SCARLESS WOUND HEALING IN THE MAMMALIAN FETUS

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SURGEONS are often required to manage patients with pathologic processes in which abnormal healing occurs. Such conditions exist in virtually all surgical specialties and include inadequate tissue repair that causes dehiscence or chronic, nonhealing open wounds, cutaneous keloids and hypertrophic scars, adhesions limiting function after tendon repair, failed nerve repair because of scar, biliary and intestinal tract strictures, intra-abdominal adhesions and visceral scarring, such as cirrhosis and atherosclerotic vascular disease. All these conditions are associated with significant morbidity and mortality rates, or both, and are often difficult and challenging to treat. A search for improved management of these conditions is a continuous endeavor of investigators of wound healing and connective tissue metabolism.

Tissue repair in the mammalian fetus is fundamentally different than normal adult healing. In adult humans, injured tissue is repaired by collagen deposition, collagen remodeling and eventual scar formation, whereas fetal wound healing seems to be more of a regenerative process with minimal or no scar formation. Therefore, fetal healing provides an accessible mammalian animal model of an optimal healing response. Understanding the mechanisms of such scarless healing may provide new treatment modalities by which therapy of many of the aforementioned conditions can be greatly improved. Additionally, understanding fetal healing may be invaluable to the clinician as the new age of human fetal surgery is being entered. Current knowledge concerning the remarkable process of mammalian fetal wound healing is presented in this study.

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ASPECTS OF DEVELOPMENT THAT MAY AFFECT HEALING

The fetus is a developing organism in which cellular migration, proliferation and differentiation occur at an unparalleled magnitude. Because healing of damaged tissue is a process of cellular interaction, the mechanisms underlying fetal tissue repair cannot be fully understood without an awareness of the unique physiologic and biochemical processes within the fetus, as well as the unique intrauterine environment in which healing occurs. Several aspects of fetal development have the potential to affect the cellular and extracellular processes necessary for healing.

The hormonal milieu of an organism has direct effects on the cellular activity within various organ systems. In adults, the endocrine system is integrally involved in the response to traumatic injury (1). Compared with adults, the fetus contains a functionally different endocrine system, for example, the fetal adrenal gland is probably second only to the liver in size. The majority of the gland is composed of a "fetal zone" that quickly involutes after birth. Fetal adrenal steroid secretion is enormous, approximately ten times that of the nonstressed adult (2). It is quite possible that the different state of hormonal flux in the fetus may affect the processes of tissue repair.

The immune system and its associated inflammatory mediators are intimately involved in adult tissue repair. The fetus is immunologically immature and less competent than the adult. Neutropenia and a lack of antigenic stimulation may contribute to the "immaturity" (3, 4). Humoral immunity is comprised mostly of immunoglobulin G obtained passively from the maternal circulation, but the fetus is capable of producing immunoglobulin M when provoked (2). The complement system is also underdeveloped. Components are present in reduced quantities compared with adults (5, 6). It is possible that the immunologic differences in the fetus may relate to the differences in wound healing observed in the fetal system.

Oxygen is essential for normal adult wound healing. The fetus is hypoxic, however, and has

an oxygen partial pressure of approximately 20 torr, reflecting an oxygen partial pressure of 30 to 35 torr of maternal blood in the placental intervillous spaces (2). Obviously, this factor has potential effects on tissue repair, although the fetus seems to compensate fairly well; the lactic acid level (an indicator of anaerobic glycolysis) is only slightly higher in umbilical cord blood, compared with maternal blood (2).

The external fetal environment consists of the placenta and amniotic fluid. Fetal survival is dependent on a symbiosis between mother and fetus through the placenta. As a transfer organ the placenta acts as surrogate lungs, kidneys and intestinal tract for the fetus (7). Functional impairment of the placenta, such as decreased uteroplacental blood flow as encountered in maternal hypertension, may directly impair fetal functions. This includes healing.

Amniotic fluid provides a sterile, weightless environment that is protective and thermally stable. Factors within amniotic fluid can locally affect the healing process. Systemic effects of amniotic fluid are also possible because the fetus swallows the fluid (8), thus allowing the absorption of a variety of potential mediators. In fact, absorption of various nutrients has been demonstrated in isolated intestines of fetal rabbits (9). An effect on fetal cellular function has been demonstrated by the observation that amniotic fluid has a trophic effect in supporting fetal gastric cells in culture (10, 11). Obviously, such interactions may affect tissue repair.

