An Anti-Tumor Necrosis Factor-α Antibody Inhibits the Development of Experimental Skin Tumors

Kate A. Scott,[1,2] Robert J. Moore,[1] Caroline H. Arnott,[1] Nick East, Richard G. Thompson, Bernard J. Scallon, David J. Shealy, and Frances R. Balkwill

Cancer Research UK Translational Oncology Laboratory, John Vane Science Centre, Barts & The London, Queen Mary's School of Medicine and Dentistry, London EC1M 6BQ, United Kingdom [K. A. S., R. J. M., C. H. A., N. E., R. G. T., F. R. B.], and Centocor, Malvern, Pennsylvania 19355 [B. J. S., D. J. S.]

Abstract

The proinflammatory cytokine tumor necrosis factor-α (TNF-α) was originally considered to have activity against malignant disease. However, recent studies suggest TNF-α may also act as an endogenous tumor promoter. In the present work, mice deficient in TNF-α either genetically (*TNF-α*$^{-/-}$) or after blockade with a neutralizing antibody (cV1q) were used to investigate the role of TNF-α in skin tumor development. Papillomas were induced in wild-type (wt) mice after treatment of skin with the initiating agent 9,10-dimethyl-1,2-benzanthracene followed by promotion with 12-*O*-tetradecanoylphorbol-13-acetate (TPA) for 15 weeks. *TNF-α*$^{-/-}$ mice were resistant to papilloma development when compared with wt mice on C57Bl/6J, 129/SvEv, and BALB/c genetic backgrounds. Primary murine keratinocytes (newborn keratinocytes) and skin homogenates were used to characterize TPA-stimulated TNF-α expression. TPA induced TNF-α protein in newborn keratinocytes *in vitro* and epidermis *in vivo*. Neutralization of TNF-α protein with cV1q *in vivo* for 0–15 weeks of promotion significantly decreased skin tumor development after 9,10-dimethyl-1,2-benzanthracene/TPA treatment. cV1q treatment during the early stages of tumor promotion (0–6 weeks) was equally effective. These data suggest that early induction of TNF-α is critical for skin tumor promotion. cV1q also reduced TPA-stimulated expression of matrix metalloproteinase 9 and granulocyte macrophage colony-stimulating factor, proteins that are differentially regulated in wt and *TNF-α*$^{-/-}$ epidermis. Treatment of the 410.4 transplantable breast carcinoma with cV1q reduced tumor growth *in vivo*, illustrating that inhibition of tumor growth through neutralization of TNF-α is not limited to skin carcinogenesis. These results provide further evidence for procancer actions of TNF-α and give some rationale for use of TNF-α antagonists in cancer prevention and treatment.

Introduction

The cytokine TNF-α[3] is a major mediator of inflammation. A key role for this cytokine in the pathogenesis of diseases such as rheumatoid arthritis, Crohn's disease, and psoriasis has been demonstrated by the successful treatment of such conditions with antibodies to TNF-α or with a soluble TNF-α receptor fusion protein (1, 2).

In malignant disease, TNF-α is classically considered as an anticancer agent. In both animal models and clinical trial, high-dose local TNF-α administration selectively destroys tumor blood vessels (reviewed in Ref. 3). TNF-α treatment can also stimulate T-cell-mediated antitumor immunity in mouse models (4). These actions have raised fears that patients receiving TNF-α antagonist therapies for inflammatory conditions may have an increased incidence of malignancy. However, there is also substantial evidence that TNF-α may act as an endogenous tumor promoter, contributing to the tissue remodeling and stromal development necessary for tumor growth and spread (reviewed in Refs. 3 and 5).

Direct evidence for the involvement of TNF-α in malignancy comes from observations that TNF-α knockout mice (*TNF-α*$^{-/-}$) on a mixed genetic background are resistant to chemical carcinogenesis of the skin (6). To extend this observation, we have studied skin tumor development in *TNF-α*$^{-/-}$ mice on three different genetic backgrounds and in wt mice treated with a neutralizing TNF-α antibody. This work is of importance because susceptibility to tumor promotion varies in different strains of mice, and use of an anti-TNF-α antibody in experimental cancer models has not been reported. We have characterized the induction of TNF-α expression in skin during tumor promotion to establish times at which the presence of TNF-α may be important. Data presented here suggest that neutralization of TNF-α during the early stages of tumor promotion is sufficient to inhibit tumor formation, giving some rationale for use of TNF-α antagonists in cancer prevention and treatment.

