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EXPERIMENTAL ALLERGIC ENCEPHALOMYELITIS SUPERNATANT TRANSFER ACTIVITY (EAE-STA) IN LEWIS RATS: IMMUNOBIOLOGIC AND INITIAL BIOCHEMICAL PROPERTIES[1]

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Supernatants derived from incubated lymph node cells (LNC) of Lewis rats sensitized to neuroantigen-adjuvant have the capacity to transfer experimental allergic encephalomyelitis (EAE) to syngeneic recipients. Sensitization of donors with guinea pig or rat spinal cord adjuvant by either of two routes of injection was effective in generating EAE supernatant transfer activity (EAE-STA). Despite transfer of typical EAE histopathologic lesions, which were intense and disseminated throughout the neuraxis in some animals, recipient animals invariably remained clinically well. Donor sensitization with purified myelin basic protein of guinea pig or rat origin was conspicuously ineffective in generating EAE-STA. Absorption studies revealed that EAE-STA was diminished after exposure to guinea pig or rat spinal cord or guinea pig or rat kidney, but it was not demonstrably reduced after absorption with neonatal rat spinal cord lacking encephalitogenic activity or with guinea pig or rat myelin basic protein or lysozyme. EAE-STA is stable at −20°C/4 days, −70°C/2 days, 4°C/18 hr, and 56°C/1 hr, and has a m.w. in excess of 100,000 daltons.

The study of experimental allergic encephalomyelitis (EAE),[3] a prototype autoimmune disease of the central nervous system (CNS), has led to considerable knowledge of basic mechanisms responsible for neuroimmunopathologic disease of experimental animals, with possible extension of this information to patients with multiple sclerosis (1). Successful transfers of EAE first in outbred rats (2), and later in inbred strains of guinea pigs (3) and rats (4), by using relatively large numbers of viable lymphoid cells, together with generally unsuccessful attempts to accomplish transfer with immune serum (reviewed by Chase (5)), have led to the belief that this neuroautoimmune disorder is mediated primarily by a cellular immune mechanism. More recent studies (6, 7) have implicated thymus-derived (T) cells in the development of the disease and provided further evidence against involvement of antibody in the pathogenesis of EAE.

In continuing efforts to more precisely define the immunopathogenesis of EAE, we have secured evidence for a role of lymphoid cell-derived products of importance in the disease. We have previously reported that supernatants derived from incubated lymph node cells (LNC) of rats sensitized to spinal cord homogenates plus complete Freund's adjuvant (CFA) transfer histopathologic changes of EAE to syngeneic recipients (8). In continuation of our previous studies, we now report further biologic and initial biochemical findings regarding EAE supernatant transfer activity (EAE-STA).

MATERIALS AND METHODS

Animals. Male Lewis rats, 225 to 250 g (Microbiological Associates, Bethesda Md., and Simonsen Laboratories, Gilroy, Calif.) were used as donors and recipients when 8 to 12 weeks of age.

Antigens. Guinea pig spinal cord (GPSC) (Pel-Freeze, Rogers, Ark.) was obtained frozen and used within 3 months of shipment. Rat spinal cord (RSC) was obtained fresh in our laboratory and was used immediately. Both guinea pig and rat myelin basic protein (MBP) were prepared in our laboratory following the procedure of Deibler *et al.* (9) and were assayed for homogeneity by means of polyacrylamide disc gel electrophoresis carried out under acid conditions (1 M acetic acid, pH 2.5) (9). Encephalitogenicity of each MBP was demonstrated by injecting 5 to 50 μg per guinea pig or rat and noting clinical and histopathologic changes of EAE.

Sensitization of donor animals. Rats were injected with a 33% homogenate of GPSC emulsified in an equal volume of CFA containing 4 mg/ml Bacille Calmette Guerin (BCG) (obtained from Drs. Eva Leake and Quentin Myrvik, Wake Forest University, Winston-Salem, N. C.) as previously described (10). Animals were sensitized intracutaneously either over the back and neck, by injection of 0.7 ml inoculum divided equally between seven sites (six on the upper back and one on the ventral neck) or by the footpad route in which 0.1 ml inoculum was injected into each hind footpad. In some experiments, 50 μg of MBP in combination with CFA administered by the footpad route were used for the sensitizing regimen. *Bordetella pertussis*, known to markedly augment and accelerate EAE in actively sensitized animals (11), served as a supplemental adjuvant in some experiments by using 20×10^9 organisms in 0.1 ml introduced into the dorsum of each hind foot immediately after footpad inoculation with neuroantigen-CFA.

