

Endogenous TNF α Suppression of Neovascularization in Corneal Stroma in Mice

Shuko Fujita,¹ Shizuya Saika,¹ Winston Whei-Yang Kao,² Kyoko Fujita,¹ Takeshi Miyamoto,¹ Kazuo Ikeda,³ Yuji Nakajima,³ and Yoshitaka Ohnishi¹

PURPOSE. To examine the role of tumor necrosis factor α (TNF α) in stromal neovascularization in injured cornea in vivo and in cytokine-enhanced vessel-like endothelial cell tube formation in vitro.

METHODS. An in vitro model of angiogenesis was used to examine the roles of TNF α on tube formation by human umbilical vein endothelial cells (HUVECs) cocultured with fibroblasts on induction by transforming growth factor β 1 (TGF β 1) and vascular endothelial growth factor (VEGF). Central cauterization was used to induce stromal neovascularization in corneas of wild-type (WT) and TNF α -null (*Tnfa*^{-/-}) mice. At 7, 14, or 21 days of injury, experimental mice were killed, and the eyes were enucleated and subjected to histologic and immunohistochemical examination and real-time reverse transcription-polymerase chain reaction.

RESULTS. HUVECs formed a vessel-like tube structure on the fibroblast feeder layer. Adding TGF β 1, VEGF, or both augmented vessel-like tube formation by HUVECs cocultured with fibroblasts. Adding TNF α (5 ng/mL) completely abolished the formation of tube-like structures despite the presence or absence of TGF β 1 or VEGF in coculture. In vivo, cauterization of the central cornea induced the formation of CD31⁺ new vessels surrounding the limbus in WT mice. More prominent central stromal neovascularization accompanied by increased expression of TGF β 1 and VEGF was found in *Tnfa*^{-/-} mice compared with WT mice.

CONCLUSIONS. In addition to inhibiting TGF β 1 and VEGF expression by fibroblasts, endogenous TNF α may counter the induction effects of TGF β 1 and VEGF on vascular endothelial cells and may block neovascularization. (*Invest Ophthalmol Vis Sci*. 2007;48:3051-3055) DOI:10.1167/iovs.06-1083

Cornea is an avascular tissue and must remain transparent to refract light properly. Neovascularization in cornea resulting from various inflammatory disorders such as trauma, microbial infection, alkali burn, and limbal stem cell deficiency

can impair vision. Cytokines and growth factors orchestrate cell behaviors in the development of corneal neovascularization.^{1,2} Vascular endothelial growth factor (VEGF) and transforming growth factor β (TGF β) are two major cytokines involved in injury-induced neovascularization.³⁻⁶ These cytokines are upregulated in corneal stroma (inflammatory cells and resident corneal cells) during wound healing and inflammatory disorders.⁷

Tumor necrosis factor α (TNF α) is a pleiotropic proinflammatory cytokine.⁸ However, the role of TNF α in the development of neovascularization in injured tissues remains largely elusive because results of experiments that examined the roles of TNF α on the pathogenesis of different fibrotic and inflammatory disorders were controversial. For example, it has been shown that the suppression of TNF α by the administration of neutralizing antibody yields a favorable clinical outcome by reducing inflammation.^{9,10} This observation is substantiated by the finding that the ablation of TNF α -receptor (*Tnfr*^{-/-}) in mice is beneficial in pulmonary fibrosis caused by asbestos.¹¹ Studies using TNF α -null (*Tnfa*^{-/-}) and *Tnfr*^{-/-} mice demonstrated more severe bleomycin-induced pulmonary fibrosis than did wild-type (WT) mice. It has also been reported that the overexpression of TNF α suppresses such bleomycin-induced pulmonary fibrosis.¹²⁻¹⁴ These findings imply that TNF α signaling may have a role in modulating inflammatory responses and fibrosis. Therefore, adding TNF α may have beneficial effects on reducing certain types of pathogenic fibrosis while producing adverse effects on others. The pleiotropic roles of TNF α on various pathogenic disorders are further substantiated by the results of studies with collagen-induced arthritis (CIA) in *Tnfa*^{-/-} mice that exhibited some reduction in the clinical parameters of CIA and, on histologic examination, significantly more normal joints. However, severe disease was evident in 54% of arthritic *Tnfa*^{-/-} joints. Interestingly, collagen-immunized *Tnfa*^{-/-} mice developed lymphadenopathy and splenomegaly.¹⁵

