DENATURED MUSCLE GRAFTS FOR NERVE REPAIR

AN EXPERIMENTAL MODEL OF NERVE DAMAGE IN LEPROSY

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About 20% of patients with leprosy develop localised granulomatous lesions in peripheral nerves. We report experiments in guinea-pigs in which freeze-thawed autogenous muscle grafts were used for the treatment of such mycobacterial granulomas.

Granulomas were induced in guinea-pig tibial nerves and the animals were left for 7 to 100 days in order to assess maximal damage. The local area of nerve damage was then excised and the gap filled with denatured muscle grafts. Clinical assessment after periods up to 150 days showed good sensory and motor recovery which correlated well with the histological findings.

The muscle graft technique may be of value for the treatment of chronic nerve lesions in selected cases of leprosy.

About 15 million patients in the world have leprosy. The causative organism, Mycobacterium leprae, has a predilection for peripheral nerves and is the most common cause of chronic peripheral neuropathy (Dastur 1978).

According to their immunological status, susceptible patients may develop tuberculoid, or lepromatous borderline (intermediate) forms of the disease. Although drug therapy is effectively bactericidal, a number of patients with each form of the disease develop irreversible nerve damage. This is seen especially in borderline patients undergoing the acute exacerbations of the disease known as reactions.

About 20% of patients develop localised granulomatous damage at certain points along the course of medium-sized peripheral nerves, resulting in some degree of paralysis and loss of sensation (Antia, Pandya and Dastur 1970). The common sites are, in descending order of frequency, the ulnar nerve at the medial aspect of the elbow; the median nerve immediately above the wrist; the lateral popliteal nerve near the neck of the fibula; the posterior tibial nerve above the medial malleolus; the zygomatic branch of the facial nerve where it crosses the zygoma; and the radial nerve in the posterior compartment of the arm (Enna and Delgado 1981). The current management of these lesions includes the use of antileprosy drugs, steroids and analgesics. If medical treatment fails, nerve decompression by internal or external neurolysis, or by transposition of the ulnar nerve may be attempted, but have proved of limited value (Antia et al 1970; Chaise and Roger 1985). Excision of the granulomatous lesions and bridging with nerve autografts has been unsuccessful (Sunderland 1973). The reasons for this are, first, that major nerve trunks are involved, needing long cable graft repairs with multiple cutaneous nerves. Limited donor material means that large lesions cannot be repaired. Secondly, the loss even of one sensory nerve is detrimental to a patient already suffering from some anaesthesia. Finally the donor nerve may itself be diseased. At present, surger, can only correct paralytic deformities, debride trophic ulcers and drain abscesses.

In 1984 Keynes, Hopkins and Huang showed that mouse peripheral nerve regenerated into degenerating skeletal muscle. Since then, Glasby and his colleagues have carried out extensive studies on the use of freeze-thawed autogenous muscle grafts to repair transected peripheral nerves (Glasby et al 1986 a,b,c,d; Davies et al 1987; Gattuso, Glasby and Gschmeissner 1988; Gattuso et al 1981; 1989; Glasby, Gattuso and Huang 1988; Gschmeissner et al 1988). They showed that, in rats and

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marmosets, regenerating axons could pass through degenerating muscle to enter and populate the distal nerve, and a pilot study on repair of human digital nerves was also successful (Norris et al 1988).

It seemed to us that the localised nerve lesions listed above might respond to muscle grafting and we therefore investigated this technique on a guinea-pig model of neural involvement in leprosy. This model was chosen because consistent granulomatous nerve damage, resembling that found in the natural disease is easily reproduced (Cowley et al 1988).

MATERIALS AND METHODS

Outbred Hartley strain female guinea-pigs weighing 250 to 300 g were used. All animals were fed a standard diet and housed in similar conditions. Groups of five animals were used throughout, experiments being designed to include extensive controls as there are few data on nerve regeneration in guinea-pigs. Controls included end-to-end repair and grafts with nerve or with fresh or denatured muscle (see Table II). Animals were anaesthetised with 0.05 ml/kg hypnorm (Janssen Pharmaceuticals) and 0.25 mg/kg midazolam (Roche), given intramuscularly into the forelimb.

**Induction of intraneural granulomas.** A saline suspension of Pasteur strain, live *M. bovis* BCG was obtained from the Pasteur Institute, Paris. Cobalt-irradiated (2.5 Mrad) armadillo-derived *M. leprae* was provided by Dr R. J. W. Rees, (National Institute for Medical Research, London).

