

Cellular Proliferation and Activation of NF κ B Are Induced by Autocrine Production of Tumor Necrosis Factor α in the Human T Lymphoma Line HuT 78*

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Tumor necrosis factor (TNF) is a pleiotropic cytokine which has both cytotoxic and proliferative effects. HuT 78, a T-cell line derived from a Sezary lymphoma, is resistant to the cytotoxic effects of TNF, suggesting that TNF may be a growth factor for this cell line. The aim of this study was to determine whether autocrine TNF production could function as a growth factor for HuT 78. Resting HuT 78 and K-4 cells, a protein kinase C- β -deficient clone of HuT 78, both produced significant amounts of TNF compared with Jurkat cells. Thymidine incorporation by HuT 78 and K-4 cells was inhibited by 90.5 and 73.2%, respectively, with addition of a neutralizing monoclonal antibody to TNF α , suggesting that TNF is an autocrine growth factor for these cells. HuT 78 and K-4 cells also expressed high levels of constitutively active NF κ B, unlike Jurkat cells, which expressed high levels only upon activation with TNF or phorbol 12-myristate 13-acetate. p50 was the major component in the NF κ B complexes in HuT 78 and K-4 cells. Anti-TNF α antibody dramatically decreased levels of NF κ B in both HuT 78 and K-4 cells. As the TNF gene has an NF κ B binding motif, an autocrine loop involving TNF induction of NF κ B is therefore likely in these cells. These findings in a neoplastic T-cell line suggest that therapy directed against TNF could be effective in a subset of T-cell lymphomas.

Tumor necrosis factor α (TNF α)¹ is a potent cytokine with a wide range of biological activities (1). It was initially described in serum of endotoxin-treated mice as the mediator of the necrosis of some transplantable tumors (2). It has since been reported to be cytotoxic to certain transformed cell lines (3–5) and may act either as a mediator in beneficial processes of host

defense, immunity, and tissue homeostasis or in the pathogenesis of infection, tissue injury, and inflammation (6). *In vitro*, TNF has been shown to be involved in mediating a wide range of biological activities, including differentiation, antiviral responses, and proliferation. TNF stimulates proliferation of many cell types, both lymphoid and non-lymphoid, including thymocytes (7), fibroblasts (5), chondrocytes (8), and some human cancers, including chronic lymphoid leukemia and ovarian cancer (9, 10). TNF is synthesized by a broad range of cells, including macrophages/monocytes, T and B lymphocytes, NK cells (1), mast cells (11), some transformed cell lines (12, 13), and breast and epithelial tumor cells (14, 15). The activated macrophage is considered to be the main producer of TNF *in vivo* (16). The most potent inducer of TNF production in these cells is lipopolysaccharide (LPS) derived from the cell wall of Gram-negative bacteria (1). Transformed myeloid cell lines such as U937 and HL60 can also be induced to produce TNF with LPS and such agents as phorbol esters and the calcium ionophore A23187 (17, 18). Similarly, T lymphocytes produce TNF in response to phorbol esters and calcium ionophore or mitogens (19). Certain transformed cell lines produce TNF constitutively (12, 13), although the basis for such constitutive production has not been determined.

The regulation of production of TNF is complex. There are likely to be four levels of control: the transcriptional level, the mRNA abundance level, the level of translation and, finally, posttranslationally. Most attention has been focussed on regulatory elements responsible for transcriptional activation. Elements responsive to the transcription factor NF κ B are especially important to confer LPS inducibility (20). Determining how NF κ B becomes activated in cells is therefore likely to be important for our understanding of TNF induction. The NF κ B family of proteins currently comprises three main groups of molecules. The first includes NFKB1 p105/p50 and NFKB2 p100/p52 (21, 22). p105 and p100 represent precursor proteins which are proteolytically cleaved to form p50 and p52, respectively, which are transcriptionally active (23, 24). The second subclass includes RelA (formerly p65) (25), c-Rel (26), v-Rel (27), and RelB (28), none of which require processing in order to be active. The third subclass includes I κ B α (29), I κ B γ (30), and Bcl-3 (31) which regulate the cytoplasmic retention, DNA binding, and transcriptional activity of NF κ B complexes. The predominant form of NF κ B in cells appears to comprise a heterodimer of p50 and RelA complexed to I κ B α , although other arrangements of NF κ B occur and are likely to play a role in NF κ B-driven gene expression (32). NF κ B is constitutively present and active in the nucleus only in a restricted subset of cells such as B cells (33) and certain T-cell lines (34). Latent NF κ B is the predominant form in other cell types where it is maintained in the cytoplasm complexed to an inhibitory subunit I κ B (35). Upon activation with agents such as LPS, interleukin 1

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¹ The abbreviations used are: TNF, tumor necrosis factor; DTT, dithiothreitol; IL, interleukin; NF κ B, nuclear factor κ B; PKC, protein kinase C; PMA, phorbol 12-myristate 13-acetate; PMS, phenazine methosulfate; MTS, 3-(4,5-dimethylthiazol-2-yl)-5-(3-carboxymethoxyphenyl)-2-(4-sulphophenyl)-2H-tetrazolium.