OVERVIEW OF ADULT (POSTNATAL) WOUND HEALING

Normal healing is essentially the same at all postnatal ages. Because the adult repair response is the norm, it is essential to be familiar with the biologic features of normal postnatal healing before discussion of fetal repair. Adult healing occurs by a sequence of events in which cellular and matrix components act in concert to repair a tissue defect. Based on Schilling's categorization, the healing response can be described in four broad phases—hemostasis, inflammation, proliferation and remodeling (12). At the time of injury, hemostatic mechanisms, which include vasoconstriction, platelet aggregation and fibrin deposition, are initiated to control local hemorrhage. The initial wound matrix of fibrin acts as a scaffold on which inflammatory cells enter the wound (12, 13). Platelet alpha degranulation results in the release of platelet derived growth factor (PDGF) (14, 15) and transforming growth factor beta (TGF-β) (16), which are chemoattractants, mitogens and stimulators of collagen deposition for cells that will enter the wound.

Acute inflammation is established 12 to 24 hours postoperatively as vasoconstriction is followed by vasodilation and increased capillary permeability. This results in the influx of polymorphonuclear leukocytes (neutrophils) into the wound site. The neutrophils control bacterial contamination, but are probably not necessary for healing, because neutropenic patients and animals usually heal normally (17). Neutrophils are gradually replaced by macrophages and then lymphocytes, as a chronic inflammatory infiltrate is established. Macrophages are thought to be essential because macrophage depletion inhibits normal fibroplasia (18). Similar to platelets, macrophages secrete various growth factors, such as PDGF and TGF-β, which enhance fibroplasia (18, 19). The redundancy of these factors further stimulates the influx of fibroblasts into the wound where they are stimulated to proliferate and produce collagen (16). In addition to the enhanced fibroblast response, epithelialization occurs and neovascularization is prominent as endothelial cells proliferate. As fibroblast activity continues, the initial fibrin matrix is replaced by collagen.

Remodeling of the matrix occurs years after injury because there is continued synthesis and degradation of collagen. The resultant collagenous scar represents a fine balance between the two processes. Crosslinking and structural modifications of collagen fibrils are largely responsible for the increase in tensile strength of the healing tissue during this time (20–22). The final result of adult healing is a replacement of normal tissue by a collagenous scar, which lacks the ordered structure of normal skin and is an imperfect process as exemplified by the observation that healed wounds never regain the full tensile strength of uninjured tissue (20).

A distinct feature of postnatal open wounds is the ability to concentrically decrease the area of lost tissue by the process of contraction. The forces generated during contraction are thought to arise from myofibroblasts within the granulation tissue of the wound. The specialized fibroblasts contain the contractile protein actin (23). Evidence also exists that direct fibroblast-collagen matrix interaction may cause contraction of open wounds (24). By reducing the area of tissue defect, contraction facilitates repair of open wounds in which tissue loss is a prominent feature.

MODELS OF FETAL HEALING

Monkeys (25), rats (26–28), sheep (29) and rabbits (4, 30–32) have been used in investigations of fetal healing. The latter two have been used most frequently. The South American opossum has also been studied in an attempt to gain easier access to the fetus that continues to develop on the exterior of the mother (33, 34). The use of an animal model is imperative because of unique physiologic features and environment of the fetus that would be nearly impossible to replicate in a culture dish or other *in vitro* systems. Generally, the procedures for studies of fetal healing involve a maternal laparotomy during which a hysterotomy is created, thus providing access to the fetus. Wounding takes place while the fetus remains within the uterus. Most commonly, wounds that are incisional and excisional are created, although burning and freezing have also been performed. The hysterotomy and maternal incision are then closed. Usually, cesarean section is performed at specified times postoperatively so that fetal wound tissue can be sampled before parturition and consequent violation of intrauterine conditions (35).

