Neutralization of Tumor Necrosis Factor Bioactivity Ameliorates Urethane-Induced Pulmonary Oncogenesis in Mice


*Applied Biomedical Research & Training Center “Marianthi Simou,” Department of Critical Care & Pulmonary Services, General Hospital “Evangelismos,” School of Medicine, National and Kapodistrian University of Athens, Athens, Greece; †Department of Physiology, School of Medicine, University of Patras, Rio, Greece

Abstract

Tumor necrosis factor (TNF) has been implicated in inflammation-associated tumor progression. Although multiple reports identified a role for TNF signaling in established cancers, few studies have assessed the impact of TNF blockade on early tumor formation promotion. We aimed at exploring the effects of TNF neutralization in a preclinical mouse model of lung carcinogenesis. For this, Balb/c mice (n = 42) received four weekly intraperitoneal urethane injections (1 g/kg) and twice-weekly intraperitoneal soluble TNF receptor (etanercept; 10 mg/kg) administered during tumor initiation/promotion, tumor progression, or continuously (months 1, 6, and 1-8 after urethane start, respectively). Lung oncogenesis was assessed after 8 months. In separate short-term studies, Balb/c mice (n = 21) received a single control or urethane injection followed by twice-weekly intraperitoneal control or sTNFR:Fc injections. Lung inflammation was assessed after 1 week. We found that sTNFR:Fc treatment during tumor initiation/promotion resulted in a significant reduction of tumor number but not dimensions. However, sTNFR:Fc administered during tumor progression did not impact tumor multiplicity but significantly decreased tumor diameter. Continued sTNFR:Fc administration was effective in halting both respiratory tumor formation and progression in response to urethane. This favorable impact was associated with impaired cellular proliferation and new vessel formation in lung tumors. In addition, TNF neutralization altered the lung inflammatory response to urethane, evidenced by reductions in TNF and macrophage and increases in interferon γ and interleukin 10 content of the air spaces. sTNFR:Fc treatment of RAW264.7 macrophages downregulated TNF and enhanced interferon γ and interleukin 10 expression. In conclusion, TNF neutralization is effective against urethane-induced lung oncogenesis in mice and could present a lung chemoprevention strategy worth testing clinically.

Neoplasia (2011) 13, 1143–1151
Introduction
Lung cancer presents a contemporary global pandemic responsible for an estimated 13% (1.6 million) of cancer cases and 18% (1.4 million) of cancer deaths worldwide in 2008 [1]. Current evidence indicates that the death toll of lung cancer is expected to rise further, especially in developing countries [2]. Moreover, adenocarcinoma, a histologic subtype of non–small cell lung carcinoma, presents the bulk of new disease and is steeply rising in incidence, in particular among women [3]. Although smoking cessation constitutes the mainstay of lung cancer prevention, most lung cancers are currently diagnosed in former smokers [4], and many cases occur in never smokers [5]. Moreover, chronic inflammatory diseases of the respiratory tract, such as chronic obstructive pulmonary disease (COPD), promote carcinogenesis independent of smoking [3,6]. These highlight the need for the development of strategies for early detection and chemoprevention of lung cancer, in addition to tobacco control [7,8].

Tumor necrosis factor (TNF), a multifunctional proinflammatory cytokine initially found to inhibit tumor growth through hemorrhagic necrosis [9], has recently been implicated as a key promoter of inflammation-associated malignant tumor progression and metastasis [10,11]. For example, TNF participates in an autocrine growth-promoting signaling network enabling the intraperitoneal spread of established ovarian cancer [12]. However, far less is known on the functions of the cytokine in early phases of carcinogenesis, such as tumor initiation and promotion. In the lungs, TNF and its receptors (TNFRs) are expressed by lung cancer cells [13,14] and TNF signals integrated by cancer cell nuclear factor (NF)–κB transcriptional activation function to accelerate tumor cell growth [11]. In a mouse model of multistage lung oncogenesis induced by the carcinogen urethane, we have previously shown that epithelial NF-κB drives both carcinogen-induced inflammation and neoplasia starting from early stages of oncogenesis, with TNF presenting a possible downstream effector of NF-κB signaling [15]. Earlier elegant work that used genetic targeting of the murine Tnf gene showed that Tnf-deficient mice are protected from urethane-induced tumorigenesis [16]. Although this evidence directly implicates TNF as an important mediator of lung tumor initiation and promotion, no effort has been undertaken to pharmacologically block TNF signaling in preclinical lung cancer models.

