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*REVIEW*

# IMMUNOPATHOLOGICAL COMPARISONS BETWEEN EXPERIMENTAL AUTOIMMUNE ENCEPHALOMYELITIS AND MULTIPLE SCLEROSIS*

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## INTRODUCTION

The premise that experimental autoimmune encephalomyelitis (EAE) is a valid experimental model for human multiple sclerosis (MS) is the keystone of the concept of MS as a disease in the realm of immunoneurology, and has represented one justification for the expenditure of considerable research effort on EAE (Paterson, 1969). It therefore seems appropriate to examine progress in the area of the comparative immunology of EAE and MS by applying questions relevant to both diseases. It will be recognized that these are answered by a rather selective choice of current references. Additional discussions on the 'EAE-MS controversy' are contained in reviews by Nilsson (1972) and Paterson (1972).

## IS A RELEVANT IMMUNOGEN (ANTIGEN) KNOWN AND CHARACTERIZED?

*For EAE*, the answer is 'yes'. This immunogen is contained in the basic protein molecule of myelin, of known sequence (Carnegie, 1971; Eylar *et al.*, 1971). Notably, different peptide-regions of this molecule can be immunogenic, depending on species, e.g. peptide region 112–122 in the guinea-pig (Lennon, Wilks & Carnegie, 1970), region 45–86 in the rat (Dunkley, Coates & Carnegie, 1973) and rabbit (Shapira *et al.*, 1971), and a C-terminal region in the monkey (Eylar *et al.*, 1972a).

*For multiples sclerosis*, the answer is 'no'. The immunogen, if such exists, is neither known nor characterized.

## IS THERE EVIDENCE FOR A HUMORAL IMMUNE RESPONSE TO A BRAIN-SPECIFIC ANTIGEN?

*For EAE*, the answer is 'yes'. This specific humoral immune response is directed against determinant(s) of the basic protein of myelin, and is demonstrable by at least three methods:

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radioimmunoassay (McPherson & Carnegie, 1968; Lennon *et al.*, 1971); immunofluorescence (Whittingham *et al.*, 1972); and haemolysis of antigen-coated sheep erythrocytes (Lennon & Feldmann, 1972); it follows the usual rules, a primary IgM and a secondary IgG response (Lennon *et al.*, 1971). The humoral immune response requires the whole basic protein as immunogen in all species; although peptides from the basic protein molecule are encephalitogenic, they do not include antibody (Lennon *et al.*, 1970; Dunkley *et al.*, 1973).

The humoral immune response is demonstrable also by more elaborate assays using cellular systems. These include, firstly, the use of lymphoid cell suspensions from spleen or lymph nodes of immunized animals to show an increase in count of lymphocytes capable of binding iodine-labelled basic protein (Coates & Lennon, 1973; Yung *et al.*, 1973). Secondly, using sheep red cells coated with basic protein antigen (Lennon & Feldmann, 1972), the formation of haemolytic plaques occurs when lymphoid cells from immunized animals are introduced into a medium containing such coated red cells; this technique was extended to demonstrate lysis by cells in sections of lymph nodes of animals immunized with basic protein, and even by cells in perivascular areas of the brain of animals with EAE (Lennon, Feldmann & Crawford, 1972). Similarly, specific binding of myelin basic protein labelled with peroxidase was shown for lymphoid cells in the lesions of EAE (Johnson *et al.*, 1971).

Finally, serum from animals with EAE contains biologically active neurotoxic factors of immunoglobulin character which are presumed to be antibodies, although this has not been unequivocally demonstrated. When added to cultured neural tissue, these factors cause (a) demyelination (Raine & Bornstein, 1970) and (b) suppression of polysynaptic electrical activity (Bornstein & Crain, 1965). It is of interest that immunization with whole brain tissue induces both (a) and (b), whereas basic protein induces only (b) (Seil *et al.*, 1968; Bornstein & Crain, 1971). Data are not yet available on the co-identity, or otherwise, of antibodies to basic protein demonstrable by radioimmunoassay and neurotoxic factors; nor has there been established any correlation between titre of antibody to basic protein antigen and activity of neurotoxic factors in sera of animals immunized with basic protein.

*For multiple sclerosis*, the answer is 'no'. There is no demonstrable humoral antibody to any defined autoantigen of neural or non-neural origin and, in particular, no antibody to basic protein antigen is demonstrable in serum by sensitive radioimmunoassay (Lennon & Mackay, 1972). Moreover counts of antigen-binding lymphocytes, using iodine-labelled basic protein, are not increased among blood lymphocytes of patients with MS (Coates, 1972). The opportunity has not arisen to test spleen cells or neural lesions of patients with MS for the presence of lymphocytes capable of causing haemolysis of basic protein-coated sheep red cells, as has been done in the case of EAE (Lennon *et al.*, 1972).

There are neurotoxic factors in serum of patients with MS which, as in EAE, are immunoglobulins (Dowling *et al.*, 1968) but not fully characterized antibodies. These give positive results in bioassays using cultured neural tissue, causing demyelination (reviewed by Alvord, 1970; Lumsden, 1969), suppression of polysynaptic electrical activity (Bornstein & Crain, 1965; Cerf & Carels, 1966), and inhibition of myelination (Kies *et al.*, 1973). In view of the wholly negative results for specific antibody to basic protein of myelin in MS with conventional immunological assays, these bioassays demonstrating a damaging component in serum in MS assume critical importance. Again, as in EAE, the antibody character, or otherwise, of these neurotoxic factors needs to be established, as does the target site of their activity.