(IL1), and TNF, I κ B dissociates from NF κ B, which then translocates to the nucleus where it binds its decameric DNA recognition sequence. Phosphorylation and proteolysis of I κ B have been implicated in the dissociation process (36, 37).

Here, we describe for the first time the constitutive production of TNF and high levels of constitutively active NF κ B in a human T lymphoma cell line, HuT 78, which is resistant to cytotoxicity by TNF. In addition, we demonstrate that a monoclonal antibody to TNF blocks proliferation of the cells and constitutive NF κ B. The results suggest that an autocrine loop involving TNF production and NF κ B activation may be operating in the cells which leads to proliferation.

EXPERIMENTAL PROCEDURES

Materials—Recombinant human TNF α was a generous gift from Cetus Corp. (Emeryville, CA). The monoclonal antibody to human TNF α (Clone 4H31) was obtained from Sanbio (Uden, The Netherlands). The anti-IE hybridoma, Y-17, was obtained from ATCC. T4 polynucleotide kinase, the 22-base pair oligonucleotide containing the consensus sequence (underlined)(5'-AGTTGAGGGGACTTCCCA-GGC-3'), the 22-base pair oligonucleotide containing the consensus sequence (underlined) for the immunoglobulin enhancer oligonucleotide factor 1 (OCT1) (5'-TGTCGAATGCAAAATCACTAGAA-3') and poly(dI-dC) were from Promega (Madison, WI). [γ -³²P]ATP (10 mCi/mmol) was obtained from Amersham International (Amersham, Buckinghamshire, United Kingdom). Antibodies to p50, RelA, and c-rel were from Santa Cruz Biotechnology, Inc. (Santa Cruz, CA).

Cell Lines—The L929 murine fibrosarcoma and Jurkat T-cell lymphoma cell lines were obtained from ECACC (Porton Down, Salisbury, Wiltshire, UK). HuT 78, a T-cell lymphoma derived from peripheral blood of a Sezary lymphoma patient, was obtained from ATCC (Rockville, MD). K-4 cells were generated from HuT 78 as described previously (38). Cells were cultured in RPMI 1640 containing 10% fetal calf serum, 2 mM L-glutamine, penicillin, and streptomycin and 5×10^{-5} M 2-mercaptoethanol (complete medium) (Life Technologies, Inc., Paisley, Scotland).

TNF Production—HuT 78, K-4 and Jurkat cells (1×10^6 cells/ml) were incubated at 37 °C for 48 h in medium. Supernatants were then removed and stored in 0.5-ml aliquots at -70 °C. All samples were assayed for TNF by both bioassay (L929 cytotoxicity assay) and enzyme-linked immunosorbent assay (ELISA) within 2 months of collection. The L929 cytotoxicity assay was a modification of that described by Green *et al.* (39). L929 cells between the 5th and 10th passage were incubated in 96-well flat-bottomed microtiter plates at a concentration of 8×10^3 cells/well overnight at 37 °C. Titrations of the recombinant human TNF α standard or undiluted test samples were then diluted (1/2) in medium with actinomycin D (2 μ g/ml final concentration) (Sigma, Poole, Dorset, UK) and incubated for 24 h at 37 °C. 20 μ l of 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) (Sigma) at a concentration of 6 mg/ml was added to the wells and plates incubated for 4 h at 37 °C. Dimethyl sulfoxide (British Drug House, Poole, UK) was used to dissolve the formazan crystals formed in the wells. Absorbance was measured at 570 nm on an ELISA reader.

TNF α was also measured with a commercially available ELISA (Innogenetics NV, Antwerp, Belgium). The detection limit for this assay is 4 pg/ml. The intraassay and interassay coefficients of variation are 5.5 and 7.9% respectively.

Interleukin 2 Production—Interleukin 2 (IL2) was also measured in the supernatants of resting HuT 78, K-4, and Jurkats incubated for 48 hr in medium alone, as above. IL2 was measured by an ELISA obtained from R & D Systems, Inc. (Minneapolis, MN). The sensitivity of the assay was 6 pg/ml. The intraassay and interassay coefficients of variation were 5.9 and 7.7%, respectively.