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[1] K. A. S., R. J. M., and C. H. A. contributed equally to this work.

[2] To whom requests for reprints should be addressed, at Cancer Research UK Translational Oncology Laboratory, Barts & The London, Queen Mary's School of Medicine and Dentistry, 3rd Floor, John Vane Science Centre, Charterhouse Square, London EC1M 6BQ, United Kingdom. Phone: 44-207-882-5797; Fax: 44-207-882-6110; E-mail: kate.scott@cancer.org.uk.

[3] The abbreviations used are: TNF-α, tumor necrosis factor-α; wt, wild-type; DMBA, 9,10-dimethyl-1,2-benzanthracene; TPA, 12-*O*-tetradecanoylphorbol-13-acetate; NBK, newborn keratinocyte; MMP, matrix metalloproteinase; GM-CSF, granulocyte macrophage colony-stimulating factor; AUC, area under the curve.

Materials and Methods

Mice. Mice homozygous for the mutant TNF-α knockout allele (*TNF-α*$^{-/-}$) and wt mice were maintained on BALB/c, 129/SvEv, and C57Bl/6J backgrounds after at least six backcrosses from the original 129Sv/C57Bl6 founder mice (7). In every experiment, 6-week-old wt and *TNF-α*$^{-/-}$ female mice were age-matched to within 3 days.

Tumor Induction in Mouse Skin. Tumors were induced by DMBA (25 μg in 100 μl of acetone) initiation and TPA tumor promotion as described previously (6). TPA (4 μg in 100 μl of acetone; Sigma, Poole, United Kingdom) was applied to the shaved area twice weekly for 15 weeks for BALB/c or 129/SvEv mice and 3 times weekly for 15 weeks for C57Bl/6J mice. Control mice were treated with acetone. Microchips were implanted dorsally, and the tumor history of individual mice was recorded. Tumors were defined as raised lesions of at least 1 mm diameter that had been present for at least 2 weeks.

Treatment of Mice with cV1q. Anti-murine TNF-α antibody (cV1q) is a chimeric (rat variable regions, mouse constant regions) IgG2a,k monoclonal antibody originally derived from the rat IgD antibody V1q. cV1q (0.5 mg) was given by i.p. injection to C57Bl/6J wt mice 1 day before DMBA treatment and then given once a week during TPA promotion for the time indicated. Control C57Bl/6J wt mice were either uninjected or received injection with control diluent. cV1q dosing was based on previous activity of this antibody in human/murine severe combined immunodeficient arthritis model and a murine model of congestive heart failure (8).

Primary Murine Keratinocytes. NBKs were isolated from 2–4-day-old wt mice as described in Ref. 9. Briefly, mice were killed, and the skin was removed. The skin was stretched out epidermis side up and incubated in 2.5% (w/v) trypsin in Tris saline solution overnight at 4°C. The following day, epidermis was separated from dermis, and epidermal cells were isolated by disaggregation. The cell suspension was passed through a 70-μm cell strainer and then centrifuged at 250 × *g* for 10 min. Viable cells were counted using trypan blue exclusion and plated at 1 × 10^7 cells/collagen I-coated 10-cm dish (Becton Dickinson, Oxford, United Kingdom). Cells were cultured for 7–10 days in FAD medium with 0.1 mM calcium (JRH Biosciences, Andover, United Kingdom) containing 10% fetal bovine serum (chelex-treated to remove Ca^{2+}) and HICE (0.5 μg/ml hydrocortisone, 5 μg/ml insulin, 0.1 nM cholera toxin, and 10 ng/ml EGF). NBKs were trypsinized, plated at 7.5 × 10^5 cells/well, and treated with 100 nM TPA in the presence of 4% fetal bovine serum and HICE.

Preparation of Epidermal Homogenates. The skin of mice was treated as specified. Mice were killed, and skin was removed, spread on card, and snap frozen in liquid nitrogen. Epidermis was subsequently scraped from dermis using a surgical scalpel and placed in ice-cold cell lysis buffer [1% (v/v) Triton X-100, 0.5% (w/v) sodium deoxycholate, and 0.1% (w/v) SDS in PBS]. Samples were homogenized using an Ultra-Turrax T25 homogenizer and incubated on ice for 10 min. Undigested tissue was removed by centrifugation at 15,000 × *g* for 10 min. The supernatant was collected, and total protein concentration was determined using the BCA protein assay kit (Sigma).

