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The Impact of Cyclooxygenase-2 Mediated Inflammation on Scarless Fetal Wound Healing

Traci A. Wilgus,* Valerie K. Bergdall,† Kathleen L. Tober,* Kara J. Hill,* Srabani Mitra,‡ Nicholas A. Flavahan,‡ and Tatiana M. Oberyszyn*

From the Departments of Pathology, Veterinary Preventive Medicine/University Laboratory Animal Resources,† and Heart and Lung Research Institute,‡ The Ohio State University, Columbus, Ohio*

Cyclooxygenase-2 (COX-2) and the prostaglandin products generated as a result of COX-2 activity mediate a variety of biological and pathological processes. Scarless healing occurs in fetal skin in the first and second trimesters of development. This scarless healing process is known to proceed without a significant inflammatory response, which appears to be important for the lack of scarring. Because the COX-2 pathway is an integral component of inflammation, we investigated its role in the fetal repair process using a mouse model of scarless fetal wound healing. COX-2 expression in scarless and fibrotic fetal wounds was examined. In addition, the ability of exogenous prostaglandin E_2 to alter scarless fetal healing was evaluated. The results suggest that the COX-2 pathway is involved in scar production in fetal skin and that targeting COX-2 may be useful for limiting scar formation in adult skin. ***(Am J Pathol 2004, 165:753–761)***

The cutaneous wound healing process is known to differ between fetal and adult skin. Wound repair in adult skin begins with an acute inflammatory phase and ends with the formation of a permanent scar. In contrast, early gestation fetal wounds (first and second trimester) heal in a near perfect fashion, rapidly and without the production of a scar.[1–4] There has been much interest in characterizing the key factors responsible for the switch from scarless healing to an adult-like, scar-producing phenotype typical of skin past the second trimester of gestation. Identification of differences in the two types of healing could identify factors that promote scar tissue generation. This correlation between factors identified as reduced in scarless healing and the inhibition of those factors in adult wounds to reduce scarring has been especially true for transforming growth factor-β (TGF-β). This cytokine was one of the first mediators found to be differentially regulated in scarless healing and was shown to promote scar tissue deposition when introduced into scarless wounds.[5–12] As a result of these findings and others implicating TGF-β in fibrosis,[13] the effect of down-regulating this molecule was tested in adult skin and found to reduce scar formation.[14–16]

A key feature of scarless fetal healing appears to be a lack of inflammation in response to the wounding event.[6–8,17–25] In contrast, the early phases of wound healing in late fetal and adult skin are characterized by a robust inflammatory response, and eventually a permanent scar in the wound area.[26,27] While the interleukins IL-6, IL-8, and IL-10 have been studied in fetal wound repair,[23–25] the role of other classic inflammatory mediators in scarless healing is not known.

Metabolites and enzymes of the arachidonic acid cascade, including the cyclooxygenase-2 (COX-2) enzyme and its enzymatic product prostaglandin E_2 (PGE_2), are known to be critical mediators of the inflammatory response. COX-2 has received much attention recently as it is involved in diseases associated with dysregulated inflammatory conditions, such as rheumatoid and osteoarthritis, cardiovascular disease, and the carcinogenesis process.[28–32] COX-2 undergoes immediate-early up-regulation in response to an inflammatory stimulus,[33] such as a wound. It functions by producing prostaglandins that control many aspects of the resulting inflammation, including the induction of vascular permeability and the infiltration and activation of inflammatory cells.[34] Interest in the role of the COX-2 pathway and other aspects of inflammation in the adult wound repair process is increasing[35] as these early events have been shown to regulate the outcome of repair.[36–38]

Based on the involvement of COX-2 in inflammation and the recent demonstration that it contributes to several aspects of adult wound repair,[36,39,40] we examined the role of COX-2 in the fetal wound healing process. These studies demonstrate differential expression of the COX-2 enzyme in early and late gestation fetal wounds. Furthermore, PGE_2, a COX-2 product shown to mediate many processes in the skin, caused a delay in healing and the production of a scar when introduced into early fetal wounds. These data further our understanding about the fundamental differences between scarless healing and

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Address reprint requests to Tatiana M. Oberyszyn, Ph.D., The Ohio State University, Department of Pathology, 1645 Neil Avenue, 129 Hamilton Hall, Columbus, OH 43210. E-mail: oberyszyn.1@osu.edu.

