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TRANCE, a TNF Family Member, Is Differentially Expressed on T Cell Subsets and Induces Cytokine Production in Dendritic Cells[1]

Régis Josien,[2,3]* Brian R. Wong,[2†] Hong-Li Li,* Ralph M. Steinman,* and Yongwon Choi[4†‡]

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.   *The Journal of Immunology,* 1999, 162: 2562–2568.

T he TNF and TNF receptor families of proteins play critical roles in the initiation and regulation of the immune response (1). These proteins enable a complex dialogue to occur between cells within the immune system and with cells of other tissues. Despite the apparent redundancy of the TNF/TNF receptor family as evidenced by the continually growing number of discovered ligand/receptor pairs and by the common signaling transducers used by the receptors, the specific function of these family members clearly exist, as shown by gene knockout studies in which the deletion of one family member cannot be fully compensated by the others (1). Specificity may be achieved by restricting their expression to particular cells and/or by linking their signal transducing effectors to cell-specific signaling pathways.

T cells can modulate the function of dendritic cells (DC),[5] APCs specialized in the activation of naive T cells (2), via TNF-related

molecules. CD40L, a CD4$^+$ T cell-restricted molecule, was shown to induce differentiation, cytokine production (TNF-α, IL-8, IL-12, and MIPα), and protection from spontaneous apoptosis in DC (3–6). TNF was also shown to enhance DC survival in vitro (7). IL-12-producing DC were shown to skew the response of T cells toward the Th1 phenotype (8, 9), suggesting that CD4$^+$ T cells express CD40L to adjust the type of response (Th1 vs Th2) by controlling DC function.

Recently, we showed that TRANCE (TNF-related activation-induced cytokine), a member of the TNF family (10) also called receptor-activating NF-κB-ligand (11), is a DC survival factor that regulates the expression of the anti-apoptotic molecule, Bcl-x$_L$ (12). We and others initially reported that TRANCE expression appears restricted to T cells, whereas high levels of TRANCE-R are expressed on mature DC, suggesting that TRANCE/TRANCE-R interactions are involved with T cell-DC communication (10–12). Recently, however, TRANCE expression was also detected on osteoblasts and was shown to be required for osteoclast differentiation from myeloid progenitors (13, 14) (our unpublished observations). In addition, a soluble decoy receptor (osteoprotegerin (OPG)/osteoclastogenesis inhibitory factor (OCIF)) for TRANCE can block TRANCE-mediated osteoclast differentiation and may therefore modulate T cell-DC interactions (15).

In this paper we show that both CD4$^+$ and CD8$^+$ T cells, when activated through the TCR/CD3 complex, express high levels of TRANCE, and its expression is strongly enhanced by CD28-mediated costimulation on CD4$^+$ T cells. In addition, we show that TRANCE has no significant effect on activated T and B cells, although they can express low levels of TRANCE-R when activated. TRANCE can up-regulate both proinflammatory cytokines and factors in DCs that mediate T cell growth and differentiation, a property shared with CD40L. Moreover, TRANCE cooperates with CD40L or TNF-α to enhance the survival of DCs. Therefore, TRANCE is likely to play an important role in the regulation of T cell responses by controlling the lymphocyte stimulatory capacity of DC.

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 hIgG1 was fused to the C-terminal end of the extracellular domain of the murine TRANCE-R (also called receptor-activating NF-κB) (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.

Mice

C57BL/6 (H-2^b) and BALB/c (H-2^d) mice were obtained from Taconic Farms (Germantown, NY).

Medium

The culture medium used was RPMI 1640 supplemented with heat-inactivated 5% FCS, 2 mM L-glutamine, 100 U/ml penicillin, 0.1 mg/ml streptomycin, 10 mM HEPES, and 5×10^{-5} M 2-ME.

