Transfer of Allergic Encephalomyelitis in Rats by Intracerebral Injection of Lymphoid Cells.* (30155)

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Allergic encephalomyelitis (AE) is widely accepted as a promising model system for incisive studies of auto-immunity(1,2). All evidence suggests that the disease results from an immune response called forth by injected nervous tissue emulsified in Freund's adjuvant and directed against unique antigenic constituents in the nervous system of the sensitized host. Still unsettled, however, is the nature of the immune response and precisely how it interacts with its "target"—the neuraxis(3,4). The strongest evidence that sensitized cells, rather than circulating antibodies, are the immunological instruments of tissue injury has been the successful transfer of AE in rats and other animals by means of sensitized lymphoid cells, in contrast to the lack of transfer with immune serum(5-8).

In previous AE-transfer studies reported from this laboratory, the donor lymphoid cells were injected into the recipient rats *via* the intravenous route(3-5,9). The purpose of this paper is to describe transfer of the disease in rats using the intracerebral route. In circumventing the undefined role of the blood-brain-barrier and insuring immediate and direct contact between donor cells and "target," the intracerebral route offers an additional and particularly advantageous system for the study of AE.

Methods. Donor rats and cell suspensions. Rats of the Lewis strain were employed as donors.‡ This is a highly inbred and isohistogenic strain based on skin grafting criteria (10). The donors were sensitized by intracutaneous injection of 0.7 ml (0.1 ml in each of 7 sites) of a standard guinea pig spinal cord-adjuvant inoculum as described previously(5,9). The donors were sacrificed 9 days after sensitization—a time previously found to be optimal for intravenous transfer of AE within the Lewis rat strain(4). Lymph nodes draining injection sites (cervical, paratracheal, axillary and pectoral) were processed following previously described methods(5,9). In brief, lymph node cells were dissociated through cyto-sieves into Hanks' solution and centrifuged in the cold to yield from 0.1 to 0.4 ml of packed cells per donor rat. The cells were resuspended in fresh Hanks' solution yielding a slightly viscous preparation, consisting largely of monodispersed lymph node cells, which provided the cell suspension for intracerebral transfer.

Recipient rats and intracerebral injections. Either Lewis rats or Wistar rats—an allogenic strain—were employed.§ Under ether anesthesia, hair was removed over the dorsal skull and the exposed skin prepared with hexachlorophene and tincture of merthiolate.‖ With attention to aseptic technique throughout, a 3-6 mm incision was made in the skin over the longitudinal suture of the dorsal skull. The volume of cell suspension to be injected was drawn into a 1.0 ml syringe fitted with a 25 g needle. The syringe was held perpendicular to the skull with the needle tip in position just to one side of the midline and overlying the frontal lobe, well ahead of the motor cortex. With a rapid rotary motion of the syringe and gentle, steady pressure the needle acted as a drill and pierced the outer and inner tables of the skull coming to rest well within the

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‡ Lewis rats obtained from Microbiological Associates, Washington, D.C. For work described, males weighing between 150-200 g were used and maintained on commercial pellets and water *ad lib.*

§ Wistar "CFN—Specific pathogen free" rats obtained from Carworth Farms, New City, N. Y. Females weighing 125-175 g were used and maintained on water and commercial pellets *ad lib.*

‖ "Phisohex," Winthrop Laboratories, New York.

TABLE I. Transfer of Allergic Encephalomyelitis (AE) into Lewis or Wistar Rats with Lewis Sensitized Lymphoid Cells *via* Intracerebral Route.

Transfer No.	Lewis donor No.*	Lewis (L) or Wistar (W) recipient No.	Lesions of AE in recipients: † Perivascular cuffing	Microglial reaction plus cuffing
1	582	L-587	+	++
		W-575	+	++
2	589	L-599	+	+
		W-600	+	+++
3	770	W-782	+	++
4	771	W-787	+	+
5	772	L-784	0	0
6	776	L-789	+	0
7	777	L-790	+	0
8	778	W-792	0	0

* Lewis donors sacrificed 9 days after sensitization to spinal cord plus adjuvant and dissociated lymph node cells prepared (see *Methods*). Total of 0.12 to 0.15 ml of cell suspension injected intracerebrally into each recipient.

† Recipients sacrificed 6 days after cell transfers for histological study. Grading of lesions shown based on number and intensity of lesions present.

underlying brain tissue. After 0.06 to 0.08 ml of cell suspension was slowly injected, the needle was carefully withdrawn. A new needle was fitted to the syringe which was then reloaded with the same volume of cell suspension and the entire procedure was repeated through the same midline skin incision at a corresponding site on the contralateral side. Very slight, if any, leaking of inoculum from the hole in the calvarium was noted.

