Role of Elevated Plasma Transforming Growth Factor- β 1 Levels in Wound Healing

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Transforming growth factor (TGF)- β 1 plays a central role in wound healing. Wounds treated with neutralizing antibody to TGF- β 1 have a lower inflammatory response, reduced early extracellular matrix deposition, and reduced later cutaneous scarring, indicating the importance of local tissue TGF- β 1. By contrast, increasing the local, tissue levels of TGF- β 1 increases the early extracellular matrix deposition but does not alter scar formation. Increased levels of plasma TGF- β 1 correlate with increased fibrogenesis in the lung, kidneys, and liver. The aim of the present study was to investigate the role of elevated systemic levels of TGF- β 1 on wound healing. We used transgenic mice that express high levels of active TGF- β 1 and have elevated plasma levels of TGF- β 1 and wild-type mice of the same strain as controls. Incisional wounds and subcutaneously implanted polyvinyl alcohol (PVA) sponges were analyzed. Surprisingly, cutaneous wounds in transgenic, TGF- β 1-overexpressing mice healed with reduced scarring accompanied by an increase in the immunostaining for TGF- β 3 and TGF- β -receptor RII and a decrease in immunostaining for TGF- β 1 compared with wounds in control mice. By contrast, the PVA sponges showed the opposite response, with PVA sponges from transgenic mice demonstrating an enhanced rate of cellular influx and matrix deposition into the sponges accompanied by an increase in the immunostaining for all three TGF- β isoforms and their receptors compared with PVA sponges from control mice. Together, the data demonstrate that increased circulating levels of TGF- β 1 do not always result in increased expression or activity in selected target tissues such as the skin. The two wound models, subcutaneously implanted PVA sponges and cutaneous incisional wounds, differ significantly in terms of host response patterns. Finally, the data reinforce our previous observations that the relative ratios of the three TGF- β isoforms is critical for control of scarring. (*Am J Pathol* 1999, 154:1115–1124)

Transforming growth factor (TGF)- β 1 plays a central role in wound healing. Released by degranulating platelets at the site of injury, TGF- β 1 influences the inflammatory response, angiogenesis,¹ re-epithelialization, extracellular matrix deposition, and remodeling.^{2,3} We have previously demonstrated the role of local TGF- β 1 in cutaneous scarring by exogenous addition of neutralizing antibody to TGF- β 1 at the wound site.⁴ Anti-TGF- β 1-treated wounds had a lower inflammatory response, less extracellular matrix deposition in the early stages of wound healing, and reduced scar formation. By contrast, increasing the tissue levels of TGF- β 1 increased early extracellular matrix deposition but did not alter subsequent scar quality when compared with untreated control wounds in adult rodents.

TGF- β 1 has also been implicated in various fibrotic disorders, such as glomerulonephritis⁵ and pulmonary fibrosis.⁶ Increased levels of plasma TGF- β have been found to correlate with increased fibrogenesis after bone marrow transplantation therapy in patients with advanced breast cancer.⁷ Intravenous administration of recombinant TGF- β 1 to rats induces fibrotic lesions in the liver, kidneys, pancreas, and testes,⁸ suggesting an endocrine-like effect of TGF- β 1.

We have used the recently developed transgenic mouse lines that express high levels of active TGF- β 1⁹ to investigate the role of elevated systemic levels of active TGF- β 1 on wound healing. The liver fibrosis and delayed liver regeneration after partial hepatectomy characteristic of these transgenic lines has been shown to result directly from the overexpression of TGF- β 1,¹⁰ and in line 25 mice, the characteristic kidney fibrosis and kidney failure has also been shown to be due to the high circulating levels of TGF- β 1 driven by the transgene.¹¹ Based on these observations, we wished to test the hypothesis that elevated plasma TGF- β 1 would enhance scarring in cutaneous wounds. As polyvinyl alcohol (PVA) sponges have frequently been used to assess wound healing, we evaluated healing in both incisional and PVA sponges. Surprisingly, cutaneous wounds in transgenic, TGF- β 1-overexpressing mice healed with less scarring than control mice, whereas the sponges showed the opposite response, with the transgenic mice demonstrating an

