

Cells, Matrix, Growth Factors, and the Surgeon

The Biology of Scarless Fetal Wound Repair

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Objective

This review updates the surgeon about the cellular, matrix, and growth factor components of scarless fetal wound repair.

Summary Background Data

Fetal skin wound healing is characterized by the absence of scar tissue formation. This unique repair process is not dependent on the sterile, aqueous intrauterine environment. The differences between fetal and adult skin wound healing appear to reflect processes intrinsic to fetal tissue, such as the unique fetal fibroblasts, a more rapid and ordered deposition and turnover of tissue components, and, particularly, a markedly reduced inflammatory infiltrate and cytokine profile. Scarless fetal wounds are relatively deficient in the inflammatory cytokine, transforming growth factor β (TGF- β). In contrast, the fibrosis characteristic of adult wound repair may be associated with TGF- β excess. Recent experimental studies suggest that specific anti-TGF- β therapeutic strategies can ameliorate scar formation in adult wound repair and fibrotic diseases. Inhibitors of TGF- β may be important future drugs to control scar.

Conclusions

Based on the scarless fetal wound repair model, a number of ways in which the matrix and cellular response of the healing adult wound might be manipulated to reduce scarring are reviewed.

Fetal surgical skin wounds heal rapidly and without the scarring and inflammation that accompany adult skin wounds. In all species examined (mice, rats, rabbits, pigs, sheep, and monkeys), the prenatal wound healing process is faster and more efficient than adult repair and produces new tissue rather than scar.¹ Similarly, human fetal surgery has shown that the younger the fetus is at

the time of surgery, the less likely he/she will be born with surgical scars. How does this happen? A summary of some of the phenomenologic differences between adult and fetal repair is shown in Table 1.

We propose that scarless fetal repair is a consequence of a unique extracellular matrix produced by the fetal fibroblast in the absence of an adult-like inflammatory response to injury. Unraveling the biology of fetal repair has led to novel strategies for the prevention and treatment of scarring and fibrosis.

THE FETAL ENVIRONMENT

There are multiple differences between the fetal and adult environment that can influence wound repair.

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**Table 1. COMPARISON OF
ADULT AND FETAL SKIN WOUND
HEALING CHARACTERISTICS**

Wound Healing Characteristics	Adult	Fetus
Scar	Present	Absent
Cell Proliferation	Slower	Faster
Speed to closure	Slower	Faster
Scab	Present	Absent
Oxygen tension	Greater	Lesser
Fluid environment	Absent	Present
Sterile environment	Absent	Present
Skin temperature	Cooler	Warmer
Acute inflammation	Greater	Lesser
Matrix deposition	Slower, disorganized	Faster, organized
TGF β & bFGF	Greater	Lesser
Angiogenesis	Greater	Lesser
Epithelialization	Slower	Faster
Keratinization	Present	Immature

First, fetal skin wounds are continuously bathed in warm, sterile amniotic fluid that is rich in growth factors. Amniotic fluid also is a fertile source of extracellular matrix molecules, such as hyaluronic acid and fibronectin, which are important components in fetal skin wounds.² Second, fetal tissue oxygenation is much less than that of adult tissue. We have measured tissue pO_2 in fetal sheep at midgestation, a time at which scarless skin healing occurs. Using a miniaturized oximeter probe, we found that fetal sheep tissue pO_2 is only 16 mm Hg, whereas adult tissue pO_2 is 45 mm Hg to 60 mm Hg. These findings seem paradoxical because adult wound studies have shown that wound hypoxia can result in delayed healing, impaired leucocyte function, and increased infection.³ Finally, the profile of growth factors in fetal and adult serum are different — e.g., fetal serum contains much higher levels of insulin-like growth factor II and hyaluronic acid stimulating factor.^{4,5}

Basically, there are two ways to evaluate the role of the environment in the fetal wound healing process — either put adult skin in the fetal environment, or place fetal skin in the adult environment, then determine the effect of the environment on healing. To investigate the influence of the fetal environment in modulating postnatal wound healing, we transplanted adult sheep skin onto 60-day gestation (term = 145 days) fetal lambs.⁶ In this experiment, the engrafted adult skin is bathed in amniotic fluid, perfused by fetal blood, and the immature fetal immune system will not reject the adult skin graft. The adult grafts were wounded 40 days later (100 days gestation), at a time at which scarless repair occurs in fetal sheep. The wounds were analyzed by collagen immunohistochemistry and were shown to heal with scar forma-

tion. Neither an amniotic fluid environment nor perfusion by fetal blood prevented scar formation in the wounded adult skin graft. This study suggests that scarless fetal skin healing properties are intrinsic to fetal skin and are not caused by the fetal environment.

Support for this concept comes from the flip side experiment — fetal tissue healing in the adult environment. Ferguson and colleagues at the University of Manchester in England have done these experiments using an opossum called the *Monodelphus domesticus*. At birth, the opossum is physiologically and functionally a fetus and the pouch young remain attached to the mother's nipple for 4 weeks after birth. So the "fetal" development of this marsupial continues in this nonsterile environment and in the absence of amniotic fluid. Wounded 2-day old pouch young heal rapidly and scarlessly outside of the sterile, fluid uterine environment, whereas older animals show extensive scarring.⁷ Similarly, using novel *in vitro* systems, both Ihara and Martin have shown that isolated fetal rat or mouse tissue grown in organ culture media can heal wounds without scar formation.^{8,9} Thus, amniotic fluid and fetal blood components, such as platelets, are not required for scarless repair.

