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New monoclonal antibodies reactive with defined sequential epitopes in human myelin basic protein

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Summary

Three new IgG monoclonal antibodies are described which recognise sequential epitopes of the human myelin basic protein (MBP) molecule in amino acid sequences 36–50, 64–75 and 80–89. Two of the secreting hybridomas were prepared by immunisation of mice with synthetic peptides. This procedure appears to generate antibodies of similar affinities to those made using intact myelin basic protein as the immunogen. It has the advantage that antibodies to preselected regions of the molecule can be made at will and the problem of subsequent epitope localisation is simplified. It is possible with synthetic peptides to generate antibodies of specificities which it would be impossible to achieve by immunisation with intact myelin basic protein. The monoclonal antibodies described here should be useful tools in studies of myelin catabolism in vivo and in vitro. Of particular interest is our Clone 22, making an antibody which reacts equally well with intact human MBP and synthetic peptide sequence 80–89 in liquid phase assays. Antibodies of this rare specificity have been claimed to be able to react with the peptides of myelin basic protein found in the spinal fluid of patients with multiple sclerosis.

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Introduction

Myelin basic protein (MBP) is digested in active multiple sclerosis (MS) plaques and some of the peptides resulting from this breakdown reach spinal fluid and possibly blood (Whitaker, 1984). It is of interest to study this process in detail for at least two reasons. Firstly, characterisation of the cleavage products may indicate which of many potential sources of proteases are quantitatively of most importance in MBP proteolysis in vivo, e.g. macrophages, intrinsic myelin proteases, blood. Secondly, characterisation of some of the MBP peptide fragments reaching the spinal fluid and blood is necessary in order to optimise ultrasensitive immunoassays for them. Such assays might have potential application in monitoring disease activity and its response to therapy (Whitaker, 1984). A recent report suggests that some MBP peptides may also reach urine in multiple sclerosis patients (Whitaker, 1987).

It has been claimed that only antibodies of certain rare specificities are capable of detecting the MBP peptides in the spinal fluid of MS patients. One such specificity is an antibody which reacts well with both intact human MBP and human MBP peptide 80–89 in solution (Whitaker et al., 1986). Recent attempts to produce antibodies of this specificity using synthetic peptides as antigens yielded antibodies only reacting with intact MBP in a conformation adopted when it was adsorbed to plastic (Price et al., 1986).

Monoclonal antibodies (MAb) to sequential epitopes are particularly useful in following the fate of an epitope from the intact MBP molecule to oligopeptides. In the present paper we describe the preparation and characterisation of three new monoclonal antibodies recognising sequential epitopes of human MBP including an epitope in the sequence 80–89.

Materials and methods

Preparation of purified MBPs and large MBP fragments. Intact MBPs and proteolytically prepared fragments were obtained as described previously (Groome et al., 1985; Hruby et al., 1987).

Preparation of synthetic peptides. Peptides were synthesised using the Fmoc method developed by Dr. R. Sheppard and his colleagues at the University of Cambridge (Atherton and Sheppard, 1984). Peptides were synthesised on a manual Pepsynthesiser obtained from Cambridge Research Biochemicals, Button End Industrial Estate, Harston, Cambridge, U.K. Amino acids were purchased as Fmoc pentafluorophenyl esters ready for use. Each cycle was completed with a 3-fold molar excess for 1 h using 1-hydroxybenzotriazole catalysis. The completion of each cycle was tested by ninhydrin reaction on a sample of resin. Cleavage from the resin was achieved by treatment with 5% (v/v) ethanedithiol in trifluoroacetic acid for 7 h at room temperature. The liquids were removed under vacuum and the peptide precipitated and washed thoroughly with ether. The peptides were thoroughly dried

over P_2O_5 and NaOH pellets and stored at $-20\,^{\circ}C$ in a desiccated box. The purity and composition of peptides was checked by high pressure liquid chromatography (HPLC) and amino acid analysis. Each peptide was greater than 90% homogeneous as judged by HPLC. Peptides containing the amino acid arginine had traces of a form of the peptide still carrying the Mtr side chain blocking group as this is more slowly removed by trifluoroacetic acid than other blocking groups. This was the only significant contaminant in any of the peptides used here. Immediately prior to use for coupling procedures the peptides were dissolved in 0.01 M HCl and shaken with ether to remove excess ethanedithiol. Solutions for inhibition experiments were made in 0.1 M phosphate buffer pH 7.0 and stored in aliquots at $-80\,^{\circ}C$.

