Irradiated Experimental Nerve Heterografts Pretreated with Specific Antiserum

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The immunologic mechanisms involved in the rejection of transplanted tissue have received considerable attention since Medawar\textsuperscript{6,10} established the importance of immunologic phenomena in the rejection of homografts. There has been little reported, however, regarding nerve grafts. Alvord\textsuperscript{4} in 1949 studied meningoencephalomyelitis using peripheral nerve with adjuvant to produce antiserum and found that it would react against peripheral nerve tissue. Studies of anti-peripheral nerve serum by complement fixation have been reported in reference to disseminated encephalomyelitis using monkey peripheral nerve combined with adjuvant to develop antiserum in rabbits.\textsuperscript{6} Waksman and Adams\textsuperscript{14} in 1955 reported similar work without adjuvant, using human and dog peripheral nerve. They found rabbits inoculated with human nerve to have greater titers with heterologous nerve than with homologous, while with dog nerve, the reverse was true.

Previous study using the double immunodiffusion method of Ouchterlony revealed uniformly negative results when postoperative serum was tested after nerve grafting with homografts or heterografts. When nerve extracts in Freund's adjuvant were injected into rabbits, the antiserum gave dense, clear lines of precipitation with the corresponding nerve extracts. When the antiserum was absorbed with normal serum from the immunizing species, however, no precipitation lines developed between the nerve extract and the corresponding antiserum. It seemed probable that antibodies to serum protein were involved.

Immunologic enhancement of tumors was defined by Kaliss\textsuperscript{5} in 1958 as the progressive growth of homografts produced by contact with specific antiserum in the host. In 1960 Snell, et al.,\textsuperscript{13} tested two hypotheses of the mechanism: the physiologic alteration as proposed by Kaliss, and the possible blockage of immunity in lymphoid tissue induced by contact with antiserum. They concluded that cellular immunity, rather than humoral antibody, inhibited the growth of most grafts and the depression of this immunity by antibody was favorable to the growth of a homograft.

The purpose of this paper was first to evaluate the cellular changes in the regional lymph nodes draining the sites of irradiated implanted heterografts and then to explore the effect on the response of the host to heterologous nerve pretreated with graft-specific antiserum.

Materials and Methods

The nerve grafts were removed from fresh human cadavers under conditions as aseptic as possible and immediately packaged in heat-sealed polyethylene bags and frozen to \(-12^\circ\text{F}\). The grafts were maintained in a frozen state and irradiated with \(2 \times 10^8\) rep by a Van de Graaff generator. The grafts were then stored in a freezer at \(-12^\circ\text{F}\) until used.\textsuperscript{7,8}

Regional Lymph Node Study. A study was made of the cellular changes in the regional lymph nodes draining the sites of implanted grafts in the ear of the rabbit. These changes were correlated with the response of the rabbit immune serum to homologous nerve extracts by means of immunodiffusion plates.

Eight albino New Zealand rabbits, 2 months old, were used. Irradiated and non-irradiated human nerves were cut into 1-cm segments and subcutaneously implanted through a 1-cm incision in the rabbit ear. The wound was closed with 3-0 chromic suture. The rabbits were not anesthetized. Pairs of rabbits were sacrificed at 1, 2, and 4 weeks after implantation. One rabbit was used as a normal control, and one rabbit had the 1-cm incision but no implant. The regional lymph nodes draining the implants and the contralateral lymph nodes were removed and the size and gross findings compared. The nodes

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were placed in neutral formalin, sectioned, and stained with methyl green-pyronin. In addition, sera were removed from the rabbits to be tested against extracts of nerves in double immunodiffusion plates.

Specific Antiserum Study of Nerve Graft. The nerve was thawed, trimmed of extraneous fat and tissue, washed in cold buffered saline, and weighed. The tissue was minced with scissors and added to an equal volume of cold phosphate-buffered saline at pH 7.2. This mixture was homogenized in a Ten Broeck homogenizer, left to stand overnight at 5°C, filtered through gauze, and centrifuged at 2000 rpm for 10 minutes. The resulting extract was dispensed in small aliquots and stored at −12°C. The amount of antigen was estimated on the basis of the weight of the nerve in milligrams per milliliter of solution.

Immunizing Emulsion and Procedure. Landers-Contes rabbits, weighing 2 to 2.6 kg, were immunized. Each rabbit was injected with approximately 500 mg of nerve tissue contained in phosphate buffered saline and an equal volume of Freund's incomplete adjuvant. Subcutaneous injections of 0.5 ml were made in each of four sites in the nuchal region on either side of the vertebral column, making a total dose of 2 ml. The animals were bled by cardiac puncture at 30 days. The undiluted antiserum was used for the tests.

Pretreatment of Grafts. The nerve was cut into 0.5-cm and 4-cm segments, covered with the undiluted antiserum, and incubated overnight at 5°C. It was then centrifuged for 10 minutes at 2000 rpm, and the serum removed and retained for serologic study. The nerve was washed in sterile saline and the procedure repeated twice more. The nerve was covered with serum and then frozen at −12°C. Nerves were washed in sterile saline before use (Fig. 1).

Implants in Rats. Twenty-four rats were implanted with 0.5-cm segments of treated and untreated human nerve through a 1-cm subcutaneous incision in the back. Implants were placed approximately 3 cm away from the wound to avoid local scar tissue. The wound was closed with metal clips. The rats were explored at 1, 2, 3, and 6 weeks after surgery. Gross examination and microscopic study of the implants were performed to evaluate the reactions produced.

Grafts in Dogs. Six adult mongrel dogs were divided into two groups. The first group received 4-cm treated grafts and the second group received 4-cm untreated grafts. The nerves were washed in sterile saline before grafting. A 10-cm longitudinal incision was made along the peroneal nerve at the lateral knee joint. After the peroneal nerve was identified by a Medtron stimulator, a 1-cm portion of the nerve was removed and the graft sutured into the defect with three 6-0 nylon sutures at each end. One dog of each group was explored and killed at 2, 3, and 6 months. Gross observations and nerve action potentials were recorded and sections of the nerve graft examined microscopically.

The action potential was measured in a Lucite nerve chamber, using a Tektronix pulse generator. A Tektronix a-c preamplifier was used to record diphasic action potentials. Signals were displayed on an oscilloscope, negativity downwards.

Results

Lymph Node Study. Gross observations after 1 week showed the rabbit wounds to have small linear scar formation and to be completely healed. The rabbits appeared healthy and showed no weight loss. Upon exploration at 1, 2, and 4 weeks, the regional lymph nodes removed from sites draining the implants were always twice the size of the contralateral lymph node and of the normal node. At 1 week there was gross evidence of edema around the nonirradiated implant. Microscopically, there were scattered foci of hemorrhage in the graft and inflammatory round cells with small lymphocytes which invaded from the periphery to the center part of the