Despite a relatively constant intrauterine environment, fetuses heal without scar early in gestation and begin to scar late in gestation.¹⁰ We have studied the temporal sequence of repair outcomes in early, mid, and late gestation fetal rhesus monkeys. Early gestation primate lip wounds heal with regeneration of all skin elements, including collagen, hair follicles, sebaceous glands, and even muscle in the deeper tissue layers. However, as gestation proceeds, the primate fetus first loses its ability to regenerate normal hair follicle and other appendage patterns, but remains able to restore a normal, reticular collagen pattern after wounding. This pattern of "transition" wound repair is not regeneration or classic scar because the wound collagen organization is unchanged from skin that is not wounded. By the early third trimester, a complete switch to adult-type repair occurs with wounds showing densely packed, disorganized collagen deposition characteristic of scar.¹¹

The experiments described in this section led to the hypothesis that fetal healing must involve different cellular and connective tissue events than adult repair, and that this process is independent of the unique fetal environment. In addition, we have learned that the type of healing result in fetal animals also depends on 1) the extent of tissue damage — large excisional fetal skin wounds scar earlier in gestation than incisional wounds¹²; 2) the type of tissue that is injured — internal fetal tissues, such as the diaphragm muscle, stomach, and peritoneum, heal with scar formation¹³; and 3) the species of animal — fetal rabbits do not heal excisional skin wounds.¹⁴

A MODEL OF HUMAN FETAL SKIN REPAIR

An exciting new technique to study human fetal skin wound healing in the postnatal environment has been developed, and this model has helped delineate the relative importance of fetal tissue properties and the absence of certain inflammatory cytokines in scarless repair. Grafts of human fetal skin placed onto adult athymic mice retain the morphologic features of normal human fetal skin development. Full-thickness skin grafts from human fetuses at 15 to 22 weeks gestational age were placed onto athymic mice in two locations — cutaneously onto a fascial bed and thereby exposed to air, or subcutaneously in a pocket under the murine panniculus carnosus (Fig. 1).¹⁵ Linear wounds were made in each graft 1 week after transplantation, and grafts were harvested 30 minutes to 30 days after wounding. Wounds made in identical gestational age human fetal skin grafts healed with scar in cutaneous grafts and without scar in subcutaneous grafts (Fig. 2).

Why does the same gestational age human fetal skin heal with scar in a cutaneous location and without scar in a subcutaneous location? Differences in species-specific extracellular matrix, adult mouse fibroblasts *versus* fetal human fibroblasts, graft neovascularization, growth factor profile, inflammatory cell recruitment, differentiation, and the presence of an air-tissue interface may each contribute to this divergent healing response. To determine if murine collagens are present in the cutaneous graft scars, thereby indicating the presence of functioning adult murine wound fibroblasts, we performed indirect immunohistochemistry for murine and human collagen types I and III. We found that the subcutaneous grafts healed with human collagen types I and III in a scarless pattern.¹⁶ The wound collagen pattern was reticular and indistinguishable from the surrounding undamaged dermis. Conversely, the cutaneous grafts healed with murine collagen in a scar pattern with disorganized collagen fibers that streamed in from the murine wound base. Murine collagen scar was present along the base of the cutaneous grafts and as a thin capsule around the subcutaneous grafts. These findings suggest that adult fibroblasts deposit collagen in a scar pattern within fetal tissue. In contrast, fetal fibroblasts deposit collagen without scar formation in fetal skin placed in an adult subcutaneous environment. The fetal fibroblast appears to be a critical effector cell for scarless repair.

THE FETAL FIBROBLAST

In the fetal wound, fibroblasts deposit matrix in an organized fashion similar to normal skin, so that the fetal fibroblast can function autonomously. However, this

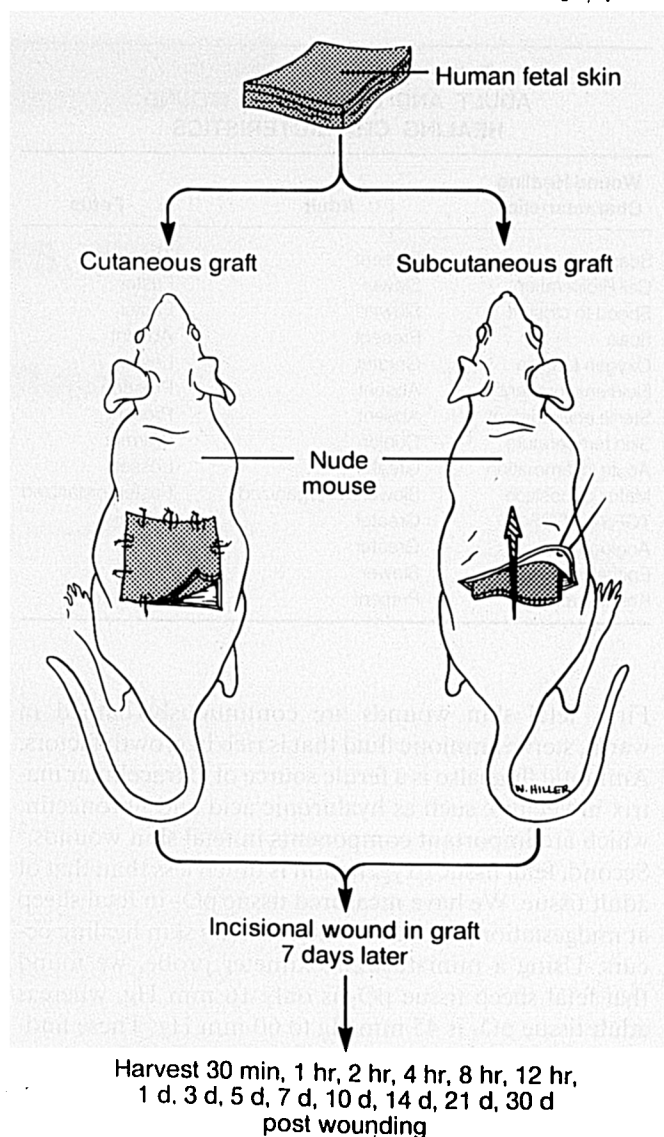


Figure 1. Schematic of experimental algorithm of human fetal skin transplantation onto the nude mouse. Each mouse received either two cutaneous or two subcutaneous grafts in dorsolateral flank locations.

