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Antiglycolipid Immunity
Possible Viral Etiology of Multiple Sclerosis

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1. INTRODUCTION

The concept that infection plays a part in the pathogenesis of Multiple Sclerosis (MS) has been considered for over 100 years.[1] It is therefore appropriate to review the evidence to see why this concept is still very much in the forefront of present-day investigation into the etiology of this disease and to look at new ideas that might contribute to solving this elusive problem.

2. EPIDEMIOLOGIC EVIDENCE

Full reviews of the important factors in the epidemiology of MS are available.[2] In summary, over 200 prevalence studies of MS indicate that high-incidence areas of >30 cases/100,000 population are present throughout northern and central Europe, southern Canada, the northern United States, New Zealand, and southeast Australia, with some evidence of clustering that remains static. Rates of 5–30/100,000 are medium-frequency areas and include those areas around the Mediterranean, Israel, the Siberian and Ural areas of the U.S.S.R., southwest Norway, northern Scandinavia, most of Australia, and pos-

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sibly Tunisia. Incidence less than 5/100,000 is seen in areas around the equator, the Caribbean, Mexico, Asia, Alaska, and Greenland.

The Caucasian populations of the high- and medium-risk areas are particularly prone to this disease. Migrants from high- to low-risk areas moving after the age of 15 years appear to carry with them the risk of their birthplace. Similarly, migrants under the age of 15 going from a high- to a low-risk area tend to acquire the incidence of the low-risk area. There is also evidence that those going from the low- to the high-risk areas increase their chance of acquiring MS. Epidemics of MS may have occurred in two areas, the Faroe Islands and Iceland.[2] In this report it was also established that there is a susceptibility related to genetic determinants, particularly as some families in which several cases of MS have occurred possessed a common gene. HLA typing suggests that people with the HLA class II antigen D/DR2 particularly may have a higher incidence of MS in the white population of North and Central Europe, North America, and Australia. Class I antigens A3 and B7 may not be as important as originally indicated. Thus, the geographical distribution and possibly the migrant data suggest that MS may be caused by acquired, exogenous environmental factors, the most likely being some sort of infection. The findings also suggest a long incubation period for the disease to manifest itself.

An etiologic role for virus(es) in the pathogenesis of this disease would seem to implicate a long-term, perhaps immunologic, damaging process rather than any direct cytolytic effect viruses may have. It also seems likely, if a long incubation period is relevant, that the immunologic stimulus may be subtle rather than obvious, so that a longer period is necessary for a damaging immunopathological reaction to occur.

3. EVIDENCE FROM ANTIVIRAL ANTIBODY STUDIES AND VIRUS ISOLATION

Many antiviral antibody studies have been done in both sera and cerebrospinal fluid (CSF). What has become quite clear is that no single virus is particularly associated with MS. Adams *et al.*[3,4] and Salmi *et al.*[5] report that measles virus might be important in relation to MS. Haire[6] discussed the significance of antiviral antibodies in MS, particularly those to measles. She discussed the significance of IgM activity to the membranes of measles-virus-infected cells. Elevated serum antibodies also have been found against herpes simplex virus (HSV),[7] canine distemper,[8] rubella,[9] and corona[10] viruses. Antimeasles antibodies, as well as antibodies against rubella and vaccinia, have been shown to be synthesized within the central nervous system (CNS) of MS patients.[11,12] Antibodies to simian virus 5 (SV 5) have been found in MS patients.[13]

Salmi *et al.*[14] showed evidence of intrathecal antibody synthesis to a far wider range of viruses. These include rubella, measles, parainfluenza 2, respiratory syncytial, influenza A and B, mumps, adeno-, HS and varicella–zoster, parainfluenza 3, corona OC43 and 229E, rota-, polio, and cytomegaloviruses. This has been confirmed for the first nine of these viruses (up to HSV).[15] Antibodies

against up to 11 different viruses were synthesized simultaneously in the CNS in the same patients in the study of Salmi *et al.*[14] The intrathecal IgG index indicated that the antibody was being produced within the CNS. When they tried to relate intrathecal antibody synthesis to the clinical data in MS patients, they came to the conclusion that the bulk of the intrathecal synthesis of immunoglobulins and specific viral antibodies is not relevant to the pathogenesis of MS. However, they also concluded that random and continuous intrathecal antibody synthesis is a characteristic and unique feature in MS patients and that possibly a minor fraction of the antibody specificities may play a pathogenic role in the disease process. Vandvik *et al.*[16] showed that a small fraction of oligoclonal IgG bands in MS carry measles-specific activity. Nordal *et al.*[17] showed local synthesis of antibodies in the CNS of MS patients against measles, mumps, rubella viruses, and HSV, with antibodies to more than one virus present at the same time. However, they also found these antibodies in normal patients.

Viruses isolated from MS material include HSV,[18] parainfluenza virus type 1,[19] and coronavirus.[20] Paramyxovirus-like inclusions in MS brains have been seen by electron microscopy.[21,22]

The frequency of all these findings concerning the possible roles of different viruses as causes of MS tended to detract from the concept that they were involved in the etiology. However, if there was some common factor present that could produce CNS inflammation and demyelination among the many viruses incriminated in MS, then it might be possible to correlate disease with an etiology involving infection with more than one virus.

Many of the viruses that have been related to MS are enveloped budding viruses (Table 14-1). Enveloped budding viruses take host cell glycolipid into their coat (Fig. 14-1). This glycolipid presented in the virus coat to the immune system may be much more antigenic than glycolipid on its own. The fact that it can be derived from the cells of the CNS, including oligodendrocytes, and can be

TABLE 14-1
Enveloped Budding Viruses that Have Been Reported in Relation to MS

Herpes simplex	DNA
Varicella zoster	DNA
Vaccinia	DNA
Measles	RNA
Canine distemper	RNA
Mumps	RNA
Parainfluenza	RNA
Influenza	RNA
Corona	RNA
Rubella	RNA
Retroviruses	RNA

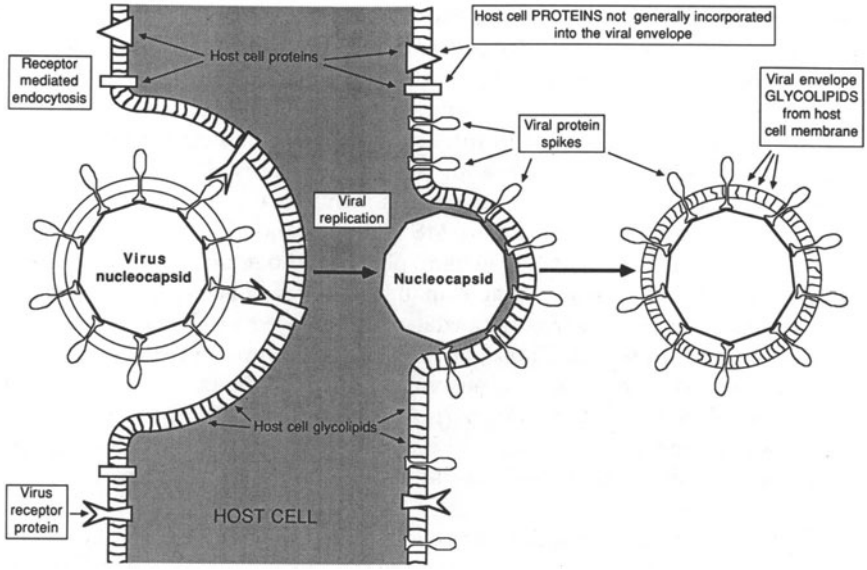

FIGURE 14-1. Incorporation of host cell glycolipid into the envelope of a budding virus.

presented from this partially immunologically privileged site to the peripheral immune system might provoke both cell-mediated and humoral antibody attacks on glycolipid in CNS cells and, in MS particularly, oligodendrocytes and myelin. The concept of this type of autoimmune damage has been put forward previously[23] and is discussed in detail later.

For viruses to cause the damage seen in MS, they have to be able to get to the brain. This may be accomplished with little difficulty, as most virus infections have a viremia, and the virus is likely to get into the brain transported in coated vesicles through endothelial cells.[24] Whether these viruses then do any harm is dependent on many factors, such as suitable cells for replication, the immune state of the host at the time of infection, and probably the HLA type of the individual infected. This latter point has been made clear in animal models in which the pathology may differ considerably depending on the host species and strains and their different major histocompatibility complex genes, some showing complete resistance to the disease process and others showing intense pathology. Theiler's murine encephalomyelitis virus is a good example in that there are susceptible and resistant strains of mice to this infection.[25] Viruses can also enter the CNS along nerves, e.g., HSV and rabies virus, but this is probably not relevant in the context of MS. It is of interest that Rogers *et al.*[26] isolated several viruses from kuru-infected chimpanzee brains. These animals were kept in very strict isolation and certainly were not expected to have latent virus infections in their brain cells in addition to the kuru agent, which had been the only pathogen inoculated. This

indicates the vulnerability of the brain to virus infection but does not tell us how many of the viruses that may be present might be associated with a disease process.

4. GENERAL MECHANISMS BY WHICH VIRUS DAMAGE COULD OCCUR

Once in the brain, viruses may cause damage directly by cytolysis. This is unlikely in MS, as virus would be much easier to find in the brain, and the damage would be more acute and inflammatory than that usually seen.

Viruses may set up a chronic infection of cells, altering their metabolism, e.g., of oligodendrocytes, which, as a result, might cause demyelination. Once myelin is broken down, secondary inflammation is likely to occur in relation to the removal of breakdown products. If this mechanism was relevant, virus would certainly have been found with the availability of modern probing and isolation techniques.

Viruses might induce an immune response to virus antigen that is presented at the host cell membrane surface. This response also would be likely to be inflammatory in nature, destroying virus and perhaps the cell from which the virus is originating. Secondary inflammation also would occur. Again, this is unlikely in MS for the reasons stated above.

Virus could persist in cells in an unusual form, causing metabolic disturbances, for example in oligodendrocytes, which would upset the production and support for the myelin they produce. The form of these viruses may be such that we have not as yet developed the technology to recognize them. Once myelin breaks down, a secondary, inflammatory reaction would occur.

