Recombinant Basic Fibroblast Growth Factor Stimulates Wound Healing in Healing-impaired *db/db* Mice

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Summary

The stimulatory effect of recombinant basic fibroblast growth factor (bFGF) on wound healing was assessed using healing-impaired (*db/db*) mice. Full-thickness wounds were made in female diabetic C57BL/KsJ *db/db* mice, and their normal (*db/+*) littermates with a punch biopsy instrument. Recombinant bFGF was applied locally to the open wound once a day. The mice were later killed and histological sections of the wounds were prepared. The degree of wound healing was evaluated using several histological parameters such as degree of reepithelialization, granulation tissue thickness, matrix density, number of infiltrated cells, and number of capillaries. Wounds from normal mice displayed good reepithelialization rates and granulation tissue formation, while wounds from *db/db* mice had poor responses, especially in the dermal parameters. Although the application of bFGF to wounds in the normal (*db/+*) mice had little effect, application of bFGF to wounds in *db/db* mice induced significant responses in all of the dermal parameters compared with nontreated *db/db* mice ($p < 0.001$). In the presence of bFGF, these parameters approximated those observed in nontreated littermates. A minimum of 0.5 μg bFGF in either single or multiple applications was required for a significant effect. bFGF that was either boiled or pretreated with neutralizing antibody had little stimulatory effect. Time-course experiments indicated that the granulation response in bFGF-treated mice peaked between 8 and 12 d, and decreased after 12 d, while matrix density continued to increase until the 18th day ($p < 0.05$). The breaking strength of healed linear wounds in *db/db* mice was also decreased when compared with heterozygous littermates. This parameter was also improved by the administration of bFGF to the wounds ($p < 0.05$).

The healing of wounds proceeds in three overlapping phases: inflammation, granulation tissue formation, and matrix formation and remodeling (1). This process is believed to require the interaction of cells in the dermis and epidermis as well as the activities of chemical mediators released from inflammatory cells, fibroblasts, and keratinocytes. Although the precise interactions of the components comprising normal wound healing are not understood, a number of conditions such as immunodeficiency, chemotherapy, venous stasis, and diabetic ulcers result in delayed wound healing (2). One of the obvious features of full thickness wound healing is the formation of granulation tissue in the dermis. The proliferation of mesenchymal cells and capillaries, and the influx of macrophages that compose the granulation tissue serve to replace the lost dermis and may also provide substrates and inducers for reepithelialization.

It has been postulated that the application of agents that induce fibroblast and/or endothelial cell proliferation to healing-impaired wounds might increase the rate and degree of granulation tissue formation and stimulate wound repair. Several fibroblast mitogens have been tested and appear to increase wound healing in certain animal systems (3–14). Recently a number of proteins have been characterized as being inducers of angiogenesis when applied to specific tissues (15). The best characterized of these is basic fibroblast growth factor (bFGF)[1] (16, 17). bFGF is a potent mitogen and chemoattractant for endothelial cells as well as fibroblasts. Therefore, bFGF appears to be a strong candidate as a potentiating agent for wound healing. Previous reports (4–7, 10) indicated that the injection of bFGF increased granulation tissue formation in implanted sponges and increased the breaking strength of incisional wounds in normal rats. However, the effects of bFGF on the cellular parameters of wound healing were not evaluated.

Since the effects of bFGF on potentiating wound healing

[1] *Abbreviation used in this paper:* bFGF, basic fibroblast growth factor.

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in animals displaying impaired healing had not been examined, we undertook to characterize this phenomenon. We describe here a histological evaluation of the effect of bFGF on wound healing using healing-impaired mice. Mutant diabetic mice (*db/db*) show a delayed healing response in the dermis compared with their heterozygous *db/+* littermates. The application of recombinant bFGF to wounds in *db/db* mice increases the number of infiltrated cells, capillary number, matrix formation, and wound breaking strength almost to the levels observed in normal *db/+* littermates.

Materials and Methods

Animals. Female mutant diabetic mice, C57BL/ksJ *db/db*, and their normal littermates (*db/+*) were purchased from The Jackson Laboratory (Bar Harbor, ME). All mice were maintained on a standard laboratory diet and water ad libitum, and were used at 8 wk of age. At the initiation of experiments, mice were housed individually and checked for urinary glucose by reagent strips (Miles Laboratories Inc., Elkhart, IN). All *db/db* mice were judged to be mildly to severely diabetic. Mice were maintained in individual cages throughout the experiment.