ELISA for Cytokines. GM-CSF and TNF-α protein were measured in epidermal homogenates by ELISA (R&D Systems, Oxfordshire, United Kingdom) at 1 mg/ml total protein for GM-CSF and 3 mg/ml for TNF-α according to the manufacturer's instructions.

Zymography. Semiquantitative zymography for MMP-9 was carried out on epidermal homogenates as described previously (10). Protein identity was confirmed using a MMP-9 standard (R&D Systems). Samples were run in triplicate, and MMP-9 band intensity was quantified using NIH Image 1.58 image analysis program.

Measurement of cV1q Levels. A murine monoclonal antibody specific for the rat variable domain of cV1q (CNTO 222) was generated using standard hybridoma techniques. This antibody was conjugated to a CM-5 sensor chip (Biacore, Piscataway, NJ) and used to capture cV1q from mouse plasma samples on a Biacore 3000 instrument. The response units obtained from experimental samples were converted to μg/ml by comparison with a standard curve of cV1q spiked into normal mouse serum.

Growth of Syngeneic 410.4 Tumor *in Vivo* and Treatment with cV1q. Female BALB/c mice (6–8 weeks of age) were treated with either cV1q (0.5 mg) or control (PBS) by i.p. injection (10 mice/group). The following day, mice were injected with 1 × 10^6 410.4 cells into the right flank. cV1q or control treatment was administered once weekly for the duration of the experiment. The growth of the 410.4 mammary tumor was evaluated by measuring the tumor dimensions three times weekly.

Statistical Evaluation of Tumor Development. The AUC method was used to compare the total papilloma incidence or 410.4 tumor growth in cV1q-treated and control mice over the duration of each study. Statistical comparisons were made using the Mann-Whitney *t* test.

Results

TNF-α$^{-/-}$ Mice Are Resistant to Skin Carcinogenesis on Three Different Genetic Backgrounds. The development of papillomas after DMBA/TPA two-stage carcinogenesis was studied in wt and *TNF-α*$^{-/-}$ mice on BALB/c, C57Bl/6J, or 129/SvEv backgrounds (Fig. 1). The average number of papillomas/mouse was recorded on a weekly basis (tumor multiplicity). Papillomas were first observed 7–8 weeks after the start of TPA treatment in all three backgrounds of wt mice. The induction of papilloma formation was delayed in all strains of *TNF-α*$^{-/-}$ mice, with the first tumors appearing after 10–12 weeks. Tumor multiplicity peaked at 16 weeks for C57Bl/6J wt mice and 18 weeks for both BALB/c and 129/SvEv wt mice (Fig. 1). *TNF-α*$^{-/-}$ mice developed significantly fewer tumors than wt mice (Fig. 1). A comparison of maximum tumor multiplicity in wt and *TNF-α*$^{-/-}$ mice is shown in Table 1. The difference between development of skin tumors in wt and *TNF-α*$^{-/-}$ mice was significant on all three backgrounds (Table 1). These data indicate that resistance of *TNF-α*$^{-/-}$ mice to tumor promotion is not related to differences in susceptibility between strains. Moreover, because the TNF-α gene is located within the MHC locus, there

