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Injury induces *in vivo* expression of platelet-derived growth factor (PDGF) and PDGF receptor mRNAs in skin epithelial cells and PDGF mRNA in connective tissue fibroblasts

(gene expression/chronic injury/wound healing/chronic ulcers/epithelium)

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ABSTRACT **Platelet-derived growth factor (PDGF) stimulates many of the processes important in tissue repair, including proliferation of fibroblasts and synthesis of extracellular matrices. In this study we have demonstrated with *in situ* hybridization and immunocytochemistry the reversible expression of c-*sis*/PDGF-2 and PDGF receptor (PDGF-R) *b* mRNAs and their respective protein products in epithelial cells and fibroblasts following cutaneous injury in pigs. Epithelial cells in control, unwounded skin did not express c-*sis* and PDGF-R mRNAs, and fibroblasts expressed only PDGF-R mRNA. The expression levels in the injured site were correlated with the stage of tissue repair, being highest during the initial stages of the repair process and declining at the time of complete re-epithelialization and tissue remodeling. It is suggested that the controlled, reversible expression of a potent mitogen and its receptor induced by injury may function in an autocrine/paracrine manner on both epithelial cells and fibroblasts to bring about their sustained proliferation during the normal healing process. These studies provide a molecular basis for understanding the mechanisms contributing to normal tissue repair. We suggest the possibility that a defect in these mechanisms may be associated with defective wound healing. It is also conceivable that "chronic" injury may induce irreversible gene expression leading to pathologic, unregulated cell growth.**

Cutaneous injury initiates a series of events that normally result in tissue repair and healing of the wound. Major events in the healing process include proliferation of connective tissue cells, production of extracellular matrices, including collagen synthesis, and epithelial cell migration and proliferation leading to re-epithelialization of the wounded tissue. The mechanisms that regulate these repair processes remain largely unknown. Cell proliferation, migration, and protein synthesis can be stimulated by growth factors that act on responsive cells expressing specific growth factor receptors. *In vivo* studies have shown that the local application of exogenous single growth factors (1–4) or a combination of growth factors (5, 6) can enhance the healing process following experimental wounding in animals.

Platelet-derived growth factor (PDGF) has been suggested to play an important role in wound healing. It is a potent mitogen and chemoattractant for connective tissue cells and a stimulator of collagen synthesis by fibroblasts (for a review see ref. 7). Thus, PDGF can contribute to connective tissue repair and remodeling. PDGF does not have a direct effect on normal epithelial cells since these cells do not express surface receptors for PDGF.

We now report that acute skin injury by surgical excision in swine induces the strong expression of both the PDGF receptor (PDGF-R) *b* mRNA and the c-*sis*/PDGF-2 mRNA in skin epithelial cells and the c-*sis* mRNA in connective tissue fibroblasts. This was accompanied by the expression of their respective protein products. The expression levels were correlated with the stage of wound healing, being highest during the time of rapid cell ingrowth, proliferation, and matrix synthesis and declining at the time of complete re-epithelialization and remodeling. These striking findings are unexpected, since epithelial cells do not normally express c-*sis* and PDGF-R mRNAs, and normal fibroblasts do not express c-*sis* mRNA.

The studies reported here provide direct evidence that injury can induce the expression of mRNAs encoding a potent mitogen and its receptor. The controlled, reversible induction of gene expression initiated by injury may play an important role in the regulation of normal tissue repair. Chronic injury, on the other hand, may induce an irreversible gene expression that contributes to pathologic, unregulated cell growth.

MATERIALS AND METHODS

Collection of Biopsy Specimens Before and After Skin Injury. Total excisional skin biopsy specimens were obtained from normal skin prior to wounding (control) and daily for 9 days after wounding. Partial thickness wounds (10 × 15 mm) were surgically induced in the back of young white Yorkshire pigs (10–15 kg) at a depth of about 1.0 mm by using a modified Castroviejo electrokeratome (Storz, St. Louis; modified by Brownells, Montezume, IA) (5, 6).