Healing wounds are evaluated by histologic and biochemical analysis, or both, of wound tissue. There are many studies in which excisional biopsy specimens of primarily closed wounds have been analyzed. Although useful data are obtained in this manner, limitations arise in the biopsy of primarily closed skin wounds because of the inability to accurately identify the boundary between healing and uninjured tissue. Additionally, "contamination" of specimens with normal tissue renders biochemical assays less specific. Because of the limitations, as well as the relative paucity or difficulty in obtaining fetal wound tissue, wound implant devices have been used, including the Hunt-Schilling chamber, the viscose cellulose sponge, Goodson's and Hunt's expanded polytetrafluoroethylene (Gore-tex™) tube and the polyvinyl alcohol (PVA) implant (36). The ideal implant should be as inert as possible within the wounds, so that observed cellular and matrix events closely correlate with those of wound biopsies.

CHARACTERISTICS OF THE FETAL HEALING RESPONSE

Closed linear wounds. The most striking features of primarily closed, linear wounds are the rapidity with which they heal and the absence of obvious scarring. After injury, linear wounds in fetal rabbits appear macroscopically healed by five to seven days (32). Scar formation has been noted to be grossly and microscopically absent in both experimentally created wounds (29, 32, 37) and surgical wounds after *in utero* repair of cleft lips in mice (38), monkeys (39) and rabbits (40). Evaluation of the processes responsible for these phenomena can be descriptively divided into cellular and extracellular matrix events.

Cellular events in fetal closed wounds. A striking difference between postnatal and fetal repair is the absence of acute inflammation in fetal wounds of several species. Only a few neutrophils were noted within incised fetal sheep wounds (29). Similar observations were made in the monkey (25), while an absence of acute inflammation was reported in fetal rat wounds (41). Tissue sampling with a variety of implants has confirmed the absence of acute inflammation within wounds of fetal rabbits (4, 32, 42).

Several hypotheses could explain the lack of acute inflammation in fetal wounds. The sterile, intrauterine environment may contribute by failing to provide antigenic stimulation for polymorphonuclear infiltration (25). However, "sterile" wounds in germ-free adult guinea pigs demonstrated acute inflammation, although in lesser quantities than in control animals (43). This suggests that bacterial contamination may not be the sole initiator of acute inflammation in adult wounds. Immunologic immaturity and neutropenia have also been implicated as being causative (4, 29), indicating that the fetus is unable to mount an inflammatory response despite provocation. In an experimental model for biliary atresia, acute inflammation within the liver resulted after injection of periportal sodium morrhuate in fetal dogs (44). Thus, it seems that the underdeveloped fetal immune system may be capable of an adult-like acute inflammatory response after stimulation.

Similar to differences in acute inflammation in fetal and postnatal healing, the chronic inflammatory response also differs. Although macrophages have been identified in fetal rabbit wounds by nonspecific esterase staining (4) and a slight increase in macrophages has been described in fetal sheep wounds (29), lymphocytes have been rarely encountered in fetal wounds. Mesenchymal cell infiltration in fetal sheep wounds has been temporally associated with the appearance of macrophages (29) and likewise, mesenchymal cells have been noted in wounds of fetal rabbits (32). Mononuclear cells have also been repeatedly observed in fetal rabbit wounds

using PVA implants, but the specific identity is still unknown (32).

The hallmark of the proliferative phase in adult healing is fibroplasia. Results of studies of different fetal species suggest that there is interspecies variation in the fetal wound fibroblastic response, although the response seems to be significantly reduced when compared with adult wounds. Biopsies of healed fetal sheep wounds were observed to contain a mild fibroblastic infiltration (29). Similarly, fetal rabbit wounds that were evaluated by simple biopsy (45) or PVA implant histologic features (32) contained only a scant presence of fibroblasts. However, when Gore-tex implants were used as the means of tissue sampling in fetal rabbit wounds, fibroblastic encapsulation of the implant was observed (4). The biomaterial differences between Gore-tex and polyvinyl alcohol could account for these varied observations.

Epithelialization is also a prominent proliferative response in postnatal wound healing. This process occurs quite rapidly in primarily closed fetal wounds. Within 72 hours of injury, fetal rat wounds were completely epithelialized with an epidermis that was similar in structure and thickness compared with that of adjacent uninjured skin (41). The epidermis of fetal rat cheek wounds was considered completely normal with the presence of rete pegs after 24 hours of healing (27). Healed wounds in fetal rabbits were covered with epithelium that appeared to have normal cell density, depth and cell structure (37). Wounds in fetal sheep seemed grossly healed by three days, including an epithelial covering, although the epithelium was slightly thickened compared with normal skin. As in adults, the crypts of hair follicles adjacent to the wounds seemed to be the origin of the rapid epithelialization (29). Fetal maturation seems to slow epithelialization in fetal sheep, although the fetal rate (48 to 72 hours) remained significantly more rapid than adult sheep, which required seven days for complete wound epithelialization (46).

