Brief Definitive Report

TRANSFER OF EXPERIMENTAL ALLERGIC ENCEPHALOMYELITIS IN LEWIS RATS USING SUPERNATES OF INCUBATED SENSITIZED LYMPH NODE CELLS*

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Experimental allergic encephalomyelitis (EAE), a prototype autoimmune disease and a promising model system for study of the human demyelinating diseases, can be induced by a single injection of either nervous tissue homogenates or a myelin basic protein (MBP) constituent of nervous tissue, combined with adjuvant (1, 2). A cell-mediated immune mechanism has been implicated in the pathogenesis of EAE since the transfer of this disease has been accomplished with lymphoid cells from sensitized donors (3-7), in contrast to unsuccessful attempts using immune serum (8). Support for a T-cell-mediated immunopathogenesis has been reported recently by two groups of workers. Gonatas and Howard (9) showed an inhibition of EAE development in B rats, viz., rats depleted of T cells by thymectomy, irradiation, and reconstitution with bone marrow B cells. Ortiz-Ortiz and Weigle (10) extended this observation showing an inhibition of EAE in rats rendered devoid of MBP-reactive T cells, while disease could be induced in rats devoid of MBP-reactive B cells. The precise lymphoid cell subpopulation and/or cellular product(s) responsible for this disease, however, are as yet undefined.

In earlier investigations from this laboratory, lymph node cells (LNC) from sensitized Lewis donor rats were incubated at 37°C for 1-4 h, washed, and injected into Lewis recipients. After incubation, the capacity of these LNC to transfer EAE was variably diminished – an effect best recognized by lack of disease in those recipients receiving small numbers of incubated donor LNC in contrast to successful transfer in other animals injected with equivalent numbers of unincubated cells (11, 12). This finding suggested that during incubation of sensitized LNC, EAE transfer activity was either destroyed or such activity conceivably was released into the medium in which the suspended cells had been incubated.

In preliminary efforts to search for EAE transfer activity in sensitized LNC supernates, such activity was only demonstrated irregularly and occasionally (M. M. Ginsberg, and P. Y. Paterson, unpublished data). The purpose of this report is to briefly summarize a relatively large number of experiments now completed, including key control groups of animals, which provide unequivocal

evidence for transfer activity in supernates derived from sensitized lymphoid cells prepared and incubated under the conditions described.[1] For purposes of convenience, we have termed such activity EAE supernatant transfer activity (EAE-STA).

Materials and Methods

Animals. Male Lewis rats, 8-12 wk of age (Microbiological Associates, Bethesda, Md. and Simonson Laboratories, Gilroy, Calif.) were used as donors and recipients. All animals were maintained on Purina Laboratory Chow (Ralston Purina Co., St. Louis, Mo.) and water ad libitum.

Sensitization of Donor Animals. Rats were injected intracutaneously over the back and neck with guinea pig spinal cord (GPSC) (Pel Freeze Bio-Animals, Inc., Rogers, Ark., and Dutchland Lab. Animals; Denver, Pa.) plus complete Freund's adjuvant (CFA) as previously described (13, 14). Control donor rats included unsensitized rats and others sensitized with CFA only or guinea pig kidney and CFA.

LNC Suspensions. 9 days after sensitization, the draining lymph nodes were excised, trimmed of fat, and expressed through a stainless steel screen (120 mesh) with moderate pressure using a plastic syringe plunger. The dissociated LNC, suspended in Hanks' balanced salt solution (HBSS) without serum, were washed once and resuspended at the desired concentration in fresh HBSS. Control recipients received 5×10^6 unincubated LNC via the lateral tail vein.

Incubation of LNC Suspensions. LNC suspensions marked for generation of EAE-STA were incubated at two arbitrary cell concentrations: 2.5×10^8 and 5.0×10^8 LNC/ml. These cell suspensions were incubated in a 37°C waterbath for 1 h with gentle mixing at 10-min intervals. After incubation, supernates were obtained by centrifugation ($200\,g$, 20 min) of the LNC suspension and 2-4 ml supernate injected intravenously into syngeneic recipients. In two experiments, the LNC incubation was carried out in the presence of lyophilized reconstituted GPSC (0.5 mg protein/ml) or GPSC was added at the same concentration to the supernate after incubation.

Criterion for Transfer of EAE. Although recipient animals were observed for clinical signs of EAE, none appeared. Lack of such signs was consistent with the previously reported rarity of neurologic signs in recipients of unincubated LNC (1, 3, 15, 16). 14-16 days after transfer, recipient animals were sacrificed, and their brains and spinal cords processed and sectioned for routine hematoxylin-eosin staining as described previously (15). In eight recipients of LNC supernate, other organs, e.g. spleen, kidney, heart, and liver, also were removed and processed for microscopic observation.

