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J Clin Invest. 1992;90(1):1-7. https://doi.org/10.1172/JCI115821.

Research Article

Transforming Growth Factor-β in Disease: The Dark Side of Tissue Repair

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Introduction

Inflammatory, immune, and tissue repair responses protect us against a hostile and dangerous environment. However, these responses sometimes fail, mistarget, or overshoot and harm what they were meant to protect. For example, prostaglandins are important proinflammatory mediators that can also cause unwanted, painful reactions. As a result, we spend a part of our lives taking aspirin and other inhibitors of prostaglandin synthesis. The theme we would like to develop in this review is that, while a growing body of evidence implicates transforming growth factor-β (TGF-β)[1] as a cytokine vital to tissue repair, it also is one whose excessive action may be responsible for the tissue damage caused by scarring in many serious diseases. We propose that the pathological consequences of the action of TGF-β be termed the "dark side" of tissue repair. Inhibitors of TGF-β may be important future drugs in controlling this condition.

TGF-β: basic biology

TGF-β, a multifunctional cytokine, plays an important role in embryonal development and in regulating repair and regeneration following tissue injury (1–3). It consists of a family of three isoforms, TGF-β1, 2, and 3, that are structurally and functionally closely related to one another. The TGF-βs are members of a superfamily of cytokines that includes other regulators of differentiation and tissue repair such as activin, Müllerian inhibitory substance, and bone morphogenetic proteins (4). In their active form, all of these substances are dimers of a 12-kD polypeptide that arises from a larger precursor through proteolytic processing.

Multiple events involving TGF-β take place in tissue repair after injury. Platelets contain high concentrations of TGF-β and upon degranulation at a site of injury release TGF-β into the surrounding tissue (5). TGF-β then initiates a complex sequence of events that promotes healing including: chemoattraction of monocytes and leukocytes (6–8); induction of angiogenesis (9); and control of the production of cytokines and other inflammatory mediators (10–12).

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Received for publication 29 April 1992.

1. *Abbreviation used in this paper:* TGF-β, transforming growth factor-β.

J. Clin. Invest.

0021-9738/92/07/0001/07 $2.00
Volume 90, July 1992, 1–7

Two additional features of the TGF-β response in injury may be the most important for the topic of this review: autoinduction of TGF-β production (13) and induction by TGF-β of increased deposition of extracellular matrix (9, 14, 15). TGF-β stimulates the synthesis of individual matrix components including fibronectin, in particular one of its variant forms, as well as tenascin, collagens, and proteoglycans (9, 16–20). It simultaneously blocks matrix degradation by decreasing the synthesis of proteases and increasing the levels of protease inhibitors (21, 22). TGF-β increases the expression of integrins and changes their relative proportions on the surface of cells in a manner that could facilitate adhesion to matrix (23). All these events can be beneficial in tissue repair. However, the dark side to the TGF-β effects is that the TGF-β-induced deposition of extracellular matrix at a site of tissue injury can lead to scarring and fibrosis. Furthermore, the ability of TGF-β to induce its own production may be the key to development of the scarring and fibrosis into chronic, progressive conditions that will in time obliterate the tissue structure.

TGF-β in kidney disease

Studies in a model of acute mesangial proliferative glomerulonephritis induced by injecting rats with antithymocyte serum show that production of TGF-β underlies the accumulation of glomerular extracellular matrix in this disease. The injured glomeruli express more TGF-β mRNA, synthesize more TGF-β protein, and produce many-fold more fibronectin and proteoglycans than do normal glomeruli (24). Simultaneous with increased matrix production is a striking decrease in plasminogen activator activity and a parallel increase in production and deposition of plasminogen activator inhibitor-1 in the nephritic glomeruli (Tomooka, S., W. A. Border, B. C. Marshall, and N. A. Noble, manuscript submitted for publication). The plasminogen/plasmin system is thought to play an important role in the normal degradation and turnover of matrix (25, 26). Thus both increased production and decreased removal are equally likely to contribute to the accumulation of pathological matrix in the disease. Fig. 1 illustrates the induction of TGF-β1 mRNA in the glomeruli of the nephritic kidney.

