Somatic gene therapy is a potentially useful strategy for the delivery of growth factors or cytokines to enhance wound healing. Experimental excisional and incisional wounds in impaired-healing diabetic mice (db/db) were treated with aFGF and with a plasmid coding for aFGF. A eukaryotic expression plasmid composed of the Hst signal peptide sequence in-frame with the human aFGF sequence was used. Transfection of tissues was accomplished either by direct plasmid uptake or by uptake facilitated with cationic liposomes. The results show that the closure of excisional wounds was significantly accelerated (p < 0.05) by topical application of human recombinant aFGF or by transfection with the aFGF plasmid but not by vehicle or control plasmid not containing the aFGF sequence. In incisional wounds, aFGF or transfection with the plasmid significantly increased the wound-breaking strength compared to their corresponding controls (p < 0.05). Quantitative histology of the plasmid-treated incisional wound sections revealed improved wound quality. The transcription of mRNA from human aFGF cDNA in the incisional wound tissue extracts was confirmed by RT-PCR, and the expressed aFGF was detected by immune dot blot and immunohistochemistry assays. The transfection was a transient process with a peak at 9 d in db/+ (littermates of the diabetic mice) incisional wounds, at 36 d in db/db incisional wounds, and at 27 d in db/db excisional wounds. Cells transfected with human aFGF occupied up to 6.4% of the transactional area in the wound sites. Thus, aFGF gene delivery resulted in both gene expression and a functional improvement in healing. Key words: gene therapy/diabetic mice/cytokine expression. J Invest Dermatol 108:313–318, 1997

Acidic fibroblast growth factor (aFGF or FGF-1) is one of the most promising cytokines for treating impaired wound healing. aFGF is a short polypeptide that influences the general proliferative capacity of a wide range of mesoderm- and neuroectoderm-derived cells in vitro and is also a potent angiogenesis inducer in vivo (Imamura et al., 1990; Mason, 1994). In rats and mice, exogenously applied aFGF promoted healing of full-thickness dermal wounds and produced a transient increase of tensile strength in incisional wounds (Mellin et al., 1992, 1995). Applications of aFGF have been limited, however, because of the necessity to administer relatively large amounts of recombinant aFGF that is often blocked from reaching the target tissue by eschar. The short half-life of aFGF is another drawback of topical administration. Skin wounds represent an attractive target for therapeutic gene manipulation because wounds are readily accessible. Major skin-cell types (such as fibroblasts, keratinocytes, and endothelial cells) are usually easy to infect with currently used vectors (Krueger et al., 1994). Skin cells are highly responsive to the aFGF stimulus, and the results are easy to observe (Krueger et al., 1994). To establish a practical somatic gene therapy for wound healing, this study was designed (i) to use recombinant human aFGF to demonstrate its therapeutic effect on wounds in genetically healing-impaired diabetic mice, (ii) to apply a plasmid containing an insert of human aFGF cDNA to the wounds and to confirm the in vivo transcription and translation of the aFGF cDNA, and (iii) to determine whether the expressed aFGF can result in a local effect with potential therapeutic significance.

**MATERIALS AND METHODS**

**Construction of the Expression Vector for aFGF** The plasmid pMEXneo-sp-aFGF, which is composed of the cDNA for human signal peptide and human aFGF, was a gift from X. Zhan and T. Maciag, American Red Cross, Rockville, MD (Foroughi et al., 1993). Briefly, an Hst signal peptide (sp) sequence was joined in-frame at the 5' end of the aFGF cDNA to facilitate the secretion of the peptide, and the full-length cDNA of the sp-aFGF was constructed between the restriction sites of SalI and EcoRI in the plasmid pMEXneo. A Kozak sequence with the initial codon within it preceded the 5' region of the sp-aFGF cDNA for maximal eukaryotic translation efficiency. A sequence of murine sarcoma virus long terminal repeat and a simian virus 40 polyadenylation site were, respectively, located up- and downstream of the insert (Fig 1). The pMEXneo plasmid without

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*Transfection with aFGF cDNA Improves Wound Healing* 
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Manuscript received February 6, 1996; revised September 28, 1996; accepted for publication November 5, 1996.