phenomenon changes late in gestation, when adult-like healing with scar formation begins. Important differences in gene regulation have been found between fetal and adult cells, including dermal fibroblasts. For example, prolyl hydroxylase controls an important rate-limiting step in collagen production. Studies comparing early passage human fetal and adult fibroblasts show that prolyl hydroxylase activity in fetal fibroblasts is much greater until approximately 20 weeks gestation (term = 40 weeks), after which it gradually falls toward adult levels.¹⁷ Regulation of prolyl hydroxylase in fetal cells, unlike adult cells, is controlled by poly-ADP-ribose synthetase, an enzyme that has been implicated in cell repair and tumorigenesis. Furthermore, early fetal tissues have

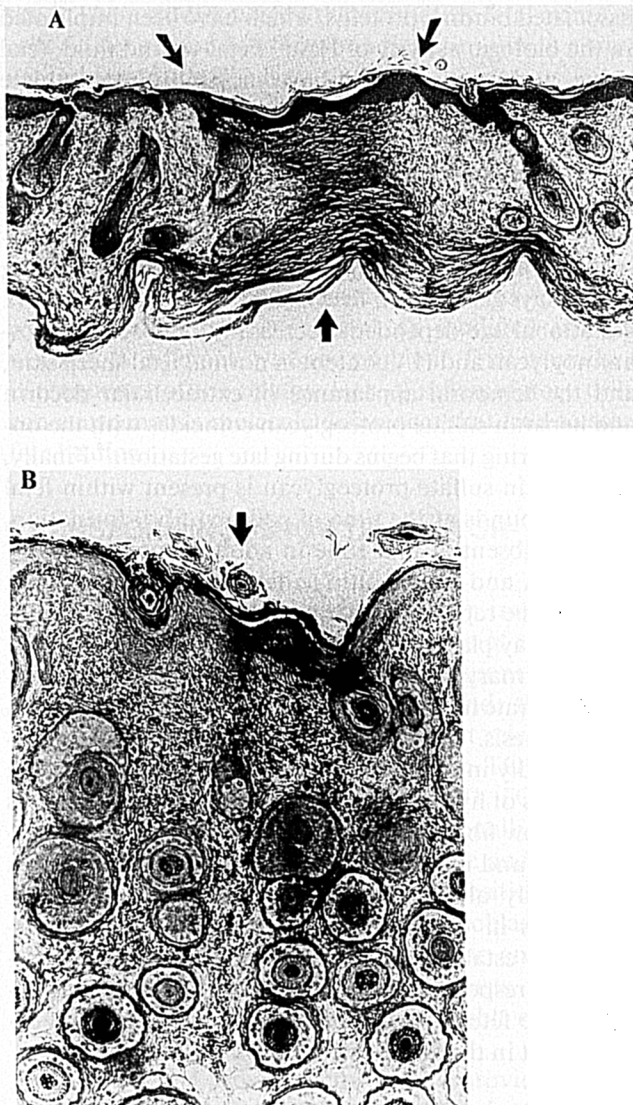


Figure 2. Human fetal skin at 19 weeks gestational age was transplanted onto athymic mice, wounded by incision, and stained with Mallory's trichrome. Cutaneous graft (A) healed with scar formation (closed arrows). Subcutaneous graft (B) healed without scar (closed arrow). The hair follicle and collagen patterns are unchanged from the surrounding undamaged dermis, demonstrating scarless human fetal skin repair (original magnification $\times 50$).

high endogenous levels of transcription factors called homeobox genes.¹⁸ Because regeneration-specific homeobox genes are expressed during regeneration of newt limbs, their presence in early fetal cells can have a marked effect on the response to wound stimuli.¹⁹

The identification of cytoskeletal differentiation markers in fibroblasts has led to the recognition of their phenotypic heterogeneity. During wound healing and fibrocontractive diseases in the adult, some fibroblasts acquire morphologic and biochemical features of smooth

muscle cells (myofibroblasts), including the expression of alpha-smooth muscle actin (ASMA).²⁰ In turn, ASMA expression and myofibroblast function can be modulated by the wound matrix and growth factors,²¹ and these interactions change during development.²²

We have investigated the role of the myofibroblast in fetal sheep tissue repair using ASMA immunohistochemistry and transmission electron microscopy for myofibroblast detection. Small excisional wounds in the fetal lamb heal without scar formation or contraction at 75 days gestation (term = 145 days), when ASMA-positive cells are absent, but excisional wounds begin to contract and show some scar formation beginning at 100 days gestation, when ASMA-positive myofibroblasts first appear. Transmission electron microscopy studies show that early in development, microfilament bundles in fibroblasts are sparse and disorganized, but as gestation progresses, the bundles became more prevalent and form tightly parallel arrangements characteristic of the contractile machinery of myofibroblasts.²³ The acquisition of more smooth muscle-like elements in wound fibroblasts later in gestation implies a relationship to the development of scar seen at that time. Within either the late gestation fetal wound or the adult wound, the forces of wound contraction generated by myofibroblasts and the "stiffer" matrix that is present can alter the alignment of collagen fibrils leading to the early establishment of an abnormal, scar-like orientation. Recent studies have focused on the principal components of the fetal dermal matrix — collagen and proteoglycans produced by the fetal fibroblast.

