Peripheral neuropathy is a common, irreversible complication of diabetes. We investigated whether gene transfer of an engineered zinc finger protein transcription factor (ZFP-TF) designed to upregulate expression of the endogenous vascular endothelial growth factor (VEGF)-A gene could protect against experimental diabetic neuropathy. ZFP-TF–driven activation of the endogenous VEGF-A gene results in expression of all of the VEGF-A isoforms, a fact that may be of significance for recapitulation of the proper biological responses stimulated by this potent neuroprotective growth factor. We show here that this engineered ZFP-TF activates VEGF-A in appropriate cells in culture and that the secreted VEGF-A protein induced by the ZFP protects neuroblastoma cell lines from a serum starvation insult in vitro. Importantly, single and repeat intramuscular injections of formulated plasmid DNA encoding the VEGF-A–activating ZFP-TF resulted in protection of both sensory and motor nerve conduction velocities in a streptozotocin-induced rat model of diabetes. These data suggest that VEGF-A–activating ZFP-TFs may ultimately be of clinical utility in the treatment of this disease. Diabetes 55: 1847–1854, 2006

Peripheral neuropathy, characterized by a progressive loss of sensation in the extremities, occurs in ~50% of diabetic individuals after 25 years of disease (1,2). Despite the prevalence of this condition, beyond the careful management of the diabetes itself via glycemic control, no treatment for diabetic neuropathy exists.

In the present study, we examined whether gene transfer of an engineered zinc finger protein transcription factor (ZFP-TF) for the activation of the endogenous vascular endothelial growth factor (VEGF)-A gene could be used to prevent progression of neuropathy in streptozotocin (STZ)-induced diabetic rats. VEGF-A has shown particular promise in this regard. In vitro, the addition of the VEGF_{165}^A isoform alone has been shown to have both direct neuroprotective (3–5) as well as neuronal growth-promoting (6–8) effects. In rat and rabbit models of diabetic neuropathy, VEGF_{165}^A gene transfer conferred a complete reversal of the deficits in nerve conduction velocities (NCVs) characteristic of diabetes. Preliminary results from clinical studies have indicated improvements in the signs and symptoms of sensory neuropathy in diabetic patients after intramuscular injection of a plasmid DNA encoding VEGF_{165}^A (9). Collectively, these studies have provided support for the development of therapies for peripheral neuropathy based on VEGF_{165}^A as well as other VEGF gene family members, and human gene therapy clinical trials testing this concept have either been proposed or are underway (10).

Given the potential demonstrated by VEGF-A in experimental as well as clinical diabetic neuropathy, we chose to investigate an alternative strategy for the therapeutic application of this powerful growth factor, namely, the activation of the endogenous VEGF-A gene through the action of an engineered ZFP-TF. ZFP-TFs can be designed to control the expression of any desired gene (rev. in 11). We have previously reported on a ZFP-TF engineered to upregulate VEGF-A mRNA and protein expression in human cells in culture (12) as well as in animal models of disease (13,14). In all cases studied, this regulation results in the expression of all of the different VEGF-A isoforms (12,14), a fact that appears (at least in the context of angiogenesis) to provide for more complete and robust biological function (13–15). Here, we demonstrate that this VEGF-A–activating ZFP-TF is capable of stimulating VEGF-A expression that can protect neuronal-derived SK-N-MC cells from growth arrest in response to serum starvation in vitro. Moreover, in an experimental model of diabetic neuropathy, a single administration by intramuscular injection of a plasmid DNA encoding the VEGF-A–activating ZFP-TF showed significant and dose-related protection of both motor and sensory NCVs. Repeat administration of plasmid DNA encoding the VEGF-A–activating ZFP-TF resulted in sustained robust protection against STZ-induced measurements of diabetic neuropathy. These data suggest that a VEGF-A–activating ZFP-TF may ultimately be of clinical utility in the treatment of diabetic neuropathy.
VEGF-ZFP GENE THERAPY FOR DIABETIC NEUROPATHY

VZ+434, encodes the designed three-finger ZFP DNA-binding domain, the nuclear translocation signal from simian virus 40 large T antigen, and the transactivation domain from the p65 subunit of the human nuclear factor-KB, subcloned into pVAX-1 (Invitrogen, San Diego, CA) with expression driven by the human cytomegalovirus (CMV) promoter. Plasmids were formulated at a concentration of 2 mg/ml in 5% poloxamer 188 (BASF, Washington, New Jersey), 150 mM NaCl, and 2 mM Tris (pH 8.0).

