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Delivery of Interleukin-4–Encoding Lentivirus Using Multiple-Channel Bridges Enhances Nerve Regeneration

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Objectives/Hypothesis: Facial nerve injury is a source of major morbidity. This study investigated the neuroregenerative effects of inducing an anti-inflammatory environment when reconstructing a facial nerve defect with a multichannel bridge containing interleukin-4 (IL-4)–encoding lentivirus.

Study Design: Animal study.

Methods: Eighteen adult Sprague-Dawley rats were divided into three groups, all of which sustained a facial nerve gap defect. Group I had reconstruction performed via an IL-4 multichannel bridge, group II had a multichannel bridge with saline placed, and group III had no reconstruction.

Results: Quantitative histomorphometric data were assessed 10 weeks after injury. On post hoc analysis, the IL-4 bridge group demonstrated superior regeneration compared to bridge alone on fiber density (mean = 2,380 ± 297 vs. 1,680 ± 441 fibers/mm², P = .05) and latency time (mean = 2.9 ms ± 0.6 ms vs. 3.6 ms ± 0.3 ms, P < .001). There was significantly greater regeneration in the IL-4 bridge group versus unreconstructed defect for total fiber and density measurements (P ≤ .05). Comparison of facial motor-evoked distal latencies between the IL-4 bridge group versus bridge alone revealed significant electrophysiologic improvement at week 8 (P = .02).

Conclusions: Inflammation has been implicated in a variety of otolaryngologic disorders. This study demonstrates that placement of a multichannel bridge with lentivirus encoding IL-4 improves regenerative outcomes following facial nerve gap injury in rodents. This effect is likely mediated by promotion of an anti-inflammatory environment, and these findings may inform future therapeutic approaches to facial nerve injury.

Key Words: Facial nerve injury, axotomy, motoneuron regeneration, interleukin-4, anti-inflammatory.

Level of Evidence: NA

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synkinesis, and the need for concerted effort to achieve complete eye closure.⁶

There are three clinically relevant injury patterns to nerves, including crush-induced injury, simple transection without a gap defect, and transection with resultant gap defect. Existing studies show that promoting an anti-inflammatory regenerative environment after crush injury enhances facial motoneuron survival and functional recovery in the mouse model.⁷ Specifically, modulation of the interleukin-4 (IL-4)/STAT-6 pathway has demonstrated promising results for motoneuron survival and recovery.⁷—⁹ Given its small-scale anatomy, it is difficult to model more clinically relevant injury patterns—particularly gap defect injury—in the mouse. We hypothesized that inducing an anti-inflammatory environment via promotion of the IL-4/STAT-6 pathway would result in improved regenerative outcomes following gap defect injury. This study compared histomorphometric, functional, and electrophysiological outcomes in rats with IL-4 embedded multichannel bridges and those with multichannel bridges without the addition of IL-4-encoded lentivirus. The long-term goal of this work was to influence management of facial nerve injury in human patients.

**MATERIALS AND METHODS**

**Animal Housing**

This experimental protocol was performed in strict accordance with National Institutes of Health guidelines and was approved by the University of Michigan Institutional Animal Care and Use Committee prior to initiation (PRO00008431). All animals used in this study were female Sprague-Dawley rats (8 weeks old, weight = 250–350 g at time of surgery). They were quarantined and housed in a central animal care facility following purchase from Envigo (Franklin Township, NJ) and allowed sufficient time to acclimate. They were provided with a 12-hour light/dark cycle with food and water available ad libitum.

**Animal Treatment and Experimental Design**

Eighteen rats were randomly divided into three groups as shown in Table I. For all of the animals, a 5-mm nerve gap defect was created in the animal's left facial nerve main trunk. The non-operated right side of the face of the rat served as a negative control.

**TABLE I.**

<table>
<thead>
<tr>
<th>Group</th>
<th>No. of Animals</th>
<th>Intervention Performed</th>
</tr>
</thead>
<tbody>
<tr>
<td>I</td>
<td>6</td>
<td>Gap defect created, interleukin-4 multichannel bridge placed</td>
</tr>
<tr>
<td>II</td>
<td>6</td>
<td>Gap defect created, saline with multichannel bridge placed</td>
</tr>
<tr>
<td>III</td>
<td>6</td>
<td>Gap defect created, no repair or reconstruction performed</td>
</tr>
</tbody>
</table>