For completeness, reference can be made to two other 'factors' of uncertain significance described in serum of patients with MS. One of thses is 'toxic' to lymphocytes (Paterson, 1972) and could therefore suppress phytohaemagglutinin-induced transformation of human lymphocytes (Hughes, Caspary & Field, 1968; Noort & Stjerholm, 1971) (*vide infra*), and the other factor suppresses development of EAE in appropriately immunized animals (Tsaregorodtseva & Chernigovskaya, 1969).

## IS THERE EVIDENCE FOR A HUMORAL IMMUNE RESPONSE TO UNIDENTIFIED ANTIGENS?

*For EAE*, there is no necessity to consider this in view of the specificity of the immune response to basic protein (*vide supra*).

*For multiple sclerosis*, the answer is 'yes'. The evidence, discussed fully by Prineas (1970) and Tourtellotte (1972), is as follows. There are many reports of an increased level of IgG in the cerebrospinal fluid (CSF) (Prineas, 1970). This IgG is of limited heterogeneity (oligoclonal), as indicated by abnormal kappa:lamda light chain ratios in immunoglobulin molecules in the CSF and discrete bands on agar gel electrophoresis of CSF (Link, 1972). Also IgG is demonstrable in MS plaques in the CNS (Tourtellotte, 1970), oligoclonal IgG protein bands were isolated from MS brains (Link, 1972) and plasma cells are present in fresh plaque lesions in the CNS (Lumsden, 1971); Lumsden (1971) and Tourtellotte (1972) disagree as to the origin of IgG in plaque lesions. Such findings, although used at times to support concepts of autoimmunity in MS (Prineas, 1970; Lennon & Mackay, 1972), point merely to an immune response of limited heterogeneity to an antigen in the CNS. This antigen could be of extrinsic origin, e.g. a viral component, or of intrinsic origin, i.e. an autoantigen. For example, there is evidence for marked immunoglobulin synthesis in the CNS in the virus induced disease subacute sclerosing panencephalitis (SSPE) (Link, 1972).

## IS THERE EVIDENCE FOR CELL-MEDIATED IMMUNE RESPONSE TO ANTIGENS SPECIFIC TO BRAIN?

*For EAE*, the answer is 'yes'. This specific cell-mediated immune response is directed against determinant(s) of the basic protein of myelin, and has been demonstrated by the following methods.

(i) Delayed hypersensitivity by skin testing was demonstrated with whole neural tissue (Waksman, 1956), basic protein (Lennon *et al.*, 1970) and peptides from basic protein (Lennon *et al.*, 1970).

(ii) Macrophage migration inhibition factor (MIF) was produced after exposure of cells from peritoneal exudates to antigen, including basic protein and peptides from basic protein, in different species, including the guinea-pig (Lennon *et al.*, 1970; Spitler *et al.*, 1972) and rat (Lennon & Dunkley, 1973).

(iii) Macrophage slowing factor (MSF), demonstrable by the cytopherometer assay, was produced after exposure of cells, from peritoneal exudates of guinea-pigs with EAE, to basic protein of myelin (Caspary, Hughes & Field, 1970).

(iv) Transformation–mitosis responses have been demonstrated in EAE in several studies using lymphoid cells and neural components (Paterson, 1972). Splenic lymphocytes from guinea-pigs immunized with basic protein of myelin or tryptophan peptide and exposed in

tissue culture to the immunogen, basic protein of myelin or tryptophan peptide underwent transformation (Lamoureux *et al.*, 1972; Lennon & Carnegie, 1973) indicative of the immunogenic capacity of the tryptophan peptide in this species.

(v) Cytotoxicity of lymphocytes from animals with EAE was demonstrable by exposing lymphocytes to cultured neural tissue; three such studies were cited by Nilsson (1972).

Lymphoid cells were shown to transfer EAE to syngeneic animals (rats) (Paterson, 1960), but this does not necessarily point to cellular, as opposed to humoral, immune mechanisms in that there was no indication from such experiments whether thymus-derived T cells or bone marrow-derived B cells were the actual effector cells in the recipient. Knowledge on the contribution of T and B cells to EAE has been retarded by the difficulty experienced by our own and other laboratories in inducing EAE in the mouse; the information provided by Levine & Sowinski (1973) on degrees of susceptibility of mouse strains to EAE should prove most helpful to investigators wishing to examine T–B interactions in EAE using, for example, *in vitro* techniques and anti-theta serum.

*For multiple sclerosis*, the answer to this question is a reserved 'yes'. This is because the evidence for a cell-mediated immune response to basic protein of myelin is conflicting and inconsistent; also similar reactivity can occur in other neurological disorders as well as MS.

(i) Skin-testing for delayed hypersensitivity using myelin basic protein gives negative results (Field, 1965; Lisak *et al.*, 1968).

(ii) MIF is produced by blood lymphocytes when exposed to myelin basic protein, particularly in 'active' MS, according to two studies (Bartfeld & Atoynatan, 1970a; Rocklin *et al.*, 1971), but two studies have given negative results (Behan *et al.*, 1972; Strandgaard & Jorgenson, 1972). Moreover, of the two affirmative studies, there were discrepancies in regard to the incidence of reactivity of lymphocytes to myelin basic protein in diseases other than MS, particularly cerebrovascular disease; these discrepancies are discussed in detail by Bartfeld, Atoynatan & Donnenfeld (1972).

(iii) MSF, demonstrable by the cytopherometer assay, is produced by blood lymphocytes when exposed to myelin basic protein (Field & Caspary, 1970; Minderhoud & Smith, 1972) but, as with MIF assays, positive results were obtained in MS and also in cerebrovascular disease (Field, 1972).