We previously reported that endogenous TNF α could subdue TGF β 1-mediated tissue damage by alkali burn to the ocular surface, as exemplified by the greater severity of tissue damage in *Tnfa*^{-/-} than in WT mice. The tissue damage caused by alkali burn in *Tnfa*^{-/-} mice was accompanied by excess inflammation, myofibroblast generation, and marked neovascularization.¹⁶ Results of our previous studies of *Smad7* gene transfer¹⁷ to alkali-burned cornea and bone marrow transplantation from WT mice to *Tnfa*^{-/-} mice and in vitro coculture experiments revealed that macrophage-derived TNF α could counteract the effect of TGF β on fibrotic or inflammatory reaction of the alkali-burned cornea.^{16,17} However, in our previous study, an alkali burn with sodium hydroxide eyedrop damaged large areas of ocular tissue, including cornea, limbus, and bulbar conjunctiva. Thus, healed corneal surfaces were covered by conjunctival epithelium in WT and *Tnfa*^{-/-} mice.^{7,16,17} Therefore, it remains unknown whether such marked corneal neovascularization observed in *Tnfa*^{-/-} mice is associated with an invasion of conjunctival epithelium into the affected cornea or by a lack of TNF α alone. In the present study, to uncover the role of endogenous TNF α in the development of corneal neovascularization, we compared in vivo neovascularization by

From the ¹Department of Ophthalmology, Wakayama Medical University, Wakayama, Japan; the ²Department of Ophthalmology, University of Cincinnati Medical Center, Cincinnati, Ohio; and the ³Department of Anatomy, Graduate School of Medicine, Osaka City University, Osaka, Japan.

Presented at the annual meeting of the Association for Research in Vision and Ophthalmology, Fort Lauderdale, Florida, April 2006.

Supported by Grants C11591871 (SS) and C16590150 (KD) from the Ministry of Education, Science, Sports and Culture of Japan.

Submitted for publication September 12, 2006; revised October 15 and December 25, 2006 and January 22, 2007; accepted May 1, 2007.

Disclosure: S. Fujita, None; S. Saika, None; W. Whei-Yang Kao, None; K. Fujita, None; T. Miyamoto, None; K. Ikeda, None; Y. Nakajima, None; Y. Ohnishi, None

The publication costs of this article were defrayed in part by page charge payment. This article must therefore be marked "advertisement" in accordance with 18 U.S.C. §1734 solely to indicate this fact.

Corresponding author: Shuko Fujita, Department of Ophthalmology, Wakayama Medical University, 811-1 Kimiidera, Wakayama, 641-0012, Japan; shuko@wakayama-med.ac.jp.

TABLE 1. Sequences of Primers and Probes

Transcript	Sequence
mTGFβ1	F: 5'-gca aca tgt gga act cta cca gaa-3' R: 5'-gac gtc aaa aga cag cca ctc-3' P: 5'-acc ttg gta acc ggc tgc tga ccc-3'
mVEGF	F: 5'-agc gga gaa agc att tgt ttg-3' R: 5'-caa cgc gag tct gtg ttt ttg-3' P: 5'-cca aga tcc gca gac gtg taa atg ttc c-3

F, forward primer; R, reverse primer; P, probe.

cauterization in WT and *Tnfa*^{-/-} mice and also examined the effects of TNFα on neovascularization using an in vitro model of cultured vascular endothelial cells. Our data indicated that TNFα might directly block neovascularization while it suppressed TGFβ1 and VEGF expression by fibroblasts in situ.

MATERIALS AND METHODS

Experiments were approved by the DNA Recombination Experiment Committee and the Animal Care and Use Committee of Wakayama Medical University and were conducted in accordance with the ARVO Statement for the Use of Animals in Ophthalmic and Vision Research.

Effects of TNFα and TGFβ1 on VEGF Expression in Ocular Fibroblasts

Real-time reverse transcription-polymerase chain reaction (RT-PCR) with TaqMan probes was used to examine the effect of TNFα on VEGF expression by corneal fibroblasts using protocols previously described.¹⁶ Corneal stromal cells derived from explant outgrowth of 2-day-old mice were obtained. Confluent cells were treated with human recombinant TGFβ1 (2 ng/mL; R&D Systems, Minneapolis, MN) or combination TGFβ1 (2 ng/mL) and TNFα (5 or 10 ng/mL; R&D Systems) for 24 hours. Six wells were used for each culture condition. Total RNA extracted was subjected to real-time RT-PCR analysis for VEGF mRNA (Table 1). Data were analyzed by unpaired *t*-test.