**Virus Production**

Lentivirus was utilized to promote expression of secreted IL-4, under a cytomegalovirus promoter, in the multichannel bridge for experimental group I. The lentivirus was produced from HEK293T cells via a previously established protocol.¹⁰ Briefly, the lentivirus was produced via cotransfecting HEK-293 T cells, purified via the Lenti-X Maxi Purification Kit (Clontech Laboratories, Mountain View, CA) and then concentrated.¹⁰,¹¹ Viral titers (2E10 IU/mL) were determined by Lentivirus qPCR Titer Kit (Applied Biological Materials, Richmond, British Columbia, Canada).¹¹ The multichannel bridges were created via a previously described technique.¹¹ Briefly, sugar strands were drawn and coated with a 1:1 mixture of poly(lactide-co-glycolide) (75:25 ratio of D,L-lactide to L-glycolide; Lakeshore Biomaterials, Birmingham, AL) microspheres and NaCl (63–106 μm granules; Sigma-Aldrich, St. Louis, MO). Coated strands were packed into molds, equilibrated to 800 psi of CO₂ for 16 hours, and subsequently released at 60 psi/min to foam into final structure. Bridges were cut into 5-mm sections for a final dimension of 5 × 0.75 × 1.25 mm. Bridges were dried overnight on a lyophilizer and stored in a desiccator. The bridges were then disinfected with 70% ethanol and washed with sterile water, following which iterative loading of the lentivirus was performed.¹¹ After the loading cycles and subsequent drying, the bridges were then stored in –80°C until utilized for the interventions.

**Surgical Techniques**

Experimental manipulations were performed with sterilized equipment in a dedicated procedure room. General anesthesia was induced and maintained via 1.8% isoflurane. The operative sites were shaved, prepped, and draped in accordance with proper aseptic technique. An operating microscope (Wilde M690; Leica Microsystems, Heerbrugg, Switzerland) was utilized for the dissection. A postauricular incision was fashioned, and the posterior belly of the digastric muscle was traced proximally to its insertion on the skull base. There, the main trunk of the facial nerve was identified, and a gap defect was created via sharp microscissors; then the group-specific intervention was performed. The nerve gap length was standardized via measuring calipers. For groups I and II, the multichannel bridge was placed in contact with the proximal and distal cut end of the nerve. For group III, no reconstruction was performed. The skin edges were reapproximated with absorbable 4–0 Monocryl subcuticular sutures, and skin glue was utilized for superficial reapproximation. The animals were given 0.5 mg/kg carprofen injections for postoperative analgesia. To ensure adequate postoperative recovery, animals were closely monitored for return of spontaneous activity and oral intake.

At the conclusion of the experiment at 10 weeks, the rats were again anesthetized. The left facial nerve was reexposed and the dissection was carried distally to identify the left marginal mandibular nerve. The main trunk and marginal mandibular nerves were resected with microscissors, frozen, and processed for histologic analysis. The incision was extended toward the animals' whisker pad and the zygomaticus, and associated facial musculature was dissected from the underlying tissue sharply. Following conclusion of the harvest, the animals were euthanized in accordance with institutional protocol.

**Electrophysiological Analysis**

At weeks 2, 4, 6, 8, and 10 from surgery, electrophysiological assessments were performed via Viking Quest (CareFusion, San Diego, CA) software and hardware. The measurements were performed under inhaled anesthetic. Briefly, 28-gauge needle electrodes were placed proximally parallel to the main trunk at the level of exit of the facial nerve from the animal's skull base.
and distally in the vibrissal musculature. Latency of signal responses and motor unit action potential values were obtained, and standard error of means are reported. Repetitive stimulation was performed until a biphasic recording was obtained. Amplitude values (in millivolts) were calculated by measuring the magnitude between the maximum positive and negative turnaround point of the signal in millivolts. Latency of signal response was measured in milliseconds from the stimulus artifact to the first reproducible change in the baseline.

Muscle Histomorphometric Analysis
At the conclusion of the experiment, muscle was harvested from the animal’s whisker pad, frozen in liquid nitrogen, and stored in a −80 °C freezer. Cross-sections of 14 μm were obtained. Muscle sections were processed with acetylcholinesterase (motor endplates), α-bungarotoxin (acetylcholine receptors), and a neurofilament antibody (nerve fibers) as described in our previously published protocol. Images were taken at 40× magnification using a Nikon A1 confocal microscope (Nikon, Tokyo, Japan); the microscopic images were converted to digital images for analysis.

