Search for the Cause of Multiple Sclerosis and other Chronic Diseases of the Central Nervous System

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LOCALIZATION OF SPECIFIC BRAIN ANTIGENS

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SUMMARY

Recent developments in immunocytochemical methods (specifically horseradish peroxidase labelling) have greatly increased the sensitivity and specificity of immunohistology. The peroxidase-antiperoxidase (PAP) method of Sternberger and the conjugated-peroxidase (CP) method of Nakane permit retrospective analysis of formalin-fixed, paraffin-embedded tissue, the staining of tissue embedded in plastic, and the examination of peroxidase-labelled tissue at both the light and electron microscopic (EM) levels. For molecules which retain their antigenicity after fixation and tissue processing, the immunoperoxidase methods are far superior to the immunofluorescence techniques since the stained sections are permanent, can be used at the EM level, and can be counterstained by conventional histochemical procedures. Examples of specific staining for the glial fibrillary acidic (GFA) protein (the major protein component of glial filaments), tubulin, myelin basic protein from central myelin, and the P-2 protein from peripheral myelin in human and animal tissues are presented. Because of its high specificity for glial filaments in astrocytes, antibody to the GFA protein has been used to demonstrate fibrous gliosis in brains of multiple sclerosis, aged, and Alzheimer's disease patients. Antibody to the GFA protein is routinely being used with the PAP method to assist in the diagnosis of human brain tumors.

I. INTRODUCTION

Availability of monospecific antisera and the development of new immunohistochemical horseradish peroxidase methods--the peroxidase-antiperoxidase (PAP) method of Sternberger (1) and the conjugated-peroxidase (CP) method of Nakane (2)--have generated many immunocytochemical studies (see ref. 3 and 4 for reviews). This presentation will give examples of how we have and continue to utilize immunocytochemical methods in the study of multiple sclerosis (MS), Alzheimer's Disease (AD), aging, and Alexander's disease.

Treatment and cure of patients with neurologic disorders of unknown etiology, MS, aging, and dementia, as well as those of known origin, spinal cord injury and ischemic vascular disease, are limited because the mechanisms of degeneration and regeneration of the central nervous system (CNS) are largely unknown. Gliosis is a principal event in CNS trauma and a common feature of many neurological disorders. Gliosis is characterized by extensive proliferation and hypertrophy of astrocytes and their processes in cortical and subcortical regions frequently with preservation of unaffected nerve cells and their processes. While fibrous gliosis may be a beneficial reaction to CNS injury, it may also be deleterious to the functioning of residual neuronal circuits and prevent remyelination and axonal regeneration.

The most prominent characteristic of fibrous gliosis is extensive glial filament synthesis. A putative function of fibrous gliosis is to provide mechanical support, and glial filaments are believed to be the framework for this support. In addition to a passive structural role, astrocytes are thought to actively monitor and control the contents of the extracellular space of the CNS including the amounts of ions, transmitters, trophic factors, nutrients, and waste materials. Our continued studies of the astrocyte and their glial filaments are based on the central role the astrocytes may play in the normal and disease state of the CNS.

II. GLIAL FIBRILLARY ACIDIC PROTEIN FROM MULTIPLE SCLEROSIS PLAQUES

Since our initial isolation and characterization of the glial fibrillary acidic (GFA) protein from MS plaques (5,6, see ref. 3 and 7 for reviews), several lines of evidence have accumulated which support our view that the GFA protein is the major protein of glial filaments. These data are summarized in TABLE 1.

OLIGODENDROCYTE - MYELIN SHEATH INTERRELATIONSHIPS

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SUMMARY

It is emphasised that continuity of surface membrane between an oligodendrocyte and its myelin sheath creates a large compartment which is separated from the rest of the central nervous system by its own permeability barrier. Interrelationships existing between oligodendrocytes and their myelin sheaths are stressed from two points of view. At a structural level the disposition of lipids and proteins in the membrane around the oligodendrocyte - myelin compartment is defined with an emphasis on those antigenic constituents which are located at the external surface of the system. At a metabolic level the oligodendrocyte cell body is considered to be the key site of metabolic and dynamic activity in the compartment especially with regard to the synthesis of membrane components. Features of the oligodendrocyte - myelin compartment are discussed in relation to demyelination and multiple sclerosis.

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1. INTRODUCTION

The glial compartment of the central nervous system [CNS] comprises a number of different cell types (1,2) which interact closely together but which, in the main, have not had their functions fully defined. It is, however, generally agreed that most interfasicular oligodendrocytes of the white matter (3) are responsible for the huge burst of membrane synthesis which occurs at myelination and leads to the formation of myelin sheaths around axons in the CNS. The same oligodendrocytes probably then maintain the integrity of myelin throughout life. Perineuronal satellite oligodendrocytes in the grey matter, while closely resembling oligodendrocytes in the white matter, are not involved in the main phase of myelination but may, later on, remyelinate axons which lose myelin sheaths due to damage (4).

Axons are not completely covered with a myelin sheath along their whole length [Fig.1]. Separate myelin sheaths provide insulation around axons between successive nodes of Ranvier where the axolemma is exposed to the extracellular environment. Oligodendrocytes are capable of producing a considerable number of internodal myelin sheaths. The distance between a myelin sheath and its parent oligodendrocyte can be over 30 μ (5) but generally the formation of sheaths occurs around axons which are closer, or sometimes adjacent, to the oligodendrocyte cell body. Myelin sheaths are in continuity with the oligodendrocyte cell body through the surface membrane and cytoplasm-filled processes (2,3) as shown in Figs.1 and 3.This oligodendrocyte-myelin compartment is isolated from the rest of the CNS by its own permeability barrier which must be breached before damage such as demyelination can be initiated.

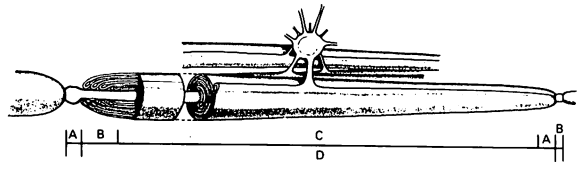

FIG.1. DIAGRAM OF AN OLIGODENDROCYTE, SOME OF ITS PROCESSES AND AN INTERNODAL MYELIN SHEATH IN THE CNS. A, node; B, paranodal region; C, perinodal region; D, internodal sheath.

Degeneration of myelin sheaths, a characteristic feature of multiple sclerosis [MS], leads to impaired impulse transmission along axons. In severe demyelination the passage of an impulse is blocked completely. The demyelination observed in the brain and spinal cord in

MS could result from a direct attack on some specific component of the myelin sheath or it may follow from an attack on the oligodendrocyte causing cell death and the loss of metabolic support for the myelin. Another possibility is that an attack at some different site in the CNS may spill over to cause 'bystander' demyelination (6). What ever the pathogenetic mechanism for myelin destruction it is important to define at a structural level the orientation of lipid and protein components in the surface membrane of the oligodendrocyte-myelin compartment especially with regard to the distribution of antigenic constituents at the external surface. At a metabolic level it is necessary to discover how the integrity of the compartment is maintained and how it reacts following viral infection, exposure to antibody against surface antigens or to degradative enzymes such as proteinases and phospholipases which may be released from invading, sensitised cells.

2. STRUCTURAL ASPECTS OF THE OLIGODENDROCYTE-MYELIN COMPARTMENT

The multilamellar membrane system of the myelin sheath is derived from, and is continuous with, the plasma membrane of the parent oligodendrocyte (3). The repeating unit of compact myelin is a double membrane structure derived from the association at their cytoplasmic surfaces of plasma membrane from the oligodendrocyte process (7). In compact myelin membrane surfaces at the cytoplasmic apposition [main dense line in electron micrographs] do not readily separate under a variety of treatments (8). Surfaces at the external apposition [the intraperiod dense line in electron micrographs] separate readily in, for example, hypotonic conditions and are clearly less intimately associated.

The membrane of the myelin sheath region of the oligodendrocyte-myelin compartment is highly enriched in lipid relative to protein [Table 1] in keeping with the role of the sheath as insulation (9). The lipids of compact myelin are of the usual amphipathic type which will spontaneously aggregate into bilayer structures when dispersed in an aqueous environment. Compact myelin is unusual in that glycosphingolipids, mainly cerebroside, account for some 20% of the total lipids of the membrane. These glycosphingolipids contain long [C24] saturated and monoenoic fatty acids of which about 50% have an α- hydroxyl group. Cerebrosides have a high endothermic phase transition temperature even in the hydrated state (10). Another notable feature of myelin lipids is that some 80% of the ethanolamine-containing phospholipids are in the plasmalogenic form (9).