Finally viruses could set up an autoimmune reaction against cells of the CNS, e.g., against oligodendrocytes and/or myelin, thus producing demyelination and subsequent secondary inflammation. This may be an example of "molecular mimicry." Another way might be by the enveloped budding viruses,mentioned previously, presenting glycolipid host cell membrane from an immunologically privileged site to the peripheral immune system and setting up an antiglycolipid immunopathogenic response. It is possible that both mechanisms might act together.

5. MOLECULAR MIMICRY AND AUTOIMMUNITY RELATED TO VIRUSES AND THEIR POSSIBLE ROLE IN THE IMMUNOPATHOGENESIS OF DEMYELINATION

By direct comparisons of amino acid sequences, viruses have been shown to share common polypeptide sequences with certain host cell components. These studies have been done using computer analysis but do not indicate whether the sequences are at a site that might have biological significance. These polypeptide

sequences could produce autoimmune reactions. Relevant examples of autoimmunity related to both the CNS and other organs of the body may provide insight into mechanisms of MS pathogenesis. It is presently uncertain, in some cases, whether the mechanism involved in each case is molecular mimicry or presentation of host cell membrane in the envelope of budding viruses. Fujinami and Oldstone[27] showed that hepatitis B virus polymerase (HBVP) shares six consecutive amino acids with the encephalitogenic site of rabbit myelin basic protein (MBP). Rabbits immunized with selected peptides from HBVP produced antibody that reacted with the predetermined sequences of HBVP and MBP. Peripheral blood mononuclear cells from these rabbits show a proliferative response when incubated with either MBP or HBVP. The rabbits develop a pathological change in their CNS somewhat similar to experimental allergic encephalomyelitis (EAE) following immunization with MBP.

Tardieu *et al.*,[28] using reovirus type I, report evidence of an autoimmune reaction. they confirm that many autoantibodies are produced that react with a large variety of normal tissues and that there are antigenic structures shared between viral determinants and normal tissue. Lane and Hoeffler[29] have shown that the SV 40 T antigen mimics a structure on a host cell protein. This cross-reactive protein is located within the nucleus of all of the mammalian cell types examined. Fujinami *et al.*,[30] using monoclonal antibodies (MAb), showed that the phosphoprotein of measles virus and a protein of HSV type 1 cross-react with an intermediate-filament protein, probably vimentin of human cells. It is difficult for autoantibody to react with intracellular antigens; although prior disruption of the cell by some other lytic action might expose antigens, subsequently an autoantibody reaction could result in further damage. Jahnke *et al.*[31] discuss sequence homology between viral proteins and the proteins MBP and P_2 related to encephalomyelitis and neuritis, particularly mentioning measles, Epstein–Barr, influenza A and B, and other viruses that cause upper respiratory infections. They point out that postinfectious or postvaccinial neuritis may be caused by immunologic cross-reactions evoked by specific viral antigenic determinants that are homologous to regions in the target myelins of the central and peripheral nervous systems (PNS).

Kagnoff *et al.*,[32] working on the pathogenesis of celiac disease, suggested that a human adenovirus type 12 (Ad 12) might be involved. They showed that α-gliadin, a component of wheat and an activator of celiac disease, shares a region of amino acid sequence homology with the 54-kDa E_1G protein of Ad 12, which is usually isolated from the intestinal tract. They proposed that this Ad 12 amino acid sequence could act similarly to the α-gliadin in wheat and activate the disease process.

Srinivasappa *et al.*,[33] in an analysis of over 600 MAbs raised against many DNA and RNA viruses, found that approximately 4% showed some cross-reaction with host determinants expressed on uninfected tissues. Several MAbs reacted with antigens in more than one organ. Although this may be an example of autoimmunity occurring through molecular mimicry, some MAbs are likely to have been produced by the presentation of host cell membranes by the viruses themselves because of their mode of replication by budding. Many of these would

be directed against the glycolipid component of the host cell, because this is what a budding virus mainly picks up when it leaves the cell.

Miller *et al.*,[34] using the nonenveloped Theiler's murine encephalomyelitis virus, has shown by using functional T-cell analysis that there was a correlation with the extent of exact amino acid homology among the viral capsid proteins, the neuroantigens, purified rat and guinea pig MBP, human proteolipid protein, and related picornaviruses. Antigenic mimicry between measles virus and human T lymphocytes has also been shown.[35] The authors suggest that this might play a part in the immune suppression seen in measles.

Haspel *et al.*[36] have shown that mice inoculated with reovirus type 1 (nonenveloped virus) develop an autoimmune polyendocrine disease. They produced a large panel of hybridomas making monoclonal autoantibodies that reacted with cells in the islets of Langerhans, anterior pituitary, gastric mucosa, and with cell nuclei. Several of the autoantibodies recognized hormones, e.g., glucagon, growth hormone, and insulin. The exact antigenic determinants that were being recognized, i.e., protein, carbohydrate, or lipid, were not determined.

Huber and Lodge[37] have shown in mice that a coxsackievirus B type 3 (CVB-3) causes an extensive myocarditis. This work demonstrates that two distinct cytolytic T-lymphocyte (CTL) populations are present. One lyses uninfected myocytes (autoreactive), and the other lyses CVB-3-infected myocytes (virus specific). The lesions caused by the autoreactive CTL are more extensive and necrotizing than those caused by the virus-specific CTL. It is interesting that autoreactive CTL are not demonstrated in animals infected with a nonmyocarditic CVB-3 strain. Athymic nude mice do not develop myocarditis unless reconstituted with autoreactive CTL sensitized against the CVB-3 myocarditic strain, showing that the lesions are directly T-cell dependent. All these examples show how viruses can behave within and outside the nervous system. It is reasonable to consider the application of these findings to demyelinating nervous system pathology.

6. EXPERIMENTAL ALLERGIC ENCEPHALITIS, EXPERIMENTAL ALLERGIC NEURITIS, VIRUSES, AND DEMYELINATION

Reference in the previous section to viruses being able to mimic portions of MBP and P_2 raises the question of whether EAE and/or experimental allergic neuritis (EAN) induced by viruses could be an important mechanism of demyelination. The EAN is included because I believe the mechanism for the demyelination in the PNS is the same as that seen after virus infections. A similar viral etiologic mechanism could result in MS. The EAE model has been used in many laboratories for many years because it has been felt that the mechanisms involved in this disease might play a part in the etiology of MS. There are problems related to this concept, the first being that highly purified MBP produces inflammation with very little demyelination,[38–41] which is the major feature of MS. In fact, in the EAE model significant demyelination is more prominent and better seen when whole CNS white matter, particularly that of the

spinal cord,[42] or MBP with cerebrosides (i.e., galactocerebroside) is inoculated.[43] Dubois-Dalcq *et al.*[44] and Fry *et al.*[45] show that rabbits immunized with galactocerebroside produce antiglial and demyelinating antibodies. Maggio and Cumar[46] find that only sulfatide antibodies are demonstrable after EAE has been induced by inoculation of MBP and adjuvant in animals.

Seil *et al.*[47] show that animals sensitized against MBP only usually lacked the *in vitro* demyelinating factor commonly found in animals given whole white matter. Similarly, Raine *et al.*[41] show that in experiments concerned with demyelination *in vitro* using sera against whole white matter, MBP, and galactocerebroside, the damage to myelin is associated with antigalactocerebroside activity and not anti-MBP antibody. Paterson[43] suggests that more attention needs to be paid to the role of cerebrosides not only in the production of EAE but also in demyelination. However, Zamvil *et al.*,[49] using T-cell clones specific for MBP, have induced chronic relapsing paralysis and demyelination in PL/SJ F_1 mice. Watanabe *et al.*[50] have shown that EAE-like lesions in rats can be induced by lymphocytes taken from Lewis rats infected with a coronavirus, but demyelination is not a feature. Prior to transfer these lymphocytes are restimulated with MBP. This model demonstrates that a virus infection of CNS tissue can initiate a pathological autoimmune response. This study would have been of greater interest had they used glycolipids as well as MBP to stimulate the lymphocytes, particularly with reference to causing demyelination.

As in demyelination in some animal virus models, the strain of animal has been of paramount importance in the production of EAE. Gasser *et al.*[51] and Williams and Moore,[52] by comparing the EAE-sensitive Lewis strain of rats to the resistant BN strain, show that it is likely that an autosomal dominant gene linked to the histocompatibility locus determines susceptibility to EAE by acting as an immune response gene. However, EAE can be produced in BN rats if rat or guinea pig spinal cord is used rather than MBP.[53] Perhaps this could be explained because some other constituent, possibly cerebrosides (glycolipids), in spinal cord tissue is contributing significantly to the EAE and the demyelination. Tsukada *et al.*[54] describe a chronic EAE-type lesion with demyelination induced by inoculation of guinea pigs with cerebral endothelial cell membrane known to be entirely free of MBP and proteolipid protein. They do not identify the factor that provokes this demyelinating EAE, nor do they appear to consider the possible role of membrane glycolipids as an etiologic agent.

In MS it seems that MBP is unlikely to be the significant factor for the demyelinating aspect of this disease. The MBP may contribute to the inflammation seen if molecular mimicry of MBP through virus infections can be established. Certainly for EAE-type pathogenesis to be relevant in MS, there has to be an initial "trigger factor" for the process to occur in humans. It appears that viruses could possibly do this, and if they do, it could be by molecular mimicry of MBP amino acid peptides. However, because molecular mimicry of MBP is unlikely to produce demyelination, there may be a simpler and more understandable mechanism, i.e., the presentation of nervous system host cell glycolipid by a variety of enveloped budding viruses (previously mentioned) and other organisms that are known to have cerebrosides in their envelope, e.g., mycoplasma,[55]

causing an autoimmune demyelinating disease. This hypothesis is appealing and is deserving of further examination.

7. NERVOUS SYSTEM GLYCOLIPIDS AND MULTIPLE SCLEROSIS

Ideas on the etiology of MS are still fairly poorly developed. Whether glycolipids from the CNS presented to the peripheral immune system on virus envelopes can produce a response that leads to demyelinating damage that could account for the MS lesions seen in the CNS should be addressed. Most of the work to date concerning viruses has been centered on reactions against virus proteins and MBP, including molecular mimicry. Proteins are known to be very immunogenic, and the example of MBP producing EAE has been a major model for MS research.