Microscopic sections and classification of lesions. From 12 to 14 blocks of formalin-fixed brain and spinal cord from each recipient sacrificed 6 days after transfer were embedded in paraffin, sectioned, and then stained with hematoxylin-eosin (and occasionally with luxol fast blue-PAS for evaluation of myelin sheaths) as described elsewhere(11). Guidelines for interpretation and classifying histological changes were as follows: Subarachnoid and meningeal cellular infiltrations were largely ignored. Similarly, changes in the immediate vicinity of the needle tract were excluded from serious consideration. Special attention, however, was given to remote focal areas of inflammation, each oriented around a small vessel, usually a venule. Such lesions, consisting only of perivascular cuffing of mononuclear cells without associated parenchymal changes, were set down in one category. More intense lesions, consisting not only of perivascular cuffing, but, in addition, showing a conspicuous microglial reaction in the immediately adjacent parenchyma were placed in a second category. Based on the number and severity of lesions found in routine microscopic sections studied, an arbitrary grading system of 1+ to 3+ was used for scoring.

Results. The results of 8 intracerebral transfer experiments with Lewis donor cells are recorded in Table I. Of the 5 Lewis recipients, 2, (789 and 790) were found to have lesions consisting only of perivascular cuffing with round cells resembling small lymphocytes. Small veins or venules were most commonly involved and 2 to 4 concentric layers of cells were not uncommon. Two other Lewis recipients (587 and 599) showed more severe lesions which were scattered in fair numbers throughout the neuraxis. These lesions consisted of perivascular cuffing and a conspicuous microglial reaction in the immediately adjacent nervous tissue parenchyma. The remaining Lewis recipient (784) showed neither type of lesion. Injection of sensitized Lewis lymphoid cells into 5 Wistar recipients gave comparable, if not more striking results. In 4 recipients (575, 600, 782 and 787) areas of microglial proliferation plus perivascular cuffing were readily demonstrable in the brain and cord. The lesions were especially intense and nu-

TABLE II. Results of Intracerebral Transfer of Cells from Sensitized or Normal Lewis Donors into Lewis or Wistar Rats.

Lewis donors*		Recipients		No. of recipients with†	
Sensitized with	Status of cells transferred	Strain used	No. inj	Perivascular cuffing	Microglial reaction & cuffing
Cord-adjuvant	Killed LN cells‡	Wistar	2	0	0
" "	Living liver cells	Wistar	2	0	0
Adjuvant only	Living LN cells	Lewis	7	4	0
" "	*Idem*	Wistar	1	1	0
Nil	"	Lewis	9	2	0
Nil	"	Wistar	3	2	0

* Donors sacrificed 9 days after sensitization with spinal cord plus adjuvant (Cord-adjuvant) or adjuvant only and dissociated cell suspension prepared from lymph nodes (LN) or liver. Total of 0.12 to 0.16 ml of cell suspension injected into each recipient.

† Recipients sacrificed 6 days after cell transfers for histological studies.

‡ Lymph node (LN) cells killed by exposure to 56°C for 30 min just prior to transfer.

merous in recipient no. 600, as indicated by the 3+ rating. The 5th Wistar rat (792) showed neither type of lesion.

The lesions found in the brain and spinal cord after intracerebral transfer were indistinguishable from those previously described in Lewis and Wistar rats with actively induced AE or in both strains following intravenous injection of appropriately sensitized donor cells(4,5,11). Luxol fast blue-PAS stains revealed definite, although minimal, perivascular demyelination to be associated with those lesions having both parenchymal microglial and perivascular lymphocytic infiltrative components.

None of the recipients exhibited clinical signs of AE. The relatively infrequent occurrence of such signs as hindleg paralysis and ataxia in rats following intravenous injection of donor cells also was observed in prior transfer studies reported from this laboratory (5,9). It is assumed that the absence of clinical signs is a reflection of the tendency for the lesions associated with the transfer form of AE to be fewer in number and milder, than those associated with actively induced disease.

Additional intracerebral transfers were carried out with lymphoid cells from "control-donors" consisting of normal Lewis rats or those sensitized to adjuvant alone. The results of these transfers together with those carried out with killed, sensitized lymphoid cells or living liver cells are recorded in Table II. Recipients injected with either lymphoid cells which had been killed by heating (56°C for 30 minutes) or viable liver cells from donors sensitized to spinal cord plus adjuvant were completely devoid of any focal inflammatory changes. In contrast, occasional focal areas of perivascular cuffing were seen in many of the recipients which received living lymph node cells, be they from normal Lewis donors or Lewis donors sensitized to adjuvant alone. However, none of the recipients had parenchymal microglial lesions. This last finding underlines the importance of living lymphoid cells sensitized to nervous tissue in the development of this histological hallmark of AE.