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[3] Abbreviations used in this paper: EAE, experimental allergic encephalomyelitis; CNS, central nervous system; LNC, lymph node cells; EAE-STA, experimental allergic encephalomyelitis supernatant transfer activity; GPSC, guinea pig spinal cord; MBP, myelin basic protein; BCG, Bacille Calmette Guerin; HBSS, Hanks' balanced salt solution; GPK, guinea pig kidney; RK, rat kidney.

LNC suspensions and generation of EAE-STA. Nine days after sensitization, the draining lymph nodes were removed from donor animals and the resulting LNC were processed for generation of EAE-STA as previously described (8). When *B. pertussis* was used as a supplemental adjuvant, donor animals were sacrificed, and lymph nodes were taken 7 days after sensitization. Briefly, axillary, pectoral, peritracheal, and cervical nodes were used for EAE-STA generation from back-neck sensitized animals, whereas popliteal, inguinal, and periaortic nodes were taken from footpad sensitized donors. The respective lymph nodes were expressed through a stainless steel screen (120 mesh) with Hanks' balanced salt solution (HBSS), washed once, and resuspended to a concentration of either 2.5×10^8 or 5×10^8 LNC/ml in fresh HBSS. Control syngeneic recipients received 5×10^8 unincubated LNC injected i.v. via the lateral tail vein.

LNC suspensions, for generation of EAE-STA, were incubated for 1 hr in a 37°C water bath with frequent mixing. After incubation, the LNC suspensions were centrifuged at 200 × G for 20 min, and 2 to 4 ml of the supernatant were injected i.v. into syngeneic recipient rats. In some experiments, LNC suspensions were centrifuged at 20,000 × G for 20 min before injection of supernatants.[4]

Absorption studies. LNC supernatants were absorbed with various nervous tissue and control antigens for 16 to 20 hr at 4°C followed by injection into syngeneic recipients. GPSC, RSC, neonatal RSC, guinea pig kidney (GPK), and rat kidney (RK) antigen preparations were made up as follows: a 33% homogenate in water was lyophilized and stored at 4°C over desiccant. On the day of the experiment, an appropriate amount was resuspended in HBSS by means of a Dounce homogenizer, and the suspension was centrifuged at 220 × G for 10 min. The pellet of this centrifugation was discarded and the antigen supernatant was diluted with HBSS so that 1 ml of this antigen preparation, when mixed with the LNC supernatant, contained 0.5 mg/ml of absorbing antigen protein. The protein concentration of each antigen preparation had been previously determined by means of the Lowry method (12). Absorption with guinea pig or rat MBP as well as lysozyme, a protein similar in m.w. and charge to MBP, was carried out by first suspending an appropriate amount of protein in 1 ml of HBSS so that mixture of the protein solution with the LNC supernatant would result in a 40 μg/ml or 80 μg/ml final concentration of MBP or lysozyme. The use of 80 μg MBP/ml supernatant represented an MBP concentration in excess of that contained in the spinal cord antigen preparation used for absorption. In some experiments, all absorption mixtures were centrifuged at 20,000 × G for 20 min immediately before injection.

Characteristics of EAE-STA. The temperature stability of EAE-STA was determined by using active supernatants after removal of incubated LNC. Supernatants were exposed to −20°C for 4 days, −70°C for 2 days, 4°C for 18 hr, and 56°C for 1 hr, and thereafter allowed to come to room temperature and injected into syngeneic recipients.

[4] In an attempt to determine if EAE-STA was demonstrable in the medium in which the dissociated LNC were initially suspended before incubation, the single wash fluids (200 × G) of several LNC preparations were assayed for EAE-STA by injection into syngeneic recipients. Histopathologic changes of EAE were observed in these recipients indicating that EAE-STA is present upon dissociation of LNC from the intact lymph node. However, in the interest of maintaining a standardized reproducible system for generation of EAE-STA, all experiments reported here were done with LNC supernatants derived from a 1 hr 37°C incubation of 2.5 to 5×10^8 LNC/ml.

An estimation of the m.w. of EAE-STA was made by ultrafiltration using an Amicon XM100A membrane (Amicon Corp., Lexington, Mass.). Active supernatants (about 7 ml) were applied to an Amicon-stirred cell (Model 12) and concentrated by positive pressure to 1 ml. The material retained by the XM100A membrane was reconstituted to the starting volume (7 ml) with HBSS and injected into recipient animals. The effluent without further dilution was injected into separate recipient rats.