In Vitro Coculture Experiment

A commercial coculture system of human vascular endothelial cells (HUVECs) and fibroblasts (NV kit; Kurabo, Tokyo, Japan) was used to examine effects of cytokines on tube-like structure formation. Using this coculture of HUVECs and fibroblasts, we examined the effect of TNFα (5 ng/mL; R&D Systems) on TGFβ1 (0.5 or 1 ng/mL) and VEGF-A (10 ng/mL; Kurabo) stimulation of vessel-like tube formation according to the protocol provided by the manufacturer. Tube tissue was detected by immunostaining with anti-CD31, an endothelium marker. Immune reactivity was visualized by diaminobenzidine color reaction, as previously reported.⁷

Induction of Stromal Neovascularization by Central Corneal Cauterization in Mice

Tnfa^{-/-} mouse in C57BL/6 genetic background was a generous gift from H. Tsutsui (Hyogo Medical University, Hyogo, Japan).¹⁸ Corneal neovascularization from the limbal vessels was induced by central corneal cauterization in one eye of individual WT or *Tnfa*^{-/-} mice using a disposable tool (Optemp; Mod-Tronic Instruments; Brampton, ON, Canada). Experimental mice were killed after 7, 14, or 21 days of injury. Eyes were then enucleated and subjected to cryosection for immunohistochemistry or extraction of total RNA. Ten WT and 10 *Tnfa*^{-/-} corneas were used for histologic examination at each time point. The same numbers of corneas were used for the preparation of total RNA.

Immunohistochemistry

Immunohistochemical examination was performed to detect stromal neovascularization with anti-CD31 and to measure the production of

TGFβ1 and VEGF with respective antibodies. Cryosections (7 μm) were fixed in cold acetone and processed for immunohistochemical examination, as previously reported.^{19,20} The following antibodies were diluted in PBS: rat monoclonal anti-CD31 (PECAM) antibody, rabbit polyclonal anti-TGFβ1 antibody, and rabbit polyclonal anti-VEGF antibody (all 1:100 in phosphate-buffered saline, Santa Cruz Biotechnology, Santa Cruz, CA). After fluorescein-conjugated secondary antibody reaction and DAPI nuclear counterstaining, the specimens were observed under a fluorescent microscope. Negative control staining was performed by omission of primary antibodies, which did not yield specific staining (data not shown).

Detection of mRNAs of TGFβ1 and VEGF in Burned Corneas

Total RNA extracted from two corneas was subjected to analysis of TGFβ1 and VEGF mRNA by real time RT-PCR. Average values from five specimens (10 corneas) at each time point were analyzed by unpaired *t*-test using procedures previously reported (Table 1).^{16,17}

RESULTS

Effects of TGFβ1 and TNFα on VEGF Expression by Cultured Fibroblasts

Healing of injured corneas is often complicated by neovascularization with the upregulation of VEGF, a major angiogenic factor in physiological and pathologic conditions. Expression of VEGF might be modulated by various cytokines, such as TGFβ1. In the present study, stromal fibroblast cultures were used to examine whether TNFα affects the expression of VEGF. Adding 2 ng/mL recombinant TGFβ1 caused a twofold increase of VEGF mRNA expression by cultured cornea fibroblasts. Upregulation of VEGF mRNA by TGFβ1 was abolished by the addition of TNFα (5 and 10 ng/mL) to the medium (Fig. 1).

Effects of TGFβ1 and TNFα on Formation of Vessel-like Structure by HUVECs In Vitro

As shown in Figure 1, TNFα antagonized the effects of TGFβ1 on VEGF expression by cultured fibroblasts. Thus, it is likely

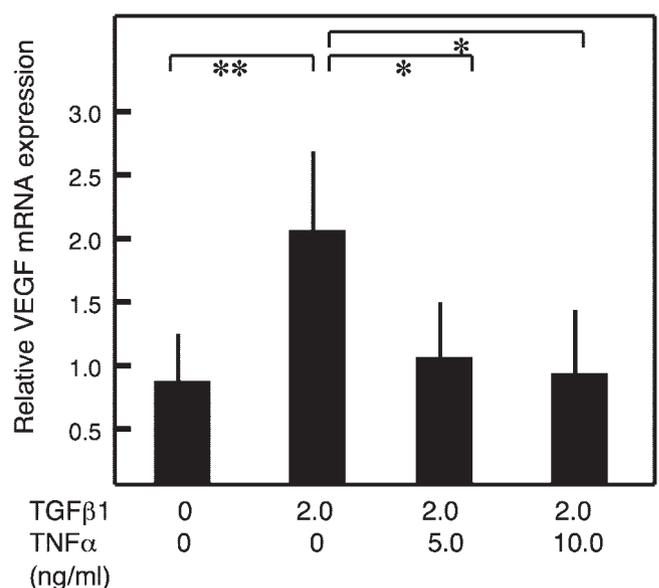


FIGURE 1. VEGF expression in mouse corneal fibroblast culture in the presence of TGFβ1, TNFα, or both was examined by TaqMan real-time RT-PCR. TGFβ1 (2 ng/mL) upregulated VEGF mRNA expression, and this was counteracted by the further addition of 5 or 10 ng/mL TNFα. Bar, SD. **P* < 0.05. ***P* < 0.01, respectively.

