

# 行政院國家科學委員會專題研究計畫 期中進度報告

TRAIL 所傳導的逆向訊息在 T 細胞活化之角色及分子機轉

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# 行政院國家科學委員會專題研究計劃成果 報告

中文：TRAIL 所傳導的逆向訊息在 T 細胞活化之角色及  
分子機轉

英文：Role and molecular mechanism of TRAIL  
transduced reverse signal in T cells

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## Abstract

Key words: TRAIL; reverse signal; human CD4 T cells; tyrosine kinase.

TRAIL, a novel member of TNF superfamily, induces apoptosis in transformed cell lines of diverse origin. TRAIL is expressed in most of the cells and the expression is upregulated in activated T cells. The actual biological function of TRAIL/TRAIL receptor is still not clear. Previous studies in our laboratory, we demonstrated that cross-linking of TRAIL by plate-bound recombinant TRAIL receptor, DR4-Fc fusion protein enhanced T cell proliferation and increased IFN- $\gamma$  production in conjunction with immobilized sub-optimal anti-CD3 stimulation in mouse splenocytes. The increase of T cell proliferation by DR4-Fc was dose-dependent and this effect could be blocked by soluble recombinant TRAIL proteins, indicating the occurrence of co-stimulation effects on T cells via signals transduced through TRAIL (**Chou et al., J. Immunol. 167: 1347, 2001**). Thus, in addition to its role in inducing apoptosis by binding to the death receptors, TRAIL itself can enhance T cell proliferation after TCR engagement and signal the augmentation of IFN- $\gamma$  secretion via a p38-dependent pathway. Our finding further implied the possibility that TRAIL-induced T cell co-stimulation may be involved in T cell activation. The significance of TRAIL co-stimulation and other co-stimulatory molecules in T cell activation is still not clear. Therefore, we further explore the role of TRAIL co-stimulation on T cells activation and the molecular mechanism of signal transduction through TRAIL in T cells. We have characterized the T cell subsets responding to TRAIL co-stimulation in T cell activation and to further investigate role of TRAIL induced co-stimulation in the pathogenesis of human autoimmune diseases and we demonstrated that TRAIL costimulate human CD4 T cells and also enhanced the proliferation and IFN- $\gamma$  production in SLE patients CD4 T cells (**Tsai et al. Arthritis & Rheumatism 50:629, 2004**). For further exploration of the possible molecular mechanisms of TRAIL-induced T cell co-stimulation, we are studying the possible signaling pathway and the TRAIL associated molecules in transduction of TRAIL reverse signal as well as other co-stimulation signals in T cell activation by using proteomics approach for probing the protein kinase activation in signal transduction during the T cell activation in TRAIL-co-stimulation. We have identified the p38 MAPK and the PI-3K/Akt pathways were critically involved in TRAIL-induced costimulation of T cells. We also have identified three possible candidate tyrosine phosphorylated proteins in the 2D gel

electrophoresis proteomics approach. This study will provide a new approach to address the role and molecular mechanisms of TRAIL induced co-stimulation in T cell activation.

## **Introduction**

The TNF family of cytokines include physiological death factors and influence a variety of immunological functions, such as cell activation and death (1). TNF and Fas ligand have received the most intense study and were shown to participate in activation-induced cell death, immune privilege, autoimmune disorders, and tumor evasion from the immune system (2-5). TNF-related apoptosis-inducing ligand (TRAIL, also called Apo2L) is another family member of TNF superfamily that is capable of inducing apoptosis (6). Four receptors for TRAIL have been identified. The ability to transduce death signals is restricted to death receptor 4 (DR4/TRAIL-R1) and death receptor 5 (DR5/TRAIL-R2) (7-10). In contrast, TRID/DcR1/TRAIL-R3 and TRUNDD/DcR2/TRAIL-R4 lack functional death domains and are unable to activate apoptosis (7, 9, 11-13). Furthermore, it has been shown that these two inhibitory receptors could inhibit TRAIL-mediated apoptosis, and TRAIL-R3 and TRAIL-R4 were suggested to act as "decoy receptors" to protect normal tissues from cell death (11-13). There is complex interplay between TRAIL and TRAIL receptors in vivo and the actual biological function of TRAIL/TRAIL receptor is still not clear.

