TALL-1 is a novel member of the TNF family that is down-regulated by mitogens

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Abstract: Members of the tumor necrosis factor (TNF) family play important roles in modulation of immune responses. We describe the identification and cloning of a novel TNF family member that has been designated as TALL-1. TALL-1 is a 285-amino acid type II transmembrane protein. Its carboxy terminus shares ~35% sequence identity with the recently identified APRIL and ~20–25% with TNF, FasL, TRAIL, and lymphotoxin-α, suggesting that TALL-1 and APRIL belong to a subfamily of the TNF family of ligands. Northern blot analysis suggests that TALL-1 is expressed abundantly in peripheral blood leukocytes and weakly in spleen but is barely detectable in all other tissues examined. Reverse transcriptase-polymerase chain reaction analysis indicates that TALL-1 is specifically expressed in monocytes and macrophages but is undetectable in T and B lymphocytes. Furthermore, TALL-1 expression is dramatically down-regulated by phorbol myristate acetate/ionomycin. *J. Leukoc. Biol.* 65: 680–683; 1999.

Key Words: *cytokines · signal transduction · leukocytes*

Members of the tumor necrosis factor (TNF) family of ligands play important roles in various physiological and pathological processes, including cell proliferation, differentiation, death, modulation of immune responses, and induction of inflammatory and autoimmune diseases [1, 2]. So far, at least 15 members have been identified in the TNF family, including TNF, FasL, lymphotoxin-α, lymphotoxin-β, TRAIL/APO-2L, CD27L, CD30L, CD40L, 4-1BBL, OX40L, and the recently identified TRANCE/RANKL [3, 4], LIGHT [5], TWEAK [6], TL1 [7], and APRIL [8]. Most TNF family members are synthesized as type II transmembrane precursors. The amino terminus of the type II transmembrane precursors is localized in the cytoplasm and the carboxy terminus extends into the extracellular space. A conserved ~150-amino acid region (20–25% identity) at the carboxy terminus of the extracellular domain, with which the ligand binds to its cognate receptor, is the structural hallmark of the TNF family. Except for the receptor binding region, the other domains are unrelated among the TNF family members.

Members of the TNF family interact with their cognate receptors either through cell-cell interactions or cleavage of their extracellular domains by metalloproteinases to form soluble cytokines [1, 2]. The receptors for TNF family members belong to the TNF receptor family, which contains about 20 members. Stimulation of TNF receptor family members by their ligands triggers overlapping and divergent intracellular signal transduction pathways, including those that lead to apoptosis and nuclear factor-κB (NF-κB) and aprotinin-1 (AP1) activation [1, 2].

To identify novel members of the TNF family, we searched the GeneBank EST database with the TBLASTN program for genes homologous to the extracellular domains of human TNF and FasL/ApoL. This search identified multiple EST clones that encode two different human genes. We have designated the first gene as TALL-1 (for **T**NF- and **A**poL-related **l**eukocyte-expressed **l**igand 1, see below). Sequence analysis of the EST clones encoding TALL-1 suggests that they are partial clones. To clone the full-length TALL-1 cDNA, we screened a human peripheral blood leukocyte cDNA library with one of the EST clones as probe (GeneBank accession number AA682460). Subsequent sequence analysis of the longest five positive clones indicates that TALL-1 encodes a 285-amino acid protein (**Fig. 1A**). Structural analysis suggests that TALL-1 is a type II transmembrane protein. The carboxy-terminal part of the extracellular domain of TALL-1 shares approximately 20–25% sequence identity with the corresponding domains of TNF, FasL, TRAIL, and lymphotoxin-α (Fig. 1B). Sequence homology is primarily limited to the residues forming several β-strands (Fig. 1B). These data suggest that TALL-1 is a member of the TNF family and, like some other members of the TNF family, folds into an anti-parallel β-sandwich structure [9, 10].

Sequence analysis of the EST clones encoding the second TNF/FasL-like gene, which we have designated TALL-2, indicates that this gene is identical to the recently described molecule APRIL [8]. The carboxy-terminal region of the extracellular domain of TALL-2/APRIL also shares about 20–25% sequence identity with those of TNF, FasL, TRAIL, and lymphotoxin-α (Fig. 1B). It is interesting that the carboxy-terminal regions of the extracellular domains of TALL-1 and TALL-2/APRIL share ~35% sequence identity with each

Abbreviations: TNF, tumor necrosis factor; AP1, activator protein; PBL, peripheral blood leukocytes; PMA, phorbol myristate acetate; IL, interleukin; IFN-γ, interferon-γ, NF-κB, nuclear factor κB.