A preliminary report of this work has been presented at the Fifth Annual Meeting of the Wound Healing Society, Minneapolis, MN, April 27–30, 1995.

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Abbreviations: aFGF, acidic fibroblast growth factor; RT-PCR, reverse transcription-coupled polymerase chain reaction.
the insert was prepared by double digestion with SalI/EcoRI, gel purification of the large fragment, then blunting, and ligation of the ends of the linear plasmids with mung bean nuclease and T4 ligase.

aFGF and aFGF cDNA Delivery to Excisional Wounds The experiments reported herein were conducted according to the principles set forth in the Guide for the Care and Use of Laboratory Animals (1985). Genetically healing-impaired female diabetic mice (8-wk-old C57BL/Kj db/db, from The Jackson Laboratory, Bar Harbor, ME) that exhibit several characteristics of human adult-onset diabetes, including obesity and markedly delayed wound closure, and are widely accepted as an in vivo model to study wound healing (Greenhalgh et al, 1990; Brown et al, 1994; Sun et al, 1996) were used to evaluate the in vivo effect of aFGF and aFGF cDNA. A single full-thickness 6-mm diameter (0.28 cm²) excisional wound was made on the mid-dorsum with a biopsy punch (Acuderm Inc., Ft. Lauderdale, FL) and left undressed. In each experiment, groups of five animals were treated with either 1.0 μg of human recombinant aFGF (GIBCO BRL, Gaithersburg, MD) in 10 μL of phosphate-buffered saline (PBS) containing 0.3 μg of heparin or the PBS vehicle as control once daily for 15 consecutive days after wounding (two experiments). For gene therapy groups, 2.5 μg of pMXneo-sp-aFGF in 10 μL of a Lipofectamine/Dulbecco's modified Eagle's medium (1:1, vol/vol) mixture with 0.3 μg of heparin or 2.5 μg of pMXneo in 10 μL of Lipofectamine/Dulbecco's modified Eagle's medium (1:1) mixture (as a control) were topically applied to the wounds once daily for three consecutive days after wounding (five experiments). Scabs were gently removed on days 3, 5, 7, 9, 11, 13, and 15 after wounding to accurately visualize the wound margin and to facilitate access of the tested reagent to the wound. The wound area was recorded and analyzed at 1 h after wounding (day 0), on day 1 and alternate days thereafter by using an image analysis system (BioScan Inc., Edmonds, WA).

Application of aFGF and aFGF Gene to Incisional Wounds The diabetic animals were divided into ten groups of five mice, and a linear incision 2 cm long was made transversely on the shaved dorsum and closed by sutures. In the aFGF groups, each mouse in two groups of mice received 1.5 mg of human recombinant aFGF in 15 μL of PBS containing 0.3 μg of heparin once daily for 12 d (5 μL topical on incision margins and 10 μL injected subcutaneously at the base of the incision) and each mouse in another three groups received 130 μg of pMXneo (as control) once daily for 12 d (three experiments). All groups treated with aFGF or PBS, one group treated with pMXneo-sp-aFGF, and one group treated with pMXneo placebo were sacrificed after wounding. The remaining groups were sacrificed on day 27 after wounding. The wound strip was excised from the dorsal skin and cut into an 8-mm-wide strip by using a template. Breaking strength was measured immediately after excision by using a custom-made tensiometer. Tension was applied at a constant rate of 1 cm/min by using a 1.0-kg force transducer. Breaking strength, the point of maximal stress before wound separation, was recorded and analyzed on a computer supplied with custom-made software.

To prevent disturbance of wounds by licking, all animals were individually housed after wounding.