THE FETAL WOUND MATRIX

The extracellular matrix is a complex, cross-linked structure of proteins and polysaccharides that surrounds cells and organizes the geometry of normal tissues. Fetal wounds synthesize most of the matrix molecules present in adult wounds, but there are differences in the timing and pattern of these molecules in fetal wounds. For example, the cell adhesion molecule called tenascin is deposited much more rapidly and persists longer in fetal wounds, perhaps accounting for the rapid epithelialization and cellular ingrowth that occurs in fetal wound healing.²⁴

Collagen is an important component of the extracellular matrix; a scar is defined as abnormal collagen organization after wound repair compared with normal surrounding tissue. Thus, the pattern of collagen deposition in fetal and adult wounds is of particular interest. The orderly deposition of collagen in fetal animal models has been demonstrated using histologic, immunohistochemical, biochemical, and biophysical techniques.²⁵ The collagen pattern in fetal wounds is reticular and indistin-

guishable from adjacent normal tissue, whereas the adult wound contains large, parallel collagen bundles that are oriented perpendicular to the wound surface.¹⁰ Thus, scarless fetal wound healing must reflect the organization of collagen, not the absence of collagen in the fetal wound matrix.

Although collagen types I, III, V, and VI are present in both fetal and adult wounds,^{10,26} there are a few known differences in dermal collagen between the fetus and the adult. The presence of aminopropeptides of type I collagen in heterogeneous collagen fibrils is more common in the fetus compared with the adult.²⁷ The fetal dermis also contains a preponderance of type III collagen,²⁸ and as the fetus develops, the ratio of type III/type I collagen decreases, which may influence collagen fibril size. Small diameter collagen I fibrils with a high turnover and a similar configuration to fetal collagen (in terms of attached type III collagen and presence of type I aminopropeptide) occur adjacent to the epidermal/dermal junction in adult skin,²⁷ which is the region that shows minimal scarring in adult wounds. These minor differences between collagen in the fetal and adult dermis may be important in modulating the nature of the wounding response.

Alterations in the synthesis of proteoglycans and their constituent glycosaminoglycans correlate with the cell proliferation, migration, and collagen synthesis that accompany adult wound healing.²⁹ Proteoglycans are a heterogeneous group of polyanionic macromolecules that consist of a protein core, to which a variable number of linear, sulfated glycosaminoglycan chains are bound covalently. These macromolecules include versican, a large chondroitin sulfate proteoglycan; decorin, a small dermatan sulfate proteoglycan; and heparan sulfate proteoglycan. Proteoglycans and glycosaminoglycans have been shown to affect wound collagen organization and fibrillogenesis, and by binding to specific binding sites on collagen, control its rate of degradation.³⁰ The sulfated glycosaminoglycans temporally follow hyaluronic acid in the adult healing process, and the decline of hyaluronic acid levels and the appearance of sulfated glycosaminoglycans have been shown in several embryologic systems to correlate with the onset of cytodifferentiation.³¹

The fetal wound matrix is rich in glycosaminoglycans — in fetal rabbit wounds, the glycosaminoglycan content is approximately 3 times that of the adult wound during the same time period, and approximately 10 times that found in fetal skin that is not wounded.³² Hyaluronic acid (HA), the principal glycosaminoglycan present in fetal wounds, is a large molecule composed of alternating units of glucuronic acid and N-acetylglucosamine. An HA-rich matrix is permissive for cell motility and proliferation, and a prolonged presence of HA in fetal wounds may provide the matrix signal that orchestrates healing by regeneration. Mammalian HA also has

associated binding proteins, which have been implicated in the biologic activity of HA.³³ Fetal wound fluid, fetal urine, and amniotic fluid have the ability to stimulate and sustain HA synthesis because of a unique hyaluronic acid-stimulating activity. Recent support for the important role of HA in the fetal wound healing process is that levels of both HA and hyaluronic acid-stimulating activity in fetal lamb wound fluid decrease significantly during the transition period from fetal-like to adult healing at 120 days gestation in fetal sheep.³⁴ Similarly, there is a gestational age-dependent decrease in both total glycosaminoglycan and HA content in normal fetal sheep skin, and the temporal appearance of extracellular decorin and heparan sulfate proteoglycan coincides with the onset of scarring that begins during late gestation.³⁴ Finally, chondroitin sulfate proteoglycan is present within fetal mouse wounds at the time of collagen fibril formation, but it is absent at that time in adult mouse wounds.²⁶ Thus, HA and chondroitin sulfate are likely important for scar-free repair, whereas other sulfated glycosaminoglycans may play a role in scar formation.

The primary wound “scaffolding” into which fibroblasts migrate has a very important influence on collagen fibrillogenesis. In the fetal wound, fibroblasts can migrate rapidly into a loose honeycomb matrix containing high levels of hyaluronic acid. In contrast, adult wounds exhibit slow fibroblast migration into a denser, more resistant wound matrix where fibroblast migration occurs more easily along the wound margins. These initial differences in the migration and orientation of fibroblasts may establish the pattern of collagen fibrils deposited in the respective wounds, with a loose reticular structure in the fetus and a closely packed, disorganized arrangement in the adult.

THE ROLE OF INFLAMMATION AND CYTOKINES

There are major differences in the degree of inflammation in fetal and adult wounds. Until mid-gestation, the fetus is significantly neutropenic and has not developed self/non-self immunologic identity.³⁶ Histologically, there are very few, if any, polymorphonuclear leukocytes in fetal wounds,³⁷ and there may be a defect in immature polymorphonuclear leukocyte chemotactic ability.³⁸ Recent studies have correlated the absence of scarring in fetal wounds with the sparse inflammatory response, as evidenced by markedly reduced macrophage and monocyte infiltrates,⁷ absence of endogenous immunoglobulins at the wound site,^{10,25} reduced angiogenesis, and altered levels of peptide growth factors.³⁹ The transition of the fetal healing phenotype to a scarring, adult phenotype in the marsupial correlates directly with the amount of inflammatory reaction at the wound

site.⁴⁰ These studies suggest that immature fetal immune cells do not respond to the wounding stimulus in a similar fashion to adult cells.