Preparation of Reagents. Recombinant bFGF was a gift from Synergen, Inc., (Boulder, CO). Carboxymethylcellulose (mol mass 250 kD) was purchased from Polysciences Inc. (Warrington, PA). The vehicle solution consisted of 1.5% carboxymethylcellulose and 0.5% mouse serum in sterilized PBS solution. Different bFGF solutions were prepared by adding concentrated recombinant bFGF to the vehicle solution. Polyclonal antibodies against recombinant bFGF were raised in rabbits as described (18), and IgG fractions were prepared by protein A–Sepharose column chromatography and lyophilized.

Wounding. Mice were anesthetized with sodium pentobarbital solution (40 mg/kg, i.p.), and their dorsal hair was clipped. Two full-thickness round wounds were prepared on the upper back of each mouse using a punch biopsy instrument (6 mm diameter; George Tiemann and Co., Long Island City, NY). The two wounds were separated by 1 cm in the anterior-posterior direction. After the operation, 20 μl of test solution was applied to each wound. A single mouse received the same test solution in both wounds to prevent possible effects from leakage from one wound to the other. The mice were kept anesthetized until the wound was almost dry. The wounds were left open during the experiment. In some experiments, as noted, mice were given daily applications of reagent until the 5th day.

Sample Preparation and Histological Evaluation. On the indicated day, the mice were killed by cervical dislocation, using care not to pull back the skin. The wounds were excised and fixed in 10% buffered formalin solution. After overnight fixation, the tissue was trimmed and cut through at the widest margin. The tissue was embedded in paraffin and sectioned in 5-μm increments. The sections were made perpendicular to the anterior-posterior axis and perpendicular to the surface of the wound. Six sections were placed on a slide, and stained with hematoxylin and eosin.

Histological evaluation was performed by one of us (R.T.), with the identity of the samples masked. Of the six sections on any one slide, the section with the widest original wound margin was used for scoring. Sections that had abscesses were not evaluated. The parameters measured were degree of reepithelialization, granulation tissue thickness, matrix density, number of infiltrated cells, and number of capillaries for the entire wound area. Each of the parameters was graded numerically as described below to permit average scores to be compiled.

Reepithelialization. The degree of reepithelialization was measured and given a value of 0 to 10; 0 was equivalent to no closure and 10 was equivalent to complete reepithelialization by keratinocytes.

Granulation Tissue Thickness. A value of 1 equals a thin granulation layer, 2 equals moderate granulation, 3 equals a thick granulation layer, and 4 equals a very thick granulation layer.

Matrix Density. The degree of dermal matrix deposition was scored as 1 equals edematous with little matrix, 2 equals a small amount of coarse matrix, 3 equals a moderate amount of matrix, and 4 equals dense matrix.

Infiltrated Cells. As an index of the degree of cellular infiltration, the number of fibroblasts and macrophages was estimated. Polymorphonuclear cells and lymphocytes were excluded from the counting. A score of 1 equals few cells, 2 equals a moderate number, 3 equals many cells, 4 equals very many cells.

Capillary Number. The number of capillary lumens was counted in the complete wound cross-section at ×100 magnification. A score of 0 equals 0–4 capillaries per wound, 1 equals 5–14 capillaries per wound, 2 equals 15–24 capillaries per wound, etc. Only mature vessels that contained erythrocytes were counted.

Breaking Strength. Full thickness, anterior-posterior incisional wounds (4 cm) were made on the back with a scalpel. After application of bFGF or vehicle solution, the incision was closed using with four monofilament nylon sutures (5-0; Ethicon Inc., Sommerville, NJ) placed at 1 cm intervals. Mice were killed on postwounding day 9, sutures were removed, and three strips of skin (0.8 cm wide) were taken perpendicular to the wound and between the sutures. The edges of the original wound were not used. The first strip was used for histological evaluation, and the other two strips were kept wet with PBS and used for tensile strength measurements. Breaking strength was measured using a spring balance (maximum 250 *g*; Ohaus Scale Corp., Florham Park, NJ). The ends of the wound were secured by binder clips (1/2 inch; Swingline, Inc., Long Island City, NY). A force was applied across the incision at a constant speed (1 cm/s). The breaking strength was the point of maximal stress before wound separation, and was expressed as g/mm incisional width. Measurements were done with the investigator blinded as to sample origin.