Synthetic peptides used in the present study. The corrected amino acid sequence for human MBP was that recently published by Gibson et al. (1984). The one-letter code system for amino acids is that described by Dayhoff (1972).

- Peptide 1 corresponds to human MBP sequence 80–89: TQDENPVVHF.
- Peptide 10 corresponds to human sequence 75–89: KSHGRTQDENPVVHF.
- Peptide 12 corresponds to human sequence 80–97 with a cysteine added at the NH_2 terminal and a glycine added at the COOH terminal: CTQDENPVVHFFK-NIVTPRG.
- Peptide 13 corresponds to human sequence 36–50: GILDSIGRFFGGDRG.
- Peptide 16 corresponds to human sequence 64–78 with a cysteine added at the NH_2 terminal: CARTAHYGSLPQKSHG.
- Peptide 21 corresponds to human sequence 69–83 with a cysteine added at the NH_2 terminal: CYGSLPQKSHGRTQDE.
- Peptide 27 corresponds to human sequence 61–75 with a cysteine added at the NH_2 terminal: CHHPARTAHYGSLPQK.

Preparation of peptide 10/tuberculin conjugate. Peptide 10 was coupled to tuberculin (Central Veterinary Laboratories, Weybridge, U.K.) using glutaraldehyde. The peptide and tuberculin, each at a concentration of 5 mg/ml in 0.1 M phosphate buffer pH 7, were coupled by the addition of glutaraldehyde (Sigma grade 1) to a final concentration of 0.06% (w/v). The solution was stirred overnight and then dialysed in benzoylated dialysis tubing exhaustively against phosphate-buffered saline. The conjugate was filter sterilised and stored in liquid nitrogen.

Immunisation procedure for preparation of Clone 22. Female BALB/c mice were primed subcutaneously with one human dose of BCG vaccine (Glaxo) as described previously (Lachmann et al., 1986). After 1 month each animal received 25 μg of peptide 10 as a tuberculin conjugate subcutaneously in Freund's incomplete adjuvant. This dose was repeated after a further month. Finally the mice were rested for at least 6 months and a selected mouse was boosted on each of the 3 days prior to fusion with 100 μg of peptide/tuberculin conjugate in saline.

Preparation of peptide 16/tuberculin conjugate. Peptide 16 was coupled to tuberculin via the -SH of cysteine using a heterobifunctional ester as previously described (Morrison et al., 1987). The final conjugate was filter sterilised and stored in liquid nitrogen.

Immunisation procedure for preparation of Clone 26. The immunisation protocol used was similar to that for Clone 22 but using the peptide 16/tuberculin conjugate.

Immunisation procedure for preparation of Clone 14. This clone was prepared in the same fusion as Clone 12 previously described (Groome et al., 1986). The spleen cells came from an outbred rat immunised with a bovine MBP/ovalbumin conjugate made with carbodiimide (Groome et al., 1986).

Cell fusion procedure. This was carried out as described previously (Groome et al., 1985, 1986). Isotyping of the rat and mouse monoclonals was carried out with reagents obtained from Serotec, Industrial Estate, Kidlington, Oxfordshire, U.K.

Inhibition ELISA. The Clone 14 MAb was characterised by inhibition enzyme-linked immunosorbent assay (ELISA) as described previously (Hruby et al., 1985). As noted previously, relative binding reactivities determined by this method must differ by a factor of 2 to be considered significant.

Inhibition radioimmunoassay. The Clone 22 and Clone 26 antibodies were characterised by inhibition radioimmunoassay. One advantage of this procedure is the greater precision which can be achieved and freedom from the plate to plate variation which can affect ELISA results.