In the present work, we aimed at investigating the effects of TNF neutralization in a preclinical mouse model of lung carcinogenesis induced by repeated administration of the respiratory carcinogen urethane to sensitive Balb/c mice. TNF bioactivity was blocked using soluble TNF receptors (sTNFR) coupled to constant immunoglobulin fragment (Fc) during distinct time frames of tumor initiation/promotion and/or progression. Our findings indicate that this strategy is effective in ameliorating experimental chemical lung oncogenesis by favorably impacting both tumor initiation/promotion and progression.

Materials and Methods
Reagents
sTNFR:Fc (etanercept, Enbrel; Wyeth Hellas SA, Athens, Greece), a dimeric fusion protein consisting of the extracellular ligand-binding portion of the human 75-kDa TNF receptor linked to the Fc portion of human IgG1, was purchased from the pharmacy [13]. Urethane (ethyl carbamate) was from Sigma-Aldrich (St Louis, MO). 3-(4,5-Dimethylthiazol-2-yl)-5-(3-carboxymethoxyphenyl)-2-(4-sulfophenyl)-2H-tetrazolium, inner salt (MTS) assay was from Promega (Madison, WI), anti–proliferating cell nuclear antigen (PCNA) antibody from Santa Cruz Biotechnology (Santa Cruz, CA), terminal deoxynucleotidyl nick-end labeling (TUNEL) kit was from Roche Molecular Biochemicals (Penzberg, Germany), anti–factor VIII–associated protein (F8A) antibody was from Invitrogen (San Francisco, CA), and mouse cytometric bead array (CBA) assaying TNF, interferon (IFN) γ, monocyte chemotactic protein 1 (C-C motif chemokine ligand 2), interleukin 6 (IL-6), IL-10, and IL-12p70 (detection limits: 7.3, 2.5, 52.7, 5.0, 17.5, and 10.7 pg/ml, respectively) was from BD Biosciences (San Jose, CA).

Assessment of Lung Inflammation
Bronchoalveolar lavage (BAL) was performed with three aliquots of 1000 μl of sterile ice-cold PBS. Fluid was combined and centrifuged at 260g for 10 minutes to separate cells from supernatant. The cell pellet was resuspended in 1 ml of PBS with 1% bovine serum albumin, and the total cell count was determined using a grid hemocytometer. Cell differentials were obtained by counting at least 400 cells on Wright-Giemsa–stained cytospin (cytospin) (Three Step Stain Set; Richard-Allen Scientific, Kalamazoo, MI). Total cell numbers in BAL were then calculated by multiplying the percentage of each cell type by the total number of BAL cells. Inflammatory cytokines were determined in cell-free BAL supernatants by CBA as described previously [15]. Protein levels were determined using the albumin protein assay (Hercules, CA). Cytokine levels were corrected for BAL protein and given as nanograms per gram of protein.