Neovascularization and its associated endothelial proliferation is a conspicuous aspect of healing tissue. Most studies have found that adult-like angiogenesis is absent in primarily closed, linear fetal wounds. Angiogenesis is absent in wound biopsies from fetal sheep (29), monkeys (25), rabbits (45) and rats (26, 27), as well as PVA implants removed from wounds in fetal rabbits (32). However, as encountered with observations concerning fibroplasia, apparently conflicting data exist from studies in which Gore-tex (4) or viscose cellulose (31, 42) implants were used in fetal rabbits. In those experiments, granulation tissue containing capillary loop formation was seen infiltrating the wound devices.

Extracellular matrix of the fetal wound. Healing adult wounds have matrices of fibrin, fibronectin, proteoglycans and collagen. The healed adult wound matrix is composed almost entirely of collagen. Excessive, disorganized collagen deposition replaces the normal dermis in wounds as a mature scar is formed. The absence of appreciable scarring in fetal wounds suggests that marked differences exist in the fetal wound matrix, compared with the adult matrix. Histologically, fetal sheep wounds appear to be lacking an abundance of collagen (29) and wounds through muscle in fetal rats also are lacking collagen (26). PVA implants removed from wounds of mid-third trimester fetal rabbits after five to seven days of healing contained no infiltrating collagen by histologic examination using Masson's trichrome staining, whereas adult rabbit wound implants contained abundant collagen. High pressure liquid chromatography with a sensitivity to the level of 40 picamoles, also failed to detect collagen hydroxyproline within the fetal implants (32). The extracellular matrix of the fetal wound tissue within the implants, however, stained intensely with alcian blue, which was eliminated after pretreatment with hyaluronidase. This suggests that the glycosaminoglycan (GAG) hyaluronic acid (HA) was the predominant noncollagenous component of the matrix of wounds in fetal rabbit tissue (32).

Further characterization of the GAG present within the fetal wound matrix was achieved after biochemical extraction of the GAG within PVA implants removed from wound sites in fetal rabbits (47). It was shown that there was a progressive accumulation of GAG within fetal wounds and confirmation was provided that HA was the predominant GAG. Fetal wounds contained significantly more GAG compared with adult wounds, and HA predominated as the sole GAG in fetal wounds for a longer period (47). Subsequently, HA was detected in wound fluid from fetal sheep as long as three weeks after wounding, whereas HA could not be detected in adult sheep wound fluid at seven days after injury (48). The results of these studies demonstrate that a striking feature of the fetal wound matrix is a persistent abundance of HA.

Clearly, histologic examination shows that col-

lagen is present in fetal wounds, although infiltration into PVA implants does not seem to occur. Therefore, the absence of obvious fetal scarring may also be attributed to a highly organized deposition of collagen, as well as a lack of excessive collagen deposition. Light microscopic examination of healed wounds in fetal rabbits showed that the dermal matrix contained a reticular collagenous pattern (32, 37), rather than bundles of collagen and fibroblasts as seen in adult wounds. Type-specific collagen immunohistochemical staining of wounds from fetal sheep in the second and early part of the third trimester has shown that 14 days after wounding, the conformation of dermal collagen within the wounds was similar to that of unwounded skin, in other words, there was no formation of scar. Interestingly, as fetal maturation occurred, a transition to postnatal healing became apparent. Collagen deposition became more adult-like in wounds in fetal sheep during the middle part of the third trimester as demonstrated by the deposition of packed collagen fibers resulting in minimal scar formation 14 days after injury (46).

Although collagen deposition in fetal wounds is highly organized and nonexcessive, paradoxically, collagen appears in fetal wounds more rapidly than in adult wounds. Hydroxyproline accumulation, which is indicative of collagen production, is detectable earlier in wounds in fetal rabbits than in adult wounds (4). Similarly, in polyvinyl alcohol sponges, hydroxyproline accumulation in wounds in fetal sheep is greater and more rapid than in adult wounds (49). Immunohistochemical staining has also demonstrated an earlier deposition of collagen in wounds in fetal sheep compared with adult wounds (46). These observations correlate well with the *in vitro* finding that fetal dermal fibroblasts have a greater capacity for collagen production than do adult fibroblasts (50). The aforementioned findings suggest that matrix formation in the fetal wound is highly efficient and organized.