Reverse Transcription–Coupled Polymerase Chain Reaction (RT-PCR) Assay of the aFGF Gene Transcription To examine the transcription of sp-aFGF cDNA in the db/db incision specimens harvested 27 d after wounding, total RNA was extracted from the tissue and digested with RNase-free DNase (GIBCO BRL, Gaithersburg, MD). Then, RT-PCR was performed according to the protocols provided by manufacturer (Invitrogen, San Diego, CA). Two primer sets were designed (Fig 1): primer set 1 for amplifying the full-length human aFGF sequence (sense sequence, 5'-ATGGCTGAAGGGGAAATCACCACCTTACGCCACT-3'; antisense sequence, 5'-TTAATCAGAAGACTGGCAGGGGGAGGAAACAGAG-3') and primer set 2 for the spliced full-length sequence of the signal peptide and human aFGF (sense sequence, 5'-GGGAGACGTCTTAGTGCCGGGGGCCCCAGAGG-3'; antisense sequence, 5'-GTCGAGCTTAAAGTTAATCAAGAAGACTCGGAGGG-3').

Dot Blot Assay of the Expression Product of the aFGF Gene The skin margin along the incisional wound treated with pMXneo-sp-aFGF was harvested, trimmed to about 3 mm in width, chopped into small pieces in a dry ice box, homogenized at 4°C in NaCl/Tris(hydroxymethyl)aminomethane (Tris) buffer (pH 7.6) in the presence of 100 μg of phenylenediamine fluoride per ml and 2 μg of aprotinin per ml (Sigma, St. Louis, MO), and centrifuged at 10,000 × g for 20 min. The supernatants were centrifuged with Centricon 100 (Amicon, Danvers, MA) at 1000 X g to remove molecules larger than 100 kDa and then concentrated with a Centricon 3. The skin containing the incision treated with pMXneo only and a piece of normal skin from each animal were harvested, processed identically, and used as controls 1 and 2, respectively. Immune dot blot was performed with 3 or 6 μl of the concentrated filtrate with an equivalent amount of protein (1 μg/ml) loaded on nitrocellulose filters (Millipore, Bedford, MA). The blots were probed with polyclonal antibodies (R & D Systems, Minneapolis, MN) against bovine aFGF and developed according to the manufacturer's instructions (Vector Laboratories, Burlingame, CA).

Immunohistochemical Assay of the Gene Transfection Efficiency and Morphometric Assay of Wound Healing To determine the time course of gene transfection efficiency, the diabetic mice and their lean littermates (C57BL/KJ db/+ , The Jackson Laboratory, Bar Harbor, ME) were used. A full-thickness excisional or incisional wound was made and treated with pMXneo-sp-aFGF or pMXneo as described above. The wound was harvested at the designated intervals, fixed in 10% neutral-buffered formalin for 24 h, embedded in paraffin, and sectioned at 5 μm thickness. After being deparaffinized and hydrated in water, the sections were digested with 0.1% trypsin for 1 h, blocked with goat nonimmune serum for 20 min, probed with the primary antibodies, and stained with Fast Red by following the procedures of the manufacturer (Sigma, St. Louis, MO). The tissue sections were observed with a Nikon Optiphot microscope with a color video camera connected to an image analysis system. The in vivo transfection efficiency of the aFGF gene was defined in the following way: under the X40 objective lens, the region of cell transfection adjacent to the wound was located and the anti-aFGF stained areas were measured in sample areas by setting the threshold range of the red staining color. The transfected areas were then expressed as percentages of the sampled areas. The value of the transfection efficiency is the mean of measurements from two fields per section of two incisions per each group from three or four mice in each group. An image analysis system was used to evaluate transcriptional scar area, cellular density, hair follicle number, and dermal thickness as measures of the quality of wound healing.

Statistical Analysis Data are presented as means ± SD except where indicated. Statistical analysis of the data were performed by using the one-way analysis of variance test with significance at 0.05 level.

