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ANNOTATION

CAN VIRAL ENVELOPE GLYCOLIPIDS PRODUCE AUTO-IMMUNITY, WITH REFERENCE TO THE CNS AND MULTIPLE SCLEROSIS?

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Can viral envelope glycolipids produce auto-immunity, with reference to the CNS and multiple sclerosis?

Many viruses, with lipid envelopes derived from the host cell membranes, have been implicated in the aetiology of multiple sclerosis (MS), and epidemiological studies support an infectious agent. Alternatively the disease is thought by other workers to be auto-immune in nature, and recently much attention has been focused on immunological sensitivity to glycolipids in MS patients. In this paper it is proposed that CNS demyelination could arise in susceptible individuals (HLA type) from an immune response to glycolipids, triggered by the carrier effect of one or more enveloped neurotropic viruses.

Introduction

Viruses have been thought to be involved in such diseases as multiple sclerosis (MS) for many years. The proof of this still escapes the medical profession. Many different viruses including measles (Adams *et al.*, 1970; Salmi *et al.*, 1973; Miyamoto *et al.*, 1976), rubella (Horikawa, Tsubaki & Nakajima, 1973), herpes (Catalano, 1972), coronavirus (Burks *et al.*, 1979), and canine distemper virus (Cook, Dowling & Russell, 1979) have been implicated by the presence of anti-viral antibodies. Several viruses have been isolated including Herpes simplex (Gudnadottir *et al.*, 1964) and para-influenza type 1 (ter Meulen *et al.*, 1972). Paramyxo-like virus inclusions have been seen (Prineas, 1972; Pathak & Webb, 1976) and canine distemper virus is implicated epidemiologically. The number of viruses implicated in MS has,

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if anything, confused the issue of a viral aetiology. The possible role of viruses in MS is well reviewed by ter Meulen & Stephenson (1983).

Rogers *et al.* (1967) isolated several viruses from kuru infected chimpanzee brains. From such observations it has become quite clear over the years that viruses enter the brain easily, can remain there throughout life, and in many cases produce no disturbance. In any virus infection with a viraemia, virus from the cerebral capillaries can be transported easily across the basement membrane by pinocytosis and enter the brain parenchyma (Pathak & Webb, 1974). Neurotropic viruses enter the brain before there is any inflammation. The fact that these viruses cross the blood-brain barrier, whereas smaller particles such as ferritin do not, is of interest. Pathak & Webb (1974) suggested that this was because the virus in the blood represented virus which had replicated in the host, and so would have host membrane in its envelope and be considered as 'self'. The cells of the immune response, lymphocytes, plasma cells, macrophages, and occasional polymorphonuclear cells, enter later by diapedesis across the basement membrane and an inflammatory response develops.

Membrane glycolipids and 'budding' viruses

Many of the neurotropic viruses are 'budding' viruses. They incorporate lipids from the membranes of the host cell into their envelope. On return to the peripheral circulation, such virions could present the CNS glycolipids in their envelopes in an actively antigenic form to the immune system, and so trigger an immune response, resulting in CNS auto-immunity. This mechanism would encompass both those of the virus and the auto-immune (experimental allergic encephalomyelitis, EAE) models of CNS demyelination, reconciling the two schools of thought in relation to such diseases as MS.

To date most physico-chemical analyses of viral antigenicity have concentrated on viral proteins. Some workers have also looked for proteins or glycoproteins of the host cell which may have become incorporated into the viral envelope, but these have proved to be negligible. Viruses represent virally coded proteins in their envelope and do not make use of host coded membrane protein. Glycolipids may be highly antigenic when incorporated into the envelope of budding viruses and by contrast these are host membrane glycolipids.

Many of the viruses, which at one time or another have been thought to have been involved in MS pathogenesis, are capable of multiplying in the cells of the CNS, mature by budding through the cell membranes and take host cell glycolipids into their envelope. In the paramyxoviridae which includes measles, mumps, canine distemper, parainfluenza types 1 to 4 and Sendai virus, release of mature virions takes place by budding and 20–40% of the dry weight of the virion is lipid (Nakajima & Obara, 1967). Klenk & Chopin (1969, 1970 a,b) cultured para-influenza virus type SV5 in four different host cells with different lipid compositions, and determined that the lipid composition of the viral envelope closely resembled that of each of the host cell membranes from which it was derived. That the lipids of the viral envelope resemble those of the host cell from which the virus is derived has been demonstrated for many viruses including, mumps virus (Soule, Marinetti & Morgan, 1959), influenza virus (Kates *et al.*, 1961, Blough & Merlie, 1970), Sinbis virus (Hirschberg & Robbins, 1974; Quigley, Rifkin & Reich, 1971), Semliki Forest virus (Renkonen *et al.*, 1971; Laine *et al.*, 1972) and Venezuelan equine encephalomyelitis virus (Heydrick, Comer & Wachter, 1971).

