by using immobilized 4-1BB costimulates B cell proliferation and Ig production (48). 4-1BBL is also a monocyte activation factor, because cross-linking 4-1BBL on monocytes by immobilized 4-1BB induces expression of IL-6, IL-8, and TNF-α and inhibits expression of IL-10 (49). Furthermore, 4-1BBL reverse signaling enhances the migration and infiltration of monocytes in vitro and in vivo (50). It has recently been shown that 4-1BBL can also act as a coreceptor for TLRs to enhance TNF production in macrophages (51). In this case, 4-1BBL-mediated reverse signaling is not initiated by ligand binding and cannot be defined as co-stimulation because it does not boost TCR signaling. The underlying mechanism of 4-1BBL reverse signaling is unknown.

**Membrane-bound TNF-α (mTNF)**

Since the identification of the soluble form of TNF-α >30 years ago, this molecule has been recognized as a pleiotropic cytokine. It is mainly produced by macrophages but also by a broad variety of other cells and tissues, including lymphoid cells, mast cells, endothelial cells, fibroblasts, and neuronal tissue. Membrane-integrated and soluble TNF-α bind to two receptors, TNFR1 and TNFR2, and induce multiple signals (52). TNF-α induces cell death in certain circumstances through a TNFR1-mediated, death domain and caspase-dependent pathway, although in most scenarios TNF-TNFR1 ligation induces inflammatory responses by NF-κB activation via TNFR-associated factor 2 (TRAF2). In contrast, TNFR2 mainly mediates nonapoptotic signaling upon binding to TNF-α. For example, TNFR2 is a costimulator for CD8+ T cells (53). mTNF can also deliver reverse signals initiated by anti-TNF mediated cross-linking, which costimulate T cells to express cytokines and adhesion molecules (54–56). Reverse signaling by mTNF differentially regulates CD4+ and CD8+ T cell activity against...
allogeneic endothelial cells by attenuating the proliferative potential of Th2 cells and increasing the cytotoxicity of CD8+ T cells (57). Surprisingly, reverse signaling initiated by engaging mTNF with soluble TNFRs also leads to the desensitization of monocytes and macrophages to LPS by down-regulating the production of soluble TNF, IL-6, IL-1, and IL-10 (58, 59). It is unclear how these soluble TNFRs cross-link mTNF to induce reverse signals instead of inhibiting this interaction. It is possible that soluble TNFRs transiently interact with proteins on the cell surface or the extracellular matrix to form oligomeric structures. These data suggest that soluble TNFR shed during inflammation might have the potential to bind mTNF and thereby down-regulate inflammation. Although more evidence is needed to identify the molecular mechanism of mTNF-mediated costimulation, CKI is likely involved in the signaling pathway because it phosphorylates serine residue 5 of the mTNF cytoplasmic domain upon cell activation in vitro (60).

FasL (CD178)

FasL has been well characterized for its induction of cell death through the Fas receptor (65). Its expression is mainly induced on hemopoietic cells such as activated T cells and NK cells. However, some nonhemopoietic cells involved in immune privilege and tumor escape also express FasL (66). A naturally arising point mutation in the FasL gene in gld mice results in the expression of nonfunctional FasL that can no longer bind Fas (67). Exploration of the reverse signaling capacity of FasL has provided both in vivo and in vitro evidence that this molecule costimulates thymocyte maturation (68) and mature T cell proliferation with a stronger influence on CD8+ than on CD4+ T cells (69–71). The differential sensitivity of CD4+ and CD8+

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**Table I. TNF family members that transduce reverse signals**

<table>
<thead>
<tr>
<th>Counter-Receptor</th>
<th>Expression</th>
<th>Receptor</th>
<th>Expression</th>
<th>Readouts of Reverse Signaling</th>
</tr>
</thead>
<tbody>
<tr>
<td>CD40L</td>
<td>Activated T cells</td>
<td>CD40</td>
<td>B cells, DCs</td>
<td>Costimulation, direct stimulation</td>
</tr>
<tr>
<td>LIGHT</td>
<td>Activated T cells, immature DCs, monocytes</td>
<td>HVEM</td>
<td>T, B, NK cells, DCs, myeloid cells</td>
<td>Costimulation</td>
</tr>
<tr>
<td>TRANCE</td>
<td>Activated T cells</td>
<td>RANK</td>
<td>Most cell types</td>
<td>Costimulation</td>
</tr>
<tr>
<td>TRAIL</td>
<td>Activated NK cells, T cells</td>
<td>TRAILR1</td>
<td>Most cell types</td>
<td>Costimulation</td>
</tr>
<tr>
<td>CD30L</td>
<td>Neutrophils, B cells, macrophages, neoplastic cells, activated T cells</td>
<td>CD30</td>
<td>Resting CD8 T cells, activated T and B cells</td>
<td>Costimulation, direct stimulation, inhibition</td>
</tr>
<tr>
<td>FasL</td>
<td>Activated NK cells, T cells</td>
<td>Fas</td>
<td>Most cell types</td>
<td>Costimulation</td>
</tr>
<tr>
<td>mTNF</td>
<td>Macrophages, monocytes</td>
<td>TNFR2</td>
<td>Immune cells, endothelial cells</td>
<td>Costimulation, desensitization</td>
</tr>
<tr>
<td>4-1BBL</td>
<td>Activated T cells, B cells, DCs, activated monocytes</td>
<td>4-1BB</td>
<td>Activated T cells, B cells, activated DCs</td>
<td>Costimulation of B cells, activation, migration, and infiltration of monocytes</td>
</tr>
<tr>
<td>OX40L</td>
<td>Activated T cells, B cells, DCs, activated monocytes</td>
<td>OX40</td>
<td>Activated T cells, B cells, activated DCs</td>
<td>Direct stimulation</td>
</tr>
<tr>
<td>CD70</td>
<td>Activated T cells, B cells, DCs, activated monocytes</td>
<td>CD27</td>
<td>T cells, activated B cells</td>
<td>Direct stimulation</td>
</tr>
</tbody>
</table>