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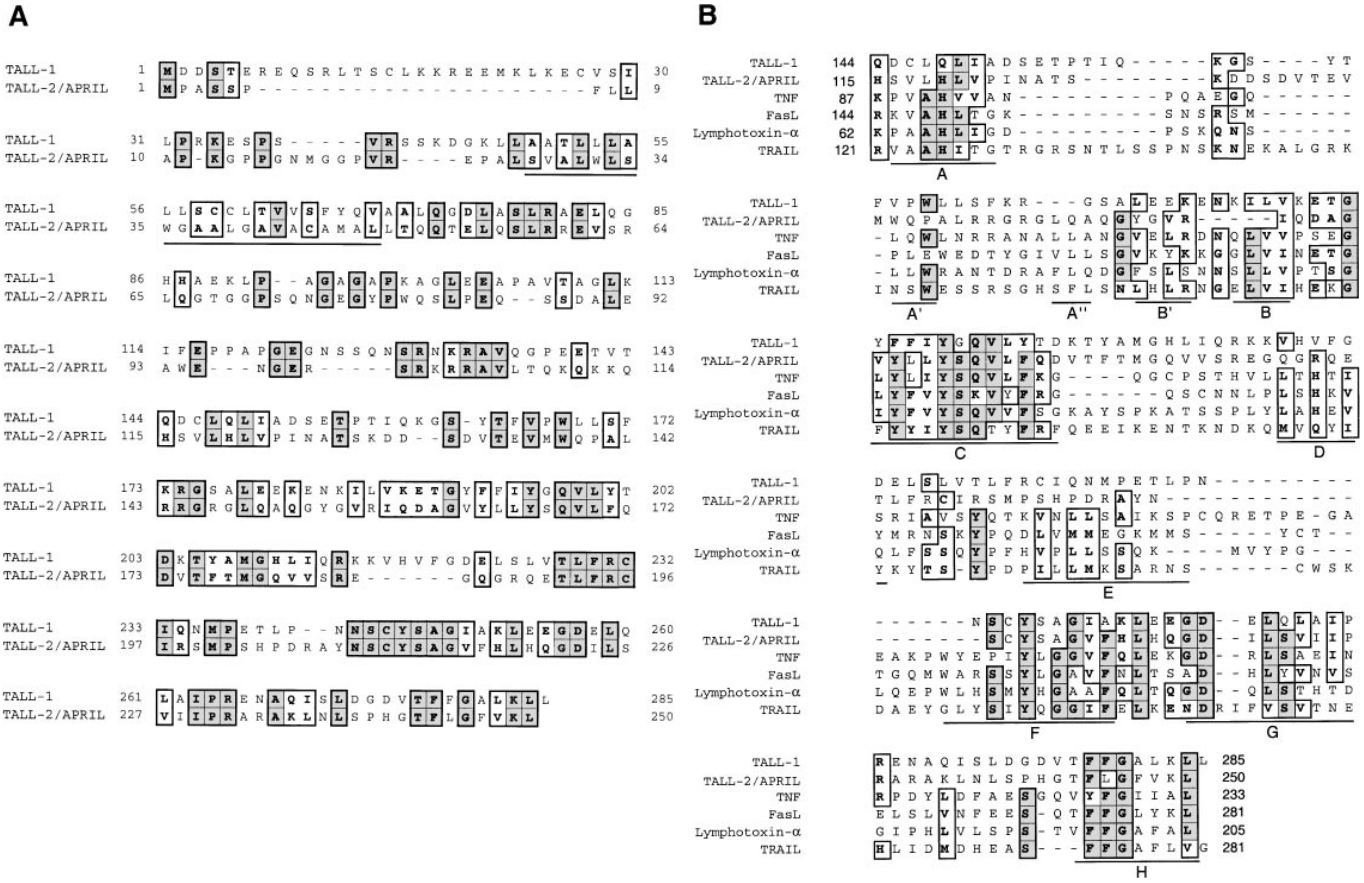

Fig. 1. Sequences of human TALL-1 and TALL-2/APRIL and alignment with other TNF family members. (A) Sequence alignment between full-length human TALL-1 and TALL-2/APRIL. The putative transmembrane domains of TALL-1 and TALL-2/APRIL are underlined. Homology regions are boxed with identical residues shaded. (B) Sequence alignment of the carboxy-terminal domains of human TALL-1 and TALL-2/APRIL with some other TNF family members. The conserved β-strands are underlined. Homology regions are boxed with identical residues shaded. The GeneBank accession numbers for TALL-1 and TALL-2 sequences are AF136293 and AF136294, respectively.