Cross-reactivity between viruses and host membrane components

This has been shown by many workers. Harboe, Schoyen & Bye-Hansen (1966) showed that fowl plague and influenza virus grown in entodermal cells of the chick chorio-allantoic membrane could be inhibited by antibody prepared in rabbits against normal uninfected allantoic membrane. Feinsod, Spielman & Swaner (1975) showed that Sindbis virus which had replicated in *Aedes aegypti* mosquitoes was neutralized by immune serum made against whole body extracts of uninfected *A. aegypti*. This serum did not neutralize Sindbis virus grown in vero cells. Steck, Tschannen & Schaefer (1981) showed that the 'neurotropic' strain of vaccinia virus when given to mice would produce an immune reaction, in which antibodies binding to normal uninfected myelin and oligodendrocytes were detectable. However, if the 'dermatotropic' strain of virus was used, no antibodies against CNS tissue were produced. Reilly & Schloss (1971) demonstrated that Friend leukaemia virus buds from erythrocyte membranes taking red blood cell membrane components into its envelope. Cox & Keast (1973) showed that such a virus could trigger a reaction leading to the haemolysis of normal uninfected erythrocytes. Almeida & Waterson (1969) using a corona virus which had grown in chicken fibroblasts, showed that immune sera made against this virus in chickens labelled only the viral protein spikes. If, however, the immune serum was made in rabbits, against the 'chicken fibroblast' derived virus, the resulting antiserum labelled both the viral protein spikes and the intermediate envelope lipids, and thus demonstrated the chicken host cell origin of the viral envelope. In addition the 'chick fibroblast' derived virus could be neutralized by immune serum made in rabbits against uninfected chick fibroblasts. Rook & Webb (1970) observed that lymphocytes of mice which were reactive against tick borne encephalitis virus (Langat TP21) destroyed both Langat TP21 infected, and to a lesser but significant extent, uninfected cultured mouse glial cells suggesting that, in the process of immunizing the donor mouse with Langat TP21, cell mediated immunity to some component of normal brain membranes had been produced.

Semliki Forest virus as a model for CNS demyelination

Semliki Forest virus (SFV) is an alphavirus of the Togaviridae, and is an enveloped virus that matures by budding from the cell membranes. The virus derives its envelope lipids from these membranes (Renkonen *et al.*, 1971; Luukkonen, Kaariainen & Renkonen, 1976). Mice infected intra-peritoneally develop demyelination which is maximal between days 14 and 21 post-infection, after the immune response has cleared detectable virus from both blood and brain. The demyelination is focal and can occur throughout the CNS (Kelly *et al.*, 1982) including the optic nerves and the spinal cord (Illavia, Webb & Pathak, 1982; Pathak, Illavia & Webb, 1983). The demyelination is dependent upon T-lymphocytes probably cytotoxic cells (Jagelman *et al.*, 1978; Fazakerley, Amor & Webb 1983; Pathak *et al.*, 1983) and probably results from an immune reaction against viral antigens on the surface of oligodendrocytes or myelin. Oligodendrocytes do not appear to be destroyed during an avirulent SFV (A7) infection although an immune attack could change the cellular activity from myelin maintainance to that of cell repair, and by default allow degeneration of the myelin. Good remyelination occurs by day 35 post-infection, although the myelin does not return completely to normal. Zlotnik, Grant & Batter-Hatton (1972)

have shown chronic active gliosis with spongiform change in mouse brain 2 years after avirulent SF virus infection. A possible explanation is that brain-derived SFV returns to the peripheral circulation and initiates an immune reaction against CNS glycolipids which contributes to this long term pathological change.

In support of this hypothesis, brain derived SFV has been shown to react significantly in an ELISA against immune serum raised to galactocerebroside, gangliosides and particularly to glucocerebroside (Webb *et al.*, 1981). In addition, the antiglucocerebroside serum coupled to either ferritin or protein-A gold, successfully labels SFV budding from brain cell cultures (N. Evans, personal communication). The host cell plasma membranes are not labelled by this antiserum, which is appropriate since SFV grown *in vitro* buds extensively from the internal cell membranes (Erlandson *et al.*, 1967) and glucocerebroside is a marker of internal but not external membranes.

