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TRANCE Is a Novel Ligand of the Tumor Necrosis Factor Receptor Family That Activates c-Jun N-terminal Kinase in T Cells*

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A novel member of the tumor necrosis factor (TNF) cytokine family, designated TRANCE, was cloned during a search for apoptosis-regulatory genes using a somatic cell genetic approach in T cell hybridomas. The *TRANCE* gene encodes a type II membrane protein of 316 amino acids with a predicted molecular mass of 35 kDa. Its extracellular domain is most closely related to TRAIL, FasL, and TNF. *TRANCE* is an immediate early gene up-regulated by TCR stimulation and is controlled by calcineurin-regulated transcription factors. TRANCE is most highly expressed in thymus and lymph nodes but not in nonlymphoid tissues and is abundantly expressed in T cells but not in B cells. Cross-hybridization of the mouse cDNA to a human thymus library yielded the human homolog, which encodes a protein 83% identical to the mouse ectodomain. Human *TRANCE* was mapped to chromosome 13q14 while mouse *TRANCE* was located to the portion of mouse chromosome 14 syntenic with human chromosome 13q14. A recombinant soluble form of TRANCE composed of the entire ectodomain induced c-Jun N-terminal kinase (JNK) activation in T cells but not in splenic B cells or in bone marrow-derived dendritic cells. These results suggest a role for this TNF-related ligand in the regulation of the T cell-dependent immune response.

The TNF[1] cytokine family currently includes TNF, LT-α, LT-β, FasL, CD40L, CD30L, CD27L, 4–1BBL, OX40L (1), and TRAIL/APO-2L (2, 3), which exhibit the highest homology between their C-terminal, receptor binding domains. The family members are type II membrane proteins that act in an autocrine, paracrine, or endocrine manner either as integral membrane proteins or as proteolytically processed soluble effectors. Binding to their cognate receptors leads to the activation of several signal transduction pathways: the cascade of caspase/interleukin-1β-converting enzyme-like proteases, the nuclear factor-κB (NF-κB) family of transcription factors, and the mitogen-activated protein kinases including the c-Jun N-terminal protein kinases (JNK) and the extracellular signal-regulated kinases (ERK) (4–6).

The biochemical pathways activated by the TNF-related ligands are coordinated to effect a diverse set of biological responses including apoptosis, differentiation, proliferation, and survival (1). Caspases execute the biochemical events leading to apoptosis (4) whereas NF-κB appears to inhibit cell death (7). In addition to its anti-apoptotic role, NF-κB regulates numerous genes, such as cytokines and adhesion molecules, that are critical in triggering and maintaining immune-mediated inflammatory responses (8). TNFR1, TNFR2, CD30, CD40, DR3/wsl-1/TRAMP/Apo-3, and the TRAIL receptor, when stimulated or overexpressed, recruit TRAF2, a signal-transducing protein that activates JNK *in vitro* (9). Fas can activate JNK by recruiting the protein Daxx to its death domain (10). Thus, JNK activation appears to be a common signaling event downstream of TNF-related ligand/receptor binding. JNK is linked to lymphocyte activation and proliferation since it can activate c-Jun, a component of the nuclear factor of activated T cells (NFAT) and activator protein-1 (11). Emerging evidence suggests that JNK is also critical in mediating apoptosis in nonlymphoid cells in response to some (10, 12–14), but not all, physiologic agonists, *e.g.* TNFR1-mediated cell death (9, 15).

The expression of TNF-related ligands on T cells is regulated by signaling from the T cell receptor (TCR) and mediates many of its biological effects. FasL, TNF, and CD30L are responsible for TCR-mediated apoptosis of T cells and immature thymocytes (16, 17). Seven of the TNF family members, in conjunction with TCR stimulation, can enhance T cell proliferation (1). Therefore, up-regulation of TNF cytokine members and their receptors by the TCR may provide an autocrine costimulatory mechanism to enhance the cells' own proliferation after stimulation with antigen (1). The TCR also up-regulates TNF-related ligands for the purposes of B cell co-stimulation, protection against Ig antigen/receptor-induced apoptosis and antibody isotype switching (18–20), dendritic cell activation and differentiation (21), and inducing apoptosis in virally infected or transformed cells (22).