Nerve Histomorphometric Analysis
At the conclusion of the experiment, the animals’ bilateral facial nerve main trunks and marginal mandibular nerves were harvested and fixed in 4% paraformaldehyde. After placement in a phosphate-buffered solution, the nerves were embedded in a Cryomold with optimal cutting temperature compound (Fisher Healthcare, Waltham, MA), frozen with liquid nitrogen, and stored in a −80 °C freezer. Cross-sections of 14 μm were obtained, and the sections were stained with a neurofilament antibody as previously described.

Fluorescent microscopy at 20× magnification was performed via a Nikon Microphot FXA fluorescent microscope (Nikon). The microscopic images were converted to digital images for quantification. Manual counts were performed by an experienced individual blinded to the experimental groups, and counting was performed across the entire cross-sectional area at 20× magnification. The architecture, quality, and quantity of the regenerating nerve fibers were systematically assessed. Utilizing Photoshop software

<table>
<thead>
<tr>
<th>Group</th>
<th>Mean Background Intensity (Fluorescent Intensity Units)</th>
<th>Mean Nerve Intensity (Fluorescent Intensity Units)</th>
</tr>
</thead>
<tbody>
<tr>
<td>I</td>
<td>0.87</td>
<td>27.2</td>
</tr>
<tr>
<td>II</td>
<td>1.07</td>
<td>2.51</td>
</tr>
<tr>
<td>III</td>
<td>1.02</td>
<td>1.73</td>
</tr>
<tr>
<td>Intact</td>
<td>1.03</td>
<td>3.23</td>
</tr>
</tbody>
</table>

Fig. 1. Immunohistochemistry testing of interleukin (IL)-4 expression in the experimental groups, demonstrating robust IL-4 expression (green) in animals implanted with a multichannel bridge seeded with IL-4–expressing lentivirus. Experimental groups include IL-4 group (A–D), gap defect group (E–H), and the empty bridge group (I–L). IL-4 antibody is represented by green fluorescence, arginase by red fluorescence, and Hoechst by blue fluorescence. The fluorescent signals are merged for each of the experimental groups for (A), (E), and (I). White arrow heads in (A) and (B) denote regions of maximal IL-4 signal, corresponding to higher density of inflammatory cellular infiltrate, with basal expression of IL-4 present throughout neural tissue in the IL-4 group. The human IL-4 signal is absent in animals implanted with a multichannel bridge with saline and animals with gap defect. Scale bar represents 20 μm.
the area of the nerve was determined to obtain nerve density (axonal fibers/mm²) and total neural area represented by axon fibers or percent nerve measurements. This value was determined for all of the animals in our experimental groups, and mean and standard error of difference were calculated for each cohort for direct comparative analysis.

**Confirmation of IL-4 Expression via Immunohistochemistry**

Implementing a previously established protocol, 14-μm cross-sections of the of the animals’ facial nerves were obtained and stained with Hoechst, arginase, and human IL-4 antibodies. Human IL-4 antibodies were utilized to avoid detection of endogenous IL-4 expression. The sections were permeabilized in 0.5% Triton-X 100 (Sigma-Aldrich) and 1x TRIS (Thermo Fisher Scientific, Waltham, MA) and then blocked. Following the blocking solution, the sections received arginase (polyclonal, arginase I, 1:100, #sc-18351; Santa Cruz Biotechnology, Dallas, TX) and IL-4 primary antibodies (polyclonal, IL-4, 1:100, #LS-C408858; LSBio, Seattle, WA) and were incubated overnight. The following day, the sections were rinsed, and the secondary antibodies were applied. The sections were rinsed again and then received a counterstain of Hoechst 33342 (1:200, 1 mg/mL; DNA, Thermo Fisher Scientific, Waltham, MA). They were then mounted with ProLong Gold antifade reagent and imaged at 60× magnification via Nikon A1 Confocal Microscope (Nikon). Quantitative data on signal fluorescent intensity were then derived from random sampling of all specimens and controls (Table II) using MetaMorph Microscopy Automation and Image Analysis Software (Molecular Devices LLC, San Jose, CA) per a previously published protocol.15

**Statistical Analysis**

All statistical analyses were performed in GraphPad Prism 7.0 (GraphPad, San Diego, CA). All results for quantitative analysis are reported as mean ± standard error. Histomorphometric and electrophysiological data were analyzed via one-way analysis of variance (ANOVA) followed by Tukey post hoc analysis. All statistical tests of significance were two-sided, with an α of .05. Sample size analysis was based on previously established parameters and data analysis from a prior investigation using a similar model.16

**RESULTS**

All animals enrolled in the study survived to the established endpoint. The rats gained weight appropriately throughout the study period. There were no adverse effects from any of the performed procedures, and there was appropriate wound healing following surgery.

