Overexpression of Transforming Growth Factor Beta-2 and Its Receptor in Rhinophyma: An Alternative Mechanism of Pathobiology

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Proliferative scarring is one of the clinical features of rhinophyma. The following study was undertaken to test the authors' hypothesis that fibrosis might also play an important role in the pathobiology of rhinophyma. The rhinophyma specimens were obtained from 5 white men (mean age, 67.8 years). Normal skin biopsies near benign facial lesions from 5 additional white men of similar age were obtained to serve as controls. Peroxidase-labeled immunohistochemical staining was performed in the rhinophyma and normal skin specimens for the presence of transforming growth factor (TGF) beta-2 and/or TGF-β II receptor. Histological slides were then measured for the intensity of staining for TGF-β2 and TGF-β II receptor using a computer-aided imaging system. The dermis of the rhinophyma tissue displayed stronger immunoreactivity of TGF-β2 ($p = 0.014$) and TGF-β II receptor ($p = 0.006$) compared with the normal skin. The results of this study demonstrate the overexpression of the fibrogenic protein TGF-β 2 and TGF-β II receptor in rhinophyma tissues. These findings support the authors' hypothesis that fibrosis may also play an important role in the pathobiology of rhinophyma.

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Rhinophyma is a form of end-stage acne rosacea of the nasal skin with unclear etiology. It distorts both appearance and function of the nose. Because the pathobiology of rhinophyma is not fully elucidated, its treatment remains problematic. The medical therapy of rhinophyma is limited to treating obvious stimulating factors for acne rosacea. However, the mainstay in treatment for rhinophyma is still surgical. Many surgical modalities, such as simple scalpel excision, cryosurgery, dermabrasion, electrocautery, and laser therapy, have been used to treat rhinophyma but no single modality is superior.[1,2]

The proliferation of sebaceous glands have been thought to be the main mechanism in the pathobiology of rhinophyma.[3] However, several physical characteristics of the disease are also consistent with proliferative scarring.[1] In a recent report, Tope and Sangueza[4] found a second histopathological pattern of rhinophyma, a fibrous variant, in a 71-year-old man. Therefore, more than one mechanism could possibly be postulated in the pathobiology of rhinophyma.

Regardless of the exact etiology for rhinophyma, the dense fibrosis and increased collagen production are not unlike other fibrotic conditions that occur in other organs. These other fibrotic and proliferative disorders have been associated with overproduction of transforming growth factor beta (TGF-β).[5] This is particularly true of the isoforms TGF-β1 and TGF-β2.[6,7] TGF-β3 trends to decrease fibrosis and scarring.[6,8] Data from our laboratory and others suggest that TGF-β2 is the most fibrogenic of the isoforms.[9–11]

The purpose of the current study was to test our hypothesis that fibrosis might also play an important role in the pathobiology of rhinophyma,

based on our clinical observation of rhinophyma and our understanding of proliferative scars. To do this, we investigated the expressions of the fibrogenic cytokine TGF-β2 and the TGF-β II receptor in excised rhinophyma tissue.

Patients and Methods

Patients

From October 1997 to September 1998, 5 white men with primary rhinophyma who were treated at the Bay Pines VA Medical Center, Bay Pines, FL, were enrolled in this study, which was approved by the institutional review board. The mean age of these patients at the time of treatment was 67.8 years (age range, 59–74 years), and the duration of the disease was from 10 to 25 years. Rhinophyma tissues were obtained from 5 consecutive patients who underwent "cold" knife excisions of their grossly disfiguring lesions. Five additional white men of similar age undergoing simple excision of benign nasal skin lesions during the study period were chosen as control subjects. Normal skin specimens adjacent to the lesions were obtained from these 5 patients.

Tissue Collection and Processing

Rhinophyma or normal skin specimens were collected at the time of operation. The tissue sample was frozen immediately in liquid nitrogen, embedded in optimal cutting temperature compound (Miles Laboratories, Inc., Naperville, IL), and then stored at −70°C until it was used. Cryostat sections 8-μm thick were cut transversely from all specimens and were prepared for immunohistochemical staining.

An additional tissue sample from each patient was also fixed in 10% buffered formalin solution, embedded in paraffin, and cut in 10-μm cross-sections. Each of these sections was then stained with hematoxylin–eosin stain for routine histological evaluation.

