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TRANCE, a TNF Family Member, Is Differentially Expressed on T Cell Subsets and Induces Cytokine Production in Dendritic Cells

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TNF-related activation-induced cytokine (TRANCE) is a member of the TNF family recently identified in activated T cells. We report here that TRANCE mRNA is constitutively expressed in memory, but not naive, T cells and in single-positive thymocytes. Upon TCR/Cd3 stimulation, TRANCE mRNA and surface protein expression are rapidly up-regulated in CD4+ and CD8+ T cells, which can be further enhanced on CD4+ T cells by CD28-mediated costimulation. However, TRANCE induction is significantly suppressed when cells are stimulated in the presence of IL-4, but is not modified in the presence of IFN-α, IFN-γ, TGF-β, TNF-α, or IL-2. High levels of TRANCE receptor expression are found on mature dendritic cells (DCs). In this study we show that activated T and B cells also express TRANCE receptor, but only at low levels. TRANCE, however, does not exert any significant effect on the proliferation, activation, or survival of those cells. In DCs, TRANCE induces the expression of proinflammatory cytokines (IL-6, IL-1) and T cell growth and differentiation factors (IL-12, IL-15) in addition to enhancing DC survival. Moreover, TRANCE cooperates with CD40 ligand or TNF-α to further increase the viability of DCs, suggesting that several TNF-related molecules on activated T cells may cooperatively regulate the function and survival of DCs to enhance T cell-mediated immune responses. 

Materials and Methods

Expression and purification of soluble TRANCE-R-Fc and hCD8-TRANCE

To create a TRANCE-R-Fc recombinant molecule (TR-Fc), the Fc portion of hlgG1 was fused to the C-terminal end of the extracellular domain of the murine TRANCE-R (also called receptor-activating NF-kB) (11) and produced in a baculovirus expression system according to the manufacturer’s instructions (BaculoGold, Pharmingen, San Diego, CA). TR-Fc was purified from the culture supernatants on protein A-Sepharose bead (Pharmacia, Piscataway, NJ). hCD8-TRANCE was prepared as previously described (12).

Determination of the specificity of hCD8-TRANCE and TR-Fc

293T cells grown in DMEM/10% FCS were transfected with expression vectors containing mTRANCE cDNA, mTRANCE-R, or mFas cDNA by calcium phosphate precipitation. Cells were incubated with 10 μg/ml of hCD8-TRANCE or 5 μg/ml of TR-Fc, and binding was revealed by FACS as described below.

Results

Regulation of TRANCE mRNA expression in T cells

TRANCE mRNA expression was measured in sorted naive (CD44low) and memory (CD44high) lymph node T cell subsets and in various thymocyte populations (Fig. 1). Purified T cells and thymocytes were stimulated with anti-CD3 mAb or were left unstimulated for 3.5 h, and levels of TRANCE mRNA were assessed by semiquantitative RT-PCR analysis (Fig. 1). Resting CD8+ and CD4+ memory cells expressed high levels of TRANCE, whereas resting naive CD8+ and CD4+ T cells did not express TRANCE mRNA. Upon CD3 stimulation, all T cell subsets up-regulated TRANCE, with the highest levels observed in CD3-stimulated memory CD4+ and CD8+ T cell subsets. CD40L mRNA expression was also examined and, consistent with a previous report, was up-regulated in activated CD4+ naive and memory T cells (18). In contrast, CD40L mRNA expression was very weak in CD8+ T cells (Fig. 1).

Although TRANCE was not expressed in resting peripheral T cells, we could detect substantial levels of TRANCE mRNA in single-positive CD4+CD8- and CD4+CD8+ thymocytes. In contrast, CD40L was restricted to CD4+CD8- thymocytes (Fig. 1). This suggests that TRANCE and CD40L are transiently expressed upon maturation of thymocytes (19).