Human MBP was radioiodinated to a specific activity of 20 μCi/μg using Iodogen catalysis (Johnston and Thorpe, 1982). Desalting of MBP was carried out on a short column of Sephadex G25 equilibrated with 0.01 M HCl containing 0,5 mg/ml calf thymus histones (Sigma type 2S). An initial titration of each antibody tissue culture supernatant was carried out to determine a dilution which bound ~30% of the total 20000 cpm of MBP under the conditions of the assay below in the absence of any inhibitor. This varied from a 1 in 50 dilution of tissue culture supernatant up to 1 in 500 for different MAbs. Dilutions were made in 0.05 M phosphate buffer pH 7 containing 1.2% (w/v) sodium chloride, 0.05% (w/v) Tween 80 and 0.5 mg/ml calf thymus histones. This is referred to as 'buffer A'. 100 μl of antibody were mixed with 50 μl of standard containing different amounts of MBPs or peptides as inhibitors and incubated overnight at +4°C. Then, 20000 cpm of ^{125}I-MBP in 50 μl of buffer A was added to each tube. After a further overnight incubation in the cold room 50 μl of SacCell (Wellcome) anti-mouse IgG (diluted 1 in 2 in buffer A) was added to each tube followed by a vortex mixing. After 30 min, 1000 μl of water were added to each tube and the SacCell deposited by centrifugation. The supernatant was carefully tipped off from each tube and the bound radioactivity was counted on a gamma counter. The results could be curve fitted on the gamma counter (Berthold) which provided estimates of the amount of each inhibitor needed to give 50% inhibition. This, however, requires zero inhibitor and non-specific binding tubes to be placed at the start of each group of inhibited samples. Alternatively, the tubes can all run through the counter together and the data then be plotted on logit/log graph paper. The object is to determine the concentration of each inhibitor which gives 50% inhibition of binding. The data are then converted into relative reactivities as for ELISA (Hruby et al., 1987). Percentage of reactivity for a putative antigen is calculated as:

$$\% = \frac{100 \times \text{moles of standard (immunising) antigen giving 50\% inhibition}}{\text{moles of putative antigen giving 50\% inhibition}}$$

As with ELISA the relative binding reactivity must differ by a factor of 2 to be considered significant.

Results

Clone 22

The reactions of the MAb from Clone 22 are shown in Table 1 and Fig. 1. The mouse IgG2b MAb from this clone has maximum reactivity with the immunising

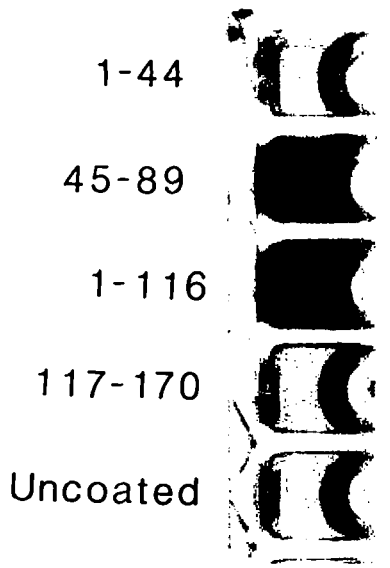

Fig. 1. Reactivity of MAb from Clone 22 with human MBP peptides by ELISA.

TABLE 1

REACTIVITY OF CLONE 22 MAb (DETERMINED BY INHIBITION RADIOIMMUNOASSAY)

Percent reactivities are recorded as c100 if they are in the range 90–110%.

Antigen/competitor	% Reactivity
Bovine MBP	60
Human MBP	58
Human 45–89	c100
Synthetic peptide Cys–80–97–Gly	50
Synthetic peptide 80–89	41
Synthetic peptide 75–89 [a]	100
Probable epitope	Thr^{80}–Phe^{89} TQDENPVVHF

[a] Immunising peptide.

TABLE 2

REACTIVITY OF CLONE 26 MAb (DETERMINED BY INHIBITION RADIOIMMUNOASSAY)

Percent reactivities are recorded as c100 if they are in the range 90–110%.

Antigen/competitor	% Reactivity
Bovine MBP	c100
Human MBP	c100
Sheep MBP	120
Guinea pig MBP	80
Rabbit MBP	c100
Synthetic peptide Cys–64–78 [a]	100
Synthetic peptide Cys–61–75	c100
Synthetic peptide Cys–69–83	1.5
Synthetic peptide 75–89	0
Probable epitope	Tyr^{69}–Lys^{75} YGSLPQK

[a] Immunising peptide.

peptide human 75–89 and with human 45–89. Human MBP, human MBP peptides 80–97 and 80–89 appear to react slightly less well (around 50% in each case). The equal reactivity of human (58%) and bovine (60%) MBPs suggests that Thr^{80} is not an important component of the epitope as this residue is replaced by proline in bovine MBP.

Direct ELISA on wells coated with human MBP peptides 1–44, 45–89, 1–116 and 117–170 showed reactivity only with peptides 45–89 and 1–116 (Fig. 1). These results are consistent with a sequential epitope for the Clone 22 MAb located in human MBP amino acid sequence 80–89.

