Tumor-Induced Oxidative Stress Perturbs Nuclear Factor-κB Activity-Augmenting Tumor Necrosis Factor-α–Mediated T-Cell Death: Protection by Curcumin

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Abstract

Cancer patients often exhibit loss of proper cell-mediated immunity and reduced effector T-cell population in the circulation. Thymus is a major site of T-cell maturation, and tumors induce thymic atrophy to evade cellular immune response. Here, we report severe thymic hypocellularity along with decreased thymic integrity in tumor bearer. In an effort to delineate the mechanisms behind such thymic atrophy, we observed that tumor-induced oxidative stress played a critical role, as it perturbed nuclear factor-κB (NF-κB) activity. Tumor-induced oxidative stress increased cytosolic IκBα retention and inhibited NF-κB nuclear translocation in thymic T cells. These NF-κB–perturbed cells became vulnerable to tumor-secreted tumor necrosis factor (TNF)-α (TNF-α)–mediated apoptosis through the activation of TNF receptor-associated protein death domain–associated Fas-associated protein death domain and caspase-8. Interestingly, TNF-α–depleted tumor supernatants, either by antibody neutralization or by TNF-α-small interfering RNA transfection of tumor cells, were unable to kill T cell effectively. When T cells were overexpressed with NF-κB, the cells became resistant to tumor-induced apoptosis. In contrast, when degradation-defective IκBα (IκBα super-repressor) was introduced into T cells, the cells became more vulnerable, indicating that inhibition of NF-κB is the reason behind such tumor/TNF-α–mediated apoptosis. Curcumin could prevent tumor-induced thymic atrophy by restoring the activity of NF-κB. Further investigations suggest that neutralization of tumor-induced oxidative stress and restoration of NF-κB activity along with the reeducation of the TNF-α signaling pathway can be the mechanism behind curcumin-mediated thymic protection. Thus, our results suggest that unlike many other anticancer agents, curcumin is not only devoid of immunosuppressive effects but also acts as immunorestorer in tumor-bearing host. [Cancer Res 2007;67(1):362–70]

Introduction

Transcription factor nuclear factor-κB (NF-κB) is one of the major antiapoptotic transcription factors that regulate ~150 genes, including several cytokine genes. NF-κB consists of multiple proteins belonging to Rel family that includes proteins, such as p105/p50 (NF-κB1), RelA (p65), RelB, cRel, etc. (1, 2). In resting cells, NF-κB proteins are retained in the cytoplasm as inactive forms via the association with inhibitory IκB molecules. The classic induction of p50-p65 NF-κB heterodimers involves activation of the IκB kinase (IKK) complex, the large multisubunit complexes that phosphorylate two NH_2-terminal serine residues of IκB, resulting in phosphorylation- and ubiquitin-dependent degradation of IκB, with IκBα being most predominant (3). As a consequence, NF-κB complexes translocate to the nucleus and regulate expression of κB target genes (4, 5). DNA-binding activity of NF-κB is rapidly induced in all cell types in response to proinflammatory cytokines and the byproducts of microbial and viral infections. It is therefore anticipated that NF-κB–induced transcription would play a central role in host defense and inflammatory responses (3). In fact, importance of NF-κB in host immunity, lymphoid organ development, and antiapoptotic gene expression has been well established (6, 7).

Thymus is the major site for T-cell maturation, which plays a pivotal role in the internal defense system. $CD8^+$ CTLs are major effector cells involved in immunologically specific tumor destruction, and $CD4^+$ T cells are essential for "helping" $CD8^+$ T cell–dependent tumor eradication (8). Recently, tumor-induced thymic atrophy is being considered as a part of the immune evasion strategy of the developing tumor (9, 10). Because T-cell proliferation, differentiation, and apoptosis take place in thymus, aberration in one or more of these processes due to tumor may therefore result in thymic atrophy. Several observations indicate that a chronic inflammatory condition develops in patients with advanced cancer, causing oxidative stress that can shut off immune functions. Increased oxidative stress has further been detected as one of the causes of tumor-induced T-cell depletion (11, 12). There are contradictory reports about the effect of reactive oxygen species (ROS) on NF-κB activation. Some reports claim that ROS is a common intermediate in the activation of NF-κB by diverse agents (1, 13), whereas others claim oxidative stress–induced NF-κB inhibition (14–17). The latter reports are further supported by the fact that NF-κB is down-regulated/inhibited in T cells of tumor-bearing mice and cancer patients (18, 19). It is acknowledged that many tumors secrete tumor necrosis factor (TNF)-α (TNF-α), which is cytocidal for thymic T cells (20). TNF-α regulates immune responses, inflammation, and apoptosis by exerting its diverse biological activities by activating multiple signaling pathways, including IKKs, c-Jun NH_2-terminal kinase (JNK), and caspases (21). IKK activation inhibits TNF-α–induced apoptosis through the transcription factor NF-κB, whose target genes include those that encode inhibitors of caspases and JNK (22). Moreover, NF-κB not only antagonizes apoptotic signals of TNF-α but also activates its prosurvival pathway (3). Thus, any perturbation in

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doi:10.1158/0008-5472.CAN-06-2583

NF-κB activity by developing tumor may make T cells susceptible to tumor-secreted TNF-α–induced apoptosis. Because such tumor-induced thymic disorder can abate the cellular defense mechanism, any therapeutic regimen that can protect thymus from tumor assault will be helpful to protect substantial peripheral T-cell pull in the tumor-bearing host.

The present study was conducted to delineate the mechanisms of tumor-induced thymic T-cell depletion and the effect of curcumin (diferuloylmethane, a known antioxidant with proven antitumor activity; ref. 23) in prevention of tumor-induced thymic degeneration. We observed severe hypocellularity and structural disintegration of the thymus of tumor-bearing animals. A search for the mechanisms of tumor-induced thymic demise revealed critical roles of elevated oxidative stress and down-regulated NF-κB activity. Earlier, we reported that curcumin induces apoptosis in tumor cells (23–25). Here, we evaluated the effectiveness of curcumin in the prevention of tumor-induced thymic atrophy. In fact, curcumin administration restored tumor-induced depression in NF-κB activity in thymic T cells through the reduction of oxidative stress. Simultaneously, it normalized TNF receptor 1 (TNFR1) expression in thymic T cells and decreased TNF-α production by tumor cells. Concerted effects of all these finally resulted in T-cell survival. Our study suggests the therapeutic possibility of curcumin, as it is a known anticancer agent with strong immunomodulatory effect.

Materials and Methods

Treatment of animals. Animal experiments were done following the 'Principles of laboratory animal care' (NIH publication no. 85-23, revised in 1985) as well as Indian laws on 'Protection of Animals' under the prevision of authorized investigators. Swiss albino mice (National Center for Laboratory Animal Sciences, Hyderabad, India) weighing 25 to 27 g were divided into four groups of 10 animals each including normal set (non–tumor bearing), tumor-bearing set (which were i.p. injected with 1×10^5 exponentially grown ascites carcinoma), curcumin-treated set (non–tumor bearing), and curcumin-treated tumor-bearing set. Curcumin (treatment started 7 days after tumor inoculation) was fed orally (50 mg/kg body weight every alternate day; ref. 24). For low-TNF-α–secreting tumor, 1×10^5 exponentially grown sarcoma-180 tumor cells were introduced ascitically.

Cell culture. At day 21 of tumor inoculation, thymus was removed, and single-cell suspension was made in RPMI 1640. Macrophages were allowed to adhere at 37°C for 1 h. Peripheral blood collected from mice and from healthy human volunteers with informed consent (Institutional Review Board 1382) were centrifuged over Ficoll-Hypaque density gradient (Amersham Pharmacia, Uppsala, Sweden) to obtain total leukocytes. T cells were purified from total leukocytes and thymocytes by negative magnetic selection using human T-cell enrichment cocktail (Stemcell Technologies, Vancouver, British Columbia, Canada). Viable cell numbers were determined by trypan blue exclusion test. T cell isolated procedure yielded >97% positive for CD3 cells as defined by immunocytometry. Isolated cells ware maintained in complete RPMI 1640 supplemented with 10% fetal bovine serum (Sigma, St. Louis, MO) at 37°C in humidified incubator containing 5% CO_2.

