Interaction between the Insulin-like Growth Factor Family and the Integrin Receptor Family in Tissue Repair Processes

Evidence in a Rabbit Ear Dermal Ulcer Model

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Abstract

We have determined previously that IGF-I is dependent on the presence of IGF binding protein-1 (IGFBP-1) to act as a wound healing agent. We sought to determine the mechanism whereby IGFBP-1 is able to enhance IGF-I bioactivity. As IGFBP-1 binds both the α5β1 integrin as well as IGF-I in vitro, we asked which of the following interactions were important: (*a*) the ability of IGFBP-1 to interact with an integrin receptor, and/or (*b*) the binding of IGF-I by IGFBP-1. We used an IGF-1 analogue (des(1-3)IGF-I) with a > 100-fold reduction in affinity for IGFBP-1 as well as an IGFBP-1 mutant (WGD-IGFBP-1) which does not associate with the α5β1 integrin to selectively abrogate each of these interactions. We also tested the ability of IGFBP-2, a related binding protein which has an arginine-glycine-aspartate sequence but does not associate with integrin family members, to enhance IGF-I bioactivity. Full-thickness dermal wounds were created on rabbit ears; various combinations of native IGF-I, native IGFBP-1, native IGFBP-2, and their respective analogues/mutants were applied to each wound. Wounds were harvested 7 d later for analysis. Only native IGF-I in combination with native IGFBP-1 was effective as a wound healing agent, enhancing reepithelialization and granulation tissue deposition by 64±5 and 83±12% over controls ($P = 0.008$ and 0.016, respectively). The same doses of IGF-I/WGD-IGFBP-1, des(1-3)IGF-I/IGFBP-1, and IGF-I/IGFBP-2 were ineffective. We propose that IGF-I physically interacts with IGFBP-1 and that IGFBP-1 also binds to an integrin receptor, most likely the α5β1 integrin. This interaction is unique to IGFBP-1 as the closely related IGFBP-2 had no effect, a finding consistent with its inability to bind to integrin receptors. Our results suggest that activation of both the IGF-I receptor and the α5β1 integrin is required for IGF-I to stimulate wound healing. (*J. Clin. Invest.* 1996. 98:2462–2468.) Key words: wound healing • insulin-like growth factor-I • integrins • extracellular matrix • growth factors

Introduction

Wound repair is a complex biological phenomenon. The normal repair process is dependent upon protein synthesis, matrix deposition, cellular migration, and replication (1, 2). These diverse processes are orchestrated by environmental signals such as extracellular matrix molecules and peptide growth factors. While an interaction between these different components has been assumed, only recently have specific relationships linking growth factors and the extracellular environment begun to be elucidated (3–8).

Insulin-like growth factor-I (IGF-I) is a peptide present in wounds and is hypothesized to play a major role in tissue repair (9–12) since it can stimulate extracellular matrix deposition and fibroblast growth in vitro. IGF-I's actions are modulated by proteins found in the pericellular space that have been termed the IGF binding proteins (IGFBPs).[1] The six members of the IGFBP family have become increasingly appreciated as powerful determinants of IGF-I bioactivity (13–15). However, much remains to be learned about the exact mechanisms whereby they modulate IGF-I actions.

IGFBP-1 and IGFBP-2 are two of these binding proteins; they are unique among members of the IGFBP family in having an arg-gly-asp (RGD) sequence. IGFBP-1 has been demonstrated to have both inhibitory as well as potentiating effects on IGF-I actions in vitro (15). Interestingly, it has been shown recently to be a ligand for the α5β1 integrin receptor (16). IGFBP-2, although less thoroughly researched than IGFBP-1, is also known to both enhance as well as diminish the effects of IGF-I (17–19); an important characteristic distinguishing it from IGFBP-1 is that IGFBP-2 is not known to bind to integrin receptors despite the fact that it contains an RGD motif (16). The effects of IGFBP-2 on wound healing have not been studied.

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1. *Abbreviations used in this paper:* CHO, Chinese hamster ovary; des(1-3)IGF-I, destripeptide IGF-I; GAG, glycosaminoglycan; IGFBP, IGF binding protein; RGD, arginine-glycine-aspartate; WGD-IGFBP-I, ^{221}Tryptophan-IGF-I.

Integrins are classically considered the archetypal matrix adhesion receptors. However, the past few years have brought about a greater appreciation of the functional repertoire of the integrins, and they are now known to be involved not only in adhesion, but also in such processes as morphogenesis, cell migration, and signal transduction (7, 20). In particular, their roles in wound repair are areas of active investigation, and currently it is known that expression of the integrin subtypes is a dynamically regulated process throughout the various stages of dermal repair (21–24). Growth factors have been shown to influence expression of integrin family members in tissue repair (25–27). However, a direct interaction between growth factor receptor family members and integrin receptors has not been demonstrated in in vivo wound healing assays.

Our lab has previously shown that IGF-I is as effective a vulnerary agent as TGF-β1 and PDGF-BB in our wound healing models, but it requires the presence of IGFBP-1 to have an effect (28, 29). Doses of up to 100 μg per wound of IGF-I by itself were ineffective, but using as little as 1 μg per wound in the presence of IGFBP-1 enhanced wound healing 80–100% over control wounds. We have attempted to determine a mechanistic explanation for these prior observations. We used a naturally occurring IGF-I analogue [destripeptide IGF-I, or des(1-3)IGF-I] to disrupt binding protein/IGF-I interactions. This analogue binds to the IGF-I receptor with normal affinity yet associates very poorly with IGFBP-1 (17, 30–32), allowing us to determine whether an actual physical association between IGF-I and IGFBP-1 was necessary. We also tested the importance of the IGFBP-1/integrin interaction by using a synthetic mutant form of IGFBP-1 which has its RGD binding site converted to WGD. This amino acid substitution renders it unable to interact with the α5β1 integrin in vitro while maintaining its normal affinity for IGF-I (16). Further analysis of the requirement for integrin binding was obtained by testing the ability of IGFBP-2 to enhance IGF-I's vulnerary activity. Our results suggest a novel role for IGFBP-1, as a conduit modulating IGF-I receptor and integrin receptor activities in reparative processes.