Statistical Analysis. All the data were analyzed by Student's paired *t*-test. Results were expressed as means ± SEM. The differences in measurements between the two wounds on a single mouse were never greater than the differences observed between identical mice.

Results

Our initial attempts to measure the effect of bFGF on full-thickness wounds in normal BALB/c and C57BL mice indicated that the application of the growth factor had only small positive effects (data not shown). As shown in Table 1, normal C57BL/ksJ *db/+* mice had good wound closure rates and granulation tissue formation. The application of bFGF to these wounds had a minimal effect on the wound healing parameters (Table 1). Typical examples of the histology of the wounds from treated vs. nontreated *db/+* mice are illustrated in Fig. 1, *a* and *b*. It is apparent that reepithelialization was completed by 8 d after wounding and thick granulation tissue was formed in both specimens. It should be noted that al-

Table 1. *Effects of bFGF on Wound Healing in Normal and db/db Mice*

Treatment	*n*	Reepithelialization	Granulation tissue thickness	Matrix density	Infiltrated cells	Capillary number
Normal Mice (*db/+*)						
0 μg bFGF × 5 d	10	8.8 ± 0.6	3.3 ± 0.1	3.3 ± 0.3	3.2 ± 0.2	8.1 ± 0.8
Normal Mice (*db/+*)						
5 μg × 5 d	9	8.6 ± 0.7	3.6 ± 0.2	3.4 ± 0.3	3.8 ± 0.1*	10.6 ± 1.3
db/db Mice						
0 μg × 5 d	10	7.1 ± 0.7	1.4 ± 0.2	2.1 ± 0.4	1.7 ± 0.1	2.4 ± 0.4
db/db Mice						
5 μg × 5 d	10	8.4 ± 0.6	2.8 ± 0.2‡	3.1 ± 0.2§	2.8 ± 0.2‡	8.2 ± 0.9‡

Samples were taken at 8 days post-wounding. Vehicle solutions plus and minus bFGF as indicated were applied each day for five days beginning with the day of wounding. Scoring of each parameter was graded by the method described in the text. μg refers to the amount of bFGF applied. *n*, Number of wounds analyzed.

* Value is different from that of control *db/+* mice, 0 μg × 5 d, $p < 0.02$.

‡ $p < 0.001$.

§ Asterisks indicate that the values are significantly different from that of *db/db* mice, 0 μg × 5 d, $p < 0.05$.

though reepithelialization was complete in the samples illustrated in Fig. 1, *a* and *b*, in the majority of the samples (Table 1), reepithelialization was only 80–90% at this time. The dermal layer appears to be somewhat thicker in the treated wound illustrated, but this was not always observed. At the higher magnification, infiltrated macrophages, fibroblasts, and lymphoid cells as well as capillaries were evident in both nontreated and treated wounds. Thus, no major differences were