Cells

Mature bone-marrow derived DC (BMDC) were generated as described previously (16) and were used on day 8 of culture. Splenic DC were isolated as previously described (17) and cultured overnight to induce maturation. Lymph node T cells (≥99% CD3$^+$ as assessed by flow cytometry) were prepared by magnetic bead depletion (Dynal, Oslo, Norway) of class II-, B220-, NK1.1-, and F4/80-positive cells. Th1 and Th2 clones derived from DO11.10 TCR transgenic mice, tested by intracellular staining of IL-4, IL-10, and IFN-γ, were provided by Drs. Dan Littman and Isabelle Riviere (The Skirball Institute, New York University Medical Center, New York, NY). The Th1 clones were IL-4$^-$ IL-10$^-$ IFNγ$^+$, and the Th2 clones were IL-4$^+$ IL-10$^+$ IFN-γ$^-$. B cells (≥94% B220$^+$) were prepared from spleen cells by magnetic bead depletion of Thy-1.2-positive cells (Dynal). Cell viability was assayed by trypan blue exclusion or propidium iodide uptake.

Flow cytometry

The expression of TRANCE on activated CD4$^+$ and CD8$^+$ T cells was assessed using the TR-Fc fusion protein at 5 μg/ml followed by FITC-conjugated goat anti-hIgG (Fc-specific) F(ab')$_2$ fragment (Jackson ImmunoResearch Laboratories, West Grove, PA). The negative control consisted of normal hIgG1 (Sigma, St. Louis, MO). T cell and thymocyte subsets were sorted using a FACS Vantage (Becton Dickinson, Mountain View, CA).

RT-PCR analysis

For semiquantitative PCR analysis, total RNA was extracted from FACS-sorted T cell subsets (RNA Isolation Kit, Stratagene, CA) cultured in 24-well plates coated with or without anti-CD3 (145-2C11; 10 μg/ml) for 3.5 h and subjected to RT-PCR as previously described (10). The following primers were used: hypoxanthine phosphoribosyltransferase (HPRT): sense, 5'-GTA ATG ATC AGT CAA CGG GGG AC-3'; antisense, 5'-CCA GCA AGC TTG CAA CCT TAA CCA-3'; TRANCE: sense, 5'-CCT GAG ACT CCA TGA AAA CGC-3'; antisense, 5'-TAA CCC TTA GTT TTC CGT TGC-3'; and CD40L: sense, 5'-GTG GCA ACT GGA CTT CCA GCG CG-3'; antisense, 5'-GCG TTG ACT CGA AGG CTC CCG-3'. The PCR products were analyzed by Southern blot as previously described (10).

Ribonuclease protection assays

Total RNA was obtained from 1×10^7 BMDC (RNA isolation kit, Stratagene, CA) treated for 12 h with hCD8-TRANCE (1–5 μg/ml), a 1/100 dilution of murine CD8-CD40L baculoviral supernatants, or an equivalent volume of PBS. Five micrograms of RNA from each sample was hybridized to a ^{32}P-labeled antisense RNA probe set (mCK-1, mCK-2, mCK-3, mAPO-2; PharMingen) and digested with RNase and T1 nuclease, and the
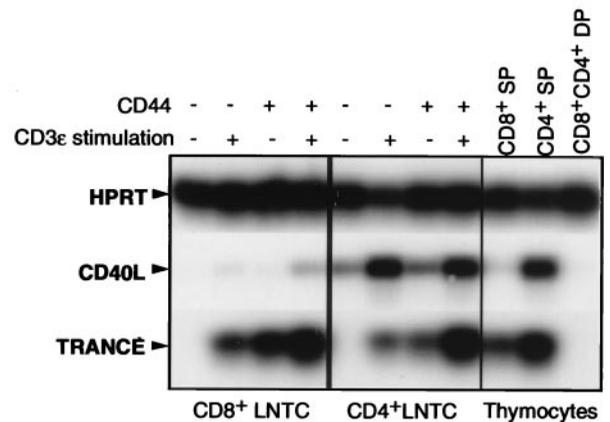