Renal cell carcinoma (RCC) cell lines, SK-RC-45 and SK-RC-26B, were obtained from Dr. N. Bander (The New York Hospital, Cornell University Medical College, New York, NY). The normal kidney epithelial (NKE) cell line was established from the uninvolved kidney tissue of a patient with RCC and immortalized by transfection with the gene for telomerase (obtained from Dr. A.V. Gudkov of The Cleveland Clinic Foundation, Cleveland, OH). Tissue from primary lesions of RCC and glioblastoma (GBM) were provided by the Cooperative Human Tissue Network. Informed consent was obtained from all patients with localized disease. Tissues were digested and primary RCC and GBM cells were allowed to adhere overnight and the adherent cells were maintained in complete RPMI 1640 (RCC) or DMEM (GBM) and allowed to reach confluence before use.

Tumor supernatants freed from cellular components were used in 1:1 ratio with RPMI 1640 to study the effect of tumor supernatant on T cells in the absence or presence of 10 μmol/L curcumin. To study the role of TNF-α exposure and inhibition of NF-κB in tumor-induced T-cell killing, 1×10^6 cells were treated with neutralizing TNF-α antibody (2 μg/mL; Santa Cruz Biotechnology, Santa Cruz, CA) and/or NF-κB inhibitor SN50 (50 ng/mL; Calbiochem, Minneapolis, MN) and/or recombinant TNF-α (10 ng/mL; R&D Systems, La Zola, CA). In separate experiments, to test the role of preexposure to oxidative stress in NF-κB inhibition, T cells were treated with low dose of H_2O_2 (100 μmol/L) for longer time (12 h) to induce oxidative stress followed by 10 ng/mL TNF-α (26), and after 24 h, cells were harvested for further experiments.

Plasmids, small interfering RNA, and transfections. The cDNA encoding p65 subunit of human NF-κB, IκBα, IκBα-32A/36A [IκBα super-repressor (IκBα-SR), kind gift from Dr. J. Didonato, The Cleveland Clinic Foundation], TNFR1, and TNF-α was subcloned into the pcDNA3.1 vector (Invitrogen, Carlsbad, CA). The resulting HA-NF-κB, HA-IκBα, HA-IκBα-SR, and HA-TNFR1-pcDNA3.1 plasmids (4 μg each/million cells) were introduced separately into isolated T cells using T-cell nucleofector kit (Amaxa, Koein, Germany). TNF-α-pcDNA3.1 plasmid (2 μg/million cells) was added to SK-RC-45 cell lines for transfection of stable cell lines with LipofectAMINE 2000 (Invitrogen). Isolation of stably expressing clones were obtained by limiting dilution and selection with G418 sulphate (Cellgro, Kansas City, MO) at a concentration of 1 μg/mL and cells surviving this treatment were cloned and assessed for NF-κB, IκBα, IκBα-SR, TNFR1, and TNF-α expression by immunofluorescence and Western blot analysis. Ehrlich's ascites carcinoma (EAC), SK-RC-26B (RCC), and CCF-52 (GBM) cells were transfected with 300 pmol TNF-α/control double-stranded small interfering RNA (ds-siRNA; active 5′-GACAACCAACUAGUGGUGCdTdT-3′ or inactive 5′-GACAACCAGGGCGUGGUGC-dTdT-3′; Dharmacon, Lafayette, CO) and LipofectAMINE 2000 separately for 24 h. TNF-α expression in mRNA as well as protein level were estimated by reverse transcription-PCR, Western blotting, and quantitative immunofluorescence.

Flow cytometry. For the determination of cell death, T cells were stained with propidium iodide (PI) and Annexin V-FITC and analyzed on flow cytometer (FACSCalibur, Becton Dickinson, Mountain View, CA), equipped with 488 nm argon laser light source, 515 nm bandpass filter for FITC fluorescence, and 623 nm bandpass filter for PI fluorescence, using CellQuest software (23). For the determination of cell surface TNFR1 expression, thymic T cells were incubated with FITC-conjugated TNFR1 antibody or isotype-matched IgG (2 μg/mL; BD PharMingen, San Diego, CA) followed by flow cytometric analysis. For determination of intracellular TNF-α level, tumor cells were incubated with phorbol 12-myristate 13-acetate (10 ng/mL), ionomycin (1 μmol/L), and brefeldin A (10 μg/mL; Sigma) for 4 h. Cells were fixed, labeled with FITC-conjugated TNF-α (2 μg/mL; BD PharMingen), and analyzed on flow cytometer. For the measurement of intracellular ROS, thymic T cells were incubated with 10 μmol/L 5,6-carboxy-2′,7′-dichlorofluorescein-diacetate (CM-H_2DCFDA; Molecular Probes, Carlsbad, CA) for 1 h. Cells were analyzed flow cytometrically for DCFDA fluorescence.

Western blotting and electrophoretic mobility shift assay. Nuclear and cytosolic fractions as well as whole-cell lysates were prepared as described (23–25). The protein of interest was visualized by direct Western blot analysis. For the determination of direct interaction between two proteins, IKKα/IKKβ–associated phospho-IκBα or TNFR-associated protein death domain (TRADD)–associated Fas-associated protein death domain (FADD)/caspase-8 was immunopurified from cell lysates with anti-TRADD/IKKα/IKKβ antibodies (Santa Cruz Biotechnology) and protein A-Sepharose beads. The immunopurified protein was detected by Western blot using specific antibodies. Equal protein loading was confirmed by reprobing the blots with α-actin/histone H1 antibody (Santa Cruz Biotechnology; ref. 23). For electrophoretic mobility shift assay (EMSA), double-stranded NF-κB nuclear binding consensus oligonucleotides (5′-AGTTGAGGGGACTTTCCCAGGC-3′) were end labeled by T_4-polynucleotide kinase. Nuclear protein-DNA complexes were separated by 4% PAGE. Twenty-fold unlabeled oligonucleotide competitor was used to confirm specific protein-DNA binding (25). The gel was dried and exposed to a PhosphorImager screen (Bio-Rad, Hercules, CA).

ELISA, histology, and enzyme assay. TNF-α levels were quantified using ELISA kit (BD PharMingen). For histology sections, similar thymic lobes were fixed in formalin, embedded in paraffin, cut in 5-μm sections, and stained with H&E. In-gel (nondenaturing PAGE) enzyme assay was used to determine the activities of superoxide dismutases (SOD) and catalase. The achromatic band intensities indicated the enzyme activities (27).

Statistical analysis. Values are shown as SE, except otherwise indicated. Data were analyzed and, when appropriate, significance ($P < 0.05$) of the differences between mean values was determined by a Student's t test.

Results

Tumor induces disruption of thymic integrity. Thymus of tumor-bearing mice was reduced in size and showed severe hypocellularity compared with its normal counterparts as was evident from total cell counts (Fig. 1*A*). Histologic pattern of thymus from tumor-bearing mice revealed a severe disintegration of thymic architecture with massive depletion of cortical region and disappearance of corticomedullary junctions. Curcumin given at a dose of 50 mg/kg body weight (the optimum dose with least toxicity and maximum tumoricidal activity; ref. 24) restored thymic cellularity along with improved thymic architectural integrity (Fig. 1*B*). Higher doses of curcumin not only failed to protect thymus from tumor insult but also caused immunotoxicity to normal mice where as lower doses were ineffective. Similarly, a significant decrease in $CD3^+$ T-cell population was also observed in peripheral blood of tumor bearer that could be restored back to normal level by curcumin (Fig. 1*A*). Our flow cytometry data also revealed that in tumor-bearing mice, the number of Annexin V/PI–positive cells was significantly increased ($P < 0.005$) in T cells, indicating increased cellular death. Administration of curcumin to tumor-bearing animals reduced the percentage of cell death in this organ (Fig. 1*C*). All these results suggested that curcumin has immunoprotective ability during carcinogenesis.