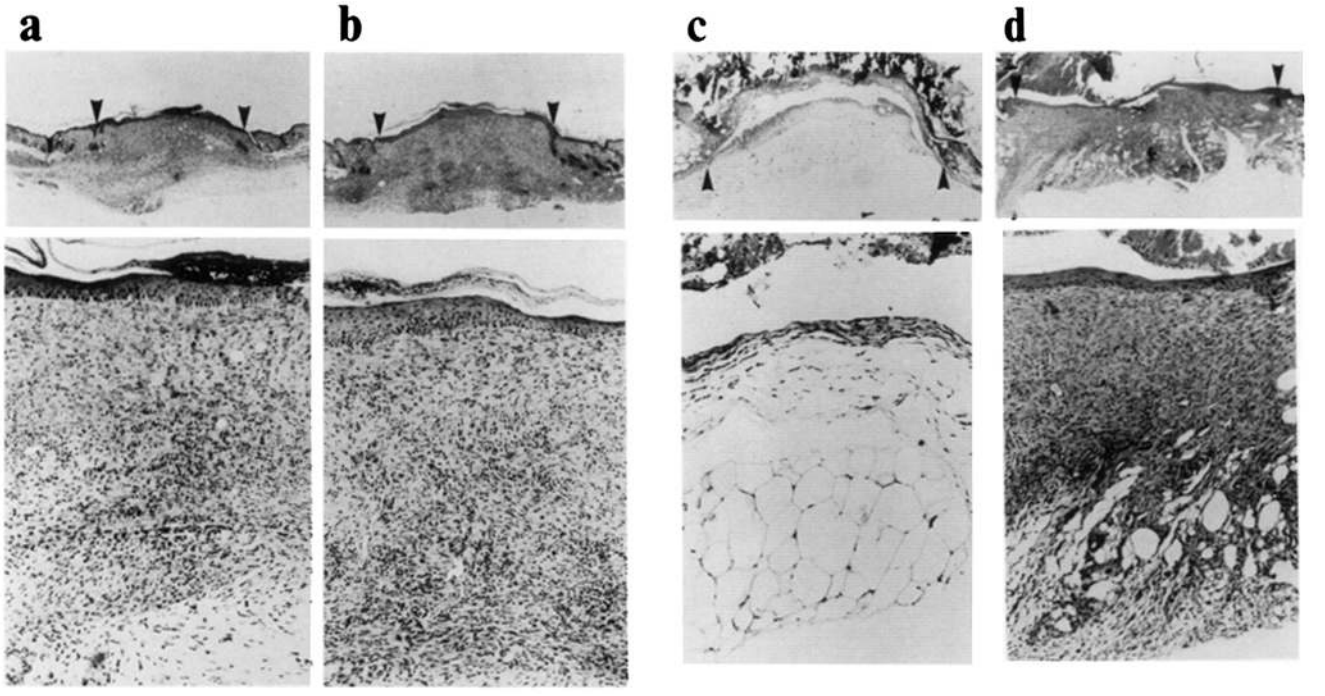

Figure 1. Photomicrographs of wound specimens from *db/db* and *db/+* littermates. Sections were prepared and stained as described in Materials and Methods. *Top panels*, ×30; *bottom panels*, ×140. Each photomicrograph was taken from a section prepared for the experiments presented in Table 1. Arrowheads indicate the original wound margins. (*a*) Section from a wound from a nontreated normal mouse (*db/+*) at 8 d after wounding. (*b*) Section from a wound from a normal mouse (*db/+*) at 8 d after the treatment with 5 μg of bFGF per day for 5 d. (*c*) Section from a wound from a nontreated *db/db* mouse at 8 d after wounding. (*d*) Section from wound from a *db/db* mouse at 8 d after treatment with 5 μg of bFGF per day for 5 d. The scoring for the healing parameters for these four samples with respect to reepithelialization, granulation tissue thickness, matrix density, infiltrated cells, and capillary numbers were, respectively: (*a*) 10, 3, 4, 3, and 6; (*b*) 10, 2, 4, 4, and 11; (*c*) 4, 1, 1, 1, and 2; (*d*) 10, 3, 4, 3, and 12.

observed between the control and the treated wounds in *db/+* mice.

When wound healing in the *db/+* mice was compared with that in the homozygous *db/db* littermates, an impairment in dermal wound healing was obvious in the *db/db* mice (Table 1 and Fig. 1 *c*). At 8 d after wounding there was a slight decrease in the degree of reepithelialization, and significantly less granulation tissue formation, cellular infiltration, and neovascularization under normal conditions of wound repair when the *db/db* mice were compared with *db/+* mice (Table 1). The decrease of the healing response is quite evident by the absence of granulation tissue at this time (Fig. 1 *c*). Instead of an influx of fibroblasts, macrophages, and blood vessels, the dermal layer is occupied by adipose tissue. The incompletely reepithelialized epidermis is covered by a thick crust. The wider wound border seen in the *db/db* animals (Fig. 1 *c*) when compared with those in the *db/+* animals (Fig. 1 *a*) probably results from the distension of the skin in the obese *db/db* animals.