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Figure 1. Experimental model. Male transgenic mice line 25 containing the fusion gene (Alb/TGF- β 1) consisting of a modified porcine TGF- β 1 cDNA under the control of the regulatory elements of the mouse albumin gene were used for this investigation.⁹ Male wild-type mice of the same strain (C57B/L6J $\times$ CBA) were used as the control group. Four 0.75-cm, full-thickness, incisional wounds were made on the dorsum of the animal and left to heal by secondary intention (a). Two PVA sponges were implanted into subcutaneous pockets on the ventral surface of the animals, and the transverse wounds were sutured to prevent extrusion of the implants (b). Cutaneous wounds and PVA sponges were harvested on days 7, 14, and 80 after wounding.

enhanced rate of cellular influx and matrix deposition into the sponges compared with controls.

Materials and Methods

The recently developed transgenic mouse line (line 25) containing a fusion gene (Alb/TGF- β 1) consisting of a modified porcine TGF- β 1 cDNA (producing active TGF- β 1) under the control of the regulatory elements of the mouse albumin gene were used for this investigation.⁹ These transgenic mice have elevated circulating plasma levels of active TGF- β 1. Wild-type mice of the same hybrid strain (C57BL/6J $\times$ CBA) were used as the control group.

Experimental Model

Animals were anesthetized by intraperitoneal injections of avertin. All animals were males between 4 and 6 weeks of age. Four 0.75-cm full-thickness incisional wounds were placed on the dorsal skin, equidistant from the midline and adjacent to the four limbs. These wounds were left to heal by secondary intention (Figure 1a). Two PVA sponges (0.5 cm $\times$ 0.5 cm and weighing 8 mg each) were inserted into subcutaneous pockets on the ventral wall of the abdomen through two ventral incisions that

were subsequently sutured with 5.0 ethilon (Ethicon) to prevent extrusion of the sponges (Figure 1b). Animals were recovered in their cages and were fed mouse chow and water *ad libitum*. In total, 29 control mice and 26 mice from line 25 were wounded. Four mice from line 25 died within 2 weeks of surgery. The remaining animals were killed on days 7, 14, and 80 after wounding by carbon dioxide narcosis, and the cutaneous wounds and sponges were harvested. From each animal, the dorsal cutaneous wounds and sponges were processed for routine histology, immunocytochemistry, and hydroxyproline assay as described previously.^{12,13} The sponges were carefully dissected free of the surrounding capsule before processing. All animal protocols were approved by institutional (National Institutes of Health) review.

Histology

The wounds and sponges were fixed in 10% formal saline, processed for paraffin embedding, sectioned at 7-mm thickness, and stained with picrosirius red to enhance polarization of collagen fibers.¹⁴ The differences in the architecture of the neodermis of wounds in the control and transgenic mice were assessed using a polarizing microscope (Zeiss, Oberkochen, Germany) as described previously.¹³

Histological sections of the PVA sponges were also stained with hematoxylin and eosin to assess the cellularity and presence of granulation tissue within the sponges. A graticule (Leitz) was used in the eye-piece of the microscope, and cells present within two randomly selected defined areas (0.05 mm²) at 40 $\times$ magnification were counted. The cellularity of three sections from each PVA sponge was quantified. Note the randomly defined areas did not include the capsules surrounding the sponges but the spaces within the sponges.

Figure 2. Macroscopic appearance of cutaneous scars. At 80 days after wounding, there is a firm, raised obvious cutaneous scar (arrowheads) in the control mouse (a). By contrast, the wound in the transgenic mouse has healed with a very fine linear scar (arrowheads), which is barely discernible (b).