**Immunohistochemistry Confirmation of IL-4 Expression**

Immunofluorescence staining of tissue sections was undertaken to confirm expression of IL-4 in the appropriate experimental group. There was preferential...
immunofluorescence of the anti–IL-4 antibody in nerve sections from group I in comparison to groups II and III (Fig. 1). The IL-4 group demonstrated mean IL-4 signal intensity of 27.2 units (Table II). The empty multichannel bridge demonstrated mean 2.51 units, the gap defect 1.73 units, and the intact nerve 3.23 units, values which were similar to mean background levels.

**Histology**

Qualitative analysis of cross-sections of the peripheral marginal mandibular branch demonstrated evidence of varying degrees of neural regeneration for the experimental groups. Representative histology for the intact nerve, multichannel bridge with IL-4 encoding lentivirus, multichannel bridge with saline, and gap defect groups are demonstrated in Figure 2. Representative histology for the muscle sections is demonstrated in Figure 3.

**Histomorphometry**

We performed quantitative histomorphometric analysis of the facial nerve marginal mandibular branch, including total nerve fiber count, fiber density (measured in fibers/mm²), and percentage of total neural area represented by axon fibers (measured as percent nerve) (Fig. 4). One-way ANOVA results demonstrated a significant difference when analyzing between groups for the measures of total nerve count ($P = .0001$), axonal fiber density ($P < .001$), and percent nerve ($P < .0001$). Post hoc analysis demonstrated a significant increase in total nerve count measurements for IL-4 encoding Lentivirus with bridge group (group I) versus gap defect group (group III) (mean = 1,369 ± 408 vs. 687 ± 367 fibers,

![Histology images](image)

Fig. 3. Immunohistologic analysis within the vibrissal pad musculature. Images are merged to show axonal fibers (red neurofilament stain), motor end plates (blue α-bungarotoxin stain), and presynaptic terminals (green synaptophysin stain). Groups include (A) intact, (B) interleukin (IL)-4 with multichannel bridge, (C) saline with multichannel bridge, and (D) gap defect group. Scale bar represents 20 μm.
There was no significant difference in total axon count between group I and intact nerve groups (mean = 1,369 ± 408 vs. 1,690 ± 323 fibers, \( P = .3 \)). The intact nerve group demonstrated significantly greater nerve fiber counts than groups II and III (all \( P < .01 \)). For nerve density measurements, group I had mean nerve fiber 2,380 ± 297 versus 1,680 ± 441 nerves/mm² for group II (\( P = .05 \)) and 1,500 ± 374 nerves/mm² for group III (\( P = .01 \)). There was no difference in nerve density measurements for group I and intact nerve groups (mean = 2,380 nerves/mm² vs. 2,685 ± 641 nerves/mm², \( P = .6 \)). There was significantly greater density represented within the intact group in comparison to groups II or III (all \( P < .01 \)). For percent nerve measurements, the intact nerve group (mean = 16.1% ± 5.3%) demonstrated significantly greater percent neural tissue than all other experimental groups (all \( P < .01 \)).

Electrophysiological Analysis

Electrophysiological assessments were completed every other week until the study endpoint (Fig. 5). There was improved return of electrophysiological response as measured via motor unit action potential values for group I compared to group III at 10 weeks (1.4 mV ± 0.5 mV vs. 0.17 mV ± 0.1 mV, \( P < .0001 \)) on post hoc analysis. There was no difference when comparing group I to group II

\( P < .01 \). There was no significant difference in total fiber counts between the interleukin (IL)-4 and gap defect groups (mean = 1,369 ± 408 vs. 687 ± 367 fibers, \( P = .008 \)). The IL-4 group demonstrated greater axonal density than the empty multichannel bridge (mean = 2,380 nerves/mm² ± 297 vs. 1,680 nerves/mm² ± 441, \( P = .05 \)) or the gap defect (mean = 2,380 nerves/mm² ± 297 vs. 1,500 nerves/mm² ± 374, \( P = .01 \)). The intact group demonstrated greater percent neural tissue than the other experimental groups (all \( P < .01 \)). **P < .05. ***P < .005. **P < .0005.