A good short review of brain glycolipids as cell surface antigens is that by Leibowitz and Gregson.[56] Glycolipids, although a major constituent of myelin and indeed most host cell membranes, have not been properly examined because, compared with proteins, they are less immunogenic and more difficult to study. It is very difficult to get an optimum composition of a glycolipid antigenic mixture, as glycolipids are not soluble in water. The amount of antigen has to be determined empirically. Sensitization with glycolipids to produce a demyelinating disease has been considered only recently.

That glycolipids are immunogenic is unquestionable. Landsteiner[57] reports that the ceramide glycolipid of Forsmann antigen derived from type 1 pneumococci is antigenic without the aid of added protein. Heterologous anti-Forsmann antibodies introduced into a carotid artery produce a severe vascular lesion with hemorrhage, edema, and necrosis on the same side.[58] Landsteiner[57] also demonstrates that injecting brain into animals produces two sorts of immune sera: some react with proteins; others react with emulsions of an alcohol-soluble extract of brain and testicular tissue. One brain hapten described is soluble in hot alcohol. This suggests that the immune serum contains antibodies not only to proteins but against lipid material. This phenomenon of organ-specific antibodies reacting with alcoholic extracts is also obtained by injection of liver, lung, or leukocytes, often together with Wasserman antibodies. In each case it is likely that glycolipids are the antigens producing immunity.

Cerebrosides, sulfatides, and gangliosides constitute the major glycolipids of the brain.[59] The first two are myelin lipids, whereas ganglioside is mainly associated with the neuronal elements and only a little is present in glia and myelin. Niedieck and Palacios[60] state that these glycolipids are not complete antigens but are haptens and must be introduced with an immunogenic carrier to produce antibody. Czeonkowska and Leibowitz[61] show that a homologous carrier to which the animal is tolerant is ineffective in inducing antibodies to glycolipids, although generally they are immunogenic when presented in an intact membrane. Thus, it seems likely that the host cell glycolipid membrane taken by budding virus for its envelope and presented with highly antigenic virus protein will be highly immunogenic. Rapport *et al.*[62] state that antibody to glycolipid is

directed to the carbohydrate part of the molecule, but it seems likely that the lipid plays some part in the reaction.[56]

8. EVIDENCE THAT ANTIGLYCOLIPID ACTIVITY OCCURS IN MULTIPLE SCLEROSIS

Arnon *et al.*[63] found antibodies to glycolipids present in 40% of MS patients. Since only a limited number of glycolipid antigens were used to test this, the percentage of positives might have been higher if more glycolipid antigens had been tested. Kasai *et al.*[64] found that anti-G_{M4} and antigalactocerebroside antibody titers were significantly raised in the CSF of MS patients as measured by a solid-phase radioimmunoassay but not in the sera. G_{M4} is a ganglioside with a long base chain that occurs mainly in human myelin. There was no rise of antibodies in the CSF to G_{M1} or MBP. A significant number of the anti-G_{M4} and antigalactocerebroside antibodies existed as immune complexes within the CNS. Duponey[65] has shown the presence of antigalactocerebroside antibodies in the serum of patients suffering from MS. Evidence of T-cell activity against gangliosides also has been shown in MS. Offner *et al.*[66] show that G_{T1} and G_{Q1b} are powerful stimulators of active E-rosetting lymphocytes from MS patients. Sela *et al.*[67] show raised ganglioside levels in serum and peripheral blood lymphocytes from MS patients in remission compared with controls. Ilyas and Davison[68] show hypersensitivity to gangliosides in MS with an E-rosetting technique. This reaction to glycolipids seems to be much more specific in the MS patients than the reaction to MBP, which also occurs in patients with other CNS disturbances. Davison and Ilyas[69] report inhibition of E- rosette formation by gangliosides by cyclosporine A, which blocks receptors for HLA-DR antigens on T lymphocytes. Bellamy *et al.*[70] show that the gangliosides G_{M1}, G_{D1A}, G_{D1B}, and G_{Q1b} stimulate T_4 and T_8 lymphocytes from the CSF of MS patients. All these studies suggest both humoral and cell-mediated immunity to CNS glycolipids. The reaction against glycolipids seems more specific to MS than that found against MBP.

9. EVIDENCE THAT HOST-DERIVED ENVELOPE MEMBRANE IN VIRUSES IS ANTIGENIC

Some 25 years ago Harboe *et al.*[71] showed that fowl plague and influenza viruses derived from the entodermal cells of chick chorioallantoic membrane can be neutralized by serum from rabbits that had been immunized against normal, uninfected chick chorioallantoic membrane. The biologically active nature of this rabbit-derived immune serum against the chick-derived virus is important. Feinsod *et al.*[72] show that Sindbis virus derived from *Aedes aegypti* mosquitoes is neutralized by immune serum made against whole-body extracts of uninfected *A. aegypti*, i.e., the mosquito cell line in which the virus replicates. This same serum does not neutralize Sindbis virus derived from Vero cells, indicating the potency and specificity of the serum made against the uninfected mosquito cell line on the

virus that had grown in the same cells. Almeida and Waterson[73] show that only the viral protein spikes of corona virus derived from chicken fibroblasts can be labeled by immune serum made to the virus in chickens. However, when the coronavirus derived from chickens is used to make immune serum in rabbits, both the viral protein spikes and the intermediate membrane envelope of the virus are able to be labeled, showing that the chicken-derived virus is able to produce in the rabbit very considerable antichicken host-membrane antibody. This is an excellent example of the immunogenicity of host-cell membrane when presented in a viral envelope. Friend leukemia virus (a retrovirus) budding from erythrocyte membrane takes erythrocyte membrane antigen into its coat[74] and can lead to an immune reaction that causes hemolysis of normal, uninfected erythrocytes.[75]

Steck *et al.*[76] inoculated mice with the neurotropic strain of vaccinia virus and produced antibodies that bound to normal, uninfected myelin and oligodendrocytes, indicating that the virus presents to the immune system myelin and oligodendrocytic membrane components that are antigenic. This does not happen if the dermatropic strain of virus is used. Lindenmann and Klein[77] made use of viruses to present tumor tissue so that it became more immunogenic. They homogenized and lyophilized Ehrlich's ascites tumor cells (EAC) and gave them to Swiss A_2G mice and showed that they are not immunogenic when presents in this manner, and no protection to the mice occurs following challenge with live EAC. However, if the neurotropic strain (WSA) of influenza virus is inoculated into the EAC cells and the resulting progeny virus are used to immunize mice, strong immunity against EAC is produced, and the mice are protected from challenge with these neoplastic cells. However, if the same virus is cultured in eggs and given to mice, no protection occurs to the EAC. This is an example of how well viruses can present host cell membrane (in this case EAC tumor cell membrane) so as to be very specifically immunogenic as compared to the inoculation of the cells only.

Rook and Webb[78] showed that lymphocytes sensitized to tick-borne encephalitis virus (TBE) kill not only TBE-infected glial cells but also a significant percentage of normal, uninfected glial cells. This indicates that cytotoxic lymphocytes may destroy normal cells directly, including oligodendrocytes, the producers of myelin, which might be very important in the pathology of MS. There are many other examples in the literature of anti-host-cell-membrane effects induced by the glycolipid envelope membrane of budding viruses.

10. IS THERE EVIDENCE THAT ANTIGLYCOLIPID ACTIVITY PRODUCES DEMYELINATION OF THE CENTRAL OR PERIPHERAL NERVOUS SYSTEM?

It is worth quoting some of the evidence that this can occur. Anticerebroside antibodies have been shown to produce demyelination of myelinated axons in cell cultures.[41,44,45,48] Raine *et al.*[41] showed that antibody against galactocerebroside or against whole white matter produces demyelination in mouse spinal cord cul-

tures, whereas anti-MBP antibody does not. The demyelinating factor could be absorbed out by galactocerebroside. These workers feel that galactocerebroside (a marker for oligodendrocytes) is a major target for antibody-mediated demyelination. As stated previously, it seems to be true that pure anti-MBP antibody does not produce demyelination, and similarly, pure MBP, when used to produce EAE, produces inflammation and minimal demyelination. However, if glycolipids are inoculated with MBP there is good demyelination.[43,79]

Nagai *et al.*[80] report lesions of the CNS and PNS of rabbits and guinea pigs after immunization with ganglioside G_{M1} and G_{D1a}. Saida *et al.*[81] produce an EAN using purified galactocerebroside. Konat *et al.*[82] produce an MS-like disease in rabbits using bovine brain gangliosides. Saida *et al.*[81] show *in vivo* demyelination by the intraneural injection of antigalactocerebroside serum. Hughes and Powell[83] show enhancement of P_2-induced demyelination in Lewis rats by galactocerebroside and glucocerebroside added to the immunizing emulsion. Carroll *et al.*[84] produce demyelination of the optic nerve by intraneural injection of antigalactocerebroside serum. Roth *et al.*[85] show that cultures of spinal cord, when exposed to galactocerebroside and whole white matter antiserum, show myelin damage. Also, but to a lesser extent, there is some damage from antibodies to gangliosides G_{M1} and G_{M4}. Sergott *et al.*[86] show that antigalactocerebroside serum demyelinates the optic nerve *in vivo*.

11. CAN VIRUS INFECTIONS OF THE NERVOUS SYSTEM PRESENT GLYCOLIPIDS IN SUCH A WAY AS TO BE IMMUNOGENIC?

The viruses that have at one time or another been thought to be involved in MS have been mentioned previously. All of them are budding viruses and incorporate lipids from the membrane of the host cell into their envelopes. Certainly all the viruses mentioned can enter the CNS, replicate there, and, because of their mode of replication, take CNS host-cell glycolipid into their envelope. In the *Paramyxoviridae*, which include measles, canine distemper, mumps, parainfluenza types 1–4, and Sendai virus, release of mature virus takes place by budding, and 20–40% of the dry weight of the virion is lipid.[87] Particular interest must center on measles, since in acute measles encephalitis there is perivascular demyelination. This virus has the capacity for latency, and it buds from cells taking host-cell glycolipid into its coat.

