T Cell Leukemia–associated Human Notch/ Translocation-associated Notch Homologue Has IκB-like Activity and Physically Interacts with Nuclear Factor–κB Proteins in T Cells

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Summary

Translocation-associated Notch homologue (TAN-1), a gene originally cloned from the translocation breakpoint of a human T cell leukemia carrying a 9:7(q34.3) translocation, encodes a protein belonging to the Notch/Lin-12/Glp-1 receptor family. These receptors mediate the specification of numerous cell fates during development in invertebrates and vertebrates. The intracellular portion of Notch/TAN-1 contains six ankyrin repeats that are similar to those found in cytoplasmic IκB proteins. IκB proteins are specific inhibitors of nuclear factor (NF)–κB/Rel transcription factors. Here we show that TAN-1 has functional properties of an IκB-like regulator with specificity for the NF-κB p50 subunit. A recombinant polypeptide corresponding to the cytoplasmic portion of TAN-1 (TAN-1_C) specifically inhibited the DNA binding of p50-containing NF-κB complexes. When overexpressed in an appropriate cell line, TAN-1_C prevented κB-dependent transactivation in transient reporter gene assays in a fashion similar to the structurally related protein, Bcl-3. TAN-1_C could activate κB-dependent gene expression by attenuating the inhibitory effect of an excess of p50 homodimers. Immunoprecipitation experiments showed that the TAN-1 from a T cell line is associated with NF-κB containing p50 and p65 subunits. These observations indicate that TAN-1_C may directly engage NF-κB transcription factors and modulate nuclear gene expression.

The Notch/Lin-12 family of transmembrane receptors is believed to play a pivotal role in development by regulating cell-fate decisions (1–5). Extensive genetic studies of the Notch locus in invertebrates have revealed its roles during embryonic development of eyes, bristles, central and peripheral nervous system, muscles, and wings (6, 7). These observations have led to the hypothesis that Notch may not directly specify cell fate but rather inhibits specific signals required for cell-type specification. Translocation-associated Notch homologue (TAN-1)[1], the human counterpart of the *Drosophila* Notch, was discovered as a t(7;9) (q34.3) reciprocal translocation in the TCR-β locus in certain T lymphocytic leukemias (8). Altered expression of Notch proteins is also associated with cell fate abnormalities such as cervical and other epithelial neoplasias (9).

TAN-1 is expressed in a broad range of human tissues and is relatively highly expressed in lymphoid organs and the central nervous system. TAN-1 consists of an extracellular region containing 36 epidermal growth factor (EGF)–like segments, 3 Notch/Lin-12 repeats, and an intracellular portion containing 6 tandemly arranged ankyrin repeats, in addition to other functional domains (8). The extracellular portion of Notch proteins is thought to mediate receptor–ligand interactions (10, 11). The intracellular ankyrin repeats of Notch may be involved in protein–protein interactions that mediate transmission of the Notch signal to the nucleus (12–14).

Nuclear Factor (NF)-κB/Rel transcription factors are involved in the inducible expression of many genes containing *cis*-acting κB-binding motifs, including certain genes with important functions in immune, inflammatory, and acute phase responses (15, 16). In higher vertebrates, the DNA-binding subunits of NF-κB/Rel proteins encompass p50, p65 (RelA), c-Rel, p52, and RelB (17–22). These can to a limited extent homo- and heterodimerize, resulting in complexes with distinct DNA-binding specificity (23–26). Multiple stimuli cause a translocation of cytosolic NF-κB/Rel factors to the nucleus where they transactivate κB-dependent genes. This process is controlled by a class of inhibi-

[1] *Abbreviations used in this paper:* EMSA, electrophoretic mobility shift assay; GST, glutathione-S-transferase; NF, nuclear factor; OTF1, octamer-binding transcription factor 1; TAN-1, translocation-associated Notch homologue; TAN-1_C, cytoplasmic portion of TAN-1.

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tory molecules, termed IκB proteins, that retain NF-κB/Rel factors in the cytoplasm and suppress their DNA-binding activity (26–28). These include IκB-α, IκB-β, Bcl-3, and the NF-κB precursor proteins p100 and p105 (29–35). Upon activation, IκB proteins undergo a phosphorylation-controlled proteolysis that allows the released NF-κB/Rel factor to enter the nucleus and transactivate genes (24, 36–38). A common structural feature of IκB proteins is the presence of five to seven closely adjacent ankyrin repeats. Mutational analysis has shown that each of these repeats is required for the functional integrity of the proteins (32, 39).