Chemical and structural details of the compact myelin sheath have been reviewed recently (9,11,12,13,14). Results from X-ray diffraction and electron microscopy are best interpreted in terms of a bilayer arrangement for the lipid phase of the membrane. Thus, in basic structure, myelin is similar to other biological membranes. Thermal analyses reveal that the lipid phase of myelin is in an intermediate liquid-crystalline state (10) maintained by the hydration of the system and by the high levels of cholesterol present. This result suggests that lipid and protein species in myelin should be mobile in the plane of the membrane unless specific constraints operate. The absence of a phase transition for myelin suggests that cerebrosides probably do not exist as separated patches of single lipid species within the bulk lipid phase but are dispersed in the membrane with other lipids.

Antisera to glycosphingolipids and gangliosides aggregate isolated myelin sheath preparations (15). Cerebroside in isolated myelin is accessible to attack by galactose oxidase and sodium periodate (16). These studies indicate that the glycosphingolipids are arranged on the external membrane surface of compact myelin where, as hydrogen bond donors as well as acceptors (9) they may serve to 'tighten-up' the hydrophilic-hydrophobic boundary of this protein-poor membrane. With their uncharged yet polar head group cerebrosides may be very suitable molecules to have at the surface of the myelin membrane system to allow the close approach of surfaces. A small part of the ethanolamine-containing phospholipids may be externally-disposed in compact myelin (17). Cholesterol in the membrane system may preferentially interact with sphingolipids (9). Little else is known about the orientation of the lipids in compact myelin but, by extrapolation from asymmetry results with other membrane systems (18) choline-containing phospholipids may be preferentially located on the external membrane surface.

Two proteins account for some 80-85% of the total protein of compact myelin (9,11,12,13). These are the proteolipid protein [PLP] and the myelin basic protein [MBP] which are present in the membrane system in about equal amounts in brain tissue. Proteolipid proteins are widely distributed in other biological membranes (19) and the biochemistry of this protein in myelin has been reviewed (11,12,19). The PLP in myelin is highly hydrophobic and contains covalently bound fatty acid chains. PLP interacts with acyl chains of lipids in a bilayer to restrict their mobility preventing them from taking part in the bulk lipid phase transition (20). There is evidence that PLP interacts with uncharged, zwitterionic and negatively charged amphipathic lipid species (19).

component	composition in myelin (% dry wt.)			composition in oligodendrocyte plasma membrane (% dry wt.)
	man	ox	rat	calf
protein	21.3	22.3	36.0	54.2
lipid	78.7	77.7	64.0	42.5
ganglioside	0.5	0.5	0.5	0.5
	mol.% total lipid			mol.% total lipid
cholesterol	40.9	44.4	42.7	36.4
cerebroside + sulphatide	19.7	19.8	16.9	12.4
phosphatidylcholine	10.9	8.2	10.5	25.4
ethanolamine phospholipids	13.6	15.1	18.0	7.3
serine phospholipids	5.1	4.9	6.2	5.1
sphingomyelin	4.7	5.1	3.4	5.4
others including phosphatidylinositol	5.1	2.5	2.3	7.7

TABLE 1. COMPOSITION OF MYELIN SHEATH PREPARATIONS FOR THREE ANIMAL SPECIES AND FOR OLIGODENDROCYTE PLASMA MEMBRANE PREPARATIONS FROM CALF BRAIN. DATA ARE TAKEN FROM A NUMBER OF SOURCES.

MBP has been studied in depth from structural (9,11,12,13,21) and immunological (22) aspects because of its encephalitogenic properties. When injected with adjuvant into susceptible animals it causes a cell-mediated inflammatory reaction in the CNS. The disease condition which ensues, experimental allergic encephalomyelitis, is used as a model for MS. MBP is readily removed from myelin by aqueous extraction and the structure of the protein in solution has been studied (21, 23). MBP interacts with negatively-charged lipid species and these ionic interactions may be concentrated on the C-terminal region of the protein (24). However, the protein also shows evidence of hydrophobic interactions where regions of the polypeptide chain penetrate into the lipid bilayer and influence the mobility of the acyl chains of the lipids (25). Recent data suggests that sites for hydrophobic interactions on the MBP may occur on both the N- and C- terminal regions of the protein (26).

There are other minor protein constituents attributed to compact myelin. Some of these arise from contamination of the preparation (9). Others such as the Wolfgram doublet, DM-20 and some glycoproteins are genuine components of the myelin sheath. A major glycoprotein of myelin (27) accounts for some 0.4% of the total protein and carries a variety of saccharide residues. A newly identified protein, the pre-MBP (28), is structurally similar to MBP but has an extra sequence of polypeptide chain at its N-terminus. The pre-MBP is only a minor component of myelin

ability of the oligodendrocyte to react rapidly and effectively to insult. Plasma membranes can generally protect themselves against lysolipids by reacylation or deacylation reactions. Compact myelin, however, seems particularly susceptible to lysolipid attack (40,41) which leads to demyelination. This is perhaps because the necessary enzymes to metabolise the lysolipid are lacking but may also be because of the distance over which essential cofactors and metabolites have to be removed. The surface membrane of long oligodendrocyte processes may also be especially vulnerable in the same way. Damage to the permeability barrier of the processes or the myelin sheath could lead to the death of the whole compartment (40,41). The reverse situation where death of the oligodendrocyte cell body leads to subsequent demyelination through loss of metabolic support, is also true (40,42).

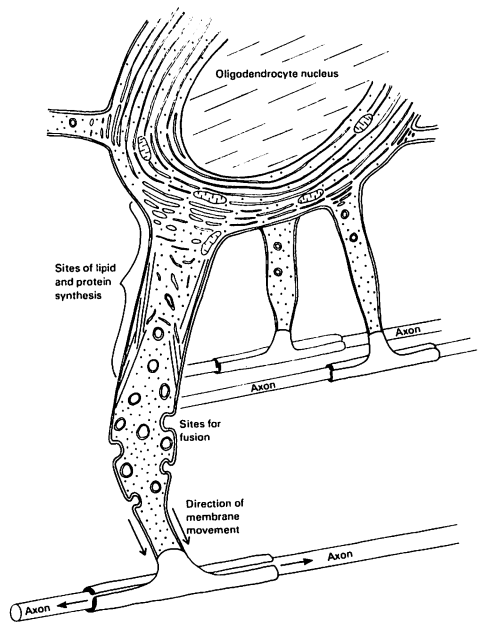

FIG.3. OLIGODENDROCYTE AND PROCESSES SHOWING MOVEMENT OF LIPID AND PROTEIN VESICLES FROM SITES OF SYNTHESIS AT THE ENDOPLASMIC RETICULUM AND GOLGI APPARATUS IN THE BODY OF THE CELL TO SITES OF FUSION WITH THE SURFACE MEMBRANE OF THE PROCESS. IT HAS BEEN SUGGESTED (9) THAT THE CONTINUOUS FLOW OF LIPID VESICLES INTO THE SURFACE MEMBRANE NEAR SITES OF SHEATH FORMATION COULD PROVIDE THE DRIVING FORCE FOR MYELINATION. THE MYELIN SHEATH EXPANDS ALONG THE AXON AS WELL AS AROUND IT.

The action of phospholipases, especially phospholipase A_2, which generate lysolipids by their action on a membrane surface may be crucial in bringing about initial damage to the oligodendrocyte-myelin compartment. Proteinases released from invading inflammatory cells (14)

will be active in degrading proteins and glycoproteins on the compartment surface but would not necessarily induce a breach of the permeability barrier. The effectiveness of a mixture of phospholipase A_2 in combination with trypsin, compared with trypsin alone, in bringing about the degradation of isolated myelin has been described (43).

3. MULTIPLE SCLEROSIS AND THE OLIGODENDROCYTE-MYELIN COMPARTMENT

Several ideas have been advanced to account for the degeneration of the myelin sheath which occurs in multiple sclerosis and include dietary, autoimmune and viral mechanisms (14). It has been suggested (summarised in 44) that alterations in dietary lipids can influence membrane fluidity perhaps leading to an unstable myelin sheath or predisposing the membrane system to viral attack. The high content of cholesterol which is present in the plasma membranes of all animal cells is believed to preserve an intermediate fluid environment in the lipid phase. The sterol can increase the fluidity of saturated acyl chains on lipids and it can decrease the fluidity of unsaturated acyl chains (45). Myelin is very rich in cholesterol and the presence of the sterol will therefore protect the membrane against changes in fluidity introduced by abnormal amounts of saturated fatty acids on complex lipids or by uncommon fatty acyl chains.