Experimentally, Klenk and Choppin[88] cultured the paramyxovirus SV 5 in four different host cells with different lipid compositions and determined that the lipid composition of the viral envelope very closely resembled that of each of the host-cell membranes from which it was derived. Lipids in the viral envelope have been shown to resemble closely those of the host cells from which they were derived in mumps,[89] influenza,[90,91] Sindbis,[92,93] Venezuelan equine encephalomyelitis,[94] and Semliki Forest virus (SFV).[95–97] The SFV [the avirulent A7(74) strain] has been used by us as a model for virus-induced demyelination in mice.[98] Mice infected with this virus show a pronounced development of antiglycolipid activity, which aroused our interest.[99,100] The mechanism involved in the produc-

tion of this antiglycolipid activity could be relevant to what is seen in MS. It is therefore worthwhile to point out some of the relevance of this model to the immunogenicity of glycolipids.

In the SFV model demyelination occurs throughout the CNS including the optic nerve and spinal cord, with maximal effects noted between days 14 and 21 after infection.[98,101,102] Remyelination is well in progress by day 35. The demyelination is dependent on T lymphocytes, as nude (T-cell-deficient) mice, in spite of high virus titers, do not demyelinate until they are given normal T cells from their *nude*/+ littermates.[103,104] Natural-killer-cell-deficient mice demyelinate well when SFV is used, and demyelination continues to occur normally in complement depleted mice. Repeated inoculation of immune sera, either intraperitoneally or intracerebrally, does not produce demyelination.[105] The first cells to enter the white matter from the perivascular cuffs are activated lymphoblastic-type cells, the majority of which do not stain with anti-IgG, -IgM, or -IgA. They can be seen in direct contact with the myelin just prior to the onset of demyelination and may be CTL.[106]

Amor and Webb[100] show a rise in antiglycolipid activity against both total neutral glycolipids and galactocerebrosides with SFV. This appears to give some protection to the mouse from further challenge with an antigenically unrelated encephalitogenic enveloped budding virus, TBE virus (Langat strain), which replicates in the same glial cells as SFV. The results suggest that cross-protection arises from immunity to common host glycolipid contained in the envelope of both viruses. However, further challenge with Langat virus not only increases the demyelination if given within 2 weeks of the SFV but delays the remyelination significantly if given later. Antiglucocerebroside, -ganglioside, and -galactocerebroside sera react with brain-derived SFV in an immunoenzymatic assay ELISA[107] and label brain-derived SFV budding virus[108] as observed by electron microscopy. Khalili-Shirazi *et al.*[99] have raised many MAbs against SFV, and these have been found to be against glycolipids and to label brain-derived "budding" SFV both by immunoelectron microscopy and by immunocytochemistry.[109] One of these anti-SFV glycolipid MAbs (308) also labels TBE virus in mouse brain, which is antigenically unrelated to SFV but buds from CNS cell membranes, again indicating a common host antigen.[110]

Khalili-Shirazi *et al.*[99] also raised MAbs against whole myelin. Some of these were against myelin proteins, and some against myelin glycolipids. One anti-myelin-glycolipid MAb (212) reacted with brain-derived SFV in an ELISA. Both the anti-SFV glycolipid MAb and myelin glycolipid MAb had some biological activity against SFV, producing either neutralization or steric hindrance. A recent MAb (555) raised against SFV, an antigalactocerebroside antibody, has very significant neutralizing activity against SFV and labels SFV as observed using electron microscopy. A further MAb (373), raised against brain-derived "inactivated" SFV, cross-reacts with sulfatides and galactocerebroside and also neutralizes SFV significantly.[111] This MAb 373 is of special interest as it labels SFV, influenza, and measles virus, which replicate in the same brain cell cultures from which the SFV is derived.[112]

This indicates that budding viruses take similar glycolipid into their coat if

the original host cell of replication is the same. Influenza virus derived from mouse brain cell cultures is also labeled by antiglucocerebroside serum (S. Pathak, personal communication).

The immune response to glycolipids is thought to be T-cell independent, and most antiglycolipid antibodies are IgM, as are the antiglycolipid MAbs described above; IgG antiglycolipid antibodies are occasionally reported. Very little work has been done on the significance of IgM antibodies in MS.

The demyelination in this SFV model can be made significantly worse if a second inoculation of the avirulent strain of SFV, A7(74), is given within 14 days of the first injection,[113] even though the A7(74)-SFV-infected mice are completely protected against challenge with a lethal dose of the virulent L_{10} strain of SFV within 24 hr following the original A7(74) infection. This indicates that immunity to the lethal effect of the virulent virus develops very early.[114]

Even in the severely demyelinated mice following two injections of the avirulent strain, considerable repair of myelin takes place by day 45. For this to be able to occur, it seems likely that the oligodendrocytes are not destroyed, and this is confirmed by pathological examination. However, clearly, this cell or the myelin it supports must be under attack and physiologically not functioning properly. As yet, it is not clear whether this may be a result of persistent replication of virus within the cell or viral antigen present on the surface of oligodendrocytes reacting with anti-SFV antibody causing low-grade inflammation or of a direct attack on the oligodendrocyte/myelin membrane combination by CTL as mentioned previously.

Other mechanisms might be, in view of the rapid rise of antiglycolipid activity, a T-cell-mediated and/or antibody attack on glycolipids in the oligodendrocytic/myelin membrane. Antiglycolipid activity is easily detectable by day 14.[100] Some research workers have suggested that the antiglycolipid activity seen is secondary to CNS damage and demyelination both in MS and in this model. We do not believe that to be the case in the SFV model, as mice given one dose of an inactivated brain-derived SFV vaccine develop very significant antiglycolipid activity in the blood by postvaccination day 18 without CNS damage. There is now some evidence that subsequent doses of SFV brain-derived vaccine will produce inflammation and demyelination.[110] Gliosis eventually occurs in the mouse brain following SFV infection.[115]

12. CONCLUSION

There now appears to be very considerable evidence that viruses can act as carriers for host-cell glycolipids and present them in an immunogenic manner. It seems a reasonable possibility that enveloped budding viruses can evoke an autoimmune reaction against nervous system glycolipid simply by the method of their replication, i.e., replicating in the brain and presenting host CNS glycolipid to the peripheral immune system, which in turn produces a cell-mediated and antibody response against nervous system glycolipids. This could apply not only to CNS glycolipids but also to PNS glycolipids. The Guillain–Barré syndrome

(GBS) following virus infection has still to be explained. Antiglycolipid antibodies occur in both GBS[116] and MS.[63] Many workers feel that an antiglycolipid immune response is probably occurring in these situations as a secondary reaction to damage of the nervous system and to myelin in particular. This is not necessarily the correct explanation. In fact, it is unlikely that glycolipids released in this way would produce an immune response with autoantibodies.

Both Allison[117] and Roitt[118] stress that in most cases release of tissue components directly into the circulation does not stimulate the production of autoantibodies. To produce autoimmunity the antigen must be presented in a manner acceptable to the immune system. Thyroiditis can be produced in rabbits if thyroid antigens are injected with Freund's adjuvant.[119] Konat *et al.*[82] produce EAE in rabbits by presenting the glycolipids in an inoculum with Freund's adjuvant. Although glycolipids are immunogenic, they can behave as haptens, and a carrier will increase their immunogenicity.[120,121] Although the carbohydrate moiety of the glycolipid is the most immunogenic, it must remain inconclusive at the moment whether the carbohydrate and/or the lipid is the most important immunogenic factor in the pathogenesis of demyelination. However, viruses can facilitate immunologic reactions in other ways, which may assist immunopathogenesis. In the ordinary state of events, budding viruses coated with host-cell glycolipids are known to be taken up by antigen-presenting cells. In this process they become uncoated, and their envelope, containing the glycolipid, is likely to be processed in the same way as viral proteins, thus allowing sensitization to the glycolipid of the cell of origin.

Viruses are known to induce the production of interferons α and β,[122] which in turn may stimulate class I antigen expression on brain cells.[123] For CTL attack on virally infected cells to occur, class I antigen expression is essential.[124] Suzumura *et al.*[125] show induction of class I antigens on oligodendrocytes and astrocytes following coronavirus infection; thus, these nervous system cells could be susceptible to CTL attack. Viruses, indirectly, can help to induce interferon γ by stimulating a T-cell immune response. It is activated T cells that induce interferon γ. Wong *et al.*[126] show that interferon γ induces a dramatic increase both in class I antigens on astrocytes, oligodendrocytes, microglia, and some neurons and class II antigens on some astrocytes. Coronavirus has been shown to induce class II antigens in astrocytes as well as class I.[127] Furthermore, class II antigens have been shown to be present on the surface of human oligodendrocytes and astrocytes.[128] Such activated cells could act as antigen-presenting cells. This function of viruses may indirectly render cells more vulnerable to immunologic recognition and damage. The retrovirus feline leukemia virus has been shown to incorporate class II antigens into its envelope, and therefore virions could present antigen.[129] If other viruses were to be shown to incorporate class II MHC antigens during the budding process, this would be a very significant finding.

The CNS, particularly, is considered a partially immunologically privileged site, as it lies within the blood–brain barrier. Foreign cells inoculated into the brain there are less easily rejected. Certainly tolerance to brain antigens by the vertebrate host is less highly developed than in many other tissues. Brain tissue is often damagingly immunogenic even if it is "self," as shown by Kabat *et al.*,[130] who

took six individual monkeys and removed from each a portion of brain. The brain tissue was emulsified and reinoculated back peripherally with adjuvants into the monkey from which the respective brain had come. All five monkeys that survived the operation developed acute CNS lesions with demyelination. This sensitivity to brain tissue has also been a factor in the development of vaccines. The history of the brain-derived virus vaccines has been notoriously associated with paralytic incidences, e.g., the Pasteur and Semple-type rabies vaccines. This emphasizes that any mechanism that presents host-derived nervous tissue to the peripheral immune system can be dangerous in respect to neuroparalytic accidents with demyelination.

Enveloped viruses could theoretically incorporate MBP from some cell membranes, but it has never been shown to occur. However, the amino acid sequences of MBP associated with mimicry caused by viruses may be important if the site at which this occurs is appropriate to provoking a pathological immune response.

Myelin basic protein remains unlikely to be the cause of demyelination in MS for reasons previously stated. perhaps a combination of mimicry and glycolipid presentation by viruses may be the crucial factor in the inflammation and demyelination seen. However, to me the evidence tends toward the concept that nervous system glycolipids presented in budding virus envelope form a more likely initiator of this disease process. For a simple diagrammatic representation of the concept, see Fig. 14-2.