When bFGF (5 μg) was applied to wounds in *db/db* mice, significant responses were observed in granulation tissue thickness ($p < 0.001$), matrix density ($p < 0.05$), infiltrated cells ($p < 0.001$), and capillary numbers ($p < 0.001$). The increase in granulation tissue was obvious when the excised treated and nontreated wounds were observed from the dermal side before sectioning. There was a slight increase in the degree of reepithelialization (Table 1), but this was probably not statistically significant (see below). A typical cross-section of a bFGF-treated wound in *db/db* mice (Fig. 1 *d*) illustrates a significant increase of granulation tissue when compared with the untreated *db/db* wound (Fig. 1 *c*). The dermis contains large numbers of fibroblasts and macrophages, and numerous capillaries are evident. Thus, with the application of bFGF to wounds in *db/db* mice, the degree of wound healing approximated that observed in the control heterozygous (*db/+*) mice.

The dose of bFGF required to stimulate healing in *db/db* mice was next established. Doses of 0.05, 0.5, and 5 μg of bFGF were applied to the wounds once a day for 5 d. The wounds from *db/db* mice that received no bFGF again demonstrated impaired wound repair with thin granulation tissue, few infiltrated cells, and decreased capillary numbers. With the application of 0.05 μg/d of bFGF, there was no statistically valid effect on these parameters when compared with the nontreated mice. However, with the application of 0.5 or 5 μg/d of bFGF, strong increases in all of the dermal parameters were observed. The number of capillaries was significantly higher than that found in nontreated mice ($p < 0.001$ at 0.5 μg/d and $p < 0.01$ at 5 μg/d). Thus, the effective dose of bFGF required for a significant increase in wound healing is between 0.05 and 0.5 μg/d when applied multiple times. These treatments had little or no effect on the degree of reepithelialization (Table 2). It is not clear why the average control values in this experiment differed from those observed in the experiment presented in Table 1. However, although the absolute values were different, the effect of the bFGF treatment was qualitatively equivalent between experiments. Wounds in heterozygous *db/+* littermates did not show a response to these concentrations of bFGF in the parameters measured (data not shown) presumably because the normal healing could not be accelerated.

Since Davidson et al. (7) reported that iodinated cartilage-derived growth factor, which appears to be a form of bFGF, disappeared from wounds within 24 h after injection, we next tested whether there were significant differences in healing between single vs. multiple applications of growth factor. As shown in Table 3, single dosing at 0.5 as well as 5 μg gave responses essentially equivalent to multiple doses at 5 μg with the exception of the increase in matrix density.

To insure that measured effects actually resulted from the bFGF in the preparations, two additional experiments were conducted. Boiled bFGF (5 μg/d) failed to induce statistically significant increases in the numbers of infiltrated cells or capillaries (Table 3). Furthermore, pretreatment of the bFGF with anti-bFGF IgG blocked the stimulation of wound healing, but treatment with nonimmune IgG did not (Table 3). These data clearly showed that bFGF was responsible for the observed histological responses.

To measure the effects of bFGF on wound healing in *db/db* mice as a function of time, histological sections of treated

Table 2. *Dose Effect on Wound Healing in db/db Mice*

Treatment	*n*	Reepithelialization	Granulation tissue thickness	Matrix density	Infiltrated cells	Capillary number
0 μg × 5 d	10	5.0 ± 0.6	1.6 ± 0.2	1.8 ± 0.2	1.8 ± 0.2	4.2 ± 1.0
0.05 μg × 5 d	10	5.0 ± 0.7	1.7 ± 0.1	1.9 ± 0.2	2.6 ± 0.3	6.8 ± 1.2
0.5 μg × 5 d	5	5.6 ± 1.1	2.8 ± 0.2*	2.2 ± 0.3	3.0 ± 0*	12.8 ± 1.4‡
5 μg × 5 d	8	4.3 ± 0.9	3.0 ± 0.3*	3.6 ± 0.2‡	3.8 ± 0.2‡	13.8 ± 2.9*

Samples were taken at 8 d after wounding. Solutions were applied once a day each day for 5 d beginning with the day of wounding. μg, the amount of bFGF applied. Scoring of each parameter was graded by the method described in the text. *n*, number of wounds analyzed. Asterisks indicate that the values are significantly different from that of control (0 μg × 5 d).

* $p < 0.01$.

‡ $p < 0.001$.