***In Situ* Hybridization and Immunocytochemistry.** Fresh biopsy specimens were cut into 2-mm sections and immersed in ice-cold 4% paraformaldehyde for 2–8 hr and subsequently allowed to sink in 30% sucrose/phosphate-buffered saline (PBS) overnight at 4°C to decrease freezing artifacts. The fixed tissues were then embedded in O.C.T. compound (Miles) for cryostat serial sectioning (8 μm; 30 sections per tissue) and *in situ* hybridization with ^{35}S-labeled complementary RNA (cRNA) probes as described previously (8). The specificity of the cRNA probes was determined by hybridization of serial sections with noncomplementary sense RNA probes. Triplicate sections from each tissue were hybridized with either complementary antisense or noncomplementary sense probes and were developed at weekly intervals over a period of 3 weeks. To identify the cells expressing the mRNA, after hybridization the tissues were stained for a

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Abbreviations: PDGF, platelet-derived growth factor; PDGF-R, PDGF receptor.

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tissue-specific marker and then counterstained with hematoxylin (9). To identify epithelial cells the tissues were stained with a polyclonal antibody to bovine keratin (Sigma). To identify connective tissue fibroblasts the tissues were stained with monoclonal antibody to human vimentin (Zymed Laboratories). The probes used in these studies were the human c-*sis* cDNA (10) and the mouse PDGF-R *b* cDNA (11), the latter having binding specificity for mRNA encoding the *sis*/PDGF-2 homodimer (12).

Detection of PDGF-Like and PDGF-R-Like Proteins. For the detection of PDGF-like proteins the tissues were stained with anti-PDGF antibody specific for the c-*sis*/PDGF-2 homodimer and the human heterodimer but not the PDGF-1 homodimer (Institute of Molecular Biology, Boston). For the detection of PDGF-R-like proteins the tissues were stained with antiserum to a synthetic peptide corresponding to the kinase polypeptide region 958–980 of the sequence reported by Yarden *et al.* (11). This region has no homology to other known receptors.

RESULTS

Expression of PDGF-R *b* and c-*sis* mRNAs in Skin Epithelial Cells. Fig. 1 demonstrates the sequential expression of PDGF-R *b* mRNA in skin epithelial cells following injury. Epithelial cells in normal, nonwounded, control tissue did not express PDGF-R *b* mRNA. In contrast, a strong expression of PDGF-R *b* mRNA was evident 1 and 2 days following injury (Fig. 1). The level of PDGF-R mRNA declined following day 2 and was significantly lower at day 5 (data not shown) and no longer detectable at day 9 (Fig. 1). Complete re-epithelialization of the wounds had occurred by day 5.

Fig. 2 shows the expression of c-*sis* mRNA in skin epithelial cells following injury. There was no significant expression in the epithelial cells of the control tissue obtained prior to injury. By day 2 following injury significant expression of c-*sis* mRNA was detected. The level of c-*sis* mRNA then declined, but weak expression was still detectable 5 and 9 days after injury (Fig. 2). The expression demonstrated in Figs. 1 and 2 could be seen throughout the entire length of the new epidermis as well as the epidermis immediately adjacent to the wound.

Expression of c-*sis* and PDGF-R *b* mRNAs in Connective Tissue Fibroblasts. Fig. 3 *Upper* shows the expression of c-*sis* mRNA in connective tissue fibroblasts following injury. Normally, fibroblasts do not express c-*sis* mRNA. Indeed, the fibroblasts in the control, nonwounded tissue specimens did not express c-*sis* mRNA. In contrast, a strong expression of c-*sis* mRNA was seen in both the fibroblasts and the epithelial cells 3 days after injury. In these studies, the fibroblasts in the hybridized tissue were stained with anti-vimentin antibody and the epithelial cells were counterstained with hematoxylin.