Thymidine Incorporation—HuT 78 or K-4 cells were incubated in 96-well flat-bottomed microtiter plates at a concentration of 1×10^5 cells/well for 24 h and pulsed with tritiated thymidine (0.5 μ Ci/well) for the last 6 h of incubation. Titrations of the TNF α standard and/or TNF α monoclonal antibody (3.5 mg/ml) or anti-IE antibody (3.5 mg/ml) were added to the wells at the start of incubation. Samples were harvested on a multiple automated cell harvester and counted in a Beckman liquid scintillation counter. In addition, to examine cell viability and numbers, we performed a non-radioactive cell proliferation assay, using MTS, a tetrazolium salt (3-(4,5-dimethylthiazol-2-yl)-5-(3-carboxymethoxyphenyl)-2-(4-sulfophenyl)-2H-tetrazolium) and an electron coupling reagent, PMS (phenazine methosulfate) (Promega). Both solutions were stored protected from light at -20 °C, and immediately before use, a

working solution containing a 1/20 dilution of PMS in MTS was made. HuT 78 cells were grown as above with the exception that PMS/MTS was added for the last 4 h of culture. Absorbance was measured at 490 nm on an ELISA reader.

Preparation of Subcellular Fractions—Subcellular fractions were prepared as described before (40). HuT 78, K-4, or Jurkat T lymphomas (1×10^6 /ml, 1 ml/sample) were treated with IL1, phorbol myristate acetate (PMA), or TNF (all at 10 ng/ml) as indicated for various times or were left untreated. Cells were then washed with ice-cold phosphate-buffered saline and resuspended into 1 ml of hypotonic buffer (10 mM Hepes buffer, pH 7.9, containing 1.5 mM MgCl₂, 10 mM KCl, 0.5 mM dithiothreitol (DTT) and 0.5 mM phenylmethylsulfonyl fluoride). Cells were pelleted in hypotonic buffer by centrifugation at $13,000 \times g$ for 10 min at 4 °C and then lysed for 10 min on ice in 20 μ l of hypotonic buffer containing 0.1% (v/v) Nonidet P-40. Lysates were centrifuged ($13,000 \times g$, 10 min, 4 °C), and the supernatant (nuclear extract) was removed into 75 μ l of storage buffer (10 mM Hepes, pH 7.9, containing 50 mM KCl, 0.2 mM EDTA, 10% (v/v) glycerol, 0.5 mM DTT, and 0.5 mM phenylmethylsulfonyl fluoride). Protein concentrations were determined (41) and the extracts were assayed immediately for NF κ B activity or stored at -86 °C until further use. All of the steps in the above procedure were performed at 4 °C unless otherwise stated.

Electrophoretic Mobility Shift Assay—Nuclear extracts (2–4 μ g of protein) were incubated with 10,000 cpm of the 22-base pair oligonucleotide containing the NF κ B binding motif which had been previously labeled with [γ -³²P]ATP by T4 polynucleotide kinase (42). Incubations were performed for 30 min at room temperature in the presence of 2 μ g of poly(dI-dC) as nonspecific competitor and 100 mM Tris buffer, pH 7.5, containing 100 mM NaCl, 1 mM EDTA, 5 mM DTT, 4% glycerol, and 100 μ g/ml nuclease-free bovine serum albumin. In some experiments, unlabeled oligonucleotides containing either the NF κ B or the OCT1 consensus sequence were added to the extracts before incubating with labeled oligonucleotide. In experiments involving antisera to NF κ B subunits, 0.5 μ l of a specific antiserum to p50, p65, or c-rel was incubated with 4 μ g nuclear extract 30 min prior to the binding reaction. All incubations were subjected to electrophoresis on native 4% (w/v) polyacrylamide gels that were subsequently dried and autoradiographed in autoradiography cassettes with intensifying screens for 16–48 h at -86 °C.

RESULTS

TNF Production by HuT 78, K-4, and Jurkat Cells—We were prompted to measure spontaneous TNF production by HuT 78 and K-4, as initial experiments demonstrated that they were resistant to cytotoxicity by TNF (data not shown). Other studies have suggested that exposure to low concentrations of TNF induce resistance to subsequent TNF-mediated cytotoxicity and that cells resistant to TNF-mediated cytotoxicity produce low levels of TNF (14, 43). Supernatants of resting HuT 78, K-4, and Jurkat cells incubated for 48 h were analyzed for TNF production by L929 bioassay which measures both biologically active TNF and lymphotoxin and by ELISA, which measures immunoreactive TNF only. Fig. 1A demonstrates that HuT 78 and K-4 produce substantial amounts of TNF α constitutively (HuT 78, 136.6 ± 64.3 pg/ml; K-4, 425.1 ± 122.1 pg/ml, mean $\pm$ S.E.), measured by L929 bioassay: Jurkats, however, produce very little TNF (19.8 ± 11.8 pg/ml TNF, mean $\pm$ S.E.). TNF levels in supernatants were also measured by a TNF α -specific ELISA. Fig. 1B demonstrates that the ELISA similarly detected constitutive TNF α production by HuT 78 and K-4 (HuT 78: 56.3 ± 2.3 and K-4: 75.2 ± 16.9 pg/ml TNF, mean $\pm$ S.E.): resting Jurkat cells, however, do not produce TNF α levels detectable by ELISA. Differences between the L929 bioassay and ELISA methods for measuring TNF have been documented previously (44, 45).