Adenoviral vector constructs and production. Recombinant adenoviral vectors, AdEGFPp65-flag, and AdVZ+434-flag were created as follows: the Mlu I-Afl I fragment of the plasmid pCDA/T5 (Invitrogen), which consists of the human CMV immediate early promoter/enhancer and two tetracycline operator sequences (TetO), and the Afl I-Hind III fragment of the plasmid pCDA3-EGFPp65-flag, or pCDNA-VZ+434-flag, were ligated. To disrupt the ZFP expression cassette, were simultaneously cloned into the Mlu I and XhoI restriction sites upstream of a bovine growth hormone polyadenylation signal (BGH polyA) in the plasmid pShuttle (Clontech, Palo Alto, CA). The CMV-TetO-ZFP-BGH polyA cassette was then excised via the unique I-Ceu I and Pl-Sce I restriction sites in the pShuttle and were ligated to the Adeno-X viral packaging vector (Clontech). All cloned sequences were verified by DNA sequencing.

Recombinant adenoviral vectors were packaged by transfecting T-REx-293 cells (Invitrogen). Adenoviruses were harvested from transfected T-REx-293 cells lysed with three consecutive freeze-thaw cycles and were amplified in T-REx-293 cells before purification by double cesium chloride gradient centrifugation (Qbiogene). Purified recombinant adenoviruses were dialyzed against PBS, lyophilized, and passed once on 293T cells. Viral titers were determined by using the Adeno-X Rapid Titer Kit (Clontech). Diffrerentiated human skeletal myoblasts and normal rat kidney (NRK) cells were plated in 24-well cell culture plates and infected with recombinant adenoviruses at 100–250 pfu/cell for 24 h at 37°C. At 48 h postinfection, cells were collected for RNA and protein analyses.

Production and transduction of differentiated skeletal muscle cells. Human cultured skeletal myocytes (Clonetics) were maintained as recommended by the manufacturer and were differentiated via treatment with a low-serum medium for 5 days (16). This procedure results in growth cessation, fusion of many myocytes to form multinucleated fibers, and upregulation of muscle-specific genes such as creatine kinase (data not shown). After differentiation, myocytes were treated with adenovirus expressing either VZ+434-FLAG or EGFP-p65-FLAG or left untreated.

Retroviral constructs, virus preparation, and generation of stable lines. A self-inactivating retroviral vector containing a tetracycline-inducible ZFP expression cassette was constructed and used for virus generation. Briefly, the pSIR vector (Clontech) was modified to contain the CMV promoter. Plasmids were formulated at a concentration of 2 mg/ml in 5% poloxamer 188 (BASF, Washington, New Jersey), 150 mM NaCl, and 2 mM Tris (pH 8.0). Two mM Tris, 4% sucrose; aliquoted; and stored at 4°C for 24 h. Twelve hours later, conditioned media after a 24-h accumulation period (i.e., 24 h after fresh media was applied to the cells). Medium was immediately analyzed upon harvesting for 24 h. The conditioned medium was removed and replaced with serum-free medium, containing 0.1 M glucose. Twenty-four hours later, the conditioned serum-free medium was removed and fresh media containing 10% fetal bovine serum was added to the cell cultures. The concentration of VEGF-A in this medium was determined by an enzyme-linked immunosorbent assay (ELISA) kit with horseradish peroxidase conjugate detection, according to the manufacturer's recommended conditions.

Preparation of serum-free conditioned medium from HEK293 cells expressing VZ+434. VEGF-A–containing conditioned medium was produced from cultured HEK293 T-REx VZ+434 cells. Cells were plated in complete growth medium (DMEM, 10% fetal bovine serum, and 2 mM t-glutamine, in 15-cm dishes, at a density of 1.5 x 10⁶ cells per dish, resulting in 80–90% confluency 24 h after plating. The cells were induced by the addition of 5 mg/ml doxycycline. Induction was allowed to proceed for 24 h, and then the complete growth medium was removed and replaced with 10 ml of serum-free medium containing 0.1 M glucose. Twenty-four hours later, the conditioned serum-free medium was removed and fresh media containing 10% fetal bovine serum was added to the cell cultures. The concentration of VEGF-A in this medium was determined by an enzyme-linked immunosorbent assay, and the medium was stored at −20°C until ready to be used in neuroprotection experiments. Conditioned medium from noninduced cells (i.e., by omitting doxycycline from all media) served as a control.