Figure 3. Microscopic appearance of cutaneous scars. Sections of wounds harvested 80 days after wounding were stained with picrosirius red and visualized through a polarized microscope. The architecture of the neodermis in the control wound (a) demonstrates parallel arrangement of the collagen fibers lacking the open basket-weave pattern of the normal dermis (b): scar formation. By contrast, the neodermis of wound in the transgenic mouse (c) more closely resembles the basket-weave pattern of normal dermis (d): better quality of scarring. Scale bar, 100 μ m.

Immunocytochemistry

TGF- β isoforms and their receptors were immunodetected in paraffin-embedded tissue sections using the immunoperoxidase staining technique. Briefly, sections were dewaxed and treated with 0.3% Triton X-100 in Tris-buffered saline (TBS) for 15 minutes. Endogenous peroxidase was quenched with 0.6% hydrogen peroxide in methanol for 1 hour at room temperature, followed by digestion with sheep testicular hyaluronidase (Sigma Chemical Co., Poole, UK) at a concentration of 1 mg/ml in sodium acetate buffer, pH 5.5, with 0.85% sodium chloride for 0.5 hour at 37°C. Normal goat serum (Vector Laboratories, Burlingame, CA) was used to block non-specific antibody binding. Sections were then incubated with the primary antibody diluted in the blocking serum overnight at 4°C. After thorough washing in TBS/0.1% bovine serum albumin, the sections were incubated with

the secondary antibody for 1 hour at room temperature followed by incubation with the ABC complex for 1 hour at room temperature. Sections were thoroughly washed after each step except after blocking with normal goat serum. Sections were then incubated with 0.05% diaminobenzidine (DAB) until the brown substrate was formed, rinsed in distilled water, counterstained with Harris's hematoxylin, rinsed in water, dehydrated, and mounted in Praxtamount. The Vectastain elite ABC kit (Vector Laboratories) was used for this protocol. For all primary antibodies, biotinylated goat anti-rabbit IgG from the Vectastain ABC kit was used at a dilution of 1/200 as the secondary antibody. Rabbit polyclonal antibodies to TGF- β s¹⁵ and their receptors (Santa Cruz Biotechnologies, Santa Cruz, CA) were used as follows: TGF- β 1, anti-P(1–30) LC, at 7 mg/ml; TGF- β 2, anti-P(50–75), at 1.5 mg/ml; TGF- β 3, anti-P(50–60), at 2.4 mg/ml; TGF- β

Table 1. Cellular Response to Sponge Implantation

Time after wounding	Geometric mean number of cells/field (95% confidence interval; <i>n</i> = number of samples)		Test statistic (<i>P</i> value)
	Control mice	Transgenic mice line 25	
7 days	51.1 (79.9, 32.6; <i>n</i> = 4)	120.2 (181.8, 79.4; <i>n</i> = 7)	<i>t</i> (9) = 2.7 (<i>P</i> = 0.027)
14 days	127.4 (166.6, 97.5; <i>n</i> = 10)	246.7 (311.1, 195.6; <i>n</i> = 5)	<i>t</i> (13) = 3.2 (<i>P</i> = 0.007)

Table 2. Hydroxyproline Content of Wounds

Time after wounding	Geometric mean $\mu\text{g}/\text{mg}$ dry tissue (95% confidence interval; n = number of samples)		Test statistic (P value)
	Control mice	Transgenic mice line 25	
7 days	17.4 (12.6, 23.9; n = 4)	6.2 (4.2, 9.0; n = 6)	$t(8) = 3.84$ ($P = 0.005$)
14 days	40.0 (31.5, 50.8; n = 10)	34.9 (24.2, 50.2; n = 6)	$t(14) = 0.66$ ($P = 0.521$)
80 days	30.2 (21.0, 43.5; n = 7)	48.5 (38.4, 61.3; n = 3)	$t(8) = 1.60$ ($P = 0.148$)