IL2 was not detected in supernatants of resting HuT 78, K-4, or Jurkat cells (data not shown).

Inhibition of Thymidine Incorporation of HuT 78 and K-4 by Anti-TNF α —HuT 78 and K-4 cells were incubated for 24 h either alone or with 3 ng/ml TNF or a neutralizing monoclonal antibody to TNF α and proliferation measured by thymidine incorporation (Fig. 2). An irrelevant control antibody (anti-IE)

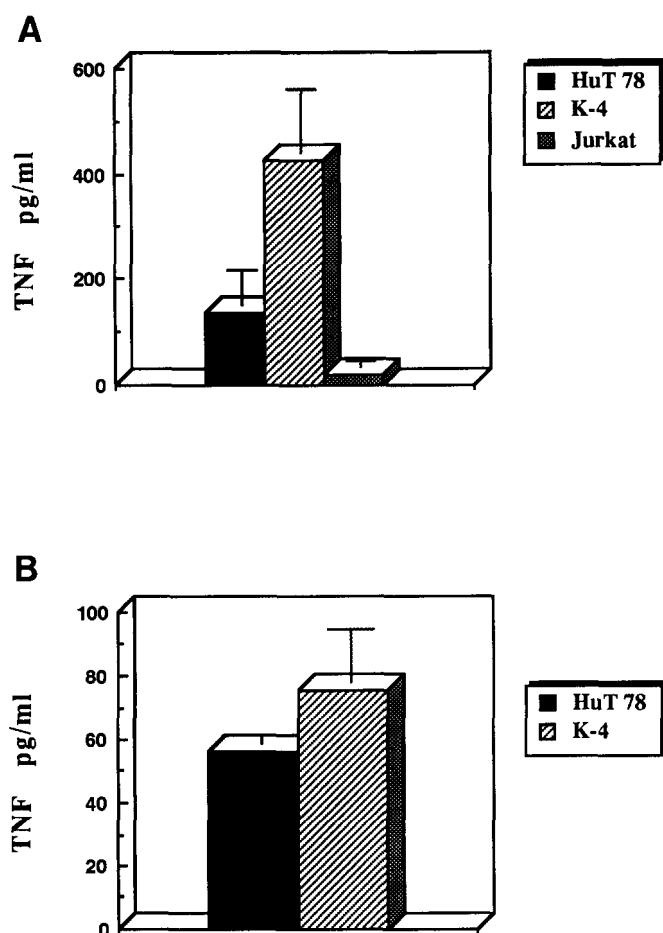


FIG. 1. **TNF production by T-cell clones.** HuT 78 and K-4 cells were incubated for 48 h in medium and TNF levels in supernatants measured by L929 bioassay (A) or ELISA (B). Mean $\pm$ S.E. of three experiments.

was also included. 3 ng/ml TNF α had no effect on proliferation; higher TNF concentrations (30 ng/ml) also had no effect (data not shown). Proliferation of cells treated with the anti-TNF α antibody (1/20 dilution) was dramatically inhibited on average by $90.5 \pm 10.1\%$ and $73.2 \pm 9.2\%$ for HuT 78 and K-4, respectively (mean $\pm$ S.E., three experiments). A similar concentration of control antibody had no effect. In addition, we examined cell viability and numbers using PMS/MTS. Culture of HuT 78 cells alone produced an absorbance of 0.573 ± 0.042 (mean $\pm$ S.D., three experiments). Culture in the presence of anti-TNF α antibody resulted in absorbance of 0.333 ± 0.012 (mean $\pm$ S.D., three experiments). Hence, these data confirm that anti-TNF α treatment is associated with significant reduction in number of viable cells.

HuT 78 and K-4 Contain Constitutive NF κ B—We next examined the cells for the presence of NF κ B. As can be seen in Fig. 3, A and B, both HuT 78 and K-4 contained high levels of constitutive NF κ B which could not be further enhanced with IL1, PMA, or TNF. This was in contrast to another human T-lymphoma line, Jurkat, which had no constitutive NF κ B (Fig. 3C). Upon stimulation with either PMA or TNF, however, NF κ B could be activated in Jurkat in a time-dependent manner.