Under aseptic conditions, the right sciatic nerve was exposed by dividing the biceps femoris muscle, and the tibial nerve fascicle was carefully isolated. This nerve was chosen because it runs as a single large bundle within the sciatic nerve sheath and was therefore suitable for endoneural injection. Using a microsyringe and a 30G needle, 0.01 ml of mycobacterial suspension (containing either 10⁶ BCG or 10⁸ *M. leprae* organisms) was injected into the fascicle. The muscle and skin were closed with Vicryl (Ethicon, UK). All injections were carried out by the same operator (SAC). The animals were left for seven to 100 days to allow granulomatous damage to develop.

**Muscle graft operation.** The sciatic nerve was exposed as before. The granuloma-infiltrated tibial nerve fascicle was separated from the peroneal component by microdissection and the area of granulomatous damage was excised, producing a gap of at least 1 cm, about 5 mm distal to the greater sciatic foramen. The gap was filled with a freeze-thawed, co-axially aligned, muscle autograft produced by excising a portion of vastus lateralis (where the fibres lie in parallel bundles), freezing it completely with the instant freezing aerosol dichlorodifluoromethane (−30°C), then thawing it in sterile distilled water. The ends of the tailored graft were then sutured to the perineurium of the nerve with 4 to 6 10/0 prolene sutures (Ethicon) and the wound was closed. All the operations were carried out by the same surgeon (JHP).

**Clinical assessment.** Detailed background studies of the functional anatomy of the guinea-pig lower limb were made. These included dissection and electrical stimulation of the sciatic nerve and its branches. Detailed observations were made of the clinical damage and pattern of recovery after crushing or transection of the sciatic, femoral, tibial and peroneal nerves. These showed no evidence of double innervation. A scoring system for assessment of motor and sensory recovery was developed (Table I). Postoperatively the animals were assessed daily.

**Histological and ultrastructural assessment.** The animals were killed at 50 and 150 days after grafting. At 150 days, electrophysiological studies were carried out on anaesthetised animals (to be reported elsewhere) and muscle

### Table I. Scoring system for clinical assessment of recovery

<table>
<thead>
<tr>
<th>Motor recovery</th>
<th>Sensory recovery</th>
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<tr>
<td>M0: complete paralysis (walks on heel)</td>
<td>S0: complete sensory loss</td>
</tr>
<tr>
<td>M1: slight resistance to dorsiflexion</td>
<td>S1: slow withdrawal of foot on firm pinch</td>
</tr>
<tr>
<td>M2: weak curling of toes</td>
<td>S2: rapid withdrawal, comparable to control</td>
</tr>
<tr>
<td>M3: strong curling of toes</td>
<td></td>
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<tr>
<td>M4: normal resistance to dorsiflexion, gait restored</td>
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### Table II. Time to early functional recovery recorded as days after operation

<table>
<thead>
<tr>
<th></th>
<th>Sensory (S1)</th>
<th>Motor (M1)</th>
<th>Trophic ulcers (healed)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control*</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>End-to-end suture</td>
<td>40 to 50</td>
<td>50 to 60</td>
<td>no ulcers</td>
</tr>
<tr>
<td>Nerve autograft</td>
<td>35 to 40</td>
<td>50 to 60</td>
<td>no ulcers</td>
</tr>
<tr>
<td>Fresh muscle graft</td>
<td>45 to 50</td>
<td>50 to 60</td>
<td>no ulcers</td>
</tr>
<tr>
<td>Denatured muscle graft</td>
<td>21 to 30</td>
<td>28 to 35</td>
<td>no ulcers</td>
</tr>
<tr>
<td>Experimental*</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Denatured muscle graft: BCG</td>
<td>28 to 35</td>
<td>50 to 60</td>
<td>70 to 80</td>
</tr>
<tr>
<td>Denatured muscle graft: <em>M. leprae</em></td>
<td>32 to 40</td>
<td>50 to 60</td>
<td>70 to 80</td>
</tr>
</tbody>
</table>

*there were five animals in each control group and 15 in the experimental groups*
and skin specimens were taken both from the experimental and from the contralateral sides. Sections of graft and of the proximal and distal nerve trunks were fixed in 2.5% cacodylate buffered glutaraldehyde for one hour, trimmed and cut to give approximately 1 mm transverse sections, fixed for another hour then washed in buffer. After fixation overnight with 1% cacodylate buffered osmium tetroxide, the tissues were washed in 70% ethanol, dehydrated through graded ethanols and impregnated and embedded in Araldite. One-micron transverse sections of the whole nerve were stained with toluidine blue.

Ultrathin sections were cut at 70 to 90 nanometres, stained with Reynold's lead citrate followed by saturated uranyl acetate in methanol and viewed on an AEI Corinth transmission electron microscope. The soleus and interossei muscles and the footpad skin were stored in formal saline for further staining procedures for the assessment of motor and sensory re-innervation, and the flexor digitorum longus was snap-frozen for muscle fibre-typing. The results of these studies will be reported elsewhere.