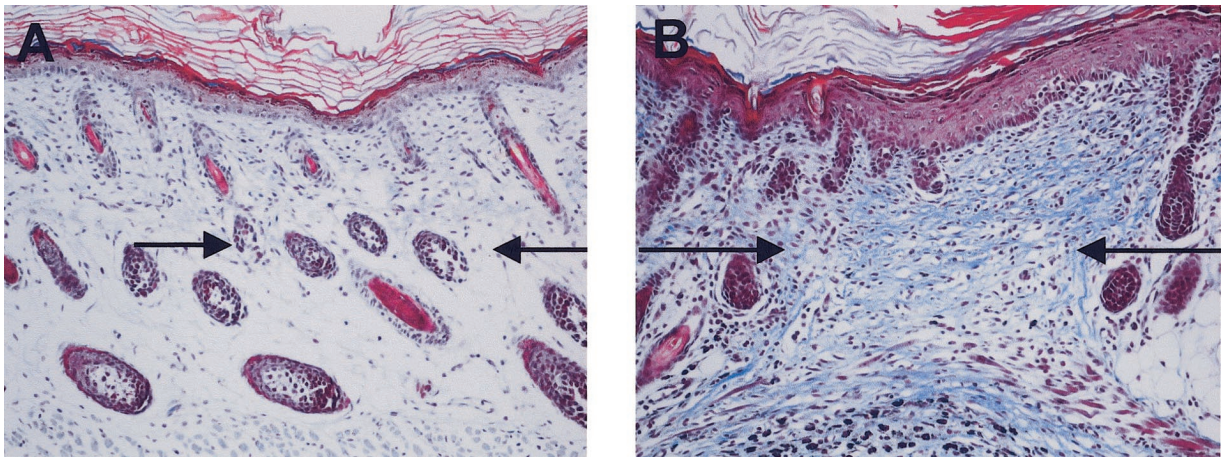

Figure 1. Differential scar formation in fetal skin. Masson's trichrome stains were performed on 7 day skin wounds that had been generated at either 15 (**A**, E15) or 18 (**B**, E18) days of gestation. The wound/scar margins are marked with **arrows** (magnification, ×20).

normal repair, and suggest the involvement of COX-2 in the production of scar tissue.

Materials and Methods

Fetal Surgeries

A murine model of fetal wound healing was used to examine the COX-2 pathway in scarless and fibrotic repair. All work was approved by the Ohio State University animal use committee. Female and male mice were mated, and surgery was performed on the pregnant female 15 or 18 days after the detection of a vaginal plug, designated day 0. These time points represent ages at which scarless (embryonic day 15, E15) or fibrotic (embryonic day 18, E18) healing take place (see Figure 1). After preparation of the abdomen for aseptic surgery, a midline laparotomy was performed under isoflurane anesthesia. Incisions were made in the uterine wall and amniotic sac overlying each fetus, and a full-thickness incisional wound, approximately 2 mm in length, was made on the dorsum of the fetus using microsurgical scissors. One μl of India ink (Fisher Scientific, Pittsburgh, PA) diluted to 10% in sterile phosphate-buffered saline (PBS) (Invitrogen Corporation, Carlsbad, CA) was introduced subcutaneously into the wound site to allow visualization of the area after healing had occurred.[10] The uterine incision was closed using 6−0 nylon suture. After approximately four fetuses were wounded in this manner, the muscle and skin layers of the pregnant mouse were sutured closed. Xylazine (3 mg/kg, Phoenix Scientific, St. Joseph, MO) was given subcutaneously for sedation and analgesia and to preclude cannibalism of the pups by the dam. Fetal skin was harvested at 6 hours, 24 hours, or 7 days post-wounding to examine inflammation and scar tissue formation. The skin was either fixed in formalin for subsequent histological analysis or snap-frozen for protein or RNA isolation. Due to the small size of the wounds, several wounds had to be pooled for each protein and RNA sample to obtain a sufficient yield for analysis. Normal E15 and E18 skin were harvested as controls. A minimum of 10 wounds for each experimental group at each time point were analyzed for histology and two pooled samples per group were used for protein and RNA analysis.

Using the same surgical techniques, E15 fetuses were wounded and injected with 1 μl of the ink solution (PBS) or the ink solution containing 2 μg PGE_2 (Sigma, St. Louis, MO). Wounds were harvested at 24 hours, 3 days, or 7 days post-wounding. The skin was either fixed in formalin for subsequent histological analysis or snap-frozen for protein isolation. Digital photographs using a Nikon Coolpix 2100 camera (Nikon Corp., Japan) were taken before sacrifice to document scarring. Due to the small size of the wounds, several wounds were pooled for each protein sample. At least 10 wounds for each experimental group at each time point were analyzed for histology and two pooled samples per treatment group were used for enzyme immunoassays.

Protein Analysis and Enzyme Immunoassays

For Western blot analysis, tissue samples were ground in liquid nitrogen using a mortar and pestle, and sonicated in Laemmli buffer (BioRad, Hercules, CA). For cultured fibroblasts, cell pellets were dispersed in Laemmli buffer and sonicated. After centrifugation, the total protein concentration of each sample was determined using the BCA (bicinchoninic acid) protein assay kit (Pierce Biotechnology, Rockford, IL). Twenty-five μg of sample or 0.5 μg of COX-2 protein standard (Cayman Chemical Company, Ann Arbor, MI) were separated on 10% SDS-polyacrylamide gels in the presence of 0.1% SDS. Proteins were then electroblotted onto Immobilon-P membranes (Millipore, Bellerica, MA). The membranes were blocked with 10% nonfat dry milk in TBST (Tris-buffered saline containing 0.1% Tween 20). The membranes were incubated with affinity-purified COX-2 rabbit polyclonal antibody (1:500 dilution, Cayman Chemical) for 1 hour at room temperature. Bound antibody was then probed with a horseradish peroxidase-conjugated secondary antibody (1:2000 dilution) for 1 hour at room temperature. After

extensive washes with TBST, proteins were visualized using an ECL chemiluminescence kit (Amersham Pharmacia Biotech, Piscataway, NJ). The developed films were digitized and the bands were quantitated using Kodak 1D image analysis software (Eastman Kodak, Rochester, NY).