Injection of an antiserum capable of neutralizing the activity of TGF-β into the nephritic rats suppresses the production of matrix components by the glomeruli and prevents the buildup of mesangial matrix (27). Two of the proteoglycans induced by TGF-β in the glomerulonephritis model, decorin and biglycan, are inhibitors of TGF-β (28); their elevated expression under the influence of TGF-β may be not only a reflection of increased extracellular matrix production, but also a response to limit further activity of TGF-β. We have taken advantage of the ability of decorin to suppress TGF-β activity and have found that injections of decorin can also suppress the glomerulonephritic disease in the rats (Border, W. A., N. A.

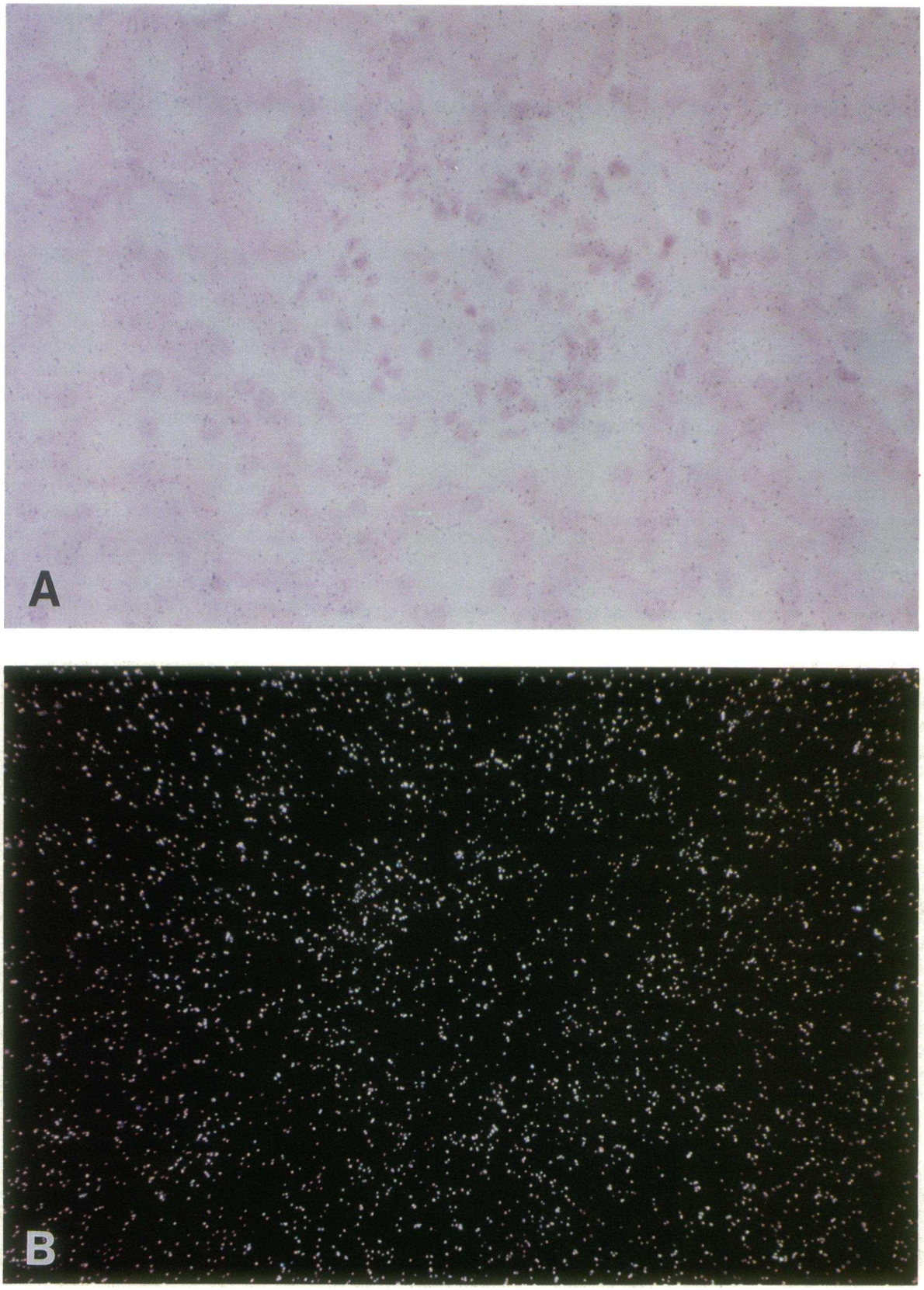
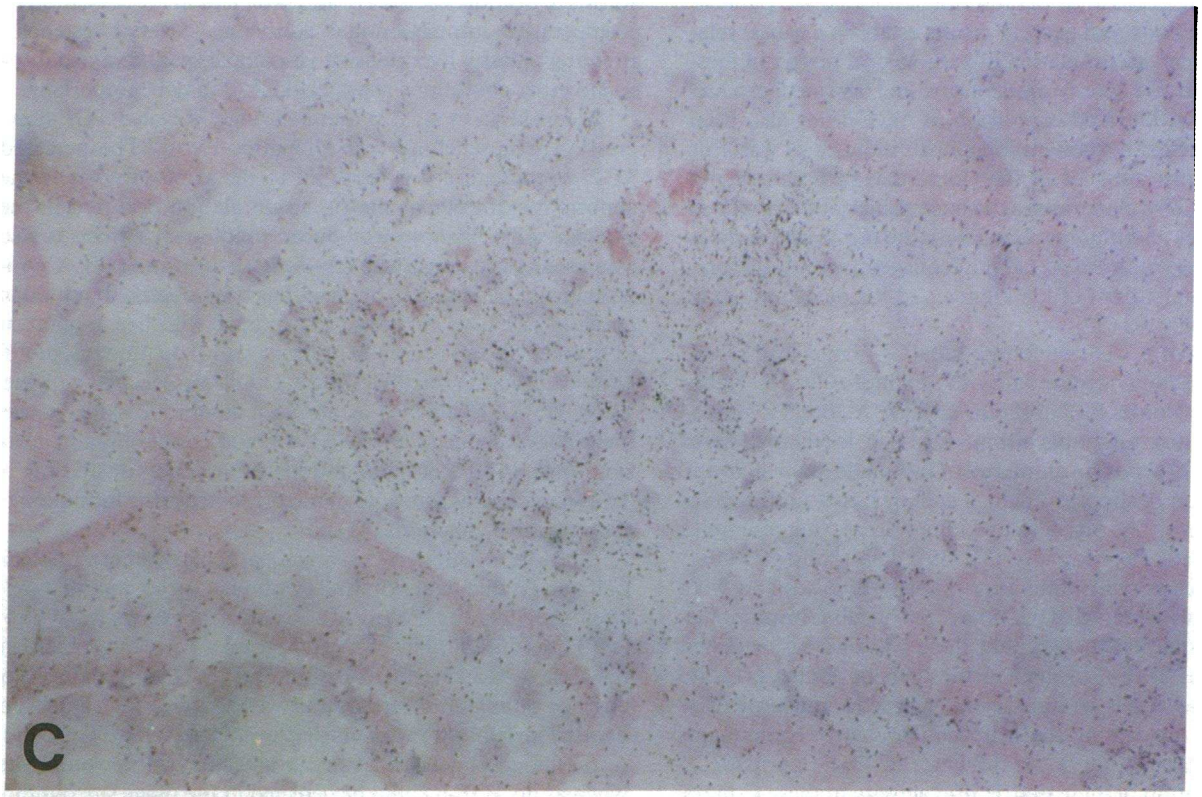