Tumor induces perturbation of T-cell NF-κB activity. NF-κB plays an important role in maintaining the integrity of thymus (28, 29). Our observations that tumor induced thymus atrophy prompted us to investigate NF-κB status in this organ. Western blot analysis with p65 and p50 NF-κB antibodies showed severe impairment in NF-κB nuclear translocation and activity in thymus of tumor bearers (Fig. 2*A*). Gel shift assay with NF-κB oligoprobe supported the immunoblot data because tumor-induced reduction in DNA-binding activity of thymic nuclear extract also indicated perturbation in NF-κB nuclear translocation (Fig. 2*B*). It is known that IκBα inhibits NF-κB nuclear translocation and proteosomal degradation of IκBα leads to NF-κB activation. In our assay system, we observed that the level of IκBα in the cytosolic fraction of thymic T cells was increased in tumor-bearing mice compared with its normal counterpart, indicating blockage in IκBα degradation leading to inhibition of NF-κB activity. The phosphorylation status of IκBα was also impaired in these cells by growing tumor (Fig. 2*A*). Among two IκBα kinases (i.e., IKKα and IKKβ), IKKβ phosphorylation as well as its association with phospho-IκBα were more reduced in T cells of tumor-bearing mice (Fig. 2*B*). All these results indicate that decreased IKK activity can be the cause behind tumor-induced NF-κB perturbation. Interestingly, although optimum dose of curcumin had little or no effect on basal NF-κB activity in normal thymus, it restored nuclear translocation as well as DNA-binding activity of NF-κB in the thymus of tumor-bearing mice (Fig. 2*A* and *C*). In this experiment, higher doses of curcumin failed to improve NF-κB activity in T cells of tumor bearers while inhibiting the same in normal T cells, indicating the existence of a dose window in curcumin-induced NF-κB regulation (data not shown). All these results restricted us to use 50 mg/kg body weight dose for further studies. We also observed that curcumin normalized the level of IκBα, phospho-IκBα, and IKK activity (Fig. 2*A* and *B*), thus improving the nuclear translocation of NF-κB compared with untreated tumor bearers where increased level of IκBα inhibits NF-κB activity. Similar results were obtained when thymic T cells were exposed to cell-free tumor supernatants *in vitro* (data not shown).

Tumor-induced oxidative stress neutralization in T cell by curcumin. To further understand the mechanism by which

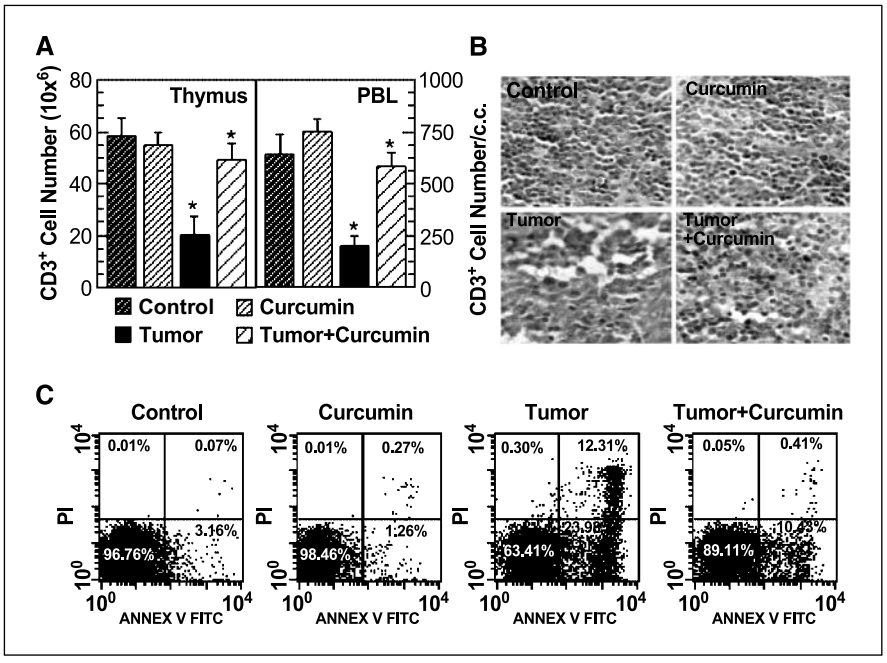

Figure 1. Curcumin protects thymic atrophy in tumor-bearing mice. *A,* $CD3^+$ T cells from thymus and peripheral blood (*PBL*) and from untreated or curcumin-treated normal and tumor-bearing mice were counted. *Columns,* mean of five independent experiments; *bars,* SE. *B,* sections of similar thymic lobes stained with H&E. Magnification, ×400. *C,* thymic T cells labeled with Annexin V-FITC and PI were analyzed flow cytometrically. *Dot plot,* Annexin V-FITC fluorescence (*X axis*) versus PI fluorescence (*Y axis*). Annexin V/PI–positive cells were regarded as apoptotic cells. *, $P < 0.005$ for control versus tumor and tumor versus tumor + curcumin.

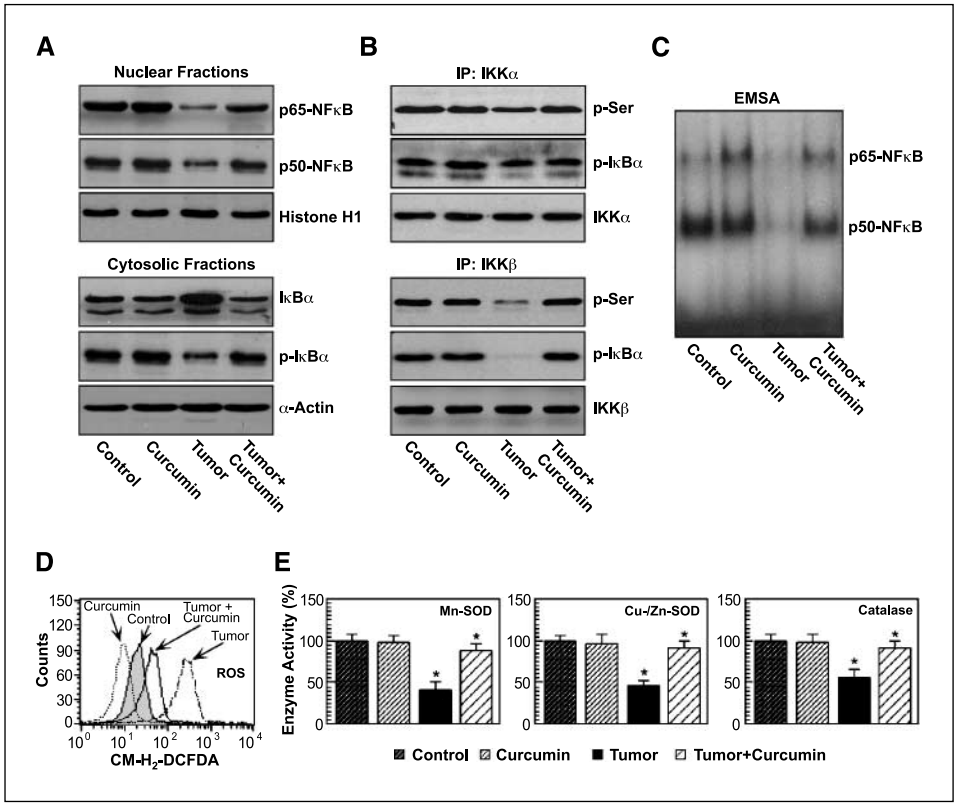

Figure 2. Curcumin prevents tumor-induced inhibition of NF-κB nuclear translocation and neutralizes oxidative stress in thymic T cells. Thymic T cells from normal and tumor-bearing mice (±curcumin) were harvested. *A,* nuclear (for p50 and p65 NF-κB) or cytosolic [for IκBα and phospho-IκBα (*p-IκBα*)] fractions from T cells were Western blotted using specific antibodies. *B,* cell lysates were immunoprecipitated (*IP*) with anti-IKKα or anti-IKKβ antibodies and Western blotted with antibodies against phospho-serine (*p-Ser*; Sigma), phospho-IκBα, IKKα, and IKKβ. *C,* nuclear extracts were subjected to EMSA analysis using NF-κB DNA-binding sequence. *D,* intracellular ROS in T cells was measured by DCFDA-dependent flow cytometry. *E,* cytosolic fractions of T cells were subjected to in-gel enzyme assay for Mn and Cu/Zn SODs and catalase. *, $P < 0.005$ for control versus tumor and tumor versus tumor + curcumin.

curcumin restores NF-κB activity, we investigated the status of ROS in thymic T cells of tumor-bearing mice, as reports indicate that increased and chronic oxidative stress can deactivate or perturb NF-κB activity (13). Flow cytometric study of CM-H_2DCFDA–loaded thymic T cells revealed significant increase in DCFDA fluorescence (Fig. 2*D*), indicative of the increased oxidative stress in thymic T cells due to tumor burden. Curcumin was found to reduce the oxidative stress rendered by tumor. Thymic T cells of the tumor bearer also showed decrease in the activities of two vital antioxidant enzymes (i.e., catalase and SOD), and curcumin was found to restore these depressed antioxidant enzymes (Fig. 2*E*). When isolated T cells were challenged with tumor supernatant, curcumin was found to lessen elevated ROS production and to improve NF-κB activity *in vitro* (data not shown). All these results indicate that curcumin acts as an antioxidant to neutralize chronic oxidative stress incurred by growing tumor in the thymus and thus protects NF-κB activity.