Methods

Agents. Recombinant human IGF-I, des(1-3)IGF-1, and IGFBP-1 (dephosphorylated form) were produced from bacteria and purified to > 95% homogeneity. They were generously supplied by Dr. George N. Cox (Synergen Corp., Boulder, CO). WGD-IGFBP-1 was prepared and purified as described previously (16). Briefly, a full-length human IGFBP-1 cDNA served as a template for PCR site-directed mutagenesis. A mutagenic primer was used to replace arginine^{221} in the RGD site of IGFBP-1 to a tryptophan^{221}. The amplified mutant IGFBP-1 cDNA was then purified and ligated into a pNUT expression vector. This vector was then transfected into Chinese hamster ovary (CHO) cells, which were then selected and amplified into a stable cell line with methotrexate. The mutant IGFBP-1 that was secreted into the medium was then purified and concentrated for use in the rabbit ear dermal ulcer experiments. This mutant has been shown to have an affinity for IGF-I that is similar to native IGFBP-1, yet does not bind the α5β1 integrin that is present on CHO cell surfaces (16). To ensure that equal amounts of protein were added to each wound the protein content of the purified WGD-IGFBP-1 samples was quantitated by amino acid composition analysis. Bovine IGFBP-2 was generously provided by Dr. Ted Busby (University of North Carolina, Chapel Hill).

Rabbit ear model. All experiments were performed with the approval of the Northwestern University Institutional Review Board. An established rabbit ear full-thickness dermal ulcer model was used as a wound healing assay (33). Since the wound is noncontractile it allows for accurate and reproducible histologic measurements of new granulation tissue and reepithelialization. On day 0, New Zealand White rabbits (2.5–3.5 kg) were anesthetized with ketamine and xylazine and, using a 6-mm biopsy punch, four circular full-thickness wounds that extended to the level of bare cartilage were created on the ventral surface of each ear. Different combinations of IGF-I, des(1-3)IGF-1, and the binding proteins were applied locally at the time of wounding (see below). Rabbits were then returned to their cages and provided food and water ad libitum. All wounds were harvested on postwounding day 7. Any wounds that showed signs of infection were not analyzed (< 10%).

Treatment groups. Treatment groups were divided into the following seven groups: (*a*) IGF-I alone at a dose of 5 μg per wound (n = 32 wounds); (*b*) IGFBP-1 by itself at a dose of 9 or 13.2 μg per wound (n = 44); (*c*) IGF-I with native IGFBP-1 at a combination dose of 5/1.5 μg per wound, respectively (n = 24); (*d*) IGF-I plus WGD-IGFBP-1 at a combination dose of 5/1.5 μg per wound (n = 30); (*e*) des(1-3)IGF-1 with native IGFBP-1 in a 5/1.5 μg dose mixture (n = 14); (*f*) IGF-I and IGFBP-2 at doses of 5 and 1.9 μg, respectively (n = 13); and (*g*) IGFBP-2 by itself at a dose of 1.9 μg per wound (n = 8). These doses of IGFBP-1 and IGF-I were chosen as they have been shown to cause an optimal increase in wound healing in previous experiments (28, 29). The dose of IGFBP-2 was selected to maintain a molar ratio of IGF-I to IGFBP-2 that was equal to the molar ratio of IGF-I to IGFBP-1. All proteins were concentrated and suspended in an aqueous vehicle of 100 mM Hepes buffer, pH 6.0, 44 mM NaH_2PO_4 such that 7.5 μl was dropped into each wound.

Histologic analysis. The wounds were excised, bisected, and fixed in Omnifix II® (AN-Con Genetics Inc., Melville, NY) for routine hematoxylin and eosin staining (3-μm sections). Histomorphometric measurements were taken for determination of new granulation tissue area as well as area covered by new epithelium. The wounds of some groups were also stained with Sirius red F3BA staining for fibronectin and collagen content (6-μm sections) and Alcian blue staining, at pH 2.5, for the determination of glycosaminoglycan (GAG) content (5-μm sections). All samples were analyzed under a magnification of 100. The Sirius red–stained slides were evaluated with plane-polarized light. Mature collagen bundles showed as yellow fibrils, whereas newly formed collagen and fibronectin stained green or red. The amount of newly formed collagen in the granulation tissue was then estimated as a percentage of the total granulation tissue as described previously (29, 34, 35). The percentage of GAG staining was likewise estimated using Alcian blue–stained slides (29). All determinations were done comparing treated wounds with control wounds treated with vehicle alone and are depicted as percent change over control wounds.

All histological measurements were independently estimated by two blinded observers.

Statistical methods. Statistical analysis was carried out using a Student's paired t test for each reagent and different combinations studied using Excel version 5.0 (Microsoft Corporation). All comparisons are made to paired control wounds treated with vehicle alone. $P < 0.05$ was considered significant.