Fig. 4. Bar graphs demonstrating (A) total axonal fiber counts, (B) axonal fiber density, and (C) neural tissue percentage for the experimental groups. There was a significant difference in total fiber counts between the interleukin (IL)-4 and gap defect groups (mean = 1,369 ± 408 vs. 687 ± 367 fibers, \( P = .008 \)). The IL-4 group demonstrated greater axonal density than the empty multichannel bridge (mean = 2,380 nerves/mm² ± 297 vs. 1,680 nerves/mm² ± 441, \( P = .05 \)) or the gap defect (mean = 2,380 nerves/mm² ± 297 vs. 1,500 nerves/mm² ± 374, \( P = .01 \)). The intact group demonstrated greater percent neural tissue than the other experimental groups (all \( P < .01 \)). **P < .05. ***P < .005. **P < .0005.

Fig. 5. Comparison of electrophysiological data between the experimental groups as measured via (A) amplitude measured in millivolts (mV) and (B) latency measured in milliseconds (ms). There was improved return of electrophysiological response for the IL-4 with multichannel bridge and saline with multichannel bridge in comparison to the gap defect group. Other comparisons were not significant when measuring amplitude. When examining latency response, the interleukin (IL)-4 group demonstrated shorter mean latency than the gap defect group. *P < .05. **P < .005. ***P < .0005.
(1.4 mV ± 0.5 mV vs. 1.1 mV ± 0.4 mV, P = .2). In comparing distal motor latency responses at 10 weeks, there was reduced mean latency of group I in comparison to group II (2.9 ms ± 0.6 ms vs. 3.6 ms ± 0.3 ms, P < .001) groups. The intact group (1.9 ms ± 0.4 ms) demonstrated significantly lower mean latency values than the experimental groups (all P < .001).

**DISCUSSION**

This study demonstrates that placing a multichannel bridge with lentivirus encoding IL-4 significantly enhanced axonal regeneration across rat facial nerve gap defect in comparison to a bridge with saline or unreconstructed defect for certain histomorphometric and electrophysiologic measures.

Although there is substantial literature exploring approaches to enhance regeneration and recovery after nerve injury, comparatively less work has specifically examined effects of modulating the inflammatory milieu, particularly as in relation to facial nerve gap injury.17–23 This study was able to demonstrate a quantitative improvement in nerve histomorphometric parameters (particularly in total fiber count, fiber density, and percent neural tissue) when utilizing IL-4–encoding lentivirus with a multichannel bridge in comparison to a multichannel bridge with saline and an unreconstructed gap defect. Further analysis demonstrated that the IL-4 group demonstrated similar total fiber count and fiber density measurements as the intact nerve group.

Following peripheral nerve injury patterns such as the gap defect injury, it is known that the immune system and nervous systems engage in cross-talk; the responses can be neuroprotective, neurodestructive, or both.24,25 Previous studies report improvements in hyperalgesia via down-regulation of proinflammatory cytokines,26 improved survival of axotomized retinal ganglion cells through utilization of anti-inflammatory cytokines,27 and improvement of functional recovery after traumatic spinal cord injury in rats via reduction of neuroinflammation after injury.28 Although this study was able to demonstrate histomorphometric improvement with the IL-4–encoding lentivirus group, evidence of improvement of functional recovery was mixed. Motor unit action potential values between the IL-4 group and the empty multichannel bridge were similar, whereas latency values were significantly prolonged in the empty multichannel bridge cohort. Although motor unit action potential and latency are part of neurophysiological diagnostic testing, abnormalities in their measures can reflect various physiological insults. Whereas reduced amplitude values indicate lower motor neuron involvement, prolonged latency may reflect selective death of fast-conducting axons or decreased myelination of regenerating axons.29 Thus, it is possible that the lack of correlation between these measures reflects selective regeneration of fast-conducting axons in the IL-4 group and thereby a reduction in mean latency.

The immune system also plays a role in facial motoneuron survival as evidenced by the fact that recovery is delayed after crush injury in immunodeficient mice.30 This relationship relates specifically to T lymphocytes, which mediate facial motoneuron survival and even rescue motoneurons from cell death after injury.31,32 Utilizing an immunodeficient mouse model, Jones and colleagues demonstrated a specific role for CD4+ T cells in mediating facial motoneuron survival following facial nerve injury.3 Follow-up studies from their group report that the IL-4 and STAT-6 mediated pathway is essential for maintaining facial motoneuron survival following nerve injury.8 IL-4 works to induce differentiation of Th2 cells from naïve helper T cells, allowing for promotion of an anti-inflammatory environment. This anti-inflammatory milieu has demonstrated potential as a novel therapeutic agent for central nervous system repair in demyelinating diseases.33,34 For peripheral nerve injury, activated Th2 cells may improve motoneuron cell body survival by providing direct neurotrophic support and/or by promoting an anti-inflammatory environment.