To investigate the molecular regulation of TCR-mediated apoptosis a cloning strategy based on somatic cell genetics (23) was used, in which gene expression in mutant T cell hybridomas, resistant to TCR-mediated cell death yet capable of other

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[1] The abbreviations used are: TNF, tumor necrosis factor; LT, lymphotoxin; NFAT, nuclear factor of activated T cells; TCR, T cell receptor; JNK, c-Jun N-terminal kinase; PCR, polymerase chain reaction; BMDC, bone marrow-derived cells; LNTC, lymph node-derived T cells; ConA, concanavalin A; PBS, phosphate-buffered saline; bp, base pair(s); CHX, cycloheximide; GAPDH, glyceraldehyde-3-phosphate dehydrogenase; IL, interleukin; Ab, antibody.

receptor-associated functions (*e.g.* IL-2 secretion), is compared with gene expression in wild-type cells sensitive to TCR-mediated cell death. Such a strategy should yield genes associated with apoptosis and not activation although it is possible to obtain genes involved in other processes. This technique was used successfully to clone the gene *TDAG51*, a gene required for Fas expression and TCR-mediated cell death (24). Using similar methods we cloned a new member of the TNF cytokine family, designated TRANCE (TNF-related activation-induced cytokine), which is predominantly expressed on T cells and in lymphoid organs and is controlled by the TCR through a calcineurin-regulated pathway. A soluble form of the ligand consisting only of the extracellular domain can activate c-Jun N-terminal kinase (JNK) specifically in T cells but not in B cells or bone marrow-derived dendritic cells. These results suggest that TRANCE plays a specific role in regulating T cell functions.

MATERIALS AND METHODS

Subtractive Hybridization and Differential Screening

1×10^8 KMls8.3.5.1 or KIT50.1.9.1 T cell hybridomas, were incubated on 15-cm plates coated with 5 μg/ml H57–597 (α-TCR Ab) as described previously (24). Poly(A)$^+$ RNA was extracted using a FastTrack 2.0 mRNA isolation kit (Invitrogen), and 2 μg from TCR-stimulated KMls8.3.5.1 (KMls8.3.5.1+) and TCR-stimulated KIT50.1.9.1 (KIT50.1.9.1+) was used to make tester and driver cDNA, respectively. Suppression subtractive hybridization was performed using the PCR-select cDNA subtraction kit according to the manufacturer's protocol (CLONTECH). Briefly, tester and driver were digested with *Rsa*I, and the tester was ligated to adapter DNA. After two hybridizations with the tester and driver cDNA (20 h and 8 h) the resulting mixture was diluted 1:1000 and amplified by PCR using flanking and nested primers to produce a subtracted and normalized PCR fragment library. The efficiency of subtraction was verified via Southern blot analysis of the unsubtracted and subtracted PCR products using a ^{32}P-labeled GAPDH cDNA probe. 26 primary cycles and 18 secondary cycles of PCR amplification yielded the greatest signal:noise ratio estimated by comparing the amount of PCR product synthesized to the amount of GAPDH in the subtracted product (data not shown). Using these conditions, subtracted PCR products were TA-cloned into the pCR2.1 plasmid (Invitrogen). To screen differentially expressed products 100 ng of plasmid DNA containing the subtracted fragments were immobilized on duplicate nitrocellulose filters using a slot-blot apparatus (Schleicher and Schuell) and hybridized to cDNA probes (1×10^7 cpm/ml) derived from either KIT50.1.9.1+ or KMls8.3.5.1+ poly(A)$^+$ RNA. Signals were quantified using a PhosphorImager (Molecular Dynamics).

Full-length Cloning of Murine and Human TRANCE cDNA

A subtracted cDNA fragment, designated 8-50.51, which scored positive in the differential screening assay, was used to screen a λZAP cDNA library derived from KMls8.3.5.1+ (24). The longest clone (2.2 kilobases) was sequenced with a Sequenase 2.0 kit (U. S. Biochemical Corp.) over both sense and antisense directions using a series of oligonucleotide primers. To clone the human homolog a *Bam*HI-*Bam*HI fragment corresponding to TRANCE (nucleotides 366–1035) was used to screen 1×10^6 phage from a λgt11 5′-Stretch Plus human leukemia library (CLONTECH) using low stringency hybridization conditions. A partial human clone was sequenced using the same method described for murine TRANCE.