All of the complexes detected in each of the cell types appeared to constitute protein components which interacted specifically with the NF κ B recognition sequence, as their formation was inhibited by addition of unlabeled oligonucleotide, containing the NF κ B binding site, to nuclear extracts before

incubation with labeled probe (Fig. 4A). An unlabeled oligonucleotide containing the OCT1 sequence motif failed to exhibit any such inhibitory effect. We also examined the extracts for the presence of p50, RelA, and c-Rel, as shown in Fig. 4B. Samples prepared from TNF-treated Jurkats were shown to contain p50 and RelA but no c-Rel, as indicated by the ability of p50 and RelA antisera to cause a further retardation in probe mobility (Fig. 4B), lanes 2 and 3). In HuT 78 and K-4, a similar result was obtained, although much larger amounts of p50 were detected than RelA (Fig. 4B, lanes 6 and 7 for HuT 78 and lanes 10 and 11 for K-4). Again, no c-Rel was detected in either cell type.

A Monoclonal Antibody to TNF Inhibits NF κ B in HuT 78 and K-4—Because both HuT 78 and K-4 were shown to produce TNF constitutively, we next determined whether the monoclonal antibody to TNF, which we had found to inhibit proliferation in the cells, would also interfere with the constitutive NF κ B, which we found in both cell types. Fig. 5A demonstrates that incubating both HuT 78 and K-4 for 48 and 72 h with anti-TNF dramatically reduced the levels of NF κ B in both HuT 78 and K-4. Similar concentrations of an irrelevant antiserum had no effect, as seen in Fig. 5B. This indicates that the constitutively active NF κ B is a result of constitutive TNF production by the cells. As the TNF gene is NF κ B regulated, an autocrine loop is therefore likely to be operational in the cells.

DISCUSSION

TNF is a pleiotropic cytokine, which is produced mainly by activated macrophages and lymphocytes in response to many stimuli, including bacterial toxins and viruses, as well as cytokines, including TNF itself (1). It is also produced by a number of transformed and neoplastic cells (12, 13, 15). In this study, we demonstrated that HuT 78, a T-cell lymphoma, and K-4, a PKC β -deficient clone of HuT 78, produce TNF spontaneously but Jurkat, another T-cell line, however, does not.

TNF is a growth factor for many normal lymphoid and non-lymphoid cell types, including thymocytes, fibroblasts, chondrocytes, and lymphoid cells (T and B lymphocytes) (5, 7, 46, 47) as well as certain neoplastic cells, including ovarian cancer (10) and B-cell chronic lymphoid leukemia (9).

HuT 78 and K-4 are resistant to the cytotoxic effects of TNF, even at concentrations of up to 30 ng/ml TNF, much higher concentrations than that needed to kill L929 cells (data not shown). Jurkat, which do not produce TNF, are sensitive to TNF-mediated killing (48). TNF production by other cell types which are resistant to cytotoxicity by TNF have previously been reported (14, 43, 49), suggesting that TNF may be an autocrine growth factor for certain cell types.

A monoclonal anti-TNF α antibody significantly inhibited proliferation of HuT 78 and K-4, suggesting that TNF was a growth factor for these cells. Recently, Pimentel-Muinos *et al.* (50) suggested that PMA-induced TNF was an autocrine growth factor for T-cells, stimulating IL 2 secretion, which then stimulated proliferation. However, this does not seem to occur in HuT 78 or K-4, as IL2 was not detected in the supernatants of resting cells after 48 h, suggesting that IL2 is not involved here (data not shown). Also, K-4 produce significantly less IL2 than HuT 78 upon stimulation with PMA (38), yet the anti-TNF α antibody inhibited proliferation of K-4.

The identification of two TNF receptors on cells suggested initially that individual receptors might mediate individual functions of killing and proliferation (51). However, both receptors have since been found to mediate both proliferation and cytotoxicity (52), therefore both may trigger proliferation in HuT 78.

Once TNF binds to receptors on cells, its intracellular signaling pathways are complex. TNF has been reported to acti-

FIG. 2. Inhibition of proliferation of T-cell clones with a monoclonal anti-TNF α antibody. HuT 78 and K-4 cells were incubated for 24 h in medium alone, with 3 ng/ml TNF α , with anti-IE (1/20 dilution of 3.5 mg/ml stock solution), an irrelevant control antibody, and with anti-TNF α (1/20 dilution of 3.5 mg/ml stock solution). Proliferation was assessed by [3 H]thymidine incorporation as described under "Experimental Procedures." Representative of three experiments, mean $\pm$ S.E.