that TNF α may suppress the vascularization promoted by TGF β 1. An in vitro model of fibroblasts and HUVEC cocultures was used to examine this possibility. Dense CD31 immunoreactivity was detected in tissue where HUVECs formed a vessel-like tube structure. Without exogenous ligands, HUVECs grown on the fibroblast feeder layer formed a vessel-like tube tissue. Adding TGF β 1 (0.5 ng/mL and 1 ng/mL) promoted the formation of tube-like tissue (data not shown). Adding 5 ng/mL TNF α abolished the formation of a vessel-like tube structure by HUVECs cocultured with fibroblasts, despite the presence and absence of TGF β 1 (Fig. 2). Similarly, adding TNF α abolished the formation of a vessel-like structure in the presence of VEGF-A alone and VEGF-A combined with TGF β 1 (Fig. 3).

In Vivo Stromal Neovascularization in Cauterized Corneas

To elucidate the possible roles of TNF α on neovascularization after corneal injury, WT and *Tnf α ^{-/-}* mice were subjected to corneal cauterization, as described in Materials and Methods. In WT mouse corneas, CD31-labeled neovascularization was not detected at day 7 of cauterization (Fig. 4A). At day 14 (Fig. 4B) and day 21 (Fig. 4C) of cauterization, a few new vessels were seen in peripheral corneal stroma adjacent to the limbus. On the other hand, in *Tnf α ^{-/-}* mice, stromal neovascularization was readily detectable as early as day 7 (Fig. 4D) and then increased at day 14 (Fig. 4E). Dense CD31 immunoreactivity was detected in the central corneal stroma of a *Tnf α ^{-/-}* mouse (Fig. 4F).

Expression of Cytokines during Stromal Neovascularization

Given the results of this study, it is plausible to hypothesize that TNF α may have a pivotal role in modulating the expression of angiogenic factors, such as TGF β 1 or VEGF during corneal wound healing. Real-time RT-PCR was used to examine this possibility. Results of real-time RT-PCR showed that cauterization in the central cornea caused an increase of TGF β 1 mRNA expression that persisted until day 21 of cauterization in WT and *Tnf α ^{-/-}* mice. TGF β 1 mRNA expression was compa-

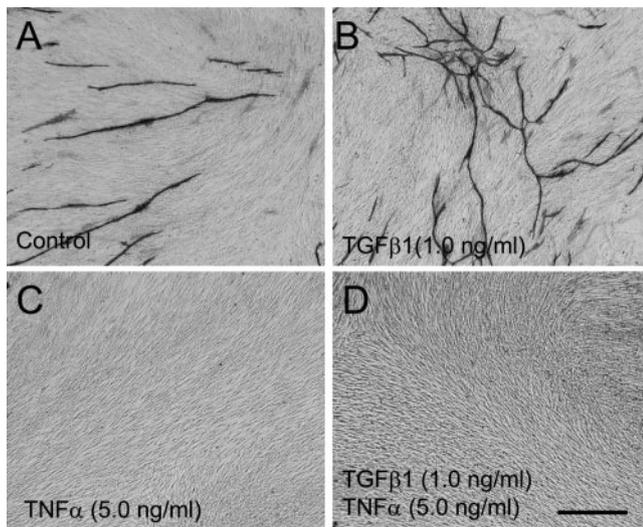


FIGURE 2. Vessel-like tube formation as detected by CD31 immunostaining in coculture of fibroblasts and human vascular endothelial cells in the presence of TGF β 1. CD31-immunoreactive vessel-like tissue was seen on the fibroblast feeder layer in the control culture without specific ligands (A). (B) More dense vessel-like tissue with more frequent branching was seen with 1 ng/mL TGF β 1. (C) Adding TNF α (5 ng/mL) blocked the formation of such CD31-labeled structures, even in the presence of TGF β 1 (D). Bar, 50 μ m.