Mouse Cell Purification

All cells were harvested from 4–8-week-old BALB/c mice (The Jackson Laboratory). T cells were purified from 5×10^7 lymph node cells using a T cell enrichment kit (Biotex). For B cells 5×10^7 splenocytes were negatively selected for T cells via magnetic beads conjugated to anti-mouse Thy 1.2 following the manufacturer's protocol (Dynabeads Thy 1.2, Dynal). Mature BMDC were isolated as described previously (25). LNTC were harvested and treated with concanavalin A (ConA; 5 μg/ml) plus IL-2 (10 units/ml) for 48 h and then with IL-2 alone (50 units/ml) for 48 h to yield proliferating T cells (17). To induce cell death, the proliferating T cells were incubated on α-CD3ϵ (145-2C11) coated plates for 6–72 h as described previously (17). Using these conditions, ~50% of the cells are dead by 48 h *versus* ~5% cell death in the cells treated with ConA plus IL-2 alone. The purity of T-, B-, and BMDC-enriched fractions was tested by fluorescence-activated cell sorter and in all cases was greater than 90%.

Northern Analysis and Semiquantitative PCR

Expression and regulation of TRANCE in T cell hybridomas was determined by Northern blot analysis of poly(A)$^+$ RNA extracted at the indicated time points from the following samples: unstimulated or TCR-stimulated cells either in the presence of media alone, FK506 (10 ng/ml; Fujisawa), or cycloheximide (1 μg/ml; Sigma). The 8-50.51 fragment was used as a probe. To determine TRANCE expression in mouse tissues or in stimulated LNTC, total RNA was extracted from various organs or cells as described previously (24), and 20 μg from each sample was analyzed by Northern blot using the TRANCE full-length cDNA as a probe. A 28 S ribosomal RNA probe (data not shown) or a GAPDH cDNA probe was used to control for RNA loading. For semiquantitative PCR analysis total RNA was extracted from T or B cell-enriched fractions using the RNA Isolation Kit (Stratagene), and first strand cDNA was transcribed from 1 μg of RNA using Superscript RT (Life Technologies, Inc.) following the protocol provided by the supplier. The first strand reaction was diluted 1:100, allowing amplification to occur as linear function of starting concentrations and was subjected to PCR using the following conditions. β-Actin: sense, 5′-ATG AAG ATC CTG ACC GAG CG-3; antisense, 5′-TAC TTG CGC TGA GGA GGA GC-3′, 94 °C 30 s, 50 °C 1 min, 72 °C 1 min for 30 cycles. TRANCE: sense, 5′-CCT GAG ACT CCA TGA AAA CGC-3′; antisense, 5′-TAA CCC TTA GTT TTC CGT TGC-3′, 94 °C 30 s, 52 °C 1 min, 72 °C 1 min for 30 cycles. The PCR products were analyzed by Southern blot as described previously (24).

Expression and Purification of Soluble TRANCE

A FLAG-tagged soluble form of TRANCE was generated by cloning a PCR product encoding the TRANCE ectodomain (amino acids 72–316) into the *Hin*dIII-*Xho*I sites in the pFLAG/CMV-1 vector (Kodak). The open reading frame and FLAG fusion was confirmed by sequencing. 293T cells were transfected with the expression construct (20 μg/10-cm plate) by the calcium phosphate method. Supernatant was harvested 72 h later, passed through a 0.45-μm filter, incubated with the α-FLAG M2 affinity gel (Kodak), and eluted with the FLAG peptide (250 μg/ml, Kodak) as outlined in the manufacturer's protocol. The eluant was dialyzed against PBS and adjusted to 10% glycerol, and the protein concentration was ascertained in a BCA protein assay (Pierce).

Chromosomal Localization of Murine and Human TRANCE

Human TRANCE Mapping—A Genebridge 4 radiation hybrid mapping panel was obtained from Research Genetics, Inc. (Huntsville, AL). Hybrid DNA was subjected to PCR (94 °C 20 s, 55 °C 15 s, 72 °C 1 min, for 30 cycles) with primers derived from the 3′-untranslated region of the human TRANCE mRNA. Analysis of the data was performed using the radiation hybrid mapping server at the Whitehead Institute/MIT Center for Genome Research as described previously (26).