Results

Fig. 1 shows epithelialization on day 7 after wounding among all the groups. Neither IGF-I nor IGFBP-1 by itself was effective in enhancing epithelialization as compared with wounds treated with vehicle alone. This result confirms our previous studies (28, 29). IGFBP-2 by itself was also ineffective. Likewise, there were no statistical differences between wounds treated with vehicle and wounds treated with the IGF-I/WGD-IGFBP-1 mixture, with the des(1-3) IGF-I/IGFBP-1 mixture,

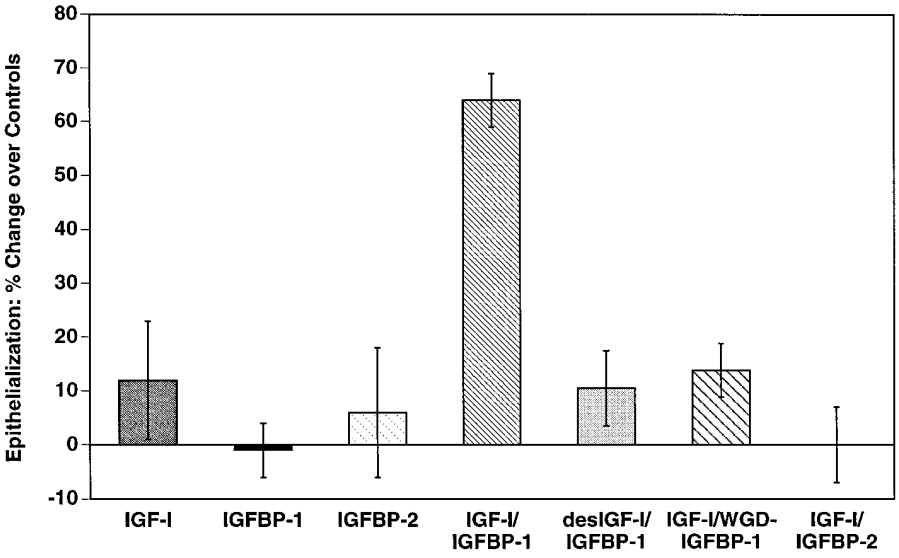

Figure 1. Only IGF-I in combination with IGFBP-1 is able to enhance epithelialization in an open wound model. The values depicted represent percent increase in epithelialization of wounds treated with the different regimens, as measured histomorphometrically, over wounds treated with vehicle alone. The error bars represent the standard error of the mean.

or with the combination of IGF-I/IGFBP-2 ($P > 0.05$). Only wounds treated with native IGF-I combined with native IGFBP-1 showed enhanced epithelialization, demonstrating a 64% increase in new epithelium over control wounds treated with vehicle alone ($n = 24$, $P < 0.05$).

A similar trend was noted in granulation tissue deposition (Fig. 2). A highly significant increase (83%, $n = 24$, $P < 0.05$) in granulation tissue volume is seen in wounds treated with the native IGF-I/native IGFBP-1 mixture. In contrast there is no increase in wounds treated with the other combinations when compared with control wounds treated with vehicle alone.

Fig. 3 is a photomicrograph comparing control wounds treated with vehicle alone (Fig. 3 *A*), wounds treated with the IGF-I/IGFBP-1 mixture (Fig. 3 *B*), wounds treated with the des(1-3)IGF-I/IGFBP-1 mixture (Fig. 3 *C*), and wounds treated with the IGF-I/WGD-IGFBP-1 mixture (Fig. 3 *D*). Note the dramatic increase in granulation tissue area and reepithelialization in the IGF-I/IGFBP-1–treated sample, with the entire frame showing the large amount of newly deposited, immature granulation tissue. Also note the large increase in the number of suprabasal keratinocytes, which is reflected in the increased height of the epidermis. Not shown are the wounds treated with IGF-I and IGFBP-2; healing in these wounds is minimal and indistinguishable from paired wounds treated with vehicle alone. By contrast, the other three groups show small amounts of healing. The mature collagen bundles of unwounded dermis can be seen on the left in each frame. Alcian blue staining of these wounds shows an increase in the amount of GAG, while Sirius red staining reveals significant new collagen deposition within the granulation tissue area and newly formed basement membrane zone. These increases are summarized in Table I.

Fig. 4 compares the magnitude of the effects of the IGF-I/IGFBP-1 mixture on granulation tissue deposition with the previously published effects of other growth factors that have been tested by this lab in the rabbit ear dermal ulcer model. As is apparent, the effects of the IGF-I/IGFBP-1 mixture are substantial and compare favorably with the vulnerary abilities of other growth factors such as TGF-β1, basic fibroblast growth factor, and PDGF-BB (33, 34).

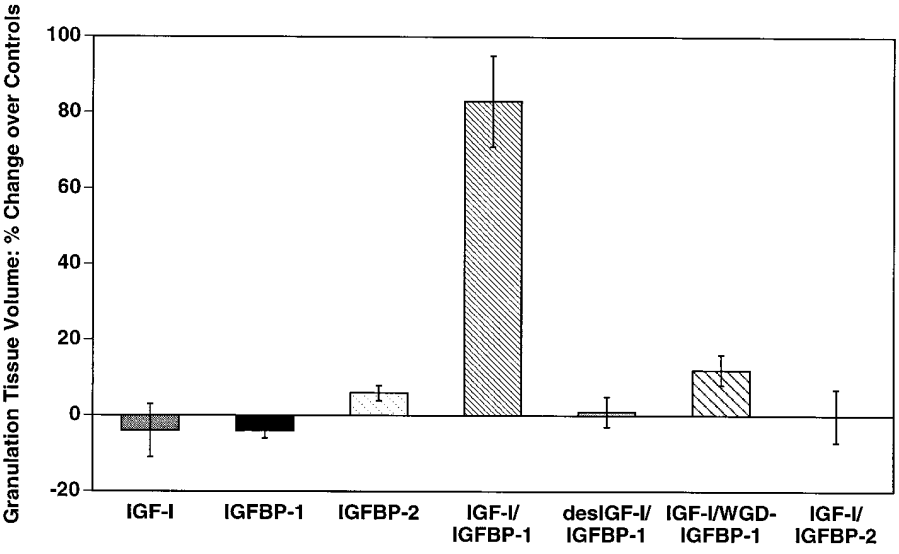

Figure 2. Only IGF-I in combination with IGFBP-1 is able to enhance granulation tissue deposition. The values depicted represent percent increase in granulation tissue volume in treated wounds over control wounds treated with vehicle alone.