13. SUMMARY OF THE EVIDENCE THAT VIRUS-INDUCED ANTIGLYCOLIPID ACTIVITY MAY BE IMPORTANT IN MULTIPLE SCLEROSIS

1. Enveloped budding viruses take the host cell membrane glycolipid into their coat.[88–91,93–97]
2. Different viruses using the same cell for replication will have similar glycolipids in their coats.
3. The principal lipid haptens of the mammalian cell are glucocer-amides,[56] and the major glycolipids of the CNS are cerebrosides, sulfatides, and gangliosides.[59]
4. Galactocerebroside is the major glycolipid of the CNS accounting for about 17% of the dry weight of adult human white matter and is an oligodendrocyte marker.[131]
5. Glycolipids are immunogenic, particularly as a component of an intact surface membrane.[56,57,132]
6. Galactocerebroside is exposed at the surface of myelin. Ganglioside is also present in myelin to a lesser extent.[56]
7. Immune reactions against both galactocerebroside and ganglioside can damage myelin and cells.[44,48,133,134]
8. Viruses can stimulate an antiglycolipid immune response.[99,100]
9. Enveloped budding viruses can be labeled by immunoelectron micros-

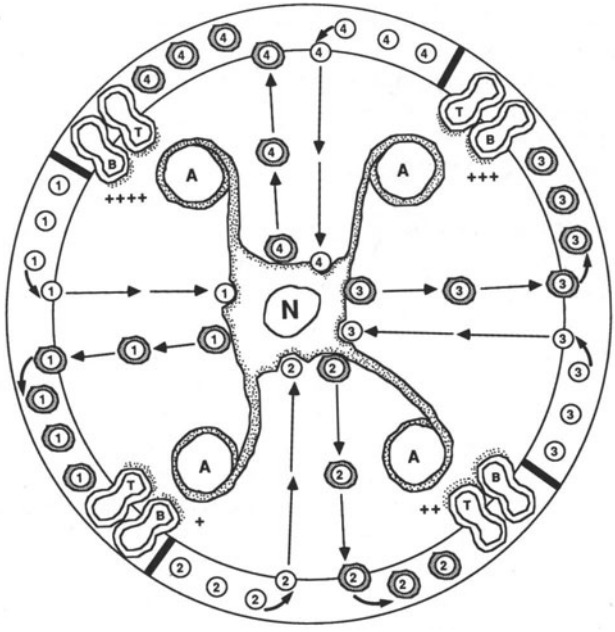

FIGURE 14-2. The possible role of recurrent infections of the CNS in MS in genetically susceptible individuals (HLA type). A neurotropic enveloped virus, for example measles (1), enters the brain and replicates in the cells of the CNS including, e.g., oligodendrocytes. The envelope of the budding virus is derived from the lipids of the host cell membranes. Glycolipids in the envelope of virions returning to the blood may be antigenic in association with the viral proteins, which may act as carrier determinants. Glycolipid-sensitized lymphocytes then enter the brian by diapedesis and attack either the myelin directly or the myelin-supporting cells. This results in demyelination and clinical relapse. After some time suppressor T cells are generated and control the reaction, resulting in remission. At a later date a second virus, e.g., a coronavirus (2) or an influenza virus (3), or a virus that has been latent and now become reactivated, enters, replicates in the brain, and returns to the circulation, presenting the same brain-specific glycolipid(s) in its envelope. The immune response is restimulated, resulting in a second, third, fourth, or fifth relapse. Remission intervenes as the T-suppressor cells control the response after each restimulation by virus. In this way any number of enveloped neurotropic viruses could be involved in initiating and restimulating an autoimmune response to the same brain cell membrane-specific glycolipid(s). Semliki forest virus (4) is included in the figure because it produces immune-mediated demyelination in experimental infection of mice. The figure represents a simplified concept of the foregoing hypothesis. The argument could be applied to other organisms, e.g., *Mycoplasma pneumoniae*, whose membrane constituents react with antibodies made against cerebrosides and indeed have been shown to react with antibodies produced in the CSF of multiple sclerosis patients.[55] Stippling, oligodendrocytic lipid membrane; N, nucleus of oligodendrocyte; T, T lymphocyte; B, B lymphocyte; A, axon.

copy and by immunocytochemistry and shown to react in an ELISA with various antiglycolipid sera and anti-RNA virus glycolipid MAb.[99,107–109,112]

10. Antiglycolipid MAb made against myelin will react in an ELISA with a demyelinating RNA enveloped budding virus.[99]
11. Patients with MS develop both humoral and cell-mediated immune reactions against glycolipids.[63,64,66–70]
12. Glycolipids inoculated into animals can produce demyelinating disease.[80,81,84–86]
13. Glycolipids and MBP produce much better demyelination than MBP alone.[41–43,47]
14. Many different enveloped budding viruses could be involved in this process, and this might account for some of the relapses in MS associated with intercurrent virus infection.

This concept would help to explain the previous findings of many different viruses being associated with MS and classify the disease as "a virus-induced antiglycolipid autoimmune disease." Once the glycolipid reaction against brain tissue glycolipid has been instigated, intercurrent infection with other organisms might also promote relapses. Husby *et al.*[135] showed that IgG antibody from children with rheumatic fever reacts with neuronal cytoplasm, particularly of the human caudate and subthalamic nuclei. This factor is removed by absorption with group A streptococci and particularly by their cell wall preparations. The antineuronal antibody appears to represent cross-reactions with antigens shared by group A streptococcal membranes and the neuronal cells.

Complement-fixing antibodies against *Mycoplasma pneumoniae* (MPN), more frequently IgM than IgG antibodies, are cross-reactive with nervous tissue, namely with cerebrosides.[136] Maida[55] has shown immunologic reactions against MPN in 18 cases of MS. The CSF titers are as high as or higher than the corresponding serum titers, indicating intrathecal antibody synthesis. I feel that his study does not rule out that these anticerebroside antibodies might be cross-reactive anti-MPN antibodies.

This is a concept that might be applied to the pathogenesis of other demyelinating diseases such as tropical spastic paraparesis associated with HTLV-I[137] and to the myelopathy found in the areas of Japan where HTLV-I leukemias are prevalent. Roman[138] draws attention to the fact that anti-HTLV-I-type antibodies have been found in patients given the definite clinical diagnosis of MS in Florida and Japan. He goes on to suggest that tropical spastic paraparesis, the Japanese myelopathy, and perhaps an MS-like neurological syndrome may represent clinical variants of the same disease, a retroviral myelopathy. The retroviruses may be of particular interest in this respect because HTLV-II can trigger transcription of mRNA for the interleukin 2 gene and the gene for its receptor.[139] This may assist stimulation of CTL.

I personally believe at this moment that the demyelinating disease multiple sclerosis occurs in people of suitable HLA type as a result of an enveloped virus infection acquired in early childhood. I believe that the initial damage to myelin may be produced by a CTL sensitized to brain myelin glycolipids migrating from

the perivascular cuffs, the perivascular cuffs representing the earliest lesion seen in MS. Few CTL would be necessary to initiate the process. Once the damage has been initiated, secondary reactions will occur as a result of the breakdown of myelin, producing further damage and finally gliosis. As to whether molecular mimicry of MBP by viruses or MBP directly plays a part remains to be seen. The evidence for MBP alone playing a part in the pathogenesis of MS and the demyelination seen is very poor. Myelin basic protein is not expressed on the surface of myelin or oligodendrocytes.[140]

The general concept described here might also be applied to postviral PNS neuritis and perhaps postviral thyroiditis, pancreatitis, oophoritis, and testiculitis, the latter seen particularly after mumps virus infection. A recent paper by Fujinami *et al.*[141] describes a MAb produced by the CNS demyelinating Theiler's murine encephalomyelitis virus (TMEV) that reacts with both galactocerebroside and TMEV. This is of particular interest, as TMEV is a nonenveloped picornavirus. They suggest possible mechanisms by which a virus of this nature might produce this antigalactocerebroside response, one being that areas on picornavirus surfaces have hydrophobic pockets that could accommodate glycolipid-like structures (J. Hogle, Research Institute of Scripps Clinic, personal communication). This indicates the possibility that even in this group of viruses glycolipids can be presented on the surface in a significantly immunogenic fashion. Although glycolipids are very difficult to work with, this must not be made an excuse for not investigating this concept further: the work should be done to prove that these ideas are relevant or misfounded.

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REFERENCES

1. Marie, P., 1884, Sclerose en plaque et maladies infectieuses, *Prog. Med.* **12**:287–289.
2. Kurtzke, J. F., 1983, Epidemiology of multiple sclerosis, in: *Multiple Sclerosis* (J. F. Hallpike, C. W. M. Adams, and W. W. Tourtellotte, eds.), Chapman and Hall, London, pp. 47–95.
3. Adams, J. M., and Imagawa, D. T., 1962, Measles antibodies in multiple sclerosis, *Proc. Soc. Exp. Biol. Med.* **111**:562–566.
4. Adams, J. M., Brooks, M. B., Fisher, E. D., and Tyler, C. S., 1970, Measles antibodies in patients with multiple sclerosis and with other neurological and non-neurological diseases, *Neurology* **20**:1039–1042.
5. Salmi, A. A., Norrby, E., and Panelius, M., 1972, Identification of different measles virus-