Bcl-3 is an exceptional IκB protein. While it inhibits the DNA binding of transcriptionally inactive p50 dimers, it can also associate with p52 dimers on DNA and provide the NF-κB/Rel protein with transactivating potential (30, 31, 33, 34, 40). Like TAN-1, Bcl-3 was identified as a gene aberrantly expressed as a consequence of a translocation in leukemic cells (41). The structural similarity between the cytoplasmic portion of TAN-1 and various IκB proteins prompted us to investigate the potential of TAN-1 to function as a membrane-bound and, eventually, cytoplasmically released form of IκB. Here we report that the cytoplasmic portion of TAN-1 ($TAN\text{-}1_C$) is a specific inhibitor of NF-κB complexes containing the p50 subunit. In cells overexpressing TAN-1, endogenous NF-κB is found associated with the receptor. We infer that the TAN-1 receptor can regulate nuclear gene expression by directly interacting with members of the NF-κB transcription factor family.

Materials and Methods

Purification of Glutathione-S-transferase–TAN-1 Fusion Protein and Antibody Production. The expression construct for gluthatione-S-transferase (GST)–TAN-1 contained a coding sequence from amino acids 1774 to 2230 cloned into the pGEX1λT vector (Pharmacia, Uppsala, Sweden). Control protein, GST, was prepared from cells containing unmodified pGEX1λT vector. GST-TAN-1 fusion protein and GST were expressed in BL21(DE3) (Novagen, Inc., Madison, WI). Soluble protein extracts were prepared by mild sonication. Cell debris were removed by centrifugation. The supernatant was loaded onto a Sepharose-glutathione affinity column, washed with solution A, 0.5% Triton X–100 in 0.5 M NaCl and 10 mM Tris, and solution B, 50 mM Tris, pH 8.0, and then eluted with 10 mM glutathione in 50 mM Tris, pH 8.0. The apparent molecular mass of GST-TAN-1 fusion protein (78 kD) was determined by 10% SDS-PAGE. An identical procedure was used for purification of GST. GST-TAN-1 and GST protein concentrations were determined with a protein assay reagent (Bio-Rad Laboratories, Richmond, CA), according to the manufacturer's protocols. This purified GST-TAN-1 fusion protein was used to immunize rabbits for antibody production.

Electrophoretic Mobility Shift Assay. Jurkat T cells were cultured in RPMI-1640 supplemented with 10% FCS and treated with 50 ng/ml PMA (Sigma, Chemical Co., St. Louis, MO) for 3 h. Nuclear extracts were prepared as described elsewhere (42). Briefly, pelleted cells were suspended in 5 vol of cold PBS and collected by centrifugation at 4°C. The cells were suspended in 5 vol packed cell pellet of buffer A containing 10 mM Hepes (pH 7.9), 1.5 mM $MgCl_2$, 10 mM KCl, and 0.5 mM dithiothreitol (DTT). The cells were allowed to stand for 10 min and were then centrifuged for 10 min at 2,000 rpm in a rotor (model HG4L; Sorval, Newtown, CT). 2 vol of buffer A was added to the pellet. The cells were lysed by a glass homogenizer (Kontes Co., Vineland, NJ). The homogenate was centrifuged at 2,000 rpm to pellet nuclei. The nuclear pellet was subjected to a second centrifugation at 25,000 *g* for 20 min. The pellet was resuspended in 3 ml of buffer C (20 mM Hepes, pH 7.9, 25% glycerol, 0.42 M NaCl, 1.5 mM $MgCl_2$, 0.2 mM EDTA, 0.5 mM PMSF, and 0.5 mM DTT) per 10^9 cells and lysed with a Kontes glass homogenizer. The resulting suspension was centrifuged at 25,000 *g* for 20 min. The supernatant was frozen at −80°C. DNA-protein–binding reactions were carried out in 10 mM Tris, pH 7.5, 10 mM EDTA, 0.5 mM DTT, 50 mM NaCl, 2 μg poly(dI-dC) (Pharmacia), and 5% glycerol in a final vol of 20 μl. The reaction contained 6 μg of nuclear extract and five-end-labeled Ig enhancer binding probes. GST-TAN-1 or GST was added directly to the binding reaction. The reaction was kept at room temperature for 30 min and loaded on a nondenaturing 6% polyacrylamide gel using 0.5 × Tris/Borate/EDTA buffer for 3 h at 150 V. P50 and p65 antibodies were purchased from Santa Cruz Biotechnology (Santa Cruz, CA).

