the finding that impressed us most has been a fall in measles antibody titre in those patients who have been most intensively treated (Table 1). Therefore we feel that further trials of TF are warranted, especially for patients with early disease.

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DISCUSSION

Dr Valdimarsson in response to questioning said that the use of measles-infected cells rather than measles virus as an antigen in lymphocyte transformation tests had been reported by Sell and his colleagues (Ahmed *et al.*, 1974, *Journal of Experimental Medicine* **139**, 902) as giving better responses. However, the response to these measles-infected cells was maximal at three days which for antigen-induced response was surprising.

The question whether repeated skin testing alone would make delayed hypersensitivity responses positive was raised. Dr Valdimarsson said that he had not found this to be the case with the antigens that he had used. Skin testing could, however, induce lymphocyte transformation reactivity.

Dr Norrby on the question whether the virus in SSPE was truly measles or measles-like, quoted work from the Wistar Institute Group claiming differences between the SSPE virus and ordinary wild-type measles (ter Meulen *et al.* 1972, *Current Topics in Microbiology and Immunology* **57**, 1–38).

Dr Norrby, however, felt that the differences claimed might be due to a host modulation of normal measles virus and that many of these differences could be mimicked *in vitro* by passage in antibody-containing media. Furthermore, he pointed out that even the defective virus was capable of giving ferrets an SSPE-like disease.

On the question whether there was any association between H-LA phenotypes and SSPE, **Dr Platz** had HL-A typed 7 patients and **Dr Valdimarsson** 8 and no obvious association had shown up in either group.

Dr K Apostolov pointed out that cultures of brain explants from SSPE did not hæmadsorb monkey erythrocytes, indicating that these cells were not capable of assembling hæmagglutinin and budding off the virus (Payne *et al.*, 1969, *New England Journal of Medicine* **281**, 858). For this reason the virus could presumably spread only from cell to cell by cell fusion. Dr Apostolov said that the whole measles-infected cell in this disease could be considered as a virus particle. He also raised the possibility that the process could be interrupted by giving complement intrathecally. There was some discussion of the mechanism of cytoxicity of measles-coated cells.

Professor Lachmann quoted the work of M B Oldstone and his colleagues (personal communication) that measles-infected HeLa cells could be lysed by antibody and complement. The antibody could be either convalescent serum from normal measles or serum from patients with SSPE. Interestingly enough it was the alternative pathway of complement activation that appeared to be exclusively involved. If the cells were grown in an antibody containing medium in the absence of complement, the measles antigen 'capped' and was then extruded from the cell. Such cells, although still showing measles antigen inside them, no longer had antigen on the surface and could no longer by lysed by antibody and complement until after a period of growth in antibody-free medium.

Dr D K Peters pointed out that since the alternative pathway of complement activation was highly concentration-dependent there might not be enough complement in the CSF to produce lysis by this mechanism.

Dr Valdimarsson stated in his *in vitro* system there was some evidence to suggest that the background killing might be K cell killing, while the specific killing might be T cell killing.

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Measles and Multiple Sclerosis[1]

Adams & Imagawa (1962) were the first to propose a possible link between measles virus infections and multiple sclerosis (MS). This was based on the demonstration of relatively higher serum titres of antibodies against measles in MS patients than in matched controls. This difference has later been confirmed in a number of studies (cf. Brody *et al.* 1972). Further it was found that measles virus antibodies occurred in a much higher frequency in the cerebrospinal fluid (CSF) of MS patients than in healthy controls (Brown *et al.* 1971).

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Significance of Increased Serum Titres of Measles Antibodies in MS Patients

It was proposed that possibly the accentuated measles virus antibody response in MS patients was based on cross-reacting antibodies (Field *et al.* 1972). However, this appeared as a less likely explanation when it was demonstrated that MS patients displayed relatively increased serum titres of antibodies against three different structural components of the virus (Salmi *et al.* 1973). The difference between MS patients and controls was particularly apparent when mean titres of hæmolysing-inhibiting (HLI) antibodies were compared. In further studies of samples from patients with optic neuritis of a type related to the MS disease (a relationship defined by the occurrence of oligoclonal IgG; *see below*) the relative increase in serum titres of HLI antibodies was strikingly pronounced (Link *et al.* 1973). The reason for this divergent measles antibody response is not known.

Immunoglobulins in the CSF of MS Patients

A prominant feature of the MS disease is the occurrence of a moderate increase of the protein content of CSF. This increase is not due to a leaking of proteins into the central nervous system (CNS) since most MS patients do not appear to have any significant damage of their blood-brain barrier (Tourtelotte & Parker 1966). The protein component predominantly responsible for this increase is IgG which to a major extent is derived from the CNS possibly due to a local synthesis of IgG in this organ (Frick & Scheid-Seydel 1958). Agar or agarose electrophoresis of concentrated CSF has revealed that in more than 90% of all MS cases this IgG has a restricted heterogeneity. IgG showing this appearance will be referred to as 'oligoclonal IgG'. This IgG generally is detectable during the earliest stages of clinically overt disease and the electrophoretic band pattern remains remarkably stable during the continued disease in individual patients.