Murine TRANCE Mapping—Murine TRANCE was mapped using an intersubspecific backcross. A TRANCE-specific genomic DNA fragment of 582 bp was amplified by PCR using synthetic oligonucleotide primers (5′-ACC CAG ATG GAC TTC TGT GG-3′, 5′-TTT CCT TCG ACG TGC TAA CG-3′), and a single-stranded conformation polymorphism between C57BL/6J and CAST/Ei mice was detected in MDE gels as described previously (27). The polymorphism was mapped on a panel of DNA from 57 C57BL/6J × (CAST/Ei)F1 × C57BL/6J backcrossed mice, donated by The Jackson Laboratory Mouse Mutant Resource, which contains a large number of previously typed markers on all chromosomes (28).

c-Jun N-terminal Kinase Assays

2–5 × 10^6 cells were incubated for 1–2 h at 37 °C in 5% CO_2 on plates coated with the α-FLAG M2 antibody (10 μg/ml). The cells were treated with either soluble TRANCE in 10% glycerol/PBS solution or an equal volume of 10% glycerol/PBS solution before being harvested at the indicated time points and frozen in a dry ice/ethanol bath. Cells were lysed with Triton lysis buffer (20 mM Tris·Cl (pH 7.5), 137 mM NaCl, 1 mM phenylmethylsulfonyl fluoride, 5 mM EDTA, 2 mM EGTA, 1 mM Na_3VO_4, 25 mM β-glycerophosphate, 50 mM NaF, 10 mM sodium pyrophosphate, 15% glycerol, 1% Triton X-100) and spun down in a microcentrifuge to remove cell debris, and supernatants were incubated with goat α-JNK1 Ab (0.3 μg; Santa Cruz Biotechnology) for 2 h at 4 °C. Protein A-Sepharose was added for 1 h, and the beads were washed twice with Triton lysis buffer then twice with JNK reaction buffer (25 mM HEPES (pH 7.4), 25 mM β-glycerophosphate, 25 mM $MgCl_2$, 2 mM dithiothreitol, 0.1 mM Na_3VO_4). For the kinase reaction, 30 μl of JNK

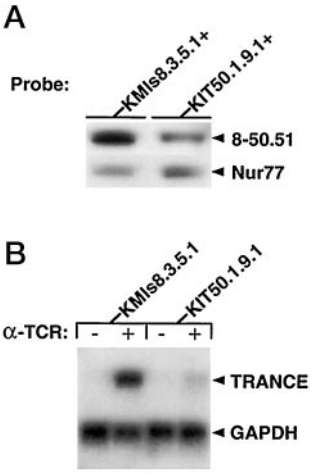

FIG. 1. **Identification of a gene defective in KIT50.1.9.1.** *A*, differential screening of the 8-50.51 gene fragment and Nur77 cDNA with probes from TCR-stimulated KMls8.3.5.1 (*KMls8.3.5.1+*) and TCR-stimulated KIT50.1.9.1 (*KIT50.1.9.1+*). *B*, Northern analysis of the TRANCE transcript in control, unstimulated (−), and TCR-stimulated (+) KMls8.3.5.1 or KIT50.1.9.1 using 8-50.51 cDNA as a probe. GAPDH was used as a control for poly(A)$^+$ RNA loading.

reaction buffer containing 1.5–3.0 μg of purified GST-c-Jun-(1–79) (generously donated by Dr. H. Hanafusa, The Rockefeller University), 0.5 μCi of [γ-^{32}P]ATP, and ATP (20 μM) was incubated with the immunoprecipitated JNK for 20 min at 30 °C. The reactions were stopped with 2 × loading buffer, boiled for 5 min, and run on a 12% SDS-PAGE gel as described previously (29).