For enzyme immunoassays, tissue samples were ground in liquid nitrogen. Protein was isolated in buffer containing protease inhibitors and total protein concentrations were determined as previously described.[36] PGE_2 (Amersham-Pharmacia, Piscataway, NJ) and TGF-β 1 (R & D Systems, Minneapolis, MN) levels were assessed using commercial kits as specified by the manufacturer. Ten μg (PGE_2) or 40 μg (TGF-β1) of total protein was used for the assays.

RNA Isolation and Semi-Quantitative Reverse Transcriptase-Polymerase Chain Reaction (RT-PCR)

Gene expression levels of COX-2 were determined using RT-PCR as previously described.[34] After separation of the PCR products in agarose gels and visualization with ethidium bromide, images were captured using a Kodak DC290 camera and bands were quantitated using Kodak 1D image analysis software (Eastman Kodak). Levels of COX-2 were normalized to the housekeeping gene HPRT, and the data are expressed as a percentage of the values in age-matched unwounded skin.

Histology and Immunostaining

Tissues were processed and subjected to Masson's trichrome staining to detect collagenous scar tissue as previously described.[36] Stained sections were photographed and assessed for the presence of scar tissue. Additionally, immunostaining for COX-2 was performed. After deparaffinization and rehydration, samples were washed in 1X Automation Buffer (Biomeda Corp., Foster City, CA). Sections were steamed in pre-warmed antigen unmasking fluid (Vector Laboratories, Burlingame, CA) for 3 minutes, washed with automation buffer, then blocked with casein solution (Vector Laboratories) for 30 minutes. Sections were then incubated overnight at 4°C with an antibody specific for COX-2 (1:200 dilution in casein, Cayman Chemical Co). After washing with automation buffer, sections were incubated with rabbit link and rabbit label solutions (BioGenex, San Ramon, CA) for 30 minutes each, then chromogenic detection was performed using DAB (Vector Laboratories). Sections were counterstained with hematoxylin, dehydrated, and mounted.

Fetal Fibroblast Culture and Proliferation Assessment

Normal embryonic day 15 and 18 skin were harvested for fibroblast cultures as outlined by Scheid et al.[41] Briefly, excised fetal skin was placed into Dulbecco's modified Eagle's medium (high glucose, Invitrogen/Life Technologies, Carlsbad, CA) supplemented with 20% heat-inactivated fetal calf serum (Gibco, Invitrogen Corp.), 100 U/ml penicillin, 100 μg/ml streptomycin, non-essential amino acids, 2 mmol/L L-glutamine, and 1 mmol/L sodium pyruvate (Life Technologies) at 37°C and 5% CO_2. Following 8 to 14 days of cultivation under daily renewal of media, sufficient fibroblasts had migrated out of the skin specimens to allow first passaging with trypsin-EDTA. Tissue culture experiments were performed within the third to eighth passage. Cells were seeded in 96-well culture plates at a density of 5×10^2 cells per well. Twenty-four hours later, cells were treated with media containing increasing doses of PGE_2 (Sigma), including 0 nmol/L (untreated), 1 nmol/L, 10 nmol/L or 100 nmol/L in the presence of a 10% total volume of alamar blue (Biosource International, Camarillo, CA) to determine proliferation rates. Plates were read spectrophotometrically at 570 and 600 nm at 8, 24, 48, and 72 hours after treatment. These values were used to calculate the percent reduction of the alamar blue dye, which corresponds to proliferation, as specified by the manufacturer.

Statistical Analysis

Statistical differences between the treatments were determined using a Student's *t*-test generated using StatView software (Abacus Concepts, Berkeley, CA).

Results

Comparison of Scar Tissue Production in Fetal Wounds During Development

Masson's trichrome staining, used to stain collagenous scar tissue blue, illustrates the healing differences between E15 and E18 skin wounds at 7 days post-wounding (Figure 1). Restoration of the normal tissue architecture can be seen in wounds generated at E15 (Figure 1A) compared to evident scars identified by the loss of hair follicles and dense collagen in wounds generated at E18 (Figure 1B). These results recapitulate what has been found previously in fetal wound healing studies and validate the murine model used in the present studies. This disparity in scar tissue production seen in late *versus* early gestation fetal wounds has been shown to correlate with differences in the levels of inflammation in these wounds.

Differential COX-2 Expression in Embryonic Wounds

Because COX-2 and its prostaglandin products, particularly PGE_2, are known to be involved in cutaneous inflammation and inflammation is thought to contribute to the switch from scarless to fibrotic healing in fetal skin, we examined the expression of COX-2 and the production of PGE_2 in early and late gestation fetal wounds. COX-2 mRNA (Figure 2) and protein (Figures 3 and 4) levels as well as PGE_2 levels were higher in E18 wound tissue