Assessment of Lung Oncogenesis
The lungs were explanted after transtracheal inflation with 10% neutral-buffered formalin under 25 cm H₂O pressure. Lung tumors were counted by four blinded readers (S.P.K., C.A.K., I.P., and G.T.S.) under a stereomicroscope (Stemi DV4; Zeiss, Jena, Germany) using surface and transillumination to visualize intrapulmonary tumors and averaged as described previously [15,16,20]. Tumor diameter (d)
was determined using microcalipers, and tumor volume \( (V) \) was determined using the formula: \( V = \pi d^3/6 \). Total tumor burden for each mouse was calculated by adding the volumes of all tumors from the lungs of each mouse. Excised mouse lungs were fixed in 10% neutral-buffered formalin for 24 hours. Lungs were embedded in paraffin based on the lung base, and 5-μm-thick sets (10 each) of serial transverse sections were cut at three levels of the lungs (apical, median, and basal). Sections were mounted on glass slides and stained with hematoxylin and eosin. The total number and the fraction of total lung lesions consisting of each type of distinct lung lesions, including hyperplasia (atypical alveolar and bronchial epithelial hyperplasia), adenoma, and adenocarcinoma, on the sections from each lung were evaluated by two blinded readers (S.P.K. and G.T.S.), according to guidelines of the Mouse Models of Human Cancers Consortium [21]. Alternatively, tissue sections were immune-labeled for PCNA, TUNEL, and F8A, as described previously [13,15,22]. The number of immunoreactive cells in lung tumors was evaluated by two blinded readers (S.P.K. and G.T.S.) in at least five high-power visual fields of at least five different tumors. The results were averaged per mouse.

**Cellular Experiments**

Mouse lung adenocarcinoma (Lewis lung carcinoma, LLC) and bone marrow–derived macrophages (RAW264.7) were purchased from the American Type Culture Collection (Manassas, VA), and experiments were done within 6 months. The cell lines were validated by the manufacturer using the short tandem repeat method. Cells were cultured at 37°C in 5% CO₂–95% air using Dulbecco modified Eagle medium 10% fetal bovine serum supplemented with glutamine and 100 mg/l penicillin/streptomycin. For experiments, cells were plated at equal densities and incubated with PBS or various concentrations of sTNFR:Fc. Cell proliferation was determined using MTS reduction [22]. Cellular mediator elaboration was assessed using CBA of cell-free cell culture supernatants and was corrected for protein content, similar to BAL samples.

**Statistics**

All values given represent mean ± SEM. To compare variables between multiple groups, one-way analysis of variance with the Tukey post hoc tests or Kruskal-Wallis test with the Dunn post hoc tests were used for normally and not normally distributed variables, respectively. All \( P \) values are 2-tailed. \( P < .05 \) was considered significant. Statistical analyses were performed, and graphs were created using Prism Software Version 5.0 (GraphPad, La Jolla, CA).

**Results**

**Early TNF Neutralization Significantly Inhibits Tumor Initiation/Promotion Induced by Urethane in Balb/c Mice**

In initial long-term tumor control experiments designed to determine the impact of TNF neutralization on urethane-induced lung carcinogenesis, 42 Balb/c mice received four consecutive weekly i.p. injections of 1 g/kg urethane. Twice-weekly i.p. drug treatment was started immediately after the first urethane dose in four different protocols: control mice received PBS continuously for 8 months, mice enrolled in a tumor initiation/promotion trial received sTNFR:Fc during the first month and PBS thereafter, mice enrolled in a tumor progression trial received sTNFR:Fc during the sixth month and PBS before and thereafter, and mice enrolled in a prolonged prevention trial received sTNFR:Fc continuously for 8 months (Figure 1A). All mice survived to termination at 8 months and did not show signs of sickness as evidenced by body mass and overall behavior. Compared with controls (mean lung tumor number = 16.0 ± 1.6 and diameter = 1.01 ± 0.07 mm), mice of the tumor initiation/promotion trial developed lung tumors of similar size (mean diameter = 1.07 ± 0.03 mm, \( P > .05 \)), but of significantly decreased multiplicity (mean number = 9.7 ± 1.1, \( P < .05 \); Figure 2, A-C). Mean lung tumor volume and overall lung tumor burden per mouse were not affected by sTNFR:Fc treatment in the tumor initiation/promotion protocol (Figure 2, D and E), but fewer adenomas developed in this group compared with controls, with a corresponding increase in the relative abundance of less progressed preneoplastic lesions (Figure 2F). These results indicated that blockade of TNF signaling during early phases of urethane-induced lung oncogenesis is effective in limiting the development of new preneoplastic lesions in the respiratory tract of Balb/c mice.