Expression Constructs and In Vitro Translation. The TAN-1 construct contains the sequence of the TAN-1 intracellular portion including amino acids 1774 to 2230, which is identical to the sequence of GST-TAN-1 fusion protein. P50 and p65 constructs have been described elsewhere (22). The expression of p50, p65, TAN-1, and Bcl-3 was directed by the expression vector Rc CMV (Invitrogen, San Diego, CA). The Bcl-3 construct was a kind gift of Dr. I.M. Verma (Salk Institute, San Diego, CA). The reporter plasmids contain a luciferase gene driven by the six reiterated κB sites. Translation of full-length p50 and p65 was performed in the presence or absence of L-[^{35}S]methionine using wheat germ extracts according to manufacturer's protocols (Promega, Corp., Madison, WI). Translated products were analyzed by SDS-PAGE (10%) followed by autoradiography.

Cell Culture and Transient Transfection Experiments. NTera-2 cells (American Type Culture Collection [ATCC], Rockville, MD) were grown in DMEM containing 10% FCS. NTera-2 cells were transfected with expression vectors in a 6-well plate in the presence of lipofectamine (GIBCO BRL, Gaithersburg, MD). The transfection was performed according to the manufacturer's standard protocols. After 48 h, the cells were harvested and extracts assayed for luciferase activity using a kit (Promega Corp.) according to the manufacturer's instructions.

Immunoprecipitation and Western Blot Analysis. Sup-T1 cells (from Dr. J. Hoxie) and U266 cells (ATCC) were extracted in 1% Triton X–100 (Sigma Chemical Co.), 50 mM Tris, pH 7.5, 1 mM PMSF, 1 μg/ml leupeptin, 1 μg/ml pepstatin, 1 μg/ml E64, and 40 μg bestatin (Boehringer Mannheim, Mannheim, Germany) at 10^8 cells/ml for 1 h on ice. Soluble detergent extracts were incubated with p50 or p65 antibodies (Santa Cruz Biotechnology) for 2 h at room temperature and then at 4°C overnight after the addition of protein G–Sepharose beads. The beads were washed five times with 140 mM NaCl, 10 mM Tris, pH 7.5, and 0.5% Triton X–100, and were then boiled in SDS sample loading buffer under reducing conditions for 10 min, and run on a 4–20% SDS-PAGE, followed by electrotransferring to polyvinylidene difluoride membranes. Membranes were blocked with 5% BSA in TBS for 2 h, and then sequentially incubated with rabbit anti-TAN-1 antibody or preimmune IgG, followed by alkaline phosphatase–conjugated anti–rabbit IgG heavy and light chains (Bio-Rad Laboratories). Alkaline phosphatase substrate was detected by Lumi-Phos™ 530 (Boehringer Mannheim).

Results

TAN-1_C Is a Specific Inhibitor of NF-κB. The mechanism by which proteins in the Notch family function is incompletely understood. A recent study showed that a construct consisting primarily of six ankyrin repeats of germline proliferation-1, a member of the Notch family, is sufficient to control downstream regulators of cell fate in *Caenorhabditus elegans* (12). Another report (14) showed that a cytoplasmic protein, Deltex, positively regulates the Notch pathway through interactions with the Notch ankyrin repeats. A GST fusion protein, corresponding to the Notch/TAN-1 intracellular domain containing six ankyrin repeats and NH_2- and COOH-terminal flanking sequences of TAN-1 (amino acids 1774-2230), was produced in *Escherichia coli* and purified by affinity chromatography. GST was also expressed for use as a control and purified in the same way. Both proteins were examined for inhibition of NF-κB DNA binding to a consensus κB site. In an electrophoretic mobility shift assay (EMSA) with nuclear extracts from Jurkat T cells, TAN-1_C inhibited DNA binding of NF-κB that had been activated by treatment of cells with PMA (Fig. 1 *A*, lane *5*; Fig. 1 *B*, lanes *2–4*). Treatment of DNA-binding reactions with specific antibodies showed that the NF-κB complex contained both p50 and p65 subunits (Fig. 1 *B*, lanes *6* and *7*). The activity of GST-TAN-1_C was specific since it did not affect the activity of endogenous octamer-binding transcription factor 1 (OTF1) (Fig. 1 *C*) and the GST portion alone showed no inhibitory effect in EMSA (Fig. 1 *A*, lane *6*; Fig. 1 *B*, lane *5*). For complete inhibition of the NF-κB complex, 4 pmol of GST-TAN-1 was required, which is comparable to the amount of IκB-α or Bcl-3 necessary for inhibition of NF-κB (29, 30).

