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*J Immunol* 2001; 166:270-276; doi: 10.4049/jimmunol.166.1.270
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Enhanced Secretion of IFN-γ by Activated Th1 Cells Occurs Via Reverse Signaling Through TNF-Related Activation-Induced Cytokine

Nien-Jung Chen,* Mei-Wen Huang,* and Shie-Liang Hsieh2*†

Growing evidence has demonstrated that members of TNF superfamily transduce signals after engagement with their receptors. TNF-related activation-induced cytokine (TRANCE), a member of TNF superfamily, is preferentially expressed on the surface of activated CD4+ Th1 cells. The soluble receptor activator of NF-κB (RANK).Fc fusion protein suppresses IFN-γ secretion by activated Th1 cells, but does not affect IL-4 secretion by Th2 cells. The suppressive effect on IFN-γ secretion is observed when Th1 cells are activated by APCs, but not by immobilized anti-TCRβ mAb. In contrast, immobilized RANK.Fc fusion protein augments IFN-γ secretion by Th1 cells, indicating the occurrence of reverse signaling through TRANCE during T cell/APC interaction. The enhanced secretion of IFN-γ mediated via TRANCE correlates with the activation of p38 mitogen-activated protein kinase and is blocked by SB203580, a p38 mitogen-activated protein kinase-specific inhibitor. Thus, in addition to its role in activating dendritic cells by binding to the receptor RANK, TRANCE itself can signal the augmentation of IFN-γ secretion via a p38-dependent pathway, and this provides yet another example of reverse signaling by a member of TNF superfamily. The Journal of Immunology, 2001, 166: 270–276.

TRANCE, primarily expressed in T cells, T cell lines, and lymphoid tissues. Upon TCR/CD3 stimulation, TRANCE mRNA and surface protein expression are rapidly up-regulated in CD4+ and CD8+ T cells, and this can be further enhanced in CD4+ T cells by CD28-mediated costimulation (8). In contrast to TRANCE, RANK is ubiquitously expressed in human tissues. High levels of RANK expression are found in mature DCs, while T and B cells only express low levels of RANK after activation. In DCs, TRANCE induces the expression of proinflammatory cytokines (IL-1, IL-6) and T cell growth and differentiation factors (IL-12, IL-15), in addition to enhancing DC survival (1–3). Moreover, TRANCE cooperates with CD40 ligand (CD40L) or TNF-α to further increase the viability of DCs (4), suggesting that these TNF-related molecules are important in the activation of DCs and in modulating the immune system.

Recently, our understanding of the signaling pathways downstream of members of TNFR superfamily has advanced dramatically. In addition, there is growing evidence that ligands of the TNF superfamily, such as CD40 ligand (CD40L/CD154) (9–11), CD30L (12), CD27 ligand (CD27L/CD70) (13), FasL (14, 15), CD137L (16), and OX40L (17), also transduce signals after engagement with their receptors. It has been shown that reverse signaling via CD40L is involved in a range of different immune processes, such as cytokine production, costimulation of T cell activation, and proper formation of germinal centers (10). Blair et al. (11) also demonstrated that CD40L could trigger short-term CD4+ T cell activation as well as mediating the secretion of immunomodulatory cytokines and apoptosis. In addition, Wiley et al. (12) showed that cross-linking of CD30L by a mAb or by CD30.Fc fusion protein induced the production of IL-8 by freshly isolated neutrophils. Moreover, reverse signaling via CD27L/CD70 has been shown to induce a subset of leukemic B cells to proliferate vigorously, an effect that is synergistically enhanced by ligation of CD40, but inhibited by the presence of IL-4 (13). Recently, Suzuki and Fink (14, 15) further demonstrated that maximal proliferation of CTL requires reverse signaling through FasL. It is also interesting to note that addition of CD137.Fc fusion protein induces a
substantial degree of proliferation in human peripheral monocytes (16). Furthermore, cross-linking of OX40L on CD40L-stimulated B cells results in a significantly enhanced proliferative response of B cells and the down-regulation of the transcription factor B cell lineage-specific activator protein (17). These studies provide evidence to demonstrate the importance of reverse signaling in the activation of the immune system. Therefore, it is interesting to know whether bidirectional signaling might also occur in other members of TNFR superfamily.

