Relation of measles virus to encephalitogenic factor with reference to the aetiopathogenesis of multiple sclerosis

J. R. McDERMOTT, E. J. FIELD,¹ AND E. A. CASPARY

From the Medical Research Council Demyelinating Diseases Unit, Newcastle General Hospital, Newcastle upon Tyne

SYNOPSIS A combination of affinity chromatography with the macrophage electrophoretic migration (MEM) test has been used to study the antigenic similarities between encephalitogenic factor (EF) and measles virus. These have determinant(s) sufficiently closely related to account for the elevated level of circulating antibody to measles in multiple sclerosis.

Recent years have seen more than 20 reports (reviewed by Brody, 1972) indicating that the level of measles antibody in blood is in general higher in patients with multiple sclerosis than in normal subjects. Since the association of measles with subacute sclerosing panencephalitis (SSPE) is now generally accepted, it has been suggested that measles virus might operate as a 'slow' agent in multiple sclerosis too. An alternative explanation of the elevated measles titre in multiple sclerosis (MS), is that breakdown products of nervous tissue (and notably encephalitogenic factor (EF)) may share antigenic determinant(s) with measles virus so that the cross reactivity of serum antibody to such breakdown products is interpreted as elevated measles titre. The present work offers direct evidence that measles virus and encephalitogenic factor (EF) do indeed share antigenic determinant(s).

METHODS

The method employed has been to estimate blood lymphocyte sensitization to measles and EF; to filter out lymphocytes with specific sensitization to either measles or EF by affinity chromatography and subsequent re-estimation of the sensitivity of the lymphocytes which have come through the chromatography column. In a typical experiment lympho-

cytes from a patient with MS were assayed for sensitization to EF and to measles. The cells were then passed down a column packed with Bio-Gel P-2 beads to which EF was linked so that lymphocytes sensitized to EF were retained. The cells issuing from the columns were re-estimated for sensitization both to EF and measles. In such an experiment the effluent cells, while showing the expected marked reduction in sensitization to EF, also showed reduced sensitization to measles.

Lymphocytes were isolated from 15 ml. venous blood by the method of Coulson and Chalmers (1967) using methyl cellulose and carbonyl iron as modified by Hughes and Caspary (1970). More than 98% pure lymphocytes were obtained with almost 100% viability (as shown by an erythrosin exclusion test). Cells from eight patients with multiple sclerosis (seven in an acute episode; one quiescent); one patient with syringomyelia, and one with stroke; two children with measles; and two normal adults have been studied.

Lymphocyte sensitization was measured by the macrophage electrophoretic mobility (MEM) test (Field and Caspary, 1970, 1971; Caspary and Field, 1971). In principle the method depends upon the observation that, when sensitized lymphocytes are brought into the presence of antigen, they elaborate a 'lymphokine' (macrophage slowing factor—MSF—which may be identical with MIF) with the property of causing normal guinea-pig macrophages to travel more slowly in an electric field. Normal macrophages may thus be used as an indicator system for the interaction of lymphocytes with antigen. A detailed

¹ Present address: Institute of Pathology, Newcastle General Hospital, Newcastle upon Tyne.

description of the test together with an experimental protocol *in extenso* has been given by Field and Caspary (1971).

If t_c = migration time of the macrophages when no antigen is present; t_e = migration time when antigen present; then in general $t_e > t_c$ and $t_e - t_c/t_c \times 100$ is a measure of the slowing induced by the interaction of lymphocytes and antigen and so of lymphocyte sensitization. It is these percentage figures which are given in the protocols. All estimations were, of course, carried out 'blind'.

In carrying out a test for lymphocyte sensitization 0.5×10^6 lymphocytes are used as a routine. This number contains sufficient lymphocytes with specific sensitization to give a maximal response (Carnegie *et al.*, 1973). By dropping the number of lymphocytes to 0.4, 0.3, ... 0.05 million it is possible to estimate the 'end point' at which there are not enough specifically sensitized cells present to give a significant result (Field and Caspary, 1972; Caspary and Field, 1974). This 'titration method' has been adopted for the lymphocytes before and after passage through specifically loaded columns.