NF-κB Subunit Specificity of TAN-1_C. Using in vitro–translated p50 or p65, we tested whether the IκB-like activity of GST-TAN-1_C was specific for NF-κB subunits. GST-TAN-1_C effectively inhibited DNA binding of p50 dimers but not that of p65 dimers (compare Fig. 2 *A*, lanes *3* and *4*). The inhibition of p50 dimer DNA binding by GST-TAN-1_C was concentration dependent (Fig. 2 *B*). This subunit specificity resembles that of the protooncogene, Bcl-3, and is different from those of IκB-α and -β, which selectively interact with p65 (29, 30, 33). Interestingly, the ankyrin repeats of TAN-1 are more closely related to Bcl-3 (50% similarity and 29% identity) than IκB-α (45% similarity and 22% identity). These results suggest that NF-κB dimers containing the p50 subunit are a target for negative regulation by human Notch/TAN-1 molecules.

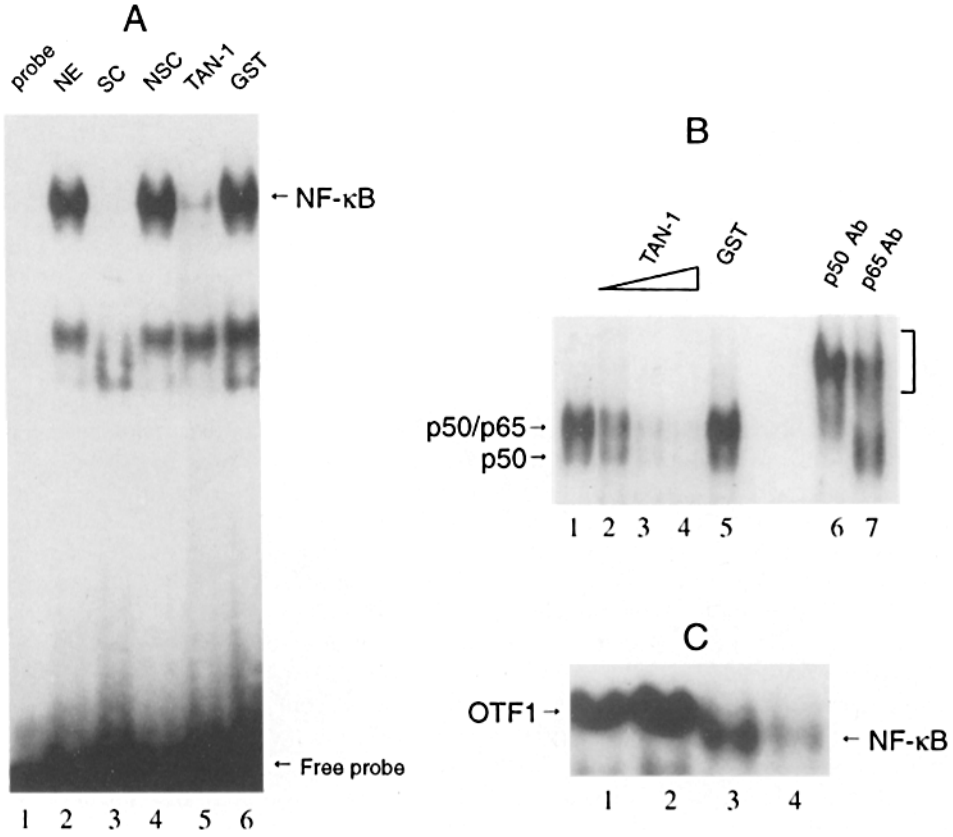

Figure 1. Specificity of inhibition of DNA binding of nuclear NF-κB by recombinant TAN-1_C. (*A*) PMA-induced nuclear extracts were assayed with bacterially expressed GST-TAN-1_C or control protein, GST, in an EMSA using radiolabeled κB DNA. Lane *1*, [^{32}P]-κB DNA probe alone; lane *2*, nuclear extracts (*NE*) with probe; lane *3*, specific competitor (*SC*) (κB DNA); lane *4*, nonspecific competitor (*NSC*) (Sp-1 DNA); lane *5*, TAN-1 (4 pmol); and lane *6*, GST (4 pmol). The faster migrating band is considered a nonspecific binding component that may cross-react with κB DNA present in the nuclear extracts, as this complex does not supershift in the presence of anti-p50 or anti-p65 antibodies (data not shown). (*B*) The same nuclear extracts were analyzed in an EMSA including in the presence of anti-p65 and p50 antibodies. Lanes *1–4*, TAN-1 (0, 1, 2, and 4 pmol); lane *5*, GST (4 pmol); and lanes *6* and *7*, p50 and p65 antibodies, respectively. (*Bracket*) Positions of immune complexes. (*C*) The same nuclear extracts were analyzed in an EMSA with radiolabeled κB and OTF1 probes. Lane *1*, nuclear extract plus GST (4 pmol) and OTF1 probe; lane *2*, nuclear extract plus TAN-1 (4 pmol) and OTF1 probe; lane *3*, nuclear extracts plus GST (4 pmol) and κB probe; and lane *4*, nuclear extract plus TAN-1 (4 pmol) and κB probe. The positions of NF-κB and OTF1 are indicated.