specific antibodies in the serum and cerebrospinal fluid from patient with subacute sclerosing panencephalitis and multiple sclerosis, *Infect. Immun.* **6**:248–254.
6. Haire, M., 1977, Significance of virus antibodies in multiple sclerosis, *Br. Med. Bull.* **33**:40–44.
7. Catalano, L. W., Jr., 1972, Herpesvirus hominis antibody in multiple sclerosis and amyotrophic lateral sclerosis, *Neurology* **22**:473–478.
8. Cook, S. D., Dowling, P. C., and Russell, W. C., 1979, Neutralizing antibodies to canine distemper and measles virus in multiple sclerosis, *J. Neurol. Sci.* **41**:61–70.
9. Horikawa, Y., Tsubaki, T., and Nakajima, M., 1973, Rubella antibody in multiple sclerosis, *Lancet* **1**:996–997.
10. Burks, J. S., Devald-Macmillan, B., Jankovsky, L., and Gerdes, J., 1979, Characterization of coronaviruses isolated using multiple sclerosis autopsy brain material, *Neurology* **29**:547.
11. Norrby, E., Link, H., Olsson, J. E., Panelius, M., Salmi, A. G., and Vandvik, B., 1974, Comparison of antibodies against different viruses in cerebrospinal fluid and serum samples from patients with multiple sclerosis, *Infect. Immun.* **10**:688–694.
12. Cremer, N. E., Johnson, K. P., Fein, G., and Litosky, W. H., 1980, Comprehensive viral immunology of multiple sclerosis II. Analysis of serum and CSF antibodies by standard serologic methods, *Arch. Neurol.* **37**:610–615.
13. Goswami, K. K. A., Randall, R. E., Lange, L. S., and Russell, W. C., 1987, Antibodies against the paramyxovirus SV5 in cerebrospinal fluids of some multiple sclerosis patients, *Nature* **327**:244–246.
14. Salmi, A., Reunanen, M., Ilonens, J., and Panelius, M., 1983, Intrathecal antibody synthesis to virus antigens in multiple sclerosis, *Clin. Exp. Immunol.* **52**:241–249.
15. Arnadottir, T., Reunanen, M., and Salmi, A., 1982, Intrathecal synthesis of virus antibodies in multiple sclerosis patients, *Infect. Immun.* **38**:399–407.
16. Vandvik, B., Norrby, E., Nordal, H., and Degre, M., 1976, Oligoclonal measles virus specific IgG antibodies isolated by virus immunoabsorption of cerebrospinal fluids, brain extracts, and sera from patients with subacute sclerosing panencephalitis and multiple sclerosis, *Scand. J. Immunol.* **5**:979–992.
17. Nordal, H. J., Vandvik, B., and Norrby, E., 1978, Multiple sclerosis: Local synthesis of electrophoretically restricted measles, rubella, mumps and herpes simplex virus antibodies in the central nervous system, *Scand. J. Immunol.* **7**(6):473–479.
18. Gudnadottir, M., Helgadottir, H., Bjarnason, O., and Jonsdottir, K., 1964, Virus isolated from the brain of a patient with multiple sclerosis, *Exp. Neurol.* **9**:85–95.
19. Meulen ter, V., Koprowski, H., Iwasaki, Y., Kackell, Y. M., and Muller, D., 1972, Fusion of cultured multiple-sclerosis brain cells with indicator cells: Presence of nucleocapsids and virion and isolation of parainfluenza-type virus, *Lancet* **1**:1–5.
20. Burks, J. S., DeVald, B. L., Jankovsky, L. D., and Gerdes, J. C., 1980, Two coronaviruses isolated from central nervous system tissue of two multiple sclerosis patients, *Science* **209**: 933–934.
21. Prineas, J., 1972, Paramyxovirus-like particles associated with acute demyelination in chronic relapsing multiple sclerosis, *Science* **178**:760–763.
22. Pathak, S., and Webb, H. E., 1976, Paramyxovirus-like inclusions in brain of patient with severe multiple sclerosis, *Lancet* **2**:311.
23. Webb, H. E., and Fazakerley, J. K., 1984, Can viral envelope glycolipids produce autoimmunity, with reference to the CNS and multiple sclerosis? *Neuropathol. Appl. Neurobiol.* **10**: 1–10.
24. Pathak, S., and Webb, H. E., 1974, Possible mechanisms for the transport of Semliki Forest virus into and within mouse brain, *J. Neurol. Sci.* **23**:175–184.
25. Rodriguez, M., Pease, L. R., and David, C. S., 1986, Immune-mediated injury of virus-infected oligodendrocytes, *Immunol. Today* **7**(12):359–362.
26. Rogers, N. G., Basnight, M., Gibbs, C. J., Jr., and Gajdusek, D. C., 1967, Latent viruses in chimpanzees with experimental kuru, *Nature* **216**:446–449.

27. Fujinami, R. S., and Oldstone, M. B. A., 1985, Amino acid homology between the encephalitogenic site of myelin basic protein and virus: Mechanism for autoimmunity, *Science* **230:**1043–1045.
28. Tardieu, M., Powers, M., Hafler, D. A., Hauser, S. L., and Weiner, H. L., 1984, Autoimmunity following viral infection: Demonstration of monoclonal antibodies against normal tissue following infection of mice with reovirus and demonstration of shared antigenicity between virus and lymphocytes, *Eur. J. Immunol.* **14:**561–565.
29. Lane, D. P., and Hoeffler, W. K., 1980, SV40 large T shares an antigenic determinant with a cellular protein of molecular weight 68,000, *Nature* **288:**167–170.
30. Fujinami, R. S., Oldstone, M. B. A., Wroblewska, Z., Frankel, M. E., and Koprowski, H., 1983, Molecular mimicry in virus infection: Cross reaction of measle virus phosphoprotein or of herpes simplex virus protein with human intermediate filaments, *Proc. Natl. Acad. Sci. U.S.A.* **80:**2346–2350.
31. Jahnke, U., Fischer, E. H., and Alvord, E. C., Jr., 1985, Sequence homology between certain viral proteins and proteins related to encephalomyelitis and neuritis, *Science* **229:**282–284.
32. Kagnoff, M. F., Austin, R. K., Hubert, J. J., Bernardin, J. E., and Kasarda, D. D., 1984, Possible role for a human adenovirus in the pathogenesis of celiac disease, *J. Exp. Med.* **160:** 1544–1557.
33. Srinivasappa, J., Saegusa, J., Prabhakar, B. S., Gentry, M. K., Buchmeier, M. J., Wiktor, T. J., Koprowski, H., Oldstone, M. B. A., and Notkins, A. L., 1986, Molecular mimicry: Frequency of reactivity of monoclonal antiviral antibodies with normal tissues, *J. Virol.* **57:** 397–401.
34. Miller, S. D., Clatch, R. J., Pevear, D. C., Trotter, J. L., and Lipton, H. L., 1987, Class II-restricted T cell responses in Theiler's murine encephalomyelitis virus (TMEV)-induced demyelinating disease, *J. Immunol.* **138:**3776–3784.
35. Bahmanyar, S., Srinivasappa, J., Casali, P., Fujinami, R. S., and Oldstone, M. B. A., 1987, Antigenic mimicry between measles and human T lymphocytes, *J. Infect. Dis.* **156:**526–527.
36. Haspel, M. V, Onodera, T., Prabhakar, B. S., Horita, M., Suzuki, H., and Notkins, A. L., 1983, Virus-induced autoimmunity: Monoclonal antibodies that react with endocrine tissues, *Science* **230:**304–306.
37. Huber, S. A., and Lodge, P. A., 1984, Coxsackie virus B-3 myocarditis in Balb/c mice. Evidence for autoimmunity to myocyte antigens, *Am. J. Pathol.* **116:**21–29.
38. Paterson, P. Y., 1976, Experimental autoimmune (allergic) encephalomyelitis. Induction, pathogenesis and suppression, in: *Textbook of Immunopathology* (P. A. Miescher and J. J. Mueller-Eberhard, eds.), Grune & Stratton, New York, pp. 225–251.
39. Raine, C. S., Traugott, V., Iqbal, K., Snyder, D. S., Cohen, S. R., Farooq, M., and Norton, W. T., 1978, Encephalitogenic properties of purified preparations of bovine oligodendrocytes tested in guinea pigs, *Brain Res.* **142:**85–96.
40. Tabara, T., and Endoh, M., 1985, Humoral immune response to myelin basic protein, cerebroside and ganglioside in chronic relapsing experimental allergic encephalomyelitis of the guinea pig, *J. Neurol. Sci.* **67:**201–212.
41. Raine, C. S., Traugott, U., Farooq, M., Bornstein, M. B., and Norton, W. T., 1981, Augmentation of immune-mediated demyelination by lipid haptens, *Lab. Invest.* **45:** 174–182.
42. Hoffman, P.M., Gaston, D. D., and Spitler, L. E., 1973, Comparison of experimental allergic encephalomyelitis induced with spinal cord, basic protein, and synthetic encephalitogenic peptide, *Clin. Immunol. Immunopathol.* **1:**364–371.
43. Paterson, P. Y., 1977, Autoimmune neurological disease: Experimental animal system and implication for multiple sclerosis, in: *Autoimmunity* (N. Talal, ed.), Academic Press, New York, p. 658.
44. Dubois-Dalcq, M., Niedieck, B., and Buyse, M., 1970, Action of anti-cerebroside sera on myelinated nervous tissue cultures, *Pathol. Eur.* **5:**331–347.