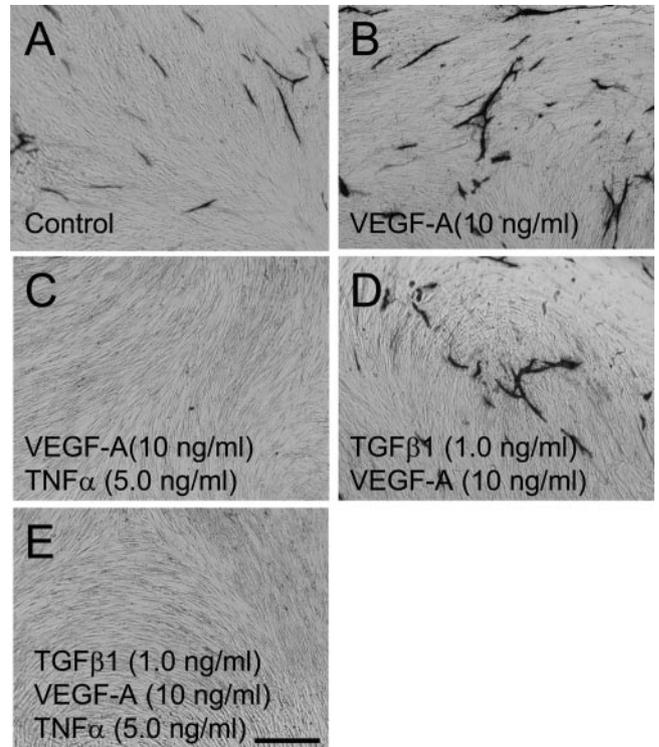


FIGURE 3. Vessel-like tube formation was detected by CD31 immunostaining in coculture of fibroblasts and human vascular endothelial cells in the presence of VEGF-A. (A) CD31-immunoreactive vessel-like tissue was seen in the control culture without TGF β 1, VEGF, or both. (B) More dense vessel-like tissue with extensive branching was seen with 10 ng/mL VEGF-A. (C) Adding TNF α blocked the formation of such CD31-labeled structures in the presence of VEGF-A. The formation of vessel-like structures in the presence of TGF β 1 and VEGF-A (D) was also abolished by the further addition of TNF α (5 ng/mL; E). Bar, 50 μ m.

table between WT and *Tnf α ^{-/-}* corneas at day 7 of injury, and it was more greatly enhanced in *Tnf α ^{-/-}* corneas than in WT corneas at day 21 (Fig. 5A). Results of immunohistochemical analysis of TGF β 1 protein were consistent with those of real-time RT-PCR in that TGF β 1 protein was faintly detectable, with a minor peak at day 14 in WT corneal stroma throughout the intervals examined. In *Tnf α ^{-/-}* mice, this cytokine was readily observed in stroma at all the time points examined (Fig. 5B).

Expression patterns of VEGF mRNA and protein were similar to those of TGF β 1. Cauterization in central corneas of WT mice induced an increase of VEGF mRNA expression that peaked at day 14 (Fig. 5C) and then declined to a lower level at day 21. In contrast, the elevated VEGF mRNA expression maintained at a higher level at days 14 and 21 in *Tnf α ^{-/-}* corneas was comparable to that of WT mice. VEGF protein was not seen at day 7 and was faintly detected in WT corneal stroma at days 14 and 21. In *Tnf α ^{-/-}* mice, this cytokine was markedly observed in all the time points (Fig. 5D). VEGF mRNA expression augmented by the lack of TNF α was more prominent than by the lack of TGF β 1.

DISCUSSION

It has been demonstrated that inhibiting TNF α signaling by blocking TNF α receptor did not prevent the development of corneal neovascularization in a rat model.¹⁹ However, the report did not address whether the suppression (or lack) of TNF α signal did not affect, or could promote, corneal neovascularization. We previously reported that *Tnf α ^{-/-}* mice experienced more severe fibrosis, inflammation, and neovascular-

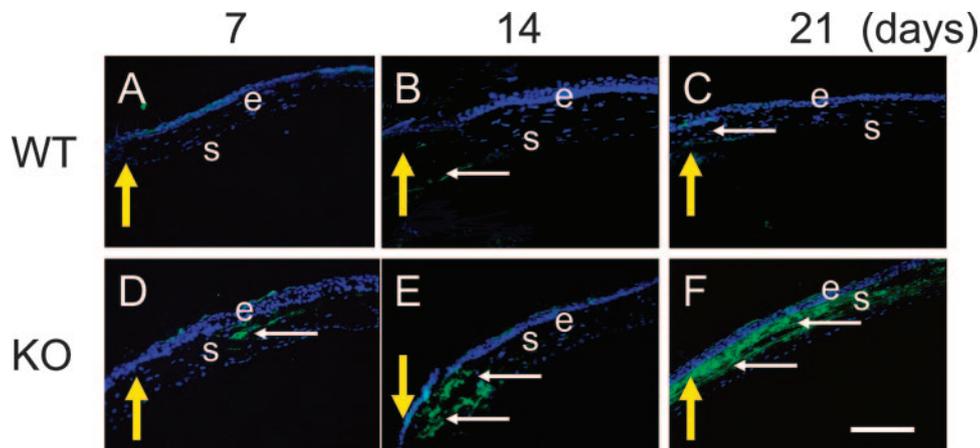


FIGURE 4. Immunohistochemical detection of CD31 for stromal neovascularization. CD31 immunoreactivity indicates the presence of neovascularization in the affected stroma. At day 7, no CD31 immunoreactivity was observed in a WT cornea (A), and then a few CD31-labeled spots were seen in the peripheral cornea close to the limbus at days 14 (B) and 21 (C). In *Tnfa*^{-/-} mice, at day 7, CD31-labeled neovascularization was readily detected in corneal stroma (D). The density of such neovascularization was increased at days 14 (E) and 21 (F) in *Tnfa*^{-/-} mice. White arrows: CD31-labeled neovascularization; yellow arrows: border between cornea and limbus. e, corneal epithelium; s, stroma; DAPI nuclear localization. Bar, 100 μ m.