Local Production of Virus Antibodies in the CNS of MS Patients

The fact that most MS patients have an unimpaired blood-brain barrier allows an analysis of the source of origin of measles antibodies present in CSF. Determinations have been made of the ratio of measles antibody titres in serum/CSF and for comparison of the same ratio of antibodies against viruses (adenovirus and poliovirus) assumed not to be of relevance for the disease (Norrby, Link & Olsson 1974). Whereas the ratio for the latter antibodies was normal (values of 300–600) in almost all cases, a significantly reduced ratio of measles antibodies was found in about 60% of all cases. This was interpreted as being due to a selective accumulation of measles antibodies in the CNS of these patients. In further studies (Norrby, Link, Olsson, Panelius, Salmi & Vandvik 1974) it has been shown that by the same criteria a relative increase in the CNS of antibodies against mumps, herpes simplex Type 1 and rubella virus can also be encountered although in a much lower frequency than measles antibodies.

Relationship of Measles Virus-specific Antibodies and Oligoclonal IgG Derived from the CNS of MS Patients

In recent investigations this problem has been attacked in two different ways. One method has been to make a preparatory agarose electrophoresis of concentrated CSF from MS patients selected to have a readily demonstrable accumulation of measles antibodies in their CSF. This technique was previously successfully employed to identify the different measles antibody activities of bands of oligoclonal IgG in CSF from cases of subacute sclerosing panencephalitis (SSPE) (Vandvik & Norrby 1973). Results obtained with MS CSF materials were somewhat less clear. This was due to the fact that the CSF even of selected MS patients contains lower titres of measles antibodies and less oligoclonal IgG relative to polyclonal IgG than CSF of SSPE patients. However, in 4 cases a difference could be shown in the distribution of antibodies against different structural components of measles virus in fractions obtained by preparatory agarose electrophoresis. This suggests an oligoclonal rather than polyclonal response of measles antibodies.

The second technique used to identify a possible measles antibody activity of oligoclonal IgG was immunoadsorption. Concentrated CSF from selected MS patients was mixed with concentrates either of cell-associated virus antigens or of purified extracellular virus particles and incubated at room temperature for one hour and at 4°C overnight. Antigen-antibody complexes were then isolated by ultracentrifugation and from these complexes IgG was consecutively eluted at pH levels of 4, 3 and 2. The results of examination of CSF material from 2 MS patients are summarized in Tables 1 and 2. Under the conditions of adsorption antibodies against structural components of the virus were effectively removed. In spite of this removal of measles antibodies only a slight reduction of IgG occurred in the adsorped material from one patient (Os; Table 1) and no significant reduction of IgG content was detectable in materials from 4 additional MS patients. Repeated adsorptions did not change this picture.

Table 1

Adsorption-elution of a CNS extract of periventricular plaque material from a multiple sclerosis patient (Os) with purified extracellular measles virus particles

Sample	Electrophoretic pattern	IgG (mg/ml)	Measles virus antibody titres in HI	HLI	NC-CF	Adenovirus antibody titre in HE
Unabsorbed	Oligoclonal IgG	6.0	6400	12 800	80	80
Supernate after adsorption	Slightly reduced oligoclonal IgG	4.6	< 4	6400	4	80
Eluate pH 4.0	Polyclonal IgG	0.11	120	240	12	< 6
Eluate pH 3.0	Oligoclonal IgG	0.31	240	960	6	< 6
Eluate pH 2.0	Oligoclonal IgG	0.36	480	960	6	< 6

HI, hæmagglutination-inhibition
HLI, hæmolysis-inhibition
NC-CF, nucleocapsid complement fixation
HE, hæmagglutination enhancement

Measles antibody activities were recovered in high titres in the low pH eluates. In most cases studied the best yield of IgG was obtained at pH values of 3 and 2 suggesting the presence of specific antibodies of high affinity. In control materials IgG was recovered at higher pH levels. The relative recovery of antibodies against different structural components of measles virus in materials from some patients varied between different low pH eluates.

Agarose electrophoretic characterization showed that the adsorption with measles virus antigen did not detectably change the pattern of oligoclonal IgG, except for slight alterations in the case of material from patient Os. However, the low pH eluates contained a varying number of bands of oligoclonal IgG. In material from some patients different bands of IgG were recovered in eluates at different pH levels. However, it was noteworthy that the IgG bands of low pH eluates in general did not correspond to the major bands of IgG, but to less pronounced oligoclonal IgG proteins of the unadsorbed CNS or CSF materials.

Adsorption of serum samples from MS patients also allowed a recovery at low pH levels of measles antibodies and of oligoclonal IgG proteins. These bands of IgG could not be detected in whole serum against the background of polyclonal IgG.