Regulation of TRANCE protein expression on the surface of T cells

TR-Fc fusion protein that could specifically recognize TRANCE-transfected 293T cells, but not 293T cells transfected with vector alone (Fig. 2A) was used to detect surface TRANCE expression on T cells. TRANCE was not detected on resting CD4+ or CD8+ T
cells (Fig. 3). On CD4⁺ T cells, surface TRANCE expression was detected as soon as 4 h after anti-CD3 and anti-CD28 stimulation, peaked around 48 h, and remained high at least until 96 h (Fig. 3). The kinetics of TRANCE expression on CD8⁺ T cells were slower than those on CD4⁺ T cells, and CD8⁺ T cells expressed lower levels of TRANCE than CD4⁺ T when stimulated with anti-CD3 and anti-CD28 mAbs (Figs. 3 and 4). However, CD4⁺ and CD8⁺ T cells stimulated with anti-CD3 in the absence of costimulation expressed similar low levels of TRANCE (Fig. 4). Indeed, anti-CD28 mAb-mediated costimulation greatly enhanced TRANCE expression on CD4⁺, but not significantly on CD8⁺ T cells (Fig. 4). To determine whether TRANCE expression is restricted to Th subsets, Th1 and Th2 clones derived from DO11.10 TCR transgenic mice were stained with TR-Fc. As shown in Fig. 5, TRANCE was not detected on resting clones, but was strongly up-regulated on both Th1 and Th2 clones after anti-CD3 stimulation, although the Th1 clones consistently expressed higher levels than the Th2 clones.

To further analyze the regulation of TRANCE expression on activated T cells, we tested the effects of several cytokines. Purified T cells were stimulated for 60 h in the presence or the absence of cytokines. Among the different cytokines tested, we found that IL-4 (20 ng/ml) significantly inhibited the expression of TRANCE on activated CD4⁺ but not CD8⁺ T cells (Fig. 6). In contrast, TGF-β1 (1 ng/ml), IFN-α (1000 U/ml), IFN-γ (100 U/ml), IL-2 (50 U/ml), TNF-α (50 ng/ml), or LT-α (50 ng/ml) had no significant effect on TRANCE expression (data not shown).

**TRANCE-R is expressed on activated T and B cells**

We previously showed that high levels of TRANCE-R are expressed on mature DC (12). Since TRANCE-R has also been detected on activated human T cells (11), and TRANCE can activate c-Jun N-terminal kinase in thymocytes (12), we analyzed its expression on murine T cells using the hCD8-mTRANCE fusion molecule (Fig. 2B) and FACS analysis (Fig. 7). As previously reported (12), resting T cells did not show any detectable TRANCE-R expression on their surfaces (Fig. 7A). However, when T cells were stimulated with anti-CD3, low levels of TRANCE-R were detected only after 48 h of simulation and were
not further increased by anti-CD28-mediated costimulation. TRANCE-R expression was not enhanced by IL-4 and/or TGF-β1 (Fig. 7A) despite a previous study showing that these cytokines enhance the expression of TRANCE-R on activated human T cells (11). In addition, TRANCE did not have any effect on the survival or primary or secondary proliferative responses of murine CD4+ or CD8+ T cells despite significant TRANCE-R expression on those cells (data not shown). TRANCE-R expression were also detected on activated B cells (Fig. 7B). TRANCE-R expression was detected 24 h after stimulation and peaked at 48 h. Moreover, TRANCE-R expression was significantly enhanced by CD40 cross-linking on B cells, but only slightly by anti-μ and IL-4. This stimulatory requirement of TRANCE-R expression on B cells was similar to that of Fas expression (Fig. 7B). The level of expression of TRANCE-R on mature DCs was consistently >10-fold the expression on activated B cells (data not shown). TRANCE had no effect on proliferation, the expression of surface activation/adhesion markers, or survival of B cells stimulated to express TRANCE-R (data not shown).