RESULTS AND DISCUSSION

Identification of Mouse and Human TRANCE—We investigated the molecular defects in KIT50.1.9.1, a mutant T cell hybridoma resistant to TCR-mediated apoptosis (23), by comparing its gene expression with that of KMls8.3.5.1, the parental cell line sensitive to TCR-mediated apoptosis. Differentially expressed genes between TCR-stimulated KIT50.1.9.1 (KIT50.1.9.1+) and TCR-stimulated KMls8.3.5.1 (KMls8.3.5.1+) were isolated using suppression subtractive hybridization, a cDNA subtraction technique based on suppression PCR that is sensitive to rare transcripts (30). After KIT50.1.9.1+ cDNA was subtracted from KMls8.3.5.1+ cDNA a mini-library was generated by randomly subcloning the subtracted PCR products. The plasmid library was then subjected to differential screening using KIT50.1.9.1+ cDNA and KMls8.3.5.1+ cDNA as probes. Of the 347 plasmids screened, 76 produced a stronger signal with the KMls8.3.5.1+ probe than with the KIT50.1.9.1+ probe. One positive, designated 8-50.51, is shown in Fig. 1*A*. In contrast, Nur77, a gene whose expression is induced normally in both cells (data not shown) produced similar signals with both probes indicating that an equivalent amount of labeled probe was used from each cell line. Sequencing of 8-50.51 revealed an 87-bp DNA fragment with no homology to any genes in the GenBank™ data base. To confirm differential expression of 8-50.51 we probed a Northern blot containing unstimulated and TCR-stimulated KMls8.3.5.1 and KIT50.1.9.1 poly(A)$^+$ RNA. The probe identified a 2.2–2.3-kilobase message that was highly induced in TCR-stimulated KMls8.3.5.1, but only weakly induced in TCR-stimulated KIT50.1.9.1 (Fig. 1*B*).

Using 8-50.51 as a probe we screened a KMls8.3.5.1+ cDNA library to obtain a full-length clone. The full-length cDNA (Fig. 2*A*) is 2237 bp in length with a canonical Kozak consensus sequence starting at 137 bp from the 5′ end of the clone. This translation initiation site permits the synthesis of a 316-amino acid protein with a hydrophobic transmembrane domain and no identifiable signal sequence strongly suggesting a type II

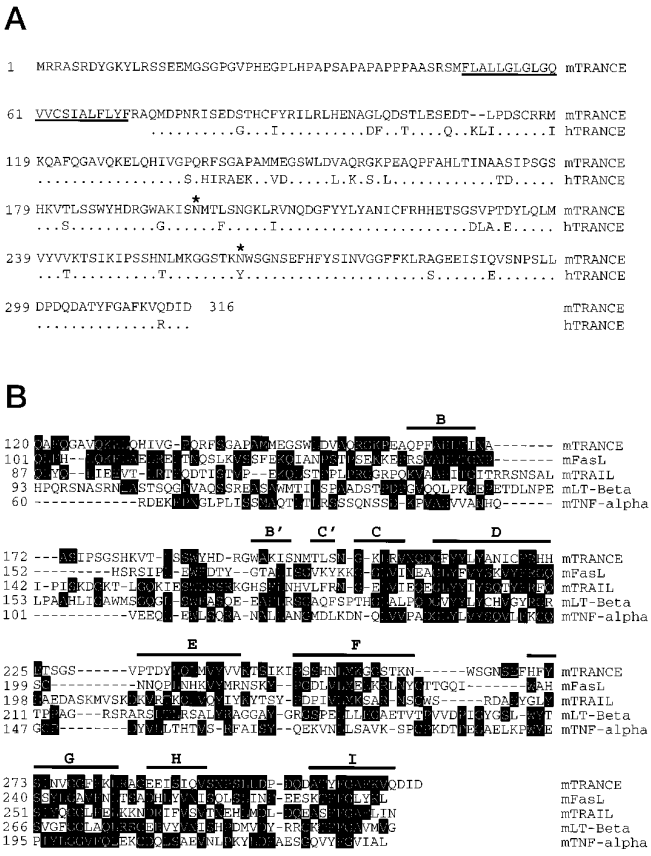

FIG. 2. **Sequence analysis of the TRANCE gene.** *A*, the predicted amino acid sequence of the full-length mouse TRANCE protein (*mTRANCE*) compared with the extracellular domain of human TRANCE (*hTRANCE*). *Dots* indicate shared identity between the mouse and human protein, and *dashes* indicate gaps between regions of homology. The transmembrane domain is *underlined*. Residues labeled with an *asterisk* (*) indicate a potential *N*-linked glycosylation sites. The *numbers in the left-hand column* indicate the amino acid residue positions in the mTRANCE protein. GenBank™ accession numbers: mTRANCE, AF013170; hTRANCE (partial), AF013171. *B*, amino acid alignment of TRANCE with other members of the TNF cytokine gene family. *Bars* represent the β sheets as estimated from the TNF crystal structure (31). *Shaded residues* are those that match the consensus sequence. The *numbers in the left-hand column* indicate the residue positions from the full-length protein sequences. *Dashes* indicate gaps between regions of homology.