Role of TNF-α/TNFR1 in tumor-induced T-cell death. Most of the cells are protected from TNF-α cytotoxicity, presumably by NF-κB–mediated induction of protective genes (30, 31), and TNF-α itself can induce NF-κB activation via IKK pathway (18). Because our data indicated impairment in NF-κB nuclear translocation and activity in thymus of tumor bearers, we next aimed at investigating the status of TNF-α and its receptor in our assay system. Our flow cytometric and immunoblotting data revealed that there was an up-regulation of TNFR1 on the surface of thymic T cell of tumor bearer that could be normalized to basal level after curcumin treatment (Fig. 3*A, left*). It was also found that tumor insult elevated TNF-α level significantly ($P < 0.001$) in serum (Fig. 3*A, middle*) through the production of high level of TNF-α by the tumor (Fig. 3*A, right*). The decrease in TNF-α level in serum might be due to the decreased production and secretion of TNF-α by the tumor cells on curcumin treatment because the levels of TNF-α in ascites fluid and in tumor cells were decreased to normal level by curcumin (Fig. 3*A*).

It is recognized that caspase-8 cascades play important role in TNFR1-mediated apoptosis (1). We, thus, next intended to find the role of this protease in tumor-induced thymic T-cell apoptosis. Caspase-8 activity, as was evident from the processing of procaspase-8, was increased in thymic T cells of tumor-bearing mice, whereas the level of caspase-8 inhibitory protein FLIP (both long and short form) was decreased in T cells (Fig. 3*B*). Thymic T cells from tumor-bearing animals also showed increased amount of TRADD-associated FADD and caspase-8 (Fig. 3*B*), suggesting that the activation of caspase-8 was due to the switching on of TNF-α–mediated apoptotic signaling. Curcumin treatment blocked TNF-α secretion, normalized TNFR1 expression, inhibited caspase-8 activation as well as amount of TRADD-associated caspase-8, reduced FADD recruitment to TRADD, and also improved FLIP levels, thereby protecting thymic T cells from tumor-induced death (Fig. 3*A* and *B*).

Our results showed that tumor-induced T-cell death and secreted high level of TNF-α. Moreover, it is known that increased TNF-α exposure can make T cell susceptible to apoptotic onslaught (1). We therefore next attempted to correlate these phenomena. To test our assertion, we first neutralized TNF-α in tumor supernatant with antibody and then used those supernatants to estimate their T-cell killing activity. Results of these experiments (Fig. 3*C, left*) supported our notion because TNF-α antibody dramatically decreased tumor-induced killing of T cells. Such inhibition of killing was consistent with curcumin-mediated inhibition, thereby raising the possibility that curcumin might be blocking tumor-induced

T-cell killing by down-regulating TNF-α production. These results were further confirmed by TNF-α-siRNA transfection experiments. When EAC, RCC, or GBM cells were transfected with ds-siRNA against TNF-α, the cell-free supernatant of these tumor cells failed to induce substantial T-cell killing compared with control ds-siRNA–transfected tumor cell supernatants (Fig. 3*C, right*). Our immunoblotting data further suggested that ds-siRNA could efficiently and specifically knocked down TNF-α in those cells. Interestingly, we observed that ascitically grown sarcoma-180 cells that secrete low level of TNF-α failed to cause severe thymic atrophy (59×10^6 cells in normal versus 45×10^6 cells) compared with high-TNF-α–secreting ascites tumor (59×10^6 cells in normal versus 20×10^6 cells), thereby further strengthening the role of TNF-α in tumor-induced thymic demise.

To reestablish that curcumin improves thymic integrity and protects T cells via down-regulation of TNF-α/TNFR1, we over-expressed TNFR1 in T cells (Fig. 3*D*) and challenged those cells with tumor supernatant. It was found that these cells became more susceptible to supernatant-induced apoptosis than the control vector-expressing cells (Fig. 3*D, left*). Interestingly, human primary tumor cells (RCC and GBM) also showed similar pattern, indicating the fact that observed results are not exclusive for murine tumor