Figure 1. In situ hybridization of rat glomeruli with a TGF-β1 antisense probe. Bright- and dark-field micrographs of a normal glomerulus (*A* and *B*) and a nephritic glomerulus (*C* and *D*). TGF-β1 probe provided by Dr. H. L. Moses, Vanderbilt University.



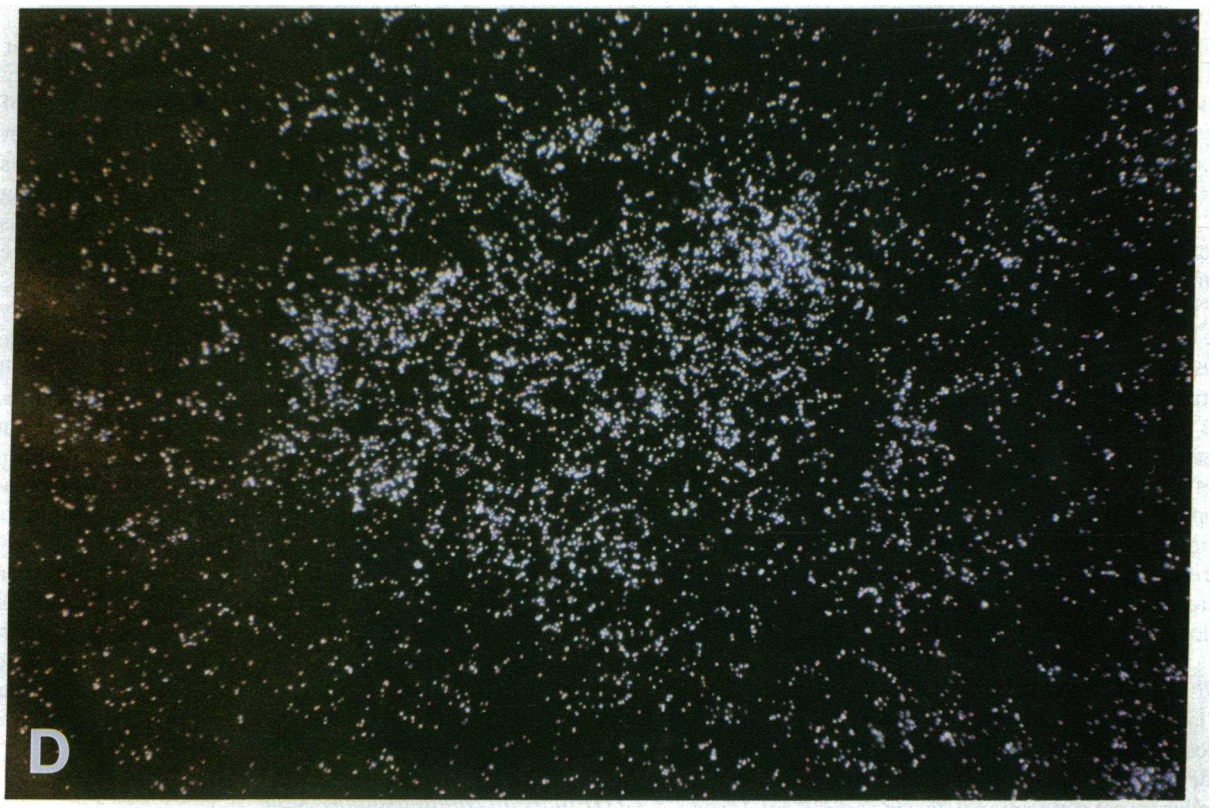


Figure 1 (*Continued*)

Noble, T. Yamamoto, Y. Yamaguchi, M. D. Pierschbacher, J. Harper, and E. Ruoslahti, manuscript submitted for publication). The antibody and decorin results establish a causal relationship between accumulation of pathological matrix in mesangial proliferative glomerulonephritis and elevated production of TGF-β. Elevated expression of TGF-β has also been reported in crescentic glomerulonephritis induced by injecting antibody against antigens of the glomerular basement membrane (29). TGF-β elaborated as a consequence of the antibody injury in the glomerulus correlates with the previously demonstrated increase in the expression of collagen mRNA and protein in the interstitium of the kidney and the development of severe renal fibrosis (30).

Quite recently, we have found that TGF-β may also be important in diabetic nephropathy (Yamamoto, T., T. Nakamura, N. A. Noble, E. Ruoslahti, and W. A. Border, manuscript submitted for publication). Elevated levels of TGF-β mRNA were observed in glomeruli of rats made diabetic by the administration of streptozotocin, a chemical that produces insulin deficiency. The rats develop kidney disease that resembles human diabetic nephropathy (31, 32). The levels of TGF-β mRNA increased with time after onset of diabetes and were highest in diabetic rats that did not receive insulin. Immunohistochemical staining showed that there was also an increased expression of TGF-β protein in the diabetic kidneys. Moreover, elevated levels of fibronectin, tenascin, and proteoglycans, which are among the extracellular matrix components typically produced under the influence of TGF-β, provided a strong indication of increased TGF-β activity in these kidneys. The relevance of these findings to human diabetes was confirmed by the demonstration of much elevated amounts of TGF-β protein in the glomeruli of patients with diabetic nephropathy. Glomeruli from normal kidneys or from other nonprogressive kidney disorders were negative for TGF-β. Thus, TGF-β may play an important role in the development of lesions in diabetic nephropathy, which is one of the most important diabetic complications that occurs despite insulin treatment.