Table 3. Hydroxyproline Content of PVA Sponges

Time after wounding	Geometric mean $\mu\text{g}/\text{mg}$ dry tissue (95% confidence interval; n = number of samples)		Test statistic (P value)
	Control mice	Transgenic mice line 25	
7 days	1.6 (1.2, 2.1; n = 4)	3.7 (2.4, 5.8; n = 7)	$t(9) = 2.67$ ($P = 0.026$)
14 days	7.9 (7.1, 8.9; n = 10)	13.4 (10.8, 16.8; n = 6)	$t(14) = 4.60$ ($P < 0.001$)
80 days	14.0 (12.6, 15.6; n = 7)	13.8 (12.5, 15.3; n = 3)	$t(8) = 0.16$ ($P = 0.88$)

type I receptor, anti-P(482–501) RI (R-20), at 1:100 dilution; TGF- β type II receptor, anti-P(550–565) RII (C-16), at 1:100 dilution.

As controls for the staining procedure, sections were incubated with an equivalent concentration of irrelevant rabbit IgG (Serotec, Oxford, UK) instead of the primary antibodies, and sections were incubated with normal goat serum (omitting the primary antibody) with the rest of the protocol unchanged. Nonspecific brown cellular staining was not observed in any of the sections used as controls for the staining procedure. The specificity of the antibodies used has been established previously by Flanders et al¹⁵ by using preincubation of the antibodies with the relevant peptides. For each primary antibody, three sections each from at least four wounds from each group of animals at each of days 7 and 14 were analyzed for immunoreactivity. Figures 4 to 8 are representative of the results obtained.

Hydroxyproline Assay

All materials were of analytical grade and purchased from BDH Chemicals (Poole, UK) or from Sigma Chemical Co. unless otherwise stated.

The cutaneous wounds were microdissected flush with the wound margin, freeze dried, weighed, and stored at -70°C until further use. The sponges were carefully dissected free from the surrounding capsule, freeze dried, weighed, and stored at -70°C until further use. Normal, unwounded skin and unused sponges were also processed similarly. Hydroxyproline content of the samples was determined using the method of Stegmann and Stalder¹⁶ and expressed as $\mu\text{g}/\text{mg}$ dry weight of samples.

Statistical Analyses

The hydroxyproline contents of sponges and wounds were compared for the wild-type and transgenic mice at three different time points. The distribution of the data was tested, and some degree of skewness was detected in most cases. Log transformation was found to be the

most appropriate and consistent normalizing transformation across all three time points for the sponges and cutaneous wounds, and t -tests were then performed to compare differences between the two groups. Results are expressed as the geometric means with their 95% confidence intervals as well as the t statistic and P values. A P value of less than 0.05 indicates a significant difference between groups. Similar statistical analyses were performed for the cell counts in the PVA sponges.

Results

A total of 204 cutaneous wounds and 102 implanted PVA sponges of transgenic and control mice were analyzed as reported.

Cutaneous Scarring: Macroscopic Appearance

Surprisingly, the cutaneous wounds in transgenic mice healed with a fine linear scar that was barely discernible 80 days after wounding (Figure 2b). By contrast, wounds in the control mice healed with firm, raised, obvious scars (Figure 2a). These differences in the macroscopic appearances of scars were apparent as early as 14 days after wounding.

Cutaneous Scarring: Microscopic Appearance

The histological appearance of wounds was consistent with the macroscopic appearance of the healed wounds. Although wounds in the control mice generally appeared wider than wounds in the transgenic mice, all wounds had re-epithelialized by 7 days after wounding. As expected, sections of wounds from control mice stained with picrosirius red and visualized through polarized light demonstrated characteristics of scarring, including obvious compact, parallel arrangement of collagen fibers in the neodermis of control wounds (Figure 3a) rather than the basket-weave appearance of normal dermis (Figure 3b). By contrast, the neodermis of wounds in transgenic mice (Figure 3c) showed some degree of basket-weave organization of collagen and more closely resembled the

Figure 4. Immunostaining for TGF- β 1. Sections of wounds and PVA sponges harvested 7 days after wounding were immunostained for TGF- β 1 using the ABC technique (brown is positive staining). Surprisingly, the epidermis and neodermis of cutaneous wounds from transgenic mice (b) stained much less intensely for TGF- β 1 than wounds from control mice (a). By contrast, PVA sponges from the transgenic mice (d) stained markedly more intensely than PVA sponges from control mice (c). Scale bar, 100 μ m. Arrows indicate the wound site as determined microscopically from the divided panniculus carnosus and the differences between adjacent normal dermis and the wound.