TRAIL exists mainly in membrane bound form, and its expression on T cells is induced after T cell activation by anti-CD3 or type I interferon (14). TRAIL and members of this ligand superfamily primarily interact with their receptors by direct cell-cell contact (15). This observation, coupled with the cross-species sequence conservation of the cytoplasmic domains of these ligands, has led to the suggestion that signaling occurs in both directions for this family of ligand/receptor pairs (15). Recently, there is growing evidence that ligands of the TNF superfamily, such as CD40 ligand (CD40L/CD154) (16-18), CD30L (19), CD27 ligand (CD27L/CD70) (20), FasL (21,22), CD137L (23), OX40L (24) and TRANCE (25) also transduce signals after engagement with their receptors. These studies provide evidence to demonstrate the importance of reverse signaling in activation of the immune system. It is interesting to know whether bi-directional signaling might also occur in other members of TNFR superfamily. Therefore we investigated the possible signal

transduction via TRAIL after engagement with its receptor on T cells. In our previous studies, we found that cross-linking of TRAIL by plate-bound DR4-Fc fusion protein enhanced T cell proliferation and increased IFN- $\gamma$  production in conjunction with immobilized sub-optimal anti-CD3 stimulation in activated T cells in a dose-dependent manner. The effect of increased IFN- $\gamma$  production could be blocked by SB203580, a p38 mitogen-activated protein kinase (MAP Kinase)-specific inhibitor (26). Thus, it appears that reverse signaling is also occurring following the interaction of TRAIL and DR4.

During T cell activation, there are two signals involved in activating T cells. One is the signal transduced through T cell receptor (TCR), the other is the co-stimulation signal transduced through CD28 or other co-stimulation molecules. Recently there are a number of T cell surface molecules with co-stimulation activity reported including TRAIL (26). The significance of these different co-stimulation molecules contributed to T cell activation is still not clear. Are these different co-stimulation molecules responsible for activation of different subsets of T cells or provide different strength of signals for T cell activation is also not understood. It is still not clear how these co-stimulation signal transduced and what is the key protein kinase activated linked to signal transduced through T cell receptor during T cell activation.

In recent studies, results obtained using recombinant soluble TRAIL receptor DR5-Fc in mice exacerbated autoimmune arthritis and led to profound hyper-proliferation of synovial cells and arthritogenic (27). Furthermore, Hilliard et al. found that chronic TRAIL blockade in mice with soluble DR5 exacerbated experimental autoimmune encephalomyelitis induced by myelin oligodendrocyte glycoprotein (28). These effects might not only resulted from the blockage of TRAIL/TRAIL receptor interaction in vivo, but it also raised the possibility that these effects might result from the DR5/TRAIL engagement to transduce a reverse signal to pre-activated T cells. Our study has clearly demonstrated that triggering of TRAIL by immobilized DR4-Fc, in conjunction with immobilized sub-optimal anti-CD3 mAb, induced maximal proliferation response and enhanced IFN- $\gamma$  secretion by activated T cells (13). Thus, the exacerbated autoimmune arthritis and hyperproliferation of synovial cells as well as promotion of experimental autoimmune encephalomyelitis in mice chronically treated with DR5 might result from the triggering of pre-activated T cells in vivo. Our study may provide a new insight into the biological function of TRAIL. Our finding also further implied the possibility that TRAIL-induced T cell co-stimulation may be involved in T cell activation, in particular, in the process of hyper-reactive T cell activation response to autoantigens in human autoimmune disease condition. We are attempting to extend this observation to explore role of TRAIL and other co-stimulation signals on T cells

activation and human autoimmune diseases. It is still not clear that whether there is any special subsets of T cells require TRAIL co-stimulation to be activated or need the TRAIL signaling to augment the signal transduce from TCR in vivo. We attempt to elucidate the role of TRAIL co-stimulation in T cell activation by characterization of the T cell subsets responding to TRAIL co-stimulation and to further investigate role of TRAIL induced co-stimulation in the pathogenesis of human autoimmune diseases.

The role of TCR engagement in conjunction with the TRAIL signal remains unclear. Other molecules known for their positive signaling capabilities have recently been implicated in the death of cells in the absence of a concomitant antigen receptor signal. For example, signaling through CD40 without concurrent engagement of the B cell receptor leads to Fas-mediated cell death (29, 30), and may serve an immunoregulatory role by removing nonspecific B cells. It will be interesting to determine if TRAIL can still signal without engagement of the CD3/TCR complex and to analyze the consequences of such uncoupled signaling.