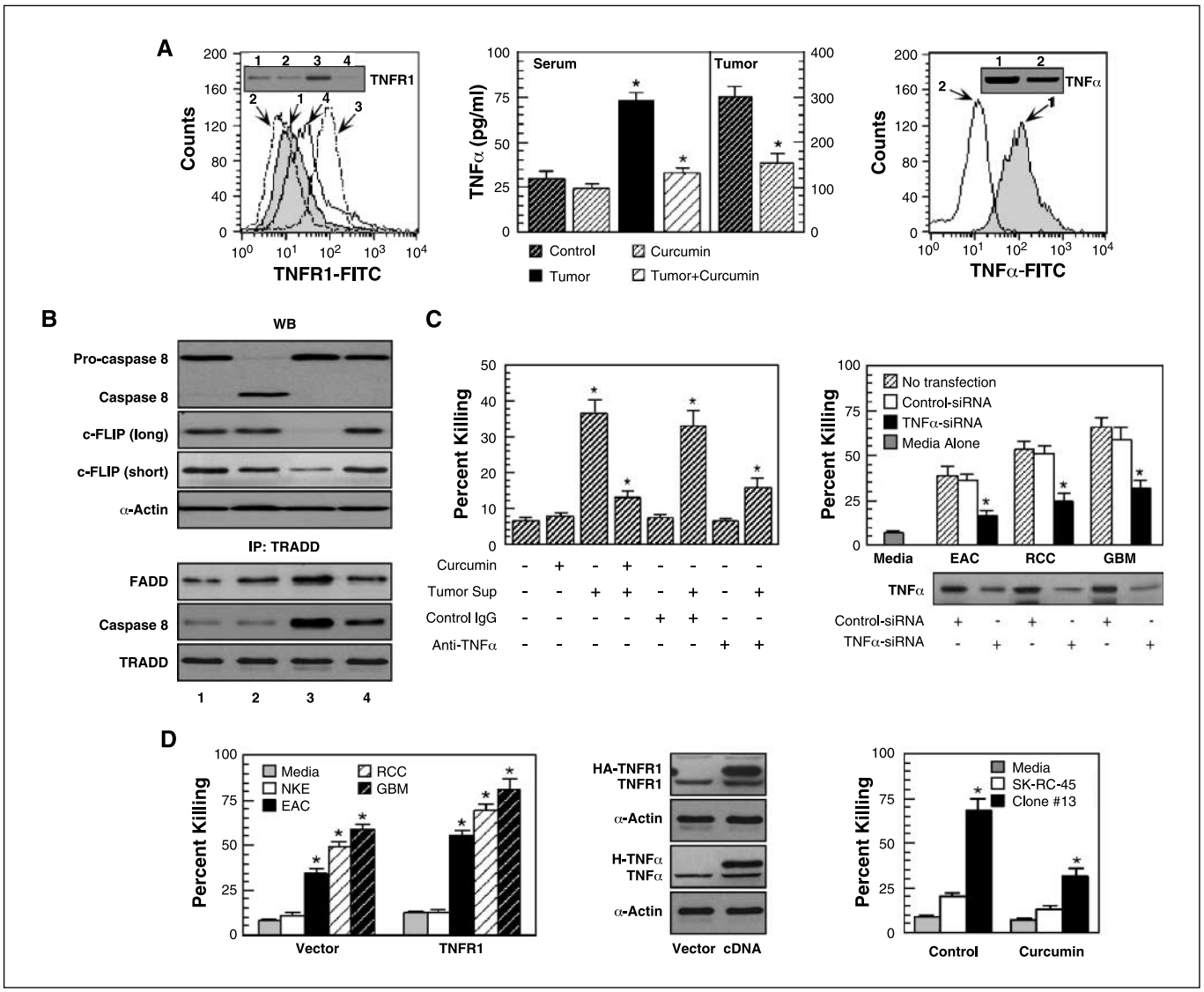

Figure 3. Inhibition of TNF-α exposure confers protection to T cell from tumor assault. Thymus from normal and tumor-bearing mice (±curcumin) was harvested. *A, left,* flow cytometric determination of TNFR1 expression on thymic T cells surface. *Inset,* immunoblotting of TNFR1 (T cells from *1*, normal mice; *2*, curcumin-treated mice; *3*, tumor-bearing mice; *4*, curcumin-treated tumor-bearing mice). *Middle,* TNF-α levels in serum and cell-free tumor supernatant were determined by ELISA. *Right,* intracellular TNF-α levels in tumor cells were determined by flow cytometry and immunoblotting. *Inset, 1,* untreated tumor cells; *2,* curcumin-treated tumor cells. *B, top,* T-cell lysates were Western blotted for the determination of caspase-8 activation and cFLIP level; *bottom,* TRADD-associated FADD and caspase-8 was detected by coimmunoprecipitation and Western blotting. *C, left,* T cells were treated with tumor supernatant in the presence or absence of neutralizing TNF-α antibody or curcumin. Percentage cell death was determined flow cytometrically. *Right,* EAC, RCC, or GBM cells were transfected with control or TNF-α-siRNA and tumor supernatants were incubated with T cells to determine percentage cell death. *Bottom,* immunobloting of TNF-α expression in siRNA-transfected cells. *D, left,* Tumor (EAC/RCC/GBM) or NKE supernatants were incubated with empty vectors or TNFR1-transfected T cells to determine percentage cells death; *middle,* immunoblotting of TNFR1/TNF-α expression in vector or TNFR1/TNF-α–transfected cells; *right,* normal T cells were incubated with SK-RC-45 or TNF-α–overexpressing clone 13 supernatants to determine percentage cell death. *, $P < 0.005$ for control versus tumor, tumor versus tumor + curcumin, control IgG versus +anti-TNF-α, control siRNA versus TNF-α-siRNA, vector versus TNFR1, SK-RC-45 versus clone 13, and clone 13 versus clone 13 + curcumin.

systems but human tumors also exploit similar mechanism to induce T-cell death. We further experimented with RCC cell lines SK-RC-45 and its TNF-α–overexpressing SK-RC-45 clone 13; the parental cell incidentally expresses low level of TNF-α. Consistent with the TNF-α expression pattern, clone 13 induced more T-cell death, indicating the role of TNF-α in tumor-mediated T-cell death (Fig. 3*D, right*). Interestingly, curcumin administration could prevent clone 13–mediated T-cell death apparently via inhibition of TNF-α secretion from tumor cells (Fig. 3*D*). All these results suggest that tumor-mediated T-cell death mechanism involves the participation of TNF-α–induced apoptotic pathways, thus reduction in tumor TNF-α level and normalization of TNFR1 expression pattern in thymus can be one of the mechanisms behind curcumin-induced thymic protection in the tumor-bearing host.

NF-κB inhibition potentiates tumor-induced T-cell death. Our earlier *in vivo* results suggested that the mechanism of tumor-induced thymic cell death might include perturbation in thymic NF-κB activity and increased exposure to TNF-α. To reconfirm the role of NF-κB inhibition in tumor-induced thymic demise, two approaches were undertaken. In the first approach, we pretreated T cells *in vitro* with cell-permeable NF-κB inhibitor peptide SN50 and then incubated the cells with tumor supernatant. Under this NF-κB–inhibited condition, tumor supernatant could kill more T cells than that of untreated or alone SN50-treated cells (Fig. 4*A*). Interestingly, when TNF-α–neutralized supernatant was added to the NF-κB–inhibited condition, the T-cell death was decreased dramatically ($P < 0.001$). In parallel experiment, the cells were pretreated with SN50 and then challenged with recombinant TNF-α. It was found that the rate of death has gone up to 55% in the cells treated with both SN50 and TNF-α from TNF-α alone (16.9%) or SN50 alone (7.8%; Fig. 4*A*), suggesting that NF-κB plays crucial role in thymic T-cell survival from TNF-α exposure.

In the second approach, T cells were overexpressed with NF-κB or IκBα/IκBα-SR (Fig. 4*C*) and then incubated with EAC/RCC/GBM cell–free supernatants. In the first case, when T cells were overexpressed with p65-NF-κB, the cells became more resistant to tumor-induced death compared with the control vector-transfected cells (Fig. 4*B*). On the other hand, when T cells were transfected with wild-type (WT) *IκBα* gene, the cells became susceptible to tumor-induced death. When IκBα-SR (IκBα-32A/36A) was introduced into T cells, this degradation-resistant IκBα subunit rendered T cells more susceptible to tumor-induced death (Fig. 4*B*) that could not be successfully prevented by curcumin administration (data not shown). All these observations indicate that perturbation in NF-κB activity made thymic T cells susceptible to tumor/TNF-α–induced death.

Preexposure to oxidative stress renders thymic T cells susceptible to TNF-α–induced cell death. Our observation from *in vivo* results raised the possibility that chronic tumor-induced oxidative stress might be the cause behind observed decrease in NF-κB activation in T cells and these unprotected T cells might have died from increased TNF-α exposure. To prove the same, T cells were preexposed for longer time to low dose of H_2O_2, in presence or absence of curcumin, and then incubated with TNF-α. A significant ($P < 0.001$) increase in the level of Annexin V/PI–positive (dead) T cells was observed when the cells were challenged with both H_2O_2 and TNF-α compared with that of control or those treated with either H_2O_2 or

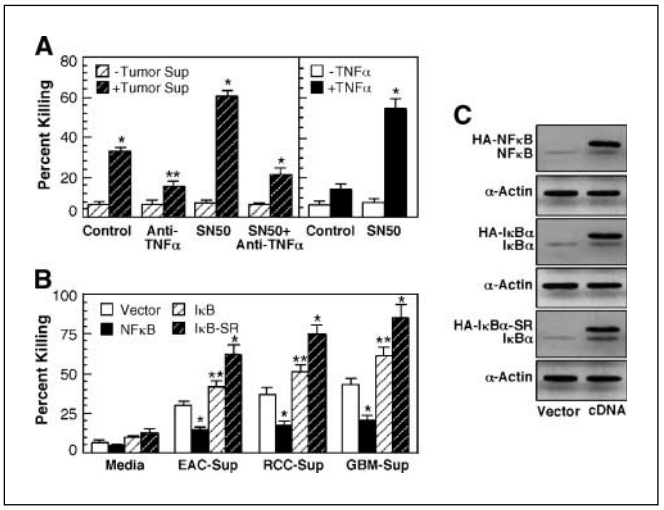

Figure 4. Tumor-induced thymic T-cell killing involves inhibition of NF-κB activity. *A,* T cells were preincubated with control vehicle or SN50 for 3 h followed by coincubation with medium alone or with tumor supernatant (*Sup*; pretreated with ±TNF-α antibody; *left*). In parallel experiments, SN50-pretreated T cells were incubated with TNF-α (*right*) followed by flow cytometric determination of percentage cell death. *B,* T cells transfected with NF-κB, IκBα, or IκBα-32A/36A (IκBα-SR) were incubated with EAC, RCC, or GBM supernatants to determine percentage cell death. *C,* Western blot representation of NF-κB and IκBα expression pattern in NF-κB–, IκBα-, and IκBα-SR–transfected T cells. *, $P < 0.005$ for tumor supernatant versus +curcumin, tumor supernatant +SN50 versus tumor supernatant +SN50+anti-TNFα, TNFα versus TNFα +SN50, and control vector versus NF-κB/IκBα-SR; **, $P < 0.05$ for tumor supernatant versus +anti-TNFα and control vector versus IκBα.