TRANCE induces cytokine production in DC
TRANCE and CD40L can up-regulate Bcl-xL expression and protect DC against spontaneous apoptosis in vitro (12). In addition to its survival-enhancing function in DC, CD40L can induce IL-12 (4, 6, 9, 20) and IL-18 expression (21), which, in turn, can promote a Th1-mediated immune response (9) and an array of cytokines involved in T cell activation (IL-1, IL-6, IL-15, and TNF-α) (21, 22). To determine whether TRANCE plays a similar role in cytokine regulation, TRANCE- or PBS-treated DC were subjected to ribonuclease protection assays with probes specific for a variety of known cytokines (Fig. 8). TRANCE induces the expression of the proinflammatory cytokines IL-1β, IL-1Ra, IL-6, and the T cell and NK cell-activating cytokine, IL-15 (Fig. 8). TRANCE also up-regulates the mRNA encoding the p40 subunit of IL-12. In this assay, IL-12 p35 mRNA was not detected, probably because the steady state level of p35 mRNA was below the limit of detection.
expression and down-regulated TGF-β up-regulated both TGF-α, IL-6, IL-9, IL-10, IL-15, IL-1β (leukotriene-α), leukotriene-β, IFN-γ, or IFN-β mRNA. Yeast transfer RNA controlled for non-specific probe protection. Representative results of two independent experiments are shown. B. The glyceraldehyde-3-phosphate dehydrogenase (GAPDH) signal was used to control for the amount of input RNA and to quantify the relative expression of cytokine mRNA by phosphorimaging.

TRANCE cooperates with CD40L and TNF-α to enhance the survival of DC

Since activated CD4+ T cells express both TRANCE and CD40L, and both TRANCE-R and CD40 can activate similar signal transducing pathways (e.g., JNK and NF-κB), we hypothesized that during a T cell-DC interaction both ligands could cooperatively enhance DC survival. As shown in Fig. 9, TRANCE or CD40L alone weakly enhanced the survival of mature splenic DC relative to their more striking effects on BMDC. The simultaneous addition of both ligands together, however, inhibited cell death to a greater degree than either ligand alone (Fig. 9). TNF could also prevent spontaneous apoptosis as previously described (23) and cooperates with TRANCE to enhance splenic DC survival (Fig. 9). Granulocyte-macrophage CSF, a cytokine required for DC differentiation, had little effect on splenic DC survival; however, its effect was significantly amplified when administered with TRANCE (Fig. 9). The cooperative effects of TRANCE, CD40L, and TNF-α on DC survival were also observed with BMDC (Fig. 9).

Discussion

The data presented in this paper further extend our investigation of the role of TRANCE/TRANCE-R in the immune system. Previously, TRANCE was shown to enhance the survival of DCs, a property shared with CD40L (3, 12). TRANCE and CD40L also appear restricted to T cells. Their similarities compelled us to compare their expression during T cell activation and their functions on DCs. We showed that TRANCE is expressed on both activated CD4+ and CD8+ T cells, with higher levels of expression observed on CD4+ T cells. In contrast, CD40L is expressed only on activated CD4+ cells. These results suggest that TRANCE may allow CD8+ T cells to modulate DC function independently of CD40L. TCR stimulation by itself is sufficient to induce TRANCE expression on T cells, which can be further increased by CD28-mediated costimulation on CD4+ T cells, but not significantly on CD8+ T cells. In contrast, CD28 costimulation does not modify the level but only the kinetics of expression of CD40L on activated CD4+ T cell (24, 25). Moreover, the kinetics of TRANCE expression during CD4+ T cell activation are different from those described for CD40L (26). Indeed, maximal levels of TRANCE expression are detected at 48 h after stimulation and persist for at least 2 days more, whereas CD40L protein has been shown to be rapidly expressed and then to wane within 16–24 h (26). Therefore, TRANCE may act at later time point than CD40L during the CD4+ T cell-mediated immune response to regulate the functions of DCs. Interestingly, CD40 is expressed on both immature and mature DC and can signal DC maturation (2), whereas TRANCE-R is only expressed on mature DC and does not appear to be important for DC maturation (12).

We previously showed that TRANCE-R was not detected on resting T cells by FACS analysis (12). Since a recent report indicates that TRANCE-R is expressed on activated human peripheral blood T cells when stimulated with IL-4 and/or TGF-β1 (11), we have examined the expression of TRANCE-R on murine T cells. Similar to human T cells, we could detect TRANCE-R when murine T cells were activated. However, we did not find an effect of TRANCE on proliferation, costimulation, survival, or cell death in these cells, which contrasts with what was observed in human T cells (11). These discrepancies could reflect functional differences between the human and mouse TRANCE-R in T cells and/or differences in culture and stimulation conditions. We recently showed that a soluble form of TRANCE can be shed from TRANCE-transfected 293 cells (data not shown). However, preliminary experiments have shown that TRANCE was not shed in vitro from