Although it is important to note that the molecules mediating these signals have yet to be identified; however, due to the short cytoplasmic domain of TRAIL, it has not been noticed that TRAIL might have the capability to transduce signal by itself. This implied that there might be other important intracellular molecules associated with TRAIL to transduce the signal. Even though the phenomenon of reverse signaling has been observed in several members of TNF superfamily, including CD40L/ CD154, CD30L, CD27L/CD70, FasL, CD137L, OX40L and TRANCE (16-25), however, the downstream signaling pathways after 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 (31). Therefore, Watts et al. (31) 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 TRAIL, and our previous study provided evidence that p38 MAPK is involved in reverse signaling via TRAIL. This raises the question as to whether MAPK signaling pathways are also initiated via other members of TNF superfamily. In a recent report, Chen et al. also demonstrated that p38 MAPK was involved in reverse signal through TRANCE (25). The presence of reverse signaling further increases the complexity to our current understanding of TNF/TNFR superfamilies.

For further explore the possible molecular mechanisms of TRAIL-induced T cell co-stimulation, we attempt to study the possible signaling pathway and the TRAIL

associated molecules in transduction of TRAIL reverse signal as well as other co-stimulation signals in T cell activation by using co-immunoprecipitation with DR4-Fc and yeast two hybridization technique to identify the molecules associated with TRAIL intra-cytoplasmic domain during TRAIL reverse signal transduction. Furthermore, we attempt to develop the proteomics approach for probing the protein kinase activation profiles in signal transduction during the T cell activation in TRAIL-co-stimulation by collaboration with Professor 周綠蘋 (Department of Biochemistry and Molecular Biology, National Taiwan University) to establish the proteomics system. This study will provide a new approach to address the role and molecular mechanisms of TRAIL induced co-stimulation in T cell activation.

## Results and Discussion

Previously, we have shown that triggering of TRAIL by immobilized TRAIL receptor, DR4-Fc, induced maximal proliferation response and enhanced IFN- $\gamma$  secretion in mouse T cells. Thus, in addition to its role in inducing apoptosis by binding to the death receptors, TRAIL itself can enhance T cell proliferation after TCR engagement. These findings suggest the possibility that TRAIL-induced costimulation of T cells may also occur in human and to trigger activation of human T cells. Enhanced reactivity of T cells to autoantigens via aberrant costimulation signals may play a role in the development of human autoimmune diseases. Therefore we investigated TRAIL-induced costimulation of T cells in patients with SLE. Here we demonstrated that TRAIL engagement induced costimulation of human CD4 T cells and T cells isolated from patients with SLE revealed enhanced reactivity to TRAIL-induced costimulation *in vitro*.

In order to demonstrate that TRAIL-induced costimulation of T cells occurs on human T cells. We purified T cells from human peripheral blood mononuclear cells (PBMC) The results in demonstrated that cross-linking of TRAIL by plate-bound DR4-Fc enhanced human T cell proliferation in conjunction with sub-optimal concentration of anti-CD3. The plates precoated with hIgG1 were used as controls. These costimulation effects are dependent on anti-CD3, because cell proliferation was not detected in the absence of anti-CD3. This proliferation effect by plate-bound DR4-Fc was dose-dependent and could be neutralized by soluble recombinant TRAIL, indicating that these effects resulted from immobilized DR4-Fc acting directly on

pre-activated T cells surface, instead of acting indirectly via Fc receptors. We then investigated the cytokine production when T cells activated by TRAIL-induced costimulation. To investigate the cytokine production profiles of T cells activated by TRAIL-induced costimulation, we measured IL-2, as well as IL-4 and IFN- $\gamma$  in supernatants released from cultures co-stimulated by immobilized anti-CD3 and DR4-Fc. The results demonstrated that the production of IL-2 by T cells was significantly enhanced when TRAIL was cross-linked by immobilized DR4-Fc compared to human IgG1. Similarly, the secretion of IFN- $\gamma$  by T cells was also significantly enhanced when TRAIL was cross-linked by immobilized DR4-Fc. Although the IL-4 secretion was also enhanced by plate-bound DR4-Fc in human T cells; the levels of IL-4 in the culture supernatant were not as significantly elevated compared to IFN- $\gamma$ .