(iv) Blast transformation of lymphocytes by neural antigens has given very conflicting results, as indicated by several representative studies presented in Table 1. Positive and negative results are cited, almost equally, and transformation was obtained in some studies in neurological diseases other than multiple sclerosis. The many sources of technical difference in this procedure (Table 1) could account for these varying findings.

(v) Cytotoxicity of blood lymphocytes from patients with MS using cultured neural tissue has been reported (Berg & Källen, 1964).

There could be no human counterpart to the experimental transfer of disease with lymphoid cells, as described in EAE.

## DOES ANTIGEN SUPPRESS DISEASE?

*For EAE*, the answer is 'yes'. This has been repeatedly demonstrated by injecting animals before or shortly after challenge with central nervous tissue or myelin basic protein and is reviewed by Alvord (1970). Protection can be achieved by various presentations of antigen, the optimal being unaltered basic protein in Freund's incomplete adjuvant (*vide*

TABLE 1. Transformation responses to neural antigens in multiple sclerosis

| Authors | Result * | Antigen | Type or stage of disease† | Culture conditions‡ | Assessment of response | Controls† | |
|---|---|---|---|---|---|---|---|
| | | | | | | Healthy | CNS diseases |
| Hashem & Barr (1963) | + | Rabies vaccine | 'Recent onset' 3/3; 'Latent' 0/2 | n.s. | Morphology | 1/6 | Encephalitis 2/2<br>Polyneuritis 1/1 |
| Fowler, Morris & Whitley (1966) | + | CSF | 'Typical' 6/6 | 5% Heterologous and autologous serum | Morphology | 0/5 | Organic nervous disease 0/5 |
| Brody *et al.* (1968) | − | CSF | n.s. 0/13 | 15% Autologous plasma | Thymidine, autoradiography | n.s. | Non-demyelinating diseases 0/11 |
| Hughes, Caspary & Field (1968) | + | BPM | 'Acute' 6/10 | 10% Autologous serum | Thymidine, counting | 2/12§ | Tumour, MND, and others 4/12 |
| Behan *et al.* (1968) | − | BPM | n.s. 0/6 | n.s. | Thymidine, counting | 0/7 | Alzheimer 0/6, polyneuritis 0/4, encephalomyelitis 2/2 |
| Jensen (1968) | − | CSF | n.s. 0/6 | 33% Autologous plasma | Thymidine, autoradiography | n.s. | None |
| Dau & Peterson (1970) | + | BPM | 'Widely different' stages 14/20 | 10% Autologous serum | Thymidine, counting | 0/21¶ | Various 0/7 |
| Bartfeld & Atoynatan (1970) | + | White matter, BPM, CSF | 'Active' 12, 'Inactive' 11 } pooled | 20% Autologous plasma | Morphology | n.s. | CVD, MND, and others 0/21<br>Wilson's disease 1/1 |
| Kibler, Paty & Sherr (1971) | − | BPM, CSF | 'Acute', and 'Chronic stable' 28 | 15% Calf serum | Thymidine, counting | n.s. | CVD, tumour, and others 0/21¶ |

* Authors' assessment. † Number positive/number tested. ‡ Source, and percentage of serum in 199 or MEM. § Laboratory workers handling brain products. ¶ 'Weak positives' were reported among these. BPM = basic protein of myelin. CSF = cerebrospinal fluid. MND = motor neurone disease. CVD = cerebrovascular disease. n.s. = Not stated or not studied.

*infra*), or by use of chemically altered basic protein (Swanborg, 1972) or 'analogues' of basic protein, e.g. basic polymers injected in combination with basic protein (Teitelbaum *et al.*, 1972). Moreover full reversal of established EAE in the monkey was possible with repetitive injections of native or chemically modified basic protein in FIA (Eylar *et al.*, 1972b).

The protection phenomenon has been studied in detail in our laboratory (Coates, Mackay & Crawford, 1973), using guinea-pigs and rats as test animals, and either native basic protein or peptides therefrom; the protective inocula were given in FIA and the challenge inocula in Freund's complete adjuvant. The degree of protection from EAE after challenge varied according to the amount of basic protein used for protection, and the time-gap between protection and challenge. Protection was not equivalent to 'conventional' tolerance in that protected animals retained capacity for production of humoral antibody to basic protein, and for production of MIF as assessed by exposure of peritoneal lymphocytes to basic protein (Coates *et al.*, 1973).

*For multiple sclerosis*, this question is unanswered. There have been few attempts to assess the protection phenomenon as demonstrable in EAE in patients with MS (Campbell *et al.*, 1973; Cendrowski, 1972; Fasanaro, Cientile & Stella, 1972), and in particular it is not known whether MS can be ameliorated by injection of basic protein of myelin in Freund's incomplete adjuvant. Clinical investigators would see considerable ethical problems in this approach in that the putative 'protecting' inoculum might well have a sensitizing (encephalitogenic) rather than a protecting effect.

## DO CYTOTOXIC DRUGS SUPPRESS DISEASE?

*For EAE*, the answer is 'yes'. Various immunosuppressive agents, particularly cyclophosphamide and methotrexate, have been shown to inhibit development of EAE, particularly when the drug is given after immunization but before onset of EAE. Some inhibition was obtained even when cyclophosphamide was given after onset of EAE (Paterson & Hanson, 1969).

*For multiple sclerosis*, the answer is probably 'no', the usual finding being that conventional immunosuppressive agents do not inhibit exacerbations (Cendrowski, 1972; Neumann & Ziegler, 1972; Silberberg, Lisak & Zweiman, 1973), although a complex regime of 'maximum immune suppression' was claimed to show a 'fulminant course' (Brendel, Seifert & Lob, 1972). Differences must be acknowledged between the immunological events associated with primary induction of EAE, which could be highly susceptible to suppression, and those possibly associated with well-established MS which, being more like a secondary immune response, would be less readily susceptible to suppression.