FIGURE 1. RT-PCR analysis of TRANCE mRNA expression in thymocytes and peripheral T cell subsets. Lymph node T cells (LNTC) were sorted by FACS based on CD4, CD8, or CD44 expression. Naive and memory T cells were identified as CD44$^-$ and CD44$^+$, respectively. Thymocytes were sorted into single positive (SP; CD4$^+$CD8$^-$ and CD4$^-$CD8$^+$) and double positive (DP; CD4$^+$CD8$^+$) populations. The LNTC-sorted populations (1×10^6) were stimulated on anti-CD3 mAb-coated plates or were left unstimulated for 3.5 h before their RNA was harvested. RT-PCR followed by Southern blot analysis with ^{32}P-labeled cDNA revealed the expression of TRANCE, CD40L, and HPRT. HPRT levels normalized the amount of cDNA template used in each PCR reaction.

protected probe fragments were resolved on 5% polyacrylamide gels according to the manufacturer's protocols. Band intensity was quantified by phosphorimaging (Molecular Imager System, Bio-Rad, Richmond, CA) and normalized to the intensity of the glyceraldehyde-3-phosphate dehydrogenase (GAPDH) probe.

Results

Regulation of TRANCE mRNA expression in T cells

TRANCE mRNA expression was measured in sorted naive (CD44^{low}) and memory (CD44^{high}) 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
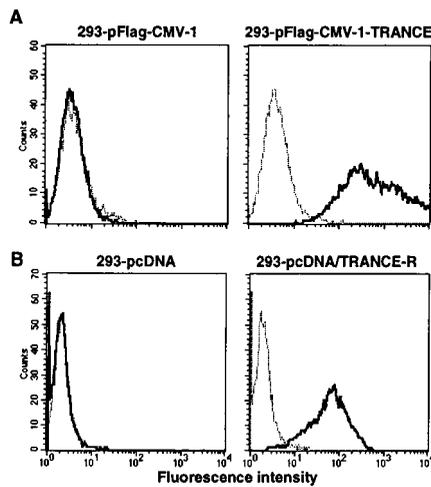
FIGURE 2. Specificity of hCD8-mTRANCE and TRANCE-R-Fc fusion molecules. 293-T cells transfected with vector alone (pcDNA), vector containing mTRANCE, or the extracellular domain of TRANCE-R and stable cell lines were cloned by limiting dilution. *A*, Cells transfected with pFlag-CMV-1 of pFlag-CMV-1/mTRANCE were incubated with 5 μg/ml of TRANCE. R-Fc (solid line) or normal hIgG1 (dotted line) followed by FITC-conjugated anti-human Fc. *B*, Cells transfected with pcDNA or pcDNA/TRANCE-R were incubated with 10 μg/ml hCD8-mTRANCE (solid line) followed by biotinylated anti-human CD8 and streptavidin-phycoerythrin. Negative control cells were incubated with the secondary Ab alone (dotted line). Cells were analyzed on a FACScan.

FIGURE 4. The effect of CD28-mediated costimulation on TRANCE expression on CD3-activated CD4$^+$ and CD8$^+$ T cells. Purified lymph node T cells were cultured as described in Fig. 3 in the presence or the absence of anti-CD28 mAb (2.5 μg/ml). TRANCE expression was assessed after 72 h of culture. One representative experiment of five is shown.

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 stimula-
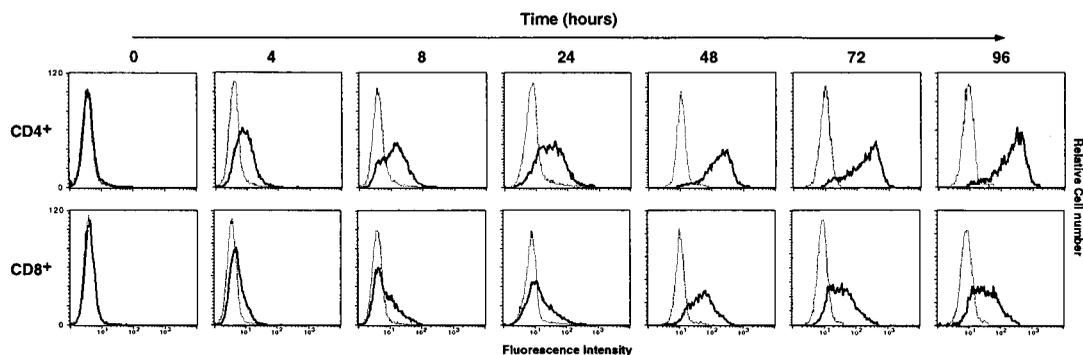
tion, although the Th1 clones consistently expressed higher levels than the Th2 clones.