To determine whether TRAIL engagement selectively activate specific population of human T cells, we investigated the T cell subsets responding to TRAIL-induced costimulation. The CD4 and CD8 T cells were purified from PBMC and to assay the T cell proliferation and cytokine response in vitro. The results demonstrated that cross-linking of TRAIL by plate-bound DR4-Fc enhanced predominantly human CD4 T cells proliferation in conjunction with immobilized sub-optimal anti-CD3. The proliferation of CD8 T cells was also enhanced by immobilized DR4-Fc; however, the enhancement of proliferation was not as significant as CD4 T cells. In addition, the results also demonstrated that secretion of IFN- $\gamma$  was significantly enhanced predominantly in human CD4 T cells when TRAIL was cross-linked by immobilized DR4-Fc. The production of IL-4 was low in both human CD4 and CD8 T cells compared to the production of IFN- $\gamma$  when TRAIL was cross-linked by immobilized DR4-F. Taken together, our results indicated that TRAIL engagement selectively induced activation of human CD4 T cell subsets. To exclude the possibility that the difference between CD4 and CD8 T cells in response to TRAIL-induced costimulation was due to different expression level of TRAIL on CD4 and CD8 T cells, we studied the expression of TRAIL on CD4 and CD8 T cells by staining with anti-TRAIL mAb and analyzed in flow cytometry. The results demonstrated that there is no significant difference in expression of TRAIL on CD4 and CD8 T cells in flow cytometry, indicating that the difference in response to TRAIL-induced costimulation was not due to the expression level of TRAIL. Our results suggested that there might be intrinsic signaling differences between CD4 and CD8 T cells in response to TRAIL engagement. Our results demonstrated that TRAIL induced activation of human CD4 T cells in conjunction with signal from TCR. It raises the possibility that TRAIL-induced costimulation of T cells may occur in human T cell activation, leading to an enhanced



reactivity to low affinity self-antigens in autoreactive T cells. Therefore we investigated TRAIL-induced costimulation of T cells in patients with SLE. The results demonstrated that T cells isolated from patients with SLE showed higher proliferation response to TRAIL-induced costimulation compared to normal healthy subjects control. In addition, upon activation by TRAIL, expression of CD25 is upregulated in human T cells; and the expression CD25 is more upregulated in T cells from SLE patients compared to normal healthy subjects after TRAIL engagement, indicating that lymphocytes from autoimmune diseases have a “hyper-reactivity” in response to low affinity autoantigen. Taken together, our results indicated that T cells from SLE patients showed enhanced reactivity to TRAIL-induced costimulation compared to normal control. It will be interesting to know that whether the TRAIL-induced costimulation of T cells reflects disease activity in SLE and lupus nephritis. We have characterized the T cell subsets responding to TRAIL co-stimulation in T cell activation and to further investigate role of TRAIL induced co-stimulation in the pathogenesis of human autoimmune diseases and we demonstrated that TRAIL costimulate human CD4 T cells and also enhanced the proliferation and IFN- $\gamma$  production in SLE patients CD4 T cells. This part of work has been published recently (Tsai et al. *Arthritis & Rheumatism* 50:629, 2004).

For further explore the possible molecular mechanisms of TRAIL-induced T cell costimulation, we are studying the possible signaling pathway in transduction of TRAIL reverse signal as well as other co-stimulation signals in T cell activation. Our results revealed that PI3K/Akt signaling pathway may play an important role in TRAIL-induced costimulation of human T cells. This part of work is writing manuscript for submission for publication. Using proteomics approach with two-dimension (2D) electrophoresis phosphoprotein profiling with anti-protein tyrosine kinases Abs for probing the protein kinase activity in signal transduction during the T cell activation, three proteins which were increasing phosphorylated in tyrosine residue when activated by DR4-Fc. These results will provide a new vision to address the role of T cell activation and TRAIL induced co-stimulation in the pathogenesis and therapy of human autoimmune diseases.

## Self-estimation

We are satisfied with the progress we have obtained in recent one year and we also have fruitful publication in this year. We will keep following the data we obtained, and the results will submit for publication in the near future.

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