Clone 26

The reactions for the MAb from Clone 26 are shown in Table 2. The mouse IgG1 MAb from this clone reacts fully with human MBP, the immunising synthetic peptide human 64–78, and peptide 61–75 but not with peptide 75–89. This suggests a sequential epitope within the sequence 64–75 of human MBP. The slight reactivity of peptide 69–83 (1.5%) suggests that a significant portion of the epitope lies with the amino acid sequence 69–75 (YGSLPQK).

This conclusion is supported by full reactivity of the antibody with bovine, sheep, guinea pig and rabbit MBPs. The sequences corresponding to human 69–75 are all identical in these species. Differences from human MBP occur at position 63 where human has a proline and all the other species have an alanine, and at position 67 where human MBP has alanine and all the other have threonine. Major differences occur in the amino acid sequences corresponding to human 76–78 (SHG) in bovine (AQH) and guinea pig (SQR). Thus it is unlikely that the contact residues for the epitope extend to Pro^{63} or Ser^{76}.

Direct ELISA of the Clone 26 MAb on wells coated with human MBP peptides 1–44, 45–89, 1–116 and 117–170 showed reactivity only with peptides 45–89 and

TABLE 3

REACTIVITY OF CLONE 14 MAb (DETERMINED BY INHIBITION ELISA)

Percent reactivities are recorded as c100 if they are in the range 90–110%.

Antigen/competitor	% Reactivity
Bovine MBP [a]	100
Pig MBP	85
Chicken MBP	c100
Human MBP	45
Guinea pig MBP	23
Rat L MBP	0–0.1
Rabbit MBP	0.1
Bovine 1–116	150
Bovine 1–89	c100
Bovine, guinea pig 39–89	1
Bovine, guinea pig 45–89	0
Bovine 1–44	0
Synthetic 36–50	25
Probable epitope	Gly^{36}–Gly^{46} GILDSLGIFFG

[a] Immunising antigen.

1–116 as expected if the antibody recognises an epitope in the human MBP sequence 64–75 (not shown).

Clone 14

The reactions of the MAb from Clone 14 are shown in Table 3. The rat IgG from this clone reacts well with bovine, pig, chicken, human and guinea pig BP but very little with rabbit and rat large BP. The amino-terminal half of bovine MBP also

TABLE 4

SUMMARY OF PREPARATION AND PROPERTIES OF NEW MONOCLONAL ANTIBODIES

	Species/isotype	Immunogen	Epitope (human sequence)
MAb Clone 14	Rat IgG	Bovine MBP/ovalbumin conjugate made with carbodiimide	36–50
MAb Clone 22	Mouse IgG2b	Human synthetic peptide 75–89 coupled to tuberculin with glutaraldehyde	80–89
MAb Clone 26	Mouse IgG1	Human synthetic peptide 64–78 coupled to tuberculin with heterobifunctional ester	69–75

reacts fully but when the Phe-Phe bond at 44–45 is split, as in fragments 1–44 and 45–89, reactivity disappears, an observation suggesting that this bond is in the epitope. The next residue, Gly^{46}, is also important because rabbit and rat BP, which have substitutions at that position, are practically non-reactive. The epitope probably extends towards the amino terminal beyond Asp^{39} because bovine and guinea pig 39–89 are only slightly reactive. The slightly weaker binding of guinea pig and human BP suggests some importance of position 41, where guinea pig and human have isoleucine instead of the bovine leucine. Confirmation of this epitope localisation is provided by human synthetic peptide 36–50 which reacts almost as well as human MBP. The preparation and properties of all three new MAbs are summarised in Table 4.

Discussion

Several groups have reported the preparation and characterisation of MAbs to MBP (Groome et al., 1985; Hruby et al., 1985, 1987; Chou et al., 1986). Most of these MAbs were made using intact MBP or large peptides derived from MBP by proteolysis as the immunogen. Since the epitope specificity of each antibody was then unknown detailed inhibition experiments had to be completed in order to localise the epitope for each antibody. An ELISA inhibition method for this purpose was described by Hruby et al. (1985).

Most of the MBP MAbs so far described recognise sequential epitopes, i.e. small peptides can adopt a conformation similar to the intact MBP molecule and thus have full reactivity with the antibody. A few MAbs appear to recognise conformational epitopes (Alvord et al., 1986). For the purpose of following MBP catabolism in human diseases and identifying the peptides derived from MBP breakdown in blood and spinal fluid, MAbs to sequential epitopes are generally the more useful reagents.