## OTHER CONSIDERATIONS

There are other types of evidence, not previously covered in this paper which are often cited in relation to resemblances between EAE and MS.

It is argued by the 'resemblist' school that whereas lesions of acute EAE may differ from those of MS, 'chronic' forms of EAE can be induced with lesions more analagous to those of MS, allowing for species differences (Prineas, Raine & Wisniewski, 1969). The 'non-resemblist' school claims that there are histological differences, e.g. minimal and infrequent demyelination, which distinguish all forms of EAE from MS (Field, 1969).

EAE is in most species a 'single event' process which is followed by either death or full recovery, whereas MS is a chronic relapsing disease. Chronic recurrent types of EAE are not well developed and such models would seem desirable. Post-rabies vaccination paralysis is a valid human analogue of EAE, but this disease does not appear to run a course similar to that of 'spontaneous' MS (Field, 1969).

A much used 'marker' for autoimmune processes in man has been co-existences and clustering of other disease of autoimmune character, but this marker is not well fulfilled in regard to MS. Some such clustering was reported in a recent study, but the likelihood of ascertainment error was conceded (Baker *et al.*, 1972).

Epidemiological data including age-specific incidence rates, the prevalence of MS in colder latitudes, and the retention of this high prevalence in adult but not juvenile migrants to low prevalence areas, are consistent neither with entirely immunological concepts of pathogenesis, nor with infective concepts (Brody, 1972; Leibowitz, Kahana & Alter, 1972).

Viral infection is claiming increasing consideration as a contributory pathogenetic process in MS (Weiner, Johnson and Herndon, 1973), based on (a) epidemiological considerations (Brody, 1972); (b) the presence of abnormal levels of antibody to viral antigens, particularly measles in patients with MS (Brody *et al.*, 1968; Millar *et al.*, 1971); (c) the demonstration of virus by electron-microscopy in sections of brain from a patient with multiple sclerosis (Prineas, 1972); (d) data from cell fusion studies in which a parainfluenza agent was 'rescued' from brain tissue of two patients with MS (Meulen *et al.*, 1972); and (e) analogies perceived between MS and neurological diseases recognized as attributable to damaging virus–host interactions (Riekkinen *et al.*, 1971), occurring possibly in the setting of a relative immuno-deficiency. For example, kuru has a known association with a transmissible neurotropic 'slow' virus, and SSPE has a known association with measles infection in early life (Riekkinen *et al.*, 1971). Whether the putative virus(es) act(s) indirectly by stimulating an autoimmune response, or directly as a cytopathogenic agent, is debatable (Field, 1973).

A non-immunological point of contact between EAE and MS is provided by the observations (Einstein *et al.*, 1972) on selective enzymic degradation of the basic protein around active plaques of MS. Thus autoimmune processes in MS would be secondary to release of peptide antigen liberated by a proteolytic attack, on myelin, of uncertain nature.

## CONCLUSIONS

Because of the promise developing from biochemical sequence studies on the basic protein molecule over the past several years, it was inevitable that a primary effort in MS research would be in relation to EAE. Certainly, the studies on the immunogenicity of various peptides from the basic protein have been of fundamental interest, yet these have not brought us any further towards an understanding of the essential 'cause' of MS, not towards its cure or prevention. Hence, there is uncertainty as to whether the immunological approach to MS is the right pathway to be following. Two points could be made concerning this.

The first point is that EAE in small animals injected with encephalitogenic material is a very 'clean' immunological model in which the usual aim is for maximal 'attack rates' of unequivocal disease. By contrast, as recently emphasized by Dixon (1972), human immunopathology is an end-result of a highly complex set of interactions which may involve immuno-

genic stimuli by extrinsic (viral) antigens or intrinsic (auto) antigens, past immunological experience, and various determinants of tolerance–response decisions. These determinants could be genetic, with possible links to genes for histocompatibility (Mackay & Morris, 1972; Naito *et al.*, 1972), physiological, including hormonal changes, or environmental, including stress, malnutrition and other effects. Hence the tenuous immunological resemblances between EAE and MS speak not so much against immunopathic disorder *per se* in the genesis of MS as against direct extrapolation of the 'simple' laboratory model EAE to the complex disease-mosaic expressed as human multiple sclerosis.

The second point is that preoccupation with immunological aspects of EAE has not been accompanied by a correspondingly deep immunological analysis of human multiple sclerosis, so that there are still certain fundamental questions to be answered.

(i) Are the biologically active factors, the demyelinating and polysynaptic blocking factor demonstrable in MS and EAE, of immunoglobulin and antibody character and what is their relationship, if any, to antibody or basic protein demonstrable in EAE by immunoassay? In what range of diseases are these factors demonstrable? Going further, does the polysynaptic blocking factor react with serotonergic neurones (Lennon & Carnegie, 1971)? The concept of 'immunopharmacological block' has been strengthened by demonstrable antibody blockade in experimentally-induced myasthenia (Patrick & Lindström, 1973).

(ii) What is the status in man of cell-mediated immunity to basic protein antigen, and to what degree is this demonstrable in health?—in miscellaneous non-neural diseases?—in neural disease excluding MS?—and in MS itself? What is the most reliable *in vitro* technique for demonstrating this—transformation of lymphocytes *in vitro*, production of macrophage or leucocyte migration inhibition factor, or production of macrophage slowing factor (Field & Caspary, 1970)? Would there be any consistent pattern of results using each of these different *in vitro* techniques to measure cell-mediated immunity to neural antigens?