Peptide growth factors are released by inflammatory cells and help provide a cell-to-cell and cell-to-matrix communication system. These cytokines can affect matrix synthesis, matrix degradation, cell proliferation, and cell recruitment to the wound site. Because of the prominent role that inflammation plays in adult tissue repair, the characteristic inflammatory mediators of adult wound healing may be absent or modified in fetal wounds. A different cytokine profile in fetal wounds, a consequence of the relative lack of inflammatory cells, can cause matrix molecule differences that lead to scar-free healing.

TRANSFORMING GROWTH FACTOR-BETA (TGF- β)

Of the many cytokines that have been implicated in wound healing, TGF- β affects all phases of the healing process, including the inflammatory response and matrix accumulation.⁴¹ In the adult wound, the macrophage is a crucial inflammatory cell that releases TGF- β and other cytokines. The mammalian TGF- β family consists of three known isoforms, TGF- β 1, TGF- β 2, and TGF- β 3, that are structurally and functionally closely related to one another. Through autocrine and paracrine mechanisms, TGF- β stimulates the deposition of collagen and other matrix components by fibroblasts, inhibits collagenase, blocks plasminogen inhibitor, enhances angiogenesis, and is chemotactic for fibroblasts, monocytes and macrophages.⁴² Thus, TGF- β is capable of stimulating fibrogenesis by the fibroblast.

Transforming growth factor- β may provide the link between injury, inflammation, and fibrosis. After tissue injury, activated macrophage-derived TGF- β disturbs the balance of synthesis and degradation of collagens and causes accumulation of extracellular matrix. Transforming growth factor- β induces alpha-smooth muscle actin expression in fibroblasts, and this finding correlates with scar formation and pathologic wound contraction.⁴³ The ability of TGF- β to induce its own production may be crucial for the development of progressive scarring in chronic diseases that lead to eventual obliteration of normal tissue architecture. The correlation between TGF- β levels and scar formation holds true for a variety of fibrotic diseases. Experimental intraperitoneal administration of TGF- β results in adhesion formation.⁴⁴ There is enhanced expression of TGF- β 1 at the site of scar in the rat brain after localized cerebral injury.⁴⁵ In proliferative vitreoretinopathy, total TGF- β levels in intraocular fluid increase as the retinal scarring progresses from mild to moderate to severe.⁴⁶ Markedly increased

amounts of TGF- β are present in fibrogenic diseases such as cirrhosis,⁴⁷ interstitial pulmonary fibrosis,⁴⁸ glomerulonephritis,^{49,50} and scleroderma.⁵¹

Exogenously applied TGF- β promotes scar formation in both adult and fetal wounds. Specifically, the addition of TGF- β to polyvinyl alcohol sponges implanted in fetal rabbits produces fibrosis.⁵² We have demonstrated that when TGF- β is added to human fetal skin wounds via a slow release disk, scar formation results.⁵³ In vitro studies have shown that exposure of fetal dermal fibroblasts to TGF- β results in marked upregulation of collagen gene expression.⁵⁴ Thus, the cellular and matrix machinery that is necessary for scar formation exists in fetal wounds.

Fibroblasts are not only capable of responding to the numerous cytokines produced by the immune system, but they can synthesize and secrete, on their own, growth factors with pleiotrophic effects. The technique of reverse transcription-polymerase chain reaction was used to detect differences in TGF- β -specific mRNA production by fetal and adult sheep fibroblasts.⁵⁵ To test whether fetal and adult fibroblasts have different responses to the low tissue pO₂ in the fetal wound environment, we examined TGF- β 1 gene expression in these cells under normoxic and hypoxic conditions. Both fetal and adult fibroblasts showed the potential to produce TGF- β 1 at normoxia, but fetal fibroblasts responded to hypoxia with a decrease in TGF- β 1 transcription, whereas adult fibroblasts were stimulated by hypoxia to increase TGF- β transcription. Thus, the low fetal wound pO₂ may markedly downregulate TGF- β 1 gene expression.

This series of studies led to the hypothesis that scarless fetal wounds may be relatively TGF- β deficient. Whitby has performed TGF- β immunostaining studies in fetal mouse wounds and has found that TGF- β was absent, whereas TGF- β staining was abundant in neonatal and adult mouse wounds.³⁹ We have shown an absence of immunostaining for either TGF- β 1 or TGF- β 2 from 1 hour to 28 days after wounding human fetal skin.⁵³ In contrast, wounds in adult human skin grafts demonstrated TGF- β 1 at the wound edge at 6 hours through 21 days and TGF- β 2 at 12 hours through 7 days. It is possible that TGF- β levels in fetal wounds are below the threshold for detection by immunohistochemical techniques, but compared with adult wounds, fetal wounds are at least relatively TGF- β deficient. Although we have detected significant amounts of TGF- β in fetal sheep wound fluid, there may be an alteration or limitation of TGF- β biologic activity by the local fetal wound environment.⁵⁶

Macrophages are the principal source of TGF- β in adult wounds, and the reduced TGF- β level in fetal wounds can reflect the absent or minimal macrophage infiltrate in these wounds. In adult wounds, TGF- β also

is released from the alpha-granules of activated platelets, so it also is possible that fetal platelets may not synthesize or release TGF- β at the fetal wound site. The relative lack of TGF- β , a cytokine known to induce fibrosis, may be an important reason the fetus heals by regeneration rather than by scarring. Transforming growth factor- β may be important in adult tissue repair, but excessive action of this cytokine may be responsible for the tissue damage caused by scarring in many serious diseases. These findings suggest that anti-TGF- β therapeutic strategies may ameliorate scar formation in adult fibrotic diseases.