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**CD30L** (CD153)

CD30L was first found on activated peripheral blood T cells (61) but is also constitutively expressed on neutrophils, B cells, macrophages, and neoplastic cells (61, 62). CD30-CD30L interactions generate bidirectional signals; CD30L cross-linking by anti-CD30 costimulates T cells to induce proliferation and cytokine production and stimulates neutrophils to enhance cell proliferation, IL-8 production, and oxidative burst (62). In addition, viral CD30, a soluble CD30 homologue encoded by the ectromelia virus, binds to CD30L and transduces a negative reverse signal by blocking Th1 but not Th2 cytokine-mediated T cell responses to viral infection (63). Furthermore, CD30L expressed on B cells can also mediate a negative reverse signal to inhibit class switch recombination as well as Ig production (64). However, these experiments are not entirely clear, as forward signals through CD30 could also be blocked (63). The mechanism of CD30L-mediated reverse signaling is still unknown.
FasL is sufficient to costimulate CD8

tivation, and the proline-rich region in the FasL cytoplasmic tail

to promote the nuclear export of NFAT (81). Recently, it has

T cells to FasL costimulation and Fas-mediated death, in con-

ting via an unknown pathway.

The molecular mechanism of FasL costimulation has been

FIGURE 2. Distinct motifs within the cytoplasmic domain of FasL regulate costimulation. Diagrammed is a summary of the motifs identified within the cytoplas-

FIGURE 3. Summary of the pathway of signal transduction mediated by FasL costimulation. Diagrammed is a schematic summary of the signaling events driving transcription factor activation initiated upon TCR cross-linking and FasL costimulation. FasL is localized in lipid rafts (labeled in green) upon FasL costimulation. The arrows between the protein components reflect either direct interactions or enzymatic activity of one protein directed against another. The readouts of the signaling pathways are cytokine production and cell proliferation (in pink). Proteins identified to associate physically with FasL are marked in orange. Proteins that can be phosphorylated upon FasL costimulation are marked in red. Rectangles mark the transcription factors (in dark blue) translocated to the nucleus upon FasL costimulation.
unstable fragment consisting mainly of the FasL intracellular domain. This small fragment can enter the nucleus, perhaps providing an alternate mechanism for reverse signaling through FasL in the absence of “ligand” to stimulate the cells or cross-link FasL. (82, 83).

Concluding remarks

In summary, the in vitro data obtained when recombinant TNFR family proteins, anti-TNF family Abs, or TNFR family protein-transfected cells are used to cross-link TNF family molecules and the in vivo data obtained from knockout mice lacking TNFR or TNF family members clearly indicate that many TNF family members can serve as counter-receptors and transduce costimulatory signals. Although some studies using knockout mice or antagonists can be interpreted as blocking either forward or reverse signaling, a cascade initiated from the FasL cytoplasmic domain confirms the concept of costimulation through reverse signaling by TNF family molecules (Fig. 3). Although the identification of costimulatory signaling induced by FasL establishes FasL as a model for reverse signaling induced by other TNF family molecules, many more features of Fas-FasL interactions remain to be elucidated. For example, how is information delivered through Fas to determine the outcome of this forward signal, given that Fas transduces not only apoptotic but also diverse nonapoptotic functions including costimulation (84)? How can one single molecule play two roles by sending and receiving bidirectional signals and by transducing both positive and negative signals? How are both self-costimulation and suicide prevented, given that most FasL+ immune cells also express Fas? Perhaps the sequestration of FasL into secretory lysosomes for targeted release onto the T cell surface or NK cell surface plays a key role in segregating FasL from Fas on the same cell surface (85). Whatever the eventual answers to these questions, it is likely that bidirectional signaling through TNF-TNFR interactions helps modulate the balance between the life and death of the target cell and contributes to fine tuning of the cellular and functional interactions in the immune system.

Disclosures

The authors have no financial conflict of interest.

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