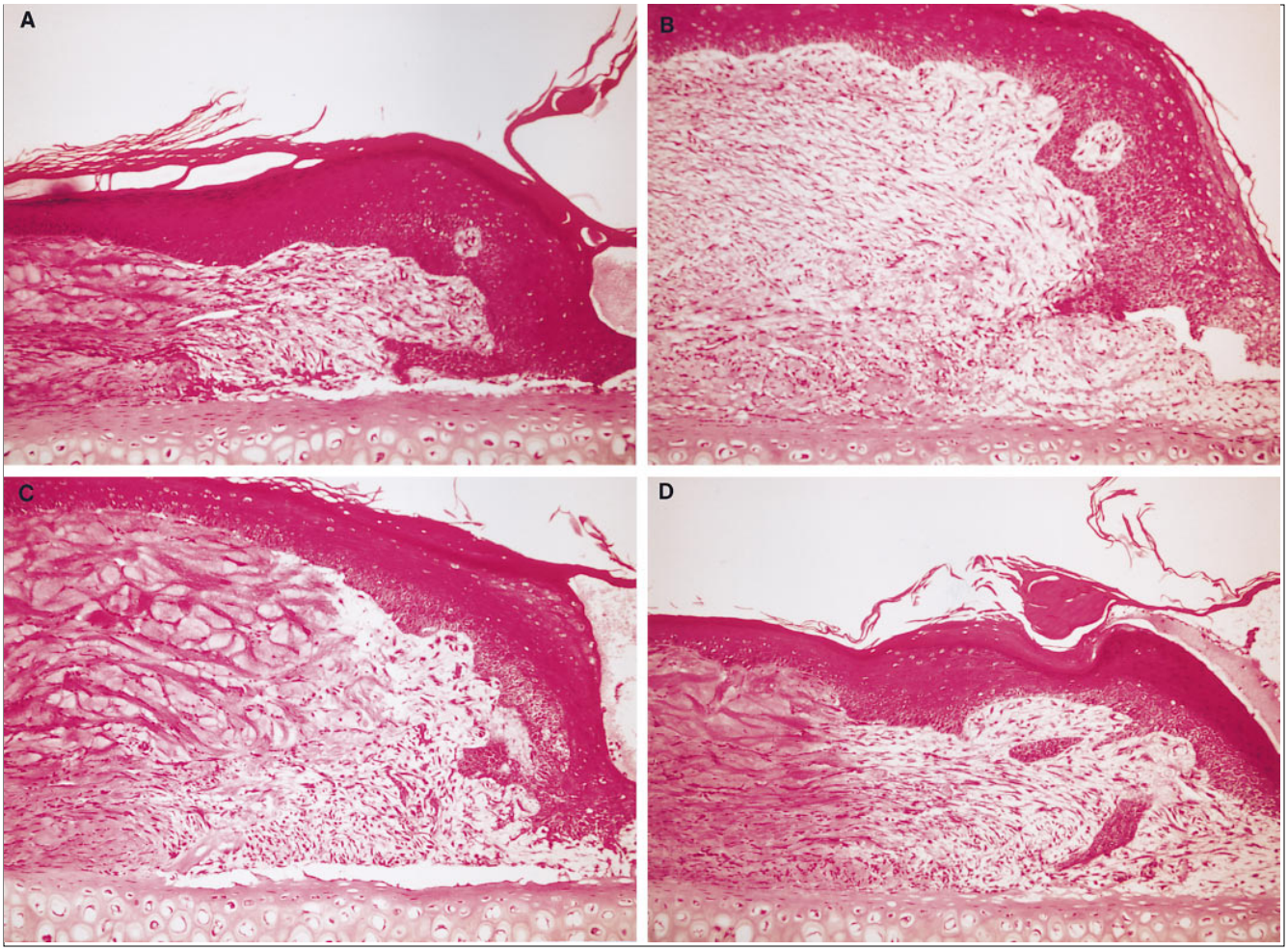

Figure 3. Photomicrograph (×200) of hematoxylin and eosin–stained slide of (*A*) an untreated wound at day 7 after wounding and (*B*) a day 7 wound treated with 5 μg of IGF-I in combination with 1.5 μg of IGFBP-1. Note the dramatic increase in the amount of new granulation tissue as well as the increase in new epithelium. Using equivalent doses of (*C*) des(1-3)IGF-I/IGFBP-1 and (*D*) IGF-I/WGD-IGFBP-1 did not produce a demonstrable effect on wound healing when compared with control wounds.

Discussion

We report in this paper an explanation for the previously described enhancement by IGFBP-1 of IGF-I bioactivity in our animal wound models (28, 29). The enhancement of IGF-I actions by binding proteins has been reported for several in vitro test systems (13, 36–40). Although many explanations have been proposed, there does not appear to be one common mechanism by which the binding proteins potentiate IGF-I activity. This variability may be a consequence of the heterogeneity in the structure of the six IGFBPs, the various cell types studied, and the experimental conditions used (for review see reference 13). For example, IGFBP-3 has been reported most commonly to inhibit IGF-I. However, pretreatment of cultured cells with IGFBP-3 enhances IGF-I actions when IGF-I is added later (37, 41). This change correlates strongly with proteolytic cleavage of IGFBP-3, which results in a decrease in the affinity of IGFBP-3 for IGF-I (42). It also correlates with the capacity of this binding protein to adhere to cell surfaces (40, 43), perhaps to an as yet uncharacterized IGFBP-3 receptor. In a more complicated system such as an experimental wound, IGFBP-3 has likewise been reported to enhance repair (44, 45), albeit in a "dead space" wound chamber model using a multiple dosing regimen. In our models, IGFBP-3 does not enhance IGF-I bioactivity (Galiano, R.D., J.D. Davidson, and T.A. Mustoe manuscript in preparation). Since IGFBP-3 does not contain an RGD sequence, it has been presumed to potentiate IGF-I's activity by protecting it from the proteases found

