Fascicular nerve allograft evaluation

Part 1: Comparison with autografts by light microscopy

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In a comparative experiment, transplantation of orthotopic nerve fascicle was performed on 100 sciatic nerves between inbred, antigenically identified rats. The authors studied cellular response, macrophage reaction, connective-tissue reaction, myelination, and distal/proximal axonal ratios, but no difference could be established between allografted and autografted fascicles after 2 months. Moreover, there was no evidence of any graded rejection phenomenon dependent on differences of tissue typing. Theories for the decreased antigenic reaction of nerve fascicles are proposed.

KEY WORDS □9 fascicular nerve grafting □9 tissue typing □9 antigenicity

DURING the century since 1878 when Alpert resected a sarcoma of the median nerve and replaced it with a 3-cm portion of nerve previously obtained from an amputation, the use of nerve allografts has continued to challenge and vex surgeons. On the one hand, the convenience and availability of this technique make them appealing; nerves can be removed from cadavers and stored until an appropriate situation presents itself. On the other hand, the disadvantage of nerve allografts has been the rejection and ultimate fibrous tissue replacement of many of the grafts.

A peripheral nerve is composed of axons that are grouped together into fascicles. These fascicles are surrounded by a connective tissue layer, the perineurium. Any given nerve may contain one or more fascicles, and these in turn are encompassed by a thick fibrous capsule, the epineurium.

Our knowledge of nerve allograft antigenicity is fragmentary; consequently, the effect of tissue typing on nerve allograft acceptance has not been adequately explored. In 1971, Palm and Black described the genetic loci in the rat (AgA, AgB, AgC, and AgD) that determine cellular antigens. The AgB histocompatibility locus of the rat, corresponding closely to the HL-A locus in man, is primarily responsible for reactions to transplantation. The other genetic loci appear to be of lesser importance in the kinetics of transplantation. The degree of difference at the AgB locus in the rat and the HL-A locus in man is of prime importance with respect to the manner in which a host animal identifies allografted tissue.

An allograft is defined as a tissue transplanted between two animals of the same species, while an autograft is a tissue that is grafted from one location to another in the
FIG. 1. Photomicrographs of sections through fascicular allografts. H & E, X 16. Upper Left: Fisher (AgB1) → Lewis (AgB2) with 2+ lymphocytes but no macrophage reaction. Upper Right: Buffalo (AgB6) → Fisher (AgB1) with no lymphocytes and minimal macrophage response. Note suture in perineural tissue. Lower Left: Buffalo (AgB6) → Wistar (AgB2) with no lymphocytes and rare macrophages. Lower Right: Lewis (AgB1) → Wistar (AgB2) with no lymphocytes and mild macrophage response.

same animal. A tissue removed from one animal and placed in another animal that is antigenically identical, that is, an identical twin or the same strain of inbred animal, comprises an isograft.

With these facts in mind, we devised the following experiment to determine the rejection response elicited by allografted nerve fascicles of known major transplantation antigen composition when compared to fascicular autografts.

Materials and Methods

We operated on 100 rat sciatic nerves. In each pair of animals, one fascicle from each limb was removed and transplanted to another limb, thus creating two allografts and two autografts between each pair of rats. Four inbred strains of rat were used: 1) Fisher (AgB1), 2) Lewis (AgB1), 3) Wistar (AgB2), and 4) Buffalo (AgB6). The groupings created by fascicular transplantation are listed in Table 1.

Male animals weighing from 300 to 500 gm were used. Their ages ranged from 3 to 5½ months, but the majority were 4 to 5 months old. The rats were anesthetized with intraperitoneal pentobarbitol 3 mg/100 gm weight. The hind limbs were clipped, and depilatory cream was applied and then removed. Clean but unsterile technique was used to expose the proximal sciatic nerve where it exited from its bone canal down to the popliteal region. At these levels, the sciatic nerve is composed of two to four
Experimental fascicular nerve grafts

fascicles, approximately 0.8 mm in diameter. The operating microscope (×16 magnification with a 200-mm lens) was used for the fascicular dissection. One small fascicle was sharply dissected away from the main nerve, with caution taken to preserve the perineurium. A 1-cm section of fascicle was then removed and immediately transplanted either to the other limb of the same animal (autograft) or to the limb of another inbred animal (allograft). The gap left by removal of the original fascicle was filled with a fascicle from either of the two aforementioned sources. The fascicular grafts were secured with one or two 10-0 nylon perineurial sutures at the proximal and distal anastomoses. Care was taken to eliminate any tension on the anastomotic sites. The muscle was approximated, and the skin was closed with 3-0 Dexon suture. The animals were returned to their cages without any external splinting.