Autoimmune mechanisms proposed for MS commonly involve MBP as the encephalitogen. However, localisation of this antigen at the cytoplasmic apposition of compact myelin effectively hides the protein from exposure to the external environment and thus to recognition and attack by invading sensitised cells or antibody. It has been noted, however, that MBP and PLP share a common antigenic site (46) and thus invading cells sensitised to MBP could react initially to PLP which is believed to be partially exposed on the external membrane surface. It is puzzling to note, though, that antisera to MBP do not aggregate isolated myelin preparations and the PLP is not noted to be encephalitogenic. As an encephalitogen MBP could be released into the circulation to perhaps initiate autoimmune mechanisms of demyelination by the normal turnover of oligodendrocytes in the CNS. However, in experimental allergic encephalomyelitis special conditions of injection of MBP involving complete Freund's adjuvant are needed to provoke a cell-mediated inflammatory response in the CNS. Further, there is no evidence that MBP released following head injury or stroke, for example, precipitates encephalitis or is even very antigenic. The situation, though, may be very different where the immune response is

IMMUNOGLOBULIN G LEVELS IN THE CENTRAL NERVOUS SYSTEM IN MULTIPLE SCLEROSIS

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A raised IgG level in the CSF is a consistent feature of multiple sclerosis(MS). In 75% of patients IgG accounts for more than 12% of the total protein (control value, 4%). A better correlation with diagnosis is found when the protein electrophoretic pattern of the CSF is examined. In more than 90% of patients with confirmed MS the IgG can be seen to be of restricted heterogeneity (1). The oligoclonal bands are relatively diffuse in themselves and probably represent immunodominant antigens, each of which has stimulated several clones of lymphocytes to produce antibody. Frick and Scheid-Seydel (2) concluded that immunoglobulins were synthesized intrathecally, on the basis of exchange of intravenous ^{131}I-labelled IgG between serum and CSF. Further confirmation comes from the results of Sandberg-Wollheim (3), who found that IgG was synthesized by CSF lymphocytes from MS patients, with the same oligoclonal distribution as the original CSF.

Origin and localization of antibody

Perivenous inflammatory cells are usually associated with demyelinating lesions in MS, and lymphocytic meningitis frequently accompanies active demyelination (4). The number of lymphocytes in the meningeal areas, which far exceeds the number of free cells in the CSF may account for the pleocytosis in the CSF in MS patients with an exacerbation (5). Structural features resembling those of lymphoid tissue have been observed in plaque and white matter tissue in MS brain (6). In an electron microscopic study, lymphocytes and macrophages were seen to be confined within thin-walled channels in the perivascular space, with plasma cells located outside. This is the same type of tissue organization as in the IgG-secreting medullary region of the lymph node.

Immunoperoxidase studies of chronic plaque tissue have revealed that reactive astrocytes and lymphoid cells contain IgG - in gliofibrils and lysosomes and bound

to myelin (7). Deposits of C1g in the same locale as IgG lends support to the view that part of the IgG is present in the form of aggregated immune complex (8).

Biochemical studies of Central Nervous System (CNS) IgG

Biochemical analyses of IgG localized in CNS tissue are complicated by the necessity to correct for the contribution of IgG from the cerebral circulation. A good approximation can be made by expressing the results as an index of IgG/albumin (9). A wide range of absolute values for IgG in MS brain have been reported, depending on the extent of washing of the tissue, prior to homogenization (Table 1). In the present preliminary study tissue slices, with approximately equal amounts of white and grey matter, were finely chopped and washed in buffered saline 5 times, prior to homogenization. The homogenate was fractionated by the method of Cuzner, Davison and Gregson (10), and membrane fractions were extracted in 1% (w/v) Triton X-100. Albumin levels were assayed by the Laurell rocket electrophoresis technique (11) and IgG by a competitive binding assay, using the reagent ^{125}I-Protein A (12).

Table 1. IgG Levels in Subcellular Fractions of Human Brain
(ug/g. wet wt.)

	FINAL SUPERNATANT	MIC. *(Triton	MIT. X-100	NUC. Extracts)	Saline Extract of white matter	Acid Extract of white matter
Normal Control n = 8	11.2	0.51	3.6	0.63	203(9),12.8(16)	$\simeq$ 0.1(15), $<$0.8(16)
Multiple Sclerosis n = 8	15.1	0.94	4.9	0.94	406(9)	0.5-1.4(15)

* % Protein extracted by Triton X-100
Microsomal fraction (MIC) = 35, crude mitochondrial fraction (MIT) = 28 and nuclear fraction (NUC) = 20

A significant proportion of IgG appears to be bound selectively to membranes, as the IgG/albumin index in the three subcellular fractions was higher than in the final supernatant (Fig. 1). The absolute levels of IgG were found to be increased in all fractions from MS brain, compared to control brain (Table 1). However, the significance of these differences was reduced when the results were calculated as an IgG/albumin index, indicating that the permeability of the blood-brain barrier was altered in the MS samples. But Schliep and Felgenhauer (13) have shown the blood-brain barrier to be essentially normal in MS, and the inflammatory response is limited in acute relapses, although in the CSF there is an increase in proteolytic enzyme activity and the appearance of myelin fragments and peptides of basic protein (14). A possible explanation of these findings may lie in the processing of antigen in the CNS. If lymphoid tissue is present in MS brain (6) antigen may be trapped and processed locally, so that little escapes to induce systemic immunity and the ensuing inflammatory reaction.

INTRODUCTION TO SESSION V

B. WAKSMAN

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Good morning everyone. We've been talking about animal models and other types of virus disease, and today we are going to address ourselves specifically to the question of viral agents in MS. I thought it would be useful if I put on the screen a slide, in which I have listed the nine viral agents incriminated at various times over the last 30 years as the possible cause of MS:

VIRUS IMPLICATED IN MS

1946	RABIES	MARGULIES
1962	SCRAPIE	FIELD
1964	HERPES SIMPLEX	GUDNADOTTIR
1972	MSAA	CARP, HENLE
1972	PARAINFLUENZA 6/94	TER MEULEN, KOPROWSKI
1976	MEASLES	PERTSCHUK, PRASAD
1976	CORONA VIRUS	BERKS
1976	DISTEMPER	COOK, DOWLING
1978	BONE MARROW AGENT	MITCHELL, PORTERFIELD

I don't propose to say anything about any of these since Dr. Sever will show you a similar slide later and discuss these agents in detail, but I thought it might be useful to have this before you as a sort of a skeleton on which to hang the morning's conversation. Now I haven't included all the names of all the authors. Dr. Lange was one of several authors who worked on the last agent listed. He is in fact going to discuss that today and Dr. Porterfield is here also to talk about it. Thank you, that's all for that slide.

I tried to think of what I as a immunologist could say other than what is already on the program, to provide a second skeleton that we might hang some additional conversation on as the morning proceeds. It seemed to me

that there was one topic that's not really elaborated in the present program and that it would be profitable for me to make a brief statement about. The most extraordinary new finding in MS, a product of the last two to three years of research, concerns the obvious presence of an abnormality of immunoregulatory lymphocytes in patients with this disease. This abnormality has been found by a number of means and its dimensions by no means have been fully explored as yet. The finding that first captured everyone's attention came originally from the laboratory of Dr. Barry Arnason and his colleagues at the University of Chicago Medical School. These investigators studied the non-specific suppressor T cell, which one can measure by stimulating the patient's peripheral blood lymphocytes with concanavalin A, taking the blasts that result from this stimulation and adding them to a fresh culture of the same person's lymphocytes, and showing that those blasts suppress DNA synthesis stimulated by any convenient means. These non-specific suppressor cells are different in MS patients from normal: in MS in remission, the level is high; during an acute attack, the level falls sharply to extremely low levels. This finding has been duplicated in several laboratories and is no longer questionable. It has been confirmed by use of an entirely different serological technique, the counting of T cells that have an Fc receptor for IgG. These are what cellular immunologists today call T-gamma cells. They represent a population which is closely similar to and probably includes the Con A suppressor cells mentioned a moment ago. The pattern observed is the same: they are high in MS in remission and fall to very low levels during an acute attack of MS. I might say that we don't know which is the chicken and which is the egg, in the sense that we don't know if the acute attack precedes this fall or whether the fall is somehow responsible for acute attack. It would be nice to think that the latter is true but we don't know that, of course. There has been no published study so far of antigen-specific suppressor cells related to any of the viruses or related to myelin antigens. In any case, the finding described above represents a real abnormality or perhaps more than one abnormality in the regulatory cell system and must play some role, therefore, in the responses of the MS patient to viral agents.