Successful transfer of EAE was determined by the presence within the brain and/or spinal cord of focal perivascular mononuclear cell infiltrates characteristic of the disease. Histopathologic changes in each recipient were scored from 1+ to 3+ based on enumerated infiltrates observed in brain and spinal cord sections: 1+, from 1-10 lesions; 2+, from 11-30 lesions; and 3+, greater than 30 lesions.

Results

The results of 22 separate experiments are represented in Table I. As shown in the upper panel, supernates derived from incubated LNC transferred EAE to 24 of 54 syngeneic recipients. These 54 recipients were employed in a total of 19 experiments, in 11 of which transfer was successfully accomplished. Sections of

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[1] For anyone wishing information concerning the experimental conditions we employed in greater detail than space permits here, a compilation of all consumable reagents, including source and lot numbers, and a detailed step-by-step description of all procedures is available. This appendix is NAPS document no. 02996 consisting of 16 pages. Order from ASIS/NAPS, Microfiche Publications, P. O. Box 3513, Grand Central Station, New York 10017. Remit in advance $3.00 for microfiche copy or for photocopy, $5.00 up to 20 pages plus 25¢ for each additional page. All orders must be prepaid. Foreign orders add $3.00 for postage and handling.

TABLE I

Transfer of EAE with Sensitized LNC or Supernates Derived from Incubated Sensitized LNC

Donor status		Proportion of recipients with lesions of EAE
Sensitization	Material transferred	
GPSC-CFA	Supernate*	24/54
GPSC-CFA	Supernate plus brain antigen‡	0/8
GPSC-CFA	5×10^8 unincubated LNC	36/40
GPSC-CFA	5×10^8 incubated LNC	9/9
CFA, kidney-CFA, or none	5×10^8 unincubated LNC or supernate	0/15

* Recipients received 2.0–4.0 ml supernate derived from a 1 h, 37°C incubation of a 2.5 × 10⁸ or 5 × 10⁸ sensitized LNC/ml suspension.

‡ Recipients received 4.0 ml supernate prepared either by incubation of donor LNC with brain antigen or by addition of brain antigen after incubation.

spleen, liver, kidney, and heart from some of these recipients injected with supernates and developing lesions of EAE, had no significant histopathologic changes. The addition of brain antigen to active supernates, also shown in the upper panel, led to EAE-STA no longer being demonstrable.

Shown in the lower panel of Table I are a variety of control transfer experiments. Transfer of unincubated LNC, from the same pools of cells used for generation of EAE-STA, served as an index for degree of sensitization of the donors. Such LNC, in relatively large numbers (500×10^6), as well as equivalent numbers of incubated LNC, transferred EAE as described previously (3, 8, 11, 12). Recipient rats receiving either LNC or supernates from donors sensitized to CFA only, guinea pig kidney and CFA, or left unsensitized developed no evidence of EAE.

Morphologically, the central nervous system perivascular infiltrates observed in recipients of supernates were indistinguishable from cellular infiltrates in recipients receiving unincubated LNC (Fig. 1). However, the total number of infiltrates in recipients injected with supernates was less than that in recipients of unincubated LNC, as judged by the average lesion scores: LNC recipients, 2.1; supernatant recipients, 1.3. The average lesion score was equally decreased in recipients of incubated LNC, viz., 1.4.

In preliminary efforts to begin characterizing the nature of EAE-STA, many questions immediately arose. For example, do active supernates contain intact LNC in numbers sufficient to account for transfer? To answer this question, portions of several supernates were ultracentrifuged at 189,000 *g* for 1 h and the pellets examined for the presence of LNC.[2] Cell counts averaged less than 1.8 × 10⁴ LNC/ml of supernate or well below the minimal number of LNC reported to transfer EAE under the conditions employed in this laboratory (3). Is EAE-STA stable? Two separate experiments have shown that after freezing of LNC