CYTOKINE EXCESS IN ADULT WOUNDS

Shah and colleagues mimicked this fetal wound situation within the healing adult rat wound by using an anti-TGF- β polyclonal neutralizing antibody to experimentally reduce TGF- β levels.⁵⁷ This manipulation resulted in markedly diminished scarring in adult wounds. The neutralizing antibody-treated wounds had normal tensile strength and a nearly normal dermal architecture compared with untreated wounds, and this salutary effect was accompanied by deposition of less collagen and fibronectin and infiltration by fewer macrophages and blood vessels. Injection of TGF- β alone had the opposite effects. Application of TGF- β neutralizing antibody at the time of wounding (and not later) was essential to reduce active TGF- β levels, prevent auto-induction of TGF- β mRNA, and limit macrophage infiltration and further TGF- β release. The effectiveness of this approach has been demonstrated in another fibrotic process, because administration of either TGF- β 1 antiserum or decorin to neutralize TGF- β biologic activity suppresses the pathologic increase in matrix synthesis that occurs in an animal model of glomerulonephritis.^{49,50} Thus, the relationship of TGF- β to scar formation fulfills Koch's postulates — TGF- β is absent in scarless fetal wounds, addition of TGF- β to fetal wounds results in scar formation, the presence of TGF- β after adult injury correlates with the degree of fibrosis observed, and blocking of TGF- β in adult wounds has a potent anti-scarring effect.

Subsequent studies by Shah and Ferguson have shown that neutralization of both TGF- β 1 and TGF- β 2 isoforms has a much greater, synergistic anti-scarring effect than neutralization of either isoform alone.⁵⁸ Neutralizing antibodies against platelet-derived growth factor also have some anti-scarring effect, but antibodies to epidermal growth factor have no effect on dermal scarring.⁵⁹ Although injection of cytokine antibodies into wounds has very limited clinical potential because of antigenicity problems, there are other promising ways to reduce wound levels of TGF- β 1 and TGF- β 2. Addition of the

Table 2. ANTI-TRANSFORMING GROWTH FACTOR-BETA THERAPEUTIC STRATEGIES

Neutralizing antibodies to TGF- β 1 and β 2
TGF- β 3
TGF- β -binding proteoglycans
Biglycan
Decorin
Soluble receptors or receptor antagonists
Prevent TGF- β activation
Block IGF-II/mannose-6-phosphate receptor
Add latent TGF- β binding protein
Antisense oligonucleotides
Anti-macrophage strategies

TGF- β 3 isoform downregulates TGF- β 1 and β 2 levels and leads to a pronounced anti-scarring result. The application to rodent wounds of the inexpensive and readily available sugar mannose-6-phosphate also limits scar formation, presumably by blocking the insulin-like growth factor-II/mannose-6-phosphate receptor that is important for TGF- β activation.⁵⁹ Various other theoretical anti-TGF- β therapeutic strategies, such as flooding the wound with soluble TGF- β receptors to compete effectively with cellular TGF- β binding sites or adding antisense oligonucleotides to inhibit TGF- β gene expression, also can make adult wounds heal in a fetal-like manner (Table 2). Inhibitors of TGF- β may be important future drugs for the control of fibrosis.

Basic fibroblast growth factor is another example of cytokine excess in postnatal wounds. Using immunohistochemical techniques, basic fibroblast growth factor is present in neonatal and adult mouse lip wounds, but is not detected in fetal mouse lip wounds.³⁹ Basic fibroblast growth factor is a powerful angiogenesis stimulator, and increased neovascularization is a normal component of adult wound repair.⁶⁰ Immunostaining for collagen IV and laminin, normal components of the endothelial basement membrane, shows profuse angiogenesis in adult wounds, whereas fetal wounds have a diminished vascular pattern similar to adjacent undamaged fetal tissue.^{10,26} A more rapid rate of adult wound neovascularization would bring earlier wound perfusion by adult serum and inflammatory cells, thereby contributing to a scar repair pattern.

In evolutionary terms, it appears that adult wounds may be optimized for speed of healing under adverse conditions (dirt, foreign bodies, etc.) and the result is an excessive inflammatory infiltrate and cytokine profile. The potential fetal regenerative response in adult wounds may be overrun by an inflammation-induced cytokine surplus leading to scar. This phenomena is an example of "cytokine poisoning," in which the inappropriate reparative response of the patient may prove det-

rimental to outcome. This paradox is analogous to the pathophysiology of toxic host mediators associated with disease states such as systemic sepsis and multiple organ failure.^{61,62}

FUTURE DIRECTIONS

Fetal wound healing studies can help surgeons understand what accounts for scarring and, perhaps more importantly, how scar formation can be prevented. There are a number of ways in which the matrix and cellular response of the healing adult wound might be manipulated to reduce scarring — i.e., inhibiting the wound inflammatory response by blocking inflammatory cytokines such as TGF- β , basic fibroblast growth factor, and platelet-derived growth factor; adding exogenous tenascin to facilitate keratinocyte and fibroblast migration into the wound; transplanting fibroblasts with fetal characteristics to the adult wound site; providing a more porous wound scaffold by addition of hyaluronic acid or hyaluronic acid-stimulating activity to enhance fibroblast migration and promote regeneration of a normal reticular collagen organization in the wounded dermis. Although all of these potential therapeutic strategies require rigorous scientific testing, adult wound healing may be altered toward a scar-free, fetal-like phenotype by modifying one or more of the components that are different between fetal and adult repair.