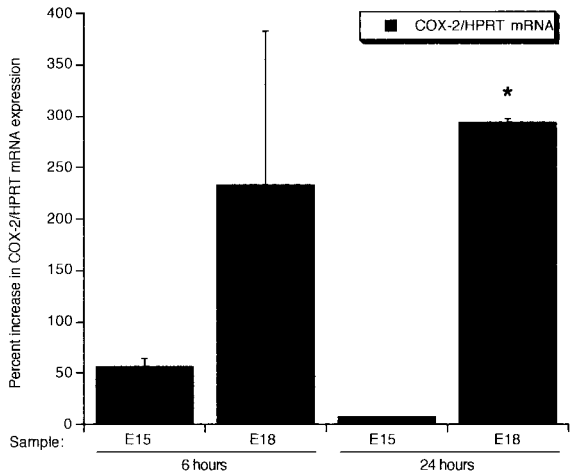

Figure 2. COX-2 mRNA expression during fetal wound healing. Semi-quantitative RT-PCR analysis was used to examine COX-2 mRNA levels in E15 and E18 wounds. The net intensity values for COX-2 bands were normalized to the housekeeping gene HPRT, and the data are presented as the percent difference in COX-2/HPRT gene expression from the age-matched unwounded values (mean ± SEM, *, $P < 0.05$).

compared to wounds introduced at E15, with 24 hours being the peak of expression (Figures 2 to 4). Twenty-four hours post-wounding, immunohistochemical staining demonstrated COX-2 protein expression in basal keratinocytes, inflammatory cells, and stromal cells in wounds made in E18 skin (Figure 4B) but not in wounds made at E15 (Figure 4A). By 7 days, COX-2 protein levels in wound tissue were undetectable, as seen in normal unwounded skin (data not shown). The differential expression of COX-2 in E15 scarless and E18 fibrotic wounds suggest that COX-2 may be involved in the regulation of scar tissue production.

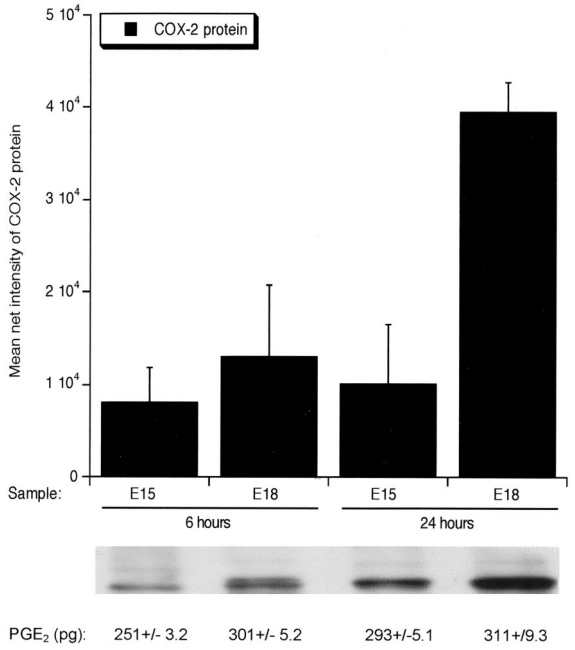

Figure 3. COX-2 protein and PGE_2 levels in fetal wounds. COX-2 protein was detected by Western blot and levels were analyzed using image analysis software. The mean net intensities of the bands ± SEM were plotted and are shown along with representative images of the protein bands. Corresponding PGE_2 levels (pg) ± SEM are also shown.

Enhanced COX-2 Expression in Late Fetal Fibroblasts

In addition to wound tissue, the levels of COX-2 protein in cultured fetal fibroblasts, the cell type responsible for collagen deposition and scar tissue production, were also examined (Figure 5). Fibroblasts were isolated from E15 or E18 skin. Net intensity values generated from Western blot analysis revealed 33% higher levels of COX-2 protein detected in fibroblasts isolated from E18 skin, which heals with a scar, compared to those from E15 skin, which heals without a scar. Taken together with the data in Figures 2 to 4, these results suggest that a baseline increase in COX-2 expression in E18 wound tissue and fibroblasts may be critical to the presence of inflammation and subsequent scar formation characteristic of late gestation fetal repair.

Effect of Exogenous PGE_2 on Scarless Healing

To further investigate the function of the COX-2 pathway in fetal wound repair, we characterized the early fetal healing response in the presence of the COX-2 enzymatic product and inflammatory modulator PGE_2. E15 wounds, which represent scarless healing, were injected with either the India ink solution alone (PBS) as a control or the India ink solution containing 2 μg of PGE_2. The ability of PGE_2 to alter the repair process was evaluated. An early increase in TGF-β 1 protein levels were detected in PGE_2-treated wounds 24 hours after wounding compared to PBS control wounds (Figure 6). While the levels of TGF-β 1 in PGE_2-treated wounds dropped by day 3 post-wounding, the levels were still twice those of control wounds. TGF-β 1 levels returned to control levels by 7 days after wounding (data not shown).

Three days after the wounds were created, a delay in healing was evident in wounds injected with PGE_2. At this point, approximately half of the PGE_2 injected wounds were not closed, compared to control wounds which were completely healed at this stage. Histological examination of the closed wounds demonstrated that those exposed to PGE_2 displayed a hyperproliferative epithelium (Figure 7, C and D), suggesting a delay in the re-epithelialization process at this stage. In contrast, the control wounds contained an intact epithelial layer similar to age-matched unwounded skin (Figure 7, A and B).