Fig. 3 *Lower* shows the expression of PDGF-R *b* mRNA in the connective tissue fibroblasts of the control tissue. This was anticipated, since normal fibroblasts express cell surface PDGF receptors (13). This expression in fibroblasts was significantly increased in tissue specimens obtained within 2 days after injury. The expression of PDGF-R *b* mRNA had declined to basal levels by 5 and 9 days after injury (data not shown). Also, 2 days after injury, strong expression of PDGF-R *b* mRNA was seen in the epithelial cells that were counterstained with hematoxylin (Fig. 3). This is consistent with the results shown in Fig. 1, which demonstrated the strong expression of PDGF-R *b* mRNA in the stained epithelial cells following injury. As shown in Figs. 1 and 3, there was no significant expression of PDGF-R *b* mRNA in the epithelial cells of control tissue.

The findings shown in Figs. 1–3 were reproducible in skin biopsy specimens obtained from three different animals. The time course of the expression was also similar in the three studies. The specificity of the *in situ* hybridization was controlled in parallel studies using noncomplementary, sense RNA probes. Under these control conditions, no significant expression was detected (data not shown).

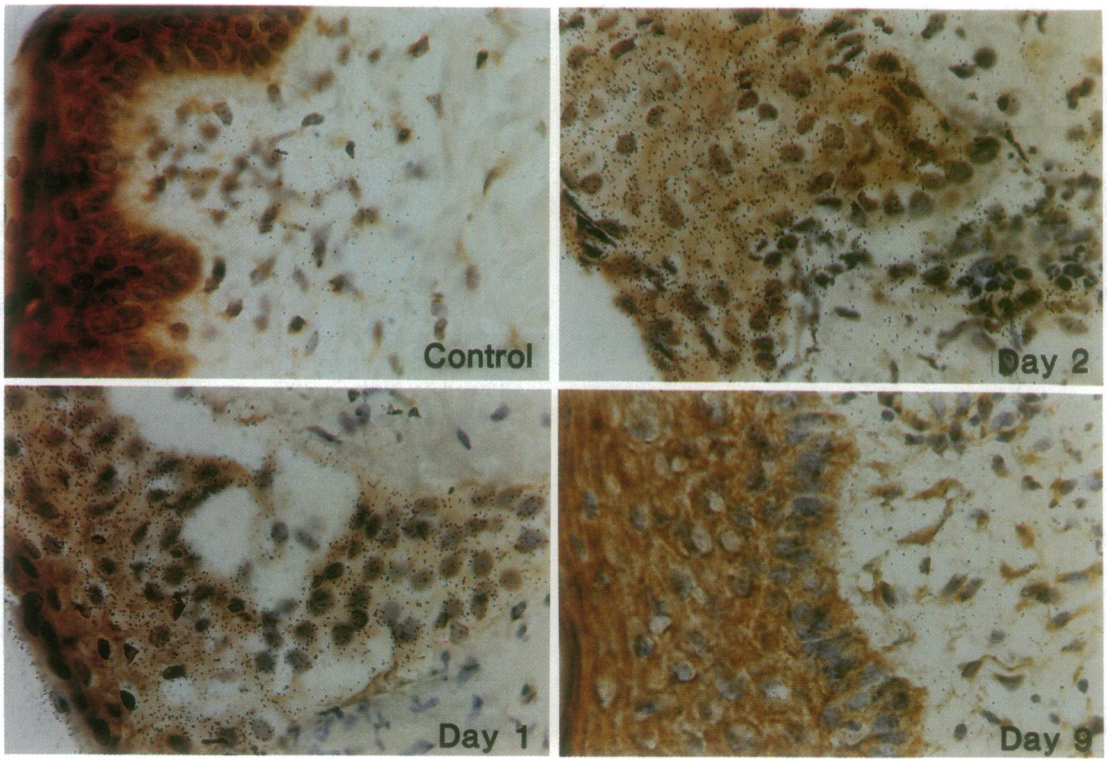

FIG. 1. Induction of PDGF-R *b* mRNA in epithelial cells following injury. Strong expression was seen at days 1 and 2 after injury. Expression ceased by day 9 after injury. Note lack of significant PDGF-R mRNA in the epithelial cells of the control, uninjured tissue. The cells were counterstained with anti-keratin antibody after *in situ* hybridization. (×550.)