Clinical and histopathologic changes of EAE. Recipients of LNC and LNC supernatants were checked daily for clinical neurologic signs indicative of EAE. Fourteen days after transfer, recipient animals were sacrificed by exsanguination and their brains and spinal cords were fixed in 10% formalin, processed, and sectioned for routine hematoxylin-eosin staining as previously described (13). Sections of the brain at the level of the thalamus, mesencephalon, and cerebellum-pons, as well as longitudinal sections of the entire spinal cord were examined for focal perivascular mononuclear infiltrates characteristic of EAE. Histopathologic changes were scored as follows: 1 to 10 discrete lesions, 1+; 11 to 30 lesions, 2+; and greater than 30 lesions, 3+.

RESULTS

Immunobiologic properties. Our total experience with the effects of EAE-STA from LNC of Lewis rats sensitized to neuroantigen combined with immunologic adjuvants is summarized in Table I. As indicated in the upper panel of Table I, successful transfer was accomplished with supernatants prepared from donors sensitized to guinea pig spinal cord in 65 of 93 experiments. In these experiments, it made little difference whether the donors were sensitized via the skin of the upper back and anterior neck or the footpads of the hind legs and, for this reason, the results have been combined. A total of 124 out of 259 recipients in these experiments developed typical lesions of EAE. Despite the fact that these lesions were very severe and disseminated throughout the neuraxis of many of the 124 positive recipients and despite the fact that in two experiments donors received supraoptimal sensitization with pertussis vaccine as a supplemental adjuvant in addition to CFA, all animals

TABLE I

Transfer of EAE in Lewis rats with LNC supernatants and LNC

Neuroantigen Used for Donor Sensitization[a]	Proportion of Successful Expts.[b]	Occurrence of EAE in Recipients		
		Clinical	Lesions	Avg. Lesion Score (Range)
Supernatant Transfers				
GPSC[c]	65/93	0/259	124/259	1.4 (1-3)
RSC	1/2	0/4	1/4	1.0 (1)
Guinea pig or rat MBP	0/7	0/18	0/18	
LNC Transfers				
GPSC or RSC	97/98	92/236	224/236	2.0 (1-3)
Guinea pig or rat MBP	6/7	11/16	13/16	1.8 (1-3)

[a] *B. pertussis* was used as a supplemental adjuvant in two experiments with GPSC sensitization resulting in successful transfer of lesions in 1/6 recipients. *B. pertussis* was also used in two experiments with GPMBP sensitization.

[b] Those experiments in which transfer recipients were observed to have histopathologic changes of EAE.

[c] With back-neck sensitization, 50 of the 113 recipients showed lesions in 23 out of 39 experiments; with footpad sensitization, 74 of 146 recipients showed lesions in 42 out of 54 experiments.

with lesions remained free of clinical neurologic signs. By using a totally syngeneic system, i.e., injection of supernatant from LNC of Lewis donor rats sensitized to Lewis rat spinal cord-CFA into normal Lewis recipient animals, EAE-STA was demonstrable in one of four recipients from two separate experiments. Production of EAE-STA, thus, can be induced with syngeneic whole nervous tissue. In contrast, EAE-STA could not be demonstrated in any of seven experiments by using donor animals sensitized to guinea pig or rat MBP. In two of these experiments, donors received supplemental pertussis vaccine, a treatment known to markedly enhance both clinical signs and histopathologic changes in actively sensitized animals as well as LNC transfer recipients (10).

To determine if active LNC supernatants contained antigen "carried over" from the LNC donors, in one experiment (data not shown), rats were sensitized with a supernatant-CFA emulsion injected either over the back and neck or via the footpad. There was no evidence of clinical or histopathologic changes of EAE after 14 days.

As set out in the lower panel of Table I, aliquots of washed but not incubated LNC suspensions used for preparing supernatants transferred EAE as expected from previous experience in our laboratory. The majority of recipients developed typical lesions of the disease; a smaller proportion developed mild, transitory clinical neurologic signs. It is important to note that LNC from donors sensitized to guinea pig or rat MBP transferred EAE to the majority of recipient animals, thereby indicating that unsuccessful transfer with supernatants from LNC of donors sensitized to MBP (third line of upper panel of Table I) was not due to lack of adequate donor sensitization with this form of neuroantigen.

In control experiments, recipients receiving either LNC or supernatants from donors sensitized with guinea pig kidney-CFA, CFA alone, or left unsensitized demonstrated no evidence of EAE. We have reported these data previously (8) (data not shown).