Immunohistochemical Staining

Peroxidase-labeled immunohistochemical staining was performed on the frozen sections of all rhinophyma and normal skin specimens for the presence of TGF-β2 and TGF-β II receptor. The specific procedure for immunohistochemical staining for localization of TGF-β2 or TGF-β II receptor antigen in the tissue sections has been described in detail elsewhere.[12,13] Briefly, the frozen sections of each tissue specimen were incubated with phosphate-buffered saline (PBS) for 30 minutes at room temperature. The sections were fixed in 4% paraformaldehyde for 20 minutes. Endogenous peroxidase was blocked by a 30-minute incubation in 1% hydrogen peroxide in PBS. After PBS washing, the sections were incubated with diluted normal goat serum (1:10) to block nonspecific protein binding, and were then incubated for 2 hours at room temperature in a humidified chamber with rabbit antibody specific for TGF-β2 or TGF-β II receptor (Santa Cruz Biotechnology Inc., Santa Cruz, CA), diluted to 1:100 in 1% bovine serum albumin in PBS. Unbound antibody was removed by washing with PBS, and bound antibody was localized with biotinylated goat antibody. After applying avidin–biotin–complex solution (Vector Labs, St. Louis, MO), 3,3-diaminobenzidine with hydrogen peroxide was added as a substrate. Finally, the sections were counterstained with hematoxylin, and dehydrated through alcohol and xylene. Positive staining was evidenced as a brown precipitate. Control staining was also performed using PBS as the primary antibody and as the secondary antibody separately.

Evaluation for Intensity of Staining

The technique used to evaluate intensity has been described previously by Wang and colleagues.[7] Sections of immunohistochemical staining were photographed using a Leitz Dialux microscope (Ernst Leitz, Wetzlar, Germany) and Kodak Ektachrome 64 ASA film (Eastman Kodak, Rochester, NY) corrected for tungsten light. The photographs were then digitized using a standard desktop flatbed scanner. The staining was changed from brown to black on a white background. The intensity of staining was measured in 10 randomly chosen, noncontiguous, nonoverlapping squares of each section using Sigma Scan (Jandel Scientific, Corte Madera, CA). The intensity was measured between 0 and 255 (0 being no light intensity or black and 255 being the greatest intensity or pure white). Because the staining was displayed as black on white, we subtracted the

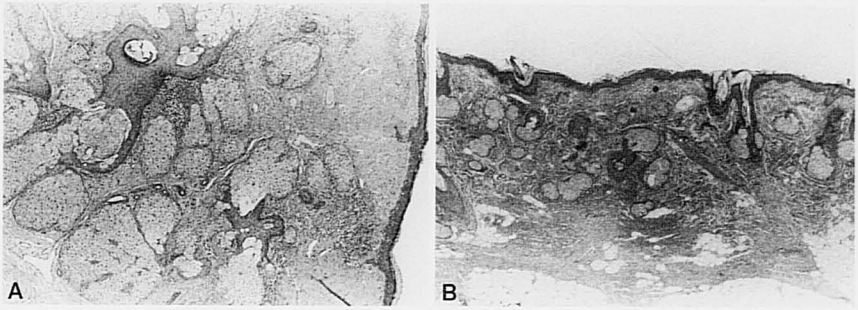

Fig 1. (A, B) *Routine histology. The rhinophyma tissue shows the hyperplasia of sebaceous glands with interstitial fibrosis and chronic nonspecific inflammation* (A) *compared with the normal nasal skin* (B). *H&E, original magnification ×100 before XX% reduction.*

Intensities of Immunohistochemical Staining in the Dermis of Rhinophyma and Normal Facial Skin Tissues

Specimen	TGF-β2	TGF-β II Receptor
Rhinophyma (N = 5)	146.8 ± 6.6[a]	141.1 ± 5.6[b]
Normal skin (N = 5)	130.6 ± 3.9	125.8 ± 1.2

p-Values between two groups.
[a] $p = 0.014$.
[b] $p = 0.006$.

intensity measurement from 255 to depict greater staining with a higher value. All data are expressed as mean ± standard deviation. An unpaired Student's *t*-test was used for statistical analysis between two groups. Significance was considered if $p < 0.05$.

Results

Histologically, hyperplasia of the sebaceous gland with variable degrees of interstitial fibrosis and chronic inflammation was demonstrated in all of the rhinophyma tissue compared with the normal skin (Fig 1) Interestingly, the differences of brown precipitates in sections between rhinophyma and normal skin tissues after immunohistochemical staining were observed principally in the dermis.

The dermis of the rhinophyma displayed stronger immunostaining of TGF-β2 compared with the normal skin (Table, Fig 2). The stronger immunostaining of TGF-β II receptor was also evidenced in the rhinophyma tissue compared with the normal skin (see the Table, Fig 3). The intensities of staining for TGF-β2 and TGF-β II receptor (summarized in the Table) were significantly higher in the rhinophyma tissues compared with the normal skin ($p < 0.05$).

Discussion

The results from this study clearly demonstrate that TGF-β2 and its receptor are overly expressed in rhinophyma tissue. These expressions are also confirmed on a quantitative basis (see the Table). Because TGF-β2 has been found to have the most significant proliferative effect in dermal proliferative diseases, such as in proliferative scars and Dupuytren's disease,[6,7,9–11] our findings support the hypothesis that fibrosis may also play an important role in the pathobiology of rhinophyma.