References

- Adzick NS, Longaker MT, eds. *Fetal Wound Healing*. New York: Elsevier Scientific Press, 1992.
- Longaker MT, Adzick NS, Hall JL, et al. Studies in fetal wound healing VII: fetal wound healing may be modulated by elevated hyaluronic acid stimulating activity in amniotic fluid. *J Pediatr Surg* 1991; 25:430–433.
- Jonsson K, Jensen JA, Goodson WH, et al. Tissue oxygenation, anemia, and perfusion in relation to wound healing in surgical patients. *Ann Surg* 1991; 214:605–613.
- Longaker MT, Chiu ES, Harrison MR, et al. Studies in fetal wound healing IV: hyaluronic acid stimulating activity distinguishes fetal from adult wound fluid. *Ann Surg* 1989; 210:667–672.
- Estes JM, Spencer EM, Longaker MT, Adzick NS. Insulin-like growth factor-II in ovine wound fluid: evidence for developmental regulation. *Surg Forum* 1991; 42:659–661.
- Longaker MT, Whitby DJ, Ferguson MWJ, et al. Adult skin wounds in the fetal environment heal with scar formation. *Ann Surg* 1994; 219:65–72.
- Ferguson MWJ, Howarth GF. Marsupial models of scarless fetal wound healing. In Adzick NS, Longaker MT, eds. *Fetal Wound Healing*. New York: Elsevier Scientific Press, 1992, pp 95–124.
- Ihara I, Motobayashi Y. Wound closure in foetal rat skin. *Development* 1992; 114:573–582.
- Martin P, Lewis J. Actin cables and epidermal movement in embryonic wound healing. *Nature* 1992; 360:179–182.
- Longaker MT, Whitby DJ, Adzick NS, et al. Studies in fetal wound healing VI: second and early third trimester fetal wounds demonstrate rapid collagen deposition without scar formation. *J Pediatr Surg* 1990; 25:63–69.
- Lorenz HP, Whitby DJ, Longaker MT, Adzick NS. The ontogeny of scar formation in the non-human primate. *Ann Surg* 1993; 217:391–396.
- Longaker MT, Burd DAR, Gown AM, et al. Midgestation fetal lamb excisional wounds contract in utero. *J Pediatr Surg* 1991; 26:942–947.
- Longaker MT, Whitby DJ, Jennings RW, et al. Fetal diaphragmatic wounds heal with scar formation. *J Surg Res* 1991; 50:375–385.
- Krummel TM, Ehrlich HP, Nelson JM, et al. In vitro and in vivo analysis of the inability of fetal rabbit wounds to contract. *Wound Rep Reg* 1993; 1:15–21.
- Lorenz HP, Longaker MT, Perkocha LA, et al. Scarless wound repair: a human fetal skin model. *Development* 1992; 114:253–259.
- Lorenz HP, Longaker MT, Whitby DJ, et al. Scarless fetal skin repair is intrinsic to the fetal fibroblast. *Surg Forum* 1992; 43:696–697.
- Duncan BW, Qian J, Liu X, Bhatnagar R. Regulation of prolyl hydroxylase activity in fetal and adult fibroblasts. In Adzick NS, Longaker MT, eds. *Fetal Wound Healing*. New York: Elsevier Scientific Press, 1992, pp 303–323.
- Mackenzie A, Leeming GL, Jowett A, et al. The homeobox gene *Hox 7.1* has specific regional and temporal expression patterns during early murine craniofacial embryogenesis, especially during tooth development in vivo and in vitro. *Development* 1991; 111:269–285.
- Brown R, Brockes JP. Identification and expression of a regeneration-specific homeobox gene in the newt limb blastema. *Development* 1991; 111:489–496.
- Sappino AP, Schurch W, Gabbiani G. Differentiation repertoire of fibroblastic cells: expression of cytoskeletal proteins as markers of phenotypic modulations. *Lab Invest* 1990; 63:144–161.
- Darby I, Skalli O, Gabbiani G. Alpha-smooth muscle actin is transiently expressed by myofibroblasts during experimental wound healing. *Lab Invest* 1990; 63:21–29.
- Sappino AP, Dietrich PY, Skalli O, et al. Colonic pericryptal fibroblasts: differentiation pattern in embryogenesis and phenotypic modulation in epithelial proliferation lesions. *Virchows Archiv A Pathol Anat Histopathol* 1990; 415:551–557.
- Estes JM, Vande Berg JS, Adzick NS, et al. Phenotypic and functional features of myofibroblasts in fetal wounds. *Differentiation* (in press).
- Whitby DJ, Longaker MT, Harrison MR, et al. Rapid epithelialization of fetal wounds is associated with early deposition of tenascin. *J Cell Sci* 1991; 99:583–586.
- Adzick NS, Longaker MT. Scarless wound healing in the fetus: the role of the extracellular matrix. *Prog Clin Biol Res* 1991; 365:177–192.
- Whitby DJ, Ferguson MWJ. The extracellular matrix of lip wounds in fetal, neonatal, and adult mice. *Development* 1991; 112:651–668.
- Fleischmajer R, Perlsh JS, Burgeson RE, et al. Type I and type III collagen interactions during fibrillogenesis. *Ann NY Acad Sci* 1990; 580:161–175.
- Merkel JR, DiPaolo BR, Hallock GG, Rice DC. Type I and type III collagen content of healing wounds in fetal and adult rats. *Proc Soc Exp Biol Med* 1988; 187:493–497.
- Yeo TK, Brown L, Dvorak HF. Alterations in proteoglycan synthesis common to healing wounds and tumors. *Amer J Pathol* 1991; 138:1437–1450.