In addition to decreasing healing rates, PGE_2 treatment of E15 wounds also resulted in the production of scar tissue at day 7 (Figure 8, D to F) compared to control E15 wounds (Figure 8, A to C) which lacked scar formation. This was evident both macroscopically, with a scar that could be seen by eye (Figure 8D), and also microscopically, where the presence of scar tissue and dense collagen fibers can be seen in trichrome-stained tissue sections (Figure 8, E and F). These results further support the idea that the COX-2 pathway can alter the outcome of wound repair.

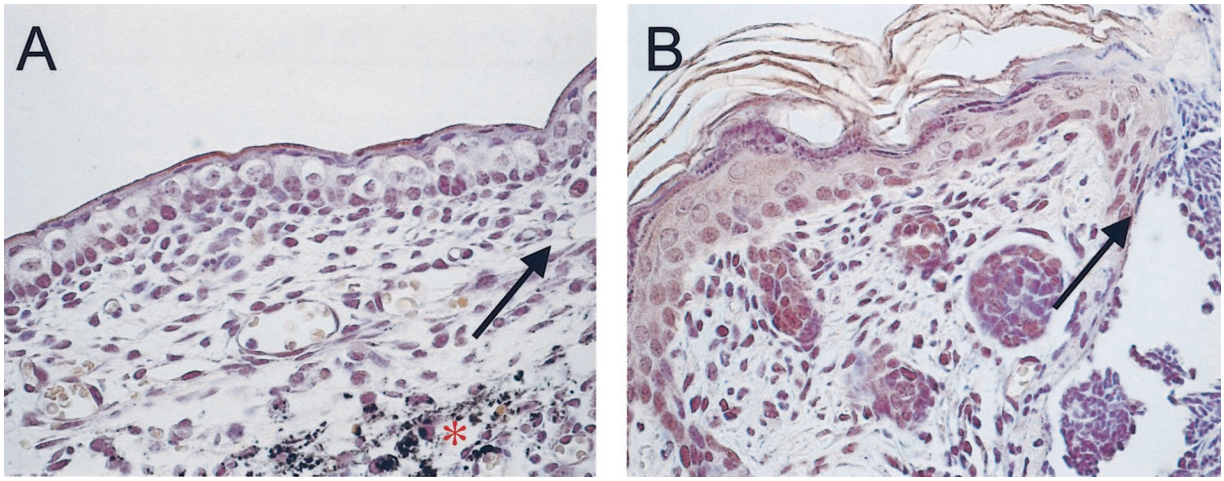

Figure 4. Immunohistochemical localization of COX-2. Localization of COX-2 protein expression in E15 (**A**) and E18 (**B**) wounds was assessed by immunohistochemistry. The wound margins are marked with **arrows** and the * denotes India ink used to mark the wound site, which can be seen in **A** (magnification, ×60).

PGE_2 Promotes Fetal Fibroblast Proliferation

One component of healing thought to regulate scar tissue deposition is the proliferative rate of dermal fibroblasts, the cell type responsible for the deposition and remodeling of scar tissue. A mechanism by which PGE_2 could be promoting scar tissue deposition in E15 scarless fetal wounds could be by increasing the rate of fibroblast proliferation. Therefore, we examined the effects of the addition of exogenous PGE_2 on cultured fetal fibroblast proliferation (Figure 9). The addition of PGE_2 resulted in a dose-dependent increase in the proliferation of fibroblasts cultured from E15 skin (Figure 9A, *, $P < 0.05$ compared to untreated cells). A similar increase in fibroblast proliferation in response to PGE_2 treatments was detected in fibroblasts cultured from E18 (Figure 9B) and adult skin (data not shown). These data implicate an increase in fibroblast proliferation as a possible mechanism for the induction of scar tissue production in fetal wounds as a consequence of PGE_2 exposure (Figure 8).

Discussion

The COX-2 enzyme and its product PGE_2 are known to modulate inflammation in the skin in response to stimuli such as ultraviolet light exposure[34,42] and topical administration of the tumor promoter TPA (12-O-tetradecanoylphorbol-13-acetate).[43,44] While there have been reports that COX-2 inhibition can be detrimental to gastric healing[45,46] and bone repair,[47] this does not appear to be true for healing in the skin. Recent studies have shown that reducing PGE_2 with the use of inhibitors specific for the COX-2 enzyme does not have an effect on wound re-epithelialization or tensile strength,[36,48–50] although it has been suggested that reducing PGE_2 by inhibiting COX-1, the "constitutive" homologue of COX-2, may have an effect.[48] While it seems that COX-2 inhibition does not deter the proper re-epithelialization of wounds of the skin, several studies now implicate the COX-2 pathway in the regulation of the inflammatory phase of cutaneous wound repair.[36,51] Inhibition of this inflammatory pathway has also been suggested to reduce scar formation.[36] This observation fits with fetal wound healing studies demonstrating a lack of inflammation in wounds that heal without a scar.

While it has been known for some time that early fetal wounds do not exhibit acute inflammation as a reaction to a cutaneous wound, most of the studies to date have focused on differences in the fetal environment, the extracellular matrix of fetal wounds, or the well-studied TGF-β, rather than differences involved in the suppression of inflammation in these wounds. Classic inflamma-

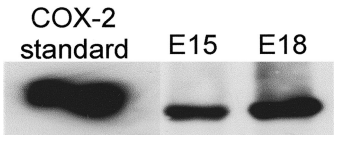

Figure 5. COX-2 protein levels in cultured fetal fibroblasts. Western blot analysis of COX-2 protein was performed on fibroblasts cultured from E15 or E18 skin. COX-2 protein is shown as a positive control.