**TNF Antagonism at Late Time Points after Urethane Significantly Inhibits Tumor Progression**

We next examined the lungs of mice enrolled in the tumor progression trial, which received sTNFR:Fc only during the sixth month after urethane start (Figure 1A). Compared with controls, these mice developed a similar number of lung tumors (mean lung tumor number = 14.3 ± 1.1, \( P > .05 \); Figure 2, A and B). However, lung tumors in this group were of markedly decreased dimensions (mean diameter = 0.71 ± 0.07 mm, \( P < .01 \)) compared with controls (Figure 2, A and C), resulting in significantly reduced mean lung tumor volume and overall lung tumor burden per mouse (Figure 2, D and E). Similar to mice enrolled in the tumor initiation/promotion trial, fewer adenomas developed in this group, with a corresponding increase in the relative abundance of hyperplastic lesions (Figure 2F). These results indicated that TNF neutralization during late phases of urethane-induced lung tumorigenesis does not impact tumor initiation/promotion but significantly retards the growth and progression of established neoplastic lesions.

**Figure 1.** Experimental setup of in vivo studies. (A) Long-term studies: 42 Balb/c mice received intraperitoneal urethane (four weekly doses of 1 g/kg) and concomitant twice-weekly intraperitoneal PBS (control) or sTNFR:Fc (10 mg/kg) during months 1, 6, or 0 to 8 after urethane start. 4EC, month of administration of four urethane (ethyl carbamate, EC) injections; \( n = \) sample size; each box represents 1 month; gray boxes indicate periods of sTNFR:Fc treatment. (B) Short-term studies: Balb/c mice received intraperitoneal PBS (control) or urethane (a single dose of 1 g/kg) followed by intraperitoneal PBS (control) or sTNFR:Fc (10 mg/kg) at days 2 and 5 after urethane. 1EC, day of administration of a single urethane (ethyl carbamate, EC) injection; \( n = \) sample size; each box represents 1 day; gray boxes indicate days of sTNFR:Fc treatment.
Continuous Inhibition of TNF Signaling Halts Both Urethane-Induced Tumor Initiation/Promotion and Progression

Finally, lungs of mice that received continuous sTNFR:Fc treatment were analyzed (prolonged prevention trial; Figure 1A4). Compared with controls, these mice displayed marked reductions in all parameters of oncogenesis examined, including lung tumor multiplicity (mean lung tumor number = 9.9 ± 1.0, \(P < .01\)), tumor size (mean diameter = 0.76 ± 0.03 mm, \(P < .01\)), mean lung tumor volume, overall lung tumor burden per mouse, as well as in the relative abundance of more progressed adenoma lesions (Figure 2). These results indicated that prolonged therapeutic TNF inhibition during the whole time course of urethane-induced lung tumorigenesis is effective in halting both tumor initiation/promotion and progression.

TNF Neutralization Inhibits Lung Tumor Cell Proliferation and New Vessel Formation within Lung Tumors

To further characterize the antitumorigenic effects of sTNFR:Fc, we immune-labeled proliferating and apoptotic tumor cells using PCNA and TUNEL, respectively. Although no immunoreactivity for TUNEL was identified in any group at the 8-month time point (data not shown), the percentage of tumor cells displaying immunoreactivity for PCNA (proliferating cells) was significantly decreased in animals that received late or continuous sTNFR:Fc treatment (tumor progression and prolonged prevention trials; Figure 3, A and B). Newly formed blood vessels within de novo developed lung tumors were then sought for using anti-F8A immune labeling. Microvascular density was equal between tumors in the control, tumor initiation/promotion, and tumor progression arms but was markedly inhibited in mice that received prolonged courses of sTNFR:Fc (prolonged prevention trial; Figure 3, C and D). These data indicated that TNF exerts tumor growth- and angiogenesis-promoting effects within chemical-induced lung tumors, which can be effectively abrogated by its therapeutic targeting.