TNF-α (Fig. 5*A*). The cells that were pretreated with curcumin and H_2O_2 and then exposed to TNF-α showed lesser number of dead cells compared with those not pretreated with curcumin (Fig. 5*A*).

Impaired NF-κB nuclear translocation activity (both p65 and p50 isotypes) was also observed in the cells treated with H_2O_2 in contrast to those of control or cells treated with TNF-α or curcumin (Fig. 5*B*). When T cells were treated with TNF-α alone, there was slight induction of NF-κB activity. However, when TNF-α was applied in combination with curcumin, activity was brought back to normal level. We also observed decreased IκBα degradation in cells treated with H_2O_2 alone or in combination with TNF-α. Interestingly, curcumin could reduce IκBα retention in all the H_2O_2-treated sets (Fig. 5*B*). In fact, H_2O_2 treatment predominantly reduced phosphorylation status of IKKβ subunit in T cells that could be successfully prevented by curcumin, indicating that oxidative stress–mediated IKK inactivation can be the cause underlying increased IκBα retention (data not shown). Oxidative stress and TNF-α exposure together showed highest amount of caspase-8 activation in T cells, whereas TNF-α activated this protease moderately. Curcumin pretreatment blocked caspase-8 activation in those cells exposed to TNF-α alone or in combination with H_2O_2 (Fig. 5*B*). All these observations indicate that preexposure to oxidative stress can render T cells more susceptible to TNF-α–mediated death as it perturbs NF-κB nuclear translocation and activity via decreased IκBα degradation. Administration of curcumin helps to neutralize the oxidative stress and prevents T cells from TNF-α–mediated apoptosis, indicating that the observed protective effects of curcumin on T cells could be due to the neutralization of oxidative stress and reduced TNF-α exposure and may not be due its direct effect on physiologic immune activity.

Discussion

Our study shows the potential mechanisms by which cancer cells induce T-cell apoptosis. Developing tumor secreted high level of TNF-α as well as induced oxidative stress that increased cytosolic IκBα retention and inhibited NF-κB nuclear translocation in T cells. Perturbation in NF-κB distribution promoted TNF-α–mediated T-cell apoptosis through TRADD-associated caspase-8 activation resulting in thymic atrophy. Tumor growth-associated changes in thymic architecture and cellularity suggest that tumor cells target thymus possibly as a mechanism to diminish antitumor immune response (32). Curcumin neutralized such tumor-induced oxidative stress, restored back NF-κB activity, and inhibited TNF-α production, thereby minimizing tumor-induced T-cell apoptosis. All these observations point toward a cross-talk between NF-κB-ROS-TNF-α in deciding the fate of T cells in tumor microenvironment and the intervening role of curcumin.

It is well recognized that NF-κB/Rel plays crucial role in immune system by controlling many cytokine genes and responding to various signals required for immune cell survival (1, 33, 34). NF-κB not only antagonizes TNF-α–induced programmed cell death (35) but also favors antiapoptotic TNF-α signaling (36). Interestingly, two cellular responses to TNF-α have been well documented, the induction of cell death through caspase cascade and the activation of gene transcription for cell survival via activation of NF-κB (18, 19), although apoptogenic activity of this cytokine also associates with a block in NF-κB–mediated cell survival signals (37). NF-κB also blunts ROS accumulation, which themselves are pivotal elements for TNF-α–induced apoptosis, whereas chronic exposure to oxidative stress inhibits NF-κB nuclear translocation activity in T cells (14, 36). On the contrary, redox regulation of NF-κB–inducing kinase is mechanistically important in cytokine induction of NF-κB activation (38) and reduction in oxidative stress was found to inhibit nuclear binding of NF-κB (39), indicating the complexity of cross-talk among TNF-α, NF-κB, and ROS. Thus, to understand the mechanisms underlying tumor-induced thymic atrophy and its protection by curcumin, we investigated the relationship between these deciding factors in our model system.

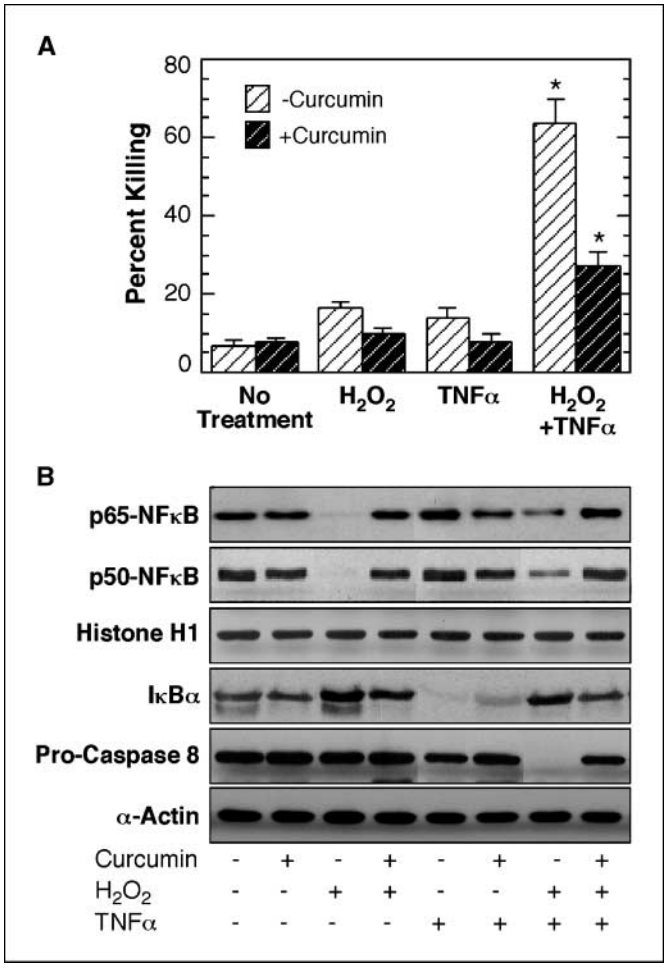

Figure 5. Preexposure to oxidative stress renders T cell more susceptible to TNF-α–mediated death. *A,* T cells, pretreated with or without curcumin, were exposed to H_2O_2 and then with TNF-α to determine percentage cell death. *B,* nuclear (for p50 and p65 NF-κB) or cytosolic (for IκBα and procaspase-8) fractions of T cells from above experimental conditions were Western blotted using specific antibodies. *, $P < 0.005$ for TNF-α versus H_2O_2 + TNF-α and H_2O_2 + TNF-α-curcumin versus H_2O_2 + TNF-α + curcumin.

Our results indicated severe impairment in NF-κB nuclear translocation in thymic T cell of tumor bearer, which might have resulted in T-cell death. Supporting our notion, p65-NF-κB–overexpressed T cells developed resistance to tumor-induced death, whereas T cells treated with cell-permeable NF-κB nuclear translocation inhibitor peptide (SN50) were vulnerable to tumor-induced death, thereby highlighting the role of NF-κB inhibition in tumor-induced thymic disruption. Next, the status of IκBα was monitored in T-cell cytosol because inhibition in proteosomal degradation of IκBα blocks NF-κB nuclear translocation and activation. In fact, dramatic increase in IκBα in thymic T-cell cytosol indicated reduced degradation of IκBα in tumor-bearing mice. To directly correlate such perturbation in NF-κB activity to tumor-induced thymic T-cell death, cells were engineered with WT IκBα or its degradation-resistant mutant IκBα-32A/36A (IκBα-SR; ref. 40). In both cases, cells became vulnerable to tumor-induced death; particularly in case of IκBα-SR, death was dramatically increased and was not successfully prevented by curcumin. There are reports showing tumor-induced block in NF-κB activation in circulatory and splenic T cells, where T cells are mature in nature (15, 16). However, this is the first report showing tumor-induced perturbation in NF-κB activity in thymus, the primary organ for T-cell development. Abnormal T-cell development and decreased thymic cellularity were reported in transgenic mice with lymphocyte-specific defect in NF-κB activation, suggesting the critical role of NF-κB in thymic development and in differentiation of $CD4^+CD8^+$ to $CD4^+$ or $CD8^+$ effector T cells (28, 29). Thus, tumor-induced perturbation in thymic NF-κB activity might be one of the causes of observed thymic atrophy. Curcumin, on the other hand, normalized IκBα status in cytosol and restored thymic NF-κB activity.