Drawing upon these observations, we sought to determine if delivery of IL-4 encoding lentivirus using multichannel bridges in a gap defect would improve regenerative outcomes. Although the robust regenerative potential of the rat model limits extrapolation to human models, the gap defect still represents a more severe injury and therefore is a greater hurdle for regenerating nerve to overcome. We therefore assessed regeneration at 2 to 3 months to allow nerve recovery, in comparison to approximately 1 month for the crush injury.25 This nerve gap model is also more clinically translatable to iatrogenic injuries or intentional creation of a gap defect for oncologic resection. IL-4 encoding lentivirus promoted recovery by 8 weeks, whereas untreated animals did not demonstrate signs of recovery until 10 weeks. At the conclusion of the experiment, IL-4 group animals demonstrated greater recovery than the gap defect group. This difference cannot be explained by the presence of the bridge alone, as the IL-4 encoding lentivirus with bridge group had increased fiber density and reduced mean latency time when compared to the multichannel bridge group with saline alone. Qualitatively, the appearance of axons was also different across the groups; with an IL-4–encoding lentivirus bridge, facial nerves displayed clearly distinct axon groups that were evenly spaced, whereas the gap defect and multichannel bridge with saline groups demonstrated erratic axonal architecture with inconsistent uptake of neurofilament stain.

A second measure of axonal integrity is the assessment of percentage neural tissue. Facial nerves from the IL-4 group had a percentage of neural tissue that most closely resembled normal uninjured facial nerves. We also discovered that the regenerated fibers in the IL-4–encoding lentivirus bridge group comprised a significantly smaller proportion of the overall area of the nerve in comparison to the intact nerve. Percentage neural tissue incorporates data on size of each axon, as opposed to the simple number. Future studies may compare the effects of local therapeutic strategies, such as those investigated here, to systemic anti-inflammatory modulation, as in management of disease processes such as Bell’s palsy and idiopathic sensorineural hearing loss.

**Limitations**

With regard to experimental protocol, the possibility that the presence of the lentivirus itself (with or without
IL-4 delivery) was responsible for the experimental results was not explored via an additional group with control lentivirus. Quantitative neural histomorphometry was performed via manual counting, where many scientists now utilize automated software. The counters were blinded to the experimental group to reduce likelihood of bias. As has been done in previous studies, our group did not seek to quantify the potential for differential infiltrating macrophage presence within the sections at a representative time point (days 14–21). Rather, sections from the week 10 time point were stained for infiltrating macrophages. Future studies could perform similar immunohistochemistry from samples taken from earlier in the experimental timeline to better assess differences within the inflammatory milieu as a result of IL-4 presence. Furthermore, the analysis of human IL-4 expression and macrophage staining was performed as a secondary outcome measure, subsequent to completion of the initial quantitative histomorphometry analyses. The number of sections available for staining from unique animals per group was limited; therefore, only those animals with residual nerve available for resectioning and staining were included. Blinded qualitative and quantitative analyses were performed, demonstrating moderate/strong human IL-4 signal in all sections from the IL-4 experimental group (e.g., the group reconstructed with a multichannel bridge seeded with lentivirus expressing human IL-4). In contrast, the human IL-4 signal was absent or weak/trace in all other groups.

Quantitative data measured were consistent with qualitative assessments noted above; however, these data were not subjected to statistical analyses due to availability of only a limited subset of animals for staining in this subsequent evaluation. Although our prior experiments have also confirmed gene expression via IL-4 immunomodulation, future studies would be beneficial to comprehensively characterize timing and expression patterns of human IL-4 in the facial nerve in a larger number of experimental animals to afford additional rigor in analyses.

CONCLUSION

Reconstruction of the facial nerve gap defect is challenging and associated with incomplete recovery. The nerve injury induces a cascade of pro- and anti-inflammatory factors that ultimately influence regenerative outcomes. In this study of a rodent facial nerve gap injury, we demonstrate that IL-4–encoding lentivirus multichannel bridges significantly improve nerve fiber density and mean latency time measurements in comparison to a multichannel bridge with saline. Although the reconstruction did not fully replicate architecture and composition of an intact nerve, this study clearly establishes the importance of modulating the inflammatory milieu following a gap defect injury. Future studies may better elucidate the regenerative potential and importance of specific outcome measures in comparison to functional measures of nerve recovery, as well as identify other factors in the inflammatory cascade to modulate.

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BIBLIOGRAPHY