(iii) Would better definition of immunological resemblances between experimental allergic neuritis and human idiopathic polyneuritis, as discussed by Nilsson (1972), contribute to understanding the relationships between EAE and MS?

(iv) What is the immunological basis of the protection afforded against EAE by modification of encephalitogenic inocula, and how should it be decided when protection regimes are sufficiently well developed and characterized to be applicable to trial as a type of 'vaccine' therapy of MS? These approaches would be contingent on the definitive demonstration in MS of a pathogenetically significant cell-mediated immune response *in vitro* to brain antigens. Further research is needed to ascertain the optimal way of developing protective inocula, i.e. whether to use native basic protein in FIA (Eylar *et al.*, 1972b; Coates *et al.*, 1973), chemically modified basic protein (Einstein, Chao & Csejtey, 1972b; Swanborg, 1972), copolymer 'analogues' of basic protein (Teitelbaum *et al.*, 1972), or encephalitogens 'inactivated' with mammalian sera (Gerstl *et al.*, 1972; Bernard & Lamoureux, 1973).

(v) The lead from Field & Caspary (1970) that there is a mutually advantageous research interface between MS and cancer has been strengthened by Pritchard *et al.*, (1972). Is there an explantion for this curious cross-reactivity between a basic protein extracted from cancer cell membrane and myelin basic protein? Are these antigens being recognized as similar by T cells on the basis of their overall electrical charge (Karniely *et al.*, 1973)?

The existence of these and other questions makes it clear that there is no scarcity of research avenues in the immunology of multiple sclerosis: the only problems seem to lie in the establishment of priorities.

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REFERENCES

ALVORD, E.C. (1970) Acute disseminated encephalomyelitis and 'allergic' neuroencephalopathies. *Handbook of Clinical Neurology*. (Ed. by P. J. Vinken and G. W. Bruyn), volume 9, p. 500. North-Holland Publishing Co., Amsterdam.

BAKER, H.W.G., BALLA, J.L., BURGER, H.T., EBELING, P. & MACKAY, I.R. (1972) Multiple sclerosis and autoimmune diseases. *Aust. N.Z. J. Med.* **2,** 256.

BARTFELD, H. & ATOYNATAN, T. (1970a) *In vitro* delayed (cellular) hypersensitivity in multiple sclerosis to central nervous system antigens. *Int. Arch. Allergy*, **39,** 361.

BARTFELD, H. & ATOYNATAN, T. (1970b) Lymphocyte transformation in multiple sclerosis. *Brit. med. J.* **2,** 91.

BARTFELD, H., ATOYNATAN, T. & DONNENFELD, H. (1972) *In vitro* cellular immunity to central nervous system antigens in multiple sclerosis. *Multiple Sclerosis—Immunology, Virology and Ultrastructure*. (Ed. by F. Wolfgram, G. W. Ellison, V. G. Stevens and J. M. Andrews), p. 333. Academic Press, New York.

BEHAN, P.O., BEHAN, W.M.H., FELDMAN, R.G. & KIES, M.W. (1972) Cell-mediated hypersensitivity to neural antigens. *Arch. Neurol. (Chic.)*, **27,** 145.

BEHAN, P.O., GESCHWIND, N., LAMARCHE, J.B., LISAK, R.P. & KIES, M.W. (1968) Delayed hypersensitivity to encephalitogenic protein in disseminated encephalomyelitis. *Lancet*, **ii,** 1009.

BERG, O. & KÄLLEN, B. (1964) Effect of mononuclear blood cells from multiple sclerosis patients on neuroglia in tissue culture. *J. Neuropath. exp. Neurol.* **23,** 550.

BERNARD, C.C. & LAMOUREUX, G. (1973) Inhibitory effect of normal sera in experimental allergic encephalomyelitis. (In press.)

BORNSTEIN, M.B. & CRAIN, S.M. (1965) Functional studies of cultured brain tissues as related to 'demyelinative disorders'. *Science*, **148,** 1242.

BORNSTEIN, M.B. & CRAIN, S.M. (1971) Lack of correlation between changes in bioelectric functions and myelin in cultured CNS tissues chronically exposed to sera from animals with EAE. *J. Neuropath. exp. Neurol.* **30,** 129.

BRENDEL, W., SEIFERT, J. & LOB, G. (1972) Effect of 'maximum immune suppression' with thoracic duct drainage, ALG, azathioprine and cortisone in some neurological disorders. *Proc. roy. Soc. Med.* **65,** 15.

BRODY, J.A. (1972) Epidemiology of multiple sclerosis and a possible virus aetiology. *Lancet*, **ii,** 173.

BRODY, J.A., HARLEM, M.M., KURTZKE, J.F. & WHITE, L.R. (1968) Unsuccessful attempt to induce transformation by cerebrospinal fluid in cultured lymphocytes from patients with multiple sclerosis. *New Engl. J. Med.* **279,** 202.

CAMPBELL, B., VOGEL, P.J., FISHER, E. & LORENZ, R. (1973) Myelin basic protein administration in multiple sclerosis. *Arch. Neurol.* **29,** 10.

CARNEGIE, P.R. (1971) Amino acid sequence of the encephalitogenic basic protein from human myelin. *Biochem. J.* **123,** 57.

CASPARY, E.A. HUGHES D. & FIELD, E.J. (1970) On the mode of action of antilymphocytic serum: experiments on electrophoretic mobility of macrophages in experimental allergic encephalomyelitis. *Clin. exp. Immunol.* **7,** 395.

CENDROWSKI, W. (1972) Immunosuppression in multiple sclerosis: therapeutic trial of azathioprine/imuran. *Multiple Sclerosis—Progress in Research*. (Ed. by E. J. Field, T. M. Bell and P. R. Carnegie), p. 168. North-Holland Publishing Co., Amsterdam.