TRANCE had no apparent effect on the expression of IL-2, IL-4, IL-5, IL-9, IL-10, IL-1α, TNF-α, TNF-β (leukotriene-α), leukotriene-β, IFN-γ, or IFN-β (Fig. 8). By the same method, we showed that CD40L, similar to TRANCE, up-regulated the expression of IL-1β, IL-1Ra, IL-6, IL-12 p40 (but not p35), and IL-15 (data not shown). However, TRANCE and CD40L differed in the regulation of TGF-β expression; TRANCE induced TGF-β2 expression and down-regulated TGF-β1 (Fig. 8), whereas CD40L up-regulated both TGF-β1 and TGF-β2 (data not shown).
activated T cells, suggesting that the relatively low level of TRANCE-R detected on activated T cells is not due to a competitive block by soluble TRANCE produced by those cells. In addition, we showed that activated B cells express low levels of TRANCE-R. Similar to activated T cells, the proliferation, survival, and phenotype of activated B cells were not affected by TRANCE. Although it remains possible that TRANCE has other effects on activated T or B cells, our data suggest that the major immune target cells for TRANCE are DCs as previously reported (12). This is an important difference from CD40L, which has also a major effect on B cell function (27).

In addition to its ability to enhance DC survival, TRANCE promotes the production of various cytokines (e.g., IL-12, IL-15, IL-1, and IL-6) in DCs. CD40L is known to be a major stimulus inducing IL-12 production by DC (4, 6), a critical cytokine involved in Th1 differentiation (9). However, neutralizing Abs to CD40L fail to completely block IL-12 production in an MLR with T cells and DC (6), and CD40L knockout mice are still able to produce IL-12 (28). TRANCE also induces IL-12 production in DC, suggesting that it may complement CD40L in vivo to promote DC-mediated Th1 differentiation. Interestingly, IL-4, which is required for Th2 cell differentiation (29), substantially inhibits TRANCE expression on activated CD4+ T cells. It is thus possible that IL-4-producing cells down-regulate TRANCE expression on T cells during T cell priming, leading to a decreased IL-12 production by DC and therefore decreased Th1 differentiation. Consistent with the potential role of TRANCE in enhancing Th1 responses and the effect of IL-4 on TRANCE expression are the lower levels of TRANCE on activated Th2 clones compared with the Th1 clones from DO11.10 mice. However, a more extensive analysis with additional Th1 and Th2 clones must be performed. The effect of various cytokines on CD40L expression has not been assessed precisely, and it remains to be determined whether IL-4 or IFN-γ affects CD40L expression in murine T cells.

IL-15 is a cytokine that shares functional similarities and receptor chain usage with IL-2 (30). It is a mitogen for NK cells (31) and is a T cell growth factor (30) and chemoattractant (32). Similar to human CD34+-derived DC, resting murine DC expressed very low levels of IL-15 mRNA (21), which were dramatically up-regulated upon TRANCE-R or CD40 triggering. In addition, IL-15 can enhance the survival of activated T cells (33) and specifically activates memory CD8+ T cells (34). This suggests a model in which activated/memory Th cells that express high levels of TRANCE promote their own survival by interacting with DC and inducing IL-15 production. Similarly to CD40L (21), TRANCE can also trigger the production of proinflammatory cytokines such as IL-1 and IL-6, which can amplify the immune response initiated by DC. TRANCE and CD40L therefore behave similarly in their ability to enhance DC-mediated lymphocyte activation.

In this study we also showed that TRANCE and CD40L, both of which are expressed on CD4+ T cells, cooperate to enhance the survival of DCs. These results suggest that DC survival in vivo may use the combined action of several TNF family members, including TNF-α, which are likely to be provided by activated CD4+ and CD8+ T cells and those present in the local microenvironment.

In summary, our data strongly suggest that TRANCE, similarly to CD40L, is likely to regulate T cell responses by modulating the function and survival of DCs. Although CD40-CD40L interactions play a major role during T cell responses against various infectious agents, CD40L knockout mice remain able to mount protective immune response against certain pathogens (e.g., viruses) (35). Therefore, it is possible that TRANCE is required to mount optimal or CD40L-independent T cell responses, a hypothesis currently under investigation.

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References

TRANCE AND ITS RECEPTOR IN THE IMMUNE SYSTEM