Renal interstitial fibrosis occurs in all patients with progressive glomerular disease and is an excellent predictor of kidney failure (33). The link between glomerular injury and interstitial fibrosis may be TGF-β, which, when released from the glomerulus, induces its own production and matrix formation in the renal interstitium. This pattern of fibrosis is prominent in the severe renal fibrosis that occurs in transplant patients treated with cyclosporin (34); the possible role of TGF-β in this condition merits further study. Because of its intricate architecture and filtration function, the kidney may be particularly susceptible to the consequences of matrix accumulation and, therefore, may be a prime organ to be affected by elevated TGF-β. However, an increasing body of evidence implicates TGF-β in analogous pathologies of other organs.

TGF-β in fibrotic diseases of other organs

The role of excessive TGF-β activity in disease was first demonstrated at a causal level in the mesangial injury rat glomerulonephritis model discussed above. More recent studies have established a similar causal connection between experimental tissue scarring and TGF-β expression in the skin (35) and the central nervous system (Logan, A., A. M. Gonzalez, S. A. Frautschy, M. B. Sporn, M. Berry, and A. Baird, manuscript submitted for publication). Moreover, there is strong correlative evidence to suggest that TGF-β overproduction is a problem in lung fibrosis, liver cirrhosis, cardiac fibrosis after infarction, scarring and fibrosis in disorders of the eye and skin, and in the formation of postoperative intraabdominal adhesions. Arterial restenosis after angioplasty, hypertensive vasculopathy, and myelofibrosis are other conditions in which TGF-β may be important.

Broekelman et al. (36) found strongly elevated TGF-β expression in human lungs with idiopathic fibrosis. The increased TGF-β production was localized to the same sites where the abnormal extracellular matrix accumulation occurred in the alveolar walls. Bleomycin-induced pulmonary fibrosis is also associated with increased TGF-β gene expression (37). A similar increase of TGF-β expression has been observed in human patients with liver cirrhosis (38), in mice with hepatic fibrosis (39), and in the rat heart after infarction (40). In humans, proliferative vitreoretinopathy of the eye is also associated with elevated TGF-β levels (41) as are various fibrotic skin diseases including systemic sclerosis (42, 43) and eosinophilia-myalgia syndrome (44). Recently, intraperitoneal administration of TGF-β to rats was shown to markedly increase the formation of postoperative adhesions (45).

The role of TGF-β in scarring is particularly interesting. It is well known that fetal skin heals without scarring and that only after birth does the healing of a wound generate a scar. Whitby and Ferguson (46) have recently found a correlation between the lack of scarring in fetal skin and the greatly reduced or absence of a TGF-β response to wounding of the skin in rodents. The fetal skin wounds displayed detectable TGF-β only in the blood platelets that had aggregated at the wound site, whereas no TGF-β could be detected in the tissue surrounding the wound. In marked contrast, the tissue surrounding a wound in neonatal and older skin was TGF-β positive.

In ~ 30–40% of all angioplasty procedures performed for atherosclerotic obstructions of the coronary arteries, the artery will show evidence of restenosis after several weeks and half of these patients will redevelop symptoms (47, 48). The tissue that causes the restenosis consists of ingrowing smooth muscle cells and their extracellular matrix (49). Majesky et al. (50), studying a balloon catheterization model in which an arterial wall is denuded of endothelial cells in a process that resembles the procedure performed on human patients, found a strong elevation of TGF-β in the treated vessel. Since TGF-β is strongly chemotactic for many types of cells including smooth muscle cells, and since TGF-β stimulates matrix production, this result strongly suggests TGF-β involvement in the restenosis process. There is rapid expression of TGF-β, but not other cytokines, in the aortas of rats after the onset of salt-induced hypertension (51). TGF-β is a potent inducer of endothelin (52). This relationship of TGF-β and endothelin may be important (53) in a number of vascular pathologies, including hypertensive changes. Moreover, increased platelet TGF-β content has been associated with myelofibrosis, leading to the hypothesis that increased release of TGF-β from megakaryoblasts may underlie the progressive fibrosis in this disease (54). Finally, increased expression of TGF-β in injured tissue is thought to predispose the cells in the injured site to oncogenesis through a tumor promoter activity of TGF-β (55, 56).