Now there are two more findings that you should be aware of. One was reported a number of years ago by Dr. Zabriskie at the Rockefeller Institute, which is now the Rockefeller University, and was treated with a good deal of skepticism. Whether that skepticism was deserved or not is not up to me to say, but the finding was not immediately followed up. He observed that MS

patients' lymphocytes stimulated with measles antigen failed to produce the well known macrophage migration inhibitory factor (MIF): MS lymphocytes don't make MIF. The question whether it was really just measles or whether other agents would have elicited the same abnormality appeared to be the principal basis of critism. It wasn't felt that enough viral agents had really been explored. The last new finding comes from Barry Bloom's lab; I won't elaborate this because Barry Bloom is here and can tell you more about it than I can. It does however deserve mention in this brief synthesis. MS lymphocytes appear to be unable to make interferon when stimulated with a variety of agents, measles virus but also other viruses and mitogenic stimuli of various kinds. In other words there is a global abnormality, again in an immunoregulatory function of MS lymphocytes. Interferon has been shown to be a powerful stimulant of a major lymphocyte population known as natural killer cells. And we don't - even the basic cellular immunologists don't yet know enough about the biological role of natural killer cells for this to be put in some kind of perspective. This group of findings is extremely important: when we think about the problem of isolating a virus, we must also think about the host's immune response to that virus and how it is affected by this abnormality, which we do not fully comprehend as you understand from my comment. Now that is all I have to say. Let us turn to the first speech which is by Dr. Alter and has to do with epidemiologic evidence for a viral cause of MS.

IS MULTIPLE SCLEROSIS CAUSED BY A VIRUS?

M. ALTER

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SUMMARY

Neuroepidemiology has provided unique insights regarding multiple sclerosis (MS). The epidemiologic features of this disease must eventually be reconciled with any hypothesis of etiology, including the idea that MS is caused by a virus. Epidemiologists have shown that MS has an unequal geographic distribution, being more common in temperate than in tropical areas. Oriental populations and populations in extreme northerly regions appear to be at lower risk of MS. These data suggest that if a virus does cause MS, the virus is either less common in the tropics and at the extremes of latitude, or that populations living in these regions are more resistant. If "resistance" accounts for population differences in MS frequency, genetic factors related to histocompatibility markers may possibly mediate its effect. Alternatively, environmental factors may play a role, e.g. the age at which a viral infection is acquired, and diet, especially the amount of animal fat consumed. Migration data on MS patients suggest that changing one's environment early in life influences risk of acquiring MS. These data are compatible with the belief that a childhood virus infection is important in the etiopathogenesis of MS. It is postulated that infection late in childhood induces an immune response which makes individuals more susceptible to MS. Inhabitants of temperate regions, as opposed to those living in the tropics and extreme climates, tend to acquire viruses later in childhood making their risk of MS greater. Measles, an ubiquitous childhood illness, may be one of the viruses (but probably not the only one) which affects immune responsiveness and thus, age of measles infection may be important in determining risk of MS.

I. INTRODUCTION

Epidemiology is concerned with the characteristics of disease in populations. Population-based studies provide a perspective of disease which no other research approach can give. The truth of this statement is well illustrated by epidemiologic studies of multiple sclerosis (MS). Neuroepidemiologists have provided unique insights into the distribution of MS in most parts of the world (1, 2). They have identified factors which contribute to the risk of acquiring MS (3). The changing frequency of MS over time has been estimated (4) and the effect of migration on risk has been defined (5). Many environmental variables (6) from cosmic radiation (7) to dental care (8) have been studied in relation to the etiology of MS and genetic factors have also been analyzed (8, 9). In recent years, epidemiological evidence has also been offered in support of the idea that MS may be related to a viral infection.

II. THE GEOGRAPHIC DISTRIBUTION OF MS

MS is not uniformly distributed geographically. In heavily populated areas of Europe and North America, MS increases in frequency with latitude (10). In the extreme North, however, the frequency of MS apparently declines (11). The quality of medical facilities does not correlate with the frequency of MS (12). Therefore, the decreased frequency of MS at the extremes of latitude is unlikely to be simply a reporting artifact. Moreover, age differences among populations living in different regions cannot explain the geographic variation in MS frequency (13). Figure 1 is a fourth order exponential curve (x^4) fitted to data on prevalence in different parts of the world (10). It was plotted in an effort to visualize the distribution of a factor (or factors) of etiologic relevance to MS. This curve may not necessarily describe the distribution of a single variable; rather, the interaction of two variables may be involved. Each variable may be distributed like a second order exponential function (x^2). One of the variables might be environmental (x^2a) and the other, genetic (x^2b). Their interaction would yield a fourth order exponential function (i.e. $x^2a \cdot x^2b = x^4ab$).

Estimates of MS prevalence in the southern hemispheres suggest that the distribution of MS is symmetrical with respect to latitude and that MS increases in frequency with latitude in the southern hemisphere as well, (at least through the temperate zone) (10). However, there are regions in the USA and Europe at the same geographic latitude which differ in mean annual temperature. These areas have different prevalence rates of MS. Indeed, the mean annual temperature may correlate better with MS frequency than does geographic latitude (14).

Data on the geographic distribution of MS are compatible with the idea that a

virus which is temperature-sensitive may cause the disease. However, the distribution of MS could also reflect population susceptibility rather than viral characteristics. Therefore, population differences which could account for the geographic differences in MS prevalence should be investigated. Despite the relationship of geography and temperature to MS frequency, the disease does not tend to begin or exacerbate during any one season (15, 16).

The striking relationship between geographic latitude (or temperature) and MS which exists in North America and Europe, does not exist in Japan. MS is low in frequency throughout that country (17) even though the Japanese islands extend over an area which, in North America, shows a marked gradient in MS frequency. Japanese may be genetically less susceptible to those factor(s) causing MS, or possibly, the environmental factor(s) causing MS may be attenuated in Japan.

The environmental factors considered as possibly accounting for the low rate of MS in Japanese include diet, disease patterns and population density. The typical Japanese diet is richer in carbohydrates and fish than the typical Western diet which includes more animal protein. Japan's disease pattern (e.g. gastroenteritis) is more like Mexico's where MS is also rare, than like the USA, where MS is common (18). Japan's large population, small geographic size and mountainous terrain produce considerable population congestion. In this respect, Japan's population is similar to underprivileged groups of minorities (e.g. Blacks) in Western countries who often live in relatively congested communities and have a lower incidence of MS than more economically advantaged White groups in the same population (19, 20). Genetic factors also set the Japanese apart from populations with higher frequencies of MS. The HL-A types associated with MS in North European populations differ from those reported to be associated with MS in the Japanese people (21). The importance of genetic "resistance" to MS might be deduced from the fact that Japanese living in Hawaii (22, 23) under Westernized conditions, as well as Japanese living on the West Coast of the USA (24) who have an even more Westernized life style, also have a low frequency of MS.

American Indians and Eskimos (25) living in northern USA and southern Canada in zones of high MS frequency, apparently have low rates of MS. Is this due to a genetic resistance conferred by HL-A genes peculiar to the Mongoloid race, or to the relatively primitive and disadvantaged life style of these northern peoples? This question is still unresolved.

III. MIGRANT POPULATIONS

Since different parts of the world have different rates of MS, one may ask whether migration from one area to another affects risk of acquiring MS. Alter and

associates (26) were among the first to address this question. By studying immigrants, they noted that groups migrating from Afro-Asia to Israel after adolescence (about age 15 years) retained a low frequency of MS similar to that of their region of origin. On the other hand, those who migrated before age 15 demonstrated a higher risk, like that of their new residence. In an extension of this work, Alter et al (27) reported that migration before 5 years (but not infancy) might determine risk of MS. Migration between about 5 and 15 appeared to be "transitional" insofar as altering risk. Migration after age 15 did not influence risk of acquiring MS. These migration data are compatible with the notion that a childhood event occurring before age 15, or even before age 5 years, might determine the risk of developing MS. Migration data from Hawaii (22), South Africa (28, 29), Australia (30), England (31), France (32) and within the USA (33, 34), also suggested that migration early in life affects the risk of acquiring MS. Those who believe that a viral infection is the cause of MS often cite the migration data as one of the strongest foundations for their assertion. However, alternatives to a viral infection are also compatible with the migration data. For example, the amount of cows' milk consumed in early childhood (35) or the amount of calcium and vitamin D in the childhood diet (36) are factors which could also explain why migration might influence risk of acquiring MS.