Figure 5. Immunostaining for TGF- β 2. Sections of wounds and PVA sponges harvested 7 days after wounding were immunostained for TGF- β 2 using the ABC technique (brown is positive staining). Cutaneous wounds from transgenic mice (b) stained marginally more intensely for TGF- β 2 than wounds from control mice (a), principally in the epidermis. By contrast, PVA sponges from the transgenic mice (d) stained markedly more intensely than PVA sponges from control mice (c). Scale bar, 100 μ m. Arrows indicate the healing wound.

Figure 6. Immunostaining for TGF- β 3. Sections of wounds and PVA sponges harvested 7 days after wounding were immunostained for TGF- β 3 using the ABC technique (brown is positive staining). There was a marked increase in the immunostaining for TGF- β 3 in both the cutaneous wounds (b) and the PVA sponges (d) from the transgenic mice compared with wounds (a) and PVA sponges (c) from control mice. Scale bar, 100 μ m. Arrows indicate the healing wound.

normal dermis (Figure 3d), reflecting a better quality of healing with reduced scarring.

The PVA sponges from transgenic mice had more cells and granulation tissue within the sponges than the control mice on days 7 and 14 after wounding, suggesting a more rapid cellular response to the implantation of the sponges in the transgenic mice (Table 1).

Hydroxyproline Content of Cutaneous Wounds and Subcutaneous PVA Sponges

The hydroxyproline content of cutaneous wounds from transgenic mice harvested 7 days after wounding was, on average, less than half the hydroxyproline content of wounds from control mice. There were no significant differences in the hydroxyproline content of wounds from the wild-type and transgenic mice harvested at later time points (Table 2). However, it was difficult to dissect the wounds from the surrounding normal skin of transgenic mice at later times due to the better quality of healing.

By contrast, the hydroxyproline content of the PVA sponges from transgenic mice harvested 7 and 14 days after wounding was significantly higher (on average, two-fold higher) than the PVA sponges from control mice. The differences in the hydroxyproline content of PVA sponges were not significant 80 days after wounding (Table 3).

Immunostaining for TGF- β Isoforms and TGF- β Receptors I and II

The three isoforms of TGF- β have previously been shown to be expressed in unique patterns in epidermis and to be regulated independently.¹⁷ To understand the effects of the systemic overexpression of TGF- β 1 driven by the albumin promoter, which is expressed most strongly in the liver,⁹ on the expression of the TGF- β isoforms and their receptors in the skin of the transgenic mice, we assessed their patterns of immunohistochemical staining at both 7 and 14 days after wounding.

Surprisingly, the epidermis and neodermis of cutaneous wounds from transgenic mice (Figure 4b) stained much less intensely for TGF- β 1 than wounds from control mice (Figure 4a). By contrast, there was a marginal increase in the staining of TGF- β 2 in cutaneous wounds, principally in the epidermis of the transgenic mice (Figure 5b) compared with control mice (Figure 5a). Most notably, there was a marked increase in immunostaining for TGF- β 3 in the cutaneous wounds from transgenic mice (Figure 6b) compared with cutaneous wounds from control mice (Figure 6a), specifically in the cellular staining and depth of neodermal and subcutaneous staining.

The staining pattern observed in the PVA sponges differed from that observed in the cutaneous wounds, in that PVA sponges from the transgenic mice (Figures 4d,

Figure 7. Immunostaining for TGF- β receptor RI. Sections of wounds and PVA sponges harvested 7 days after wounding were immunostained for TGF- β receptor RI using the ABC technique (brown is positive staining). The cutaneous wounds from control (a) or transgenic (b) mice did not differ markedly in their immunostaining for RI. By contrast, immunostaining for RI was more marked in PVA sponges from transgenic mice (d) than from control mice (c). Scale bar, 100 μ m. Arrows indicate the healing wound.