ization in alkali-burned corneas than did WT mice.¹⁶ However, in our previous experimental model of a cornea alkali burn, large areas of ocular surfaces including conjunctiva, limbus, and cornea were injured, and the regenerated ocular surface epithelium was of conjunctival origin associated with neovascularization in the healing cornea. Moreover, in such an alkali burn model, we did not examine whether TNF α could antagonize VEGF-based neovascularization that could be greatly enhanced by TGF β 1. Therefore, we did not know the role of TNF α in corneal neovascularization or its potential in antagonizing TGF β 1 effects in pathogenesis.^{20,21}

In the present study, using *in vitro* coculture of HUVECs and fibroblasts and *in vivo* *Tnfa*^{-/-} mice, we have demon-

strated that TNF α plays a pivotal role in modulating neovascularization. Adding TNF α to the culture medium blocked the tube-like structure formation of HUVECs, even in the presence of TGF β 1 and VEGF. Our data demonstrated that TNF α can directly counteract VEGF action on tube-like structure formation and can suppress VEGF upregulation stimulated by TGF β 1. *In vivo*, central corneal cauterization caused an upregulation of TGF β 1 and VEGF (Fig. 5). Thus, the expression of TNF α might reduce neovascularization by antagonizing the effects of TGF β 1 and VEGF. This suggestion is supported by the observation that *Tnfa*^{-/-} mice exhibited more severe neovascularization than did WT mice after central corneal cauterization.

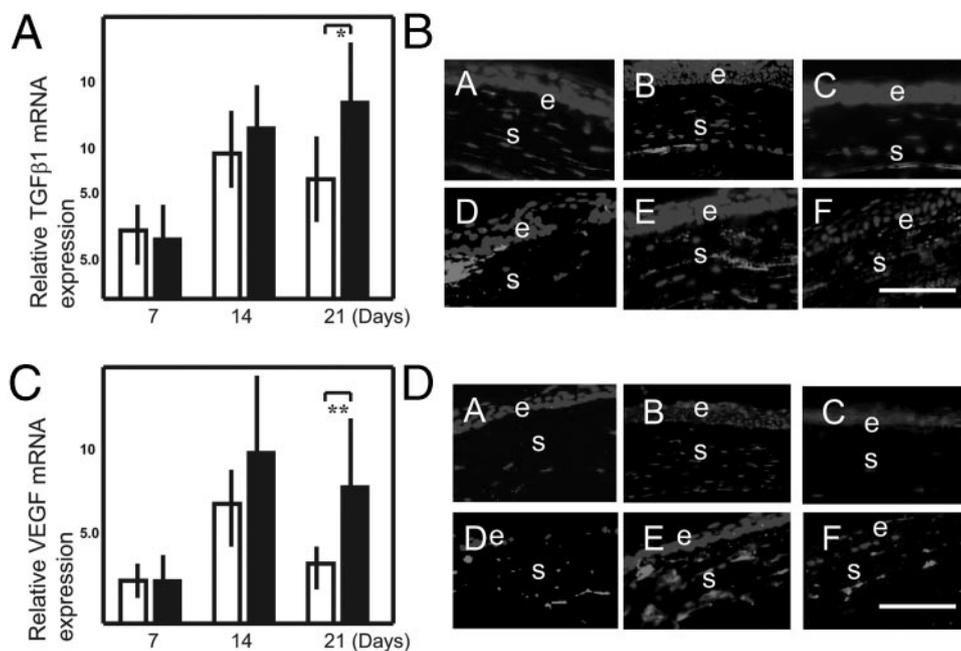


FIGURE 5. Expression of angiogenic growth factors in burned corneas. Real-time RT-PCR showed that cauterization in the central cornea caused an increase of TGF β 1 and VEGF mRNAs in the cornea until day 21 in WT and knockout mice. (A) Real-time RT-PCR shows the upregulation of TGF β 1 mRNA during the interval examined up to day 21 in WT mice (open bars). At day 21, the expression level of TGF β 1 in tissue was significantly higher in *Tnfa*^{-/-} mice (closed bar) than in WT mice. (B) Immunohistochemistry confirmed the upregulation of TGF β 1 and VEGF revealed by real-time RT-PCR. In WT corneas, TGF β 1 protein was not observed in the stroma at day 7 (BA), whereas it was detected in the corneal stroma at days 14 (BB) and 21 (BC). Overall protein level of TGF β 1 in the stroma was more marked in *Tnfa*^{-/-} corneas at days 7 (BD), 14 (BE), and 21 (BF). (C) Real-time RT-PCR reaction showed the upregulation of VEGF with a peak at