TGF-β as an immunosuppressant

TGF-β is a potent immunosuppressing agent in vivo. Both cellular and humoral immune responses are affected (2). These immunosuppressive activities are likely to underlie the beneficial effects of systemic administration of TGF-β in experimental arthritis and autoimmune disease models (57, 58). (How-

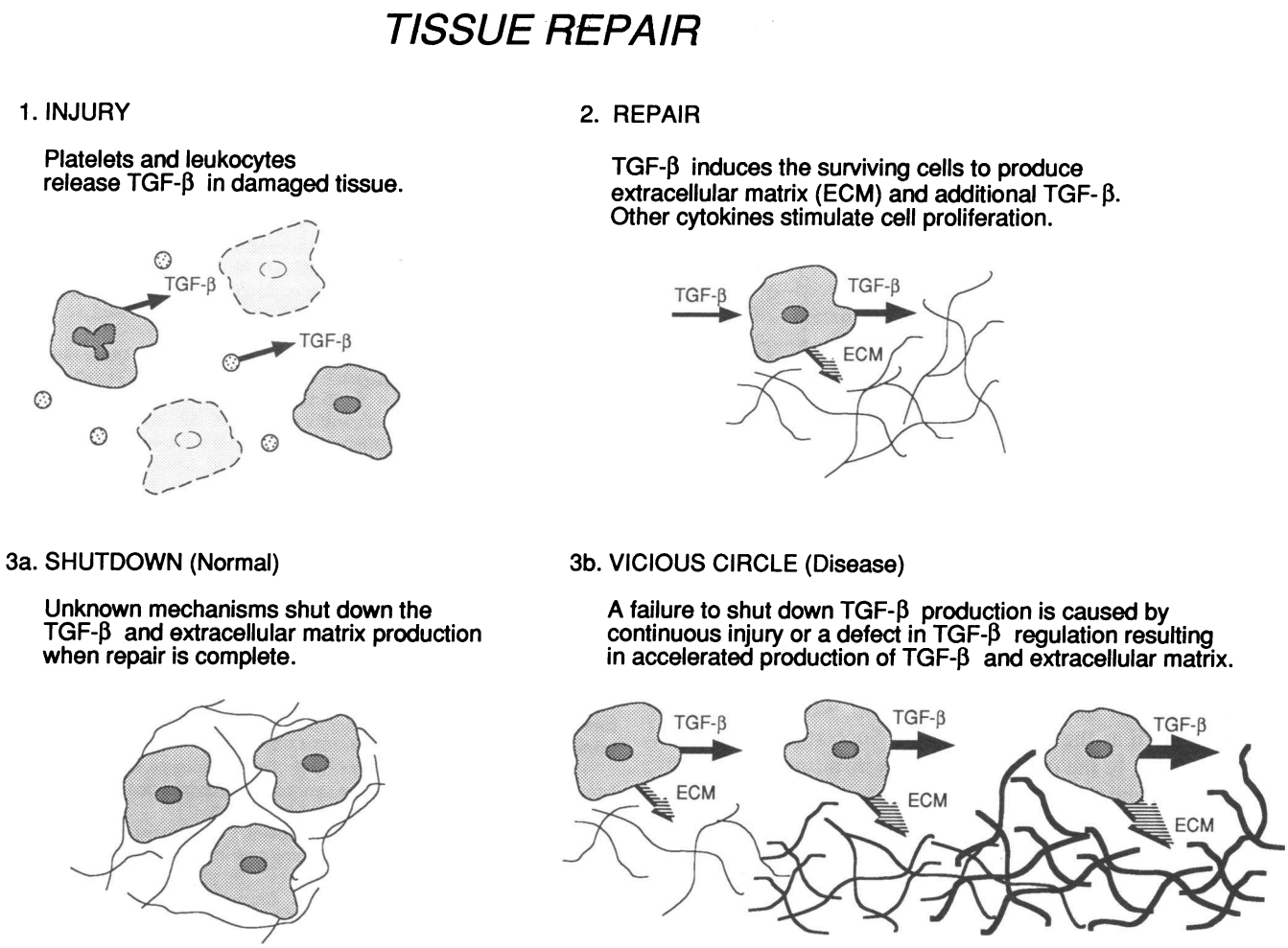


Figure 2. A schematic representation of the role TGF-β is believed to play in the repair of tissue injury and in the conversion of the repair process into a chronic fibrotic disease.

ever, when given intraarticularly, TGF-β produces a strong inflammatory reaction [59]). Another interesting effect of TGF-β is its ability to switch B cells from IgG to IgA production (60). In the most common form of human glomerulonephritis, IgA nephropathy (61), patients show a reversal of the normal balance of IgG versus IgA secretion by plasma cells (62). A recent study reported the presence of anti–mesangial cell autoantibodies in the serum of patients with IgA nephropathy (63). This finding is intriguing because the experimental model of glomerulonephritis discussed above in which elevated TGF-β expression has been demonstrated is induced by injection of antibodies reactive with the mesangial cells. The possibility that TGF-β is somehow involved in human IgA nephropathy is worthy of additional study.