In this study, we report that blockade of the interaction between T cells and APCs by soluble RANK-Fc fusion protein suppresses the secretion of IFN-γ by activated T cells. However, RANK-Fc fusion protein does not exert any effect when T cells are activated by immobilized anti-TRCβ mAb. In addition, cross-linking of TRANCE by RANK-Fc enhances the secretion of IFN-γ in activated, but not resting, CD4+ cells in a dose-dependent manner. IL-12 is not required for the enhanced secretion of IFN-γ by activated T cells, but p38 mitogen-activated protein kinase (MAPK) is apparently activated after the engagement of TRANCE. Thus, it appears that reverse signaling is also occurring following the interaction of TRANCE and RANK. This provides yet another example of reverse signaling by a member of TNF superfamily.

Materials and Methods

Generation of RANK-Fc fusion protein

Receptor-Fc fusion protein was produced as previously described (18). The coding sequence for the extracellular domain of human RANK was isolated by RT-PCR using the forward primer, 5′-CGGGATCCACCACATGGG, and the reverse primer, 5′-CGGGATCCCACCACCATGTGGTTT-3′. The amplified product was ligated into BsmHI-cut pUC19-IgG1-Fc vector containing the human IgG1 Fc coding sequence. The fusion gene was then subcloned into pBacPAK9 (HI-cut pUC19-IgG1-Fc). The expressed RANK-Fc fusion protein does not exert any effect when T cells are activated by cold PBS twice, then lysed with cold buffer A (20 mM Tris-HCl, pH 8, 200 mM NaCl, 10% glycerol, 1 mM PMSF, 2 μg/ml leupeptin, 2 μg/ml aprotonin, 0.1% 2-ME, 0.5% Nonidet P-40). The suspension lysates were separated and stored at −70°C for use in kinase assays.

Flow cytomtery analysis and Abs

The detection of intracellular cytokines was performed as described previously (19). Before staining, cells were incubated with anti-mouse CD3/CD16 mAbs (1 μg/106 cells, clone 2G42) at 4°C for 10 min to prevent nonspecific binding. Samples were then stained with biotin-labeled RANK-Fc fusion protein (1 μg/106 cells), followed by CyChrome-conjugated streptavidin (PharMingen, San Diego, CA) to detect the expression of TRANCE. To detect the intracellular IFN-γ, the same samples were fixed with 1% paraformaldehyde in PBS at 4°C for 15 min, followed by incubation with FITC-conjugated anti-mouse IFN-γ (clone XMG1.2) in 4% FCS/0.1% NaN3 (w/v) PBS at 4°C for 30 min. Finally, cells were washed and analyzed by FACSvantage (Becton Dickinson, San Jose, CA) using CellQuest software (Becton Dickinson). All the Abs used for flow cytometry analysis were purchased from PharMingen. All the Abs used for flow cytometry analysis were purchased from PharMingen.

Enzyme-linked immunosorosent assay

Cell culture supernatants were collected at various intervals, and levels of murine IFN-γ, IL-4, IL-12, IL-18, and TNF-α were quantitated using commercial ELISA kits (R&D Systems), according to the vendor’s instructions.

p38 Kinase assay

Specific protein kinase was immunoprecipitated from cell lysates by incubation for 2 h at 4°C with anti-p38 polyclonal Ab (Bio-Lab, St. Paul, MN) and protein A/G beads (Pharmacia, Piscataway, NJ). The immunoprecipitates were washed twice with buffer A and then washed with kinase buffer (25 mM HEPES, pH 7.4, 25 mM β-glycerophosphate, 25 mM MgCl2, 0.5 mM DTT, 0.1 mM sodium orthovandate). Protein kinase assays were performed for 25 min at 30°C with 3 μg of substrate (myelin basic protein, MBP) and 50 μM [γ-32P]ATP (10 Ci/mmol) in a final volume of 40 μl of kinase buffer for 30 min at 30°C. The reactions were terminated by addition of Laemmli sample buffer, and the phosphorlation status of MBP was visualized by autoradiography after fractionation on SDS-PAGE.