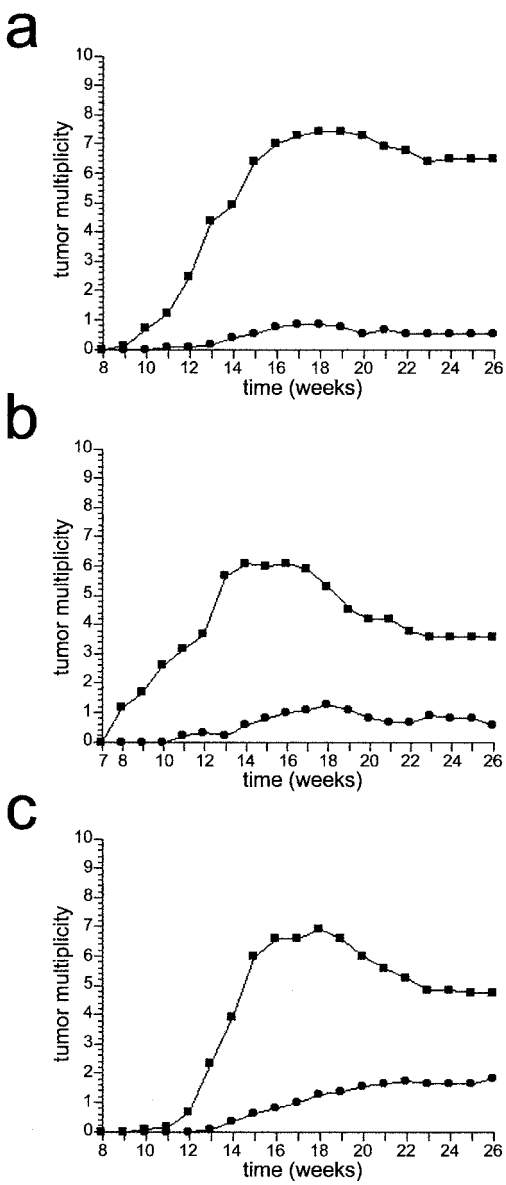

Fig. 1. Mice deficient in TNF-α are resistant to skin carcinogenesis on three genetic backgrounds. Ten wt and 10 *TNF*-$\alpha^{-/-}$ mice on each genetic background were subjected to skin carcinogenesis by DMBA initiation and TPA promotion. *TNF*-$\alpha^{-/-}$ mice (●) were resistant to papilloma formation in BALB/c (*a*), C57Bl/6J (*b*), and 129/SvEv (*c*) mice compared with wt mice (■). Tumor multiplicity equates to an average number of papillomas/mouse.

Table 1 Comparison of maximum tumor multiplicity in wt and *TNF*-$\alpha^{-/-}$ mice on three genetic backgrounds

Genetic background	Maximum average no. of papillomas/mouse		AUC method *P* value
	wt	*TNF*-$\alpha^{-/-}$	
BALB/c	7.1	0.92	0.002
C57Bl/6J	6.1	1	0.002
129/SvEv	6.9	1.28	0.001

was a possibility that antigen presentation would differ in wt and *TNF*-$\alpha^{-/-}$ mice even after several backcrosses because a small portion of the MHC locus would still be that of the original 129/SvEv ES cells. The observation that 129/SvEv *TNF*-$\alpha^{-/-}$ mice were resistant to skin carcinogenesis indicates that antigen presentation was not affected in *TNF*-$\alpha^{-/-}$ mice.

The Tumor Promoter TPA Induces TNF-α Protein in Primary Keratinocytes *in Vitro* and Epidermis *in Vivo*. To further understand the role of TNF-α in skin tumor development, we investigated the ability of TPA to induce TNF-α. When TPA was added to primary murine keratinocytes (NBKs) isolated from BALB/c wt mice, TNF-α protein was rapidly induced (Fig. 2*a*). TPA-induced TNF-α release was first observed at 60 min (68 pg/ml; Fig. 2*a*). Levels of TNF-α had increased to 253 pg/ml 2 h after TPA treatment. TNF-α induction was also observed in NBKs isolated from C57Bl/6J wt mice (Fig. 2*b*). We next studied the induction of TNF-α protein *in vivo* during skin tumor promotion. TNF-α levels in control epidermis were below the detection limits of the assay kit (5.1 pg/ml), but significant levels were detected in the epidermis after TPA treatment at all time points analyzed (Fig. 2, *c* and *d*). TNF-α was detected in both BALB/c and C57Bl/6J epidermal extracts after four applications of TPA (Fig. 2*c*). TNF-α induction was highest at 6 h (24–27 pg/mg total protein), and expression was sustained at 24 h (13–17 pg/mg total protein). The level of TPA-induced TNF-α expression was similar in BALB/c compared with C57Bl/6J wt epidermis (Fig. 2*c*). The role of TNF-α in papilloma formation may be linked to specific events occurring during the 15 weeks of tumor promotion. Thus we investigated whether the extent of TNF-α induction varies with repeated applications of TPA. TPA induced TNF-α in the epidermis of BALB/c mice at all stages of tumor promotion (Fig. 2*d*). The amount of TNF-α protein in epidermal extracts varied with the number of TPA applications. In two independent experiments, two peaks of TNF-α expression were observed, both of which occurred early in the promotion regime (Fig. 2*d*; 4 applications, 20 pg/mg; 10 applications, 24 pg/mg).