There are diverse reports about the effect of ROS in NF-κB activation (11–14, 38). In line with the growing evidences (9, 10), we observed tumor-induced increase in ROS in thymic T cells of tumor bearer with parallel inhibition in antioxidant systems. To correlate such ROS formation to NF-κB activity, oxidative stress was induced by H_2O_2 in thymic T cells. This exogenous oxidative stress also inhibited IKK activity, IκBα degradation, and NF-κB nuclear translocation. These observations are consistent with the report indicating H_2O_2-induced inhibition of NF-κB through inactivation of IKK (13) and not only suggest cell- and situation-specific effects of oxidative stress on NF-κB but also explain

why growing tumors down-regulate NF-κB in T cells. In fact, defective activation of NF-κB that affects cytokine production and functionality of T cells (1, 33) and makes it susceptible to apoptosis (30, 31) was reported in T cells from tumor-bearing mice and cancer patients (15, 16). Because many tumors secrete TNF-α that is cytocidal for thymocytes (17), next TNF-α status and its role in tumor-induced thymic T-cell apoptosis were examined. We observed high level of TNF-α in the serum and down-regulation of caspase-8 inhibitory protein FLIP in the thymic T cell of tumor bearers along with increased TNFR1 expression and TRADD-associated FADD and caspase-8 activation in thymic T cells, thereby confirming the involvement of TNF-α–mediated death pathway in thymic atrophy. To further correlate TNF-α to tumor-induced thymic regression, we treated T cells with TNF-α–depleted tumor supernatants. Interestingly, unlike untreated tumor supernatants, TNF-α–depleted tumor supernatants were unable to kill T cells effectively. In contrast, RCC variants overexpressing TNF-α instigated more T-cell death than low-TNF-α–expressing RCC, indicating that increased TNF-α exposure produced by the tumor microenvironment can make T cells susceptible to apoptosis. Thus, when TNFR1-overexpressing T cells were treated with tumor supernatant, those engineered cells became more vulnerable. Curcumin reduced serum TNF-α level and inhibited thymic T-cell TNFR1 expression, thereby preventing TRADD-mediated caspase-8 activation in these cells of tumor bearer. These findings directly correlate TNF-α status to tumor-induced thymic disruption and protective role of curcumin.

Next, to better understand the cross-talk between TNF-α and NF-κB, we treated thymic T cells with TNF-α in presence of specific NF-κB inhibitor. Results showed great enhancement in T-cell killing, indicating that impaired NF-κB activity rendered thymic T cells susceptible to TNF-α–mediated apoptosis. These findings are in line with the reports that NF-κB activation is required to counterbalance apoptotic effect of TNF-α (30, 31). Finally, to confirm directly whether oxidative stress–induced loss in NF-κB activity along with increased exposure to TNF-α are the reasons behind tumor-induced thymic T-cell death, we treated H_2O_2–preexposed thymic T cells with TNF-α and noted dramatic increase in cell death. H_2O_2 alone or in combination with TNF-α drastically inhibited IκBα degradation as well as NF-κB activation and initiated caspase-8 cascades. These results clearly show that tumor-induced ROS played an important role in blocking IκBα degradation and thereby retaining NF-κB in cytosol. Under this "NF-κB–inhibited" situation, tumor-secreted TNF-α induced T-cell apoptosis, leading to thymic atrophy. Curcumin, a known antioxidant (23, 41), at its physiologic dose counteracted these entire phenomena efficiently.

It is important to discuss in detail that in contrary to our findings, many existing reports have shown NF-κB inhibitory action of curcumin (42, 43). These reports as well as our results led us to hypothesize that curcumin may not be a universal "inhibitor" or "activator" of NF-κB activity but maintains the normal status of this important transcription factor. To prove our hypothesis, we undertook following approach. When normal T cells were treated with TNF-α, there was induction of NF-κB activity. However, when TNF-α was applied in combination with curcumin, activity was brought back almost to the normal level (Fig. 5*B*). These observations are consistent with other reports showing inhibition of stimulus-induced NF-κB activity by curcumin. In case of cancer cells, for example, where NF-κB activity is highly increased compared with normal (44), curcumin inhibits the same (42, 43). Inhibition in NF-κB activity in normal T cells at higher doses indicated the existence of an optimum dose beyond which curcumin perturbs NF-κB activity. We also observed curcumin-induced decrease in NF-κB nuclear translocation in RCC cells, in which NF-κB activity was much higher than its normal counterpart.[3] Even in normal dendritic and splenic cells, when NF-κB activity is increased/induced, curcumin brings back the level almost to normal one (45, 46). So, the effect of curcumin on NF-κB therefore may not be a universal phenomenon but might be dose specific, cell specific, and depends on the microenvironment of the concerned cell. On the other hand, in our model, because tumor-induced oxidative stress inhibited NF-κB activity in thymic T cell, curcumin released the inhibition and brought back the activity to the normal level by neutralizing the oxidative stress. Further support to our hypothesis came from the report that in untreated normal peripheral blood lymphocytes, NF-κB status remained unchanged up on curcumin treatment (47). Even in our system, optimum dose of curcumin failed to alter NF-κB activity in thymic T cells of normal mice. After analyzing all our results in the light of other reports, we came to the conclusion that the observed augmentation in thymic NF-κB activity by curcumin may be due to its antioxidant property that frees NF-κB from inhibitory effects of tumor-induced ROS. We have already reported that curcumin can successfully reduce tumor load *in vivo* (24, 48) in a dose-dependent manner. Taken together, antioxidant property of curcumin along with its role in reducing TNF-α level as well as tumor load may be the possible mechanisms behind curcumin-induced thymic protection.

Acknowledgments

Received 7/13/2006; revised 9/28/2006; accepted 11/2/2006.

Grant support: Department of Science and Technology and Council of Scientific and Industrial Research, Government of India grants.

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We thank Drs. J. Didonato and A.V. Gudkov for NF-κB and IκBα clones and NKE cells, Dr. N. Bander for various RCC cells, and P. Raymen and U. Ghosh for technical help.

[3] Unpublished data.

References

1. Dijkstra G, Moshage H, Jansen LM. Blockade of NF-κB activation and donation of nitric oxide: new treatment options in inflammatory bowel disease? Scand J Gastroenterol Suppl 2002;236:37–41.
2. Gómez J, Domingo DG, Martínez-AC, Rebollo A. Role of NF-KB in the control of apoptotic and proliferative responses in IL-2-responsive T cells. Front Biosci 1997;2:d49–60.
3. Karin M, Lin A. NF-KB at the cross roads of life and death. Nat Immunol 2002;3:221–7.
4. Pahl HL. Activators and target genes of Rel/NFκB transcription factors. Oncogene 1999;18:6853–66.
5. Karin M, Ben-Neriah Y. Phosphorylation meets ubiquitination: the control of NFκB activity. Annu Rev Immunol 2000;18:621–63.
6. Alcamo E, Mizgerd JP, Horwitz BH, et al. Targeted mutation of TNF receptor I rescues the RelA-deficient mouse and reveals a critical role for NF-κB in leukocyte recruitment. J Immunol 2001;167:1592–600.
7. Jeremias I, Kupatt C, Baumann B, Herr I, Wirth T, Debatin KM. Inhibition of nuclear factor κB activation attenuates apoptosis resistance in lymphoid cells. Blood 1998;91:4624–31.
8. Shiku H. Importance of $CD4^+$ helper T-cells in anti-tumor immunity. Int J Hematol 2003;77:435–8.
9. Yangxin FU, Paul RD, Wang Y, Lopez DM. Thymic involution and phenotypic alterations induced by