One problem which arises in building up a battery of MAbs to MBP is that only certain epitopes are immunodominant in mice when they are immunised with intact MBP. Thus after a while antibodies of similar specificities recur. The antibody specificity varies between different inbred strains of mice (Day and Potter, 1986) so that one approach to obtain new epitope specificities is to use strains of mice other than the commonly used BALB/c. A further strategy is to immunise rats rather than mice. In both these cases hybridomas of new specificities were obtained (Groome et al., 1985, 1986). The main drawback of such a strategy is that the resultant clones need to be grown in nude mice to effect ascites production (Groome et al., 1986).

A further strategy to broaden the epitopic response is to couple the MBP or the large peptide to a carrier protein such as ovalbumin in order to generate more T cell help. This helped to produce our Clone 10 (Groome et al., 1985) and Clones 12, 17, 2 (Hruby et al., 1987) and Clone 14 of the present paper. Useful as these strategies are there are some MAb epitope specificities which may be extremely difficult to make by present methods. This led us to consider to what extent synthetic peptides

could be used to raise MAbs to preselected regions of the MBP molecule, and whether the resultant MAbs would have affinities high enough to be useful in immunoassays.

One desired antibody specificity is that which Whitaker has claimed to be the optimal specificity for reacting with MBP peptides found in the spinal fluid of patients with MS (Whitaker et al., 1980, 1986). This specificity is for an antibody which reacts well with the 80–89 region of human MBP in synthetic peptide 80–89 and intact human MBP in solution. Antibodies of this specificity have been described only in certain rare rabbit sera.

In a recent attempt to make MAbs of the above specificity mice were immunised with synthetic human peptide 80–89 attached to a carrier protein (Price et al., 1986). Fusions were carried out on spleen cells from these mice, and the resultant hybridoma supernatants were screened on plates coated with human MBP. It appears, however, that none of the MAbs resulting from these fusions reacted well with intact human MBP in solution, but reacted more with a restricted conformation found in MBP absorbed to plastic (Price et al., 1986).

In an attempt to improve the chances of generating antibodies reactive with peptide 80–89 and intact human MBP in solution we have used a different immunisation and screening procedure. Firstly, rather than couple the peptide to the carrier protein with carbodiimide we have chosen to use glutaraldehyde which would be expected to mostly involve the NH_2 terminal of the peptide and the epsilon NH_2 of Lys^{75}. We conjectured that the broad reactivity of carbodiimide with amino acid side chains might have significantly altered the peptide during coupling as in the work of Price et al. (1986). Secondly, rather than use the short peptide 80–89 we used a longer peptide 75–89. It has generally been found that peptides as short as ten amino acids are below the optimum length for generating antibodies reactive with the intact molecule (Palfreyman et al., 1984). Thirdly, rather than use a normal protein carrier we used tuberculin (PPD). This has great advantages when the object is to subsequently make hybridomas because the tuberculin offers strong T cell help but does not generate a significant antibody response to itself (Lachmann et al., 1986). It has been found that sometimes most of the hybrids resulting from a fusion where ovalbumin or haemocyanin was the carrier may be reactive with the carrier and not with the peptide to which it was coupled.

Using these modifications we made Clone 22 MAb which reacts well with intact human MBP and synthetic peptide 80–89 in solution as required (Table 1). This MAb has sufficient affinity to function as the immobilised MAb in two-site immunoassays for MBP and to remove peptides containing the 80–89 region from the spinal fluid of patients with acute exacerbation of MS (data not shown).

To determine whether this synthetic approach to MAb production could also be applied to other regions of the human MBP molecule we next immunised mice with a synthetic peptide corresponding to human amino acid sequence 64–78. To the NH_2 terminal of this peptide a cysteine residue was added to allow defined coupling to tuberculin via the -SH. The mice were screened for reactivity with intact human MBP by ELISA and the highest responding mouse selected for fusion. After final boosting the spleen cells were fused as normal and the hybridoma supernatants

screened by ELISA on plates coated with intact human MBP. Many different wells secreting antibodies are found and the antibodies were ranked for affinity by inhibition ELISA. In this procedure the highest affinity antibodies are those which are susceptible to inhibition by the smallest dose of competitor MBP in solution (Groome et al., 1986). On this basis four clones were selected for recloning and further characterisation. In the event that all proved indistinguishable on the basis of their epitope specifications and affinity, moreover they are all isotyped as IgG1. Therefore, Clone 26 was selected for further use as its supernatants had the highest antibody titre. The inhibition data on the MAb from Clone 26 provides strong evidence that the epitope for this MAb is located in amino acid sequence 64 75 (Table 2) with the epitope having significant structure even in the short region 69–75. Like Clone 22, Clone 26 MAb is able to function as a solid-phase antibody in two-site assays of MBP or to remove MBP fragments containing the epitope from solutions.