Western blot analysis

Cell lysates (7.5 × 105 cells/sample) were fractionated by SDS-PAGE, then transferred to nitrocellulose paper (Hybond-C extra; Amersham, Arlington Heights, IL) by electrophoresis at 0.8 mA/cm2 for 1.5 h at room temperature. Blots were incubated with blocking buffer (5% fat-free skimmed milk/PBS) at 4°C for 12–16 h before probing. Specific anti-p38 polyclonal Ab (Bio-Lab) is diluted in the blocking buffer, and the incubation was conducted at room temperature with constant agitation for 1 h. The blot was washed and incubated with HRP-conjugated secondary Ab diluted in blocking solution for 45 min. After wash with PBS, membranes were dried and developed with an ECL system (Amersham) for 1 min, then exposed to x-ray film.
Results
Expression of TRANCE in differentiated Th1 and Th2 cells
To study the expression of TRANCE on the differentiated T cells (Th1 or Th2), we constructed a soluble fusion protein comprising the extracellular domain of human RANK and the Fc domain of human IgG1 (Fig. 1A). To determine the expression of TRANCE on activated T cells, CD4⁺ lymph node cells isolated from TCR transgenic AND mice were cultured in vitro under conditions favoring differentiation into Th1 or Th2 subsets. The AND transgenic mice express the Vα11Vβ3 transgene from a TCR specific for MCC88–103 in the context of I-Ek. After priming, T cells were restimulated by anti-TCR mAb, and the expression levels of surface TRANCE and intracellular cytokines (IFN-γ and IL-4) were determined. A significant shift in TRANCE-associated fluorescence was observed at 24 h after restimulation in Th1 cells, but not in Th2 cells (Fig. 1B, top). The surface expression of TRANCE was in accordance with the expression of TRANCE mRNA in Th1 and Th2 subsets (Fig. 1B, lower left). We then asked whether TRANCE and IFN-γ were coexpressed by the same Th1 cells. At 12 h after restimulation, 31.25% expressed both TRANCE and intracellular IFN-γ. These TRANCE⁺ IFN-γ⁺ double-positive cells reached peak at 18 h. Therefore, a large proportion of TRANCE⁺ cells is also actively producing IFN-γ at 18 h. After 24 h, most of intracellular IFN-γ was secreted into the supernatant under the stimulation conditions used. Therefore, it was difficult to study the coexpression of TRANCE and IFN-γ at this time point.

Effect of TRANCE/RANK interactions on IFN-γ secretion by Th1 cells
We then investigated the role of TRANCE in IFN-γ secretion during T cell activation. To address this question, AND CD4⁺ T cells were stimulated with MCC88–103-pulsed APCs, either in the presence or absence of soluble RANK.Fc fusion protein. After restimulation, cells were restimulated by MCC88–103-pulsed APCs for 24 h, and the cytokines secreted by T cells were quantitated by ELISA. We found that secretion of IFN-γ was dramatically suppressed by soluble RANK.Fc fusion protein, when T cells were primed in conditions favoring Th1 differentiation (Fig. 2A, top left). In contrast, RANK.Fc fusion protein only had a minimal effect on IL-4 secretion by Th1 or Th2 cells (Fig. 2A, top right). This observation suggests that inhibition of TRANCE/RANK interactions during priming suppresses the ability of Th1 cells to secrete IFN-γ following

![FIGURE 1. Expression of TRANCE on the surface of Th1 cells. A, Structure of the soluble RANK.Fc fusion protein comprising the extracellular domain (ECD) of RANK and the Fc region of human IgG1. B, CD4⁺ T cells from AND TCR transgenic mice were stimulated by Ag-pulsed APCs under conditions favoring differentiation to Th1 or Th2 cells for 5 days. After restimulation with immobilized anti-TCRβ mAb for 24 h, Cells were stained with either human IgG1 (thin line) or RANK.Fc (bold line) and analyzed by flow cytometry (top). The expression of RNA messages was determined by RT-PCR (bottom right), while the expression of IFN-γ and IL-4 in the supernatant was determined by ELISA (bottom left). C, After restimulation by immobilized anti-TCRβ mAb for 12 and 18 h, IFN-γ-secreting AND Th1 cells were detected by intracellular cytokine staining, while the surface expression of TRANCE was detected by soluble RANK.Fc.](http://www.jimmunol.org/)