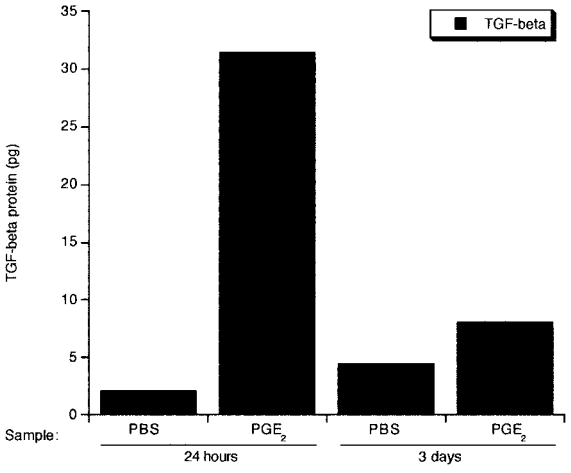

Figure 6. TGF-β 1 protein levels in E15 fetal wounds. The total protein levels of the pro-fibrotic factor TGF-β 1 in fetal wound tissues were assessed by ELISA. The mean levels of TGF-β 1 were determined in control wounds (PBS) and PGE_2-treated wounds at 24 hours and 3 days post-wounding.

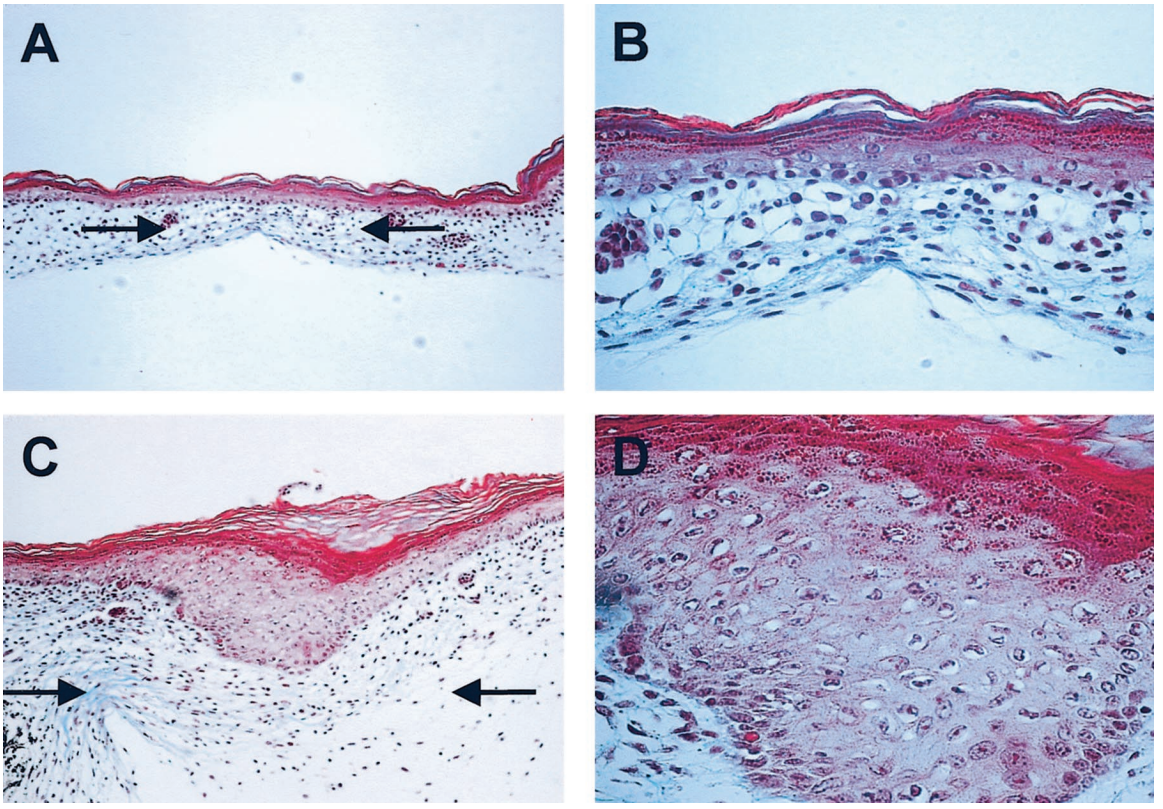

Figure 7. The effect of PGE_2 on the healing of E15 skin. Representative Masson's trichrome stained tissue sections from control wounds [magnification, ×20 (**A**); magnification, ×60 (**B**)] and PGE_2-treated wounds [magnification, ×20 (**C**); and magnification, ×60 (**D**)] 3 days after wounding demonstrates delayed healing in wounds treated with PGE_2. **Arrows** are used to indicate the wound site in **A** and **C**.