integral membrane protein topology. A comparison of extracellular domains revealed similarity of the protein with mouse TRAIL (20%), FasL (19%), and TNF (17%). Alignment with selected members of the TNF family demonstrates high identity, especially in regions forming the β strands as estimated from the TNF crystal structure (31) (Fig. 2*B*). Due to the clear similarity of this gene with the TNF family members this protein was termed TRANCE. A FLAG-tagged full-length protein with an estimated molecular mass of 35 kDa was detected by Western blotting as an ~45-kDa band suggesting that TRANCE is post-translationally modified (data not shown). Putative *N*-linked glycosylation sites are indicated (Fig. 2*A*). The FLAG-tagged TRANCE could not be immunoprecipitated with Fas, DR3/wsl-1/TRAMP/Apo-3, CD30, TNFR2, or HVEM/ATAR immunoadhesins suggesting that TRANCE does not bind to these receptors (data not shown). A partial human TRANCE cDNA, cloned from a human thymus cDNA library, is 83% identical to the mouse TRANCE ectodomain suggesting that the function of this gene is highly conserved between mouse and human.

Regulation and Tissue Distribution of TRANCE—The signaling capabilities and biological functions of the TNF family often

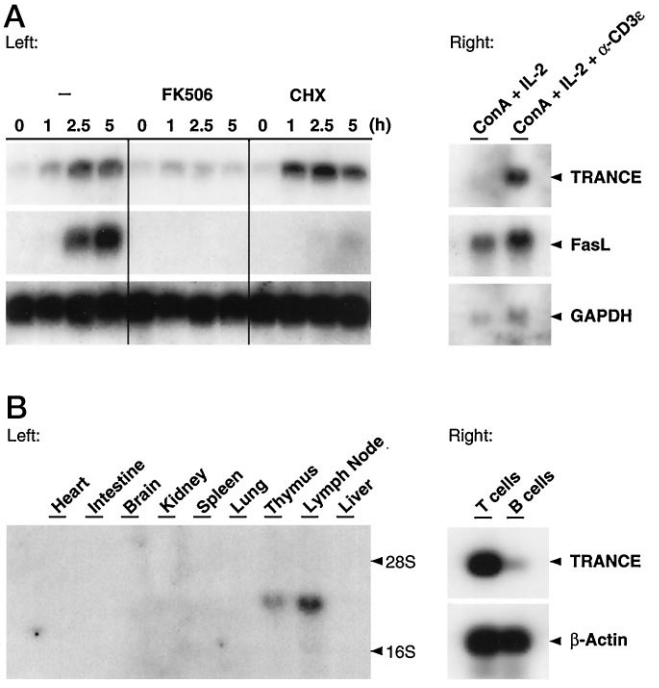

FIG. 3. **Expression and regulation of TRANCE.** *A: left*, effect of FK506 and CHX on TCR-induced up-regulation of TRANCE and FasL by Northern analysis. T cell hybridomas were stimulated on α-TCR Ab-coated plates for the indicated amount of time in the presence of media alone (−), FK506 (10 ng/ml), or CHX (1 μg/ml). *Right*, Northern blot of TRANCE and FasL expression in LNTC stimulated with ConA + IL-2 or ConA + IL-2 + α-CD3ϵ. The blots were stripped and reprobed with GAPDH to normalize for the amount of loaded RNA. *B: left*, Northern analysis of TRANCE in various mouse tissues. 28 S and 16 S ribosomal RNA is indicated. Equal amounts of RNA were loaded as determined with a 28 S ribosomal RNA probe (data not shown). *Right*, RT-PCR and Southern blot analysis of TRANCE in T and B cell-enriched populations. Amplification of β-actin was used to control for the amount of RNA template used in the PCR reaction.