If migration affects risk then genetic background cannot be the major determinant of MS. In Israel, risk of MS changed for certain young migrants within one generation, too soon to be explained by a shift in genetic susceptibility. However, a genetic factor interacting with an environmental factor could also explain the fact that migration early in life affects risk of acquiring MS.

IV. INFECTIONS AND MS

Efforts to detect an active or latent viral infection in MS have not met with much success (37, 38). In the last several years alone, agents implicated as possibly causing MS include a parainfluenza virus (39), an MS associated agent (MSAA) (37), a canine-related virus (possibly distemper) (40) and a virus isolated from bone marrow (41). However, all of these agents are now of doubtful importance in etiology. Co-cultivation techniques successful in one laboratory, have not been successful in other laboratories in revealing the elusive MS virus (39). Sensitive immunofluorescence techniques have also failed to show virus in MS tissue (42). Yet efforts to isolate a virus continue.

If an active viral infection is not the cause of MS, then latent infection needs to be considered. In 1977, Abramsky et al (43) demonstrated antibody to oligodendroglia in virtually all MS patients studied and in few controls. In 1979, an attempt to repeat this work was unsuccessful (44). The antibody response to the oligoden-

droglia was non-specific and not significantly different from the antibody response in other neurological diseases.

If neither active nor latent infection directly causes demyelinating plaques, perhaps a viral infection might do so by eliciting a host response which indirectly leads to demyelination. Zabriskie and associates (45), and more recently, Batchelor et al (46) discussed a mechanism involving a host response to viral infection which established an autoimmune reaction leading to demyelination. However, this mechanism is still speculative.

V. ALTERED IMMUNE RESPONSIVENESS TO CHILDHOOD VIRAL INFECTION

A. Measles

The idea that an infection may be involved in the etiology of MS was strongly bolstered by the observation, originally made by Adams and Imagawa (47) that measles antibody titers are increased in most MS patients. Other antibodies are also increased in some patients; for example, vaccinia (48, 49), rubella (49) and Epstein Barr (5) virus titers. However, measles is the virus against which elevated titers are reported most consistently. It has also been reported that MS patients will tend to have elevated titers to several viruses more often than controls (49, 51). Recently, elevated canine distemper virus titers were detected in MS patients (40).

B. Canine Distemper Virus (CDV)

The relationship between MS and dog exposure was recently reviewed by Alter et al (52). The notion that a canine virus might cause MS was widely discussed after Cook et al (40) reported that patients with MS had greater exposure to small household dogs than controls. Perhaps the most interesting aspect of this story was the observation by Kurtzke et al (53) that an "epidemic" of MS had occurred in the Faroes shortly after the British occupation of these Islands in World War II. MS had not been recognized in the Faroes before the British troops (and their dogs) arrived. Several years after the British left the Faroes, MS frequency decreased and now seems to have disappeared from this area (Figure 2). After the British occupation, canine distemper allegedly swept through the indigenous dog population because they were not immunized against CDV (54). Nathanson et al (55) postulated that CDV might cause MS in those individuals who had not already had measles. More recent reports (56, 57) have failed to find a significant association between exposure to dogs and risk of MS (58), and the reported elevation of CDV titers in MS patients has now been questioned (59).

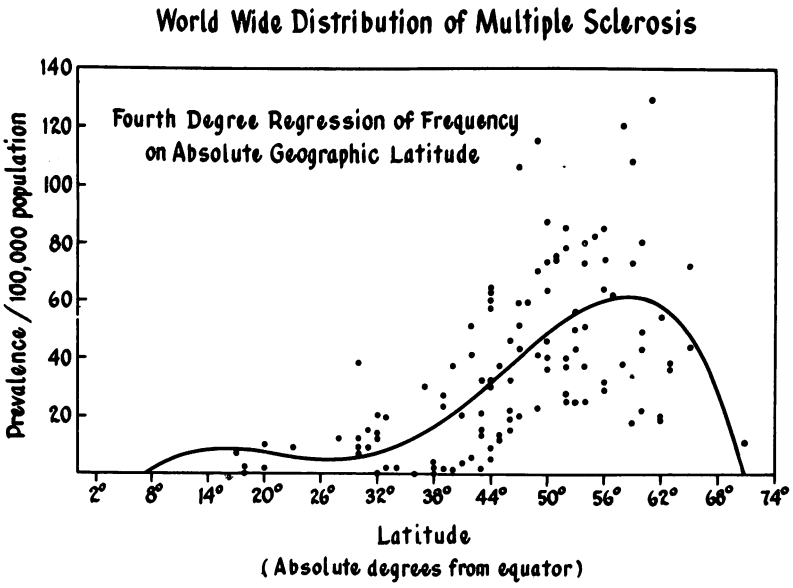

Figure 1: World-wide Distribution of Multiple Sclerosis. Fourth Degree Regression of Frequency on Absolute Geographic Latitude. From M. Alter et al.

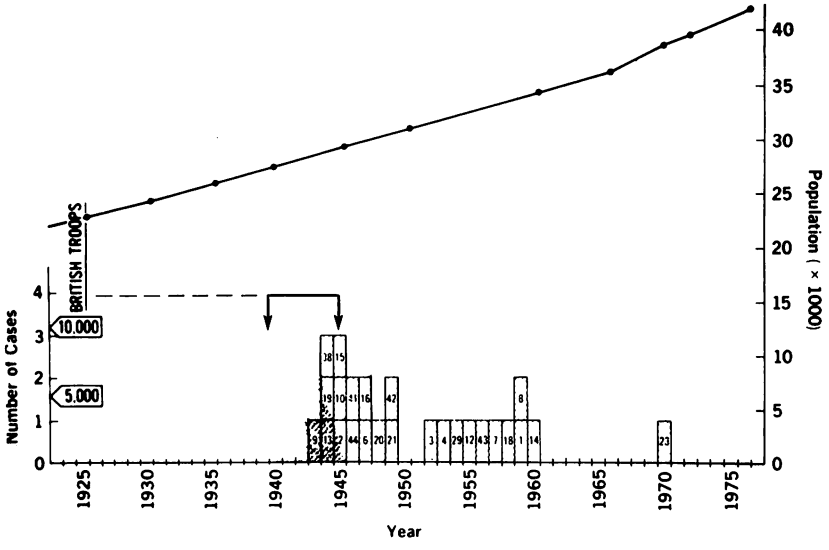

Figure 2: Distribution of Cases of Multiple Sclerosis Among Native-born Resident Faroese According to Year of Onset, 1920-1977, and of British Troops During the Occupation of the Faroes, 1940-1945. From J.E. Kurtzke and K. Hyllested.

It is interesting to note that all of the viral agents reported to be related to MS are common viruses to which immunity is usually acquired in childhood. Thus, laboratory data support the epidemiological data that a childhood event, possibly a childhood viral infection, may cause MS.

C. Host Responsiveness

Several years ago (60), I speculated that it was not the fact of infection, but rather the age at which that infection occurred which was important. I emphasized that it was the host response to a viral infection which might determine whether clinical signs of MS developed. The situation may be analogous to tuberculosis. Only a fraction of those with a positive skin test have clinical tuberculosis. It is the host response to the tubercule bacillus which determines who acquires "tuberculosis". There are, of course, limits to this analogy which relate to dose effect and virulence of a particular strain of tubercule bacillus strain, both of which are able to overcome host resistance. Thus, host susceptibility is only partially genetically determined. Susceptibility is also influenced by exogenous factors such as dose and virulence, as well as non-genetic factors, such as the host's nutritional state. The epidemiologist, even more than the clinician, is trained to think of "disease" as a model involving interaction of host, agent and the environment. However, much of the thinking in MS research today still involves a cause-effect model which is almost certainly inadequate, if not erroneous.

VI. GENETIC FACTORS

After shifting emphasis in seeking the cause of MS from a viral infection, we may ask what increases the host's risk of developing MS. Among possible risk factors, one can now list the histocompatibility (HL-A) determinants A3, B7, DW2 and DRW2, all of which are closely linked genes on the short arm of chromosome 6 in man (61, 62). The analogous locus on the mouse (H2) controls immune responsiveness (46). It is widely believed that this locus also controls immune responsiveness in man. It is conceivable that the inheritance of the particular markers A3, B7, DW2 and DRW2 increases effectiveness of an individual's immune response to viral challenge and therefore, the HL-A traits may confer a genetic selective advantage in certain regions (63). It is possible that a reciprocal association exists between the strength of the humoral immune response on the one hand, and the strength of the cellular immune response, on the other (64). Thus, in MS, increased circulating antibody to measles (and antibody to other childhood viral infections) may be associated with relative depression of the cellular immune response. Evidence of increased circulating antibody to measles in MS patients has already been cited. There are also reports which claim reduced cellular immune responsiveness in MS

(65, 66). DeVries et al (62) have discussed the interaction of humoral and cellular immunity in subjects given vaccinia. They showed that humoral and cellular responsiveness was reciprocal and related to the HL-A marker CW3.