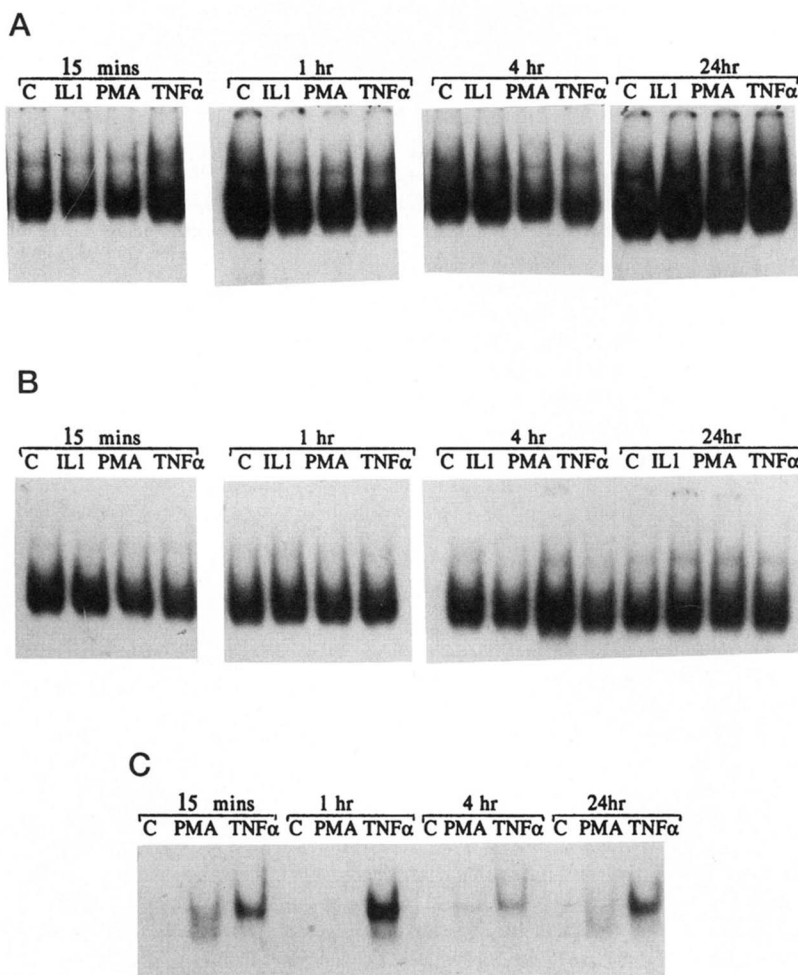
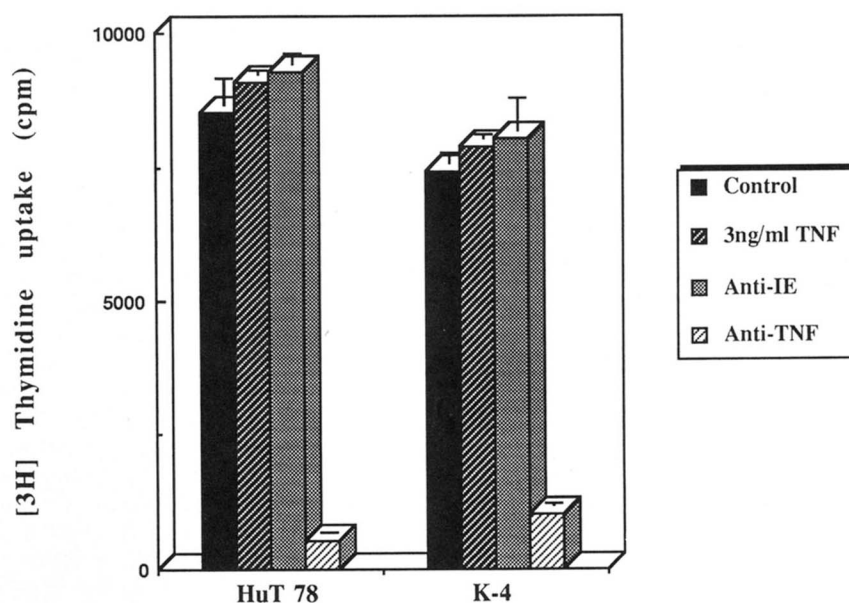


FIG. 3. Time course of activation of NF κ B in Hut 78, K-4, and Jurkat T lymphomas. Hut 78 (A), K-4 (B), or Jurkat (C) T lymphomas were incubated with IL1, PMA, or TNF α (all at 10 ng/ml) or left untreated (lanes C) for the indicated times and then assessed for NF κ B as described under "Experimental Procedures." NF κ B-DNA complexes are shown.

