Circulating antibody to myelin basic protein in relapsing-remitting multiple sclerosis?

A comparative group and sequential study by radioimmunoassay

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SUMMARY Sera from multiple sclerosis patients with relapsing-remitting disease and normal subjects were tested for antibody to myelin basic protein by a sensitive radioimmunoassay. The results showed a marginally decreased titre in multiple sclerosis superimposed on a seasonal variation. There was no correlation with the clinical state of the patients. Results are discussed briefly in relation to humoral antibody function in multiple sclerosis and experimental autoimmune encephalitis.

Evidence for increased circulating antibody to whole brain and a variety of brain fractions by various methods in multiple sclerosis (MS) has been sought for many years (reviewed by Lumsden, 1972) to try to establish a possible autoimmune or immune-mediated pathogenesis for the disease (Alvord, 1970; McAlpine *et al.*, 1972). The results of previous studies have ranged from specific elevation in MS, significant elevation but also in a number of other neurological diseases, to results where patients and control subjects did not differ (Lumsden, 1972; Caspary, 1977).

At present, experimental allergic encephalomyelitis (EAE), especially in the chronic (Stone and Lerner, 1964) and relapsing (Wisniewski and Keith, 1977) forms, presents the best animal model for the disease in man. Therefore, the specific antigen capable of initiating EAE, myelin basic protein (MBP), is the antigen of choice in the search for antibody. In animals immunised with CNS antigens to produce EAE, specific binding has been shown by radioimmunoassay by several workers (Kibler and Barnes, 1962; Caspary, 1966; Kies *et al.*, 1975; McFarlin *et al.*, 1975) but similar investigations in MS patients (Caspary, 1966; Lisak *et al.*, 1968; Lennon and Mackay, 1972) failed to demonstrate elevated antibody response. The role of such antibody remains obscure.

In the present work a further attempt has been made to seek antibody to MBP in patients with multiple sclerosis over time, using a very sensitive and specific radioimmunoassay.

Patients and methods

The patients in the MS group all had definite relapsing-remitting disease. The women in the group had a mean age of 37.9 yr and the men a mean age of 34.8 yr, with a sex ratio of 3:2. The mean age of the control subjects was 35.6 yr for women, 38.8 yr for men, with a sex ratio of 1:1.

The patients were assessed at regular intervals, and blood was taken, and separated, and the serum stored at -70°C .

Specific binding of MBP to serum was measured by radioimmunoassay using the method of Cohen *et al.* (1975) in groups of three replicates. The reagents were as follows: 5 μl serum; 500 μl radioimmunoassay (RI) buffer (0.2M Tris acetate pH 7.3; 1 $\mu\text{g/ml}$ calf thymus histone; 0.5% (v/v) calf serum; 10 μl of 25 ng MBP¹²⁵I in RI buffer (specific activity 3 $\mu\text{Ci}/\mu\text{g}$). The mixture was allowed to stand for 18 hr at 4°C and the ¹²⁵I was counted. Ethanol 500 μl at 4°C was added, left to stand at 4°C for 30 min, then centrifuged at 2500 rpm for 40 min at 4°C , and the supernatant removed. The precipitate was washed with a further 500 μl of ethanol at 4°C , and the final precipitate counted.

Results are expressed as the percentage of MBP¹²⁵I precipitated after alcohol washing minus

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that similarly precipitated in the absence of serum. Myelin basic protein was prepared by the method of McDermott and Caspary (1975).

Results

The overall results are shown in Fig. 1. While the control and MS groups overlap, there is a tendency for lower binding values in the MS patients. Comparison of the numbers above and below the mean control MBP binding value (Table) indicates a significantly raised number ($P<0.001$) of patients below the mean normal level. Comparison of the mean binding values between control subjects and patients by the t test reaches significance only at the $P=0.02$ level.

Table 2x2x2 comparison of overall MBP binding values falling above and below mean normal level

Group	> C	< C
Control	25	25
Multiple sclerosis	27	49

χ^2 11.903
 $P < 0.001$

When MBP binding values were plotted against date the results suggested a trend towards lower levels in June–July compared with those in April–May (Fig. 2), even though there was marked variation.

The values obtained on eight patients sampled sequentially are shown in Fig. 3. Three patients

Fig. 1 Individual sample binding of MBP for multiple sclerosis patients and normal subjects. Mean value and standard deviation shown by lines and broken lines respectively.

Fig. 2 Seasonal variation of MBP binding in MS patients over the period March to July 1977. Monthly means shown by broken lines.

relapsed before, during, or after the period of study but there was no obvious correlation between disease activity and antibody levels. Assay variation during the period remained within a 95% confidence limit and failed to show any trends that could be related to the clinical condition. Interpretation of the sequential results is further complicated by the seasonal fall of MBP binding between spring and summer (Fig. 2).

Discussion

The results of the present study indicate a marginally reduced serum antibody response to MBP in patients with multiple sclerosis compared with normal subjects. However, no gross changes were seen in agreement with earlier work (Lisak, 1975; Caspary, 1977). As the present findings are superimposed on a seasonal variation their precise meaning must, therefore, contain an element of uncertainty. Interpretation in pathogenetic terms is also impeded by the gross overlapping of results between MS patients and normal subjects.

In general, our limited knowledge of the function of humoral factors in MS or even in its experimental “model” (EAE) makes it difficult to assess our findings; some of the known properties are described to clarify the overall picture. Serum in the experimental disease cannot be used to transfer the disease, though passive transfer is readily achieved with cells (reviewed by Raine, 1976). Protection by serum against EAE was shown by Paterson and Harwin (1963) as well as a blocking action of serum on encephaligenicity of MBP (Bergstrand, 1976) but no parallels exist in

Fig. 3 Sequential MBP binding in eight MS patients. Arrows indicate an exacerbation (cases 3, 4, and 6).

the human disease or its treatment. Damaging effects such as myelinotoxicity and transmission block in cultures (Bornstein, 1973) have not to date been seen in vivo. Antimyelin antibody has been found by immunofluorescence in MS, amyotrophic lateral sclerosis, and in the Guillain-Barré syndrome, and appears to be specific to myelin. A lesser degree of binding occurs in normal subjects (Lisak *et al.*, 1975). Perhaps the most promising role for antibody is that shown by Brosnan *et al.* (1977) in their morphological observations on demyelination in the rabbit retina. They concluded that demyelination followed an antibody-mediated cytotoxic reaction. It, therefore, becomes apparent that conventional methods for antibody detection with MBP, the antigen of EAE but only presumptive in MS, may fail to detect a disease-specific change. In the first instance, the antigen may be different and, secondly, functional assays may be needed to detect the relevant humoral factor (antibody).

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