sTNFR:Fc Treatment Modulates Urethane-Induced Pulmonary Inflammation

Because urethane-induced lung carcinogenesis is intimately associated with a lung inflammatory response [15] and TNF is a major
proinflammatory mediator [10], we questioned whether our interventions into TNF signaling would alter inflammatory parameters during urethane-induced oncogenesis in the lungs. For this, BAL immune cell and cytokine content of our previously described experimental mice were analyzed. Compared with controls, mice enrolled in the tumor initiation/promotion trial had reduced BAL macrophages and lymphocytes; mice of the tumor progression trial decreased BAL macrophages, neutrophils, and lymphocytes; and mice that received continuous sTNFR:Fc decreased numbers of BAL macrophages only (Figure 4A). Regarding BAL inflammatory mediator content, none of the molecules tested (TNF, IFN-\(\gamma\), MCP-1, IL-6, IL-10, and IL-12p70) were downregulated by sTNFR:Fc treatment in any regimen. On the contrary, IFN-\(\gamma\) and IL-10, mediators with known anti-inflammatory and immune surveillance functions [23,24], were upregulated specifically by continuous sTNFR:Fc treatment (Figure 4). To better understand how TNF neutralization impacts urethane-induced inflammation in the lungs, we performed a short-term study. For this, Balb/c mice received a single urethane dose or PBS control, followed by twice-weekly sTNFR:Fc or PBS control and were killed after 1 week (Figure 1B). Compared with PBS-treated controls, urethane-treated mice showed increased BAL macrophage and TNF content, in accord with a previous work [15]. Mice that received both urethane and sTNFR:Fc had BAL macrophage numbers and TNF levels that were reduced compared with urethane-only–treated animals and were similar to those observed in PBS-only–treated mice. Interestingly, the latter group of mice that received urethane and sTNFR:Fc had increased BAL IFN-\(\gamma\) and IL-10 levels (Figure 5). These data indicated that sTNFR:Fc treatment downregulated monocytic inflammation and proinflammatory TNF expression in the air space compartment in response to urethane, at the same time boosting anti-inflammatory IFN-\(\gamma\) and IL-10 expression. Collectively, these findings suggested that, in addition to critical tumorigenesis-related events such as cell-autonomous proliferation and angiogenesis, TNF blockade alters yet another conditional hallmark of cancer development—cancer-related inflammation [25].

**TNF Neutralization Suppresses TNF and Augments IFN-\(\gamma\) and IL-10 Elaboration by Mouse Macrophages In Vitro**

We subsequently sought to explain the previously mentioned findings of sTNFR:Fc-induced TNF-suppression and IFN-\(\gamma\) and IL-10 up-regulation observed in our experimental animals. Because airway...
epithelial cells and alveolar macrophages are the main cell populations with secretory capabilities into the air space compartment, we hypothesized that sTNFR:Fc treatment would enhance TNF and suppress IFN-γ and IL-10 elaboration by either cell type. To model airway epithelial cells and alveolar macrophages in vitro, we used murine airway epithelial-originated carcinoma (LLC) and bone marrow-derived macrophage (RAW264.7) cells. sTNFR:Fc treatment of these cell lines had no effect on cell proliferation assessed using a MTS assay (data not shown). In addition, TNF neutralization did not impact mediator (TNF, IFN-γ, MCP-1, IL-6, IL-10, and IL-12p70) elaboration by LLC cells (data not shown). However, sTNFR:Fc treatment of RAW264.7 macrophages at concentrations above 1 μg/ml resulted in suppression of TNF secretion and in a significant induction of IFN-γ and IL-10 elaboration, with no changes in MCP-1, IL-6, and IL-12p70 expression (Figure 6), paralleling the in vivo changes observed after sTNFR:Fc treatment. These results suggested that sTNFR:Fc effects on pulmonary macrophages may mediate its immunomodulatory effects during urethane-induced lung oncogenesis and may contribute to the tumor-protective impact of TNF blockade.