Neuroprotection studies. SK-N-MC human neuroblastoma cells (American Type Culture Collection) were plated on a 96-well plate at a density of 4,000 cells per well. These cells were allowed to grow for 48 h before the growth medium was removed and replaced with serum-free medium, containing various proportions of the doxycycline-induced serum-free conditioned medium from the HEK293 T-REx VZ+434 cells. Media from HEK293 T-REx VZ+434 cells grown in the absence of doxycycline, complete medium, and serum-free medium alone served as controls. Duplicate samples were run for each set of growth conditions. The treated SK-N-MC cells were then cultured for a additional 72 h under these growth conditions, and cell number and metabolic activity were determined by using an Alamar Blue assay (described above).

Induction of diabetes and gene transfer. Diabetes was induced in male Wistar rats (Charles River, U.K.) with an intraperitoneal injection (55 mg/kg) of STZ (Sigma) given after an overnight fast. The drug was freshly dissolved in 0.1 N HCl and injected at a rate of 1 ml/100 g of body weight. Blood glucose was measured by a strip-operated reflectance photometer (MediSense Optimum; MediSense, Abingdon, Oxford, U.K.), and STZ-treated rats with blood glucose <15 mmol/l were removed from the study. Rats were group housed and were given free access to food and water in a 12-h light/dark cycle. Age- and weight-matched rats were used as nondiabetic control animals.

Quantitative RT-PCR analysis of VEGF-A mRNA expression. Total cellular RNA was isolated by using the RNeasy extraction kit (Qiagen) according to the manufacturer’s recommendations. RNA (25 ng) was used in RT-PCR analysis with TaqMan chemistry in a 96-well format on an ABI 7700 SDS machine (Applied Biosystems) as previously described (18). Reverse transcription was performed at 48°C for 30 min by using MultiScribe reverse transcriptase. After a 10-min denaturation at 95°C, PCR amplification with AmpliTaqGold DNA polymerase was conducted for 40 cycles at 95°C for 15 s and 60°C for 1 min. The primers and probes used were as previously described (12). Results (relative gene expression) are expressed as a ratio between different groups of the 18S expression. The results were analyzed with SDS Version 1.6.3 software.

Enzyme-linked immunosorbent assay analysis of VEGF-A protein expression. Secreted VEGF-A protein levels were determined in the culture media after a 24-h accumulation period (i.e., 24 h after fresh media was applied to the cells). Medium was either immediately analyzed upon harvesting or stored at −20°C for analysis at a later date. Aliquots (200 μl) of culture media were expressed, in duplicate, by using the R&D Systems sandwich enzyme-linked immunosorbent assay kit with horseradish peroxidase conjugate detection, according to the manufacturer’s recommended conditions.

Nerve conduction velocity measurements. Rats were anesthetized with isoflurane (2–4% in oxygen), and electromyograms were recorded from plantar foot muscles in response to stimulation at the sciatic notch and then at the Achilles’ tendon. Motor NVC (MNCV) was measured from latencies of the compound M waves, and sensory NVC (SNCV) was measured from the compound M waves. Results (relative gene expression) are expressed as a ratio between different groups of the 18S expression. The results were analyzed with SDS Version 1.6.3 software.

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determined to be genuine, as 1) they are obliterated by dorsal root section (data not shown) and 2) they appear at a lower stimulus voltage than M waves, a classical feature of H reflexes. Mid-thigh nerve temperature was maintained at 36 ± 0.5°C throughout the procedure. The latency difference between the two sets of M waves was calculated and related to the nerve length separating the two stimulus points (measured ex vivo) to calculate MNCV. H reflex latency differences were used similarly to calculate SNCV. NCVs were measured for both the left (injected) and right (not injected) sides.

**Statistical analyses.** The MNCV and SNCV are presented as group means ± SD. The critical testing was a comparison of left- and right-side NCVs, which was done by paired t tests; there were no multiple comparisons. For completeness, controls and pVAX-1–treated diabetic rats were compared by unpaired t tests.

**RESULTS**

**Engineered ZFP-TF VZ+434 induces endogenous VEGF-A gene expression in vitro.** The engineered ZFP-TF VZ+434 has been shown to drive activation of the endogenous VEGF-A gene in both cell culture and animal model settings (12–14). To extend these observations to an in vitro model of skeletal muscle, skeletal myocytes (C2C12) were differentiated by growth in low-serum medium for 8 days. After differentiation, the cells were infected with recombinant adenovirus vectors expressing either VZ+434-Flag (AdVZ+434-Flag) or EGFPp65-Flag (AdEGFPp65-Flag), or cells were left untreated as a control. VEGF-A protein levels determined by enzyme-linked immunosorbent assay and mRNA levels determined by TaqMan real-time PCR analysis are shown in Fig. 1B. Infection with AdVZ+434-Flag virus resulted in an ~6- and ~15-fold increase in VEGF-A mRNA and protein, respectively, relative to uninfected cells. Infection with AdEGFPp65-Flag did not result in increased levels of VEGF-A protein or mRNA, demonstrating that the increases in VEGF-A levels were a consequence of VZ+434 rather than adenoviral infection per se.