Cross-protection between antigenically unrelated Togaviruses

Although the alphaviruses, Sindbis and Semliki Forest virus, are serologically unrelated to the flaviviruses, Langat virus (TP21) and West Nile virus, they both multiply well in CNS cells (Illavia & Webb, 1968; Precious, Webb & Bowen, 1976; Herzberg, 1976). Since all are budding viruses they will have a similar host derived viral envelope provided the virus replicates in the same cell type. In our laboratory we have infected mice with the non-lethal encephalitogenic alphaviruses, Sindbis or SFV A7(74), and then challenged these animals intracerebrally at weekly intervals for 7 weeks with the normally 100% lethal flavivirus, Langat virus (TP21), or with West Nile virus. Up to and probably after 35 days following infection by either alphavirus there was a significant protection to flavivirus. Seven days after Langat or West Nile virus challenge of the alphavirus infected mice, brain virus titres were significantly lower than in mice given flavivirus alone. It was felt that protection initially might be due to interferon release but none was measureable more than 5 days after the first alphavirus infection (Oaten, Bowen & Webb, 1976; Oaten, Webb & Jagelman, 1980). At times after the second week it was considered that protection might be due to the first virus, the alphavirus, multiplying in the brain and taking brain cell membrane components into its envelope, which could be antigenic and stimulate humoral and cell mediated immunity. The second infecting virus, the flavivirus, by replicating in similar cells may also have incorporated the same cell membrane components into its envelope, and thereby be partially neutralized by the previously induced immune response. Cell membrane glycolipids are most likely to be involved in such cross-reactivity.

Relevance of glycolipids to neurological disease

In tissue culture, anticerebroside antibodies have been shown to produce demyelination of myelinated axons (Dubois-Dalcq, Niedieck & Buyse, 1970; Fry *et al.*, 1974). Raine *et al.* (1981) tested the ability of antisera against whole white-matter myelin basic protein and galactocerebroside to demyelinate myelinated cultures of mouse spinal cord. The effects of the anti-whole white matter antibody and the antigalactocerebroside antibody were identical; both produced demyelination, whilst the anti-myelin basic protein antibody had no effect. Lumsden (1972) and Leibowitz & Gregson (1979) suggested that antibody to glycolipids might be present in

patients with CNS disease. Nagai *et al.* (1976) reported that lesions of the CNS and peripheral nervous system could be produced by immunizing rabbits and guinea-pigs with ganglioside GM_1 and GD_{1a}. Saida *et al.* (1979) produced an experimental allergic neuritis by immunization with galactocerebrosides. More recently Konat *et al.* (1982) produced an experimental 'MS-like' disease in rabbits by immunizing them with bovine brain gangliosides. An immune response to glycolipids can thus result in demyelination.

Arnon *et al.* (1980) found that antibodies to glycolipids were present in 40% of MS patients' sera as tested by liposome lysis. Antibodies to GM_4 and GM_1 gangliosides were present. Offner, Konat & Sela (1981) showed that multi-sialogangliosides, particularly G_{T_1} and G_{Q_1b}, were powerful stimulators of active E-rosetting lymphocytes from MS patients. Sela, Konat & Offner (1982) demonstrated the presence of elevated ganglioside levels in serum and peripheral blood lymphocytes from MS patients in remission compared with controls. Ilyas & Davison (1983) using an E-rosette assay showed hypersensitivity to gangliosides in MS patients. Some response to myelin basic protein was also obtained, but this also occurred in patients with other CNS disturbances. However, the reactivity to gangliosides appeared to be particularly specific to the MS patients. The ganglioside-stimulated E-rosettes could be inhibited by cyclosporin A (Davison & Ilyas, 1982), which blocks receptors for HLA-DR antigens on T-cells (Palacios & Moller, 1981) and prevents interleukin production. The T-lymphocytes of MS patients thus appear to be sensitive to glycolipids.

More attention needs to be paid to the antigenicity of CNS glycolipids and in particular to the antigenicity of viral envelope glycolipids. It is an intriguing possibility that CNS demyelination in diseases such as MS, arises as a result of an auto-immune reaction against specific glycolipids, induced by the carrier effect of a budding neurotropic virus. The presence of antibodies and reactive T-lymphocytes to glycolipids in MS patients, alternatively might only reflect the release of these components during CNS damage. However, it is unlikely that glycolipids released in this way would produce an immune response as in most cases release of tissue components directly into the circulation does not provoke the production of auto-antibodies (Roitt, 1980; Allison, 1971). For example, destruction of thyroid tissue by doses of therapeutic radio-iodine, does not initiate thyroid auto-immunity, nor does damage to the liver in alchoholic cirrhosis result in the production of mitochondrial antibodies, as seen in auto-immune primary biliary cirrhosis. To initiate auto-immunity it is a prerequisite that the antigen is presented correctly to the immune system. Thus, to initiate auto-immune thyroiditis in rabbits the thyroid antigens were inoculated in Freund's adjuvant (Rose & Witebsky, 1968). Similarly to produce EAE in rabbits with glycolipids the antigen was emulsified in Freund's adjuvant (Konat *et al.*, 1982). This probably provides the required immunological carrier effect. A carrier effect is essential when considering glycolipids, since these behave immunologically as haptens, (Marcus & Schwarting, 1976; Rapport & Graf, 1969).