CERF, J.A. & CARELS, G. (1966) Multiple sclerosis: serum factor producing reversible alterations in bioelectric responses. *Science*, **152,** 1066.

COATES, A.S. (1972) *Lymphocytes binding the basic protein of myelin in disease of the nervous system.* M.D. Dissertation, University of Melbourne.

COATES, A.S. & LENNON, V.A. (1973) Lymphocytes binding basic protein of myelin: cytophilic serum antibody and effect of adjuvant. *Immunology*, **24,** 425.

COATES, A.S., MACKAY, I.R. & CRAWFORD, M. (1973) Immunoregulatory protection against experimental autoimmune encephalomyelitis. (Submitted for publication.)

DAU, P. C. & PETERSON, R.D.A. (1970) Transformation of lymphocytes from patients with multiple sclerosis: use of encephalitogen of human origin with a report of a trial of immunosuppressive therapy in multiple sclerosis. *Arch. Neurol. (Chic.)*, **23,** 32.

DIXON, F.J. (1972) Pathogenesis of immunologic disease. *J. Immunol.* **109,** 187.

DOWLING, P.C., KIM, S.U., MURRAY, M.M. & COOK, S.D. (1968) Serum 19S and 7S demyelinating antibodies in multiple sclerosis. *J. Immunol.* **101,** 1101.

DUNKLEY, P.R., COATES, A.S. & CARNEGIE, P.R. (1973) Encephalitogenic activity of peptides from the smaller basic protein of rat myelin. *J. Immunol.* **110,** 1699.

EINSTEIN, E.R., CHAO, L. & CSEJTEY, J. (1972) Suppression of experimental allergic encephalomyelitis by chemically modified encephalitogen. *Immunochemistry*, **9,** 1013.

EINSTEIN, E.R., CSEJTEY, J., DALAL, K.B., ADAMS, C.M.W., BAYLIN, O.B. & HALLPIKE, J.F. (1972) Proteolytic activity and basic protein loss in and around multiple sclerosis plaques: combined biochemical and histochemical observations. *J. Neurochem.* **19,** 653.

EYLAR, E.H., BROSTOFF, S., HASHIM, G., CACCAM, J. & BURNETT, P. (1971) Basic A1 protein of the myelin membrane. The complete amino acid sequence. *J. biol. Chem.* **246,** 5770.

EYLAR, E.H., BROSTOFF, S., JACKSON, J. & CARTER, H. (1972a) Allergic encephalomyelitis in monkeys induced by a peptide from the $A^1$ protein. *Proc. nat. Acad. Sci. (Wash.)*, **69,** 617.

EYLAR, E.H. JACKSON, J., ROTHENBERG, B. & BROSTOFF, S.W. (1972b) Suppression of the immune response: reversal of the disease state with antigen in allergic encephalomyelitis. *Nature (Lond.)*, **238,** 74.

FASANARO, G., CIENTILE, A. & STELLA, L. (1972) Prospettive per una terapia immunologica della sclerosi multipla. *Acta neurol. (Napoli)*, **27,** 367.

FIELD, E.J. (1965) Some observations on the clinical immunology of multiple sclerosis. *NINDS Monograph Number 2. Slow Latent and Temperate Virus Infections.* p. 187–192. U.S. Govt. Printing Office.

FIELD, E.J. (1969) Slow virus infections of the nervous system. *Int. Rev. exp. Path.* **8,** 129.

FIELD, E.J. (1972) Delayed hypersensitivity studies: some applications of cell electrophoresis. *J. roy. Coll. Physns (Lond.)*, **6,** 316.

FIELD, E.J. (1973) Role of viral infection and autoimmunity in aetiology and pathogenesis of multiple sclerosis. *Lancet*, **i,** 295.

FIELD, E.J. & CASPARY, E.A. (1970) Lymphocyte sensitisation: an *in vitro* test for cancer? *Lancet*, **ii,** 1337.

FOWLER, L., MORRIS, C.E. & WHITLEY, T. (1966) Lymphocyte transformation in multiple sclerosis induced by cerebrospinal fluid. *New Engl. J. Med.* **275,** 1041.

GERSTL, B., UYEDA, C.T., HUNT, H. & ENG, L.F. (1972) A physiological mechanism preventing experimental allergic encephalitis. *Immunology*, **23,** 395.

HASHEM, N. & BARR, M.L. (1963) Mitogenic effect of rabies vaccine on cultures of lymphocytes in diseases of the nervous system. *Lancet*, **ii,** 1029.

HUGHES, D., CASPARY, E.A. & FIELD, E.J. (1968) Lymphocyte transformation induced by encephalitogenic factor in multiple sclerosis and other neurological diseases. *Lancet*, **ii,** 1205.

JENSEN, M.K. (1968) Lymphocyte transformation in multiple sclerosis. *Acta neurol. scand.* **44,** 200.

JOHNSON, A.B., WISNIEWSKI, H.M., RAINE, C.S., EYLAR, E.H. & TERRY, R.D. (1971) Specific binding of peroxidase labelled myelin basic protein in allergic encephalomyelitis. *Proc. nat. Acad. Sci. (Wash.)*, **68,** 2694.

KARNIELY, Y., MOZES, E., SHEARER, G.M. & SELA, M. (1973). The role of thymocytes and bone marrow cells in defining the response to the dinitrophenyl hapten attached to positively and negatively charged synthetic polypeptide carriers. Cell fractionation over charged columns. *J. exp. Med.* **137,** 183.