The adult wound heals by the replacement of normal dermis with a scar that consists of excessive and abnormally organized collagen. In marked contrast, the fetal wound contains a persistent abundance of HA while collagen deposition is rapid and nonexcessive. Additionally, it seems that collagen deposition is highly organized, so that the normal dermal structure is restored and scarring does not occur. Fetal maturation seems to involve a transition to postnatal-like healing, but scar formation is only minimal.

Open wounds. The repair response initiated by open fetal wounds is not as uniform as the repair process in closed wounds, so that more species-related differences seem to exist. In one of the earliest studies (1958) of fetal wound healing in mammals, extensive wounds were created by the transection of the vertebral column and all surrounding tissues of fetal guinea pigs. The extensive wounds healed *in utero,* but in a postnatal manner with rapid formation of granulation tissue, fibroplasia, collagen deposition and scar formation (51). Simple open wounds in fetal sheep also healed rapidly with only a scant acute inflammatory infiltrate, followed by abundant fibroblasts and undifferentiated mesenchymal cells. In contrast to the guinea pig, exuberant granulation tissue formation did not occur. Additionally, the sheep wounds exhibited extensive contraction and epithelialization (29). Contrary to guinea pigs and sheep, excisional wounds in fetal monkeys (25) and rabbits (30, 32, 52–54) do not contract.

The healing of open wounds was studied in fetal monkeys five to seven weeks before term (25). There was no contraction of the wounds after five days, although mild epidermal proliferation at the wound edge was present. On post-wound day eight, epidermal proliferation was marked, but epithelial migration into the wound had not occurred. The wound base contained ill defined spindle shaped cells, but no granulation tissue. After 27 days, only partial epithelialization had occurred. The tissue within the wound was cellular and "rich in ground substance," but did not have any resemblance to granulation tissue in postnatal open wounds.

Linear incisions in fetal rabbits that were not closed primarily were observed to gape open without evidence of contraction (32). Using skin tattoos that were the same size as the open wounds, it was shown that the wounds did not fail to close as a consequence of an overpowering effect of rapid fetal growth (52). Therefore, not only do open wounds in fetal rabbits fail to contract, but they actually expand.

The absence of contraction of open wounds in fetal rabbits was further characterized using light and electron microscopy (53). Histologically, the wounds showed no migration of epithelium from the wound edges, no acute inflammation, no fibroblastic response and minimal collagen deposition. In the noncontracting wounds, anti-

actin antibody staining was present in the panniculus carnosus muscle, but not within the wound margin. This suggests the absence of myofibroblasts. In addition, on transmission electron microscopy only a few fibroblasts were seen and no ultrastructural characteristics of myofibroblasts were identified. Therefore, noncontracting open wounds in fetal rabbits are associated with an absence of myofibroblasts.

Noting the absence of myofibroblasts in fetal open wounds and considering the theory of adult wound contraction centering around direct fibroblast-matrix interaction, fetal fibroblasts were evaluated for the ability to generate and exert a contractile force. The cell surface areas of cultured fetal and adult rabbit fibroblasts diminished by 70 to 80 per cent after the addition of medium containing adenosine triphosphate, indicating that the cells had contracted to an equal extent (52). Fibroblast populated collagen lattices (FPCL) were then established as an *in vitro* means of studying fibroblast-matrix interaction. Lattices containing fetal or adult rabbit fibroblasts contracted equally at 24 hours, but at two and four days, the fetal FPCL demonstrated a significantly greater reduction in area. Upon addition of rabbit amniotic fluid in concentrations as low as 20 per cent, contraction of fetal and adult FPCL was significantly inhibited (52). The results of these studies provided the first *in vitro* evidence that fetal rabbit fibroblasts are capable of generating a contractile force and exerting that force onto a collagenous matrix. Furthermore, it seems that the fetal environment of amniotic fluid has an inhibitory effect on contraction.