Thus, data presented here show that the major cell type in the epidermis, keratinocytes, rapidly produced high levels of TNF-α protein in response to TPA when cultured *in vitro*. The profile of TPA-induced TNF-α expression *in vivo* suggested that early induction of TNF-α is important for papilloma development. To further dissect the role of TNF-α in tumor development and to elucidate times at which TNF-α expression is critical, we investigated the action of a neutralizing antibody to TNF-α at different stages of tumor promotion.

Neutralizing Antibodies to TNF-α Reduce Papilloma Incidence. C57Bl/6J wt mice were treated with an anti-TNF-α antibody (cV1q) once weekly throughout the skin carcinogenesis regime. The experiment shown in Fig. 3*a* combines data from three separate experiments with a total of 19 mice in the control group and 30 mice in the cV1q-treated group. Treatment of mice with cV1q significantly reduced the incidence of skin tumors compared with control mice (AUC method, $P = 0.004$ for control group *versus* cV1q-treated group during 0–15 weeks of promotion; Table

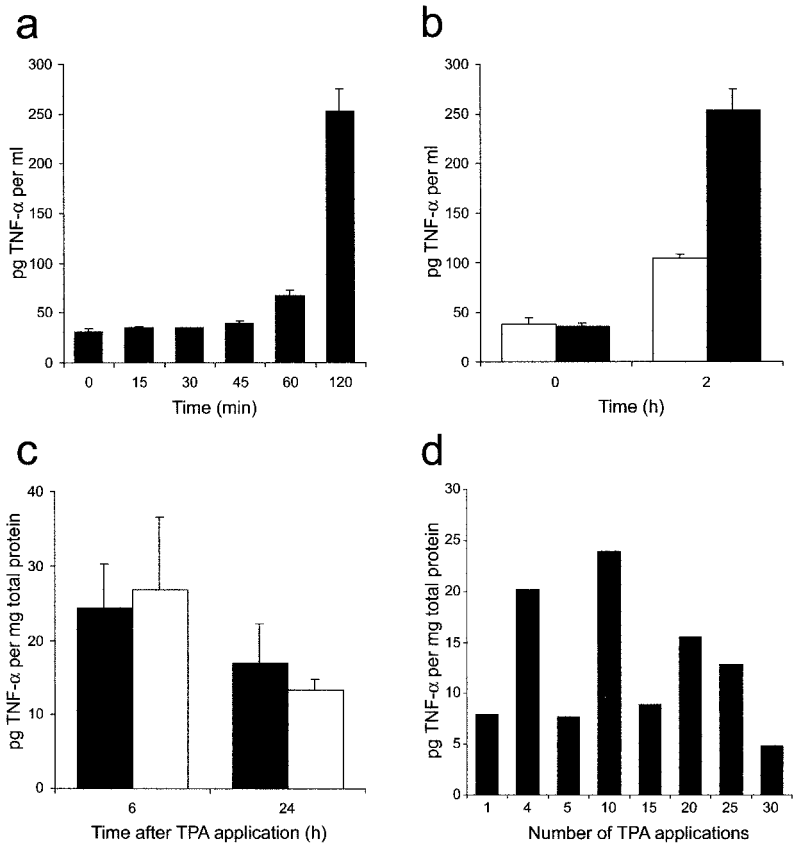

Fig. 2. TPA induces TNF-α protein expression by murine keratinocytes *in vitro* and *in vivo*. *a*, TNF-α protein production by primary murine keratinocytes (NBKs) isolated from BALB/c mice after treatment with TPA. *b*, comparison of TNF-α production in BALB/c (■) and C57Bl/6J (□) NBKs before and after TPA treatment. *c*, TNF-α protein expression in BALB/c (■) and C57Bl/6J (□) epidermis *in vivo* 6 and 24 h after four applications of TPA. Results are expressed as means ± SE; $n = 2$. *d*, TPA stimulated TNF-α production in BALB/c epidermis during 15 weeks of promotion. Samples were taken 24 h after TPA treatment. *In vivo* samples were analyzed at 3 mg/ml and corrected to pg/mg total protein. Results are representative of two independent experiments.

2). Maximal tumor multiplicity was observed at 16 weeks in control and cV1q-treated mice (6.1 and 3.4 papillomas/mouse, respectively; Fig. 3*a*).