For control purposes adsorptions of SSPE materials containing relatively large amounts of measles virus-specific oligoclonal IgG were carried out with preparations of other paramyxoviruses (Newcastle disease and parainfluenza 1 viruses). No change in the pattern of oligoclonal IgG was demonstrable and no IgG was recovered in the low pH eluates. Conversely it was shown that the measles antigen preparations used removed no oligoclonal IgG from a CNS extract from post-mortem material of a patient with herpes virus encephalitis.

Conclusion

SSPE represents an extensively studied model of a slow infection with measles virus in human CNS. In this disease there are direct signs of the presence of virus in CNS and in a certain fraction of all cases extracellular infectious virus can be isolated by co-cultivation of explant cultures of CNS tissue from the diseased patient and cells susceptible to measles virus. The CNS of patients with MS displays a completely different picture.

Table 2

Adsorption-elution of concentrated CSF from a multiple sclerosis patient (Bl) with cell-associated measles virus antigen

Sample	Electrophoretic pattern	IgG (mg/ml)	Measles virus antibody titres in HI	HLI	NC-CF	Adenovirus antibody titres in HE
Unabsorbed	Oligoclonal IgG	3.80	80	10 240	40	160
Supernate after adsorption	Unchanged oligoclonal IgG	3.45	< 10	1280	20	160
Eluate pH 4.0	One band of IgG	0.09	< 10	640	10	< 10
Eluate pH 3.0	One IgG band different from pH 4.0 eluate	0.11	40	5120	5	< 10
Eluate pH 2.0	A weaker IgG band corresponding to pH 3.0 eluate	0.05	80	2560	< 5	< 10

HI, hæmagglutination-inhibition
HLI, hæmolysis-inhibition
NC-CF, nucleocapsid complement fixation
HE, hæmagglutination enhancement

There is no convincing evidence for the presence of virus in the tissue. The proposed incrimination of virus infections in the pathogenetic events of MS therefore has to rely upon indirect evidence, i.e. serological data.

In this context the focus of interest has been the assumed antibody activity of oligoclonal IgG, which occurs in the CNS as a characteristic feature throughout the disease. The present data show on one hand that in selected MS patients oligoclonal IgG with measles antibody activity can be identified. However, on the other hand it is obvious that the overall majority of oligoclonal IgG in the CNS of MS patients represents antibodies not associating firmly with the antigen preparations employed in the immunoadsorption experiments.

The crucial problem therefore is to elucidate by further experiments the antibody activity(ies) expected to be carried by the major part of oligoclonal IgG. Several alternative explanations offer themselves:

(1) The antibodies form a readily dissociable complex with measles antigen. This appears less likely since the fact that the oligoclonal IgG eluted first at low pH levels indicates that it represents antibodies of high avidity. Further, in the case of SSPE materials, repeated adsorptions with concentrated antigen preparations allowed an almost complete removal of the oligoclonal IgG (Vandvik & Norrby, in preparation).
(2) The presence of complexes between haptens and oligoclonal IgG may prevent removal after adsorption with specific antigen. This possibility can be examined by carrying out adsorption experiments after treatments aiming at a dissociation of the hypothetical hapten-antibody complexes.
(3) The oligoclonal IgG is primarily directed against nonstructural proteins of measles virus, which possibly are not present in sufficient amounts in the antigen preparations to allow an effective immunoadsorption.
(4) The oligoclonal IgG not reacting with measles antigen is directed against antigens of other viruses or infectious agents of other kinds. Immunoadsorption may be carried out with antigen preparations of additional viruses to which a local antibody response in CSF has been identified.
(5) The oligoclonal IgG does not represent to any large extent antibodies against infectious agents, but instead to other as yet not specified antigens e.g. tissue antigens.

It appears that one important clue to the MS disease lies in the revelation of antibody activities of oligoclonal IgG produced in the CNS.

In discussing the potential importance of virus infections in MS the identification of oligoclonal IgG of presumed CNS origin carrying measles antibody activity should be emphasized. Experimental studies indicate that an oligoclonal antibody response, e.g. to bacterial polysaccharide antigens, requires the presence of sufficient amounts of antigen over extended time periods. It would seem, therefore, that oligoclonal IgG appearing in CSF in connexion with virus infections, e.g. SSPE, herpes encephalitis and mumps meningitis, should be due to a local accumulation of virus-specific antigens. By further analogy it would appear likely that measles antigen accumulates and persists in the CNS of certain MS patients, presumably by activation of latent infections and also that it is available in CNS for longer periods of time. Such an activated virus infection may represent an epiphenomenon of no significance for the disease, possibly reflecting some basic defect in the patients. Alternatively the proposed activated infection could be involved in the disease process, which may not seem unlikely in view of its assumed comprehensiveness.

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DISCUSSION

Dr Norrby, in answer to questions, stated that there appeared to be no clear relationship between the stage of multiple sclerosis and the occurrence of oligoclonal antibody in the CSF.

Dr Ten Feizi (*Clinical Research Centre, Northwick Park Hospital*) asked what was known about the clonality of the normal antibody response to measles.

Dr Norrby said that the response to normal measles was a polyclonal one but that in SSPE it was possible to find some oligoclonal anti-measles bands even in the serum.