vate multiple protein kinases and transcription factors, including NF κ B (1). We detected high levels of constitutive NF κ B in HuT 78 and K-4 cells. In most cell types, NF κ B exists in a latent form in the cytosol complexed to an inhibitory subunit I κ B (35). Only a limited number of lymphoid cells have been found to contain constitutively active nuclear NF κ B. These include mature B cells (33) and a few T lymphomas (34). In

these cells, however, the constitutive activation of NF κ B is only partial as stimulation of the cells results in a further activation. This does not appear to be the case in HuT 78 and K-4 as stimulation of the cells with agents which potently activate NF κ B in other cells failed to cause increased activation. The mechanism here appears to involve the constitutive production of TNF by the cells as a neutralizing monoclonal antibody to

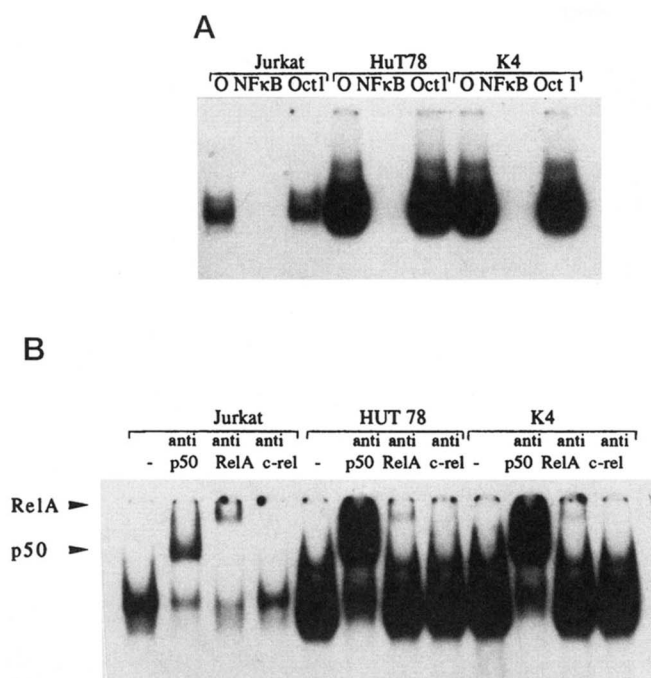


FIG. 4. Analysis of NF κ B in HuT 78, K-4, and Jurkat T lymphomas. A, 2 μ g of nuclear extract protein prepared from Jurkat cells stimulated with TNF α (10 ng/ml) for 1 h or unstimulated Hut 78 and K-4 were incubated in the absence (lanes 0) or presence of unlabeled oligonucleotides (1.75 pmol) containing the NF κ B or OCT1 consensus sequences prior to assaying for NF κ B binding activity as described under "Experimental Procedures." NF κ B-DNA complexes are shown. B, 2 μ g of nuclear extract protein prepared from Jurkat cells stimulated with TNF α (10 ng/ml) for 1 h or unstimulated Hut 78 and K-4 were incubated with the indicated antisera on ice prior to assaying for NF κ B binding activity as described under "Experimental Procedures." NF κ B-DNA complexes are shown. Supershifted complexes corresponding to p50 and RelA are indicated.

TNF completely blocked the appearance of NF κ B in the nucleus of the cells. As NF κ B is known to play a key role in the control of TNF gene transcription (20), an interesting autocrine loop may therefore be operating in the cells. The role of activated NF κ B in the proliferation of the cells through the induction of other genes is unresolved. For example, NF κ B is known to regulate the *c-myc* gene (53), which has a well established role in growth control. Kitajima *et al.* (54) have previously demonstrated that inhibiting NF κ B causes an inhibition of tumorigenesis, in experiments using antisense technology. Hence, NF κ B could have indirect effects on growth control regulation through interaction with either proto-oncogenes or growth factor genes involved in tumor growth and proliferation.

Although lymphotoxin is detected by the L929 bioassay (55), it is unlikely to be relevant here as an autocrine growth factor for HuT 78, as the monoclonal antibody producing virtually complete inhibition of cell proliferation was specific for TNF α . Also, Ware *et al.* (56) reported that lymphotoxin is not produced by HuT 78 and recently, it has been reported that lymphotoxin does not activate NF κ B (57).