day 14 and a decline at 21 days in WT corneas (open bars). At days 14 and 21, expression levels of VEGF mRNA in tissue were significantly higher in *Tnfa*^{-/-} corneas (closed bar) than in WT corneas. (D) Immunohistochemistry shows no VEGF protein expression in WT corneal stroma at day 7 (DA), whereas faint expression is seen at days 14 (DB) and 21 (DC). On the other hand, marked VEGF protein expression is observed in knockout stroma at days 7 (DD), 14 (DE), and 21 (DF). e, corneal epithelium; s, stroma; DAPI nuclear localization. Error bars, SD (A, C). * $P < 0.05$. ** $P < 0.01$. Scale bars, 50 μ m (B, D).

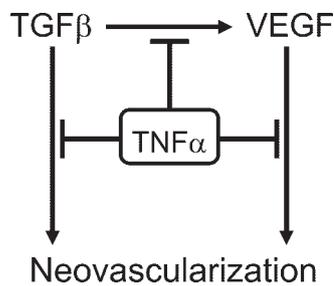


FIGURE 6. Hypothetical mechanism of TNF α on corneal neovascularization. TNF α may directly block the angiogenic activity of TGF β 1 and VEGF, and it inhibits the upregulation of TGF β 1 and VEGF by fibroblasts.

Loss of TNF α caused persistent upregulation of VEGF (Fig. 5), which might have accounted for the consequences of augmented neovascularization by central corneal cauterization in *Tnf α ^{-/-}* mice. Moreover, in vitro coculture experiments using fibroblast feeder and HUVECs showed that TNF α directly blocked the vessel-like tube formation of HUVECs in the presence of TGF β 1 and VEGF. In corneal fibroblast culture, TNF α blocked the upregulation of TGF β 1 VEGF, also contributing to the antiangiogenic effects of TNF α . Thus, the antiangiogenic effect of endogenous TNF α can be explained in part by a direct counteraction of TNF α against angiogenic reaction promoted by TGF β 1 and VEGF in addition to the suppression of upregulation of such angiogenic growth factors in vivo (Fig. 6).

Many investigators have reported pleiotropic effects of TNF α on various forms of pathogenesis. For example, systemic inflammatory diseases, such as rheumatoid arthritis, can be benefited by the administration of neutralizing anti-TNF α antibody, suggesting that reduction in the local TNF α level is favorable for suppressing inflammation.²² Such anti-TNF α antibody neutralization is effective in treating bleomycin-induced pulmonary fibrosis.¹⁴ On the other hand, experimental arthritis or pulmonary fibrosis in mice is reportedly more severe in *Tnf α ^{-/-}* mice than in WT mice.^{13,15} These findings indicate that partial reduction of TNF α and total loss of cytokine exhibit different actions to cells that are implicated in the disease process. It had been suggested that infliximab, a neutralizing antibody against TNF α used clinically for the treatment of rheumatoid arthritis, might also be effective in the treatment of inflammatory ocular diseases.²² However, our present data implicate that the use of infliximab in treating ocular inflammation involving neovascularization may produce adverse effects.

Taken together, our present findings and reports by other investigators indicate that TNF α is a two-edged sword in modulating the pathogenesis of various diseases characterized by inflammation and fibrosis. The presence of TNF α signaling can alleviate or worsen the diseases. TNF α activates a complicated signaling network that includes c-Jun N-terminal kinase (JNK) and nuclear factor- κ B (NF- κ B); for a review, see Wajant et al.⁸ JNK and NF- κ B signals further activate various pathways, such as activator protein-1 or Smads, by inducing Smad7.⁸ However, it has been well shown that signaling through TNF α receptor pathways counteract the TGF β signal at multiple steps, providing a plausible explanation of our observations. Further studies are needed to delineate the details of signaling cross-talk among soluble factors involving TNF α .