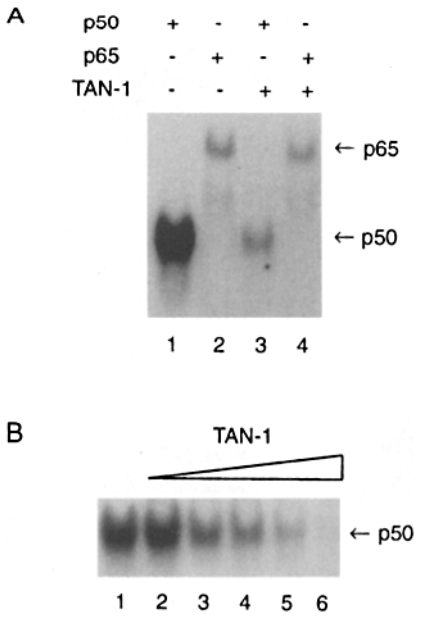

Figure 2. Subunit specificity of the inhibition of κB DNA-binding activity by GST-TAN-1_C. (*A*) Radiolabeled κB probe was incubated with: in vitro–translated p50 (1 μl), lane *1*; p65 (4 μl), lane *2*; 1 μl of p50 plus GST-TAN-1 (4 pmol), lane *3*; and 4 μl of p65 plus GST-TAN-1 (4 pmol), lane *4*. (*B*) 1 μl of p50 was incubated with TAN-1 (0, 0.3, 0.6, 1, 2, and 4 pmol), lanes *1–6*.

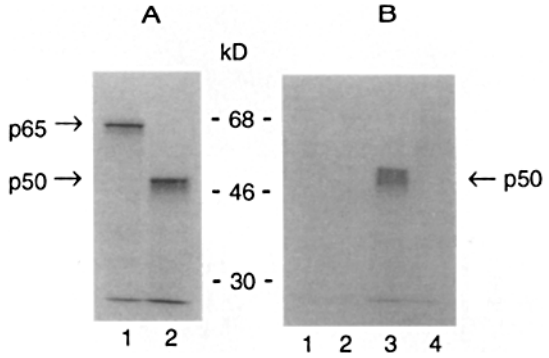

Figure 3. Association of TAN-1 with p50. (*A*) Translated product (5 μl) was analyzed by SDS-PAGE on a 10% gel, followed by autoradiography. Lane *1*, p65; lane *2*, p50. (*B*) ^{35}S-labeled in vitro–translated p50 (10 μl) or p65 (10 μl) was incubated with 10 μg of GST-TAN-1_C or 10 μg of GST bound to Sepharose-glutathione beads. The reaction mixture was rotated at 4°C for 4 h, and subsequently washed and eluted. The eluted samples were run on a 10% SDS-PAGE, followed by autoradiography. Lane *1*, p50 plus GST; lane *2*, p65 plus GST; lane *3*, p50 plus GST-TAN-1_C; lane *4*, p65 plus GST-TAN-1_C.

Direct Binding of TAN-1 to NF-κB p50 Subunit. We did not observe GST-TAN-1_C directly binding to the κB site on DNA (data not shown). Therefore, it may prevent DNA binding of p50-containing NF-κB complexes by binding to the p50 subunit. To demonstrate such a direct protein–protein interaction, GST-TAN-1_C was tested for precipitation of in vitro–translated p50 and p65 on glutathione Sepharose beads. In vitro–translated ^{35}S-labeled p50 and p65 products are shown in Fig. 3 *A*, lanes *1* and *2*. GST-TAN-1_C precipitated p50 but not p65 (Fig. 3 *B*, lanes *3* and *4*). The GST control precipitated neither p50 nor p65 (Fig. 3 *B*, lanes *1* and *2*), indicating that the inhibition of the DNA-binding activity of p50 is specific and dependent on a direct association between p50 and TAN-1. These results suggest that TAN-1 functions as a bona fide IκB protein.

