Allergic Encephalomyelitis: Passive Transfer Across Major Histocompatibility Barrier[1] (35097)

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Experimental allergic encephalomyelitis (EAE) is an autoimmune disease of the nervous system with features characteristic of delayed hypersensitivity. It has been transferred passively with living, immunized lymphoid cells. Passive transfers have succeeded when EAE cells were injected into inbred recipients that were isogenic with or genetically tolerant of the donors (Lewis → Lewis, or Lewis → (Lewis × BN)F_1 hybrids) (1, 2). Successful transfers of EAE to *non-inbred* rats have made use of parabiosis (3), neonatal tolerance procedures (4), donor splenectomy (5), or intracerebral inoculation (6). The degree of incompatibility overcome in experiments on non-inbred animals is uncertain because of lack of genetic definition and the inadvertent inbreeding attendant upon closed colony commercial breeding. In genetically defined experiments, transfers of EAE have been made despite minor histocompatibility barriers, and such transfers were enhanced by adrenalectomy of recipients or focal impairment of their blood–brain barrier (2). These methods were of no avail, however, when there were major histocompatibility differences between donor and recipient (2). In the present work a *major* histocompatibility barrier has been overcome by treatment of the recipients with an immunosuppressive drug and cells bearing donor antigens, in advance of the EAE transfer.

Methods. Donors of EAE cells were female Lewis rats (Ag-B^1). Recipients were male BN rats (Ag-B^3). The Ag-B genetic locus determines a major histocompatibility difference (7). The BN recipients were pretreated with living lymphoid and marrow cells from normal (Lewis × BN)F_1 hybrids, 4 hr after injection with the immunosuppressive drug, cyclophosphamide (8, 9). Hybrid cells were used as a source of donor-type antigens in preference to Lewis cells in order to avoid a graft-versus-host reaction that might follow injection of Lewis cells into immunologically depressed BN rats (hybrid cells are genetically incapable of mounting a graft-versus-host reaction against a parental strain). Washed hybrid spleen and bone marrow cells were injected separately into the dorsal penile vein, and washed lymph node and thymus cells were pooled and injected into the peritoneal cavity of the BN recipients. Doses of approximately 0.6×10^9, 0.5×10^9 and 1.1×10^9 cells, respectively, derived from a single hybrid, were injected into each BN recipient. In the three experiments reported, the doses of cyclophosphamide were 100, 125, and 150 mg/kg of body weight, respectively, dissolved in water and injected immediately intraperitoneally. In the experiment with the lowest dose, the hybrid cell treatment (but not the drug) was repeated 3 times at 5- or 6-day intervals. These variations did not influence the results, therefore the results were pooled in Table I.

Seventeen or 19 days later, transfer of EAE lymph node cells from Lewis donors to the pretreated BN recipients and to Lewis controls was performed (2, 10). Lewis donors were immunized with 10 mg (wet wt) of guinea pig spinal cord tissue emulsified in Freund's complete adjuvant; the dose of 0.05 ml was injected into one of the right hind

[1] Supported wholly by Grant 536-B-11 from the National Multiple Sclerosis Society.

TABLE I. Passive Transfer of EAE from Lewis Donors to BN Recipients Despite Major Histocompatibility Barrier.[a]

Recipient[b]	Pretreatment[c]	No. of rats with EAE score[d] 2+	1+	0
Lewis	Cy + hybrid cells	7	0	0
	None	12	0	0
BN	Cy + hybrid cells	7	12	4
	Cy only	0	0	6
	Hybrid cells only	0	0	4
	None	0	0	8

[a] Composite of 3 experiments, each of which included positive controls (untreated Lewis recipients) and negative controls (nontolerant BN recipients).

[b] All rats received transfer of EAE lymph node cells from Lewis donors.

[c] Cy = cyclophosphamide; hybrid cells = spleen, lymph node, marrow, and thymus cells from normal (Lewis × BN) F_1 hybrids.

[d] Histological score of perivascular EAE lesions adjacent to thermal injury of brain.

foot pads. At the same time, 0.1 ml of concentrated pertussis vaccine (about 20 billion organisms) was injected into the dorsum of the same foot as an ancillary adjuvant. Seven days later, when many donors had early signs of EAE, their lymph nodes draining the sites of inoculation were processed into a cell suspension and injected into the dorsal penile veins of the recipients. The donor:recipient ratio was 1:1. The EAE transfer was delayed for 17 or 19 days after the preliminary procedure because cyclophosphamide treatment of recipients inhibits passive transfer (11). A preliminary experiment indicated that considerable inhibition could be expected as late as 8 or 10 days after 100 mg/kg of cyclophosphamide. In order to accelerate the production of passive EAE, a focal brain injury was produced in all the recipients by applying a preheated soldering iron for 7 sec to the intact skull 2 or 3 days before the transfer (10). This added procedure induces rapid localization of EAE lesions in the region of the thermal injury and allowed us to sacrifice the recipients 1 day after lymph node cell transfer. Hematoxylin–eosin stained sections of forebrain were randomized and studied for perivascular inflammatory infiltrates of EAE near the zone of thermal coagulation necrosis, without knowledge of group of origin.