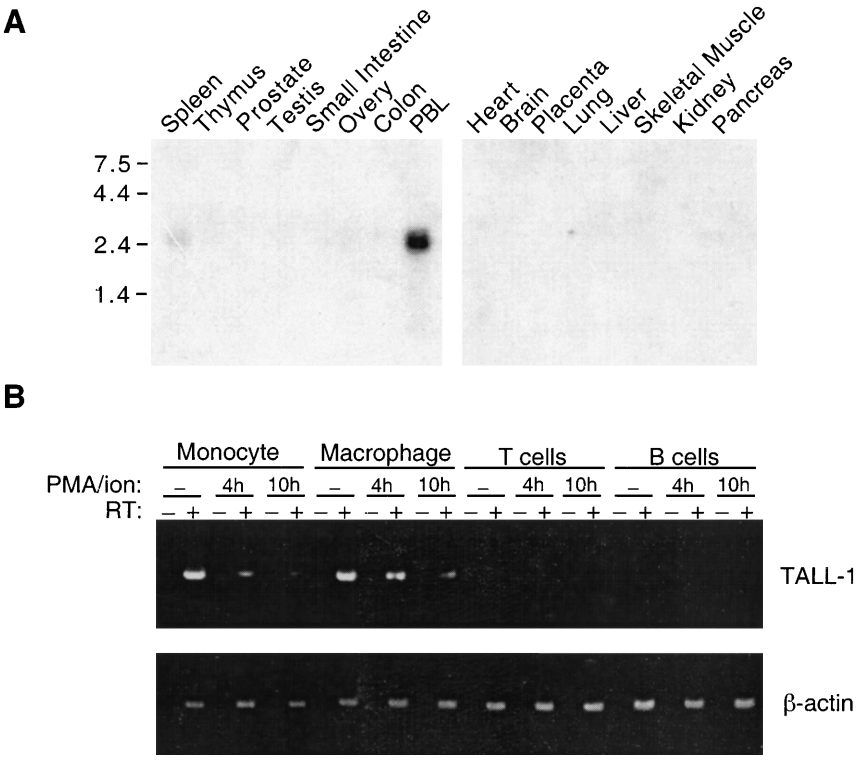

Fig. 2. Analysis of TALL-1 gene expression in human tissues and cells. (A) Northern blot analysis of TALL-1 gene expression in human tissues. Multiple human tissue blots were purchased from Clontech and were hybridized under high stringent conditions. The molecular size standards (in kilobases) are shown at the left. (B) Reverse transcriptase-polymerase chain reaction (RT-PCR) analysis of TALL-1 mRNA expression in isolated primary leukocytes. Human peripheral blood monocytes and macrophages were isolated as described [13]. Human peripheral T lymphocytes were isolated from monocyte/macrophage-depleted lymphocytes using anti-CD2 magnetic beads (Dynal, Inc.) by following procedures suggested by the manufacturer. B cells were isolated by T cell deletion of the lymphocytes. The isolated cells were treated with PMA (10 ng/mL)/ionomycin (500 ng/mL) for 4 or 10 h or left untreated. Total RNAs were then extracted and RT-PCR experiments were performed with the primers corresponding to the sequences of TALL-1 or β-actin cDNAs.

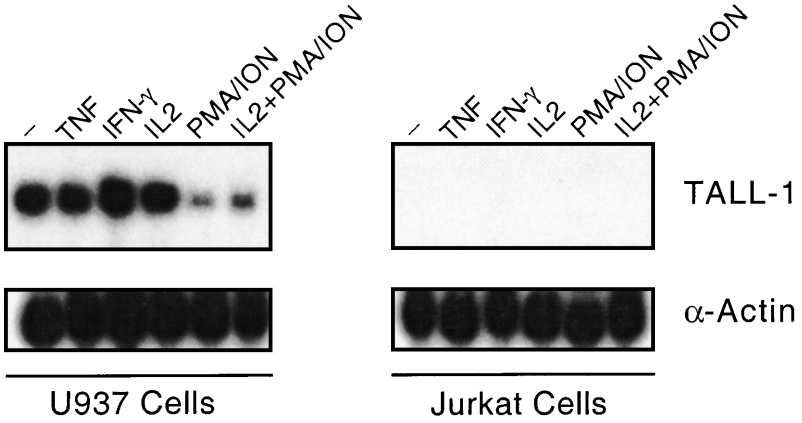

Fig. 3. TALL-1 gene expression is down-regulated by PMA/ionomycin. U937 and Jurkat cells were treated with recombinant human TNF (20 ng/mL), interferon-γ (IFN-γ; 20 ng/mL), interleukin-2 (IL-2; 20 units/mL), PMA (10 ng/mL)/ionomycin (500 ng/mL), or IL-2 plus PMA/ionomycin for 10 h or left untreated. Cells were then harvested and total RNAs were isolated for Northern blot analysis with human TALL-1 cDNA probe. The same blots were stripped and reprobed with human α-actin probe.

other, significantly higher than with other members of the TNF family (Fig. 1A). In addition to the carboxy-terminal regions of the extracellular domains, the homology between TALL-1 and TALL-2/APRIL extends to other parts of the molecules, including the amino-terminal region of the extracellular domains, the transmembrane, and the intracellular domains (Fig. 1A). These data suggest that TALL-1 and TALL-2/APRIL belong to a subfamily of the TNF family of ligands.