RESULTS

Acceleration of Healing by aFGF or aFGF Gene The exogenous application of aFGF improved healing of full-thickness skin...
Effects of aFGF on observable expression as a control or harvested
1.4 2.5

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12 16.5

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3.4 10
79

or PBS C0l1tro1).

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3.5 10
103 ± 6

of human

±

3.0 10
56 ± 9

±

8.8 10.8±2.9 10
42

±

0.8 25

f-Lg

hy

of aFGF and aFGF cDNA on Wound Areas

Table I. Time Course of Effects of aFGF and aFGF cDNA on Wound Areas

<table>
<thead>
<tr>
<th>Day after Wounding</th>
<th>aFGF (% control)</th>
<th>PBS Control (mm²)</th>
<th>n</th>
<th>aFGF cDNA (% control)</th>
<th>Control cDNA (mm²)</th>
<th>n</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>101 ± 4</td>
<td>36.4 ± 3.2</td>
<td>10</td>
<td>100 ± 2</td>
<td>30.5 ± 1.2</td>
<td>25</td>
</tr>
<tr>
<td>3</td>
<td>97 ± 6</td>
<td>36.5 ± 3.5</td>
<td>10</td>
<td>100 ± 3</td>
<td>32.6 ± 1.2</td>
<td>25</td>
</tr>
<tr>
<td>5</td>
<td>78 ± 13</td>
<td>37.2 ± 2.6</td>
<td>10</td>
<td>94 ± 3</td>
<td>32.3 ± 1.2</td>
<td>25</td>
</tr>
<tr>
<td>7</td>
<td>78 ± 12</td>
<td>28.6 ± 3.5</td>
<td>10</td>
<td>103 ± 6</td>
<td>24.3 ± 1.4</td>
<td>25</td>
</tr>
<tr>
<td>9</td>
<td>61 ± 12</td>
<td>22.9 ± 3.4</td>
<td>10</td>
<td>106 ± 12</td>
<td>16.1 ± 1.1</td>
<td>25</td>
</tr>
<tr>
<td>11</td>
<td>62 ± 12*</td>
<td>16.5 ± 3.4</td>
<td>10</td>
<td>79 ± 11</td>
<td>10.7 ± 0.8</td>
<td>25</td>
</tr>
<tr>
<td>13</td>
<td>48 ± 11*</td>
<td>15.0 ± 3.0</td>
<td>10</td>
<td>56 ± 9*</td>
<td>8.8 ± 1.0</td>
<td>25</td>
</tr>
<tr>
<td>15</td>
<td>28 ± 8*</td>
<td>10.8 ± 2.9</td>
<td>10</td>
<td>42 ± 7*</td>
<td>5.3 ± 0.8</td>
<td>25</td>
</tr>
</tbody>
</table>

* Wound areas were measured in db/db mouse excision wounds treated with 1.0 µg of human recombinant aFGF (aFGF), PBS (control of aFGF group), 2.5 µg of pMEXneo-sp-aFGF (aFGF cDNA), or 2.5 µg of pMEXneo (control cDNA, control of aFGF cDNA group).

Means ± SEM of the wound areas were expressed as a percentage of the area of control wounds.

Number of mice in each group (human recombinant aFGF or PBS control).

Number of mice in each group (pMEXneo-sp-aFGF or pMEXneo).

Each control or experimental group consisted of five animals per experiment.

p < 0.05, compared with the value at day 1 by the one-way analysis of variance test.

Figure 2. Improvement of breaking strength of incisional wounds treated with aFGF and pMEXneo-sp-aFGF. Open bar, treated with PBS as control of aFGF group; densely hatched bar, treated with 1.5 µg of human recombinant aFGF in PBS daily for 12 d; hatched bars, treated with 130 µg of pMEXneo without aFGF insert (control of pMEXneo-sp-aFGF group); cross-hatched bars, treated with 130 µg of pMEXneo-sp-aFGF daily for 12 d.