The antigens used have been encephalitogenic factor (EF) made from normal human white matter (Caspary and Field, 1965); mevillin-L (Schwartz strain) measles (Evans Medical); control culture material in which the mevillin-L is produced (kindly supplied by Dr. Fermiger of Evans Medical Ltd.); F_1 (thyroglobulin) fraction of normal human thyroid; purified protein derivative (PPD) of *M. tuberculosis*; and calf thymus histone (Sigma chemicals).

In the case of EF, PPD, and thyroglobulin the antigen has been used at a concentration of 33 $\mu\text{g/ml}$. in the MEM test. With mevillin-L 0.1 ml. has been used in a final volume of 3.1 ml.

Chromatography columns were prepared with Bio-Gel beads (50–100 mesh; Bio-Rad Labs., Richmond, Calif.) to which EF or other antigen had been coupled by the azide method of Inman and Dintzis (1969), to give a loading of approximately 2 mg antigen/g dry resin. To reduce non-specific retention of lymphocytes the column was washed with 0.5 ml. normal serum followed by 5 ml. medium 199 (Wigzell and Andersson, 1969). The column was usually 1 cm high and 2.1 cm diameter. Cells in medium 199 at pH 7.2 were layered on to the column at a concentration of 2.0×10^6 ml. and allowed to pass through under gravity at 20° C at a flow rate of 1.0 ml./min. Finally the column was washed with 5 ml. fresh medium 199 and the eluate and washings combined. Lymphocyte recovery varied between 75 and 98%.

RESULTS

MULTIPLE SCLEROSIS LYMPHOCYTES 1. Normally, 0.5 million lymphocytes are used in a test for sensitization. It can be seen from Table 1 that when the number of cells is reduced a positive reaction to EF—that is, greater than 2.5%; $P < 0.01$ —is still obtained with 0.05 m cells but not with 0.01. Passing the cells down a column of histone-Bio-Gel—that is, a non-specific basic protein—led to negligible retention of EF sensitized cells. An EF loaded column on the other hand removed so many cells that even 0.3 m failed to give a positive result. This showed that an EF loaded Bio-Gel column retains significant numbers of lymphocytes which are sensitive to EF.

TABLE 1
CHRONIC MS LYMPHOCYTES; EF ANTIGEN:
HISTONE-BIO-GEL AND EF-BIO-GEL COLUMNS

No. of cells ($\times 10^6$)	EF column		Histone column	
	Before	After	Before	After
0.5	16.6	1.2	—	—
0.2	16.3	1.3	15.5	15.4
0.1	17.7	1.5	15.3	15.4
0.05	8.0	—	1.8	1.3
0.01	1.7	—	—	—

TABLE 2
CHRONIC MS LYMPHOCYTES; EF AND MEASLES ANTIGENS:
EF-BIO-GEL COLUMN

No. of cells ($\times 10^6$)	Before EF column		After EF column	
	EF	Measles	EF	Measles
3.3	—	—	1.1	—
0.2	15.6	10.5	—	10.5
0.1	15.5	8.4	—	0.6
0.05	6.3	2.2	—	—
0.025	1.5	—	—	—

2. Table 2 shows the effect of passing lymphocytes from a patient with chronic multiple sclerosis down an EF-Bio-gel column. It can be seen that, before the cells go down the column, 0.05 m are enough to give a positive result with EF and 0.1 m with measles. After the column,

TABLE 3

MS LYMPHOCYTES; EF AND MEASLES ANTIGENS: EF-BIO-GEL COLUMN; NORMAL AND MEASLES SERA

No. of cells ($\times 10^6$)	Before column		After column/normal serum		After column/measles serum (1:512)	
	EF	Measles	EF	Measles	EF	Measles
0.2	15.1	10.3	1.5	10.4	15.4	10.2
0.15	15.4	—	1.5	—	15.0	—
0.1	15.0	6.5	1.3	3.8	12.9	6.8
0.05	5.1	—	1.2	—	3.5	—