30. Ruoslahti E, Yamaguchi Y. Proteoglycans as modulators of growth factor activities. *Cell* 1991; 64:867-869.
31. Bertolami CN, Berg S, Frymiller EG. Glycosaminoglycan processing during tissue repair: degradation of hyaluronic acid. *In* Adzick NS, Longaker MT, eds. *Fetal Wound Healing*. New York: Elsevier Scientific Press, 1992, pp 215-226.
32. DePalma RL, Krummel TM, Durham LA, et al. Characterization and quantification of wound matrix in the fetal rabbit. *Matrix* 1990; 9:224-231.
33. Toole BP. Hyaluronan and its binding proteins, the hyaladherins. *Curr Op Cell Biol* 1990; 2:839-844.
34. Estes JM, Adzick NS, Longaker MT, et al. Hyaluronate metabolism undergoes ontogenetic transition during fetal development: Implications for scarless wound healing. *J Pediatr Surg* 1993; 28: 1227-1231.
35. Freund RM, Siebert JW, Cabrera J, et al. Serial quantification of hyaluronan and sulfated glycosaminoglycans in fetal sheep skin. *Biochem Mol Biol Int* 1993; 29:813-816.
36. Flake AW, Harrison MR, Adzick NS, Zanjani ED. Transplantation of fetal hematopoietic stem cells in utero: the creation of hematopoietic chimeras. *Science* 1986; 223:776-778.
37. Adzick NS, Harrison MR, Glick PL, et al. Comparison of fetal, newborn, and adult rabbit wound healing by histologic, enzyme-histochemical, and hydroxyproline determinations. *J Pediatr Surg* 1985; 20:315-319.
38. Jennings RW, Adzick NS, Longaker MT, et al. Ontogeny of fetal sheep polymorphonuclear leukocyte phagocytosis. *J Pediatr Surg* 1991; 26:853-855.
39. Whitby DJ, Ferguson MWJ. Immunohistochemical localization of growth factors in fetal wound healing. *Dev Biol* 1991; 147:207-215.
40. Armstrong J. Healing characteristics of dermal wounds on the pouch young of the short tailed grey opossum. *Wound Rep Reg* 1993; 2:96.
41. Barnard JA, Lyons RM, Moses HL. The cell biology of transforming growth factor β . *Biochim Biophys Acta* 1990; 1032:79-87.
42. Sporn MB, Roberts AB. Transforming growth factor- β : recent progress and new challenges. *J Cell Biol* 1992; 119:1017-1021.
43. Desmouliere A, Geinoz A, Gabbiani F, Gabbiani G. Transforming growth factor- β 1 induces alpha-smooth muscle actin expression in granulation tissue myofibroblasts and in quiescent and growing fibroblasts. *J Cell Biol* 1993; 122:103-111.
44. Logan A, Frautschy SA, Gonzalez AM, et al. Enhanced expression of transforming growth factor β 1 in the rat brain after a localized cerebral injury. *Brain Res* 1992; 587:216-225.
45. Williams RS, Rossi AM, Chagini N, Schultz G. Effect of transforming growth factor β on postoperative adhesion formation and intact peritoneum. *J Surg Res* 1992; 52:65-70.
46. Connor TB, Roberts AB, Sporn MB, et al. Correlation of fibrosis and transforming growth factor- β type 2 levels in the eye. *J Clin Invest* 1989; 83:1661-1666.
47. Castilla A, Prieto J, Fausto N. Transforming growth factors beta1 and alpha in chronic liver disease: Effects of interferon alfa therapy. *N Engl J Med* 1991; 324:933-940.
48. Broekelmann TJ, Limper AH, Colby TV, McDonald JA. Transforming growth factor β 1 is present at sites of extracellular matrix gene expression in human pulmonary fibrosis. *Proc Natl Acad Sci USA* 1991; 88:6642-6646.
49. Border WA, Okuda S, Languino, et al. Suppression of experimental glomerulonephritis by antiserum against transforming growth factor β 1. *Nature* 1990; 346:371-374.
50. Border WA, Noble NA, Yamamoto T, et al. Natural inhibitor of transforming growth factor- β protects against scarring in experimental kidney disease. *Nature* 1992; 360:361-364.
51. Peltonen J, Kahari L, Jaakkola S, et al. Evaluation of transforming growth factor β and type I procollagen gene expression in fibrotic diseases by in situ hybridization. *J Invest Dermatol* 1990; 94:365-371.
52. Krummel TM, Michna BA, Thomas BL, et al. TGF β induces fibrosis in a fetal wound model. *J Pediatr Surg* 1988; 23:647-652.
53. Sullivan KM, Lorenz HP, Adzick NS. The role of transforming growth factor beta in human fetal wound healing. *Surg Forum* 1993; 44:625-627.
54. Lorenz HP, Chang J, Longaker MT, Banda MJ. Transforming growth factors β 1 and β 2 synergistically increase collagen gene expression in fetal fibroblasts but not in adult fibroblasts. *Surg Forum* 1993; 44:723-725.
55. Chang J, Longaker MT, Lorenz HP, et al. Fetal and adult sheep fibroblast TGF- β 1 gene expression *in vitro*: effects of hypoxia and gestational age. *Surg Forum* 1993; 44:720-722.
56. Longaker MT, Bouhana KS, Roberts AB, et al. Regulation of fetal wound healing. *Surg Forum* 1991; 42:654-655.
57. Shah M, Foreman DM, Ferguson MWJ. Control of scarring in adult wounds by neutralizing antibody to transforming growth factor beta. *Lancet* 1992; 1:213-214.
58. Shah M, Forman D, Ferguson M. Reduction of scar tissue formation in adult rodent wound healing and manipulation of the growth factor profile. Presented at the Second Annual Meeting of the Wound Healing Society. April 1992; Richmond, VA.
59. Shah M, Roberts AB, Gold LI, Ferguson MWJ. Immunolocalization of TGF β isoforms in normal and experimentally modulated incisional wounds in adult rodents. *Wound Rep Reg* 1993; 2:124.
60. Folkman J, Shing Y. Angiogenesis. *J Biol Chem* 1992; 267:10931-10934.
61. Baue AE. The horror autotoxicus and multiple-organ failure. *Arch Surg* 1992; 127:1451-1462.
62. Deitch EA. Multiple organ failure: pathophysiology and potential future therapy. *Ann Surg* 1992; 216:117-134.