The rats were observed two to three times a week to identify any wound or ulcer complications. Two months after transplantation, the animals were reanesthetized, and the nerves were removed with 3- to 5-mm of recipient nerve and placed in 10% formalin. Animals in representative groups also underwent lower extremity gluteraldehyde perfusion (subject of another report). All animals were then killed with intracardiac MgSO₄ (magnesium sulfate).

The nerves were then embedded and cut into proximal, graft, and distal segments. Staining was accomplished with hematoxylin and eosin, Weil’s, Bielschowsky’s, and Masson trichrome stains. A neuropathologist (W.J.B.) rated cellular responses and connective-tissue responses on a 0 to 4 scale (0 = none, 4 = severe). Axon distal/proximal ratios were approximately at 25% increments. Distal myelination was noted, and macrophage response was described.

Results

It was impossible to differentiate any allografted fascicle from its autografted mate by cellular reaction, macrophage response, connective-tissue proliferation, distal myelination, or estimated axonal ratios. Of the eight nerves that exhibited any cellular response (seven graded 1+ and one, 2+), five were nerves grafted between Fisher (AgB₁) and Lewis (AgB₁) rats, another between Wistar (AgB₂) and Buffalo (AgB₄) rats, and the last appeared in an autografted fascicle (Figs. 1-3).

The connective-tissue response was 1+ to 2+ in almost all fascicle. There was no distinction between autografted and allografted nerves. The only variable appeared to be intrafascicular suture which, when present, was associated with an increased amount of connective tissue. The macrophage response varied considerably among animals, but there was no consistent relationship between antigenic mixture and macrophage reaction.

TABLE 1

Fascicular transplantation groups

<table>
<thead>
<tr>
<th>Groups</th>
<th>No. of Rats</th>
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<tbody>
<tr>
<td>allografted fascicles</td>
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<tr>
<td>Lewis to Fisher</td>
<td>6</td>
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<tr>
<td>Fisher to Lewis</td>
<td>6</td>
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<tr>
<td>Wistar to Lewis</td>
<td>6</td>
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<tr>
<td>Lewis to Wistar</td>
<td>5</td>
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<tr>
<td>Fisher to Buffalo</td>
<td>6</td>
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<td>Buffalo to Fisher</td>
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<td>Buffalo to Wistar</td>
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<td>Wistar to Buffalo</td>
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<tr>
<td>isografted fascicles</td>
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<td>Lewis to Lewis</td>
<td>6</td>
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<tr>
<td>autografted fascicles</td>
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<tr>
<td>autografts</td>
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FIG. 3. Photomicrograph of section through fascicular isograft. Graft taken from one Lewis rat (AgB1) and transplanted to another. There was no lymphocytic reaction but a mild macrophage response was seen. Note misplaced suture within the fascicle. H & E, × 16.

Both distal myelination and approximate axonal ratio showed some variability within groups, but no relationship between these parameters and major transplantation antigen could be established.

Discussion

Fresh whole-nerve allografts have consistently produced lymphocytic and macrophage infiltration, which was easily detected upon examination at 60 days after transplantation. Various forms of treatment and/or manipulation, such as predegeneration, freeze drying, irradiation, immunosuppression, cell destruction with cialit, have reduced this cellular response. In the final evaluation, however, no treatment has abolished it. All of these therapeutic modalities bear inherent risks and undesirable effects that must also be considered. Predegeneration requires an additional surgical procedure; irradiation and cell destruction with cialit produce endoneurial fibrosis, which obstructs axonal growth, and systemic immunosuppression is accompanied by the gamut of complications associated with that form of therapy.

Marmor and Das Gupta have both suggested that tissue typing of nerve allografts might be beneficial, but the finding that the allografted fascicle was histologically indistinguishable from an autografted fascicle was unexpected. We were also unable to find any evidence of a graded rejection dependent on the degree of antigenic difference.

Antigenicity is characteristically inherent in most living tissue, although for some time it was believed that myelin was the prime antigenic offender in nerve allograft rejection. This premise now seems unlikely. Myelin proteins have definite antigenic properties that can be easily demonstrated in experiments evaluating experimental allergic neuritis. However, if they represented the major antigenic component, there is no reason why a single fascicle would then appear antigenically identical to an autograft. Moreover, volume of transplanted tissue can be a variable if weak antigens are involved, yet previous experiments utilizing small volumes of rat nerve have elicited adequate rejection responses.

Another alternative conclusion would be that the major transplantation antigens reside predominantly in the epineurial and surrounding connective tissues. If this is the case, fascicular or perineurial grafts would be less antigenic because these surrounding tissues would be removed.

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