45. Fry, J. M., Weissbarth, S., Lehrer, G. M., and Bornstein, M. B., 1974, Cerebroside antibody inhibits sulfatide synthesis and myelination and demyelinates in cord tissue cultures, *Science* **183:**540–542.
46. Maggio, B., and Cumar, F. A., 1975, Experimental allergic encephalomyelitis: Dissociation of neurological symptoms from lipid alterations in brain, *Nature* **253:**364–365.
47. Seil, F. J., Falk, G. A., Kies, M. W., and Alvord, E. C., 1968, The *in vitro* activity of sera from guinea pigs sensitized with whole CNS and with purified encephalitogen, *Exp. Neurol.* **22:** 545–555.
48. Saida, T., Saida, K., and Silberberg, D. H., 1979, Demyelination produced by experimental allergic neuritis serum and antigalactocerebroside antiserum in CNS cultures: An ultrastructural study, *Acta. Neuropathol. (Berl.)* **48:**19–25.
49. Zamvil, S. S., Nelson, P. A., Mitchell, D. J., Knobler, R. L., Fritz, R. B., and Steinman, L., 1985, Encephalitogenic T cell clones specific for myelin basic protein. An unusual bias in antigen recognition, *J. Exp. Med.* **162:**2107–2124.
50. Watanabe, R., Wege, H., and ter Meulen, V., 1983, Adoptive transfer of EAE-like lesions from rats with coronavirus-induced demyelinating encephalomyelitis, *Nature* **305:**150–153.
51. Gasser, D. L., Newlin, C. M., Palm, J., and Gomatas, N. K., 1973, Genetic control of susceptibility to experimental allergic encephalomyelitis in rats, *Science* **181:**872–873.
52. Williams, R. H., and Moore, M. J., 1973, Linkage of susceptibility to experimental allergic encephalomyelitis to the major histocompatibility locus in the rat, *J. Exp. Med.* **138:** 775–783.
53. Levine, S., and Sowinski, R., 1975, Allergic encephalomyelitis in the reputedly resistant brown Norway strain of rats, *J. Immunol.* **114:**597–601.
54. Tsukada, N., Koh, C.-S., Yanagisawa, N., Okano, A., Behan, W. M. H., and Behan, P. O., 1987, A new model for multiple sclerosis: Chronic experimental allergic encephalomyelitis induced by immunization with cerebral endothelial cell membrane, *Acta. Neuropathol. (Berl.)* **73:**259–266.
55. Maida, E., 1983, Immunological reactions against *Mycoplasma pneumoniae* in multiple sclerosis: Preliminary findings, *J. Neurol.* **229:**103–111.
56. Leibowitz, S., and Gregson, N. A., 1979, Brain glycolipids as cell-surface antigens, in: *Clinical Neuroimmunology* (F. C. Rose, ed.), Blackwell Scientific Publications, London, pp. 29–41.
57. Landsteiner, K., 1947, *The specificity of Serological Reactions*, Harvard University Press, Cambridge, pp. 210–240.
58. Leibowitz, S., Morgan, R. S., Berkinshaw-Smith, E. M. I., and Payling-Wright, G., 1961, Cerebral vascular damage in guinea-pigs induced by various heterophile antisera injected by the Forssman intracarotid technique, *Br. J. Exp. Pathol.* **42:**455–463.
59. Klenk, E., 1969, On cerebrosides and gangliosides, in: *Progress in the Chemistry of Fats and Other Lipids*, Volume 10, Part 4 (R. T. Holman, ed.), Pergamon, New York, pp. 411–431.
60. Niedieck, B., and Palacios, O., 1965, Uber die Produktion von Cerbrosidantikorpern nach intradermaler Injection v.n Cerebrosid-Protein-Adjuvans-Emulsionen, *Z. Immun. Forsch.* **129:**234–243.
61. Czeonkowska, A., and Leibowitz, S., 1974, The effect of homologous and heterologous carriers on the immunogenicity of the galactocerebroside hapten, *Immunology* **27:**1117–1126.
62. Rapport, M. M., Graft, L., and Yariv, J., 1961, Immunochemical studies of organs and tumor lipids. IX. Configuration of the carbohydrate residues in cytolipin H, *Arch. Biochem. Biophys.* **92:**438–440.
63. Arnon, R., Crisp, E., Kelly, R., Ellison, S. W., Myes, L. W., and Tourtellotte, W. W., 1980, Anti-ganglioside antibodies in multiple sclerosis, *J. Neurol. Sci.* **26:**179–186.
64. Kasai, N., Pachner, A. R., and Yu, R. K., 1986, Anti-glycolipid antibodies and their immune complexes in multiple sclerosis, *J. Neurol. Sci.* **75:**33–42.

65. Duponey, R., 1972, Role of the cerebrosides and a galactodiglyceride in the antigenic cross reaction between nerve tissue and treponema, *J. Immunol.* **109**:146–153.
66. Offner, H., Konat, G., and Sela, B.-A., 1981, Multi-sialo brain gangliosides are powerful stimulators of active E-rosetting lymphocytes from multiple sclerosis patients, *J. Neurol. Sci.* **52**:279–287.
67. Sela, B.-A., Konat, A. B., and Offner, H., 1982, Elevated ganglioside concentration in serum and peripheral blood lymphocytes from multiple sclerosis patients in remission, *J. Neurol. Sci.* **54**:143–148.
68. Ilyas, A. A., and Davison, A. N., 1983, Cellular hypersensitivity to gangliosides and myelin basic protein in multiple sclerosis, *J. Neurol. Sci.* **59**:85–95.
69. Davison, A. M., and Ilyas, A. A., 1982, Cyclosporin A inhibits ganglioside-stimulated lymphocyte rosette formation in multiple sclerosis, *Int. Arch. Allergy Appl. Immunol.* **69**:393–396.
70. Bellamy, A., Davison, A. N., and Feldmann, M., 1986, Derivation of ganglioside-specific T-cell lines of suppressor or helper phenotype from cerebrospinal fluid of multiple sclerosis patients, *J. Neuroimmunol.* **12**:107–120.
71. Harboe, A., Schoyen, R., and Bye-Hansen, A., 1966, Haemagglutination inhibition by antibody to host material of fowl plague virus grown in different tissues of chick chorioallantoic membranes, *Acta Pathol. Microbiol. Scand.* **67**:573–578.
72. Feinsod, F. M., Spielman, A., and Swaner, J. L., 1975, Neutralization of Sindbis virus by antisera to antigens of vector mosquitoes, *Am. J. Trop. Med. Hyg.* **24**:533–536.
73. Almeida, J. D., and Waterson, A. P., 1969, The morphology of virus antibody interaction, *Adv. Virus Res.* **15**:307–338.
74. Reilly, C. A., Jr., and Schloss, G. T., 1971, The erythrocyte as virus carrier in Friend and Rauscher virus leukemias, *Cancer Res.* **31**:841–846.
75. Cox, K. O., and Keast, D., 1973, Rauscher virus infection, erythrocyte clearance studies, and autoimmune phenomena, *J. Natl. Cancer Inst.* **50**:941–946.
76. Steck, A. J., Tschannen, R., and Schaefer, R., 1981, Induction of antimyelin and antioligodendrocyte antibodies by vaccinia virus—an experimental study in the mouse, *J. Neuroimmunol.* **1**:117–124.
77. Lindenmann, J., and Klein, P. A., 1967, Viral oncolysis: Increased immunogenicity of host cell antigen associated with influenza virus, *J. Exp. Med.* **126**:93–108.
78. Rook, G. A. W., and Webb, H. E., 1970, Anti-lymphocyte serum and tissue culture used to investigate the role of cell-mediated response in viral encephalitis in mice, *Br. Med. J.* **4**:210–212.
79. Madrid, R. E., Wisniewski, H. M., Iqbal, K., Pullarkat, R. K., and Lassmann, H., 1981, Relapsing experimental allergic encephalomyelitis induced with isolated myelin and with myelin basic protein plus myelin lipids, *J. Neurol. Sci.* **50**:399–411.
80. Nagai, Y., Momoi, T., Saito, M., Mitzuzawa, E., and Ohtani, S., 1976, Ganglioside syndrome, a new autoimmune neurologic disorder, experimentally induced with brain ganglioside, *Neurosci. Lett.* **2**:107–111.
81. Saida, T., Saida, K., Dorfman, S., Siblerger, D. H., Summer, A. J., Manning, M. C., Lisak, R. P., and Brown, M. N., 1979, Experimental allergic neuritis induced by sensitization with galactocerebrosides, *Science* **204**:1103–1106.
82. Konat, G., Offner, H., Lev-Ram, V., Cohen, O., Schwartz, M., Cohen, I. R., and Sela, B.-A., 1982, Abnormalities in brain myelin of rabbits with experimental auto-immune multiple sclerosis-like disease induced by immunization to gangliosides, *Acta Neurol. Scand.* **66**:568–574.
83. Hughes, R. A. C., and Powell, H. C, 1984, Experimental allergic neuritis: Demyelination induced by P_2 alone and non-specific enhancement by cerebroside, *J. Neuropathol. Exp. Neurol.* **43**:154–161.
84. Carroll, W. M., Jennings, A. R., and Mastaglia, F. L., 1984, Experimental demyelinating optic neuropathy induced by intra neural injection of galactocerebroside antiserum, *J. Neurol. Sci.* **65**:125–135.

85. Roth, G. A., Royitta, M., Yu, R. K., Raine, C. S., and Bornstein, M. B., 1984, Antisera to different glycolipids induce myelin alterations in mouse spinal cord tissue cultures, *Brain Res.* **339:**9–18.
86. Sergott, R. C., Brown, M. J., Silberberg, D., and Lisak, R. P., 1984, Antigalactocerebroside serum demyelinates optic nerve *in vivo*, *J. Neurol. Sci.* **64:**297–303.
87. Nakajima, H., and Obara, J., 1967, Physico-chemical studies of Newcastle disease virus 3. The content of virus nucleic acid and its sedimentation pattern, *Arch. Gem. Virusforsch.* **20:** 287–295.
88. Klenk, H. D., and Choppin, P. W., 1970, Glycosphingolipids of plasma membrane of cultured cells and an enveloped virus (SV5) grown in these cells, *Proc. Natl. Acad. Sci. U.S.A.* **66:**57–64.
89. Soule, D. W., Marinetti, G. V., and Morgan, H. R., 1959, Studies of the hemolysis of red blood cells by mumps virus. IV. Quantitative study of changes in red blood lipids and of virus lipids, *J. Exp. Med.* **110:**93–102.
90. Kates, M., Allison, A. C., Tyrrell, D. A. J., and James, A. T., 1961, Lipids of influenza virus and their relation to those of the host cell, *Biochim. Biophys. Acta* **52:**455–466.
91. Blough, H. A., and Merlie, J. P., 1970, The lipids of incomplete influenza virus, *Virology* **40:** 685–692.
92. Hirschberg, C. B., and Robbins, P. W., 1974, The glycolipids and phospholipids of Sindbis virus and their relation to the lipids of the host cell plasma membrane, *Virology* **61:**602–608.
93. Quigley, J. P., Rifkin, D. B., and Reich, E., 1971, Phospholipid composition of Rous sarcoma virus, host cell membranes and other enveloped RNA viruses, *Virology* **46:**106–116.
94. Heydrick, F. P., Comer, J. F., and Wachter, R. F., 1971, Phospholipid composition of Venezuelan equine encephalitis virus, *J. Virol.* **7:**642–645.
95. Renkonen, O., Kaarainen, L., Simons, K., and Gahmberg, C. G., 1971, The lipid class composition of Semliki Forest virus and of plasma membranes of the host cells, *Virology* **46:** 318–326.
96. Laine, R., Kettunen, M. L., Gahmberg, C. G., Kääriäinen, L., and Renkonen, O., 1972, Fatty chains of different lipid classes of Semliki Forest virus and host cell membranes, *J. Virol.* **10:**433–438.
97. Luukkonen, A., Kaariainen, L., and Renkonen, O., 1976, Phospholipids of Semliki Forest virus grown in cultured mosquito cells, *Biochim. Biophys. Acta* **450:**109–120.
98. Webb, H. E., Chew Lim, M., Jagelman, S., Oaten, S. W., Pathak, S., Suckling, A. J., and MacKenzie, A., 1979, Semliki Forest virus infections in mice as a model for studying acute and chronic central nervous system virus infections in mice, in: *Clinical Neuroimmunology* (F. Rose, ed.), Blackwell Scientific Publications, London, pp. 369–390.
99. Khalili-Shirazi, A., Gregson, N., and Webb, H. E., 1986, Immunological relationship between a demyelinating RNA enveloped budding virus (Semliki Forest) and brain glycolipids, *J. Neurol. Sci.* **76:**91–103.
100. Amor, S., and Webb, H. E., 1988, CNS pathogenesis following a dual virus infection with Semliki Forest (Alphavirus) and Langat (Flavivirus), *Br. J. Exp. Pathol.* **69:**197–208.
101. Kelly, W. R., Blakemore, W. F., Jagelman, S., and Webb, H. E., 1982, Demyelination induced in mice by avirulent Semliki Forest virus. II. An ultrastructural study of focal demyelination in the brain, *Neuropathol. Appl. Neurobiol.* **8:**43–53.
102. Illavia, S. J., Webb, H. E., and Pathak, S., 1982, Demyelination induced in mice by avirulent Semliki Forest virus. I. Virology and effects on optic nerve, *Neuropathol. Appl. Neurobiol.* **8:** 35–42.
103. Jagelman, S., Suckling, A. J., Webb, H. E., and Bowen, E. T. W., 1978, The pathogenesis of avirulent Semliki Forest virus infections in athymic nude mice, *J. Gen. Virol.* **41:**599–607.
104. Fazakerley, J. K., Amor, S., and Webb, H. E., 1983, Reconstitution of Semliki Forest virus infected mice, induces immune mediated pathological changes in the CNS, *Clin. Exp. Immunol.* **52:**115–120.