murine mammary adenocarcinomas. J Immunol 1989; 143:4300–7.
10. Shankar A, Singh SM, Sodhi A. Ascitic growth of a spontaneous transplantable T cell lymphoma induces thymic involution. Alterations in the CD4/CD8 distribution in thymocytes. Tumour Biol 2000;21:288–98.
11. Mellqvist UH, Hansson M, Brune M, Dahlgren C, Hermodsson S, Hellstrand K. Natural killer cell dysfunction and apoptosis induced by chronic myelogenous leukemia cells: role of reactive oxygen species and regulation by histamine. Blood 2000;96:1961–8.
12. Kono K, Salazar-Onfray F, Petersson M, et al. Hydrogen peroxide secreted by tumor-derived macrophages down-modulates signal-transducing ζ molecules and inhibits tumor-specific T cell-and natural killer cell-mediated cytotoxicity. Eur J Immunol 1996;26:1308–13.
13. Flohe ÂL. Brigelius-Flohe ÂR, Saliou C, Traber MG, Packer L. Redox regulation of NF-κB activation. Free Radic Biol Med 1997;22:1115–26.
14. Bowie A, O'Neill LA. Oxidative stress and nuclear factor κB activation: a reassessment of the evidence in the light of recent discoveries. Biochem Pharmacol 2000; 59:13–23.
15. Hayakawa M, Miyashita H, Sakamoto I, et al. Evidence that reactive oxygen species do not mediate NF-κB activation. EMBO J 2003;22:3356–66.
16. Korn SH, Wouters EF, Vos N, Janssen-Heininger YM. Cytokine-induced activation of nuclear factor-κ B is inhibited by hydrogen peroxide through oxidative inactivation of IκB kinase. J Biol Chem 2001;276: 35693–700.
17. Malmberg KJ, Arulampalam V, Ichihara F, et al. Inhibition of activated/memory ($CD45RO^+$) T cells by oxidative stress associated with block of NFκB activation. J Immunol 2001;167:2595–601.
18. Ghosh P, Sica A, Young HA, et al. Alterations in NFκB/Rel family proteins in splenic T-cells from tumor-bearing mice and reversal following therapy. Cancer Res 1994;54:2969–72.
19. Ling W, Rayman P, Uzzo RG, et al. Impaired activation of NFκB in T cells from a subset of renal cell carcinoma patients is mediated by inhibition of phosphorylation and degradation of the inhibitor IκB. Blood 1998;92:1334–41.
20. Stuelten CH, Byfield SD, Arany PR, Karpova TS, Stetler-Stevenson WG, Roberts AB. Breast cancer cells induce stromal fibroblasts to express MMP-9 via secretion of TNF-α and TGF-β. J Cell Sci 2005;118: 2143–53.
21. Varfolomeev EE, Ashkenazi A. Tumor necrosis factor: an apoptosis JuNKie? Cell 2004;104:491–7.
22. Tang G, Minemoto Y, Dibling B, et al. Inhibition of JNK activation through NF-κB target genes. Nature 2001; 414:265–6.
23. Choudhuri T, Pal S, Das T, Sa G. Curcumin selectively induces apoptosis in deregulated cyclin D1-expressed cells at G_2 phase of cell cycle in a p53-dependent manner. J Biol Chem 2005;280:20059–68.
24. Pal S, Choudhuri T, Chattopadhyay S, et al. Mechanisms of curcumin-induced apoptosis of Ehrlich's ascites carcinoma cells. Biochem Biophys Res Commun 2001;288:58–65.
25. Choudhuri T, Pal S, Agwarwal ML, Das T, Sa G. Curcumin induces apoptosis in human breast cancer cells through p53-dependent Bax induction. FEBS Lett 2002;512:334–40.
26. Liu H, Ma Y, Pagliari LJ, et al. TNFα induced apoptosis of macrophages following inhibition of NFκB: B: a central role for disruption of mitochondria. J Immunol 2004;160:1354–8.
27. Carter AB, Tephly LA, Venkataraman S, et al. High Levels of catalase and glutathione peroxidase activity dampen H_2O_2 signaling in human alveolar macrophages. Am J Respir Cell Mol Biol 2004;31:43–53.
28. Esslinger CW, Wilson A, Sordat B, Beermann F, Jongeneel CV. Abnormal T lymphocyte development induced by targeted overexpression of IκBα. J Immunol 1997;158:5075–8.
29. Hettmann T, Leiden JM. NFκB is required for the positive selection of $CD8^+$ thymocytes. J Immunol 2000; 165:5004–10.
30. Senftleben U, Li ZW, Baud V, Karin M. IKKβ is essential for protecting T cell from TNFα-induced apoptosis. Immunity 2001;14:217–30.
31. Beg A, Baltimore D. An essential role of NFκB in preventing TNFα induced cell death. Science 1996;274: 782–4.
32. Lopez DM, Charyulu V, Adkins B. Influence of breast cancer on thymic function in mice. J Mammary Gland Biol Neoplasia 2002;7:191–9.
33. Baeuerle PA, Henkel T. Function and activation of NF-κB in the immune system. Annu Rev Immunol 1994; 12:141–79.
34. Mercurio F, Manning AM. NF-κB as a primary regulator of the stress response. Oncogene 1999;18:6163–71.
35. Bubici C, Papa S, Pham CG, Zazzeroni F, Franzoso G. The NF-κB-mediated control of ROS and JNK signaling. Histol Histopathol 2006;21:69–80.
36. Feuerhake F, Kutok JL, Monti S, et al. NFκB activity, function, and target-gene signatures in primary mediastinal large B-cell lymphoma and diffuse large B-cell lymphoma subtypes. Blood 2005;106:1392–9.
37. Butt AJ, Dickson KA, Jambazov S, Baxter RC. Enhancement of tumor necrosis factor-α-induced growth inhibition by insulin-like growth factor-binding protein-5 (IGFBP-5), but not IGFBP-3 in human breast cancer cells. Endocrinology 2005;146: 3113–22.
38. Li Q, Engelhardt JF. Interleukin-1β induction of NFκB is partially regulated by H_2O_2-mediated activation of NFκB-inducing kinase. J Biol Chem 2006;281: 1495–505.
39. Shapiro H, Ashkenazi M, Weizman N, Shahmurov M, Aeed H, Bruck R. Curcumin ameliorates acute thioacetamide-induced hepatotoxicity. J Gastroenterol Hepatol 2006;21:358–66.
40. Jobin C, Panja A, Hellerbrand C, et al. Inhibition of proinflammatory molecule production by adenovirus-mediated expression of a nuclear factor κB super-repressor in human intestinal epithelial cells. J Immunol 1998;160:410–8.
41. Campbell FC, Collet GP. Chemopreventive properties of curcumin. Future Oncol 2005;1:405–14.
42. Deeb D, Jiang H, Gao X, et al. Curcumin sensitizes prostate cancer cells to tumor necrosis factor-related apoptosis-inducing ligand/Apo2L by inhibiting nuclear factor-κB through suppression of IκBα phosphorylation. Mol Cancer Ther 2004;3:803–12.
43. Bharti AC, Donato N, Singh S, Aggarwal BB. Curcumin (diferuloylmethane) down-regulates the constitutive activation of nuclear factor-κB and IκBα kinase in human multiple myeloma cells, leading to suppression of proliferation and induction of apoptosis. Blood 2003;101:1053–62.
44. Rayet B, Gelinas C. Aberrant rel/nfkb genes and activity in human cancer. Oncogene 1999;18:6938–47.
45. Kim JY, Kim KH, Lee SH, et al. Curcumin inhibits immunostimulatory function of dendritic cells: MAPKs and translocation of NF-κB as potential targets. J Immunol 2005;174:8116–24.
46. Ranjan Dl, Chen Cl, Johnston TD, Jeon Hl, Nagabhushan M. Curcumin inhibits mitogen stimulated lymphocyte proliferation, NFκB activation, and IL-2 signaling. J Surg Res 2004;121:171–7.
47. Gertsch J, Guttinger M, Heilmann J, Sticher O. Curcumin differentially modulates mRNA profiles in Jurkat T and human peripheral blood mononuclear cells. Bioorg Med Chem 2003;20:1057–63.
48. Pal S, Bhattacharyya S, Choudhuri T, Datta GK, Das T, Sa G. Amelioration of immune cell number depletion and potentiation of depressed detoxification system of tumor-bearing mice by curcumin. Cancer Detect Prev 2005;29:470–8.