Absorption of EAE-STA with tissue antigens. A series of experiments were undertaken to determine if EAE-STA derived from GPSC-CFA-sensitized donors could be absorbed from supernatants by various tissue antigens (Table II). Twenty-five of 45 recipients receiving unabsorbed supernatants showed an average of 19.1 lesions per rat. The addition of a guinea pig spinal cord antigen preparation (see *Materials and Methods*) to active supernatants almost completely abrogated their transfer capacity, as tested in six separate experiments. Of 18 recipients, only one rat showed minimal evidence (one lesion) of CNS histopathologic changes. Absorption of active supernatants with RSC resulted in a partial reduction of EAE-STA as reflected both by a decreased proportion of recipients with EAE and a decreased number of lesions per recipient. In contrast, guinea pig and rat MBP as well as neonatal RSC, obtained at a time (2 days of age) when it is known to be devoid of encephalitogenic components (14), appeared to remove very little if any transfer activity from active supernatants. Likewise, EAE-STA was left unchanged after absorption with lysozyme, which was used as a control protein for MBP. However, when guinea pig or rat kidney was used for the absorbing antigen, a reduced incidence of transfer activity was observed.

Biochemical characteristics of EAE-STA. Some preliminary characteristics of EAE-STA have been determined (Table III). EAE-STA appears to be stable at −20°C for at least 4 days, −70°C for at least 2 days, 4°C for at least 18 hr, and 56°C for at least 1 hr. In addition, activity is still present in the supernatant after 20,000 × G centrifugation of either an incubated LNC suspension or an active LNC supernatant generated by a previous 200 × G centrifugation. Ultrafiltration studies to determine the approximate m.w. of EAE-STA revealed that activity was retained by a membrane having a 100,000-dalton cutoff. The presence of transfer activity in the retentate coupled with the absence of such activity in the effluent was consistent with the m.w. of EAE-STA being greater than 100,000 daltons.

TABLE II

Results of absorption of LNC supernatants[a] with tissue antigens

Supernatant Absorbed with	No. of Expts.	Proportion of Recipients with EAE	Average No. EAE Lesions (Range)/Recipient
Nil	15	25/45 (56%)	19.1 (1–122)
GPSC	6	1/18 (6%)	1.0 (1)
RSC	3	3/9 (33%)	2.3 (1–3)
Neonatal rat spinal cord	2	3/5 (60%)	7.3 (1–20)
Guinea pig MBP	4	7/12 (58%)	10.0 (1–43)
Rat MBP	3	4/9 (44%)	16.0 (3–49)
GPK	3	1/9 (11%)	1.0 (1)
RK	1	1/3 (33%)	135.0 (135)
Lysozyme	6	10/18 (56%)	26.3 (2–93)

[a] LNC supernatants derived from donor rats sensitized with GPSC-CFA 9 days earlier.

DISCUSSION

We have recently reported that supernatants of incubated LNC derived from nervous tissue-adjuvant-sensitized Lewis rats have the ability to transfer histopathologic changes of EAE to syngeneic recipients (8). Perivascular mononuclear infiltrates were evident only within the brain and spinal cord of recipient rats receiving supernatants derived from donors sensitized with CNS tissue and adjuvant (8). In the present study, we have shown the importance of the type of neuroantigen used for donor sensitization in that whole spinal cord of guinea pig origin is most effective at inducing EAE-STA, followed by rat spinal cord, whereas donor sensitization with guinea pig or rat MBP is totally ineffective (Table I). Experiments carried out to determine the role of route of sensitization of donors indicated that intracutaneous inoculation of neuroantigen emulsion either into the skin of the upper back and neck or the hind leg footpads was equally effective in the generation of EAE-STA. Perhaps the most intriguing aspect of the present findings has to do with the results obtained when active supernatants were absorbed with various syngeneic and xenogeneic antigens. When GPSC or GPK antigen preparations were used for absorption, EAE-

TABLE III

Biochemical characteristics of EAE-STA

Property	Treatment	Incidence of EAE
Temperature stability	−70°C/2 days	2/3
	−20°C/4 days	2/2
	4°C/18 hr	21/37
	56°C/1 hr	2/3
Recovery on centrifugation	20,000 × G supernatant	14/26
Molecular size	Retentate of 100,000 m.w. membrane	2/3
	Effluent of 100,000 m.w. membrane	0/3

STA was virtually abolished, except for the appearance of one lesion in one animal of each group. Diminished EAE-STA also was observed after incubation with either RSC or RK. In contrast, absorption with guinea pig or rat MBP did not demonstrably diminish EAE-STA in agreement with lack of EAE-STA in supernatants prepared from LNC of donors sensitized to either neuroantigen.