TAN-1_C Can Modulate NF-κB–Dependent Transactivation. We further investigated the effect of overexpressing the cytoplasmic domain of TAN-1 on κB-dependent transactivation in intact cells. In NTera-2 cells, the high level expression of a chloramphenicol acetyl transferase (CAT) gene driven by the κB sites of the HIV-κB-LTR or of a luciferase gene driven by six reiterated κB sites is dependent on cotransfection of NTera-2 cells with expression vectors for NF-κB proteins (31, 33, 40). As shown in Fig. 4 *A*, coexpression of TAN-1_C or Bcl-3 decreased the κB-dependent transactivation of the luciferase reporter gene in a dose-dependent manner (Fig. 4 *A*). In this experiment, the ratio of p50 to p65 plasmids was 1:1. Similar results were obtained with a HIV-κB-LTR–driven CAT gene (data not shown). In the presence of increased concentrations of p50 expression vector (p50/p65 plasmid ratio = 6:1), a bimodal effect of TAN-1_C was observed (Fig. 4 *B*). Whereas high concentrations of TAN-1_C vector resulted in an inhibition of transactivation as seen in Fig. 4 *A*, lower concentrations of transfected TAN-1_C vector resulted in an increased transactivation (Fig. 4 *B*). This behavior resembles that of Bcl-3 (compare Fig. 4 *B*, *right*). TAN-1, like Bcl-3, appeared to be able to reverse the transcriptional inhibition imposed by high amounts of p50 dimers. Higher levels of TAN-1 expression may also interfere with p50/p65 heterodimers resulting in a net inhibition of reporter gene expression. These data indicate that TAN-1_C has the potential to facilitate NF-κB–dependent gene expression which has been shown to be an important step in T cell activation (43).

Sup-T1 T Cells Contain a NF-κB–TAN-1 Complex. The preceding experiments suggested a direct and functional interaction of TAN-1_C with NF-κB in vitro and in vivo. We therefore sought evidence for a physical association between TAN-1 and p50 containing NF-κB complexes under physiologic conditions. We analyzed anti-p50 and anti-p65 immunoprecipitates from Sup-T1, a T cell line that contains copies of both the normal and truncated TAN-1 gene and U266, a mature B cell line (plasma cell) that does not express detectable levels of Notch protein. Preimmune IgG did not detect TAN-1 in either cell line (Fig. 5, lanes *1* and *2*) and anti-TAN-1 antibody found no evidence of TAN-1–related peptides in the U266 control (Fig. 5, lane *3*). However, anti-TAN-1 antibody did reveal the presence of two peptides in anti-NF-κB immunoprecipitates from Sup-T1 cell line cells. The 90-kD species observed is consistent with the truncated protein predicted from the sequence resulting from the chromosomal rearrangement seen in this cell line and certain T cell leukemias (8). The less abundant 100-kD species likely corresponds to a proteolytic product of human Notch, in that a peptide of identical molecular weight has been consistently observed