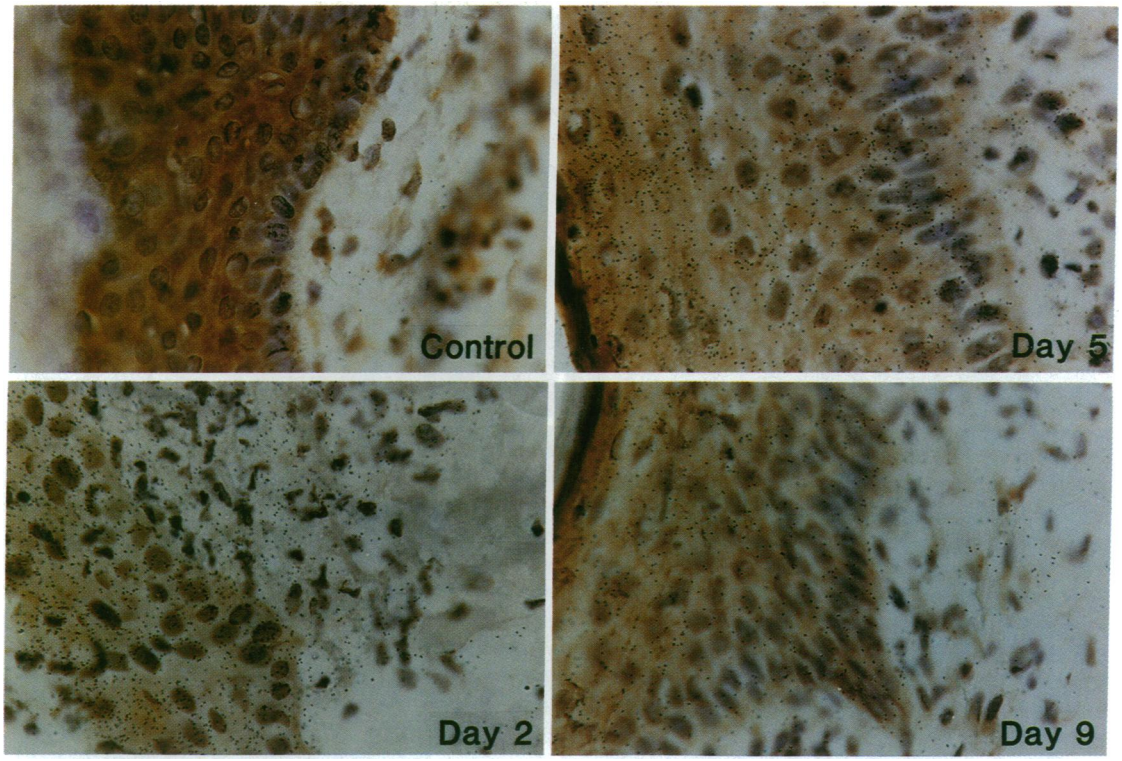

FIG. 2. Induction of c-*sis*/PDGF-2 mRNA expression in epithelial cells following injury. Strong expression was seen at day 2 after injury. Expression declined significantly at days 5 and 9 after injury. There was no significant c-*sis* mRNA in the epithelial cells of the control, uninjured tissue. The cells were counterstained with anti-keratin antibody after *in situ* hybridization. (×550.)