RESULTS

Clinical results. Using the scoring system shown in Table I, it was found that:

a) after muscle grafting of healthy guinea-pig tibial nerves, early sensory recovery (SI) was evident between 21 and 30 days and early motor recovery (M1) was seen between 30 and 35 days;

b) after muscle grafting of granulomatous tibial nerves, return of sensory function was seen between 28 and 40 days and early motor recovery was evident by 50 to 60 days. The BCG injected animals showed earlier sensory recovery;

c) the other control groups (Table II) exhibited slower sensory recovery;

d) trophic ulcers (ranging from missing toes to amputations of the foot) occurred only in experimental animals and all lesions healed spontaneously by 80 days after operation.

At 150 days, all muscle grafted animals showed excellent return of function (Table III). Early recovery was better with muscle grafts, but by 150 days end-to-end suture had resulted in more complete recovery.

Histological results

Grafting of healthy control nerves. An electron micrograph of a tibial nerve in transverse section shows myelinated axons (Fig. 1a). At 50 days, the muscle graft showed only small numbers of small diameter, thinly myelinated axons arranged into minifascicles. In a 150-day muscle graft minifascicles are seen (Fig. 2b) and there are regenerating

<table>
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<th>Table III. Functional recovery at 150 days</th>
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<td></td>
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<td>Control†</td>
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<td>Denatured muscle graft</td>
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| Experimental†               |
| Muscle graft: BCG          | 0  | 10  | 5  |    |    |    |
| Muscle graft: M. leprae    | 0  | 14  | 1  |    |    |    |

*a level of scoring – see Table I for explanation †there were five animals in each control group and 15 in the experimental groups

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**Fig. 1a**
Electron micrograph of nerves before muscle grafting. Stained with lead citrate and uranyl acetate. Magnification ×3000. Figure 1a – Normal guinea-pig tibial nerve showing normal arrangement of myelinated and unmyelinated axons. Figure 1b – Macrophage granuloma induced by intraneural injection of M. leprae showing gross destruction of nerve fibres and extensive fibrosis, arrow indicates bacilli within macrophages. Figure 1c – Epithelioid cell granuloma induced by intraneural injection of BCG, arrow shows an epithelioid cell adjacent to remaining nerve fibres.
DENATURED MUSCLE GRAFTS FOR NERVE REPAIR

Light micrographs of one-micron Araldite-embedded transverse sections of nerves, stained with toluidine blue. Magnification \( \times 180 \); a) to c) 150-day freeze-thawed muscle graft across a normal nerve a) proximal nerve b) graft showing regenerating fibres within minifascicles c) distal nerve showing regenerating nerve fibres; d) to f) 150-day muscle graft across an \( M. \) legnoe-damaged nerve d) proximal nerve e) graft showing regenerating fibres f) distal nerve showing good repopulation with myelinated fibres; g) to i) 150-day muscle graft across BCG-damaged nerve g) proximal nerve h) graft showing good regeneration i) distal nerve.

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axons in the distal nerve segment (Fig. 2c). At the ultrastructural level (Fig. 3a), the 150-day muscle graft shows large numbers of minifascicles, containing both small and larger diameter myelinated axons and many unmyelinated fibres. There is a large amount of collagen between the minifascicles. The distal segment, 150 days after grafting (Fig. 3b), shows good repopulation with regenerating fibres, with small and large diameter myelinated axons. Schwann cells are seen in characteristic relationship to the axons. At 50 days, the muscle graft showed only small numbers of small diameter, thinly myelinated axons arranged in minifascicles.

**Grafting of M. leprae-damaged nerves.** Intraneural injection of *M. leprae* produces persistent macrophage granulomas similar to those seen in lepromatous leprosy. There is destruction of most nerve fibres and extensive fibrosis (Fig. 1b). A light micrograph of a 150-day muscle graft in an *M. leprae*-damaged nerve (Fig. 2e) shows many regenerating nerve fibres in minifascicles, and an abundance of collagen. Figure 2f shows regenerating nerve fibres in the distal segment. At electron microscopic level, the 150-day graft (Fig. 4a) shows multiple minifascicles containing thinly myelinated axons of varying diameters. Scattered mononuclear cell infiltrate and fibrosis were also seen. In the distal nerve (Fig. 4b) the regenerating fibres are surrounded by much more collagen than in normal grafted nerves or in BCG-damaged grafted nerves.