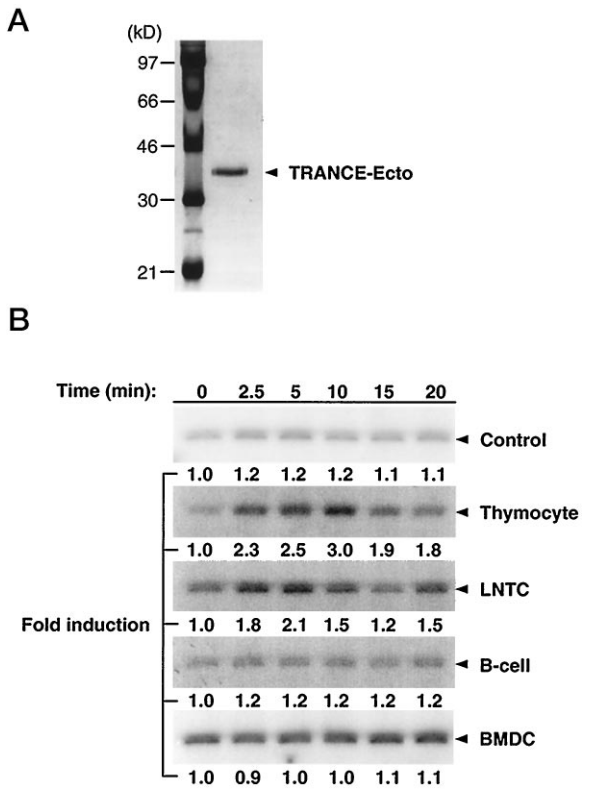

FIG. 4. **Characterization of the recombinant TRANCE-Ecto protein.** *A*, SDS-PAGE and Coomassie Brilliant Blue staining of purified TRANCE-Ecto. Molecular mass markers are indicated on the *left* of the figure. *B*, JNK activation by TRANCE. Cells were purified as described under "Materials and Methods" and stimulated with 500 ng/ml purified TRANCE-Ecto in 10% glycerol/PBS for the indicated amount of time on M2-coated plates. As a negative control, thymocytes were treated with an equivalent volume of a 10% glycerol/PBS solution on M2-coated plates (*Control*). JNK activation was assessed by incorporation of [^{32}P]ATP into purified GST-c-Jun-(1–79). The band intensities were quantified by phosphorimaging and presented as the fold induction over the unstimulated samples (0 min).

appear redundant *in vitro*. Yet specificity clearly exists, as shown by gene-knockout studies, in which the deletion of one family member cannot be fully compensated by the others. Specificity may be achieved by restricting the expression of these genes to particular cells and tissues and/or by linking their induction to different regulatory pathways. Temporal regulation of the TNF family members may also be important in properly coordinating their biological effects *in vivo* (1). The regulation of TRANCE induction by the TCR was studied in T cell hybridomas with cycloheximide (CHX), an inhibitor of translation, and FK506, a FK506-binding protein ligand that inhibits calcineurin (PP-2B) (Fig. 3*A*, *left*). Without inhibitors, TRANCE expression began 1 h after TCR stimulation and reached a maximal level at 2.5 h. FasL was also highly induced by TCR stimulation; however, its expression began at a later time point. CHX failed to inhibit the induction of TRANCE by the TCR indicating that TRANCE is an immediate early gene. In contrast, CHX completely abrogated the induction of FasL by the TCR. Thus, TRANCE, like TNF (32), is an immediate early gene with a relatively rapid onset of expression after TCR stimulation, whereas FasL induction is delayed and requires *de novo* protein expression for its synthesis. Cyclosporin A and FK506, both inhibitors of calcineurin, repress TCR-mediated TNF induction (32), and NFATp-deficient mice fail to up-regulate FasL, CD40L, and TNF expression in response to TCR stimulation (33). Therefore, FasL, CD40L, and TNF appear to be regulated by calcineurin-dependent signaling pathways involving the NFAT family of transcription factors. In the presence of FK506, the induction of TRANCE and FasL is blocked (Fig. 3*A*, *left*) suggesting that TRANCE, like several other TNF-related ligands, is controlled by NFAT proteins.

To examine TRANCE regulation in nontransformed cells, Northern analysis was performed on ConA and IL-2-stimulated LNTC, a model of antigen-mediated T cell proliferation, and on proliferating LNTC restimulated with α-CD3ϵ Ab, a model of peripheral T cell clonal deletion (17). ConA- and IL-2-stimulated T cells express relatively low amounts of TRANCE message whereas FasL expression is high (Fig. 3*A*, *right*). However, after restimulation with an α-CD3ϵ Ab TRANCE was significantly up-regulated suggesting that TRANCE may play a role in antigen-induced T cell death.

Northern blot analysis revealed that TRANCE expression is restricted to the thymus and lymph node (Fig. 3*B*, *left*). This pattern of expression differs from the pattern exhibited by TRAIL/Apo-2L and FasL, which are expressed in both lymphoid and nonlymphoid organs but is similar to the pattern exhibited by lymphotoxin-β, which is restricted to spleen. In addition, TRANCE is abundant in lymph node-derived T cells (LNTC) but not in splenic B cells (Fig. 3*B*, *right*). Thus, TRANCE is expressed specifically in T cells and in T cell-rich organs, although its expression in other cell types cannot be ruled out.