Table 3. *Effects of bFGF on Wound Healing in db/db Mice*

Treatment	*n*	Reepithelialization	Granulation tissue thickness	Matrix density	Infiltrated cells	Capillary number
Vehicle alone × 5 d	9	6.1 ± 0.9	1.9 ± 0.1	2.1 ± 0.3	1.8 ± 0.1	5.2 ± 0.6
Vehicle alone × 1 d	8	7.5 ± 0.8	1.6 ± 0.2	1.9 ± 0.2	1.9 ± 0.3	7.5 ± 1.5
0.5 μg × 1 d	10	6.4 ± 0.7	2.5 ± 0.2	2.5 ± 0.2	2.5 ± 0.2	12.1 ± 1.1
5 μg × 1 d	8	8.9 ± 0.5	2.8 ± 0.2	2.8 ± 0.2	2.9 ± 0.1	13.5 ± 1.3
5 μg × 5 d	10	5.3 ± 0.7	2.5 ± 0.2	2.2 ± 0.2	2.6 ± 0.2	12.9 ± 1.8
5 μg boiled × 5 d	9	6.2 ± 0.6	1.6 ± 0.2	2.0 ± 0.2	2.0 ± 0.2	7.1 ± 0.9
(0.5 μg + anti-bFGF IgG) × 1 d	7	7.0 ± 1.0	2.1 ± 0.1	2.0 ± 0	2.1 ± 0.1	7.9 ± 1.6
(0.5 μg + non-immune IgG) × 1 d	7	6.7 ± 0.9	2.9 ± 0.1	2.9 ± 0.2	2.9 ± 0.2	14.3 ± 2.1

Samples were taken at 8 d after wounding. Scores of each parameter were established by the method described in the text. μg, the amount of bFGF applied. *n*, number of wounds analyzed.

and nontreated wounds were prepared at 5, 8, 12, and 18 d after wounding (Table 4). All of the granulation parameters measured in bFGF-treated wounds exceeded those in nontreated mice at all days ($p < 0.1$ to $p < 0.001$). In general, the responses in the treated wounds occurred earlier and were more extensive than those observed in the nontreated wounds. However, significant differences were not seen with respect to effects on wound closure. All of the granulation parameters in bFGF-treated mice increased for the first 12 d. Interestingly, between 12 and 18 d, the granulation response appeared to begin to resolve as measured by decreases in the thickness of the granulation tissue, the number of infiltrated cells, and the number of capillaries. Meanwhile, matrix density continued to increase, suggesting that matrix maturation and remodeling were occurring.

We next attempted to measure breaking strength of bFGF-treated and control incisional wounds to determine whether increased matrix formation strengthened the healing wound. Fig. 2 illustrates the results of breaking strength measurements in *db/db* and *db/+* wounds. Wounds from mutant *db/db* mice broke at 56% of the breaking strength of wounds from normal heterozygous littermates. Application of a single dose of bFGF increased the breaking strength in wounds from *db/+* mice by 24% and that in *db/db* mice by 46% ($p < 0.05$). As a result of bFGF application, breaking strength in bFGF-treated wounds from *db/db* mice approximated that in healed wounds

Table 4. *Time Course of Wound Healing in db/db Mice With and Without bFGF*

Treatment	*n*	Reepithelialization	Granulation tissue thickness	Matrix density	Infiltrated cells	Capillary number
0 μg × 5 d	10	1.7 ± 0.2	1.3 ± 0.1	1.0 ± 0	1.3 ± 0.1	1.8 ± 0.6
8 d	9	3.6 ± 0.6	1.2 ± 0.1	1.1 ± 0.1	1.4 ± 0.2	3.4 ± 1.3
12 d	9	7.7 ± 0.6	2.0 ± 0.2	2.3 ± 0.2	2.1 ± 0.2	5.8 ± 0.8
18 d	11	10.0 ± 0	1.7 ± 0.2	3.0 ± 0.2	2.0 ± 0.2	7.0 ± 0.9
5 μg × 5 d	10	2.0 ± 0.2	1.3 ± 0.1	1.0 ± 0	1.4 ± 0.2	3.4 ± 1.1
8 d	8	3.8 ± 0.7	2.6 ± 0.2*	2.1 ± 0.2*	2.6 ± 0.2*	11.9 ± 2.7‡
12 d	10	7.9 ± 0.9	2.9 ± 0.3§	3.2 ± 0.2‡	3.2 ± 0.2‡	16.8 ± 1.9*
18 d	10	10.0 ± 0	2.3 ± 0.1‖	3.6 ± 0.2§	2.5 ± 0.2¶	10.4 ± 0.9‖

Samples were taken at designated days. Scores of each parameter were graded by the method described in the text. Statistical evaluation was done between treated and nontreated mice on the same days. Superscripts indicate that the values are significantly different. *n*, number of wounds analyzed.