The effect of the fetal environment on open wound healing has also been studied *in utero*. Exclusion of open excisional wounds from exposure to amniotic fluid in fetal rabbits was done by coverage with a sutured silicone sheet. While cellular events within the wound remained unchanged compared with control wounds, the covered wounds seemed to "contract" (30). In a similar experiment in the same species, computerized morphometry documented a 26 per cent reduction in the size of the covered wounds compared with control wounds (54). In both studies, the healing of open wounds in neonatal rabbits was also evaluated. Excisional wounds in the neonates healed in a manner similar to adult open wounds with rapid contraction and formation of granulation tissue (30, 54). Therefore, these experiments provide further evidence suggesting that amniotic fluid may be an inhibitor of open wound healing in the fetal rabbit and also show that there is an abrupt transition from fetal nonclosure of open wounds to adult-like healing upon the natural removal of amniotic fluid after parturition.

The interspecies variation that exists in open wound healing in the fetus makes it difficult to extrapolate the findings to the human condition. Some observational data in the human fetus, however, suggest that human fetal open wounds may behave more like those of monkeys and rabbits rather than sheep. A postmortem examination of a spontaneously aborted human fetus of approximately 20 weeks gestational age showed multiple digit amputations, as well as an amputated distal one-third of the right leg, presumedly from amniotic band trauma. There was also a large full thickness ulcer with exposed, underlying bone that appeared to be secondary to friction from the bone of the contralateral leg stump. The leg stump and ulcer were open, containing necrotic tissue. There was no inflammatory response and no granulation tissue formation. The amputated digits also contained necrotic debris. No inflammation or granulation tissue were present, although an abnormal, thin epithelial covering was present. A mild mesenchymal proliferation was observed in some wounds (55). These findings closely resemble the observed repair processes in open wound healing in fetal monkeys and rabbits. Therefore, compared with other species, these animals may be a more accurate model for fetal open wound healing in humans.

Burn wounds. The response initiated by electrical burn wounds was studied in fetal rats at 16.5 through 21.5 days of gestation (term is 22 days) (56). Microscopic examination of the wounds was performed up to 24 hours after injury. The wounds of younger gestational age fetuses lacked an acute inflammatory response. However, beginning at 19.5 days gestational age, acute inflammation was present. The degree of neutrophilic infiltration was correlated with gestational age from that point onward. A mesenchymal cell "filling in" was also observed in the wounds. Similar to observations in linear incisions in fetal sheep, the older fetuses had more of a postnatal repair response within their wounds.

REGULATION OF FETAL REPAIR

Adult wound mediators. Polypeptide growth factors are thought to be important mediators in embryogenesis and postnatal healing. Two such factors, TGF-β and PDGF, accelerate healing in

normal laboratory animals and are associated with the initiation of fibrosis (16, 57). PDGF is a chemoattractant for smooth muscle cells and fibroblasts (14, 15) and TGF-β has a mitogenic effect on fibroblasts and stimulates them to produce collagen (16). Considering the absence of fibroplasia and the lack of excessive collagen deposition in fetal wounds, the factors could be diminished or inactive in the fetal wound. Alternatively, fetal cells may become less responsive to the factors. Indeed, the number of TGF-β receptors on the surface of fibroblasts diminishes as fetal development proceeds, so that embryonic fibroblasts have the greatest number of receptors, fetal fibroblasts have fewer and adult fibroblasts have the least (58).

To test whether or not the fetal healing response can be altered by TGF-β, the substance was exogenously introduced into fetal rabbit wound sites using PVA implants (59). The TGF-β treated wounds demonstrated an altered, adult-like healing response in which fibroplasia and collagen deposition were markedly increased compared with untreated fetal wounds. A direct interaction of TGF-β with fetal fibroblasts was implied by the results of another study that showed cell surface binding of TGF-β to cultured fetal rabbit fibroblasts (58). The addition of PDGF to wounds in fetal rabbits similarly resulted in greater fibroblast infiltration and collagen deposition, as well as neovascularization (60).

The fetal repair response can by converted to adult-like healing after treatment with growth factors that are considered to be vital in normal postnatal healing. Thus, fetal cells are fully capable of responding to the growth factors in a manner similar to that observed in adults. The absence of features typical of adult healing in fetal wounds suggests that these growth factors may not be present within the wound sites or that their activation is inhibited.