* Statistically significant (p < 0.05).

controls (Fig 3B,D). Image analysis of the tissue sections revealed that the transfected wounds had a significantly smaller mean transectional scar area, higher cellular density, more hair follicles in the wound sites, and a tendency toward a thinner dermal layer and a more nearly normal complement of fat cells (Table II, Fig 3).

Translation of sp-aFGF cDNA In Vivo The transcription of human aFGF in the incisional wound tissue extracts was demonstrated by RT-PCR, using two primer sets. The human aFGF mRNA and the sp-aFGF chimeric mRNA were detected in vivo (Fig 4, lanes 4, 5) but only in transfected wounds. Neither the Hst signal peptide nor human aFGF should exist in a mouse. We considered the possibility that the pMEXneo-sp-aFGF plasmid injected into the wounds might be carried over to the tissue extracts and amplified in the RT-PCR, resulting in a false positive, even though there was a 15-d period between termination of the injection of pMEXneo-sp-aFGF DNA and harvesting of the tissue for the RT-PCR. When PCR was performed in the absence of reverse transcriptase, however, there was no detectable amplification of sequences in the tissue extract from pMEXneo-sp-aFGF-treated wounds (Fig 4, lane 3), indicating that there was no residual sp-aFGF DNA contamination in the extracts of pMEXneo-sp-aFGF-treated wound tissue.

Table II. aFGF cDNA Improves Wound Healing and Wound Quality

<table>
<thead>
<tr>
<th>Group</th>
<th>Scar area (mm²)</th>
<th>Cellular Density (cells/1000 µm²)</th>
<th>Dermis Thickness (µm)</th>
<th>Hair Follicles (follicles/1000 µm²)</th>
</tr>
</thead>
<tbody>
<tr>
<td>pMEXneo-sp-aFGF</td>
<td>0.22 ± 0.09a</td>
<td>5.3 ± 0.7b</td>
<td>280 ± 150</td>
<td>20 ± 10b</td>
</tr>
<tr>
<td>pMEXneo</td>
<td>0.75 ± 0.39</td>
<td>3.1 ± 0.7</td>
<td>430 ± 130</td>
<td>6 ± 7</td>
</tr>
</tbody>
</table>

* Tissue sections were from incisional wounds of db/db mice harvested at day 27 after the wound and quantified by computer-assisted image analysis as described in Materials and Methods. The value is the mean ± SD of two sections per animal of five mice in the experimental or control groups.

p < 0.05, compared to corresponding control.
increased transfection to a peak that depended on the strain of mouse (Table III). The incision wounds of diabetic mice achieved the highest mean transfection percentage, 6.4% of the sampled area at 36 d.

DISCUSSION

aFGF cDNA Mimicked the Effect of aFGF Peptide on Wound Healing Delivery of the pMEXneo-sp-aFGF plasmid to wounds led to the transcription and translation of the human aFGF gene and, most importantly, promoted excisional and incisional wound healing by the criteria of wound area, wound strength, and histology similar to the effect of recombinant human aFGF on healing. The improvement in scar area, cellular density, and hair follicle numbers suggests a relationship between acceleration of wound remodeling and improvement of the quality of wound healing in pMEXneo-sp-aFGF-treated wounds. This study and the reports of Mellin and colleagues (Mellin et al., 1992, 1995) show that recombinant aFGF improves skin healing in mice or rats. The effect in both rats and mice may result from the strong mitogenic effect of aFGF in vivo (Dabin and Courtois, 1991; Fitzpatrick et al., 1992; Matuszewska et al., 1994) and in vitro (Chen et al., 1991; Klein-Soyer et al., 1992; Tsuboi et al., 1992). Basic FGF, which has

Figure 4. Transcription of aFGF cDNA in vivo. Lane 1, normal mouse skin extracts amplified with primer set 1 (directed to the aFGF sequence); lane 2, extracts of wounds treated with the control pMEXneo and amplified with primer set 1; lane 3, tissue extract of the wounds treated with pMEXneo-sp-aFGF and amplified with primer set 1 but without reverse transcriptase; lane 4, tissue extract of the wounds treated with pMEXneo-sp-aFGF and amplified with primer set 2 (directed to the sp-aFGF fusion sequence); lane 5, tissue extract of the wounds treated with pMEXneo-sp-aFGF and amplified with primer set 1; lane 6, DNA size ladder.