KIBLER, R.F., PATY, D.W. & SHERR, V. (1971) Immunology of multiple sclerosis. *Ass. Res. nerv. Dis. Proc.* **49,** 95.

KIES, M.W., DRISCOLL, B.F., SEIL, F.J. & ALVORD, E.C. (1973) Myelination inhibition factor: dissociation from induction of experimental allergic encephalomyelitis. *Science*, **179**, 689.

LAMOUREUX, G., THIBEAULT, G., RICHER, G. & BERNARD, C. (1972) Induction de l'encéphalite allergique expérimentale avec des encéphalitogènes human de synthèse. *L'Un. Med.* **101**, 674.

LEIBOWITZ, U., KAHANA, E. & ALTER, M. (1972) Population studies of multiple sclerosis in Israel. *Multiple Sclerosis—Progress in Research* (Ed. by E. J. Field, T. M. Bell and P. R. Carnegie), p. 179–196. North-Holland Publishing Co., Amsterdam.

LENNON, V.A. & CARNEGIE, P.R. (1971) Immunopharmacological disease—a break in tolerance to receptor sites. *Lancet*, **i**, 630.

LENNON, V.A. & CARNEGIE, P.R. (1973) Immunologic responses of guinea-pigs and rabbits to a synthetic autoantigen of brain. (Submitted for publication.)

LENNON, V.A. & DUNKLEY, P.R. (1973) Humoral and cell-mediated immune responses of Lewis rats to syngeneic basic protein of myelin. (In press.)

LENNON, V.A. & FELDMANN, M. (1972) The detection of autoantibody-forming cells. I. An assay for plaque-forming cells to the basic protein of myelin in guinea-pigs. *Int. Arch. Allergy*, **42**, 627.

LENNON, V.A., FELDMANN, M. & CRAWFORD, M. (1972) The detection of autoantibody-forming cells. II. Cells in lymph nodes and central nervous system containing antibody to myelin basic protein. *Int. Arch. Allergy*, **43**, 749.

LENNON, V.A. & MACKAY, I.R. (1972) Binding of $^{125}$I myelin basic protein by serum and cerebrospinal fluid. *Clin. exp. Immunol.* **11**, 595.

LEVINE, S. & SOWINSKI, R. (1973) Experimental allergic encephalomyelitis in inbred and outbred mice. *J. Immunol.* **110**, 139.

LENNON, V.A., WHITTINGHAM, S., CARNEGIE, P.R., MCPHERSON, T.A. & MACKAY, I.R. (1971) Detection of antibodies to the basic protein of human myelin by radioimmunoassay and immunofluorescence. *J. Immunol.* **107**, 56.

LENNON, V.A., WILKS, A.V. & CARNEGIE, P.R. (1970) Immunologic properties of the main encephalitogenic peptide from the basic protein of human myelin. *J. Immunol.* **105**, 1223.

LINK, H. (1972) Oligoclonal immunoglobulin G in multiple sclerosis brains. *J. neurol. Sci.* **16**, 103.

LISAK, R.P., HEINZLE, R.G., FALK, G.A. & KIES, M.W. (1968) Search for anti-encephalitogen antibody in human demyelinative disases. *Neurology* (*Minneap.*), **18**, 122.

LUMSDEN, C.E. (1969) Properties and significance of the demyelinating antibody in multiple sclerosis. *Int. Arch. Allergy*, **36**, suppl. p. 247–275.

LUMSDEN, C.E. (1971) The immunogenesis of the multiple sclerosis plaque. *Brain Res.*, **28**, 365.

MACKAY, I.R. & MORRIS, P.J. (1972) Association of autoimmune active chronic hepatitis with HL-A1,8. *Lancet*, **ii**, 793.

MCPHERSON, T.A. & CARNEGIE, P.R. (1968) Radioimmunoassay using gel filtration for detecting antibody to basic proteins of myelin. *J. Lab. clin. Med.* **72**, 824.

MEULEN, V. TER, KOPROWSKI, H., IWASAKI, Y., KACKELL, Y.M. & MÜLLER, D. (1972) Fusion of cultured multiple sclerosis brain cells with indicator cells: presence of nucleocapsids and virions and isolation of parainfluenza-type virus. *Lancet*, **ii**, 1.

MILLAR, J.H.D., FRASER, K.B., HAIRE, M., CONNOLLY, J.H., SHIRODARIA, P.V. & HADDEN, D.S.M. (1971) Immunoglobulin M specific for measles and mumps in multiple sclerosis. *Brit. med. J.* **2**, 378.

MINDERHOUD, J.M. & SMITH, J.K. (1972) Immunological activity of blood lymphocyte fractions: a study by macrophage electrophoretic mobility method. *Clin. exp. Immunol.* **10**, 571.

NAITO, S., NAMEROW, N., MICKEY, M.R. & TERASAKI, P.I. (1972) Multiple sclerosis: association with HL-A3. *Tissue Antigens*, **2**, 1.

NEUMANN, J.W. & ZIEGLER, D.K. (1972) Therapeutic trial of immunosuppressive agents in multiple sclerosis. *Neurology* (*Minneap.*) **22**, 1268.

NILSSON, O. (1972) Immunological aspects of demyelinating diseases. *Acta neurol. scand.* **48**, suppl. 51, p. 321.

NOORT, S. VAN DEN & STJERHOLM, R.L. (1971) Lymphotoxic activity in multiple sclerosis serum. *Neurology*, **21**, 783.

PATERSON, P.Y. (1960) Transfer of allergic encephalomyelitis in rats by means of lymph node cells. *J. exp. Med.* **111**, 119.