The observation that GPK and RK absorbed EAE-STA to a degree comparable with GPSC and RSC, respectively, was a somewhat unexpected finding. However, there is substantial evidence in the older immunologic literature indicating that CNS antigens may cross-react with antigenic constituents of kidney as well as other organs. Based on serologic studies of cross-reactivity of antibodies *in vitro*, Lewis (15) in 1941 described antigenic sharing between components of brain, testicle, and corpus luteum. This observation of brain-testicle cross-reactivity was later confirmed, and brain-kidney cross-reactivity was described by Kopeloff and Kopeloff (16). These workers demonstrated the presence of antibodies reactive with kidney extracts by C fixation in several sera of monkeys immunized with monkey brain and adjuvant. More recently, Arnason et al (17) observed that LNC from rats sensitized to kidney have the capacity to demyelinate rat trigeminal ganglion organotypic cultures. This kidney-peripheral nerve cross-reaction was not surprising in view of the well-known antigenic sharing of peripheral nerve with CNS tissue (18). The central question surrounding the antigenic sharing between kidney and CNS is whether this cross-reaction has any *in vivo* relevance for disease production. Indeed, Kopeloff and Kopeloff (19) observed clinical neurologic signs in two of 10 guinea pigs after a single sensitizing injection of rabbit kidney and adjuvant. Since these animals were not studied histologically, it is not known whether the clinical evidence of neurologic disease was accompanied by lesions within the CNS. However, Arnason (17) did find mild cellular infiltrates occurring in the peripheral nerves of rats sensitized to kidney. In light of these observations, it should be emphasized that at no time have we observed neurologic manifestations in rats actively sensitized with kidney and adjuvant or animals receiving either LNC or supernatants from kidney sensitized donors (8). These findings coupled with the presently reported observation that GPK or RK can absorb EAE-STA suggest that cross-reactive kidney antigens represent a weaker immunogenic system, in terms of encephalitogenicity, when compared to the organ-specific CNS antigens directly responsible for inducing EAE. Therefore, by using this argument, the cross-reactive kidney antigens could be sufficiently immunogenic to absorb pre-formed EAE-STA while at the same time be insufficiently immunogenic to induce EAE-STA. Alternatively, the possibility cannot be excluded that the removal of EAE-STA by kidney could be due to nonspecific adsorption, i.e., a "membrane effect." The fact that neonatal rat spinal cord does not remove EAE-STA counters this argument, but admittedly there may be significant differences in the constituents of neonatal and adult tissues. To completely rule out the possibility of a "membrane effect," it will be necessary to test the absorption of EAE-STA with a noncross-reacting "membrane"-containing organ such as lung. Experiments along these lines are currently in progress.

Our findings reported previously (20), and here described in extended form, are to our knowledge, the only observations of transfer of EAE in rats by using a subcellular lymphoid cell-derived preparation. The active moiety(ies) appears to be relatively temperature stable, of a m.w. greater than 100,000 daltons, and, indeed, subcellular in nature since centrifugation in the range of 20,000 × G does not remove EAE-STA from active supernatants (Table III). Thus, EAE-STA does not appear identical to many of the presently known lymphoid cell-derived products responsible for or contributing to information transfer. For example, the fact that EAE-STA has a m.w. greater than 100,000 daltons excludes the class of molecules known as antigen-specific T cell factors, which have been reported by Taussig and Munro (21), Waltenbaugh *et al.* (22), and Takemori and Tada (23), to be in a m.w. range of 35,000 to 60,000 daltons. Furthermore, EAE-STA does not appear identical to human transfer factor as described by Lawrence (24), since transfer factor has a m.w. of less than 10,000 and is inactivated by 56°C for 30 min. At this point in our characterization of supernatant transfer activity, we cannot exclude the possibility that EAE-STA resides, at least in part, in immunoglobulin or immunoglobulin-like molecule(s). Studies to confirm or exclude the possible antibody-like nature of EAE-STA are presently ongoing.