5d, and 6d) stained markedly more intensely for all three TGF- β isoforms than the PVA sponges from control mice (Figures 4c, 5c, and 6c).

Whereas cutaneous wounds from control and transgenic mice did not differ in immunoreactivity for TGF- β receptor I (Figure 7, a and b), the immunoreactivity for the type II receptor was elevated in wounds from the transgenic mice (Figure 8b) compared with controls (Figure 8a). Similar to that observed for the TGF- β isoforms, immunostaining for both RI and RII was more marked in PVA sponges from transgenic mice (Figures 7d and 8d) compared with those from control mice (Figures 7c and 8c).

Discussion

We have used transgenic mice overexpressing active TGF- β 1 under control of the albumin promoter⁹ to test the hypothesis that elevated circulating levels of TGF- β 1 would accelerate wound healing and enhance scarring. Whereas PVA sponges implanted subcutaneously in the transgenic mice did, in fact, show increased cellularity with enhanced formation of granulation tissue and synthesis of collagen compared with control mice, unexpectedly, the opposite response was observed in cutaneous wounds in these same mice. Cutaneous wounds from transgenic mice showed reduced staining for TGF- β 1 and healed with less scarring and a better architecture of

the neodermis compared with wild-type control mice. Together, the data demonstrate that increased circulating levels of TGF- β 1 do not always result in increased expression or activity in selected target tissues, in this case the skin. Furthermore, the data demonstrate that these two models, cutaneous wounding and implantation of PVA sponges, both used routinely to evaluate wound repair, differ significantly in terms of host response patterns as assessed by altered TGF- β isoform and receptor profiles and scarring.

Increased tissue expression of TGF- β 1 has been implicated in various fibrotic disorders,^{5,6} and elevated plasma levels of TGF- β 1 have also been shown to result in fibrotic sequelae in tissues such as liver, lung, and kidney.^{7,11} Moreover, numerous studies have shown either systemic¹⁸ or topical TGF- β 1 both to accelerate the rate of healing and to enhance scarring in cutaneous wounds^{3,4,19} as well as in other injury models.²⁰ By contrast, we have shown that enhanced expression levels of TGF- β 3 relative to TGF- β 1 in wounds, achieved either by direct application of TGF- β 3 or by suppression of TGF- β 1 activity by use of isoform-specific blocking antibodies, reduces the scarring and results in a better dermal architecture.⁴ TGF- β 3 is thought to exert its anti-scarring activity in part by antagonizing TGF- β 1.²¹ In our studies presented here, cutaneous wounds from transgenic mice overexpressing TGF- β 1 showed reduced scarring accompanied by an increase in immunostaining for TGF- β 3

Figure 8. Immunostaining for TGF- β receptor RII. Sections of wounds and PVA sponges harvested 7 days after wounding were immunostained for TGF- β receptor RII using the ABC technique (brown is positive staining). There was a marked increase in the immunostaining for TGF- β receptor RII in both the cutaneous wounds (b) and the PVA sponges (d) from transgenic mice compared with wounds (a) and PVA sponges (c) from control mice. Scale bar, 100 μ m. Arrows indicate the healing wound.

and a decrease in the immunostaining for TGF- β 1 compared with wounds from control mice, reinforcing our previous observations that the relative ratio of the three TGF- β isoforms is critical for control of cutaneous scarring.⁴