![FIGURE 2. Inhibition of IFN-γ secretion from Th1 cells by RANK.Fc. CD4⁺ T cells from AND TCR transgenic mice were stimulated by Ag-pulsed APC (A) or by immobilized anti-TCRβ mAb (B), under conditions favoring Th1 or Th2 cell differentiation. RANKFc (10 μg/ml) or human IgG1 (10 μg/ml) was added during the priming. IL-12 and IL-18 were assayed at 72 h after the first stimulation. Cells were allowed to rest for 24 h and then restimulated with MCC88–103 peptide (10 μg/ml)-pulsed, mitomycin C (50 μg/ml)-treated splenocytes (I-Ek) (A) or immobilized anti-TCRβ mAb (B) for 24 h. Supernatants were harvested at 24 h after restimulation to determine the content of IFN-γ and IL-4 by ELISA. Statistical analysis by two-tailed Student’s t test revealed significant differences between IgG1-treated or RANK.Fc-treated samples (∗, p < 0.005).](http://www.jimmunol.org/)
subsequent stimulation. Because IL-12 and IL-18 have been shown to be potent inducers for IFN-γ secretion (20, 21), we asked whether blockade of TRANCE/RANK interactions during priming affected the secretion of IL-12 and IL-18 by APCs. However, the levels of IL-12 and IL-18 in the culture supernatant were not significantly affected when cells were cultured in the presence or absence of RANK.Fc fusion protein during priming (Fig. 2A, bottom). Thus, the suppression of IFN-γ secretion is not due to the depressed IL-12 and IL-18 secretion associated with mitomycin-treated APCs.

We then asked whether the same effect could be observed in the absence of APCs. We used immobilized anti-TCRβ mAb in place of Ag-pulsed APCs to elicit T cell stimulation, both in the priming and restimulation stage. Surprisingly, the secretion of IFN-γ by CD4+ T cells was not significantly affected by RANK.Fc when T cells were activated by immobilized anti-TCRβ mAb (Fig. 2B). Therefore, the inhibitory effect of RANK.Fc during T cell activation is dependent on the presence of APCs, suggesting the importance of TRANCE/RANK interactions between T cells and APC during T cell activation.

Reverse signaling via TRANCE

From the above experiments, it is clear that the interference of TRANCE/RANK interactions can suppress IFN-γ secretion. However, the effect is not due to the altered production of IL-12 and IL-18 by macrophages. Because members of TNF superfamily, e.g., CD40L (9–11), CD30L (12), CD27L (13), FasL (14, 15), CD137L (16), and OX40L (17), have been shown to deliver reverse signals, and thus modulate the activation of T cells or neutrophils, we asked whether the phenomenon observed in this study results from the blockade of signaling transduced by TRANCE on T cells, rather than by RANK on macrophages. To answer this question, Th1 cells from AND TCR transgenic mice, which had been incubated in the presence or absence of immobilized RANK.Fc fusion protein at the priming stage, were restimulated with immobilized anti-TCRβ mAb, and IFN-γ secretion was measured. We found that cross-linking of TRANCE alone had no effect on the production of IFN-γ. In contrast, when both TCR and TRANCE were cross-linked by anti-TCRβ mAb (1.25 µg/well) and RANK.Fc (20 µg/well), respectively, the secretion of IFN-γ was enhanced dramatically (Fig. 3A). The increase in IFN-γ production was proportional to the increased concentration of plate-bound RANK.Fc in a dose-dependent manner (Fig. 3B).

We also tested whether the engagement of TRANCE by RANK.Fc was cumulative. To address this question, CD4+ T cells were stimulated to differentiate into Th0, Th1, or Th2 phenotype by plate-bound anti-TCRβ mAb with or without plate-bound RANK.Fc, both in the priming and restimulation stages. Among the four groups tested, we found that the highest level of IFN-γ production was produced when Th1 cells were cross-linked by anti-TCRβ mAb and RANK.Fc fusion proteins in both the priming and restimulation stages. The levels of IFN-γ produced, from high to lowest, were RANK.Fc/RANK.Fc > RANK.Fc/IgG1 > IgG1/RANK.Fc > IgG1/IgG1, for both BALB/c (Fig. 3C) and AND T cells (Fig. 3D). This observation suggested that engagement of TRANCE could transduce a costimulatory signal to enhance IFN-γ secretion during both priming and restimulation, and this phenomenon can be observed in both TCR transgenic AND as well as non-TCR transgenic BALB/c mice (Fig. 3, C and D). In contrast, the different combinations of reagents at the priming and restimulation stages had no significant effect on IL-4 production by Th2 cells (Fig. 3, C and D).