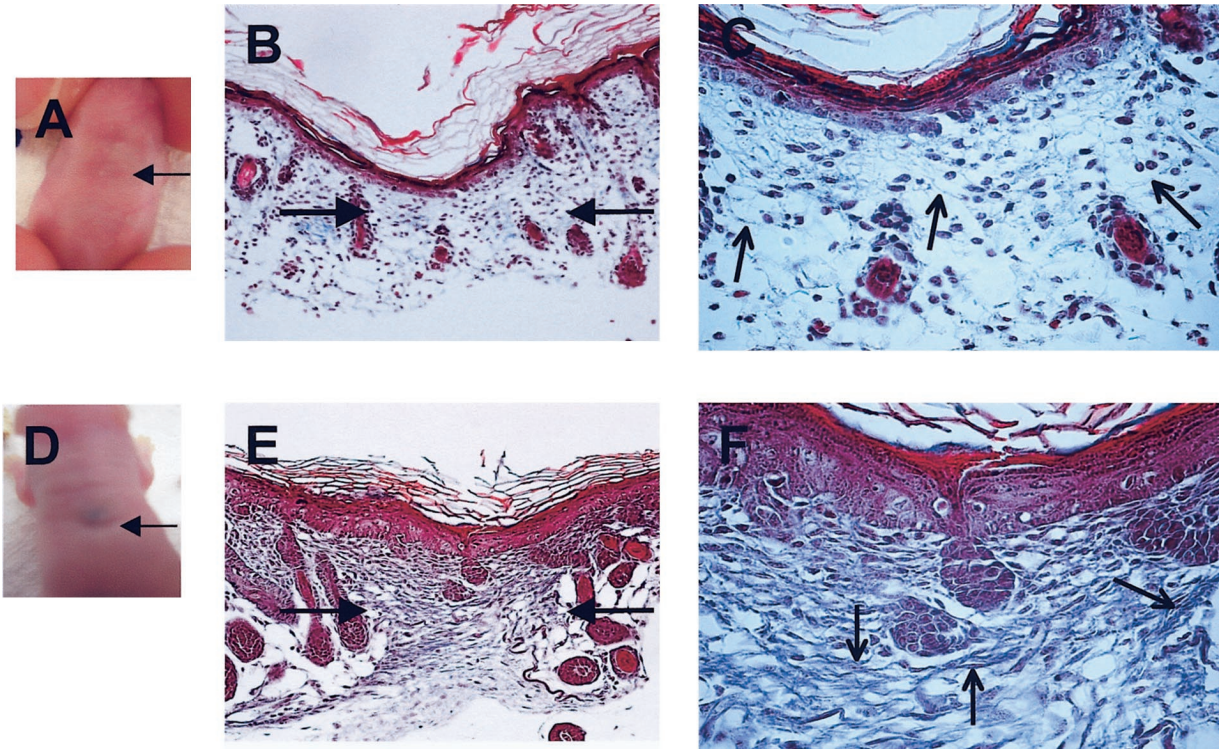

Figure 8. Gross and histological appearance of scar tissue deposition. At day 7 post-wounding, digital pictures were taken of fetuses that had either been injected with ink alone (**A**, PBS) or with 2 μg PGE_2 at age E15 (**D**). Representative Masson's trichrome-stained tissue sections also illustrate the lack of scar tissue production in control wounds [magnification, ×20 (**B**); magnification, ×60 (**C**)] and the presence of a scar in wounds injected with PGE_2 (magnification, × 20 (**E**); magnification, ×60 (**F**)). Scars/wound sites are marked with **filled arrows** (**B** and **E**). **Open arrows** highlight collagen within the wound (**C** and **F**).

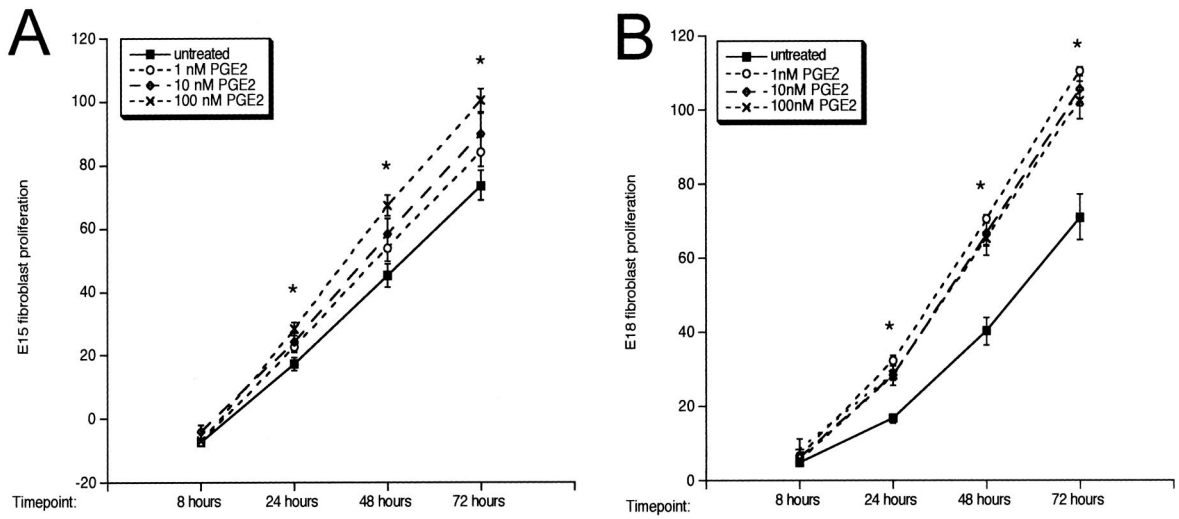

Figure 9. Fetal fibroblast proliferation. The proliferation rates of cultured E15 (**A**) and E18 (**B**) fetal fibroblasts in response to PGE_2 treatment were determined using alamar blue. The fibroblasts responded to PGE_2 in a dose-dependent manner. Treatment with 100 nmol/L PGE_2 resulted in a significant increase in proliferation compared to untreated cells (*, $P < 0.05$).