The mechanisms whereby elevated circulating levels of active TGF- β 1 in these transgenic mice lead to lower immunoreactivity for TGF- β 1 and increased levels of TGF- β 3 in cutaneous wounds compared with control mice are not known. Auto-induction and cross-regulation of the three TGF- β isoforms is complex and tissue specific.^{22,23} It may be that the high levels of systemic TGF- β 1 down-regulate subsequent expression of TGF- β 1 by desensitization of the TGF- β receptor and messenger signaling pathways. Recent data characterizing members of the Smad family of signaling proteins, which mediate signals from the serine-threonine kinase receptors of the TGF- β superfamily,²⁴ show that two of these proteins, Smad 6 and Smad 7, which inhibit signal transduction from TGF- β receptors, are up-regulated strongly by TGF- β , suggesting that they can function to truncate the downstream signal in the presence of excessive ligand.^{24,25} Such an inhibitory mechanism could potentially impact most severely on expression of TGF- β 1, which is strongly autoregulated by AP-1 sites in its promoter.²⁶

TGF- β signals through a heteromeric complex of the type I and II TGF- β receptors.^{24,27} We investigated the TGF- β receptor profiles of cutaneous wounds and found

little difference in the immunoreactivity for TGF- β receptor I between transgenic and control mice. By contrast, cutaneous wounds from transgenic mice were more immunoreactive for TGF- β receptor II than wounds from control mice. How changes in the relative ratios of these two receptors may affect downstream targets is poorly understood. However, several lines of investigation suggest that a preponderance of type II receptors favors antiproliferative responses, whereas a preponderance of type I receptors is profibrotic.^{28–30} These observations are consistent with our observations of reduced scarring and collagen synthesis in cutaneous wounds of the transgenic mice where the relative ratios of the receptors are shifted in favor of the type II receptor.

Importantly, the results of this investigation also demonstrate that host responses to subcutaneously implanted PVA sponges are different from the response to cutaneous wounding. In particular, the patterns of local induction of TGF- β receptors and cross-regulation of TGF- β isoforms observed in the cutaneous wounds are altered in the PVA sponge. This has important implications as PVA sponges are often used as models of the wound-healing response,³¹ whereas they clearly more accurately reflect the host response to a relatively inert foreign body. At 7 and 14 days after wounding, the PVA sponges from transgenic mice had more cells and a higher hydroxyproline content than sponges from control mice. It is possible that elevated levels of plasma active TGF- β 1 may lead to prestimulation of monocytes causing

the rapid infiltration of cells and increase in collagen deposition seen in the PVA sponges implanted in the transgenic mice. This is in keeping with reported data where exogenous addition of TGF- β 1 increased the cellularity and the hydroxyproline/protein content of subcutaneously implanted wound chambers/sponges in rodents.^{31,32} By contrast, the hydroxyproline content of cutaneous wounds from transgenic mice was lower than that from control mice. It may be that the PVA sponges, which are bathed in and sequester plasma, reflect the fibrogenic response of high levels of plasma active TGF- β 1 as seen in various organs, such as the liver and the kidneys.^{9,11} Differences in organ perfusion or possibly in the ability to recognize and take up distinct molecular complexes of circulating TGF- β 1 may influence the response of different tissues to high circulating levels of TGF- β 1.³³

Unlike cutaneous wounds, the immunoreactivity for TGF- β 1 in the PVA sponges from transgenic mice was higher than in the sponges from control mice. The PVA sponges from transgenic mice also stained more intensely for TGF- β 2 and TGF- β 3 as well as for TGF- β receptors I and II. Recent *in vitro* data showed the ability of TGF- β 3 to regulate collagen synthesis by dermal fibroblasts through TGF- β 1-dependent and -independent pathways. Exogenous addition of small amounts of TGF- β 3 when added in combination with TGF- β 1 stimulated procollagen and TGF- β 1 mRNA whereas greater amounts of TGF- β 3 down-regulated procollagen and TGF- β 1 mRNA.²¹ It would appear that the increased fibrogenic response seen in the PVA sponges from transgenic mice is a function both of the elevated levels of the three TGF- β isoforms and their receptors and the different ratios of both ligands and receptors, compared with that in cutaneous wounds of the same mice. Finally, these data further demonstrate the complex regulation of TGF- β s *in vivo* and tend to negate our simple hypothesis to explain individual variation in the scarring response in man, ie, that people who scar badly have higher systemic levels of circulating TGF- β 1.

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