Figure 5. Translation of aFGF cDNA in vivo. Immunoassay of wound tissue extracts. Rows A–C, tissue extract of wounds treated with pMEXneo-sp-aFGF, pMEXneo, and normal tissue, respectively. Column 1, 3 μg of total protein extract; column 2, 6 μg of protein.
60% sequence homology with aFGF and shares the capacity to stimulate proliferation in endothelial cells and fibroblasts, improved healing in diabetic mice (Greenhalgh et al, 1990; Tsuboi and Rifkin, 1990). The healing process in impaired-healing diabetic mice is complex and responsive to many different cytokines (Greenhalgh et al, 1990; Roemar and Friedmann, 1992; Brown et al, 1994), perhaps acting in a concerted temporal and spatial network.

The natural structure of the aFGF gene translation product does not possess a classical secretory signal sequence (Burgess et al, 1986), and in recent years there has been considerable debate concerning its ability to be secreted at all. Current opinion, however, is moving away from the idea that this molecule is only released from the cell when the integrity of the plasma membrane is compromised, as evidence is accumulating to suggest that it is released from the cell by novel secretory mechanisms (Jackson et al, 1992; Mignatti et al, 1992; Mason, 1994). Because the ability of aFGF to be secreted cannot be simply predicted, we used, in this study, a plasmid with the insert of the full-length aFGF sequence and a signal peptide sequence joined in-frame at the 5' end of the aFGF fragment. The transformation of cells in vitro induced by pMEXneo-sp-aFGF in this study (data not shown) and in the work of Forough and colleagues (Forough et al, 1993) demonstrated that a secretable form of aFGF was expressed in cell culture, which provided biochemical evidence to explain the acceleration of wound closure and the increase in breaking strength in vivo.

It has been hypothesized that impaired wound healing may result from lack of adequate stimulation by growth factors. In fact, growth factors have been used in clinical trials to test whether they can improve wound closure in patients with chronic nonhealing wounds (Brown et al, 1991; Robson et al, 1992; Steed et al, 1992). Cytokine gene therapy may overcome some of the shortcomings of direct application of the growth factors. For example, gene therapy encourages a continual supply of growth factor that is well dispersed within the wound site. That steady synthesis declines as the wound matures. Also, the gene product would be glycosylated by host glycosylases and dispersed deep into the wound tissue, rather than superficially. Particle-mediated transfection of human epidermal growth factor into porcine skin wound cells led to epidermal growth factor transcription and translation in the wound and resulted in a significant reduction in leaching of protein into wound fluid, a measure of epithelialization (Andree et al, 1994). Cytokine gene delivery into living animals has been shown to produce specific systemic immunologic effects (Raz et al, 1993; Wang et al, 1993), to induce antitumoral activity (Colombo et al, 1991), and to modify neuronal physiology (Federoff et al, 1992). Gene expression in muscle fiber after injection of plasmid cDNA has been demonstrated in experiments using reporter gene constructs (Wolf et al, 1990; Barr and Leiden, 1991; Danko et al, 1994). Thus, transfection with genes tailored to a specific healing deficit may permit improvement of healing impaired by that deficiency, such as insufficient vascularization or inadequate keratinocyte proliferation.