Since we planned to examine effects of induction of VEGF-A expression in a rat model of diabetes, we next sought to confirm that the VZ+434 ZFP-TF activator of VEGF-A would function robustly in cultured rat cells. As shown in Fig. 1A, the binding site of VZ+434 in the VEGF-A locus is conserved across multiple species, including human, mouse, and rat. NRK cells were infected with AdVZ+434-Flag or AdEGFPp65-Flag or they were left untreated. As shown in Fig. 1C, infection with AdVZ+434-Flag resulted in an approximately fivefold increase in VEGF-A mRNA and protein expression. As expected, no increase in VEGF-A mRNA or protein expression was seen with AdEGFPp65-Flag (Fig. 1C, left and center panels), although both vectors drive expression of their respective transgenes (Fig. 1C, right panel). These data establish that the ZFP-TF (VZ+434) activates VEGF-A expression in both cultured rat cells and in terminally differentiated human skeletal muscle cells in vitro.

**VZ+434-induced VEGF-A protects SK-N-MC human neuroblastoma cells from serum starvation-induced growth arrest.** Next, we determined whether the ZFP-TF–induced VEGF-A protein secreted from these cells, we measured the ability of conditioned medium to promote the survival of cultured neuronal cell lines after a serum starvation insult. Human neuroblastoma cells (SK-N-MC) were grown either in the presence of complete medium, in serum-free conditions, or in the serum-free medium supplemented with increasing amounts of conditioned medium (Fig. 2). Conditioned medium was prepared both in the presence and absence of the VEGF-A–activating ZFP (i.e., in the presence or absence of DOX). The media prepared in the absence of DOX serve to control for any potential neuroprotective factor(s) that may be secreted into the media by HEK293 cells alone. The results, shown in Fig. 2, indicate that only conditioned medium prepared from cells induced for expression of the VZ+434 transcription factor provided a dose-dependent protection from serum starvation. Similar data were obtained with ND8 cells, which are a fusion of primary neonatal rat dorsal-root-ganglion neurons with N18t-g2, a mouse neuroblastoma (C1300)-derived azaguanine-resistant line (data not shown). The data described above suggest that ~20–40 ng/ml of ZFP-TF–induced VEGF-A (final concentration) is sufficient to protect cultured human neuroblastoma cells from loss of viability in response to serum starvation (Fig. 2). This result is consistent with previously published studies with recombinant VEGF-A protein, wherein the effective VEGF-A dose was found to be between 10 and 100 ng/ml, with a declining response at higher doses (3,4,19).

**VZ+434 protects NCV in STZ-induced diabetic rats.** Next, we sought to determine the effect of VZ+434-driven activation of VEGF-A in an established rat model of STZ-induced diabetes (20–25). Eight weeks post-STZ treatment, diabetic rats exhibited characteristic reduction in body weight and increased plasma glucose levels (Table 1). In agreement with our expectations, neither of these indicators of diabetes was altered in any of the treatment groups (Table 1). First, a dose-ranging study using 31.25–250 μg VZ+434-driven plasmid DNA was performed to establish an efficacious dose. NCV measurements, taken 8 weeks after STZ-treatment and 4 weeks after treatment with the indicated plasmid DNAs, are shown as group mean data in Fig. 3. Treatment with the empty plasmid pVAX-1 was without effect on NCVs (Figs. 3 and 4), since no difference between values was observed between treated and contralateral limbs. Calculation of mean values (treated and untreated) for each animal in control and diabetic pVAX-1–treated groups gave highly significant reductions in both motor (controls = 50.0 ± 5.8 and pVAX-1–treated diabetic rats = 41.7 ± 5.4; P < 0.003) and sensory NCV (controls = 54.7 ± 6.7, diabetic rats = 45.5 ± 4.8; P < 0.002; Fig. 3). These NCV deficits obtained after 8 weeks of STZ treatment are consistent with, and within, the range reported elsewhere (21,22). Injection of the lowest dose of ZFP-TF expression plasmid (VZ+434 at 31.25 μg) had no effect on MNCVs or SNCVs (Fig. 3). In contrast, diabetic rats treated with all three higher doses of VZ+434 (62.5, 125, and 250 μg) demonstrated a dose-dependent, progressively increas-
ing difference between the treated versus untreated limbs with respect to both SNCVs and MNCVs (Fig. 3). In all experiments at these higher doses, the conductance velocity of the nerve on the treated side was always higher than the untreated side, and the differences were all significant ($P < 0.05$; Fig. 3). These data indicate that a single treatment of VZ434 is capable of neuroprotection in an experimental animal model of early diabetes, as determined by motor and sensory NCV measurements conducted 4 weeks posttreatment.