Theoretically, numerous neurotropic budding viruses could provide a carrier effect for CNS glycolipid haptens, thus leading to an antiglycolipid immune response and demyelination in susceptible individuals (HLA type). Such an hypothesis for the pathogenesis of MS would encompass a host of eligible budding CNS viruses (simplified in Figure 1) and the many viruses implicated by epidemiology, serology, isolation or microscopy could all be involved. This may be reflected in the finding that antibodies isolated from different plaques within the same MS

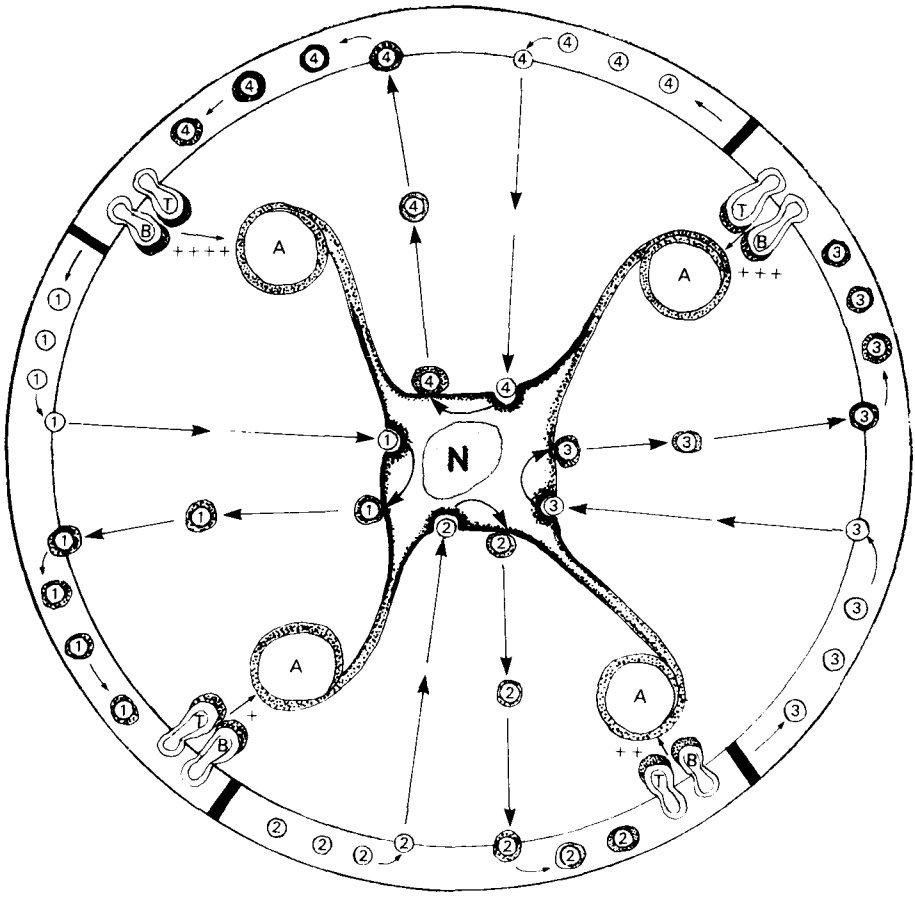

Figure 1. The possible role of recurrent infections of the CNS in MS in genetically susceptible individuals (HLA type). A neurotropic enveloped virus, for example, measles (**1**), enters the brain and replicates in the cells of the CNS including, e.g. the oligodendrocytes. The envelope of the budding 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 brain 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, e.g. a coronavirus (**2**) or a third influenza virus (**3**), or a previous virus infection which has become latent and now re-activated, 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 auto-immune response to the same brain cell membrane specific glycolipid(s). Semliki Forest virus 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. ◎ oligodendrocytic lipid membrane; ① measles; ② corona; ③ influenza; ④ arbovirus SFV. N: nucleus of oligodendrocyte; T: T-lymphocyte; B: B-lymphocyte; A: axon.

brain may be two different viruses (Nordal, Vandvik & Norrby, 1978). Relapse and remission, as seen in MS, could be a function of new virus infection (or re-activation of latent virus), and the activity of T-suppressor lymphocytes, directed against the virus induced antiglycolipid response. It is of relevance that a functional abnormality of virus induced T-suppressor lymphocytes has already been demonstrated in MS patients (Neighbour & Bloom, 1979).

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