### Technical Aspects of Gene Therapy for Wound Healing

Based on the characteristics of impaired-healing or nonhealing wounds, the strategy for application of somatic gene therapy to wound healing may differ from that currently used for therapy of cancer or inborn errors of metabolism (Anderson, 1992; Roemer and Friedmann, 1992; Greenhalgh et al, 1994; Krueger et al, 1994).

Plasmids as a vector have substantial advantages for gene delivery for wound healing. The plasmids are easy to manipulate, can accept large inserted sequences, and can be produced stably and cheaply to a high level of purity. By comparison, the vectors derived from retroviruses or adenoviruses are not recommended in wound-healing management because of potential contamination of replication-competent wild-type virus in the stock of replication-defective recombinant virus, the integration of the transferred gene into the host genome, and a potential insertional mutagenesis that may trigger cell transformation or even neoplasia (Anderson, 1992; Gutierrez et al, 1992; Mulligan, 1993; Vile and Russell, 1994). In this study, the transfection efficiency obtained from the incisional wounds indicated that even without selection pressure or any agent to facilitate the transfection, plasmids injected directly to the wound tissue resulted in not only a cell transfection in vivo but also an effective biologic response.

Simplicity should also be considered as an important component.
of the strategy. For example, topical application or injection of the expression plasmid directing the synthesis of the desired cytokine will obviate the need to express, purify, and administer the recombinant cytokine protein. Likewise, simple injection of the plasmid is more easily adapted to clinical use than alternative methods that might rely on stability of a virus vector or of a eukaryotic cell graft. The results of this study showed that either topical application or injection are effective and practical as gene delivery methods.

In the ideal case, the expression of the applied gene should be limited to the time required for the wound to heal. This goal may be reached in two ways: by using plasmid or by using heterologous (or modified) genes. Once transfected into mammalian cells, plasmids will remain extrachromosomal as nonreplacating episomes. The plasmids will be passed to daughter cells at cell division, diluted after repeated mitoses of the transfected wound cells, and eventually lost. A weak immune response may eventually inactivate the xenogeneic gene expression and remove the cells expressing the gene product. The histology of the transfected wound specimens revealed that the highest level of expression of human aFGF was located in the normal tissue adjacent to the wounds rather than the rapidly growing wound tissue itself (see Fig 3). This observation suggests that the cells in the middle of the wounds proliferated after the pMEXneo-sp-aFGF sequence had been deleted, consistent with the decrease in the number of the transfected cells. In addition, the transfection process was transient, with a slow start and an eventual decline, regardless of the strain of mice, wound type, doses of the aFGF cDNA, and delivery method.

The prospect of genetic manipulation to effect a cure is controversial but may have advantages in specific situations such as chronic human wounds or inadequate patient compliance. The enhancement of healing in these animal models should encourage further investigation for eventual clinical application.

NOTE ADDED IN PROOF

A recent report found that transfection of murine wounds with pMEX-neo-sp-aFGF increased in vivo keratinocyte proliferation in the wound site as measured immunohistochemically after incorporation of bromodeoxyuridine (Xu L, Sun L, Harmon JW, and Nielsen TB: Transfection of murine cutaneous wounds with aFGF Plasmid. Surgical Forum XLVII:699–702, 1996). This observation suggests that a part of the mechanism of action of the plasmid may be stimulation of epithelialization of the wound.

We gratefully acknowledge Dr. X. Zhan and Drs. T. Maciag (Department of Molecular Biology, Jerome H. Holland Laboratory for the Biomedical Sciences, American Red Cross, Rochester, MN) for the generous gift of the pMEXneo-sp-aFGF vector and useful suggestions. The opinions and assertions contained herein are the private ones of the authors and are not to be construed as official or reflecting the views of the Navy Department or the Naval Service at large. This manuscript was prepared by United States Government employees and, therefore, cannot be copyrighted and may be copied without restriction. L.S. held a National Research Council Research Associateship during the tenure of this work. This project was funded by the Naval Medical Research and Development Command, Work Unit 61153N MRR04120.001-1422.

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