In another study, the significance of the absence of neutrophils in fetal repair was evaluated by the introduction of adult acute inflammatory components into fetal wounds (61). PVA implants were first placed subdermally into maternal rabbits and then transferred into the respective fetuses of the does, six to 36 hours later. After five days of fetal healing, the implants from the fetal wounds contained significantly more neutrophils than present when they were removed from the maternal wounds. However, no fibroblast infiltration or collagen deposition was present in the fetal implants. Therefore, despite the apparent stimulation of neutrophilic infiltration into the site of the fetal wound by components removed from an adult wound during the phase of acute inflammation, fibroplasia did not result.

Therefore, the absence of acute inflammation in fetal wounds may not be directly related to the lack of fibrosis.

Hyaluronic acid stimulating activity. One of the substantial differences between fetal and adult wounds is the progressive and prolonged presence of HA within the fetal wound matrix. Several investigations have dealt with the hypothesis that fetal wound cells are regulated to produce HA preferentially. The studies have used a rat fibrosarcoma cell line that produces high quantities of HA as a model for fetal cells. Using the model, it was determined that fetal sheep wound fluid sampled from subcutaneous wire mesh cylinders stimulated the production of HA, whereas adult sheep wound fluid did not (62). Serum from fetal sheep (63) and amniotic fluid (64), as well as serum from fetal calves (65), were also found to stimulate the production of HA by this cell line. Biochemical analyses of a factor within serum from fetal calves that stimulates HA production has characterized it as a heat stable glycoprotein that is inactivated at extremes of acidification or alkalinization (65).

The results of these studies suggest that a factor within the fetus may regulate fibroblasts to produce HA rather than collagen. Furthermore, the presence of HA stimulating activity in serum and wound fluid indicates a possible systemic, as well as local, availability. A factor containing the stimulatory activity has been isolated *in vitro* and appears to be a glycoprotein whose activity is stable at *in vivo* temperature and acid/base conditions. However, the actual extension of these findings to the *in vivo* situation is unclear. In these studies, only HA production in a cell line that is known to produce high quantities of this substance was studied. The possible production of other GAGs has not been investigated. It is possible that a generally increased production of several GAGs may be observed in normal fetal fibroblasts or that normal cells are not stimulated to increase HA or GAG production. Nevertheless, these interesting findings warrant further study and potentially provide great insight into how fetal and adult wounds may mechanistically differ.

Hyaluronic acid. Similar to fetal skin, the fetal wound matrix is enriched in HA (66). As previously stated, it is associated with a highly organized healing response so that scarring is min-

imal or nonexistent. Observations from other biologic systems, including true regeneration (67–70), development (71) and neoplasia (72) have implicated HA as an affecter of the cellular and matrix events within these tissues. Furthermore, HA has been demonstrated to directly bind to cells (73–76) and to complex with collagen and noncollagen protein (77). Based upon these data, it was hypothesized that HA may influence the cellular and matrix events, or both, in fetal healing so that HA may have a regulatory influence on scarless repair.

This hypothesis was tested by studying the fetal healing response in rabbits after a reduction in fetal wound HA content by specific *in vivo* degradation with *Streptomyces* hyaluronidase introduced using PVA implants. A biochemically confirmed reduction in the HA content of wounds treated with the enzyme was associated with an increase in fibroplasia, collagen deposition and neovascularization (78). Therefore, the degradation of HA within fetal wounds dramatically altered the healing response so that features more characteristic of adult healing appeared.

The results of the current study are similar to those obtained after the treatment of fetal wounds with TGF-β and PDGF. A potential electrostatic interaction between the polyanionic HA and the cationic growth factors is suggested, so that a reduction in the content of HA within the wound could augment the effect of the growth factors, leading to the observed histologic results (78). A direct physicochemical effect on collagen organization by HA is also possible, so that an elevated content of HA would favor a dispersed, hydrated conformation of collagen, rather than the dense, compact organization encountered in scars (78).

The state of cellular differentiation. In regeneration and development, the accumulation and subsequent degradation of HA is associated with changes in the state of cellular differentiation. It has been proposed that HA may similarly affect the state of differentiation of immature cells involved in fetal repair. Elevated levels of HA in fetal wounds may prevent differentiation of immature mesenchymal cells present within the dermis and thereby prevent fibroplasia and scar formation. A controlled differentiation of the cells would allow the normal structure of the skin to be reformed (66, 78).