Expression of PDGF-Like and PDGF-R-Like Proteins Following Injury. The expression of c-*sis* and PDGF-R mRNAs induced by injury (Figs. 1–3) was accompanied by the expression of their respective protein products. Immunostaining studies showed the presence of PDGF-like proteins 2 days after injury (Fig. 4*A*) and the presence of PDGF-R-like proteins 1 day after injury (Fig. 4*B*). Control immunostaining in the presence of excess c-*sis*/PDGF-2 homodimer (50 ng)

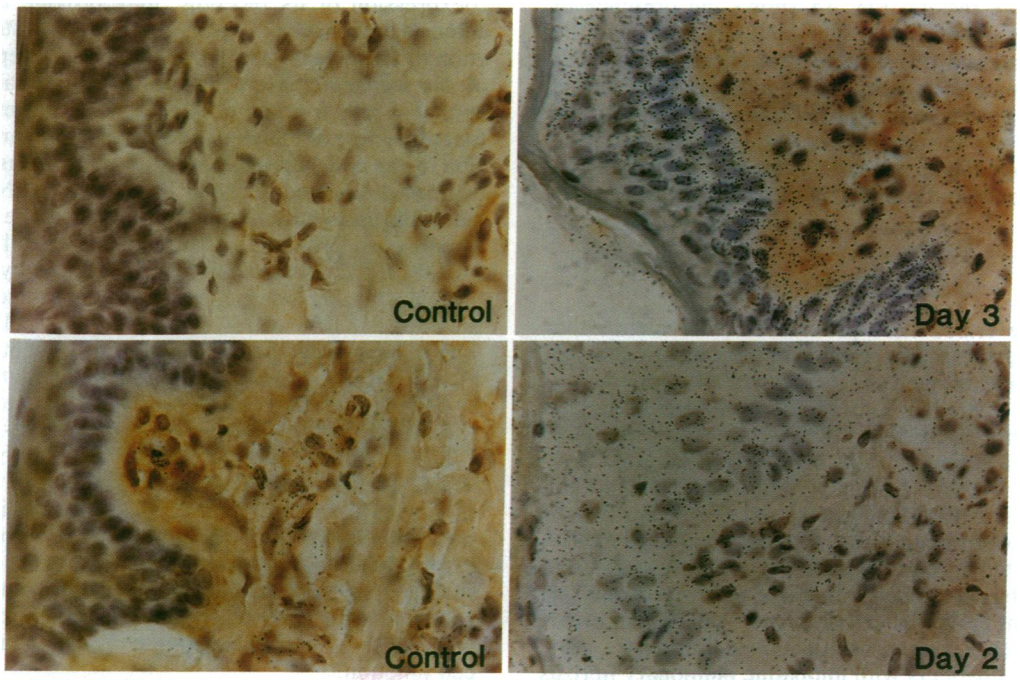

FIG. 3. (*Upper*) Induction of c-*sis* mRNA in connective tissue fibroblasts and in epithelial cells following injury. Strong expression was seen in both the stained fibroblasts and the epithelial cells 3 days after injury. Note lack of c-*sis* mRNA in the fibroblasts and epithelial cells of the control, uninjured tissue. The fibroblasts were counterstained with anti-vimentin antibody and the epithelial cells with hematoxylin. (*Lower*) PDGF-R *b* mRNA in fibroblasts and epithelial cells before and after injury. PDGF-R *b* mRNA was seen in the stained fibroblasts but not in the epithelial cells of the control, uninjured tissue. In contrast, there was a significant increase in the expression in fibroblasts, and a strong expression in the epithelial cells, 2 days after injury. (×550.)