Table I. Effects on Extracellular Matrix Components in Granulation Tissue

Regimen	GAG area	Collagen area	*n*
IGF-I	0±5%	5±3%	32
IGFBP-1	−5±6%	3±6%	44
IGF-I/IGFBP-2	−4±4%	0±5%	13
IGF-I/IGFBP-1	32±11%*	25±8%*	24
des(1–3)IGF-I/IGFBP-1	0±6%	3±5%	14
IGF-I/WGD-IGFBP-1	−4±5%	7±3%	30

GAG and collagen deposition elicited by the regimens listed are compared with control wounds treated with vehicle alone and are presented as percentage over control±SEM. *$P < 0.05$.

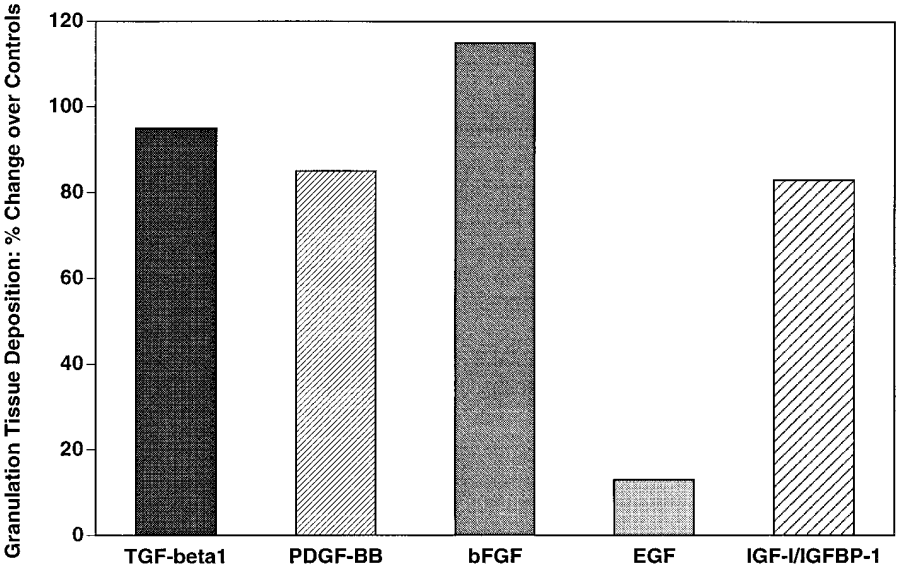

Figure 4. The effects of IGF-I/IGFBP-1 on granulation tissue deposition are compared with those of other growth factors tested in the rabbit ear dermal ulcer model.

at the wound site or from clearance or by making it more accessible to cell membrane–associated IGF-I receptors.

IGFBP-1 has also been reported to have inhibiting or potentiating activities (36, 39, 46, 47). In some instances, this appears to be attributable to a reduced affinity of dephosphorylated IGFBP-1 for IGF-I, which more nearly approximates the affinity of the type I IGF-I receptor (46). Our earliest report showed that the phosphorylated form of IGF-I inhibits IGF-I's vulnerary effects, whereas the unphosphorylated form potentiates it (28). This variable response to IGFBP-1 has also been confirmed in cell culture experiments (47). Much of the earlier confusion in the literature regarding the bimodal effects of IGFBP-1 can be explained on the basis that these early experiments often used crudely purified IGFBP-1 which had varying amounts of the phosphorylated and unphosphorylated forms. However, all the mechanisms proposed to date have suggested that IGFBP-1 has a passive role in modulating IGF-I actions, one that is dictated by its relative affinity for IGF-I as compared with other binding proteins or with the IGF-I receptor.

IGFBP-1 is unique from other binding proteins in several ways. It differs from most of the other IGFBPs in its tissue expression patterns, its sensitivity to regulation by insulin, the regulation of its affinity for IGF-I via changes in its phosphorylation state, and most interestingly in containing an RGD sequence. IGFBP-1 stimulates cell migration directly in vitro by binding to the $\alpha5\beta1$ integrin receptor (16). This effect does not appear to be mediated through other integrin receptors. As noted in prior reports, when CHO or aortic smooth muscle cell surface proteins were radiolabeled and equilibrated with an IGFBP-1 affinity column, only the $\alpha5\beta1$ integrin heterodimer bound to the column. Several radiolabeled integrins, including $\alpha1$, $\alpha2$, $\alpha3$, $\alpha5$, $\beta1$, $\beta3$, and $\beta5$ could be immunoprecipitated from the cell lysates (16, 48), but no other integrins bound to IGFBP-1. Therefore, we believe that the effects we are seeing in our model are most likely due to the binding of IGFBP-1 to the $\alpha5\beta1$ integrin. However, we cannot definitively rule out other mechanisms by which IGF-I and IGFBP-1 might function to enhance wound healing. Although we did not see any effects on wound healing when we used IGFBP-1 by itself, we are not surprised since wound healing is a complex biological phenomenon of which cell migration is but one component. A growth factor or vulnerary agent must do more than simply increase cell motility to have a demonstrable effect on wound healing parameters. However, in this study we show that the ability of IGFBP-1 to bind to an integrin family member (most likely the $\alpha5\beta1$ integrin) appears to be necessary in order for IGF-I to enhance wound repair since the WGD-IGFBP-1 mutant (which will not bind to the $\alpha5\beta1$ integrin [16]) had no effect. Moreover, IGFBP-1 must be physically complexed to IGF-I, since the des(1-3)IGF-I analogue is also ineffective. Although we have not proven definitively that a quaternary interaction is required, these observations suggest strongly that the IGF-I/IGFBP-1 mixture enhances healing by an activation of both integrin and growth factor–mediated signaling pathways.