TABLE 4

MS LYMPHOCYTES; EF AND MEASLES ANTIGENS: EF-BIO-GEL COLUMNS; MUMPS AND NORMAL SERA

No. of cells ($\times 10^6$)	Before column		After column/normal serum		After column/mumps serum	
	EF	Measles	EF	Measles	EF	Measles
0.5	—	—	1.5	—	1.6	—
0.2	14.7	10.5	1.7	10.3	1.3	10.0
0.1	14.5	5.3	—	2.2	1.3	2.3
0.05	6.2	—	—	—	—	—

even 3.3 m cells fail to give a positive result with EF, and 0.1 m no longer gives a positive result with measles (8.4% has been reduced to 0.6%). Clearly removing cells which react with EF has also removed some cells which react with measles.

To test further the possible association between measles and EF, two Bio-Gel columns were loaded with EF. One was then washed through with normal serum (control) and the other with a measles serum (titre 1:512, haemagglutination-inhibition test). The object was to test whether measles serum would bind to the EF in the column and so diminish its capacity to retain EF sensitized cells. Table 3 shows this was indeed the case. Treating the Bio-Gel-EF column with measles (but not normal) serum abolished its ability to remove EF sensitized lymphocytes and also (at the 0.1 m level) significantly reduced the retaining power of the column for lymphocytes sensitized to measles (3.8% on normal column; 6.8% on measles serum treated column). This experiment showed that measles serum of high titre could 'satisfy' the avidity of EF-Bio-Gel for lymphocytes sensitized to measles.

3. A further experiment showed that mumps serum was unable to abolish the binding capacity of Bio-Gel-EF sensitive lymphocytes (Table 4).

There was no difference in the removal of either EF or measles sensitized by normal and mumps serum.

4. Since it is known that EF shares antigenic determinant with PPD (Field *et al.*, 1963; Field *et al.*, 1971), the ability of a PPD loaded Bio-Gel column to filter out PPD, EF and measles sensitized cells was tested (Table 5). It can be

TABLE 5

CHRONIC MS LYMPHOCYTES; EF, PPD AND MEASLES ANTIGENS: PPD-BIO-GEL COLUMN

No. of cells ($\times 10^6$)	Before column			After column		
	EF	PPD	Measles	EF	PPD	Measles
1.0	—	—	—	1.6	1.4	—
0.5	15.4	17.7	—	—	—	—
0.2	—	—	10.3	—	—	10.3
0.1	—	—	9.3	—	—	8.5

seen that, while PPD filters out PPD and EF cells, it has no effect on measles (at 0.1 ml. reduction from 9.3% to 8.5%—not significant). An EF loaded column filtered out some PPD cells. PPD would appear to be too far removed

TABLE 6
MS LYMPHOCYTES; EF AND MEASLES ANTIGENS:
MEASLES-BIO-GEL COLUMN

No. of cells ($\times 10^6$)	Before column		After column	
	EF	Measles	EF	Measles
0.2	14.1	9.6	13.7	1.3
0.15	14.1	—	12.3	—
0.10	7.6	—	5.3	—

antigenically from measles to filter out cells sensitized to the latter.

5. MS lymphocytes when passed through a measles-Bio-Gel column show a large reduction in sensitivity to measles antigen, and a small but significant reduction in EF sensitivity (Table 6).

(Table 7). However, cells sensitized to PPD and thyroid were not reduced. Evidently, measles antigen(s) coupled to Bio-Gel is able to retain cells sensitized to measles and to a lesser degree EF but not to thyroid and PPD.

Measles control material—that is, the culture in which the measles virus was prepared—on the column led to no significant reduction in the number of either measles or EF sensitized cells coming through (Table 7).