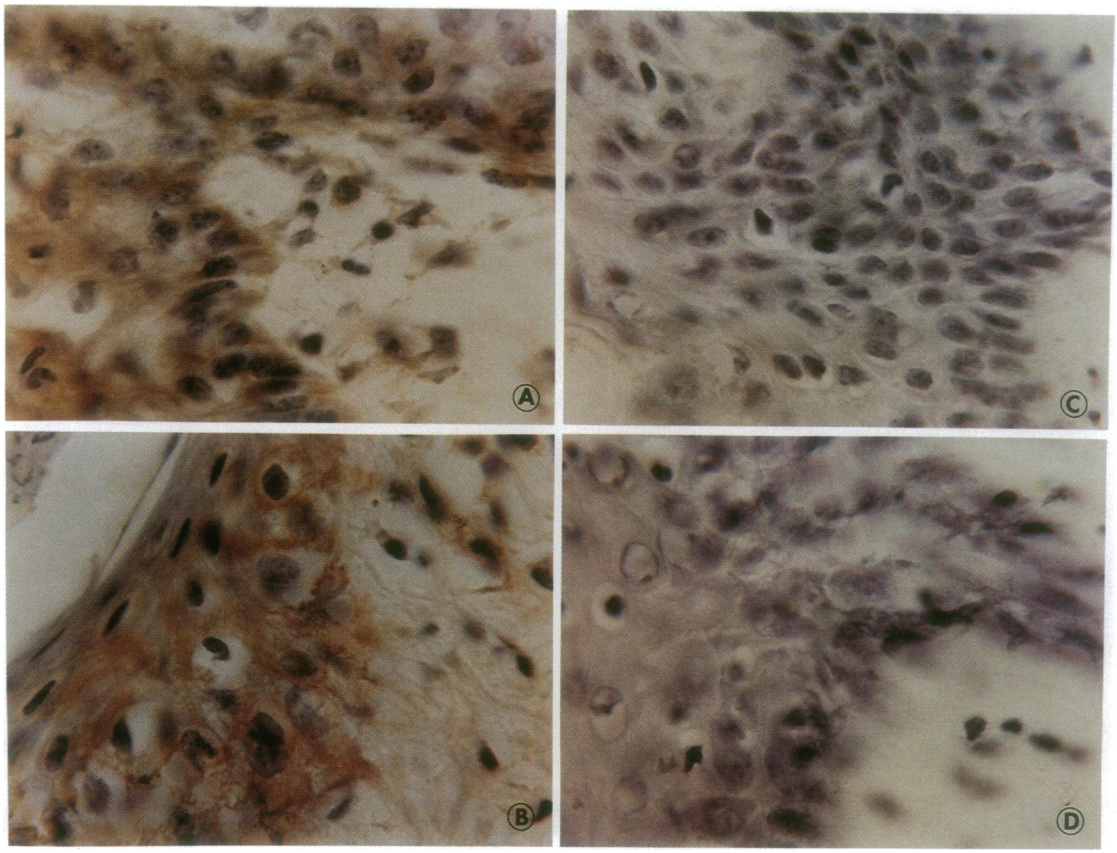

FIG. 4. Immunostaining for PDGF-like and PDGF-R-like proteins in tissue specimens obtained after injury. (*A*) Immunostaining for PDGF-like proteins in tissue specimen obtained 2 days after injury. (*B*) Immunostaining for PDGF-R-like proteins in tissue specimen obtained 1 day after injury. (*C*) Control immunostaining for PDGF-like proteins in the presence of excess c-*sis*/PDGF-2 homodimer (50 ng). (*D*) Control immunostaining for PDGF-R-like proteins in the presence of excess synthetic receptor polypeptide (100 ng). (×550.)

(Fig. 4*C*) or synthetic receptor polypeptide (100 ng) (Fig. 4*D*) produced negative results. Fig. 4*A* shows the presence of PDGF-like polypeptides in both the epithelial layer and the connective tissue, and this parallels the expression of c-*sis* mRNA in the tissue specimens shown in Figs. 2 and 3. Fig. 4*B* shows the presence of PDGF-R-like proteins in the epithelial layer and the connective tissue within 1 day of injury. This also parallels the expression of PDGF-R mRNA in the epithelial cells and connective tissue fibroblasts seen in Figs. 1 and 3.

DISCUSSION

The findings described here provide direct evidence that injury *in vivo* induces the expression of the genes encoding a potent polypeptide mitogen and its receptor. Within 2 days of injury the skin epithelial cells of the wounded tissue expressed c-*sis* and PDGF-R *b* mRNAs, and the connective tissue fibroblasts expressed c-*sis* mRNA and elevated levels of PDGF-R *b* mRNA. Normally, epithelial cells do not express PDGF and PDGF-R genes, and fibroblasts do not express the c-*sis* protooncogene. Expression of c-*sis* mRNA has been reported in certain pathologic states, such as in malignant fibroblasts derived from human fibrosarcomas (14) and in malignant epithelial cells derived from human breast (15, 16), lung (17), and prostatic (18) carcinomas. Lung epithelial cells in patients with idiopathic pulmonary fibrosis have also been found to express c-*sis* mRNA (19).