Attempts to identify proteins within the NF κ B complex in the cells revealed the presence of both p50 and RelA. Two recent studies have shown that HuT 78 nuclei contain high levels of both p52 (58) and p50 (59). Two additional proteins of p84 and p85 were also detected and represented aberrantly processed p100. The basis for these observations was found to be a rearrangement of the NF κ B2 gene in the cells, resulting in a protein which had lost an ankyrin repeat domain in its C terminus. Because ankyrin domains on p100 and p105 are

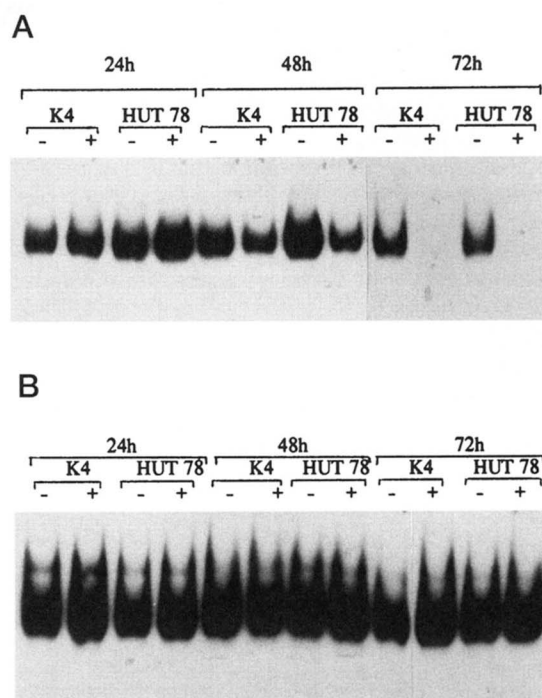


FIG. 5. Effect of a neutralizing monoclonal antibody to TNF α on NF κ B in HuT 78 and K-4. A, Hut 78 cells and K-4 were washed twice in RPMI and were then incubated in medium alone (-) or 125 μ l TNF α antibody (3.5 mg/ml stock solution) (+) for 24 h, 48 h, or 72 h as indicated. NF κ B binding activity was measured as described under "Experimental Procedures." NF κ B-DNA complexes are shown. B, Hut 78 cells and K-4 were washed twice in RPMI and were then incubated in medium alone (-) or 125 μ l of control antibody anti-IE IgG1 (3.5 mg/ml stock solution) (+) for 24 h, 48 h, or 72 h as indicated. NF κ B binding activity was measured as described under "Experimental Procedures." NF κ B-DNA complexes are shown.

important for cytoplasmic retention, the authors suggest that the lack of the C-terminal ankyrin domain results in a protein which is not processed in the normal way and escapes cytoplasmic retention, thereby localizing to the nucleus. p85 and p52 were also shown to be transcriptionally active (58). The studies did not explain why normal p52 is found in the nucleus of the cells as this would be expected to be retained in the cytoplasm complexed to I κ B. Our results indicate that constitutive TNF production by the cells is critical for the nuclear localization of both p52 and p85, along with p50 and RelA, as a neutralizing antibody to TNF dramatically decreased nuclear NF κ B levels. This suggests that the missing ankyrin repeat in p85 would not be sufficient to cause nuclear translocation, the five remaining repeats being sufficient to retain the protein in the cytoplasm. It is likely, however, that once the aberrant NF κ B has translocated to the nucleus in response to TNF, a dysregulation in TNF gene expression must occur which results in a lack of feedback inhibition. In normal cells, NF κ B activation leading to TNF production is self-limiting through mechanisms likely to involve, at least in part, I κ B induction which acts to neutralize transactivating NF κ B. Such mechanisms appear to be absent in HuT 78. This is most likely due to the presence of abnormal NF κ B2 proteins altering the functioning of the NF κ B system in the cells.

An autocrine role for TNF in NF κ B activation has also been demonstrated in a study involving PMA-stimulated T lymphocytes, where it was shown that activation of NF κ B by PMA required the induction of TNF as neutralizing antibodies to TNF blocked the response (48). Our results with HuT 78 are analogous except that no stimulus was needed to trigger TNF production. Both studies confirm the importance of autocrine

regulation in NF κ B activation in cells and suggest that once TNF is induced, it will feed back on the cells and activate NF κ B. However, the current study differs significantly from studies on peripheral blood T-cells. HuT 78 is a malignant T-cell clone derived from a cutaneous T-cell lymphoma. Inhibition of TNF α blocked proliferation of this malignant cell line, suggesting the possibility that pharmacological interventions involving inhibition of TNF production may have potential effects.

In conclusion, this study demonstrates that proliferation of HuT 78 is stimulated by the autocrine production of TNF. This also results in the activation of NF κ B in the cells, which is likely to lead to further TNF production. The HuT 78 T-cell line is derived from a cutaneous Sezary type T-cell lymphoma. The finding that proliferation of this cell line responds to inhibition of TNF production suggests the intriguing possibility that anti-TNF strategies could be employed in the treatment of these lymphomas in the clinical environment.

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