The HL-A region in man is believed also to contain genes which affect complement production (67). Complement is obviously important in the immune response. Trouillas and Betuel (67) have postulated the existence of a hypo- and hypercomplementemic group of MS patients.

Some years ago there were attempts to type the IgG molecules. The group designated gm+ showed a gradient of north-south distributions similar to that of MS (68). MS patients, however, did not have disproportionate numbers of individuals compared to controls of one type of IgG (69).

Though HL-A and complement determinants seem to be important in MS, they do not provide the whole answer to the cause of MS. There are individuals who lack the determinants associated with MS who, nonetheless, develop the disease. Other individuals who have the "correct" determinants do not seem to develop clinical signs of MS. Moreover, there are populations like that of Israel (70), Japan (17), and Jordan (71) where MS is not associated with relative excess of A3, B7, DW2 or DRW2 determinants.

Attempts to demonstrate the presence of an MS susceptibility gene (MSS) linked to a particular HL-A haplotype have been made by tracing the segregation of that haplotype in families with more than one case of MS (72, 73). By taking into account the age of individuals at risk within their family and correcting for doubtful cases of MS, it may be possible to show the existence of an MSS gene.

There are several mechanisms which could account for the association of certain histocompatibility determinants and viral disease: 1) MS associated markers could code for viral receptors; 2) these markers could enhance immune response and 3) these markers could be linked to an immune-response gene.

VII. AGE OF INFECTION

How could measles, which is a ubiquitous disease, explain the uneven geographic distribution of MS? An explanation may lie in the fact that average age of infection with measles varies geographically. As shown by Morely (74), almost all individuals in Nigeria, where MS is rare, had detectable measles antibodies by age three years, whereas in England and Wales, where MS is common, more than 40 percent of the population still had no antibodies to measles by age five years. Thus, measles tends to occur early where MS is rare and later, around school age, where MS is common.

In one study, Cendrowski and I (75) showed that MS patients tend to report having had measles at an older age, on average, than controls. This kind of study should be repeated with inquiries about measles and other childhood viral infections. Pediatric records of viral infection could be reviewed instead of relying on the memory of patients or their parents.

The age at which an infection occurs appears to be an important determinant of the clinical expression of a variety of illnesses. Subacute sclerosing panencephalitis (SSPE), which is caused by measles, provides a clear example of the effect of age of illness on risk of developing a particular disease (76). As shown in Figure 3, the majority of individuals with SSPE have had measles before age two years. Although both MS and SSPE seem to have some relationship to measles, there are interesting differences between MS and SSPE (Table 1). The population at risk for SSPE seems to be "protected" against MS and vice versa. The risk of encephalitic complications from measles is another example of the effect of age of infection upon disease expression (77). Measles encephalitis increases in frequency with age of measles, peaking in late adolescence and declining in young adulthood. The risk of respiratory complications of measles, on the other hand, appears unrelated to age. Other examples could be cited: toxoplasmosis in utero can be devastating to the fetal nervous system, whereas toxoplasmosis acquired later in life is usually a mild illness (78); poliomyelitis in infancy may manifest only as diarrhea or even be subclinical, whereas poliomyelitis acquired later in life is more likely to produce paralytic complications (79). The clinical complications of hepatitis also appears to be age-dependent (80), as do the complications of Epstein Barr virus (81). Epidemiologists would do well to examine the age pattern of childhood illnesses in MS patients.

By what mechanism could age of infection influence risk of MS? I would postulate that the immune response to viral challenges of an individual infected while young, when the immune system is still relatively "naive", differs compared to the response of those infected after the immune response repertoire is developed. More needs to be learned about the ontogeny of immune responsiveness. Is it possible that a later challenge to the immune system tends to produce conditions which are more likely to yield demyelination? If we consider the immune system as a delicately balanced cascade, like the blood clotting system, then we might conceive of the demyelination in MS as a temporary imbalance in this system caused, perhaps, by depression of a modulator cell. Indeed, Antel et al (82) showed that the proportion of T-suppressor cells declines during a flare-up of MS and increases with remission. It remains for us to show whether such a T-cell response is influenced by the age at which an individual acquired various childhood illnesses and the manner in which his immune response repertoire developed.

TABLE 1

.SOME COMPARISONS BETWEEN SSPE AND MS

factor	SSPE	MS
Sex	M > F	F > M
Socioeconomic status	poor > rich	rich > poor
Residence	rural > urban	urban > rural
Ethnic distribution	black > white Arab ≧ Sephardi > Ashkenazi	white ≧ black Ashkenazi > Sephardi > Arab
Rubeola	Acquired earlier (before age 2 years)	?Acquired later (? near adolescence)

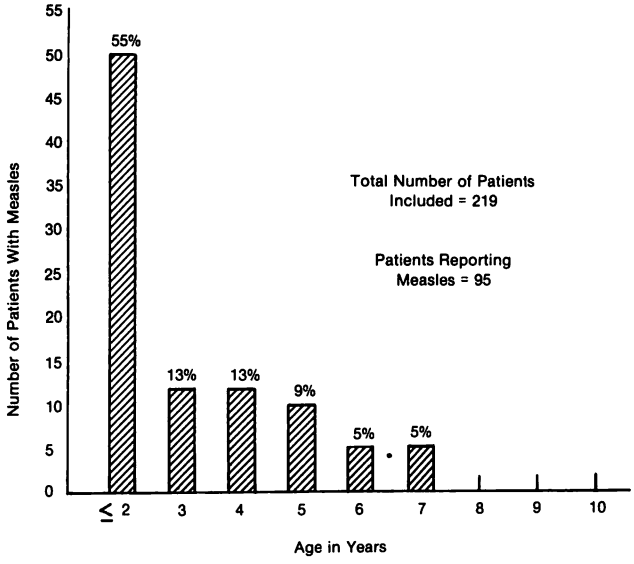

Figure 3: Subacute Sclerosing Panencephalitis: Frequency Related to Age of Measles. From J.T. Jabbour et al.

VIII. INFECTION AND IMMUNE SUPPRESSION

Some infections temporarily suppress the immune response. Measles is one of them (83, 84). Dutz (85) pointed out that in primitive environments, repeated illnesses and viral challenges occur during early life, leaving the individual relatively suppressed often in early childhood. Such individuals may have a lower risk of developing MS. In contrast, most individuals in more developed communities are protected against illnesses in early childhood. Only after starting school do these children come into intimate contact with many other children and thereby, contract illnesses. Therefore, in technologically developed environments, childhood illnesses tend to be "postponed" until an older age. On average, the children in developed countries tend to acquire immunity to the viral diseases of childhood later than children in primitive environments. If we knew more about the ontogeny of immunity, we might understand why later infection may be associated with increased risk of MS and vice versa. We might also understand why early infection with measles is associated with increased risk of SSPE, why encephalitis increases with age through adolescence, why early infection with hepatitis is associated with a longer period of viral shedding and, conversely, why individuals who develop hepatitis later are usually able to clear the viral infection more rapidly. These chapters in the story of infectious disease have yet to be written and may be relevant for MS.

IX. NUTRITION

The nutritional state of a population is another aspect of the environment which seems to be associated with increased risk of MS. The mechanism might involve an increased ability of "well-fed" individuals to form antibodies. There is evidence from cancer patients (86) and from individuals in tropical countries (87, 88) that undernutrition, especially of animal protein, impairs antibody production. The world-wide distribution of MS appears to resemble the distribution of consumption of animal protein in the diet in different parts of the world (Figure 4). An enhanced ability to form humoral antibodies due perhaps to better animal protein intake (for example, from milk), may well be a characteristic of MS patients. The elevated antibody titers to measles and other viruses in MS patients may merely reflect this characteristic.