These findings suggest that molecular mechanisms can be activated at a local tissue site and contribute to the process of normal tissue repair following injury. The c-*sis* protooncogene expressed in the epithelial cells and in the connective tissue fibroblasts encodes a potent mitogen (7). The expression of this mitogen in the epithelial cells, along with the coexpression of its receptor, may provide a basis for an autocrine mechanism contributing to the sustained proliferation of these cells during the process of re-epithelialization of the wounded tissue. Studies using primary cultures of epithelial cells from injured tissue may further elucidate the functional role of PDGF on these cells. Similarly, the expression of the c-*sis*/PDGF-2 mitogen in fibroblasts, which also expressed elevated levels of PDGF-R *b* mRNA, provides a mechanism for the autocrine stimulation of these cells leading to enhanced proliferation and collagen synthesis (20). The c-*sis*/PDGF-2 protein may also be active by the paracrine mechanism; this is suggested by the continued expression of the c-*sis*/PDGF-2 mRNA by the epithelial cells after they no longer expressed the mRNA for its receptor. The c-*sis*/PDGF-2 protein produced at the later stages of wound healing by epithelial cells may therefore have its greatest effect on the underlying fibroblasts which express PDGF-R *b* mRNA.

The local production of c-*sis*/PDGF-2 mitogen could contribute to the entire cutaneous wound healing process by enhancing the recruitment of adjacent cells into the injured site, stimulating their proliferation and production of the extracellular matrices needed to restore tissue integrity. The studies presented here demonstrate that epithelial and connective tissue cells at the site of injury can themselves provide a well-controlled source of this potent modulator of cell function.

It is important to note that the expression of the c-*sis* and PDGF-R genes induced by acute injury is reversible. The expression of the PDGF-R *b* gene is particularly highly controlled in epithelial cells; its expression is greatest in these cells 2 days following injury and below detectable limits by the time complete re-epithelialization has occurred. This

suggests the presence of control mechanisms that can initiate gene expression after injury and cause gene suppression after healing. Thus, the *in vivo* findings described here may serve as a basis of investigations for the identification of the *in vivo* signaling mechanisms involved in gene expression and suppression. Furthermore, it is possible that a defective mechanism in the induction of gene expression may be associated with defective wound healing in patients with diabetes mellitus or chronic ulcers. Alternatively, a defective mechanism in gene suppression may be associated with excessive wound healing as occurs in hypertrophic scarring and keloid formation. An increased production of insulin-like growth factor I (21) and transforming growth factor β (22) in the epithelial cells adjacent to cutaneous wounds has been reported. Immunostaining studies demonstrated an increase in the staining of these growth factors within 1 day after injury, reaching a maximum at 3 days and declining by day 7. This course of expression of immunoreactive insulin-like growth factor I and transforming growth factor β is similar to that described here for the expression of the c-*sis* and PDGF-R *b* mRNAs. The presence of PDGF-like proteins has been reported in human wound fluid (23).

As described above, acute injury induced controlled, reversible gene expression contributing to the physiologic processes of tissue repair. In contrast, "chronic" injury is considered among the causes of several proliferative disorders, including lung cancer, pulmonary fibrosis, and atherosclerosis. Similarities between the wound healing process and tumor stroma formation have been described (24). It is possible that chronic injury leads to an irreversible induction of gene expression causing pathologic unregulated growth. For example, the inappropriate expression of c-*sis* mRNA seen in the malignant epithelial cells of cancer patients (14–17), including those with lung cancer, and in patients with pulmonary fibrosis (19) may originate from exposure of the epithelial cells of these patients to chronic injury. Thus, this inappropriate expression appears to result from the subversion of a normal mechanism serving for tissue repair, when this mechanism is abused by chronic external injury.

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