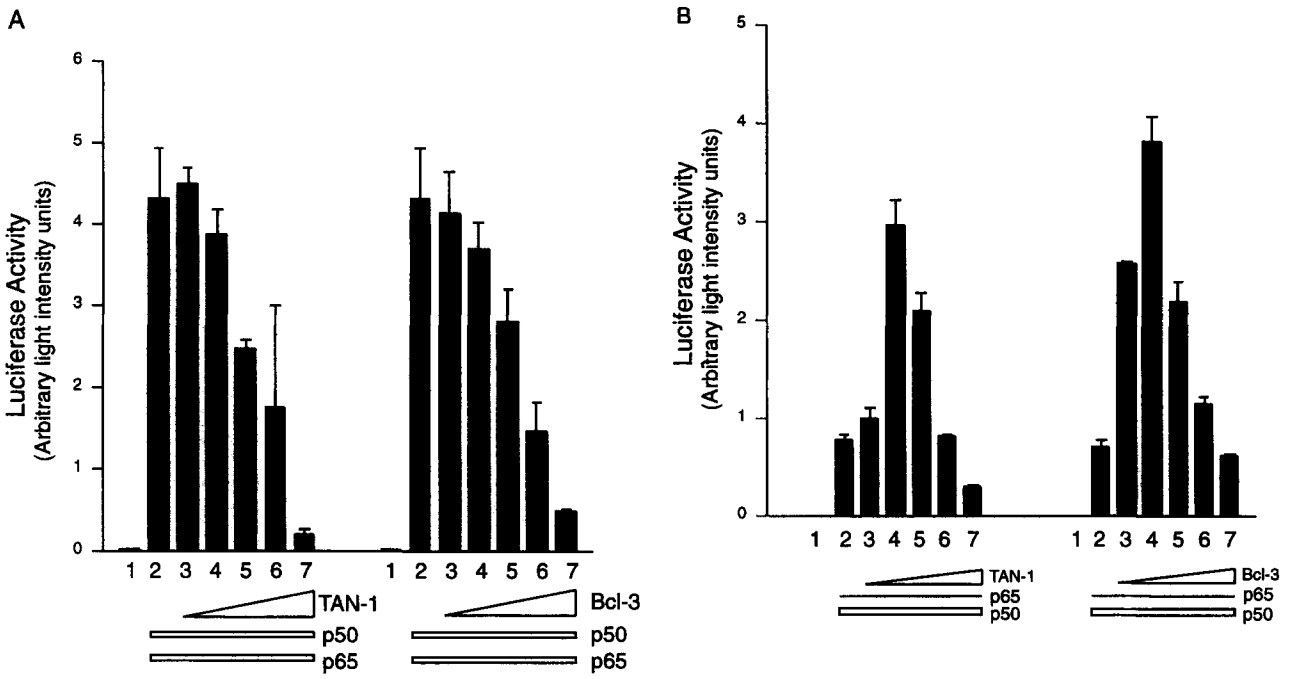

Figure 4. TAN-1 modulates transactivation mediated by NF-κB. (*A*) Transactivation of 6 κB-luciferase reporter (0.3 μg) plasmids by transfection of NTera-2 cells with expression vectors encoding columns: *1*, reporter plasmids; *2*, vectors as used in *1* plus p65 (0.5 μg) and p50 (0.5 μg); *3–7*, vectors as used in *2* plus increasing amounts of TAN-1 or Bcl-3 vector 0.1, 0.3 , 0.5, 1, and 2 μg. (*B*) Column *1*, reporter plasmid (0.3 μg); *2*, vectors as *1* plus p65 (0.05 μg) and p50 (3 μg); *3–7*, vectors as *2* plus increasing amounts of TAN-1 or Bcl-3 vector 0.1, 0.3, 0.5, 1, and 3 μg.

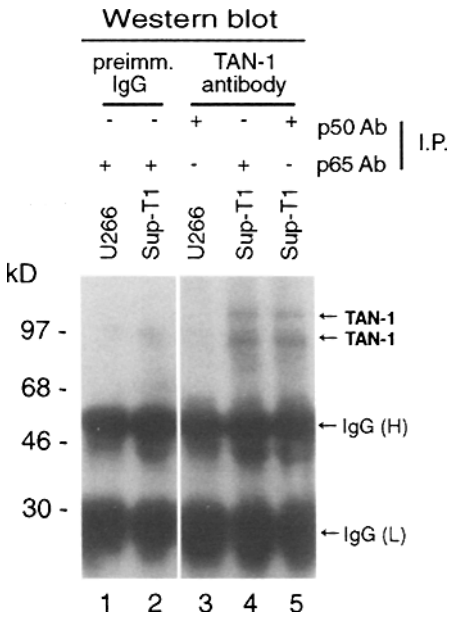

Figure 5. TAN-1 interacts with NF-κB. U266 or Sup-T1 T cells were lysed and immunoprecipitated with anti-p50 or anti-p65 antibodies. The immunoprecipitates were analyzed by SDS-PAGE (4–20%) and transferred to PVDF membranes. Lanes *1* and *2*, U266 or Sup-T1 T immunoprecipitates were probed with preimmune rabbit IgG that did not detect TAN-1 bands. Lanes *3–5*, U266 or Sup-T1 immunoprecipitates were probed with TAN-1 antibody that detected TAN-1 bands from Sup-T T cells but not from control cells, U266. Bands corresponding to IgG heavy and light chains from p50 and p65 antibodies that reacted with detection system, alkaline phosphatase–labeled anti–rabbit IgG (H+L) are also indicated.

in a variety of human cell extracts, including neuroblastoma NB5 cells (9, 11).