IGFBP-2, while containing an RGD motif, is not known to bind to integrin receptors that are expressed on the surfaces of CHO or aortic smooth muscle cells (49). This is not surprising or unexpected since the presence of an RGD sequence is in many cases neither necessary nor sufficient for the binding of other matrix molecules to integrin receptors. Our demonstration that IGF-I, at an optimal wound healing dose, is ineffective when combined with IGFBP-2 is further evidence that the binding of IGFBP-1 to an integrin receptor is the crucial link underlying its ability to enhance IGF-I bioactivity in this model.

The integrin family of receptors, although classically considered passive adhesion molecules, is known to mediate many varied effects through signal transduction pathways, many of which are shared with growth factors and cytokines. For example, integrin activation has been shown to phosphorylate not only integrin-associated focal adhesion kinase, but also downstream molecules such as IRS-1 (50), Grb2 (51), and the MAP kinases (52, 53), all of which are also part of the IGF-I–triggered signaling cascade (for reviews see references 15 and 54). Since IGF-I is considered a weak mitogen by itself, a simultaneous activation of both the IGF-I and integrin signal transduction pathways may be the mechanism accounting for the synergistic effects seen in our wound models. This conclusion is strengthened by the recent observation of Vuori and Ruos-

lahti (50) showing that activating the αvβ3 integrin receptor signals a pancreatic cell line to respond to insulin stimulation with increased DNA synthesis. In accordance with this hypothesis, recent evidence in vitro also highlights the importance of interactions between integrin and classical growth factor signaling pathways, with several groups reporting evidence showing integration of integrin and growth factor receptor signal transduction pathways (55–57). Since a physical association between IGF-I and IGFBP-1 also appears to be essential for IGFBP-1 to have potentiating effects on IGF-I activity in our model, IGFBP-1 could serve to bring the IGF-I receptor and the integrin receptor into close proximity, and perhaps thereby bring their intracellular effector molecules into close proximity. While it now appears clear that integrins and growth factors interact at the molecular level, our report is the first to demonstrate not only that there is an effector molecule that brings about this association (i.e., IGFBP-1) but also this is the first study suggesting a role for a growth factor–integrin interaction in an in vivo biological process, in this case wound healing. Since tissue repair consists of a multitude of interactions between molecules involved in migration, in proliferation, and in matrix synthesis, it would not be surprising if further interactions between these processes at the cellular level were elucidated. This would be of importance in unraveling the cellular and molecular events that determine how wound repair ensues.

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References

1. Mustoe, T.A., and R.D. Galiano. 1993. Mediators of wound healing, including the role of growth factors. *In* Advances in Wound Healing and Tissue Repair. Vol. 4. R.C. Lee, T.A. Mustoe, and J.W. Siebert, editors. World Medical Press, New York. 27–55.

2. Jyung, R.W., and T.A. Mustoe. 1993. Role of cytokines in wound repair. *In* Clinical Applications of Cytokines. A. Gearting, J. Rossio, and J. Oppenheim, editors. Oxford University, New York. 307–329.

3. Rapraeger, A.C., A. Krufka, and B.B. Olwin. 1991. Requirement of heparan sulfate for bFGF-mediated fibroblast growth and myoblast differentiation. *Science (Wash. DC).* 252:1705–1707.

4. Ruoslahti, E., and Y. Yamaguchi. 1991. Proteoglycans as modulators of growth factor activities. *Cell.* 64:867–869.

5. Ruoslahti, E., Y. Yamaguchi, A. Hildebrand, and W.A. Border. 1992. Extracellular matrix/growth factor interactions. *Cold Spring Harbor Symp. Quant. Biol.* 57:309–315.

6. Lopez-Casillas, F., H.M. Payne, J.L. Andres, and J. Massague. 1994. Betaglycan can act as a dual modulator of TGF-β access to signaling receptors. Mapping of ligand binding and GAG attachment sites. *J. Cell Biol.* 124:557–568.

7. Adams, J.C., and F.M. Watt. 1993. Regulation of development and differentiation by the extracellular matrix. *Development (Cambridge).* 117:1183–1198.

8. Streuli, C.H., C. Schmidhauser, M. Kobrin, M.J. Bissell, and R. Derynck. 1993. Extracellular matrix regulates expression of the TGF-β1 gene. *J. Cell Biol.* 120:253–260.

9. Skottner, A., M. Kanje, E. Jennische, J. Sjögren, and L. Fryklund. 1988. Tissue repair and IGF-I. *Acta Paediatr. Scand.* 347(Suppl.):110–112.

10. Steenfos, H.H., and J.Q. Janssen. 1992. Gene expression of insulin-like growth factor-I and IGF-I receptor during wound healing in rats. *Eur. J. Surg.* 158:327–331.

11. Gartner, M.H., J.D. Benson, and M.D. Caldwell. 1992. Insulin-like growth factors I and II expression in the healing wound. *J. Surg. Res.* 52:389–394.

12. Suh, D.Y., T.K. Hunt, and E.M. Spencer. 1992. Insulin-like growth factor-I reverses the impairment of wound healing induced by corticosteroids in rats. *Endocrinology.* 131:2399–2403.

13. Clemmons, D. R. 1992. IGF binding proteins: regulation of cellular actions. *Growth Regul.* 2:80–87.

14. Rechler, M.M., and A.L. Brown. 1992. Insulin-like growth factor binding proteins: gene structure and expression. *Growth Regul.* 2:55–68.

15. Jones, J.I., and D.R. Clemmons. 1995. Insulin-like growth factors and their binding proteins: biological actions. *Endocr. Rev.* 16:3–34.