An important situation involving the immunosuppressive activity of TGF-β may be AIDS. Kekow et al. (64, 65) have found elevated expression of TGF-β in lymphocytes isolated from the blood of AIDS patients. These authors suggest that the excess TGF-β may contribute to the systemic immunosuppression. Such a mechanism could explain the puzzling fact that the immunosuppression in AIDS is general and yet relatively few lymphocytes are infected by HIV. Interestingly, AIDS patients are also susceptible to a kidney disorder termed HIV-associated nephropathy in which glomerulosclerosis develops (66). In mice made transgenic for HIV there was noted a progressive buildup of glomerular extracellular matrix (67). TGF-β could provide the missing link between the infection, systemic immunosuppression, and the glomerulosclerosis.

Why is TGF-β often harmful?

TGF-β promotes wound healing. In a more primitive setting than today's world, quick wound healing responses, characterized by exuberant matrix formation and deposition, may have been all important and the possibility of deleterious side effects from such responses a tolerable price to pay. The importance of mounting a quick and effective TGF-β response upon injury may account for the unusual feature of TGF-β regulation that TGF-β can induce its own production by target cells (13, 68, 69). This feature may be responsible for the potential harm of TGF-β. Thus, positive feedback may be a mechanism whereby a TGF-β elevation can become chronic, creating a vicious circle (Fig. 2).

Prospects for TGF-β-suppressing treatments

The complex regulation of TGF-β production and activity offers a number of targets for TGF-β suppression. TGF-β is produced as an inactive precursor protein that is converted to the mature, active form by protease cleavage (1–3). In a test tube, TGF-β is commonly activated by acid treatment. Plasmin has been suggested as a protease that activates TGF-β physiologically (70), but more than one protease may be needed for effective activation (71). The activation peptide cleaved from

the TGF-β precursor and certain other proteins, including tissue proteoglycans, can inhibit TGF-β activity, presumably by competing with the receptors for the binding of TGF-β (28, 72–74). Soluble forms of the receptors (75–77) may also inhibit TGF-β activity by the same mechanism, but this has not yet been proven.

Members of the steroid receptor superfamily can regulate the TGF-β gene at the level of the gene expression (78). Curiously, a protein-restricted diet can completely suppress TGF-β gene expression in rat glomerulonephritis induced by injuring the mesangial cells (79). The molecular mechanism of this dietary effect is unknown, but it appears to offer one explanation for the alleged beneficial effect of low protein diet on the progression of various kidney diseases.

TGF-β activity has been successfully suppressed in vivo in the kidney (27), in the skin (35), and in central nervous system injury (Logan, A., A. M. Gonzalez, S. A. Frautschy, M. B. Sporn, M. Berry, and A. Baird, manuscript submitted for publication), by administering anti-TGF-β antibodies capable of preventing the binding of TGF-β to its receptors. In each case, blocking the action of TGF-β dramatically decreased the excessive deposition of extracellular matrix, but did not interfere with normal healing of the tissue. For example the dermal wounds treated with anti-TGF-β contained substantially less collagen, did not manifest a scar, but did possess the same tensile strength as the control wounds. Such studies are now being extended to the other conditions with suspected TGF-β involvement, and the use of TGF-β inhibitors more suitable for therapeutic use than antibodies is being explored. Such compounds are likely to become important therapeutics in the treatment of the diseases caused by the dark side of TGF-β.

Acknowledgments

We thank Drs. Eva Engvall and Nancy Noble for comments on the manuscript and Diane Miller for expert technical assistance.

The original work by the authors and the preparation of this manuscript were supported by grants DK43609 to W. A. Border from the National Institute of Diabetes and Digestive and Kidney Diseases—Department of Health and Human Services, DHHS and grants CA42507, CA28896, and Cancer Center Support Grant CA30199 to E. Ruoslahti from the National Cancer Institute.

Editor's note: The authors have disclosed to the Editor their interest in a company engaged in the development of TGF-β antagonists.

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