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[2] One pellet was examined by electron microscopy with the aid of Dr. Mauro Dal Canto, Departments of Neurology and Pathology, Northwestern University Medical School, Chicago, Ill., and found to contain material ranging from cell remnants to membrane fragments to intact cells. The presence of cell degradation products suggests that the intact cells present are themselves unlikely candidates for the transfer activity demonstrated by LNC supernates.
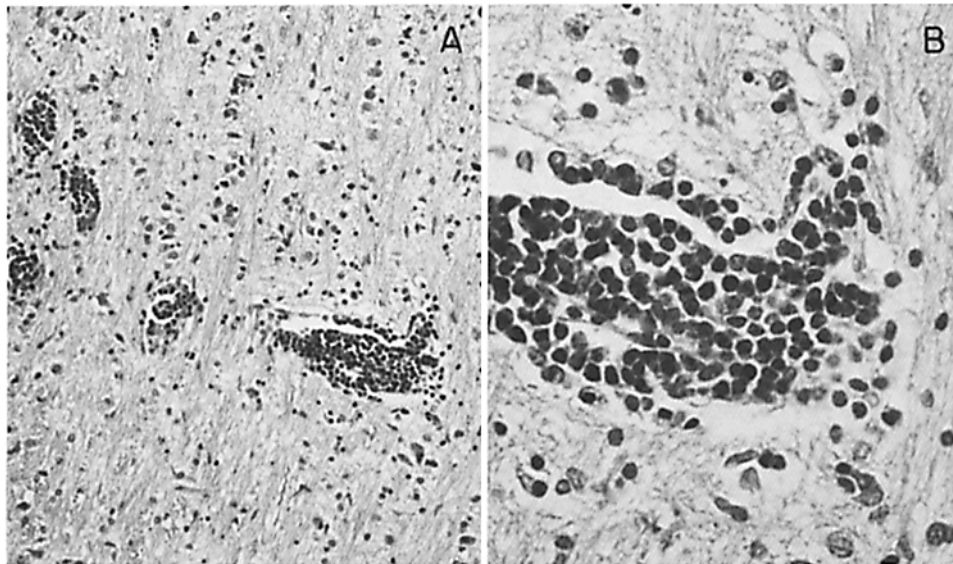
FIG. 1.   Focal perivascular mononuclear cell infiltrates indicative of EAE in the mesen-
cephalon of a Lewis rat 16 days after a single injection of sensitized LNC supernate. (A)
Hematoxylin and eosin approximately 80×. (B) The most intense infiltrate shown in A
(hematoxylin and eosin approximately 320×).

supernates ($-20°C$ for 48–72 h), EAE-STA was no longer demonstrable. An
attempt to extract EAE-STA from unincubated sensitized LNC by four freeze-
thaw cycles also proved unsuccessful. Does longer incubation time increase the
yield of EAE-STA? Supernates obtained after 2, 3, or 4 h of incubation of sensi-
tized LNC were devoid of EAE-STA.

Discussion

The study here reported indicates that supernates derived from incubated,
sensitized LNC are capable of transferring EAE in Lewis rats. EAE-STA
appears to be an immunologically specific response to nervous tissue sensitiza-
tion, since supernates derived from control donors (Table I) did not transfer.

It is noteworthy that the addition of brain antigen to active supernates
resulted in disappearance of EAE-STA. This is consistent with reports showing
that sensitized LNC suspensions, when incubated with brain antigen in vitro,
lost the capacity to transfer EAE (11, 12). The fact that the addition of brain
antigen to active supernates as well as LNC suspensions results in decreased
transfer activity suggests that "antigen carry-over" and sensitization of recipi-
ents does not account for EAE-STA.

The lack of EAE-STA in some experiments can be explained in at least two
ways. In those experiments in which supernates did not transfer, it was clear
that control recipients of unincubated sensitized LNC had markedly fewer
lesions compared to other controls in other experiments where EAE-STA was
demonstrated. Thus suboptimal sensitization of donors may well be a factor.
Second, lability of EAE-STA, suggested by preliminary characterization stud-
ies, also could be another explanation. If instability of EAE-STA is verified in

future experiments, complete characterization of the transfer activity may be a formidable undertaking.

Results similar in principle to those reported here using a different model system were described in 1967 by Guthrie et al. (17). These workers demonstrated that incubation of sensitized guinea pig peritoneal exudate cells at 37°C for 30 min effected the release of a supernatant factor which conferred upon recipients specific contact sensitivity to 1-fluoro-2,4-dinitrobenzene. Although the sensitizing antigen, the donor species, and the lymphoid cell population used by Guthrie et al. (17) differed materially from those we used for generation of EAE-STA, their observation supports the validity of our finding.

Our studies indicate that EAE, previously transferred only with viable, sensitized lymphoid cells, can now be transferred with lymphoid cell-derived preparations. Hopefully, this supernatant transfer system will permit physico-chemical studies of the factor(s) responsible for EAE-STA and provide further insight into the immunopathogenesis of EAE.

Summary

Supernates derived from incubated lymph node cells of Lewis rats sensitized to guinea pig spinal cord-Freund's adjuvant transfer experimental allergic encephalomyelitis (EAE) to syngeneic recipients. EAE supernatant transfer activity (EAE-STA) is not demonstrable in supernates derived from LNC of control donors not sensitized to nervous tissue. After addition of brain antigen to active supernates, EAE-STA is no longer demonstrable.

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