It has been shown that TRANCE induces the expression of proinflammatory cytokines (IL-1, IL-6) and T cell growth and differentiation factors (IL-12, IL-15) in DCs (8). To further confirm this phenomenon, Th1 cells from AND TCR transgenic mice, which had been incubated in the presence or absence of immobilized anti-TCRβ mAb and RANK.Fc (20 µg/well), respectively, were restimulated with plate-bound anti-TCRβ mAb plus human IgG1 or anti-TCRβ mAb plus RANK.Fc (20 µg/well) for 24 h (*, p = 0.0091). B, Resting AND Th1 cells were restimulated with immobilized anti-TCR β mAb and/or with different amount (0–20 µg/well) of immobilized RANK.Fc fusion protein for 24 h (*, p < 0.005). C and D, CD4+ T cells from BALB/c (C) or AND TCR transgenic mice (D) were stimulated to differentiate into Th0, Th1, or Th2 phenotype with anti-TCRβ mAb plus immobilized IgG1 or RANK.Fc for 5 days, followed by restimulation with plate-bound anti-TCRβ mAb in conjunction with human IgG1 or anti-TCRβ mAb (*, p < 0.005). E, CD4+ T cells from IL-12 knockout mice were stimulated to induce a Th1 phenotype with anti-TCRβ mAb in conjunction with immobilized IgG1 or RANK.Fc for 5 days, followed by restimulation with plate-bound anti-TCRβ mAb in conjunction with human IgG1 or RANK.Fc for 24 h. Supernatants were harvested and assayed for cytokine content by ELISA (*, p < 0.005).

![Figure 3. TRANCE engagement enhances IFN-γ secretion by Th1 cells. A, CD4+ T cells from AND TCR transgenic mice were stimulated in Th1-favorable conditions for 5 days. Cells were allowed to rest for 24 h and then restimulated with plate-bound anti-TCRβ mAb plus human IgG1 (20 µg/well) or anti-TCRβ mAb plus RANK.Fc (20 µg/well) for 24 h (*, p = 0.0091). B, Resting AND Th1 cells were restimulated with immobilized anti-TCR mAb and/or with different amount (0–20 µg/well) of immobilized RANK.Fc fusion protein for 24 h (*, p < 0.005). C and D, CD4+ T cells from BALB/c (C) or AND TCR transgenic mice (D) were stimulated to differentiate into Th0, Th1, or Th2 phenotype with anti-TCRβ mAb plus immobilized IgG1 or RANK.Fc for 5 days, followed by restimulation with plate-bound anti-TCRβ mAb in conjunction with human IgG1 or anti-TCRβ mAb (*, p < 0.005). E, CD4+ T cells from IL-12 knockout mice were stimulated to induce a Th1 phenotype with anti-TCRβ mAb in conjunction with immobilized IgG1 or RANK.Fc for 5 days, followed by restimulation with plate-bound anti-TCRβ mAb in conjunction with human IgG1 or RANK.Fc for 24 h. Supernatants were harvested and assayed for cytokine content by ELISA (*, p < 0.005).](http://www.jimmunol.org/).
that the enhanced secretion of IFN-γ via TRANCE is not dependent on IL-12 in this assay system. CD4+ T cells isolated from IL-12 knockout mice were stimulated with immobilized anti-TCR mAb and RANK.Fc, after which the level of secreted IFN-γ in the culture supernatant was measured. As shown in Fig. 3E, highest level of IFN-γ production was observed when the cells were cross-linked by anti-TCRβ mAb and RANK.Fc fusion proteins during both the priming and restimulation stages. This observation provides unequivocal evidence for the existence of an IL-12-independent pathway for the enhanced IFN-γ secretion via a costimulatory signal triggered by the cross-linking of TRANCE.