Evidence supporting the importance to the healing response of the differentiated state of cells present within wounded skin was provided by an experiment in which a full thickness skin allograft from a maternal ewe was transplanted to her fetus at 60 days of gestation (79). Repeat laparotomy was performed after 40 days, demonstrating complete take of the graft, but the graft retained qualities of adult skin and was wool bearing, with borders clearly distinguishable from the fetal skin. Primarily closed, full thickness incisional wounds were then created in fetal skin and the adult graft. The fetal wounds healed with a collagen staining pattern that was identical to uninjured skin, demonstrating the absence of scar formation. However, the adult graft healed by gross and microscopic scar formation. These findings show that adult wound healing is not changed into a fetal repair response despite perfusion of the injured skin by fetal blood and exposure to amniotic fluid and suggests that the differentiated state of the cellular population within the skin is vitally important in determining how the tissue will respond to injury. For instance, it is possible that mature differentiated fibroblasts can only respond to tissue injury by proliferation and exuberant collagen deposition.

APPLICATIONS OF FETAL REPAIR

There are two primary goals in elucidating the biologic processes responsible for scarless fetal healing. First, the processes of *in utero* repair must be understood so that as the fetus becomes a surgical patient (80), the healing will be manageable. The second goal is to apply the mechanisms of scarless healing to pathologic, postnatal processes in an attempt to improve clinical treatment. Such application has enormous potential impact because of reduction in the significant morbidity and mortality rates associated with conditions such as hypertrophic scars, keloids and intestinal strictures. Some examples of this extension of fetal repair to adult healing exist. For instance, because of the association of HA with cellular migration and proliferation, the effect of HA on adult wounds has been evaluated. Topical application of HA has been shown to improve the retardation in epithelialization of partial thickness linear wounds in rats that are diabetic (81).

Hyaluronic acid was also evaluated for the effect on scar formation in full thickness, linear incisions in monkeys, rabbits and guinea pigs (82). In all animal groups, there seemed to be less scar formation in wounds treated with HA compared with those treated with saline solution. However, results in regards to improved healing

with decreased scar formation are variable in studies in which HA was used for ophthalmologic applications (83, 84) or tendon repair (85). Further well-controlled studies are needed to evaluate any potential effect that HA may have upon contraction, epithelialization and collagen deposition in adult wounds.

Another approach has been to provide adult wounds with elements of the fetal environment. The totally fluid environment in which fetal repair occurs has been hypothesized to be a prominent factor in the lack of scarring and contraction of fetal wounds. To evaluate the effect of a fluid environment on adult wound healing, primarily closed incisional wounds in pigs were continually exposed to saline solution by the use of liquid-tight wound chambers (86). The wounds treated with fluid had less gross scarring, more normal epidermis and better cosmesis compared with control subjects that were exposed to air. There was no difference in hydroxyproline content of wounds from either group. The tensile strength of the control wounds was significantly greater than the wounds covered with fluid at postwound day 14, although there was no significance in the differences in strengths by day 30. This study indicates the importance of the fluid environment in fetal repair and suggests that simple manipulation of adult wound conditions may have a desired effect on healing. Further studies are required to evaluate various fluid environments, such as amniotic fluid and fluids enriched with various growth factors.

SUMMARY

Fetal wound healing is a remarkable process that is fundamentally different than postnatal healing. Healing of primarily closed, linear wounds occurs rapidly and without scarring. In late gestational age fetal sheep, a transition to adult-like healing occurs as evidenced by minimal scar formation. Acute inflammation is not involved, fibroblast recruitment and proliferation is minimal, the matrix of the wounds is enriched with HA and collagen deposition is highly organized so that scarring is minimal or nonexistent. Open wounds in several species do not contract, an observation that may be because of an inhibitor of contraction in amniotic fluid. The underlying mechanisms that regulate fetal wound repair are currently not well understood. An altered supply or activity of growth factors may be instrumental. Fibroblasts may preferentially produce HA secondary to a factor present within the fetal system, and HA may influence cellular and matrix events within the wound. As greater knowledge of the biologic factors of scarless healing in the fetus is gained, applications to abnormal adult healing may be developed. As the age of fetal surgery is approaching, it is essential to understand the injury response in these new patients.

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