16. Jones, J.I., A. Gockerman, W.H. Busby, G. Wright, and D.R. Clemmons. 1993. Insulin-like growth factor binding protein-1 stimulates cell migration and binds to the alpha 5 beta 1 integrin by means of its Arg-Gly-Asp sequence. *Proc. Natl. Acad. Sci. USA.* 90:10553–10557.

17. Ross, M., G.L. Francis, L. Szabo, J.C. Wallace, and F.J. Ballard. 1989. Insulin-like growth factor (IGF)-binding proteins inhibit the biological activities of IGF-I and IGF-II but not des(1-3)IGF-I. *Biochem. J.* 258:267–272.

18. Bar, R.S., B.A. Booth, M. Bowes, and B.L. Drake. 1989. Insulin-like growth factor binding proteins from cultured endothelial cells: purification, characterization and intrinsic biologic activities. *Endocrinology.* 125:1910–1920.

19. Bourner, M.J., W.H. Busby, N.R. Seigel, G.G. Krivi, R.H. McCusker, and D.R. Clemmons. 1992. Cloning and sequence determination of bovine insulin-like growth factor binding protein-2 (IGFBP-2): comparison of its structural and functional properties with IGFBP-1. *J. Cell. Biochem.* 48:215–226.

20. Clark, E.A., and J.S. Brugge. 1995. Integrins and signal transduction pathways: the road taken. *Science (Wash. DC).* 268:233–239.

21. Juhasz, I., G.F. Murphy, H.C. Yan, M. Herlyn, and S.M. Albelda. 1993. Regulation of extracellular matrix proteins and integrin cell substratum adhesion receptors on epithelium during cutaneous human wound healing in vivo. *Am. J. Pathol.* 143:1458–1469.

22. Gailit, J., and R.A.F. Clark. 1994. Wound repair in the context of extracellular matrix. *Curr. Opin. Cell Biol.* 6:717–725.

23. Larjava, H., T. Salo, K. Haapasalmi, R.H. Kramer, and J. Heino. 1993. Expression of integrins and basement membrane components by wound keratinocytes. *J. Clin. Invest.* 92:1425–1435.

24. Cavani, A., G. Zambruno, A. Marconi, V. Manca, M. Marchetti, and A. Giannetti. 1993. Distinctive integrin expression in the newly forming epidermis during wound healing in humans. *J. Invest. Dermatol.* 101:600–604.

25. Zambruno, G., P.C. Marchisio, A. Marconi, C. Vaschieri, A. Melchiori, A. Giannetti, and M. De Luca. 1995. Transforming growth factor-β1 modulates β1 and β5 integrin receptors and induces the de novo expression of the αvβ6 heterodimer in normal human keratinocytes. Implications for wound healing. *J. Cell Biol.* 129:853–865.

26. Danilenko, D.M., B.D. Ring, J.Z. Lu, J.E. Tarpley, D. Chang, N. Liu, D. Wen, and G.F. Pierce. 1995. Neu differentiation factor upregulates epidermal migration and integrin expression in excisional wounds. *J. Clin. Invest.* 95:842–851.

27. Gailit, J., M.P. Welch, and R.A.F. Clark. 1994. TGF-beta1 stimulates expression of keratinocyte integrins during re-epithelialization of cutaneous wounds. *J. Invest. Dermatol.* 103:221–227.

28. Jyung, R.W., T.A. Mustoe, W.H. Busby, and D.R. Clemmons. 1994. Increased wound-breaking strength induced by insulin-like growth factor I in combination with insulin-like growth factor binding protein-1. *Surgery (St. Louis).* 115:233–239.

29. Zhao, L.L., R.D. Galiano, G.N. Cox, S.I. Roth, and T.A. Mustoe. 1995. Effects of insulin-like growth factor-I and insulin-like growth factor binding protein-1 on wound healing in a dermal ulcer model. *Wound Repair Regen.* 3: 316–321.

30. Carlsson-Skwirut, C., M. Lake, M. Hartmanis, K. Hall, and V.R. Sara. 1989. A comparison of the biological activity of the recombinant intact and truncated insulin-like growth factor I (IGF-I). *Biochim. Biophys. Acta.* 1011: 192–197.

31. Clemmons, D.R., M.L. Dehoff, W.H. Busby, M.L. Bayne, and M.A. Cascieri. 1992. Competition for binding to insulin-like growth factor (IGF) binding protein-2, 3, 4, and 5 by the IGFs and IGF analogs. *Endocrinology.* 131:890–895.

32. Oh, Y., H.L. Muller, D.Y. Lee, P.J. Fielder, and R.G. Rosenfeld. 1993. Characterization of the affinities of insulin-like growth factor (IGF)-binding proteins 1-4 for IGF-I, IGF-II, IGF-I/insulin hybrid, and IGF-I analogs. *Endocrinology.* 132:1337–1344.

33. Mustoe, T.A., G.F. Pierce, C. Morishima, and T.F. Deuel. 1991. Growth factor–induced acceleration of tissue repair through direct and inductive activities in a rabbit dermal ulcer model. *J. Clin. Invest.* 87:694–703.

34. Pierce, G.F., J.E. Tarpley, D. Yanagihara, T.A. Mustoe, G.M. Fox, and A. Thomason. 1992. Platelet-derived growth factor (BB homodimer), transforming growth factor-b1 and basic fibroblast growth factor in dermal wound healing. *Am. J. Pathol.* 140:1375–1388.

35. Zhao, L., J. Davidson, S. Wee, S. Roth, and T. Mustoe. 1994. Effect of hyperbaric oxygen and growth factors on rabbit ear ischemic ulcers. *Arch Surg.* 129:1043–1049.