Engagement of TRANCE activates p38 MAPK

We wished to understand the signaling pathway transduced by TRANCE. To address this question, T cells activated by plate-bound anti-TCRβ mAb and RANK.Fc were incubated with SB203580 (p38 MAPK inhibitor), PD98059 (mitogen-activated protein/extracellular signal-related kinase inhibitor), rapamycin (p70 S6 kinase inhibitor), and D609 (phosphatidylcholine-specific phospholipase inhibitor). Among these kinase inhibitors, only SB203580 could suppress the secretion of IFN-γ in a dose-dependent manner (Fig. 4A). However, SB203580 did not affect the secretion of TNF-α from activated T cells. In contrast, PD98059, rapamycin, and D609 had only minimal effects on IFN-γ secretion (Fig. 4A). To demonstrate that the engagement of TRANCE activates p38 MAPK directly, we precipitated p38 MAPK from activated T cells to determine its activity (Fig. 4, B and C). Compared with T cells cross-linked with anti-TCRβ mAb alone, p38 MAPK activity was increased in T cells activated by immobilized anti-TCRβ mAb and RANK.Fc in a dose-dependent manner (Fig. 4C). The p38 MAPK activity increased from 1.5 h and reached peak at 24 h after stimulation. Treatment of SB203580 suppressed the p38 MAPK activity induced by TRANCE-mediated reverse signaling (lanes 5, 8, and 9 in Fig. 4C). This provided direct evidence that the engagement of TRANCE enhances the secretion of IFN-γ via the activation of p38 MAPK, with the p38 MAPK inhibitor SB203580 completely suppressing the up-regulation of IFN-γ secretion via TRANCE.

Discussion

Results obtained using TRANCE/OPGL knockout mice have shown that production of the cytokines IL-2, IFN-γ, IL-4, IL-5, and IL-6 is significantly reduced in purified opgl−/− T cells (6). Both Th1 and Th2 helper cell subtypes developed in the absence of OPGL expression, but production of both Th1 and Th2 cytokines was significantly reduced in opgl−/− T cells. These data indicate that OPGL has no apparent role in the Th1/Th2 dichotomy, but is required for optimal cytokine production following Ag-receptor activation. Our study has demonstrated that RANK.Fc inhibits the secretion of IFN-γ by Th0 or Th1 cells, but not by Th2 cells. This effect does not result from the direct inhibition of IL-12 and IL-18 secretion by macrophages, because the levels of IL-12 and IL-18 are not changed significantly. However, we have demonstrated that triggering of TRANCE by immobilized RANK.Fc, in conjunction with immobilized anti-TCRβ mAb, activates p38 MAPK to enhance IFN-γ secretion by activated CD4+ T cells. Thus, the decrease of IFN-γ production in our assay system might result from the inhibition of p38-dependent reverse signaling by TRANCE on CD4+ T cells. This observation provides an explanation for the reduced production of IFN-γ by opgl−/− T cells, which lack cell surface TRANCE and, therefore, cannot transduce the signal required to activate p38 MAPK and hence IFN-γ secretion. This could also explain why the addition of soluble rTRANCE cannot suppress the p38 MAPK activity induced by TRANCE-medi-
restore the secretion of IFN-γ by opgl−/− T cells to the same level as that of wild-type T cells (6).

Even though IL-12 is not required for activation of p38 MAPK via reverse signaling through TRANCE, we cannot rule out the possibility that other IFN-γ-modulatory factors secreted by macrophages are also impaired by soluble RANK.Fc. Prehn et al. (22) have reported that a soluble factor, which is distinct from IL-12, promotes the differentiation of CD4+ T cells. Therefore, although the levels of IL-12 and IL-18 are not affected by the addition of RANK.Fc in our assay system, it is possible that other IFN-γ-modulatory factors may be involved in the reduction of IFN-γ production by CD4+ T cells.

Many cellular functions of p38 MAPK have been defined through the use of specific p38 kinase inhibitors, the pyridinyl imidazoles. One such compound, SB203580, blocks the activity of p38α and p38β, but does not inhibit p38γ and p38δ (23), nor the extracellular signal-related kinases or c-Jun N-terminal kinases (24, 25). It has been reported that the production of IFN-γ by T cells, following activation by specific Ag-pulsed APC, is mediated by the p38 MAPK signaling pathway (26). Therefore, our observation is in accord with previous observations and further supports the essential role of p38 MAPK in IFN-γ production by activated T cells.