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

FIGURE 3. Kinetics of TRANCE expression on CD4$^+$ and CD8$^+$ T cells activated by anti-CD3 and anti-CD28. Purified lymph node T cells were cultured in anti-CD3-coated (10 μg/ml) 96-well plates for the indicated amount of time in the presence of 2.5 μg/ml of anti-CD28 mAb. Subsequently, cells were double stained with anti-CD4-phycoerythrin or CD8-phycoerythrin, and TR-Fc or control hIgG1 (5 μg/ml) followed by FITC-goat anti-hIgG F(ab')$_2$, and binding was analyzed by FACS. Histograms reveal the binding of TR-Fc (solid line) or hIgG1 (dotted line) on CD4$^+$ and CD8$^+$ gated cells. One representative experiment of four is shown.
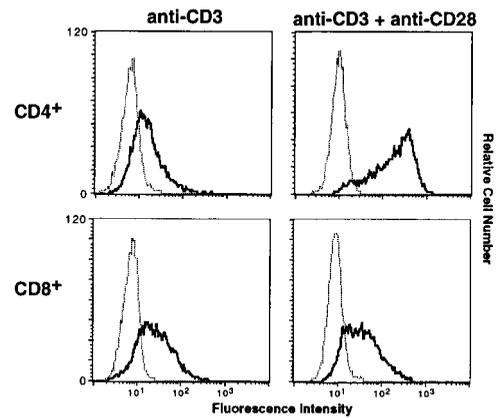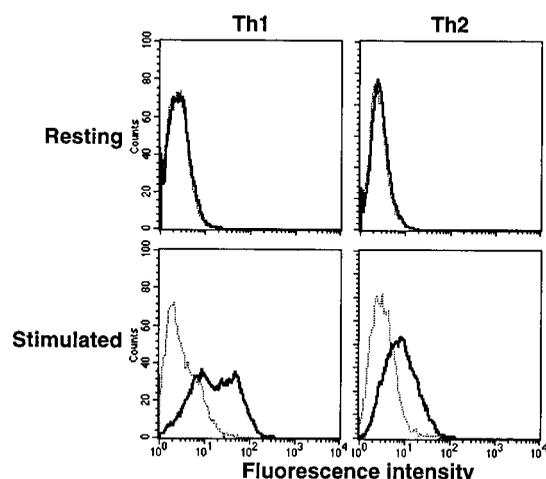
FIGURE 5. The expression of TRANCE by Th1 and Th2 clones. Resting or anti-CD3-activated (48 h) Th1 and Th2 clones derived from DO11.10 transgenic mice were stained with TR.R-Fc as described above. Representative results of two independent experiments are shown.

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
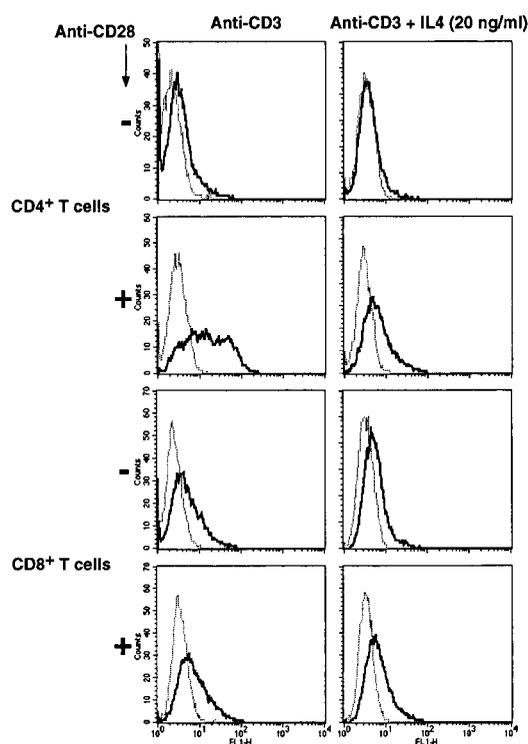