* $p < 0.001$.
‡ $p < 0.01$.
§ $p < 0.05$.
‖ $p < 0.02$.
¶ $p < 0.1$.

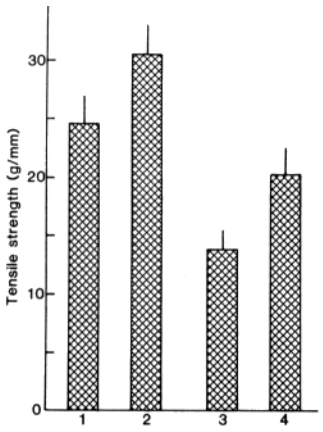

Figure 2. Tensile strength measurements. Wounds were prepared as described in the text. The breaking strength was measured and expressed as g/mm incisional width. (*1*) Wounds from nontreated *db/+* mice, (*2*) wounds from *db/+* mice treated with 5 μg of bFGF, (*3*) wounds from nontreated *db/db* mice, (*4*) wounds from *db/db* mice treated with 5 μg of bFGF. Asterisks indicate the statistical evaluation between treated and nontreated mice. $p < 0.05$. Bars: SEM.

of nontreated *db/+* littermates. Histological evaluation of wound tissues taken from the same mice showed results similar to those seen earlier in Table 1 and Fig. 1 (data not shown). These data suggest that the increased granulation tissue observed by histological evaluation contributes to an increase of the strength of the repaired wound.

Discussion

These experiments provide histological evidence that recombinant bFGF is capable of significantly improving the degree of dermal healing in *db/db* mice. The observed increases in granulation tissue thickness, infiltrated cells, capillary number, and tensile strength are consistent with the proposal that bFGF is an inducer of granulation. Besides bFGF, other polypeptide growth factors, such as platelet-derived growth factor (PDGF), TGF-α, epidermal growth factor (EGF), and TGF-β have been shown to increase various parameters of wound healing (3, 8, 9, 11–14, 19–21).

Unlike EGF, bFGF appeared to have little effect on the degree of reepithelialization. Since keratinocytes respond to bFGF (22, 23), this result was somewhat surprising. Perhaps, the presence of the large scab, which formed because we used an open wound system, affected the migration of keratinocytes. Therefore, it will be of interest to test the effect of bFGF on wounds with occlusive dressings.

It is also interesting that only a small effect of bFGF on the rate or degree of healing in normal mice was observed, although there are several reports that demonstrate positive effects of bFGF on wounds in normal rats using different assays (4–7, 10). It is possible that normal mice have sufficient amounts of growth factors to achieve a maximal rate of healing, and therefore, only minor increases in wound repair are possible upon the application of exogenous growth factors. The histological measurements that we used may not have been sensitive enough to detect these small effects.

Mutant diabetic mice (*db/db*) have elevated blood sugar levels, increased or normal insulin concentrations, and suppressed cell-mediated immunity (24). They are also obese with distended, thin skin. Thus, the reason for their impaired rate of wound healing may be complicated and multifactorial. It is known that glucocorticoid-treated rats have delayed wound healing and that TGF-β reverses this effect (13). Rats with chemically induced diabetes have a decreased rate of collagen deposition which can be restored to a normal level by the application of PDGF (8). It is also likely that the presence of macrophages has a strong effect on the formation of wound granulation tissue (25), and that macrophage accumulation is impaired in *db/db* mice. The mechanism responsible for impaired wound healing in *db/db* mice is unknown at this time and remains to be elucidated.

The effective dose of bFGF required for a significant increase in wound healing was established to be 0.5 μg/d using either single or multiple applications. The reason why a single dose was as effective as multiple doses is not clear but may relate to the recruitment of macrophages and fibroblasts by the bFGF. These migratory cells may be responsible for subsequent events at wound sites in the absence of applied bFGF. It is also interesting, and perhaps important, that multiple doses of high concentrations of bGFG did not induce unlimited granulation tissue formation, and after reepithelialization a decrease in the amount of granulation tissue and matrix occurred. This suggests that bFGF can be used as a self-limited, potent wound healing–potentiating agent.

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