Expression of TNF-α Early in Tumor Promotion Is Important for Papilloma Development. Since high levels of TNF-α expression were observed in epidermis after 4 and 10 applications of TPA, the efficacy of neutralizing TNF-α for the first 6 weeks of promotion was studied. The experiment shown in Fig. 3*b* combines data from two separate experiments with 19 mice in the control group and 18 mice in the cV1q-treated group. cV1q treatment for 0–6 weeks of promotion significantly reduced the incidence of skin tumors compared with control mice (AUC method, $P = 0.02$ for control group *versus* cV1q-treated group during 0–6 weeks of promotion; Table 2). Maximal tumor multiplicity was observed at 16 weeks, at which time control mice had developed an average of 6.1 papillomas/mouse compared with 3.4 papillomas/mouse in mice treated with cV1q for 0–6 weeks (Fig. 3*b*). Interestingly, cV1q treatment for 0–6 weeks was as effective as cV1q treatment for 0–15 weeks (Fig. 3*b* compared with Fig. 3*a*, respectively).

cV1q Stability. To measure levels of circulating antibody, a sample of blood was taken from each of 10 mice 1 week after the last dose of cV1q. A mean of 750 μg cV1q/ml plasma was detected in samples from 10 mice (range, 165-3690 μg/ml). These data confirm that dosing with cV1q at weekly intervals is sufficient for maintenance of circulating levels of cV1q.

The Expression of TPA-responsive Genes Is Decreased by cV1q Treatment. Recent work in this laboratory has shown that TPA treatment of murine skin induces GM-CSF and MMP-9 protein *in vivo* and that this induction is reduced in *TNF-α*$^{-/-}$ mice (11). Both GM-CSF and MMP-9 have been implicated in the development of skin tumors in mice (12, 13). Therefore, levels of epidermal MMP-9 and GM-CSF were measured in normal and cV1q antibody-treated mice. MMP-9 protein was not measurable before TPA treatment (Fig. 4*a, Lanes 1* and *2*). However, TPA induced MMP-9 expression in both control and cV1q-treated mice (Fig. 4*a, Lanes 3–6*). The level of MMP-9 was significantly reduced in cV1q-treated mice compared with control mice at both 6 h ($P = 0.03$) and 24 h ($P = 0.03$) after TPA treatment. Low levels of GM-CSF were detected in mouse skin before TPA treatment (Fig. 4*b*). GM-CSF expression was up-regulated by TPA, but induction was reduced in mice treated with cV1q when compared with control mice (Fig. 4*b*). GM-CSF levels were 2-fold lower 6 h after TPA treatment in cV1q-treated mice compared with control mice (Fig. 4*b*; 19 pg/mg total protein compared with 38 pg/mg total protein, respectively).

Neutralizing Antibodies to TNF-α Inhibit the Growth of a Murine Breast Carcinoma Model. To investigate whether the effect of cV1q was specific for skin tumor development, we tested the efficacy of neutralizing TNF-α in a murine breast carcinoma model. cV1q treatment significantly inhibited the growth of 410.4 tumor cells *in vivo* (Fig. 5; AUC

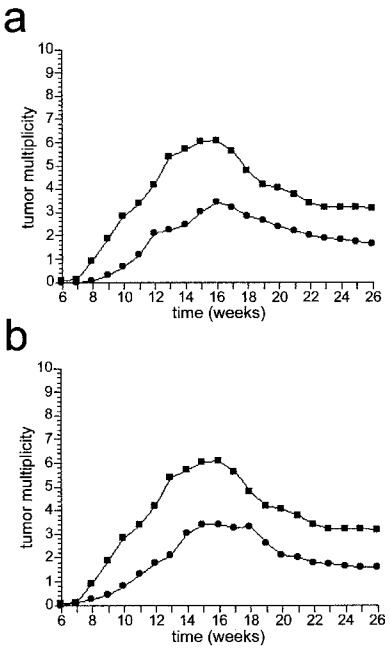

Fig. 3. Neutralization of TNF-α protein reduces susceptibility of wt mice to skin carcinogenesis. *a*, 19 control mice and 30 mice treated with cV1q (once weekly for 15 weeks of promotion) were subjected to skin carcinogenesis. Mice treated with cV1q (●) were resistant to papilloma formation compared with control (■). *b*, 19 control mice and 18 mice treated with cV1q (once weekly for 0–6 weeks of promotion) were subjected to skin carcinogenesis. Mice treated with cV1q for the first 6 weeks (●) were resistant to papilloma formation compared with control mice (■). Tumor multiplicity equates to an average number of papillomas/mouse.