Discussion

In the present work, we set out to investigate the impact of TNF bioactivity neutralization on urethane-induced lung oncogenesis in mice. Based on previous reports of tumor-protective effects of genetic TNF deficiency in this model, of pulmonary TNF expression after urethane exposure of mice, and of TNF and TNFR expression in lung cancer, we hypothesized that TNF signals promote the formation and progression of lung tumors in response to the prototype carcinogen urethane. To test this, we used different pharmacologic dosing protocols of a clinically available TNF inhibitor, designed to target distinct stages of urethane-induced respiratory carcinogenesis. We found that this strategy was effective in halting pulmonary oncogenesis across its whole timeline spectrum, ranging from tumor initiation of the naive respiratory epithelium to progression of already established neoplastic lesions. Moreover, the effects of TNF blockade were associated with decreased lung tumor cellular proliferation, impaired tumor-associated angiogenesis, and modulation of tumor-accompanying inflammation. Some of our in vivo observations regarding regulation of inflammatory signals after urethane were further recapitulated in mouse macrophages in culture, suggesting that alveolar macrophages may be involved in the favorable effects of TNF neutralization.

This report complements and corroborates previous work that showed that Tnf gene-deficient mice are protected from urethane-induced lung tumorigenesis [16]. Moreover, our results show that the protumorigenic functions of TNF signaling during chemical-induced lung carcinogenesis can be therapeutically targeted to yield clinically relevant beneficial effects. In our hands, the latter were not confined to prevention of formation of de novo neoplastic lesions, as evidenced by the reduction in tumor multiplicity in our initiation trial, but also included regression of already present preneoplastic and neoplastic lesions, as indicated by the reductions in tumor size and in the relative abundance of more progressed adenoma lesions in mice from our regression trial. Importantly, TNF neutralization could be safely applied during the whole timeline of urethane-induced respiratory oncogenesis without apparent toxicities, yielding favorable effects on both new neoplastic lesion formation and progression of preexisting tumors in the lungs.

Figure 4. Effects of TNF signaling blockade on air space inflammatory parameters in the tumor-bearing lungs of urethane-treated mice. Balb/c mice were treated as outlined under Figure 1A. (A) Bronchoalveolar lavage (BAL) absolute cell numbers. MΦ indicates macrophages; PMN, neutrophil polymorphonuclear leukocytes; LΦ, lymphocytes. (B) BAL levels of TNF, IFN-γ, and IL-10. Dots indicate raw data points; lines, mean; bars, SEM; P, overall probability. *P < .05, **P < .01, and ***P < .001 compared with control.
Inflammation, initially thought to exert antitumorigenic functions of immune surveillance and immune rejection, was, in recent years, found to accompany solid tumors starting from their development up to advanced metastatic progression stages [26]. Moreover, the inflammatory response to tumor development is not a mere bystander but seems to promote tumor formation, growth, and metastasis through blunting and co-option of, and escape from, host antitumor immunity [27]. Inflammation is so important in cancer biology that it was recently incorporated into the updated hallmarks of cancer as a conditional hallmark [25]. Our results are directly relevant with the concept of tumor-promoting inflammation. First, by targeting TNF, a cardinal proinflammatory mediator, we obtained marked suppression of chemical-induced lung tumorigenesis. Second, our interventions did not only result in cell-autonomous effects on tumor cells (reduced growth rate) but also seemed to globally impact the host milieu of lung tumor development, significantly modulating the inflammatory response to the chemical carcinogen urethane and impairing the formation of new blood vessels. In particular, sTNFR:Fc-mediated TNF neutralization augmented the local release of IFN-γ and IL-10 in the airway lumen, mediators of proven antitumorigenic functions [23,24]. Third, we provide some evidence suggesting that the effects of our interventions may not be limited to respiratory epithelium and tumor cells but may extend to inflammatory cells within the tumor microenvironment. In this regard, the changes in the inflammatory response observed in the air spaces of mice after sTNFR:Fc treatment were closely recapitulated by sTNFR:Fc treatment of macrophages in vitro.