Results. Injection of Lewis EAE cells transferred severe EAE into all Lewis recipients, as previously reported (2, 10) (Table I). Pretreatment of these isogenic recipients with cyclophosphamide and hybrid cells had no effect on the transfer. Lewis EAE cells were unable to transfer EAE into BN recipients even if these allogeneic recipients were pretreated with cyclophosphamide alone or hybrid cells alone. However, pretreatment of BN rats with both cyclophosphamide and hybrid cells permitted Lewis cells to transfer EAE across this major histocompatibility barrier (Fig. 1). The transferred EAE was not as uniform or as intense in allogeneic as in isogenic recipients. A few BN recipients had negative results, the majority had 5 to 15 lesions on the entire slide (1+ score) and even those scored 2+ did not have as many or as heavy infiltrates as the Lewis recipients. Presumably, the nonuniformity of these results reflects various degrees of success of the preliminary treatment.

To exclude the remote possibility that the observed inflammatory brain lesions were not EAE but were in some way caused by the preliminary procedure, two BN rats not included in Table I were given cyclophosphamide and hybrid cells but no EAE cells. As expected, there were no EAE lesions adjacent to their thermal brain injuries or elsewhere. Nor can the results be construed as occult active sensitization by antigen and adjuvant inadvertently carried over with the lymph node cell transfer. This possibility is excluded by the brief 1 day incubation period, the relative refractoriness of the BN strain to active sensitization, and the fact that, in the absence of immunosuppressive treatment, successful transfer depends on histocompatibility (2, 12).

Discussion. Passive transfer of EAE from Lewis to BN rats is essentially an allograft of lymphoid cells, which are "sensitive indicators of minor degrees of tissue incompatibili-

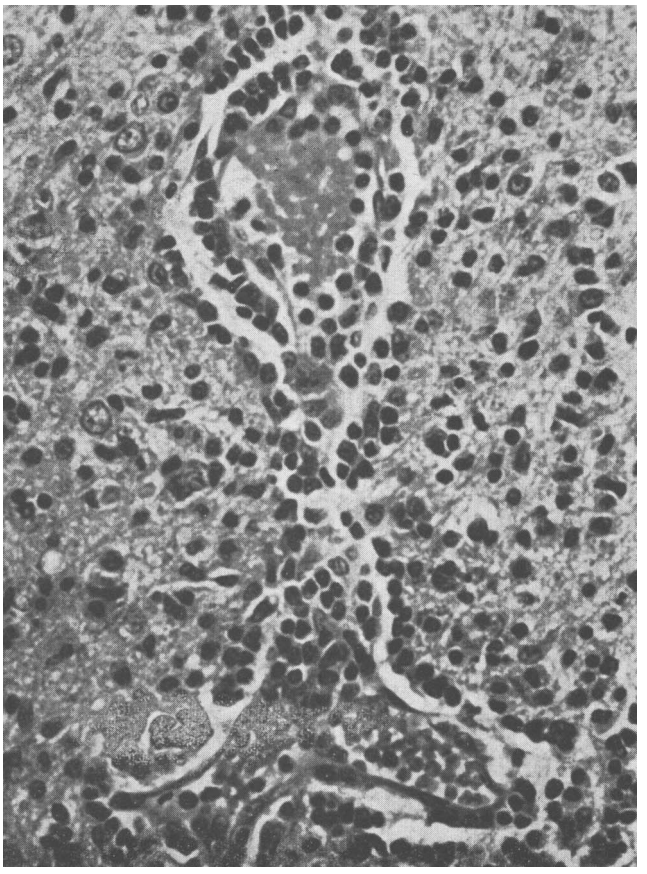

FIG. 1. EAE lesion in BN rat following transfer of Lewis cells. The vein is cut longitudinally and tangentially. There are mononuclear inflammatory cells in the lumen, wall, perivascular space and perivenous parenchyma. Hematoxylin and eosin, ×400.

ty" (13). However, the preliminary treatment with cyclophosphamide and hybrid cells has been shown by Santos to produce a chimeric state associated with tolerance to allografts (9). This technique has permitted us to breach a major histocompatibility barrier. Reports that xenogeneic grafts can survive, function, and form chimeras (14–16) give reason for hope that extension of this work may lead to transfer of EAE across species barriers and, eventually, to testing in animals of cells from human patients with presumptively autoimmune disease.

Summary. Passive transfer of allergic encephalomyelitis by injection of living, immunized lymph node cells into normal recipients constitutes a graft of lymphoid cells. It has been accomplished previously when histocompatibility barriers were absent, minor, or undefined. Treatment with an immunosuppressive drug and cells bearing donor-type antigens has now made passive transfer possible despite differences in major transplantation antigens between Lewis donor and BN recipient rats.

We are indebted to Dr. Walter Zygmunt of Mead Johnson & Co., Evansville, Indiana, for cyclophosphamide, and to Dr. H. B. Devlin of Parke Davis & Co., Detroit, for pertussis vaccine.

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Received June 22, 1970. P.S.E.B.M., 1970, Vol. 135.