PATERSON, P.Y. (1969) Immune processes and infectious factors in central nervous system disease. *Ann. Rev. Med.* **20**, 75.

PATERSON, P.Y. (1972) Experimental allergic encephalomylitis and multiple sclerosis as immunologic diseases: a critique. *Multiple Sclerosis—Immunology, Virology and Ultrastructure.* (Ed. by F. Wolfgram, G. W. Ellison, V. G. Stevens and J. M. Andrews), p. 538. Academic Press, New York.

PATERSON, P.Y. & HANSON, M.A. (1969) Cyclophosphamide inhibition of experimental allergic encephalomyelitis and cellular transfer of the disease in Lewis rats. *J. Immunol.* **103,** 1311.

PATRICK, J. & LINDSTRÖM, J. (1973) Autoimmune response to acetylcholine receptor. *Science,* **180,** 871.

PRINEAS, J.W. (1970) The etiology and pathogenesis of multiple sclerosis. *Handbook of Clinical Neurology.* (Ed. by P. J. Vinken and G. W. Bruyn), volume 9, p. 107. North-Holland Publishing Co., Amsterdam.

PRINEAS, J.W. (1972) Paramyxovirus like particles associated with acute demyelination in chronic relapsing multiple sclerosis. *Science,* **178,** 760.

PRINEAS, J.W., RAINE, C.S. & WISNIEWSKI, H. (1969) An ultrastructural study of experimental demyelination and remyelination. III. Chronic experimental allergic encephalomyelitis in the central nervous system. *Lab. Invest.* **21,** 472.

PRITCHARD, J.A.V., MOORE, J.L., SUTHERLAND, W.H. & JOSLIN, C.A.F. (1972) Macrophage-electrophoretic mobility (MEM) test for malignant disease. *Lancet,* **ii,** 627.

RAINE, C.S. & BORNSTEIN, M.B. (1970) Experimental allergic encephalomyelitis: an ultrastructural study of experimental demyelination *in vitro. J. Neuropath. exp. Neurol.* **29,** 177.

RIEKKINEN, P., RINNE, U.K., SAVOLAINEN, H., PALO, J., KOVALO, E. & ARSTILA, A. (1971) Studies on the pathogenesis of multiple sclerosis. *Europ. Neurol.* **5,** 229.

ROCKLIN, R.E., SHEREMATA, W.A., FELDMAN, R.G., KIES, M.W. & DAVID, J.R. (1971) The Guillain-Barré syndrome and multiple sclerosis. *In vitro* cellular responses to nervous tissue antigens. *New Engl. J. Med.* **284,** 803.

SEIL, F.J., FALK, G.A., KIES, M.W. & ALVORD, E.C. (1968) The *in vitro* demyelinating activity of sera from guinea-pigs sensitized with whole CNS and with purified encephalitogen. *Exp. Neurol.* **22,** 545.

SHAPIRA, R., CHOU, F.C.H., MCKNEALLY, S., URBAN, E. & KIBLER, R.F. (1971) Biological activity and synthesis of an encephalitogenic determinant. *Science,* **173,** 736.

SILBERBERG, D., LISAK, R. & ZWEIMAN, B. (1973) Multiple sclerosis unaffected by azathioprine in pilot study. *Arch. Neurol. (Chic.),* **28,** 210.

SPITLER, L.E., MULLER, C.M. VON, FUDENBERG, H.H. & EYLAR, E.H. (1972) Experimental allergic encephalitis. Dissociation of cellular immunity to brain protein and disease production. *J. exp. Med.* **136,** 156.

STRANDGAARD, S. & JORGENSON, P. (1972) Delayed hypersensitivity to myelin antigen in multiple sclerosis investigated with the leucocyte migration method. *Acta neurol. scand.* **48,** 243.

SWANBORG, R.H. (1972) Antigen-induced inhibition of experimental allergic encephalomyelitis. *J. Immunol.* **109,** 540.

TEITELBAUM, E., WEBB, C., MESHORER, A., ARNON, R. & SELA, M. (1972) Protection against experimental allergic encephalomyelitis. *Nature (Lond.),* **240,** 645.

TOURTELLOTTE, W. (1970) On cerebrospinal fluid immunoglobulin-G (IgG) quotients in multiple sclerosis and other diseases. *J. neurol. Sci,* **10,** 279.

TOURTELLOTTE, W. (1972) Interaction of local central nervous system immunity in the spread of multiple sclerosis demyelination. *Multiple Sclerosis—Immunology, Virology and Ultrastructure.* (Ed. by F. Wolfgram, G. W. Ellison, V. G. Stevens and J. M. Andrews), p. 285. Academic Press, New York.

TSAREGORODTSEVA, T.M. & CHERNIGOVSKAYA, N.V. (1969) Immunobiological properties of the serum in multiple sclerosis. *Bull. exp. Biol. Med.* **67,** 662.

WAKSMAN, B.H. (1956) Further study of skin reactions in rabbits with experimental allergic encephalomyelitis. *J. infect. Dis.* **99,** 258.

WEINER, L.P., JOHNSON, R.T. & HERNDON, R.M. (1973) Viral infections and demyelinating diseases. *New Engl. J. Med.* **288,** 1103.

WHITTINGHAM, S., BENCINA, B., CARNEGIE, P.R. & MCPHERSON, T.A. (1972) Properties of antibodies produced in rabbits to human myelin and myelin basic protein. *Int. Arch. Allergy,* **42,** 250.

YUNG, L.L.L., DIENER, E., MCPHERSON, T.A., BARTON, M.A. & HYDE, H.A. (1973). Antigen binding lymphocytes in normal man and guinea-pig to human encephalitogenic protein. *J. Immunol.* **110,** 1383.