Even though the phenomenon of reverse signaling has been observed in several members of TNF superfamily, including CD40L/CD154, CD30L, CD27L/CD70, FaSL, CD137L, and OX40L (9–17), however, the downstream signaling pathways after the cross-linking of TNF and other members of TNF family have not been elucidated until recently. It has been reported that a casein kinase I (CKI) consensus sequence is conserved in the cytoplasmic domain of 6 of 15 members of the type II integral membrane TNF ligand family (27). Therefore, Watts et al. (27) speculated that the CKI motif might be also phosphorylated in other TNF ligand family member. This represents a new insight into the mechanism of reverse signaling in this cytokine family. However, there is no CKI motif in the cytoplasmic region of TRANCE, and our study provides the first evidence that p38 MAPK is involved in reverse signaling via TRANCE. This raises the question as to whether MAPK signaling pathways are also initiated via other members of TNF superfamily.

The presence of reverse signaling further increases the complexity to our current understanding of TNF/TNF superfamily. If the reverse signaling is a general phenomenon for all the members of TNF superfamily, then it would be interesting to test whether cross-linking of TNF-related apoptosis-inducing ligand (TRAIL) can also activate signaling cascade. To date, four homologous human receptors for TRAIL have been identified, including DR4 (28), DR5/TRAIL-R2/TRAIL, receptor inducer of cell killing 2 (29, 30), TRAIL receptor without an intracellular domain/decoy receptor 1 (DcR1)/TRAIL-R3 (29, 31), and TRAIL-R4/DcR2 (32, 33), called TRAIL-R1, -R2, -R3, and -R4, respectively. Among the four TRAIL receptors, the DcR1/ TRAIL-R3 and DcR2/TRAIL-R4 do not contain death domain and are unable to transduce death signal like TRAIL.R1/DR4 and TRAIL.R2/DR3. However, DcR1 and DcR2 might be able to cross-link TRAIL to transduce the reverse signal. Thus, DcR1 and DcR2 might play another role in the reciprocal signaling between TRAIL and TRAIL receptors. Moreover, two soluble receptors of the TNFR superfamily, OPG and DcR3, have been identified as dimeric proteins, respectively (34, 35). The OPG has been shown to bind to TRANCE (7) and TRAIL (36), while DcR3 has been demonstrated to interact with FaSL (35) and LIGHT (homologous to lymphotoxins, exhibits inducible expression, and competes with HSV glycoprotein D for

HVEN, a receptor expressed by T lymphocyte (37); thus, it would be interesting to test whether OPG and DcR3 could bind to TRANCE/TRAIL and FaSL/LIGHT to trigger signaling cascade in cells expressing these ligands. In our preliminary study, we found that both the plate-bound and soluble OPG.Fc can suppress the secretion of IFN-γ from anti-TCRβ-activated T cells (unpublished data). This raised the possibility that the soluble receptors of TNFR superfamily might be able to transduce signals by cross-linking their ligands expressed on cell surface, and cannot be only regarded as decoy receptor to inhibit the interaction between membrane form ligand and receptor. Recently, Eissner et al. (38) demonstrated that reverse signaling through transmembrane TNF conferred resistance to LPS in human monocytes and macrophages by down-regulation of LPS-induced soluble TNF and IL-6 as well as IL-1 and IL-10. Based on this information, the shedding of TNFR during inflammation (39) might have the potential to bind to the membrane form TNF to down-regulate inflammation reaction.

In conclusion, our result provides another evidence to demonstrate the existence of reverse signaling in a member of TNF superfamily, suggesting bidirectional signaling might be a general phenomenon in ligand/receptor interactions of TNF/TNF superfamily.

Acknowledgments
We thank Caroline Milner for critical review of the manuscript, and Bing-Chang Chen and Wan-Wan Lin for their technical assistance. We also thank Dr. John Kung (IMB, Academia Sinica, Taipei, Taiwan) for providing the AND TCR transgenic mice and IL-12 knockout mice.

References
REVERSE SIGNALING THROUGH TRANCE