X. SANITATION

Primitive environments in which there is a paucity of MS tend to have higher frequency of diseases associated with poor sanitation (18, 60, 89), e.g. enteritis, diarrhea. They also have increased infant mortality. A high negative correlation between enteric diseases and MS has been demonstrated. Respiratory diseases showed

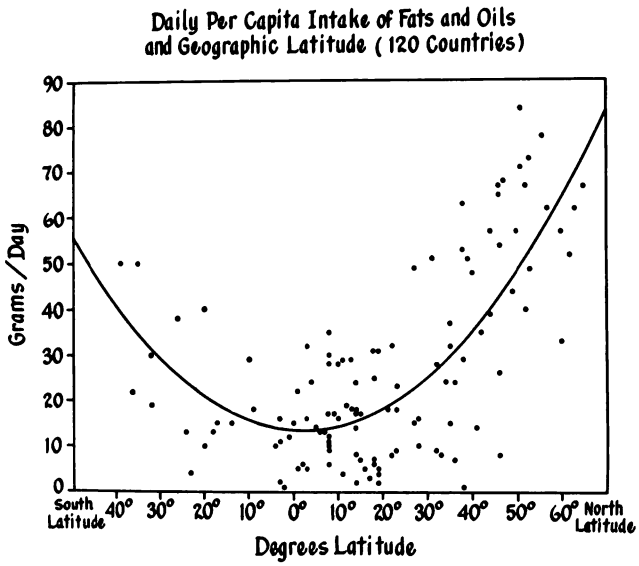

Figure 4: Daily Per Capita Intake of Fats and Oils Related to Geographic Latitude from 120 Countries. From M. Alter et al.

no significant correlation with MS frequency but tuberculosis mortality is negatively correlated with MS, i.e. the higher the mortality from tuberculosis (Figure 5), the lower the frequency of MS. This negative association suggests that a virus which

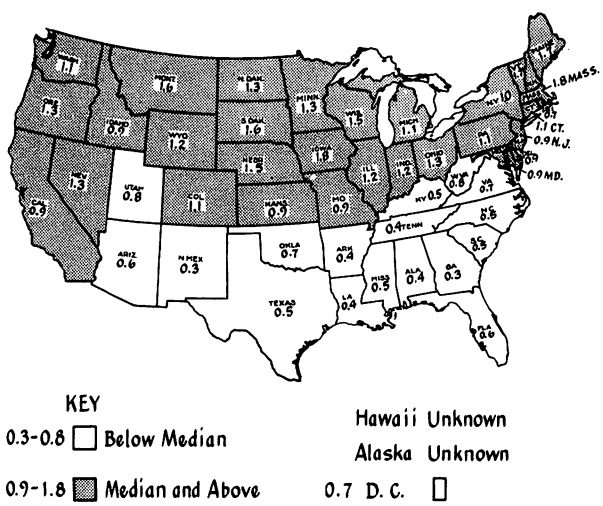

Figure 5A: Multiple Sclerosis, Mortality Rate per 100,000 Population in the U.S.A. by State, 1949-1951. From L.T. Kurland and D. Reed.

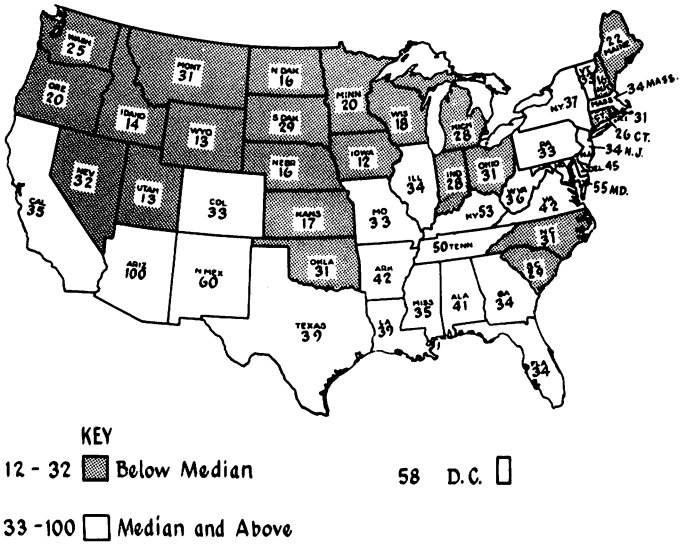

Figure 5B: Tuberculosis of the Respiratory System, Mortality Rate per 100,000 Population in the U.S.A by State, 1947. From U. Leibowitz and M. Alter.

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G. Pálffy/Pécs

The occurence of MS among the gypsies in Hungary is extremely low. In Hungarians it is about the same as in other countries of Central Europe. In the past 30 years only one gypsy patient with MS has been seen in the Department of Neurology, University of Pécs. In this period the Department has treated more than 500 cases of MS in the Hungarian population. The corresponding number of MS cases among gypsies should have been about 30, since the gypsies now make up about 6% of the population of Hungary.

Intermarriage between relatives is common among gypsies even today, but they practically never marry Hungarians. The hygienic conditions of the gypsies are poor and differ greatly from those of the Hungarian population.

One hundred healthy volunteers from a gypsy settlement were examined from HLA haplotypes. The proportion of HLA-A3 was found to be the same as in the Hungarian population, but not a single person with HLA-B7 haplotype was found in this healthy gypsy group. Our gypsy patient suffering from MS had the haplotype HLA-A3-B7.

The low frequency of MS among gypsies may be due either to their differnt genotype or to their differnt habits of hygene.

Returning to the international studies, Australia and New Zealand comprise principally a high frequency zone for 44^{o} to 34^{o} south latitude, and a medium frequency region for 33^{o} to 15^{o} south. Rates from Asia and the Pacific in the northern hemisphere are all low, except that Hawaii is likely to be in the medium zone. Even with later data, there is so far no site in Asia shown to have more than a low prevalence rate for MS. In the southern hemisphere, all rates from Asia and Africa are also low, except for English-speaking native-born whites of South Africa whose rate of 11 is within the medium range. Afrikaans-speaking native whites have the low prevalence rate of 3, while blacks and Coloured have rates of 0 (1,2).

In summation, we may consider the world-wide distribution of MS as comprising three zones of frequency or risk. The high risk zone includes northern Europe, the northern US and southern Canada, New Zealand, and southern Australia. These regions are bounded by areas of medium frequency. Asia, Latin America, and almost all of Africa are of low frequency. South America and much of Africa, though, remain largely unexplored (Figure 2).

RACE AND MS.

Note that all the high risk areas and the medium risk areas have predominantly white populations. Thus MS can be considered the white man's burden. Regardless of residence in the US, in our veteran series, blacks or Negroes have only half the risk of MS as do white males, but they still demonstrate the same marked north-south gradient in risk. With small numbers, this same series suggests a paucity as well for American Indians and for Orientals. Detels et al in California have documented a low prevalence among Japanese-Americans (10). In our series there was an apparent deficit among Spanish-Americans, but this seemed more a reflection of geography than race. Among foreign-born service men from Latin America there was, in fact, an equal deficit for whites and for "other" races (9).

MIGRATION AND MS.

The fate of migrants among these regions of differing risk is vital to our understanding of this disease. Overall, immigrants tend to retain much of the MS risk of their birthplace (Table 1).

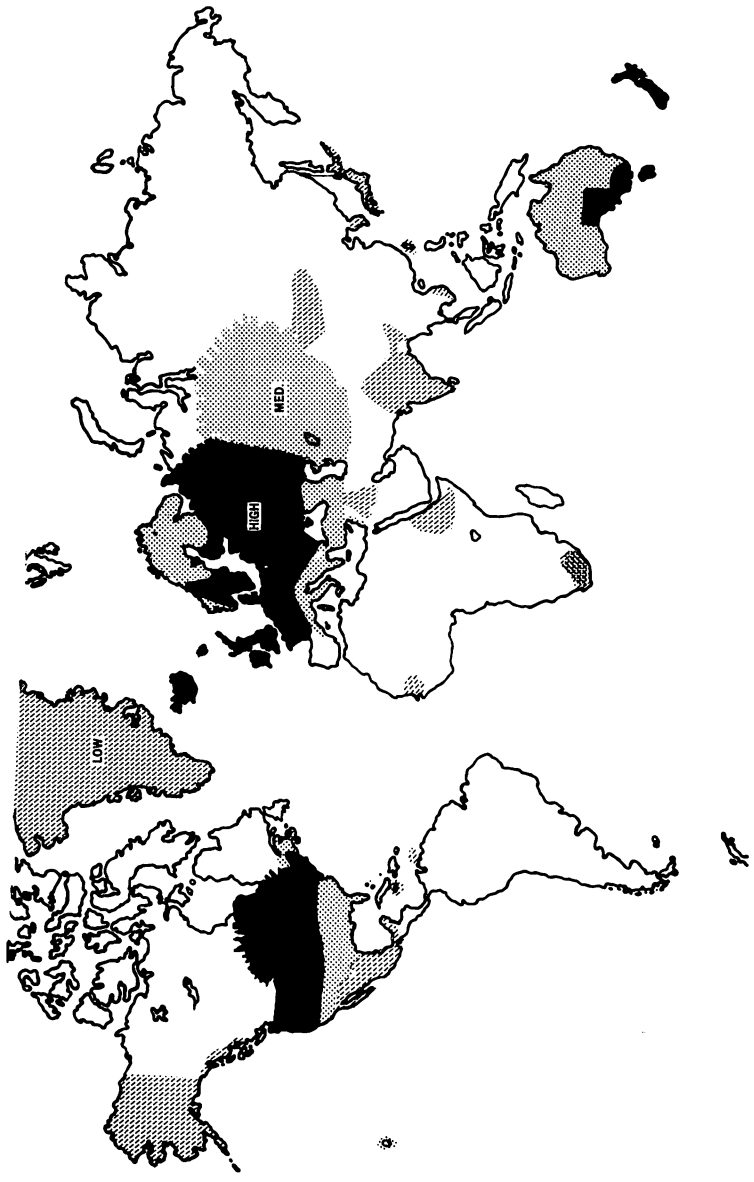

Figure 2. Worldwide distribution of MS within high (solid), medium (dotted), and low (diagonal-dashed) risk areas as of 1974. Open areas are unstudied. From Kurtzke, in press (2).