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REFERENCES

1. Paterson, P. Y. 1978. The demyelinating diseases: clinical and experimental studies in animals and man. *In* Immunological Diseases. 3rd ed. Edited by M. Samter, N. Alexander, B. Rose, W. B. Sherman, D. W. Talmage, and J. H. Vaughan. Little, Brown and Company, Boston. Pp. 1400–1438.
2. Paterson, P. Y. 1960. Transfer of allergic encephalomyelitis in rats by means of lymph node cells. J. Exp. Med. 111:119.
3. Stone, S. H. 1961. Transfer of allergic encephalomyelitis by lymph node cells in inbred guinea pigs. Science 134:619.
4. Levine, S., E. J. Wenk, and E. M. Hoenig. 1967. Passive transfer of allergic encephalomyelitis between inbred rat strains: correlation with transplantation antigens. Transplantation 5:534.
5. Chase, M. W. 1959. A critique of attempts at passive transfer of sensitivity to nervous tissue. *In* Allergic Encephalomyelitis. Edited by M. W. Kies and E. C. Alvord, Jr. Charles C Thomas, Springfield, Ill. Pp. 348–374.
6. Ortiz-Ortiz, L., and W. O. Weigle. 1976. Cellular events in the induction of experimental allergic encephalomyelitis in rats. J. Exp. Med. 144:604.
7. Ortiz-Ortiz, L., R. M. Nakamura, and W. O. Weigle. 1976. T cell requirement for experimental allergic encephalomyelitis induction in the rat. J. Immunol. 117:576.
8. Whitacre, C. C., and P. Y. Paterson. 1977. Transfer of experimental allergic encephalomyelitis in Lewis rats using supernates of incubated sensitized lymph node cells. J. Exp. Med. 145:1405.
9. Deibler, G. E., R. E. Martenson, and M. W. Kies. 1972. Large scale preparation of myelin basic protein from central nervous tissue of several mammalian species. Prep. Biochem. 2:139.
10. Paterson, P. Y. 1973. Adjuvants, cell-mediated immune responses and autoimmune disease. J. Reticuloendothel. Soc. 14:426.
11. Levine, S., and E. J. Wenk. 1965. A hyperacute form of allergic encephalomyelitis. Am J. Pathol. 47:61.
12. Lowry, O. H., N. J. Rosebrough, A. L. Farr, and E. J. Randall. 1951. Protein measurement with the Folin phenol reagent. J. Biol. Chem. 193:265.
13. Paterson, P. Y., W. P. Richardson, and D. G. Drobish. 1975. Cellular transfer of experimental allergic encephalomyelitis: altered disease pattern in irradiated recipient Lewis rats. Cell. Immunol. 16:48.
14. Fujinami, R. S., and P. Y. Paterson. 1977. Induction of experimental

allergic encephalomyelitis in suckling Lewis rats: role of age and type of sensitizing neuroantigen. J. Immunol. 119:1634.
15. Lewis, J. H. 1941. The antigenic relationship of alcohol-soluble substances of corpus luteum to those of testis and brain. Am. J. Pathol. 17:725.
16. Kopeloff, L. M., and N. Kopeloff. 1944. The production of antibrain antibodies in the monkey. J. Immunol. 48:297.
17. Arnason, B. G. W., G. F. Winkler, and N. M. Hadler. 1969. Cell-mediated demyelination of peripheral nerve in tissue culture. Lab. Invest. 21:1.
18. Arnason, B. G. W. 1975. Inflammatory polyradiculoneuropathies. *In* Peripheral Neuropathy. Edited by P. J. Dyck, P. K. Thomas, and E. H. Lambert. W. B. Saunders, Philadelphia. Pp. 1110–1148.
19. Kopeloff, L. M., and N. Kopeloff. 1947. Neurologic manifestations in laboratory animals produced by organ (adjuvant) emulsions. J. Immunol. 57:229.
20. Whitacre, C. C. 1978. Experimental allergic encephalomyelitis supernatant transfer activity (EAE-STA): further studies and initial characterization. Fed. Proc. 37:1118. (Abstr.)
21. Taussig, M. J., and A. J. Munro. 1976. Antigen-specific T cell factor in cell cooperation and genetic control of the immune response. Fed. Proc. 35:2061.
22. Waltenbaugh, C., P. Debré, J. Théze, and B. Benacerraf. 1977. Immunosuppressive factors specific for L-glutamic acid-L-tyrosine (GT). I. Production, characterization, and lack of H-2 restriction for activity in recipient strain. J. Immunol. 118:2073.
23. Takemori, T., and T. Tada. 1975. Properties of antigen-specific suppressive T cell factor in the regulation of antibody response of the mouse. I. *In vivo* activity and immunochemical characterizations. J. Exp. Med. 142:1241.
24. Lawrence, H. S. 1974. Transfer factor in cellular immunity. Harvey Lect. 68:239.