tory mediators that have been examined in fetal wound repair include, IL-6, IL-8, and IL-10,[23–25] as well as other substances that can induce inflammation in fetal skin.[6–8,20–22] As an absence of inflammation seems to be particularly important for scarless healing to take place, a better understanding of inflammatory regulation in fetal wounds is warranted. The studies described here demonstrate differential expression of COX-2 in scarless *versus* scar-forming fetal skin wounds. The involvement of the COX-2 pathway in scar formation is further highlighted by the fact that increasing PGE_2 levels in scarless wounds results in the conversion of a scarless healing process into one of repair with the generation of a scar.

Besides the wounds themselves, fetal fibroblasts, thought to be an important regulator of scarless healing,[52] were also assessed for COX-2 content. Similarly to what was found in wound tissue, higher COX-2 levels were also detected in cultured fetal fibroblasts isolated from E18 skin compared to fibroblasts isolated from E15 skin. High COX-2 expression and PGE_2 production are believed to contribute to the uncontrolled proliferation of tumor cells, as well as to mobilization and invasion, so it is conceivable that higher COX-2 expression in fibroblasts could stimulate their own activation, migration, and/or proliferation, augmenting scar tissue production. A case for COX-2 and its product, PGE_2, being important for scarring has also been made in adult wound healing studies[36,53] and in reports indicating an increase in collagen deposition and proliferation by fibroblasts in response to PGE_2.[54,55] It has been suggested that the mitogenic effects of PGE_2 on fibroblasts is mediated by signaling through the EP_1 receptor.[56] While PGE_2 induced the proliferation of fibroblasts in our studies and previous studies have shown that PGE_2 can augment proliferation and collagen deposition of fibroblasts,[54,55] it appears that fibroblasts from other organs do not respond in the same manner. For example, PGE_2 is known to reduce proliferation and collagen deposition by lung fibroblasts,[57–59] suggesting that PGE_2 can have varied effects on cells depending on the type and origin of the cell.

While the present studies are the first to report on relative COX-2 levels and the effects of PGE_2 on scarring in the murine fetus, the ability of PGE_2 to induce acute inflammation in fetal rabbit wounds has been previously documented. In these studies, the introduction of PGE_2 and another prostaglandin, $PGF_{2\alpha}$, induced inflammation in fetal wounds,[60] although their effect on collagen deposition or fibrosis was not examined. Whether PGE_2 displays immunosuppressive or anti-inflammatory properties or instead acts as a pro-inflammatory molecule most likely results from differences in the expression or activity of the receptors for PGE_2. These receptors, EP_1 to EP_4, display varied properties after binding PGE_2.[61] Interestingly, one of the PGE_2 receptors, EP_4, was found to be differentially expressed in scarless wounds.[62] Studies on the regulation and function of these receptors in the skin are just beginning, and differences in EP receptor expression and activity may very well influence not only fetal wound healing, but adult wound repair as well. Future studies will be needed to fully characterize the role of each of these receptors in the cutaneous repair process.

There are several plausible mechanisms by which PGE_2 could be inducing scar formation in fetal wounds. PGE_2 could be enhancing acute inflammation, already known to interfere with scarless healing, thereby indirectly promoting scar formation through the recruitment and activation of inflammatory cells. PGE_2 treatment could be both delaying healing and promoting scar tissue deposition through increases in the pro-fibrotic TGF-β.[13,63] Disruption of the TGF-β signaling pathway in smad3-deficient mice has been shown to speed the rate of healing,[62] and extensive data demonstrates restricted TGF-β levels are crucial to scarless healing.[5–12] Lastly, our data demonstrating increased fibroblast proliferation in response to PGE_2 suggests that PGE_2 could be directly stimulating fibroblasts to proliferate, amplifying collagen production and scarring. This idea is also sup-

ported by previous studies demonstrating an increase in collagen deposition and proliferation by fibroblasts following exposure to PGE_2.[54,55]

The data presented here further advance our understanding about the conditions necessary for scarless healing by suggesting that low levels of COX-2 expression and PGE_2 may be necessary for the scarless repair of fetal skin. We have previously demonstrated a link between decreased COX-2 activity and reduced scarring in adult murine skin.[36] The current study provides evidence that PGE_2 induces scar formation in fetal skin, further supporting a role for the COX-2 pathway in scar formation. With the demonstration that COX-2 and its products enhance scarring, the availability of COX-2 inhibitors offers a potential way to mediate scar tissue production in adult skin. The use of COX-2 inhibitors to reduce scar tissue production has implications for alleviating both the cosmetic and the functional problems associated with excessive scarring.

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