Table 3

Stimulation of Lymphocytes from Patients with Multiple Sclerosis and Matched Controls

Treatment	Measurement	MS Patient	Matched Control
Nonspecific Mitogen	Mean Response	162.1 $\pm$ 67	160.9 $\pm$ 53
	Number Positive	28/28	28/28
Measles	Mean Response	3.4 $\pm$ 1.4	2.4 $\pm$ 0.9
	Number Positive	7/28	6/28

Table 4

Suppressor Cell Activity of Lymphocytes From MS Patients and Matched Controls

Treatment	Measurement	MS Patient	Matched Control
Nonspecific Mitogen	mean response	28.8 $\pm$ 5.2%	32.7 $\pm$ 4%
	number positive	21/28 (75%)	25/28 (89%)
Measles	mean response	9.1 $\pm$ 2.4%	2.5 $\pm$ 1.5%
	number positive	9/28 (32.1%)	2/28 (7.1%)
Spontaneous	mean response	4.0 $\pm$ 0.8%	2.2 $\pm$ 0.7%
	number positive	4/28 (14.3%)	1/28 (3.6%)

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THE AMBIGUITY OF OUR CLINICO-PATHOLOGICAL, ENZYMOLOGICAL AND IMMUNOCHEMICAL EVIDENCES CONCERNING THE AGENT(S) RESPONSIBLE FOR MULTIPLE SCLEROSIS

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SUMMARY

We have so far failed to find evidence of auto-sensitization that would be specific for MS, so one or more exogenous agents would be implied. We doubt the validity of virus-like particles seen by electron microscopy in MS brains. It is relatively easy to distinguish MS patients from normal individuals, from most patients with non-neurological disease, and from many patients with non-destructive neurological illnesses; but it is difficult to distinguish MS patients from patients with destructive diseases of the nervous system if a week or two is allowed for such "neurological controls" to develop evidences of autosensitization. We raise the questions whether MS is a disease or a syndrome, and whether it is due to one or multiple causes. In addition, we have become interested in the developing of therapeutic models of EAE in three strains of guinea pigs and two strains of monkeys. The importance of an adjunct, antibiotic in macaca mulatta and steroid in macaca fascicularis, is emphasized, since only in highly inbred strain 13 guinea pigs is myelin basic protein by itself effective in treating EAE. If EAE and MS ever are shown to be related, it would be wise to have exploited such therapeutic models as fully as possible in subhuman primates so that extrapolations to humans with MS may be made as expeditiously as possible.

II. STUDIES OF MS POST MORTEM. A. BP IN MYELIN AND MACROPHAGES

Having spent many years producing specific rabbit anti-BP antisera, we could at long last use it with the immunoperoxidase technic to localize BP in tissue sections (1,14). From these tests we concluded that BP was present in CNS myelin, both in normals and in MS patients, and that fragments of myelin in MS macrophages still contained BP. Since these reactions occurred only if the lipids had been extracted, we suspected that BP is covered by lipids in vivo. The occurrence of these reactions in ordinary paraffin sections is further testimony to the long-known remarkable chemical and physical (thermal) stability of BP and at least some of its antigenic determinants, several of which have been localized to particular peptide fragments of BP (1).

From these observations we concluded that BP not only is present in MS myelin immediately adjacent to demyelinated plaques but also is relatively stable in the myelin debris being digested by macrophages. Just how to show that the MS macrophages might be processing BP in a different fashion than non-MS macrophages (e.g. those in stroke patients) has been an unresolved problem so far.

II. B. ACTIVE FOCI OF DEMYELINATION (WITHOUT LEUCOCYTES?)

For many years now we have been comparing EAE and MS, both in vivo as summarized above and post mortem (1). At the time of autopsy most MS plaques are quite inactive, with few gitter cells remaining and with abundant astrocytic gliosis. A few plaques are packed with gitter cells but careful examination of the edge reveals a sharp border between the normal myelin and the large round macrophages stuffed full of sudanophilic lipids. By analogy with other lesions, such as infarcts, where we know the time course of the histologic reactions reasonably well, such a lesion must be at least one week old, possibly even several months. In an occasional plaque, however, one can find tiny lipid droplets at the border, the normal myelin sheaths and the centripetally increasing lipid droplets overlapping over several cell diameters. One can assume from the shape of the lipid droplets and their sudanophilia that they represent cholesterol esters within microglial cells, but the number of cell nuclei does not appear to be increased at this border - and there are no perivascular lymphocytes (1), which characterize EAE (15). One can be reasonably confident that this is a currently active site, "currently" within a matter of hours to a few days at most.

Such observations cast considerable doubt on the validity of EAE as a model for demyelination in MS (1). The lack of obvious interaction between lymphocytes and macrophages is of critical importance, and raises the possibility of other myelinolytic mechanisms which are not leucocyte-dependent: genetically unstable myelin, persisting viral infections, exogenous toxins, endogenous toxins, complement-fixing antibodies, enzymes and lymphokines (some of which may also be enzymes) all come to

mind. Of course, many of these last factors are secretions of cells, but how far away may the secreting cell be? Some, such as antibodies, originate from cells which are usually extra-cerebral. Others, such as proteolytic enzymes, if secreted extra-cerebrally, would be immediately bound by large molecules, such as alpha-2 macroglobulins, and other protease inhibitors (16) continuously circulating in the plasma, and would be excluded from contact with substrates larger than several thousand daltons (17). Unless the blood-brain barrier were damaged, such molecules would not likely enter the CNS to initiate an MS plaque.

It would be easier to visualize the secreting cells as being already within the CNS, perhaps about veins within the center of the plaque, with diffusion through the plaque at times in high enough concentration to cause further demyelination in foci at the periphery, at other times not in high enough concentration to reach quite that far.

II. C. NECROTIC FOCI IN DEVIC'S AND BALO'S PATTERNS OF DEMYELINATION

Should we raise the controversy whether necrosis occurs in MS, or only pure demyelination? Almost every neuropathologist accepts the presence of necrosis as a rare event in MS, but then tends to ignore it or explain it away. The classical example, necrosis of the spinal cord and optic nerves in Devic's syndrome due to MS, has been thought to be caused by ischemia secondary to the compression of vessels by the acutely edematous MS lesions constrained by the inelastic pia mater and the absence of potentially expansible sulci in these two sites. But is this all there is to it?

Through the courtesy of Dr. Dominguez, in Caracas, Venezuela, we (18) had the opportunity a decade ago to examine several cases of Devic's syndrome, a relatively common disorder in his experience in contrast to MS, which is quite rare there. The interesting feature was the presence of necrotic foci not only in the spinal cord but also in the cerebrum, where inelasticity of the pia and subsequent ischemia could hardly be accepted as an explanation. We thought this was good evidence of Devic's syndrome being possibly due to at least two different diseases, 1) Devic's "syndrome" due to a chance distribution of MS lesions predominantly in spinal cord and optic nerves and 2) Devic's "disease," which might well have a different cause and a different geographical distribution.

More recently Dr. James McLean brought us a case of MS occurring in the daughter of two MS patients; i.e., a case from the rare conjugal MS. Her father had died at age 44 after a 9 year course of MS, her mother is still alive at age 58 after 24 years of MS, and the patient died at age 25 after only a 3 year course including recurrences of retrobulbar neuritis, ataxia and spastic hemiplegia, triplegia and finally quadriplegia. This case was interesting from several points of view, most especially because necrosis occurred in many of the concentric zones of Balo's pattern. Courville