Experimental Prevention of Nerve Homograft Rejection by Use of Immunosuppressive Drugs*

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The homotransplantation of nerves is not affected by one major problem encountered in transplantation of kidneys and other organs. These organs require survival of the actual cells in order to achieve a successful transplant, whereas the nerve homograft acts only as a tube for regeneration of the host nerve axons through it. It seems plausible, therefore, that immunosuppressive drugs could be used in nerve grafting until regeneration takes place and then discontinued. At the present time kidney homografts have succeeded for long periods of time during which immunosuppressive drugs have have been used without adverse effects on the host.5-7

The drug selected for study was 6-(1-methyl-4-nitro-5-imidazolyl)thiopurine, commonly called Imuran. During research on thiopurines in mice, the drug suppressed the formation of hemagglutinins to sheep cells and also inhibited the immune reaction to renal transplants in animals.3

Other investigators have confirmed this suppression of the immune response in a variety of animals. In investigations of organ homografts, it was apparent that Imuran would increase survival; in skin homografts, however, Imuran was much less effective.

The dosage advised for humans, according to Rundles,8 varies from 1.5 to 3 mg/kg/day. Patients were able to take 1.5-2 mg/kg/day (100-150 mg/day) for long periods without signs of toxicity. Higher doses, which are at times necessary, may produce anorexia, nausea, and malaise.

Murray9,7 set up the following protocol for his patients receiving renal transplants:

Imuran: 2-4 mg/kg/day orally.
Azaserine: 0.5 to 1.0 mg/kg intravenously for 21 days.

Prednisone: 60-80 mg orally for 6-7 days, then 15 mg thereafter if rejection does not reverse with Actinomycin C.

Actinomycin C: 6 mg/kg intravenously weekly starting at Day 21.

Toxicity has not been a major problem in organ transplantation, and, since the lower doses could be used in nerve transplantation, toxicity would not be a hindrance.

Experiment 1

Method. An experiment was set up to study the effect of an immunosuppressive drug on homotransplantation of peripheral nerves and to compare it with irradiation of the graft and with a combination of both methods.3,4 Thirty-two Sprague-Dawley rats weighing 250-300 gm were used in the study.

Imuran was selected as the immunosuppressive drug because of its wide use in renal homotransplantation. A dose of Imuran was used that would have minimal toxic effect, since the drug could only have value in nerve transplantation if toxicity was of no concern. The oral dose was therefore established as 200 mg/kg of diet, a dose found to have minimal toxicity in previous studies.

The rats were administered the drug orally in their regular laboratory feed for 5 days. A 1 cm segment of homogenous sciatic nerve was then implanted subcutaneously in the anterior thigh of half of the rats. In the other half an irradiated homograft was implanted subcutaneously. The same dose of Imuran was continued during the study, and no evidence of toxicity from the drug was observed.

Results. The animals were killed at weekly intervals and microscopic examination performed on the nerve implants using hematoxylin and eosin stain. At 2 weeks there was no evidence of destruction of the irradiated graft (Fig. 1 A). The nonirradiated graft was also intact with little evidence of inflamma-

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Fig. 3 A. A nonirradiated implant was used as a control in a rat that was not treated with Imuran. Marked destruction is taking place due to rejection at 5 weeks. H. & E., x44.

tory response (Fig. 1 B). Examination of the non-irradiated implant at 5 weeks revealed very little inflammatory response although some of the axis cylinders were disrupted (Fig. 2 A). The nonirradiated implant at 5 weeks revealed very little evidence of rejection (Fig. 2 B). The results were graded on the evidence of inflammatory response both grossly and microscopically (Table 1). The response was graded from 0 (no response) to 5 (severe destruction and replacement by scar tissue).

It appeared from these results that Imuran at nontoxic dosages has a significant immunosuppressive action on the rejection of nonirradiated homografts (Fig. 3).

Experiment 2

Method. In this experiment Imuran was used to try to prevent rejection of nerve

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<thead>
<tr>
<th>Regret response of irradiated and nonirradiated nerves</th>
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<tbody>
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<td>1 Week</td>
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<tr>
<td>Rat 1</td>
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<tr>
<td>Irradiated nerve</td>
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<td>Nonirradiated nerve</td>
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0—Well-preserved nerve.
1—Slight to moderate edema, retention of axis cylinder morphology.
2—Marked edema, some round cell infiltration, cylinder well preserved.
3—Edema, round cell infiltration, and slight collagen deposit. Cylinders slightly disrupted.
4—Scarring, early vascularization, disrupted cylinders.
5—Replaced by scar tissue. No definite axis cylinders remain.
Prevention of Nerve Homograft Rejection

**FIG. 1 A.** An irradiated subcutaneous implant homograft at 2 weeks reveals no evidence of rejection. There is no sign of inflammation about the graft or invasion of cells. The implant has also been protected by Imuran. H. & E., x 150.

**FIG. 1 B.** A nonirradiated subcutaneous homograft at 2 weeks shows little evidence of inflammatory response to the graft. Imuran appears to protect the implant from destruction. An unprotected implant at 2 weeks would show marked nerve invasion. H. & E., x 150.

**FIG. 2 A.** An irradiated implant at 5 weeks shows no evidence of rejection, but there is some loss of axis cylinders at this time. H. & E., X150.

**FIG. 2 B.** The nonirradiated implant at 5 weeks is also well-protected by the Imuran and there is no inflammatory response. H. & E., X150.
heterografts in rats. The upper and lower facial nerves in the rat were selected for study. One cm grafts of a peripheral nerve of a dog were sutured into the upper facial nerve of 12 rats. The lower facial nerve was cut and resutured as a control. The rats were then randomly divided into two groups of 6 each. Administration of Imuran (36 mg/kg day) was begun 4 days prior to surgery in Group 1 and was not started until 12 days after surgery in Group 2. All the rats were checked every 3 days for recovery of nerve function by cutaneous electrical stimulation. A whisker twitch on electrical stimulation was considered a positive result and evidence of regeneration. At 49 days the incision was opened for inspection, and the nerve was stimulated directly.

Results. All of the control nerves recovered function. The rats in Group 1 (immediate Imuran) took longer to recover than those in Group 2 (delayed Imuran). None of the heterografts recovered function, but Imuran definitely protected the grafts from immune inflammatory response in Group 1. The Group 2 rats (delayed Imuran) showed almost complete destruction of the graft.

Conclusions
It was apparent from these studies that the immune rejection response to nerve homografts can be prevented in animals by Imuran in nontoxic dosages. Comparison of implants not protected by Imuran revealed a significant difference in the inflammatory response.

Experience in patients with irradiated homografts alone has shown that regeneration does occur, but not consistently. Therefore, the addition of Imuran to the pre- and postoperative therapy may enhance the regeneration of peripheral nerves through the homograft. It is known that regeneration through a graft unprotected by irradiation or immunosuppressive drugs is prevented by an inflammatory response and scar formation in the axis cylinders. Our study has demonstrated that, in rats, Imuran can prevent the rejection phenomena in nerve homografts.

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References