**Grafting of BCG-damaged nerves.** Endoneural injection of BCG produces epithelioid cell granulomas similar to those of tuberculoid leprosy, and there is rapid destruction of most nerve fibres (Fig. 1c). Fifty days after repair of the nerve with a muscle graft, the graft and the distal nerve show large amounts of collagen and an abundance of unmyelinated fibres but a much smaller number of myelinated axons compared with a normal nerve which has been grafted. The general architecture of the graft was grossly altered by extensive mononuclear cell infiltration. By 150 days after repair, however, the graft showed much less infiltrate, less collagen and many regenerating axons in minifascicles (Fig. 2h). Figure 2i shows repopulation of the distal stump with large numbers of myelinated axons. The proximal nerve at 150 days was often abnormal (Fig. 2g).

**DISCUSSION**

The guinea-pig was chosen for this study because, unlike the rat, consistent experimental mycobacterial granulomas can be produced in its nerves. The rate of nerve regeneration is much slower in the guinea-pig than in the rat and much larger amounts of collagen are seen after grafting, both in the muscle grafts and in the distal nerve segments. There are, however, some limitations to the use of our guinea-pig as a model. First, in the guinea-pig, granulomatous damage is produced locally at the site of the endoneural injection and damage to the distal nerve only occurs secondarily, whereas, in the human disease, the distal nerve may be damaged before proximal lesions occur. Secondly, in one of the experimental groups, cobalt-irradiated *M. leprae* organisms were used. These organisms are metabolically active but unable to multiply. Despite these limitations, the guinea-pig proved to be a useful model.

The early clinical results showed that all animals treated by denatured muscle grafts (control, BCG and

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![Fig. 3a](image1.png) ![Fig. 3b](image2.png)

Electron micrographs of 150-day freeze-thawed muscle graft across normal guinea-pig tibial nerve. Stained with lead citrate and uranyl acetate. Magnification × 3000. Figure 3a – Graft showing minifascicle (arrow) formation containing both myelinated and unmyelinated axons. Figure 3b – Distal to a), showing regenerating myelinated axons surrounded by collagen.

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M. leprae) had earlier sensory recovery than those in which any other method of repair was used. Trophic ulcers, which occurred in the experimental animals, all healed spontaneously; this was a useful indication of sensory recovery.

The proximal nerve stump looked inflamed at the time of muscle grafting in most animals, and sections taken at the end of the experiment confirmed the macroscopic observations. The nerve distal to the muscle graft always looked inflamed and 'empty', both at the time of grafting and at 150 days. This compares with the clinical condition, where the distal nerve is always affected by varying degrees of fibrosis. The technique of muscle grafting was made easier in granuloma-induced nerves, because of the thickened nerve sheath.

In spite of the histological evidence of inflammation in the experimental guinea-pig muscle grafts and sometimes in the proximal nerve, fibres were able to regenerate through this environment. Importantly, our clinical and morphological studies also show that nerve regeneration can take place through the unhealthy distal nerve.

Although leprosy involves many tissues, damage to nerves causes the greatest deformity and disability (Brand and Fritschi 1985). Much of the nerve destruction is permanent; there is great need for methods of direct treatment, so that regenerating fibres can reach the target organs before the latter undergo irreversible changes.

Current surgical treatment for nerve damage aims to correct the deformities resulting from motor nerve damage, but there is no standard procedure for the sensory defects, which cause trophic ulceration and absorption of digits. Patients with successful tendon transfers may still be left with areas of anaesthesia in their hands. Some sensory recovery is possible after nerve decompression, even when carried out up to 10 years after the initial sensory loss. Theuvenet et al (1988) and Ozkan et al (1988) have reported improved sensation after the transfer of functioning sensory nerves from relatively less important areas of the hands. Muscle grafting may help to restore or improve sensory function, and we are encouraged especially by the early and consistent sensory recovery we noted in this experimental model. It seems possible that in acute neuritis, a well-timed excision of the area of granulomatous nerve damage and repair with a freeze-thawed, co-axially aligned, autogenous muscle graft could preserve both sensory and motor function. Nerve damage occurs relatively quickly, so early muscle grafting may be beneficial in allowing regenerating axons to repopulate the distal nerve before nerve fibrosis and irreversible muscle damage takes place.

Our studies have shown good correlation between clinical recovery and morphological evidence of nerve regeneration. When the results of morphometric and electrophysiological assessments become available we hope that the technique of muscle grafting will be considered for the treatment of certain types of nerve damage in leprosy.

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Fig. 4a

Electron micrographs of 150-day muscle graft across M. leprae-damaged nerve, stained as above. Magnification ×3000. Figure 4a – Graft showing similar features to Figure 3a, but with more collagen. Figure 4b – Distal to a), showing similar features to Figure 3b, but with more collagen.
REFERENCES