Northern blot analysis suggests that human TALL-1 is expressed abundantly in peripheral blood leukocytes (PBLs) and weakly in spleen as a single 2.4-kb transcript (**Fig. 2A**). TALL-1 is barely detectable in all other tissues examined (Fig. 2A). It is interesting that Northern blot analysis indicates the highest level of APRIL is also detected in peripheral blood leukocytes (data not shown) [7]. To identify the specific cell types in PBLs that express TALL-1, we performed reverse transcriptase-polymerase chain reaction analysis with RNAs extracted from isolated human peripheral monocytes, macrophages, and T and B lymphocytes. We found that TALL-1 was constitutively expressed in untreated monocytes and macrophages, but not in peripheral T and B lymphocytes (Fig. 2B). The mRNA level of TALL-1 was down-regulated by phorbol myristate acetate (PMA)/ionomycin treatment in both monocytes and macrophages (Fig. 2B). Similarly, we found that TALL-2/APRIL was also specifically expressed in monocytes and macrophages, and its expression was down-regulated by PMA/ionomycin treatment (data not shown). Consistent with these observations, we found, by Northern blot analysis, that TALL-1 was abundantly and constitutively expressed in the monocytic cell line U937 but not in the T cell line Jurkat (**Fig. 3**). In U937 cells, TALL-1 expression was also dramatically down-regulated by PMA/ionomycin, whereas TNF, interferon-γ, and interleukin-2 had no effect on TALL-1 expression (Fig. 3). These data are surprising in that many TNF family members, such as TNF, FasL, lymphotoxin-α, and LIGHT, are mostly expressed in activated cells but are not, or slightly, expressed in unstimulated immune cells and are up-regulated by mitogens [5, 11, 12].

To determine whether TALL-1 gene encodes an expressed protein, we raised a peptide-directed rabbit polyclonal antibody against a fragment of the extracellular domain of TALL-1. Immunoprecipitation and Western blot experiments with this antibody indicate that TALL-1 is expressed as an ~52-kDa protein in U937 cells (**Fig. 4**). The molecular weight of detected TALL-1 protein is larger than that deduced from amino acid sequence. This may be due to its posttranslational modification by glycosidation, which is true for most if not all members of the TNF family.

TALL-1 and TALL-2/APRIL, either alone or together, do not induce apoptosis or NF-κB and AP1 activation in several cell lines, including 293, Jurkat, and U937 (data not shown). The intracellular signaling pathways and the biological effects triggered by TALL-1 and TALL-2/APRIL are unknown at this point. However, their expression patterns and down-regulation by mitogens suggest that they are involved in monocyte/macrophage-mediated immunological processes.

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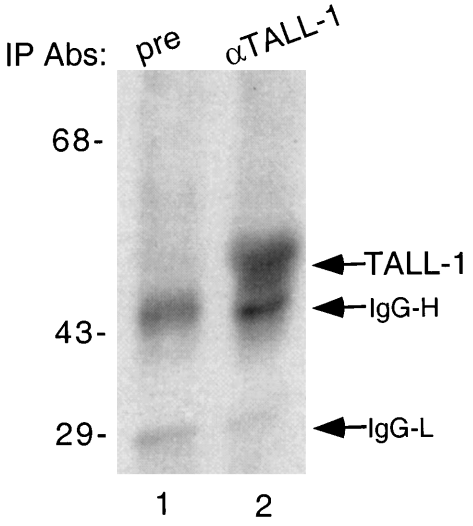

Fig. 4. Detection of TALL-1 protein in U937 cells. Lysate of U937 cells (1×10^7) were immunoprecipitated with 1 μL preimmune serum control or a rabbit polyclonal antibody against human TALL-1. The immunoprecipitates were analyzed by Western blot with the TALL-1 antibody. The molecular size standards (kDa) are shown at the left. IgG-H, IgG heavy chain; Ig-L, IgG light chain; pre, preimmune serum; aTALL-1, anti-TALL-1 antibody.

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