Having established an effective dose for VZ434, we next tested the ZFP-TF's ability to function in the context of a repeat administration study, again comparing NCV values from the treated and untreated limbs. The study design (shown in Fig. 4) called for injection of 250 μg VZ434 plasmid DNA 2, 4, and 6 weeks post-STZ treatment. Interestingly, while a single injection of 250 μg of VZ434 plasmid DNA at 4 weeks again demonstrated protection of NCVs (data not shown), the repeat intramuscular administration of VZ434 plasmid DNA at 2, 4, and 6 weeks post-STZ treatment demonstrated a robust and highly significant protection of both MNCV and SNCV (Fig. 4). As expected, no change in NCVs was observed in diabetic animals that received the control vector (pVAX). These repeat injection data may be significant given the chronic nature of the human condition.

**A**

<table>
<thead>
<tr>
<th>Species</th>
<th>VZ434 Binding Site</th>
</tr>
</thead>
<tbody>
<tr>
<td>Rat</td>
<td>AAGT-GACT-GCTTTTGGGGTGACCGCCAGACGC-</td>
</tr>
<tr>
<td>Mouse</td>
<td>AAGTTGACTTGGCTTTGGGGTGACCGCCAGACGC</td>
</tr>
<tr>
<td>Human</td>
<td>AAGT-GACTTGGGGTGACCGCCAGACGC</td>
</tr>
</tbody>
</table>

**B**

[Graph showing relative VEGF mRNA (VEGF/18S) and VEGF protein (pg/ml/24hr) for untreated, p65-FLAG, and VZ434 treated conditions.]

**C**

[Graph showing relative VEGF mRNA (VEGF/18S), VEGF protein (pg/ml/24hr), and relative transgene RNA/18S for untreated, p65-FLAG, and VZ434 treated conditions.]
cells from serum starvation–induced growth arrest (Fig. 2). An important role for VEGF-A in neuroprotection is becoming increasingly apparent. For example, gene transfer with a plasmid DNA encoding the VEGF-A165 isoform improved NCVs in several studies in vivo (20,26). Moreover, in an open-label, dose-escalation trial of the same plasmid DNA encoding the VEGF-A165 isoform, four of six patients enrolled who had diabetes showed an improvement in neuropathy in the treated limb (9). Evidence for a direct neuroprotective role for VEGF-A comes from in vitro studies, which have documented VEGF-driven protection of neuronal-derived cell lines (such as HN33 and NSC34) from serum starvation, hypoxic insult, tumor necrosis factor α, and oxidative stress (3–5). Beyond protection, VEGF-A has also been shown to promote neuronal growth with cultures of adult mouse dorsal root ganglion and superior cervical ganglion explants (6,8). In addition, VEGF-A has shown both protective and potent growth factor activity for Schwann cells (6,7), which provide myelination and support functions to neurons, suggesting an additional potential mechanism for the beneficial effects of VEGF-A action in vivo. Note that all of these studies used a single isoform of the VEGF-A growth factor (VEGF-A165). With respect to the angiogenic properties of VEGF-A, the expression of multiple isoforms has been shown to be more effective than any individual isoform alone (13,15). Engineered transcription factors via their action at the promoter of the endogenous gene generate all of the natural splice variants and protein isoforms supported by that cell type (13,14). While it remains to be determined whether the expression of the full complement of protein isoforms will be advantageous in the context of neuropathy, it is known that the isoforms differ in their effects on vascular permeability, tumor progression, receptor binding, and interaction with the extracellular matrix (27–31).