FIGURE 6. IL-4 down-regulates TRANCE expression on activated CD4$^+$ T cells. Purified lymph node T cells were cultured as described above in the presence or the absence of anti-CD28 mAb (2.5 μg/ml) and in the presence or the absence of murine rIL-4 (20 ng/ml). TRANCE expression was assessed after 72 h of culture. The results of one representative experiment of four are shown.
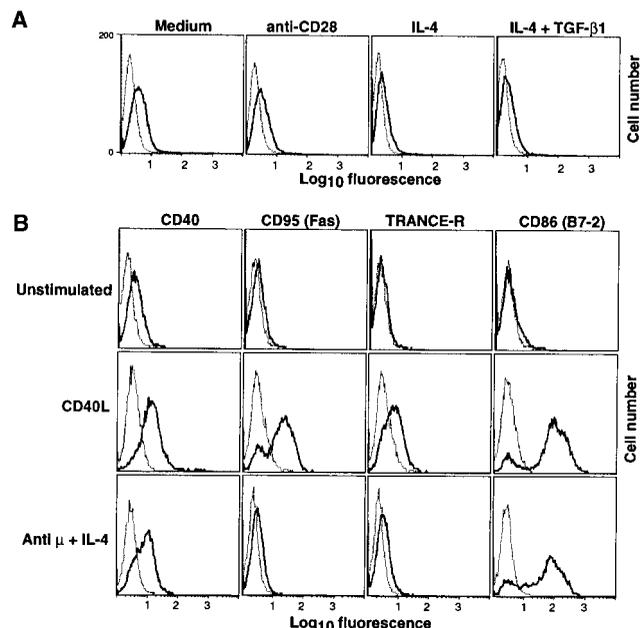

FIGURE 7. Activated T and B cells express low levels of TRANCE-R. *A*, Purified lymph node T cells were cultured in anti-CD3 coated (10 μg/ml) 96-well plate in the presence or the absence of anti-CD28 mAb (2.5 μg/ml), rIL-4 (20 ng/ml), and TGF-β1 (1 ng/ml). TRANCE-R expression was assessed after 60 h of culture using the hCD8-mTRANCE fusion molecule as described in *Materials and Methods*. TRANCE-R expression was detected only after 48 h of simulation. *B*, Purified spleen B cells were cultured in a 96-well plate in medium alone or in the presence of soluble CD40L (1/100 dilution of insect cell supernatant) or anti-μ chain Ab (0.5 μg/ml) and rIL-4 (20 ng/ml). After 48 h of culture cells were stained for CD40, CD95 (Fas), TRANCE-R, and B7-2. Maximal levels of expression were detected between 24 and 60 h of stimulation. One representative experiment of three is shown.