Chromosomal Mapping of TRANCE—The murine *TRANCE* locus was mapped to mouse chromosome 14 by use of an intersubspecific backcross (27, 28). In 57 backcross mice *TRANCE* showed two recombinants with the Rb1 locus (lod 13.4) and

nine recombinants with Rps10-rs4 (lod $\gg$ 6.4) (data not shown). After inferring marker genotypes from recombinant mice, incorporating other markers, and minimizing double crossovers, the gene order and map distances (centimorgans ± S.E.) were: Rb1-(1.5 ± 1.0)-*TRANCE*-(1.5 ± 1.1)-Rps10-rs4 (3.7 ± 1.6)-Rpl36-rs2-(6.4 ± 2.1)-Rpl7-rs2-(4.2 ± 1.7)-Dct. *TRANCE* is located on mouse chromosome 14 near a non-major histocompatibility complex locus suggestively linked to autoimmune nephritis in NZB mice (34), implicating TRANCE in the regulation of immune tolerance. Human *TRANCE* was localized by radiation hybrid mapping at 3.98 centiroentgens (approximately 800 kilobases) from the marker, CHLC.GATA6B07 (D13S325), located at 117 centiroentgens on the WI radiation hybrid framework of chromosome 13 (data not shown). Superposition of this map with the cytogenetic map of human chromosome 13 allowed the assignment of *TRANCE* to chromosomal band 13q14.

Biochemical Function of TRANCE—A soluble form of TRANCE containing the entire ectodomain fused to an N-terminal FLAG epitope (TRANCE-Ecto) was constructed to examine the biochemical function of TRANCE and to identify the cellular targets that respond to this protein. TRANCE-Ecto, when expressed in 293T cells and purified to homogeneity, has an apparent molecular mass of ~37 kDa by SDS-PAGE analysis (Fig. 4*A*). Since its calculated molecular mass is 27.5 kDa, these data suggest that the TRANCE-Ecto protein is post-translationally modified similarly to the FLAG-tagged membrane-bound protein. JNK is a signal transducing molecule commonly activated by TNF-related ligands. Therefore, we assessed JNK activation by the soluble TRANCE protein in thymocytes, LNTC, purified splenic B cells, and BMDC. JNK is rapidly activated in thymocytes (3-fold induction at 10 min), LNTC (2-fold induction at 5 min) (Fig. 4*B*), and T cell hybridomas (2-fold at 10 min; data not shown). In contrast, no effect was observed in B cells or in BMDC (Fig. 4*B*). B cells and BMDC may not be sensitive to soluble TRANCE at the concentration used in this assay due to the lack of an adequate number of cell surface receptors. Another possibility is that only certain cell types express JNK-activating signal-transducing molecules. These results suggest that the TRANCE recombinant protein is biologically active and appears to stimulate JNK specifically in cells of the T cell lineage.

In this report we have described the cloning of a novel member of the TNF cytokine gene family whose expression is restricted to T cells and lymphoid organs and can participate in signaling to T cells implicating TRANCE in the regulation of T cell-dependent immune responses. Although we obtained TRANCE through our genetic screen and it appears associated with cell death and not cell survival or proliferation (Figs. 1*B* and 3*A*, *right*), it is unclear whether TRANCE is necessary for TCR-mediated apoptosis. Stable expression of this gene in KIT50.1.9.1 failed to complement the molecular defects and rescue cell death (data not shown). However, this does not rule out the possibility that TRANCE is involved in apoptosis since more than one mutation in KIT50.1.9.1 may be acting to block the death-signaling machinery. In addition, soluble TRANCE failed to induce apoptosis in thymocytes, peripheral T cells, or BMDC (data not shown). However, other cell types and cell lines must be tested to better determine whether TRANCE is able to induce apoptosis. Due to the multifunctional role other TNF-related molecules exhibit it is likely that TRANCE plays a role in cell activation, proliferation, survival, or death depending on the context in which it is expressed and the nature of the target cell. In support of this, TRANCE activates JNK, a kinase with pleiotropic biological effects. Gene-targeting studies and further use of the soluble molecule will help elucidate its function in the immune system.

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