TNF is currently considered to be a major promoter of cancer progression [10]. Although earlier experiments pointed toward beneficial antitumor effects of the cytokine [9], it was later shown that physiologically irrelevant high doses were required for induction of tumor necrosis [28]. Instead, physiological low levels of TNF, such as produced by both tumor and host stromal cells (e.g., tumor-infiltrating macrophages), were found to promote tumor growth and metastasis in several sites [11–13,29,30]. Current evidence indicates that the tumor-promoting actions of TNF are directed toward both cancer cells and the host microenvironment in which the tumors grow. For example, TNF activates NF-κB in tumor cells, thereby promoting cell growth, preventing apoptosis, and inducing immortalization [11,13,31]. In addition, TNF impacts the host milieu by inducing angiogenesis and vascular hyperpermeability and by altering the phenotype of tumor-infiltrating macrophages and other immune cells [13,32,33]. As opposed to established cancer progression, far less is known on the effects of TNF on early stages of tumor development, that is, carcinogenesis [16,34]. Moreover, only few attempts at biologic TNF blockade in mouse models of carcinogenesis have been made [35]. We show that sTNFR:Fc treatment is effective against urethane-induced carcinogenesis, even when given during the earliest stages of respiratory epithelial tumor initiation. In combination with the existing literature, our results lend hope that TNF blockade may constitute in the future a viable strategy for tumor prevention and therapy.

A main obstacle in applying anti-TNF therapies to tumor therapeutics or even chemoprevention design is the cytokine’s name: TNF blockade may enhance carcinogenesis instead of curing it. In face of current evidence, these fears seem unsubstantiated. TNF antagonists
have been used for prolonged periods in patients with autoimmune joint disorders. The only adverse events observed were restricted to infectious disease reactivation or skewed autoimmunity, and no increased risk of epithelial malignancies was recorded [36–38]. In addition to being safe, TNF blockade seems to exert promising effects, by inducing disease stability in a significant subset of patients, or by even ameliorating the adverse effects of chemotherapy [19,39–42]. While the acquisition of a better understanding of the impact of TNF in human lung cancer and its relative importance in early versus late cancers is ongoing, our results add to the existing literature regarding the potential benefits of TNF neutralization during early stages of carcinogenesis.

In the lungs, chronic inflammatory diseases, such as COPD, are most likely causally related with lung cancer development [6]. Patients with COPD run a three to five times greater risk of being diagnosed with lung cancer independent from smoking intensity and duration [43]. In fact, although only 20% of smokers develop COPD, more than half of lung cancers coincide in patients with COPD [44]. Vice versa, more than 50% of patients with lung cancer are also diagnosed with COPD at the time of tumor diagnosis [45]. Recently, common paths to both COPD and lung cancer have been unveiled, including respiratory epithelial NF-κB activation [6,15,46]. TNF, a marked transcriptional target of NF-κB, is expressed in both COPD and lung cancer and is a lucrative candidate culprit for the inflammatory lung carcinogenesis observed in smokers and patients with COPD [14,47]. Although TNF antagonists have failed to inhibit the chronic inflammation behind COPD and to yield a clinically relevant benefit, they might be effective as chemopreventive agents in these patients [48]. In this regard, inhaled corticosteroids, the prototype respiratory anti-inflammatory drugs, have failed to halt COPD [49], but evidence suggests they might be useful for chemoprevention against lung cancer [50]. More importantly, the results from our tumor progression trial suggest that TNF antagonism may hold promise in secondary chemoprevention, that is, in patients with resected lung cancer that run increased risk for the development of a second primary tumor.

In conclusion, we showed that a clinically safe and available TNF antagonist is effective in limiting chemical respiratory carcinogenesis induced by urethane in mice. Collectively, our results and previous work that used genetic TNF targeting suggest that TNF may present an important target for lung cancer chemoprevention in the future.

References