Table 2 Comparison of maximum tumor multiplicity in control and cV1q-treated mice

Treatment	Maximum average no. of papillomas/mouse	AUC method *P* value
Control	6.1	
cV1q, 0–15 weeks	3.4	0.004
cV1q, 0–6 weeks	3.4	0.02

method, $P = 0.005$ for control *versus* cV1q treatment). This indicates that neutralization of TNF-α has widespread therapeutic potential, such that its use is not limited to inhibition of tumor formation during skin carcinogenesis.

Discussion

Chemical skin carcinogenesis in mice is a multistage process that involves initiation, promotion, and progression. Initiation is thought to involve the conversion of some epidermal cells into latent neoplastic cells (14). Promotion requires repeated exposure of skin to a tumor promoter (such as TPA), which results in dramatic morphological and biochemical changes (15). One such biochemical change is induction of TNF-α (6), which is likely to play a role in tumor promotion-related events such as epidermal hyperplasia and leukocyte infiltration into the dermis (16). Promotion ultimately results in the clonal expansion of initiated cells into papillomas (17).

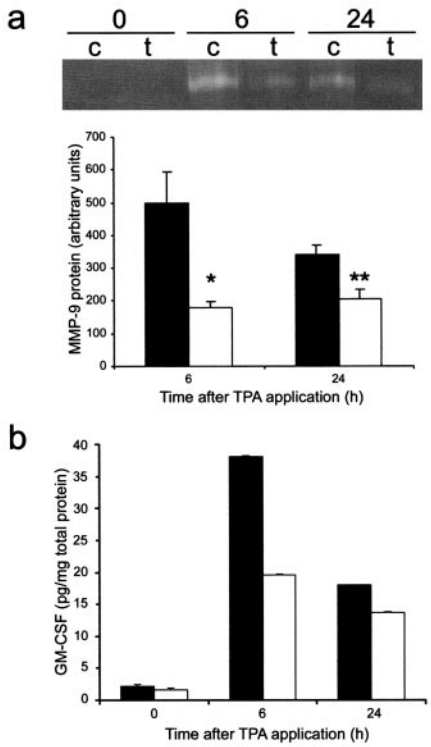

Fig. 4. Neutralization of TNF-α reduces the level of TPA-stimulated MMP-9 and GM-CSF expression *in vivo*. *a*, epidermis was collected from TPA-treated control mice (*Lanes c*) or mice treated with cV1q (*Lanes t*) at 6 and 24 h after four applications of TPA. MMP-9 was induced by TPA in control mice (■), and the level was reduced in mice treated with cV1q (□) at both 6 h (*, $P = 0.03$) and 24 h (**, $P = 0.03$). Results are expressed as means ± SE; $n = 3$. *b*, TPA stimulated the production of GM-CSF in control mice (■), and levels were reduced in cV1q-treated mice (□). Samples were taken 6 h and 24 h after four applications of TPA. Results are expressed as means ± SE; $n = 2$.

To our knowledge, this is the first time that the effects of TNF-α blockade using neutralizing antibodies have been assessed in murine models of tumor development. In this study we found that neutralization of TNF-α for the duration of tumor promotion was sufficient to inhibit skin tumor development. These data corroborate the observation that genetic deletion of *TNF*-α renders mice resistant to skin carcinogenesis. Neutralization of TNF-α protein was less effective than genetic deletion of *TNF*-α in this model. Circulating levels of anti-TNF-α antibodies were maintained throughout the experiment in treated mice; however, antibody concentration in the skin may fluctuate, allowing low levels of TNF-α activity. The presence of epidermal TNF-α early in skin tumorigenesis appears to be important because short-term anti-TNF-α treatment was equally as effective as treatment throughout tumor promotion.