36. Elgin, R.G., W.H. Busby, and D.R. Clemmons. 1987. An insulin-like growth factor (IGF) binding protein enhances the biologic response to IGF-I. *Proc. Natl. Acad. Sci. USA.* 84:3254–3258.

37. DeMellow, J.S.M., and R.C. Baxter. 1988. Growth hormone dependent

insulin-like growth factor binding protein both inhibits and potentiates IGF-I stimulated DNA synthesis in skin fibroblasts. *Biochem. Biophys. Res. Commun.* 156:199–204.

38. Blum, W.F., E.W. Jenne, F. Reppin, K. Kietzmann, M.B. Ranke, and J.R. Bierich. 1989. Insulin-like growth factor I (IGF-I) binding protein complex is a better mitogen than free IGF-I. *Endocrinology.* 125:766–772.

39. Kratz, G., M. Lake, K. Ljungstrom, G. Forsberg, A. Haegerstrand, and M. Gidlund. 1992. Effect of recombinant IGF binding protein-1 on primary cultures of human keratinocytes and fibroblasts: selective enhancement of IGF-1 but not IGF-2-induced cell proliferation. *Exp. Cell Res.* 202:381–385.

40. McCusker, R.H., W.H. Busby, M.H. Dehoff, C. Camacho-Hubner, and D.R. Clemmons. 1991. Insulin-like growth factor (IGF) binding to cell monolayers is directly modulated by the addition of IGF-binding proteins. *Endocrinology.* 129:939–949.

41. Conover, C.A., M. Ronk, F. Lombana, and D.R. Powell. 1990. Structural and biological characterization of bovine insulin-like growth factor binding protein-3. *Endocrinology.* 127:2795–2803.

42. Blat, C., J. Villaudy, and M. Binoux. 1994. In vivo proteolysis of serum insulin-like growth factor (IGF) binding protein-3 results in increased availability of IGF to target cells. *J. Clin. Invest.* 93:2286–2290.

43. McCusker, R.H., C. Camacho-Hubner, M.L. Bayne, M.A. Cascieri, and D.R. Clemmons. 1990. Insulin-like growth factor (IGF) binding to human fibroblast and glioblastoma cells: the modulating effect of cell released IGF binding proteins (IGFBPs). *J. Cell. Physiol.* 144:244–253.

44. Hamon, G.A., T.K. Hunt, and E.M. Spencer. 1993. In vivo effects of systemic insulin-like growth factor-I alone and complexed with insulin-like growth factor binding protein-3 on corticosteroid suppressed wounds. *Growth Regul.* 3: 53–56.

45. Sommer, A., C.A. Maack, S.K. Spratt, D. Mascarenhas, T.J. Tressel, E.T. Rhodes, R. Lee, M. Roumas, G.P. Tatsuno, J.A. Flynn, et al. 1991. Molecular genetics and actions of recombinant insulin-like growth factor binding protein-3. *In* Modern Concepts of Insulin-like Growth Factors. E.M. Spencer, editor. Elsevier, New York. 715–728.

46. Jones, J.I., A.J. D'Ercole, C. Camacho-Hubner, and D.R. Clemmons. 1991. Phosphorylation of insulin-like growth factor (IGF)-binding protein 1 in cell culture and in vivo: effects on affinity for IGF-I. *Proc. Natl. Acad. Sci. USA.* 88:7481–7485.

47. Busby, W.H., D.G. Klapper, and D.R. Clemmons. 1988. Purification of a 31000 dalton insulin like growth factor binding protein from human amniotic fluid. *J. Biol. Chem.* 263:14203–14210.

48. Jones, J., T. Prevette, A. Gockerman, and D. Clemmons. 1996. Ligand occupancy of the aVb3 integrin is necessary for smooth muscle cells to migrate in response to insulin-like growth factor I. *Proc. Natl. Acad. Sci. USA.* 93:2482–2487.

49. Gockerman, A., J. Jones, T. Prevette, and D. Clemmons. 1995. Insulin-like growth factor binding protein-2 inhibits smooth muscle cell migration response to IGF-I. *Endocrinology.* 136:4168–4173.

50. Vuori, K., and E. Ruoslahti. 1994. Association of insulin receptor substrate-1 with integrins. *Science (Wash. DC).* 266:1576–1578.

51. Schlaepfer, D.D., S.K. Hanks, T. Hunter, and P. van der Geer. 1994. Integrin-mediated signal transduction linked to Ras pathway by GRB2 binding to focal adhesion kinase. *Nature (Lond.).* 372:786–791.

52. Chen, Q., M.S. Kinch, T.H. Lin, K. Burridge, and R.L. Juliano. 1994. Integrin-mediated cell adhesion activates mitogen-activated protein kinases. *J. Biol. Chem.* 269:26602–26605.

53. Morino, N., T. Minura, K. Hamasaki, T. Tobe, K. Ueki, K. Kikuchi, K. Takehara, T. Kadowaki, Y. Yashaki, and Y. Nojima. 1995. Matrix/integrin interaction activates the mitogen-activated protein kinase, p44erk-1 and p42erk-2. *J. Biol. Chem.* 270:269–273.

54. Obberghen, E.V. 1994. Signalling through the insulin receptor and the insulin-like growth factor-I receptor. *Diabetologia.* 37(Suppl. 2):S125–S134.

55. Plopper, G.E., H.P. McNamee, L.E. Dike, K. Bojanowski, and D.E. Ingber. 1995. Convergence of integrin and growth factor receptor signaling pathways within the focal adhesion complex. *Mol. Biol. Cell.* 6:1349–1356.

56. Zhang, Z., K. Vuori, H.-G. Wang, J.C. Reed, and E. Ruoslahti. 1996. Integrin activation by R-ras. *Cell.* 85:61–69.

57. Schwartz, M., and D. Ingber. 1994. Integrating with integrins. *Mol. Biol. Cell.* 5:389–393.