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-x$_L$ 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.
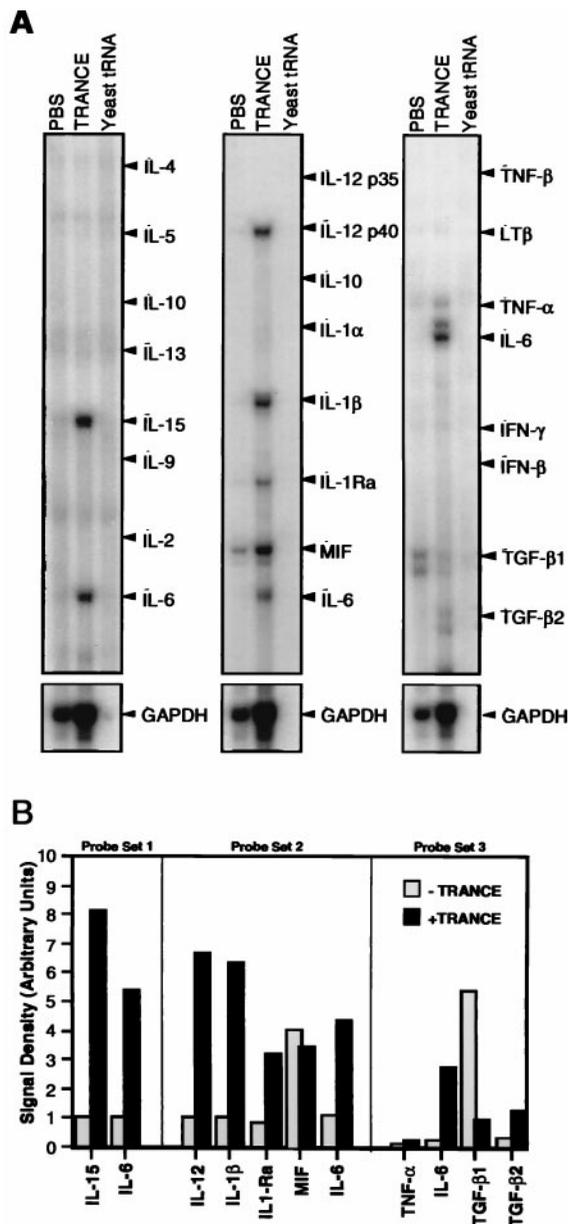
FIGURE 8. TRANCE induces an array of cytokines in BMDC. *A*, RNA was extracted from PBS- and hCD8-TRANCE (2.5 μg/ml)-treated BMDC and subjected to ribonuclease protection assays as described in *Materials and Methods* to measure levels of IL-1α, IL-1β, IL-1Ra IL-2, IL-4, IL-5, IL-6, IL-9, IL-10, IL-15, IL-1α, MIF, TNF-α, TNF-β (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 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).

*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

FIGURE 9. In vitro survival of mature DC. Mature bone marrow or splenic DC were incubated in medium with combinations of granulocyte-macrophage CSF (10 ng/ml), mCD8-CD40L (1/100 dilution), hCD8-TRANCE (1 μg/ml), hIgG1 (10 μg/ml), TR-Fc (10 μg/ml), and mTNF-α (50 ng/ml) for 72 h (BMDC) or 24 h (spleen DC), and cell viability was measured by trypan blue exclusion. These concentrations of mCD8-CD40L, hCD8-TRANCE, and mTNF-α were found to be saturating for survival of BMDC. Representative results of three independent experiments are shown.
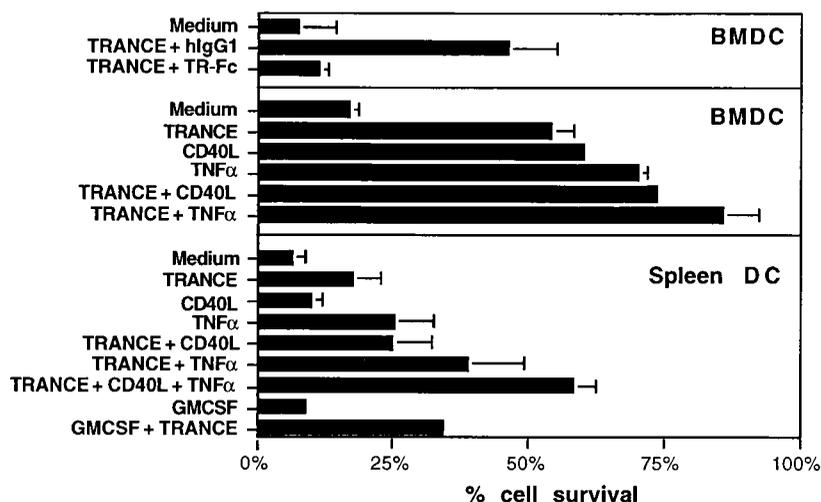
activated T cells, suggesting that the relatively low level of TRANCE-R detected on activated T cells is not due a competitive block by soluble TRANCE produced by those cells. In addition, we showed here that activated B cells express low levels of TRANCE-R. Similar to activated T cells, the proliferation, 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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