The finding that antibodies to murine TNF-α significantly inhibit the development of skin papillomas has a number of implications. First, it provides further evidence that TNF-α may act as an endogenous tumor promoter. Previously published data include observations that mice deficient in TNF-α are resistant to chemically induced skin and liver cancers, that TNF-α drives lymphadenopathy and plasmacytoid tu-

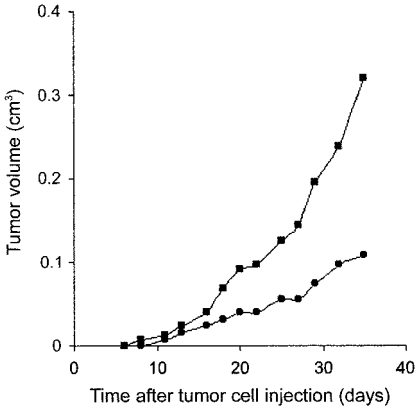

Fig. 5. Neutralization of TNF-α protein inhibits growth of 410.4 mammary tumor *in vivo*. The growth of 410.4 tumor was monitored in control and cV1q-treated mice (treatment administered once weekly for duration of experiment). Tumor growth was inhibited in mice treated with cV1q (●) compared with control (■; $P = 0.005$). Results are expressed as median tumor volume ($n = 10$).

mors in *FasL*$^{-/-}$ mice, and that TNF-α is implicated in the action of nongenotoxic liver carcinogens (reviewed in Ref. 3). In addition, in the present work we show that antibodies to TNF-α inhibit tumor development in a syngeneic transplantable breast cancer model. Local levels of TNF-α in both the skin carcinogenesis model and the transplantable breast tumor are low (pg/mg total protein) relative to many other cytokines and chemokines induced during tumor development. We propose that low levels of TNF-α in these tumor models may facilitate the establishment of a cytokine and chemokine network that contributes to disease progression, as has been observed in rheumatoid arthritis (2).

Second, our data add to the debate about the risk of malignancy in patients treated with TNF-α antagonists for inflammatory disease. Whereas this study suggests that endogenous TNF-α acts as a tumor promoter, it is clear that high doses of TNF-α given as locoregional cancer treatments have powerful antitumor activity (18). Hence, there have been concerns that long-term TNF-α blockade in the treatment of chronic inflammatory conditions may increase risk of cancer. Preliminary data from studies of malignancy in patients receiving long-term treatment with TNF-α antagonists indicate that tumor incidence is similar to that of control patients (19, 20). Moreover, in the present work there was no evidence that blockade of TNF-α with neutralizing antibodies accelerated skin tumor growth. Indeed, examination of mice 26 weeks after the start of tumor promotion revealed that tumor growth was slower in anti-TNF-α-treated mice than in control mice (fewer tumors reached a diameter of 1 cm).

Third, the data reported here provide some rationale for the use of TNF-α antagonists in cancer treatment. There is evidence that TNF-α expression in a variety of human cancers (*e.g.,* ovarian, breast, prostate, bladder, colorectal cancer, lymphoma, and leukemia) is associated with poor prognosis (reviewed in Ref. 3). TNF-α may also play a role in the development of human squamous cell carcinomas because UV light (the major carcinogen in human skin cancer) is a powerful inducer of epidermal TNF-α (21). Because TNF-α blockade significantly inhibits murine skin tumor development, it is possible that TNF-α antagonists may be useful in the treatment of human skin cancer.

Clinical evaluation of infliximab treatment in rheumatoid arthritis patients indicated an improvement in bone marrow function, inhibition of inflammatory cell infiltrate into affected joints, and reduced plasma levels of cytokines, MMPs, and angiogenic factors (2). All of these actions could be useful in biological therapy of cancer. In the current study, anti-TNF-α antibody therapy reduced levels of MMP-9 and GM-CSF, proteins that are thought to be important in the development and progression of skin cancers (12, 13). These data are in agreement with previous work from our laboratory that showed that MMP-9 and GM-CSF were differentially regulated in the epidermis of wt and *TNF-α*$^{-/-}$ mice (11). Interestingly, a decrease in MMP-9 levels has been observed in mucosal samples from Crohn's disease patients after treatment with infliximab.[4] Thus, induction of MMP-9 and GM-CSF appears to be important for the action of TNF-α in a range of pathologies. MMP-9 and GM-CSF may therefore become useful surrogate markers for response to TNF-α antagonists.

In summary, data presented here suggest that blockade of TNF-α activity may have potential use in cancer therapy. Clinical studies are currently being carried out to evaluate the efficacy of the TNF antagonists infliximab and etanercept in hematological and solid tumors with respect to therapy and supportive care.

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Molecular Cancer Therapeutics

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