These data suggest that membrane-associated Notch (TAN-1) may prevent nuclear localization of NF-κB in human T cells as has been proposed for *Drosophila* nuclear transcription factors (44).

Discussion

In this paper we show that the intracellular domain of Notch/TAN-1 specifically inhibits NF-κB activity and binds p50 in vitro. Its transfection modulates κB-mediated transactivation. TAN-1 related peptides, including that predicted from the translocation event, are found associated with NF-κB in a leukemic T cell line.

The intracellular events that follow Notch–ligand interactions are, however, unclear. In *Drosophila*, recent data suggest that Notch signaling controls nuclear events that influence the progression of uncommitted cells to a more highly differentiated state. Three loci encoding putative nuclear proteins, *Drosophila Su (H)* (homolog of mammalian C promoter binding factor), *Enhancer of split*, and *Mastermind*, have been implicated in these nuclear events (14, 45–47). Several recent studies have shown that the ankyrin repeats of the intracellular portion of Notch associate with cytoplasmic proteins. For example, *Drosophila* Notch–derived ankyrin repeat-containing peptides have been shown to bind the cytoplasmic protein, Deltex. Mouse Notch ankyrin repeats associate with the human analogue of *Su(H)*, κBF2/CBF-1, and act

as transcriptional activators through the κBF2-binding sites of the mouse *Hairy enhancer of split 1* promoter (48). Interestingly, the Deltex–Notch interaction prevents the observed cytoplasmic retention of the suppressor of Hairless protein by Notch ankyrin repeats resulting in nuclear translocation (44). These observations highlight the significance of our findings in the context of a mechanism relevant to the human system in which Notch is thought to play a potentially pathogenic role.

Adjacent ankyrin repeats have been shown to mediate protein–protein interactions (30, 32, 49, 50). Mutations disrupting single ankyrin repeats in Bcl-3, pp40, and p105 rendered these proteins incapable of suppressing the DNA-binding activity of NF-κB or associating with NF-κB/Rel proteins. This demonstrates that ankyrin motifs are absolutely required for the functional interaction between IκB and NF-κB (30, 32). So far the ankyrin repeats of human Notch have not been demonstrated to associate with intracellular proteins. Our data suggest that members of the well-characterized NF-κB/Rel transcription factor family serve, under physiologic conditions, as signal transducers for Notch1/TAN-1 receptors. The structural and pathophysiological analogy between the TAN-1 and Bcl-3 protooncogene is now further extended by a functional relationship: both proteins specifically bind the p50 NF-κB subunit and show similar effects on κB-dependent transactivation. The Bcl-3 protooncogene that shows increased expression in chronic lymphocytic leukemia cells containing the translocation t(14;19) (q32;q13.1), differs from TAN-1 in that it lacks a transmembrane domain, and is predominantly nuclear. However, earlier studies indicate that truncated, putatively activated forms of Notch without transmembrane and extracellular domains are translocated to the nucleus in transgenic flies and in transfected mammalian or *Drosophila* cells (13, 14, 47, 51). Sequence comparison and deletion analysis have identified two nuclear localization sequences that reside on either side of the Notch ankyrin repeats. Preliminary data indicate that truncated human Notch/TAN-1 is present in both the cytoplasm and nucleus (Guan, E., unpublished observations). These findings raise the possibility that the oncogenic and possibly physiologically activated forms of TAN-1 are truncated and, like Bcl-3, may act in the nucleus. Another Notch-related protein that may fall into a novel class of proteins with ankyrin repeats and a functional association with control of normal and neoplastic cell proliferation is the mouse mammary tumor gene product, int-3 (52). However, the mechanism(s) by which TAN-1 could contribute to malignancy has remained elusive. The family of Rel-related and ankyrin motif-containing proteins has been associated with tumorigenesis. v-Rel–containing viruses are highly transforming; c-Rel, p50B, Bcl-3, and TAN-1 are located at sites of recurrent translocations and genomic rearrangements (8, 40, 41, 53, 54). We are currently investigating whether TAN-1 can interact with several other Rel/NF-κB proteins and whether these alterations in p50 complexes can be consistently detected in these tumors. Inappropriately expressed human TAN-1 may contribute to tumorigenesis by regulating NF-κB gene expression (as seen for the c-Rel, p50B, and Bcl-3 genes) particularly when Notch has been affected by chromosomal translocation.

The function of Notch/TAN-1 as a regulator of NF-κB provides a preliminary understanding of its importance in human development, immunology, and oncogenesis.

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