TABLE 7
STROKE LYMPHOCYTES; EF, MEASLES, PPD AND THYROID ANTIGENS:
MEASLES-BIO-GEL AND CONTROL-BIO-GEL COLUMNS

No. of cells ($\times 10^6$)	Before column				After measles column				After control column	
	EF	Measles	PPD	Thyroid	EF	Measles	PPD	Thyroid	EF	Measles
3.0	—	—	—	—	—	3.7	—	—	—	—
1.5	—	—	—	—	—	2.3	—	—	—	—
0.2	15.4	11.0	—	—	15.8	1.8	—	—	—	10.8
0.1	15.5	9.9	17.4	18.7	15.2	—	17.6	18.7	15.5	10.2
0.075	14.7	—	—	—	11.1	—	—	—	—	—
0.05	6.2	1.3	—	12.3	3.4	—	—	12.5	6.4	—
0.025	1.3	—	—	—	1.6	—	—	—	1.6	—

OTHER NEUROLOGICAL DISEASES As with MS, lymphocytes from a patient with stroke when passed through a measles-Bio-Gel column showed retention of significant numbers of lymphocytes sensitive to EF and measles

TABLE 8
MEASLES LYMPHOCYTES (SECOND DAY OF RASH); EF
AND MEASLES ANTIGENS: EF-BIO-GEL COLUMN

No. of cells ($\times 10^6$)	Before column		After column	
	EF	Measles	EF	Measles
0.2	9.0	—	1.3	—
0.15	8.6	—	—	—
0.1	5.1	14.3	—	14.4
0.075	—	13.4	—	10.8
0.05	—	7.0	—	3.3

DISCUSSION

Specific separation of cells on affinity columns for the study of differentiation in the immune response was described by Truffa-Bachi and Wofsy (1970). Their method was a development of the cell separation on antigen-coating columns previously described by Wigzell and Anderson (1969) and which they had used for the selective elimination of specifically sensitized cells by their retention on the column. The present work uses the very sensitive and highly discriminatory macrophage electrophoretic mobility (MEM) test for studying the sensitization of cells which come through an affinity column. This marriage of affinity chromatography with the MEM test has an obvious wide application in studying immunological cross-

reactivity in general, and that between EF and cancer basic protein (Field *et al.*, 1971) has recently been explored by this method by McDermott, Dickinson, and Caspary (unpublished).

The present results indicate that measles antigen is able to attract and hold not only blood lymphocytes which have specific reactivity to measles but also to a lesser degree those which are able to react with EF. Since this ability is counteracted by measles serum but not by the unrelated mumps serum, it appears specifically related to measles. Lymphocytes from patients with other neurological diseases—for example, stroke or syringomyelia—have similar sensitization to EF and measles confirming that there is nothing specific to multiple sclerosis in this respect (Caspary and Field, 1970).

The results offer direct evidence of antigenic determinant sharing between measles virus and EF. This study has also confirmed the suggestion that PPD is antigenically related to EF (Field *et al.*, 1963; Field and Caspary, 1972) and indicates no significant determinant sharing between PPD and measles. The similarity in antigenic determinant(s) between measles and EF is great enough to account for the high antibody level to measles in multiple sclerosis patients (Field *et al.*, 1972; Field, 1973). This is supported by the fact that there is a slightly higher level of measles antibody in neurological diseases other than MS (Caspary *et al.*, 1969), especially if care is taken to study diseases with a degree of parenchymatous destruction comparable with that in MS. Few workers appear to have studied such controls which alone can offer evidence of specificity of change in multiple sclerosis.

The alternative explanation that there is a specific causal relation between measles virus and multiple sclerosis possibly as the result of infection persisting in 'slow' form has been maintained by Salmi *et al.* (1972). In support of their view, they point out that antibody to all three components of measles is raised in MS and regard it as unlikely that determinant sharing would involve them all. It would be of the greatest interest to apply the techniques described in this paper to the individual Norrby measles fractions and to study them also in other neurological diseases. However, the cellular affinity chroma-

tography technique does not seek to dissect the measles component(s) responsible for cross-reactivity (Norrby and Hammar skjöld, 1972) but merely establishes the actuality of the cross-reaction and offers it as a basis for the observed measles-MS relationship. Additional evidence comes from the observation that during an attack of measles in children lymphocyte sensitization to EF rises (Field *et al.*, 1972) and that immunization of experimental animals with measles leads to elevated lymphocyte sensitivity to EF (Field *et al.*, 1973).

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