Discussion. The finding most difficult to interpret is the most common lesion encountered after intracerebral lymphoid cell transfer, *viz.*, the focal perivascular cuffing unaccompanied by parenchymal microglial reaction. This lesion occurred after intracerebral transfer of normal lymphoid cells as well as those from donors previously exposed to nervous tissue and adjuvant. It is certainly possible that these vascular changes have no special immunological significance. For example, they may represent an inflammatory response to the trauma associated with the intracerebral injection procedure or to the mere presence of cells deposited in the brain. Similar lesions were described by Åstrom and Waksman(6) in their study employing rabbits which were injected with sensitized lymphoid cells *via* the intracisternal route. These workers believed such lesions to have little or no immunological importance. Alternatively, however, the perivas-

cular cuffings may, in fact, represent an immunological response initiated *in situ* by immunopotent Lewis lymphoid cells and directed against antigenic organ-specific constituents of either Wistar or Lewis brain tissue. In the case of Wistar recipients, strain-specific antigens recognized as "foreign" by the immunologically competent transferred Lewis cells might provide additional antigenic stimulation. The results of "control-transfers" in the present study (Table II) are in line with this thesis. Most convincing is the absence of any lesions after transfer of heat-killed sensitized cells or viable liver cells. In other work in progress in our laboratory, a similar absence of lesions after daily injections of immune serum into the brain or ventricular cavity has been noted. Together, these observations suggest that the perivascular infiltrates found after injection of living immunopotent cells are not to be explained solely on the basis of deposition of cells *per se* or the trauma of the procedure.

The results following intracerebral injection of lymphoid cells from Lewis rats sensitized to nervous tissue plus adjuvant pose no apparent difficulty with respect to their interpretation. Such cells elicit a disseminated encephalomyelitis with morphological features identical to actively induced AE, *viz.*, characteristic microglial parenchymatous reactions and perivascular myelin injury. A point deserving special emphasis is the successful transfer of AE to normal Wistar recipients with Lewis cells. This is the first time that AE has been transferred across a conspicuous strain difference without first suppressing the immunological reactivity of the recipient animals with X-irradiation(6) or performing splenectomy of the sensitized donor(9). It is assumed that success of the transfer of AE across a histocompatibility barrier using the intracerebral route is related to the relatively immunologically privileged environment of the neuraxis(12,13). The transferred cells, after deposition in brain tissue, are secure from rapid homograft rejection mechanisms of the host and enjoy extended survival and function—previously shown to be a prerequisite for the successful transfer of AE(5). Our findings in the rat lend support to preliminary observations made by Thomas and Leonard(14) that AE may be transferable in the guinea pig by intracerebral implantation of allogeneic sensitized lymphoid cells.

Many questions arise as to the precise sequence of events leading to the disseminated lesions of AE after intracerebral transfer of cells. Do the donor cells or their products, after deposition in the brain of the recipient, gain access to the subarachnoid space or the ventricular cavities and travel *via* the spinal fluid to distant areas of the neuraxis and react there with antigen? Do the donor cells or their products move through central nervous tissue tracts or natural cleavage planes, as pneumococcal polysaccharide has been shown to do by Levine(15)? Is the critical event underlying development of lesions of AE a direct interaction between sensitized donor cell and recipient tissue-fixed antigen? Or is the tissue injury caused by conventional antibrain antibody released locally in increasing amounts by the sensitized cell in response to contact with the antigen? These and other questions are under intensive study in current transfer work involving AE in this laboratory.

The studies described here are an extension of investigation of the pathogenesis of AE in this laboratory. The intracerebral method of cell transfer described here is relatively simple and straightforward and has particular promise as an additional approach for defining the relative importance of cellular and humoral immune factors in the disease. On balance, the data presented lend added weight to the concept that the interaction between cells sensitized to nervous tissue and tissue-fixed antigen in the neuraxis leads to allergic inflammatory responses. With slight modification of this view, by substituting viral antigen for antigenic constituents native to nervous tissue, the concept has potential usefulness as a working hypothesis in studies of the post-viral encephalitides and primary demyelinating diseases of man (16).

Conclusions. Rats, after intracerebral injection of lymphoid cells from donors sensitized to spinal cord-adjuvant, develop disseminated lesions of allergic encephalomye-

litis. Using the intracerebral route, the disease may be transferred across a strain barrier (Lewis to Wistar rat) in addition to transfer within an inbred rat strain (Lewis to Lewis rat). Evidence is presented that intracerebral implantation of lymphoid cells from donors sensitized to adjuvant only or from normal rats may result in injury of a type seen in allergic encephalomyelitis and believed to have an immunological basis.

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A Rapid Procedure for Staining Nissl Granules in Brain Tissue, to Be Used for Photomicrography.* (30156)

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Although the sectioning of frozen brain tissue is generally regarded as a useful technique for inspecting certain topographic and gross morphologic changes in brains after experimental surgery, several shortcomings of this technique have led many experimental neurologists to utilize more time-consuming methods for preparing their brain specimens. For example, it is widely held that frozen sections stain rather poorly and, while they may be adequate for gross microscopic inspection, they are rarely of such quality as to provide a useful material for photomicrographic illustrations. In experiments which involve the study of various changes of the nerve cell body, the use of celloidin or paraffin embedded material has been regarded as imperative, because some artifacts, such as vacuolation of normal cells, usually observed in frozen material, make interpretations of the results difficult, if not impossible.

In this laboratory it has been found, however, that frozen sections can be prepared in such a way that they are of comparable quality to celloidin embedded material in every respect. This involves dehydration of individual sections in various grades of alcohols *before* they are mounted. Such a dehydration

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