105. Fazakerley, J. K., 1985, The role of the immune system in protection and pathogenesis during Semliki Forest virus encephalitis, Ph.D. Thesis, University of London.
106. Pathak, S., Illavia, S. J., and Webb, H. E., 1983, The identification and role of cells involved in CNS demyelination in mice after Semliki Forest virus infection: An ultrastructural study, *Prog. Brain Res.* **59**:237–254.
107. Webb, H. E., Mehta, S., Leibowitz, S., and Gregson, N. A., 1984, Immunological reaction of the demyelinating Semliki Forest virus with immune serum to glycolipids and its possible importance to central nervous system viral auto-immune disease, *Neuropathol. Appl. Neurobiol.* **10**:77–84.
108. Evans, N. R. S., and Webb, H. E., 1986, Immunoelectron microscopic labeling of glycolipids in the envelope of a demyelinating brain derived RNA virus (Semliki Forest) by anti-glycolipid sera, *J. Neurol. Sci.* **74**:279–287.
109. Khalili-Shirazi, A., Gregson, N., and Webb, H. E., 1988, Immunocytochemical evidence for Semliki Forest virus antigen persistence in mouse brain, *J. Neurol. Sci.* **85**:17–26.
110. Amor, S., 1988, An investigation into mixed Toga and Flavi virus infections and their effect on the pathogenesis of CNS disease, Ph.D. Thesis, University of London.
111. Khalili-Shirazi, A., 1988, A study of the antigenicity of the host central nervous system derived glycolipids in the envelope of Semliki Forest virus, Ph.D. Thesis, University of London.
112. Pathak, S., Illavia, S. J., and Khalili-Shirazi, A., 1990, Immunoelectron microscopical labelling of a glycolipid in the envelopes of brain cell derived budding viruses, Semliki Forest, influenza and measles, using a monoclonal antibody directed chiefly against galactocerebroside made by SFV immunization, *J. Neurol. Sci.* **96**:293–302.
113. Illavia, S. J., and Webb, H. E., 1988, The pathological effect on the central nervous system of mice following single and repeated infections of the demyelinating A7(74) strain of Semliki Forest virus, *Neuropathol. Appl. Neurobiol.* **14**:207–220.
114. Bradish, C. J., and Allner, K., 1972, The early responses of mice to respiratory or intraperitoneal infection by defined virulent and avirulent strains of Semliki Forest virus, *J. Gen. Virol.* **15**:205–218.
115. Zlotnik, I., Grant, D. P., and Batter-Hatton, D., 1972, Encephalopathy in mice following inapparent Semliki Forest virus infection, *Br. J. Exp. Pathol.* **53**:125–129.
116. Hughes, R. A. C., Gray, I. A., Gregson, N. A., Kadlubowski, M., Kennedy, M., Leibowitz, S., and Thompson, H., 1984, Immune responses to myelin antigens in Guillain–Barré syndrome, *J. Neuroimmunol.* **6**:303–312.
117. Allison, A. C., 1971, Unresponsiveness to self antigens, *Lancet* **2**:401.
118. Roitt, I., 1980, *Essential Immunology*, Blackwell Scientific Publications, Oxford, pp. 306–309.
119. Rose, N. R., and Witebsky, E., 1968, Thyroid autoantibodies in thyroid disease, *Adv. Metab. Dis.* **3**:231–277.
120. Marcus, D. M., and Schwarting, G. A., 1976, Immunochemical properties of glycolipids and phospholipids, *Adv. Immunol.* **23**:203–240.
121. Rapport, M. M., and Graf, L., 1969, Immunochemical reactions of lipids, *Prog. Allergy* **13**: 273–331.
122. Toy, J. L., 1983,The interferons, *Clin. Exp. Immunol.* **54**:1–13.
123. Morris, A., Tomkins, P. T., Maudsley, D. J., and Blackman, M., 1987, Infection of cultured murine brain cells by Semliki Forest virus: Effects of interferon-β on viral replication, viral antigen display, major histocompatibility complex antigen display and lysis by cytotoxic T lymphocytes, *J. Gen. Virol.* **68**:99–106.
124. Zinkernagel, R. M., and Doherty, P. C., 1979, MHC-restricted cytotoxic T cells: Studies on the biological role of polymorphic major transplantation antigens determining T-cell restriction specificity, function and responsiveness, *Adv. Immunol.* **27**:52–180.
125. Suzumura, A., Lavi, E., Weiss, S. R., and Silberberg, D. H., 1986, Coronavirus infection induces H-2 antigen expression on oligodendrocytes and astrocytes, *Science* **232**:991–993.

126. Wong, G. H. W., Bartlett, P. F., Clark-Lewis, I., Battye, F., and Schrader, J. W, 1984, Inducible expression of H-2 and Ia antigens on brain cells, *Nature* **310**:688–691.
127. Massa, P. T., Dorries, R., and ter Meulen, V., 1986, Viral particles induce Ia antigen expression on astrocytes, *Nature* **320**:543–546.
128. Kim, S. U., Moretto, G., and Shin, D. H., 1985, Expression of Ia antigens on the surface of human oligodendrocytes and astrocytes in culture, *J. Neuroimmunol.* **10**(2):141–149.
129. Azocar, J., Essex, M., and Yunis, E. J., 1983, Cell culture density modulates the incorporation of HLA antigens by enveloped viruses, *Hum. Immunol.* **7**:59–65.
130. Kabat, E. A., Wolf, A., and Bezer, A. E., 1949, Studies on acute disseminated encephalomyelitis produced experimentally in rhesus monkeys, *J. Exp. Med.* **89**:395–398.
131. Martenson, E., 1969, Glycosphingolipids of animal tissues, in: *Progress in the Chemistry of Fats and Other Lipids*, Volume 10 (R. T. Holman, ed.), Pergaman Press, Oxford, pp. 365–407.
132. Gregson, N. A., Kennedy, M., and Leibowitz, S., 1977, Gangliosides as surface antigens of cells isolated from rat cerebellar cortex, *Nature* **266**:461–463.
133. Bornstein, M. B., and Raine, C. S., 1970, Experimental allergic encephalomyelitis in antiserum inhibition of myelination *in vitro*, *Lab. Invest.* **23**:536–542.
134. Gregson, N. A., Pytharas, M., and Leibowitz, S., 1977, the reactivity of anti-ganglioside antiserum with isolated cerebellar cells, *Biochem. Soc. Trans.* **5**:174–175.
135. Husby, G., Van De Rijn, I., Zabriskie, J. B., Abdin, Z. H., and Williams, R. C., Jr., 1976, Antibodies reacting with cytoplasm of subthalamic and caudate nuclei neurons in chorea and acute rheumatic fever, *J. Exp. Med.* **144**:1094–1110.
136. Biberfield, G., 1971, Antibodies to brain and other tissues in cases of *Mycoplasma pneumoniae* infection, *Clin. Exp. Immunol.* **8**:319–333.
137. Gessain, A., Barin, F., Varnant, J. C., Gout, O., Maurs, L., Calender, A., and De The, G., 1985, Antibodies to human T-lymphotrophic virus type I in patients with tropical spastic paraparesis, *Lancet* **2**:407–409.
138. Roman, G. C., 1987, Retrovirus associated myelopathies, *Arch. Neurol.* **44**(6):659–663.
139. Greene, W. C., Leonard, W. J., Wano, Y., Sveteik, P. B., Peffer, N. J., Sodroski, J. G., Rosen, C. A., Goh, W. C., and Haseltine, W. A., 1986, *Trans*-activator gene of HTLV-II induces IL-2 receptor and IL-2 cellular gene expression, *Science* **232**:877–880.
140. Sternberger, N. H., del Cerro, C., and Kies, M. W., 1985, Immunocytochemistry of myelin basic proteins in adult rats oligodendroglia, *J. Neuroimmunol.* **7**:355–363.
141. Fujinami, R. S., Zurbriggen, A., and Powell, H. C., 1988, Monoclonal antibody defines determinant between Theiler's virus and lipid-like structures, *J. Neuroimmunol.* **20**:25–32.