Histologically, widespread sebaceous gland hyperplasia, a mild degree of telangiectasia and fibrosis, and numerous follicular cysts are usually seen in the more common form of rhinophyma. A second, less common form usually shows considerable telangiectasia, prominent dermal fibrosis, and a marked decrease or even absence of sebaceous and appendage structures.[4] Clinically, two different types of rhinophyma are noted, but sometimes are less distinguishable. One has a scarred, fibrotic, solid appearance and the other is formed mainly by proliferated sebaceous glands.[1]

The exact pathobiology of rhinophyma remains

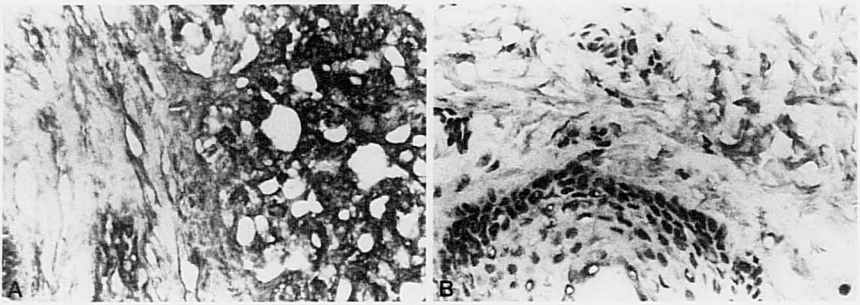

Fig 2. (A, B) *Immunohistochemical staining demonstrates a greater intensity of transforming growth factor beta-2 expression (as shown with more darkly stained patchy areas) in the dermis of rhinophyma tissue* (A) *compared with the normal skin* (B). *H&E, original magnification ×400 before XX% reduction.*

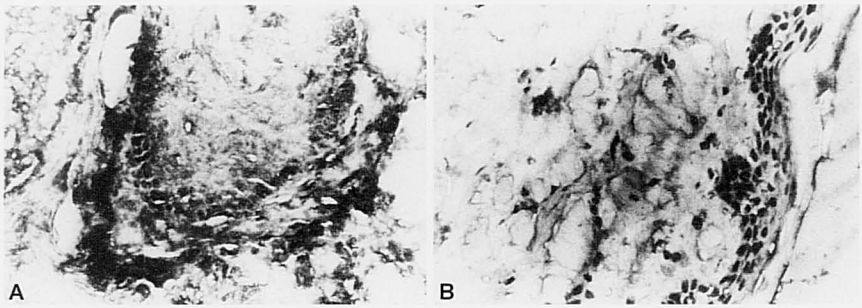

Fig 3. (A, B) *Immunohistochemical staining demonstrates a greater intensity of transforming growth factor beta II receptor expression (as shown with more darkly stained patchy areas) in the dermis of rhinophyma tissue* (A) *compared with the normal skin* (B).*H&E, original magnification ×400 before XX% reduction.*

unclear. Fibrosis has been proposed to play an important role in the pathobiology of rhinophyma.[1,4] Other factors, such as focal bacterial infection, vitamin deficiencies, endocrine abnormalities, genetics, and environmental exposure, have been proposed but are less well documented in the literature.[1,2] The histological finding of fibrosis may correlate clinically with a fibrous tumorlike hypertrophy of the rhinophyma lesion. In the case report of Tope and Sangueza,[4] the fibrosis with plump and stellate fibroblasts throughout the dermis is suggestive of a fibrous variant of rhinophyma. In the current study, TGF-β2 and its receptor were overly expressed in rhinophyma compared with the normal skin specimens. Although this series is small, these findings suggest that fibrogenesis may possibly play an important role in the pathobiology of rhinophyma.

It has been hypothesized that an abnormal response to the TGF-β2 isoform may in fact have a causative role in the formation of dermal proliferative disorders. In our recent studies, TGF-β2 has been demonstrated to increase collagen I and III synthesis and fibroblast cell kinetics in proliferative scars, and fibroblast deoxyribonucleic acid synthesis in keloids.[7,14–16] Similarly, Schmid and associates[17] have demonstrated increased expression of TGF-β type I and type II receptors in proliferative scars. Therefore, we believe that TGF-β2 plays a central role in the formation of both proliferative scars and keloids. In addition, TGF-β2 has also been demonstrated

to play an important role in other proliferative diseases, such as Dupuytren's contracture and vitreoretinopathy.[9,18]

If, in an expanded series, TGF-β2 is confirmed to play a role in the pathobiology of rhinophyma, downregulation or neutralization of this cytokine may provide a novel approach to treatment. Neutralizing antibodies to TGF-β2 have been demonstrated to reduce dermal scarring[19,20] and to decrease collagen production in explanted human proliferative scars.[7] Similarly, decreasing TGF-β2 production or secretion by compounds like mannose-6-phosphate,[6,21] decorin,[22,23] tamoxifen,[24] and interferon alpha-2b[25] have been used to treat other fibrotic diseases. This study suggests that these agents may be useful in the treatment of rhinophyma.

Conclusion

The results of this study, for the first time, demonstrate the overexpression of TGF-β2 and TGF-β II receptor in rhinophyma tissues. These findings lend support to our hypothesis that fibrogenesis may also play an important role in the pathogenesis of rhinophyma. If further studies support this hypothesis, possible treatments aimed at neutralizing or downregulating TGF-β2 may prove to be useful treatments for this condition.

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