References

1. Saika S. TGF β pathobiology in the eye. *Lab Invest.* 2006;86:106-115.

- Planck SR, Rich LF, Ansel JC, Huang XN, Rosenbaum JT. Trauma and alkali burns induce distinct patterns of cytokine gene expression in the rat cornea. *Ocul Immunol Inflamm.* 1997;5:95-100.
- Imanishi J, Kamiyama K, Iguchi I, Kita M, Sotozono C, Kinoshita S. Growth factors: importance in wound healing and maintenance of transparency of the cornea. *Prog Retin Eye Res.* 2000;19:113-129.
- Klenkler B, Sheardown H. Growth factors in the anterior segment: role in tissue maintenance, wound healing and ocular pathology. *Exp Eye Res.* 2004;79:677-688.
- Edelman JL, Castro MR, Wen Y. Correlation of VEGF expression by leukocytes with the growth and regression of blood vessels in the rat cornea. *Invest Ophthalmol Vis Sci.* 1999;40:1112-1123.
- Lai CM, Spilisbury K, Brankov M, Zaknich T, Rakoczy PE. Inhibition of corneal neovascularization by recombinant adenovirus mediated antisense VEGF RNA. *Exp Eye Res.* 2002;75:625-634.
- Saika S, Ikeda K, Yamanaka O, et al. Therapeutic effects of adenoviral gene transfer of bone morphogenic protein-7 on a corneal alkali injury model in mice. *Lab Invest.* 2005;85:474-486.
- Wajant H, Pfizenmaier K, Scheurich P. Tumor necrosis factor signaling. *Cell Death Differ.* 2003;10:45-65.
- Lipsky PE, van der Heijde DM, St Clair EW, et al. Infliximab and methotrexate in the treatment of rheumatoid arthritis: anti-tumor necrosis factor trial in rheumatoid arthritis with concomitant therapy study group. *N Engl J Med.* 2000;343:1594-1602.
- Haraoui B. The anti-tumor necrosis factor agents are a major advance in the treatment of rheumatoid arthritis. *J Rheumatoid Suppl.* 2005;72:46-47.
- Liu JY, Brass DM, Hoyle GW, Brody AR. TNF- α receptor knockout mice are protected from the fibroproliferative effects of inhaled asbestos fibers. *Am J Pathol.* 1998;153:1839-1847.
- Liu JY, Sime PJ, Wu T, et al. Transforming growth factor- β 1 overexpression in tumor necrosis factor- α receptor knockout mice induces fibroproliferative lung disease. *Am J Respir Cell Mol Biol.* 2001;25:3-7.
- Kuroki M, Noguchi Y, Shimono M, et al. Repression of bleomycin-induced pneumopathy by TNF. *J Immunol.* 2003;170:567-574.
- Fujita M, Shannon JM, Morikawa O, Gauldie J, Hara N, Mason RJ. Overexpression of tumor necrosis factor- α diminishes pulmonary fibrosis induced by bleomycin or transforming growth factor- β . *Am J Resp Cell Mol Biol.* 2003;29:669-676.
- Campbell IK, O'Donnell K, Lawlor KE, Wicks IP. Severe inflammatory arthritis and lymphadenopathy in the absence of TNF. *J Clin Invest.* 2001;107:1519-1527.
- Saika S, Ikeda K, Yamanaka O, et al. Loss of tumor necrosis factor alpha potentiates transforming growth factor beta-mediated pathogenic tissue response during wound healing. *Am J Pathol.* 2006;168:1848-1860.
- Saika S, Ikeda K, Yamanaka O, et al. Expression of Smad7 in mouse eyes accelerates healing of corneal tissue after exposure to alkali. *Am J Pathol.* 2005;166:1405-1418.
- Pasparakis M, Alexopoulou L, Episkopou V, Kollias G. Immune and inflammatory responses in TNF alpha-deficient mice: a critical requirement for TNF alpha in the formation of primary B cell follicles, follicular dendritic cell networks and germinal centers, and in the maturation of the humoral immune response. *J Exp Med.* 1996;184:1397-1411.
- Coxon A, Bolon B, Estrada J, et al. Inhibition of interleukin-1 but not tumor necrosis factor suppresses neovascularization in rat models of corneal angiogenesis and adjuvant arthritis. *Arthritis Rheum.* 2002;46:2604-2612.
- Yamane K, Ihn H, Asano Y, Jinnin M, Tamaki K. Antagonistic effects of TNF- α on TGF- β signaling through down-regulation of TGF- β receptor type II in human dermal fibroblasts. *J Immunol.* 2003;171:3855-3862.
- Abraham DJ, Shiwen X, Black CM, Sa S, Xu Y, Leask A. Tumor necrosis factor α suppresses the induction of connective tissue growth factor by transforming growth factor- β in normal and scleroderma fibroblasts. *J Biol Chem.* 2000;275:15220-15225.
- Baughman RP, Bradley DA, Lower EE. Infliximab in chronic ocular inflammation. *Int J Clin Pharmacol Ther.* 2005;43:7-11.