It is remarkable that this positive effect was observed 28 days after a single intramuscular treatment with plasmid DNA encoding VZ+434. This result is perhaps even more surprising given the short (<7 days) duration of transgene expression observed (data not shown). The second study shows that an even more robust effect may be achieved with multiple dosing. This striking result in terms of the potency and duration of effect supports earlier work with a plasmid encoding the VEGF-A165 CDNA, demonstrating sustained improvement in NCVs up to 10 weeks after gene transfer (20). While angiogenesis was proposed as a pos-

**FIG. 2.** VZ+434-induced VEGF-A protects SK-N-MC human neuroblastoma cells from serum starvation insult. SK-N-MC cells were cultured for 24 h in complete serum. Culture medium was then removed and replaced with test media: either normal culture medium with 10% fetal bovine serum, serum-free culture medium, conditioned medium from induced HEK293 cells expressing VZ+434 from a doxycycline-inducible promoter (and thus activated VEGF-A levels), or culture medium from the same cells not expressing ZFP VZ+434 (no doxycycline induction, basal VEGF-A levels). Forty-eight hours later, the cells were assayed for viability by using the Alamar Blue assay. Results are shown is means representative of at least three independent experiments. *., complete medium; ■, serum-free medium; ▲, control-conditioned medium; ■, VZ+434-conditioned medium.

**TABLE 1**

<table>
<thead>
<tr>
<th>Experimental group</th>
<th>Number of rats</th>
<th>Blood glucose (mmol/l)</th>
<th>Body weight (g)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Start</td>
<td>End</td>
</tr>
<tr>
<td>Dose-ranging study</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Controls (nondiabetic; not injected)</td>
<td>10</td>
<td>5.2 ± 0.9</td>
<td>306 ± 19</td>
</tr>
<tr>
<td>Diabetic pVAX-1</td>
<td>12</td>
<td>27.9 ± 3.5</td>
<td>297 ± 11</td>
</tr>
<tr>
<td>Diabetic VZ+434 (31.25 µg)</td>
<td>11</td>
<td>28.0 ± 4.0</td>
<td>304 ± 10</td>
</tr>
<tr>
<td>Diabetic VZ+434 (62.5 µg)</td>
<td>13</td>
<td>28.5 ± 3.9</td>
<td>300 ± 13</td>
</tr>
<tr>
<td>Diabetic VZ+434 (125 µg)</td>
<td>10</td>
<td>29.0 ± 3.0</td>
<td>302 ± 15</td>
</tr>
<tr>
<td>Diabetic VZ+434 (250 µg)</td>
<td>14</td>
<td>29.0 ± 2.7</td>
<td>295 ± 10</td>
</tr>
<tr>
<td>Repeat-dosing study</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Control (untreated)</td>
<td>12</td>
<td>7.0 ± 1.0</td>
<td>285 ± 13</td>
</tr>
<tr>
<td>Diabetic pVAX-1 (repeat injections)</td>
<td>13</td>
<td>25.8 ± 2.6</td>
<td>288 ± 15</td>
</tr>
<tr>
<td>Diabetic VZ+434 (repeat injections)</td>
<td>11</td>
<td>28.7 ± 3.9</td>
<td>292 ± 10</td>
</tr>
</tbody>
</table>

Data are means ± SD from triplicate measurements for blood glucose.
sible mechanism responsible for the therapeutic effect after delivery of the VEGF-A cDNA, the data presented here strongly suggest a direct neuroprotective effect of VEGF-A. This is further supported by work in an ALS mouse model, which showed that VEGF-A, but not the potent angiogenic factor PLGF (placental growth factor), prevented motor neuron degradation (32). Moreover, the latter studies were conducted in animals that were diabetic for 3 months before gene transfer and thus suggest that VEGF-A may be an effective treatment for the long-term effects of DN. In both the present study and the previous studies (20,26), no change in NCVs was observed on the contralateral side (uninjected side), consistent with a local effect of VEGF-A and/or the ZFP-TF treatment. Interestingly, both protection of NCVs and increased percentages of small myelinated nerve fibers were noted following local administration of insulin itself in STZ-induced models of diabetes independent of hyperglycemia (33), data further supporting the importance of direct trophic effects on the treated nerve fibers.

Thus, the current findings indicate a neuroprotective effect for the VEGF-A–activating ZFP-TF VZ+/H11001434 both in vitro and in animal models of diabetes in vivo and thus suggest that this engineered transcription factor could represent a novel therapeutic modality for the potential treatment of diabetic neuropathy.


27. Whitaker GB, Limberg BJ, Rosenbaum JS: Vascular endothelial growth factor receptor-2 and neuropilin-1 form a receptor complex that is responsible for the differential signaling potency of VEGF(165) and VEGF(121). *J Biol Chem* 276:25520–25531, 2001