The data on Clone 22 and Clone 26 in this paper show that the preparation of MAbs reactive with defined sequential epitopes of human MBP can be readily achieved using synthetic peptide immunogens. One advantage of this approach is the possibility of achieving antibody specificities which it would be extremely difficult to make by immunisations with intact MBP. Secondly, the high frequency of positive wells in tuberculin/peptide fusions allows a selection for antibodies of higher affinity. Thirdly, using the synthetic peptide approach epitope localisation is greatly speeded up because the epitope should always be located in the immunising peptide. Attention can be immediately focused on overlapping peptides in this narrow sequence.

MBP, with its considerable conformational flexibility in solution, is an ideal molecule from the viewpoint of generating protein-reactive antibodies with synthetic peptides. It is now believed that conformational flexibility is the main explanation for the ability of antibodies to synthetic peptides to react with the corresponding regions of proteins (Tainer et al., 1984). At Oxford Polytechnic, rabbits have now been immunised with over 20 different synthetic peptides corresponding to regions of human MBP. So far we do not have an example of a peptide which failed to generate an antibody response to intact human MBP. In several cases the peptide-specific polyclonal antibodies generated had high affinities for intact MBP and could be used in competitive assays more sensitive than could be achieved with any of our monoclonal antibodies. For certain purposes region-specific polyclonal antisera generated with synthetic peptides may be as useful reagents as monoclonal antibodies. Details of the preparation of region-specific polyclonal antibodies to human MBP and their use in immunoassay will be described in detail elsewhere (in preparation).

Monoclonal and polyclonal antibodies reacting with defined MBP epitopes should prove increasingly useful reagents in the study of the mechanisms of MBP digestion in multiple sclerosis and other diseases involving myelin destruction.

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References

Alvord, Jr., E.C., Hruby, S., Martenson, R.E., Deibler, G.E. and Law, M.J. (1986) Evidence for specific polypeptide chain folding in myelin basic protein from reactions between fragments of the protein and monoclonal antibodies. J. Neurochem. 47, 764–771.

Atherton, A. and Sheppard, R.C. (1985) Solid phase peptide synthesis using *N*-fluorenyl methoxycarbonyl amino acid pentafluorophenyl esters. J. Chem. Soc. Chem. Commun. 165–166.

Chou, C.H.J., Cox, A.A., Fritz, R.B., Wood, J.G. and Kibler, R.F. (1986) Monoclonal antibodies to myelin basic protein determinants. J. Neurochem. 46, 47–53.

Day, E.D. and Potter, N.T. (1986) Monoclonal and polyclonal antibodies to myelin basic protein determinants. J. Neuroimmunol. 10, 289–312.

Dayhoff, M.O. (1972) Atlas of Protein Sequence and Structure. National Biochemical Research Foundation, Washington, DC.

Gibson, B.W., Gilliom, R.D., Whitaker, J.N. and Biemann, N. (1984) Amino acid sequence of human myelin basic protein peptide 45–89 as determined by mass spectrometry. J. Biol. Chem. 259, 5028–5050.

Groome, N.P., Harland, J. and Dawkes, A. (1985) Preparation and properties of monoclonal antibodies to myelin basic protein and its peptides. Neurochem. Int. 7, 309–317.

Groome, N.P., Dawkes, A., Gales, M., Hruby, S. and Alvord, Jr., E.C. (1986) Region specific assays for myelin basic protein. J. Neuroimmunol. 12, 253–264.

Hruby, S., Alvord, Jr., E.C., Martenson, R.E., Deibler, G.E., Hickey, W.F. and Gonatas, N.K. (1985) Sites in myelin basic protein that react with monoclonal antibodies. J. Neurochem. 44, 637–650.

Hruby, S., Alvord, Jr., E.C., Groome, N.P., Dawkes, A. and Martenson, R.E. (1987) Monoclonal antibodies reactive with myelin basic protein. Mol. Immunol. 12, 1359–1364.

Johnston, A. and Thorpe, R. (1982) In: Immunochemistry in Practice, Blackwell, Oxford, pp. 109–110.

Lachmann, P.J., Strangeways, L., Vgakurum, A. and Evans, G.I. (1986) Raising antibodies by coupling peptides to PPD and immunising BCG sensitised animals. In: R. Porter and J. Whelan (Eds.), Synthetic Peptides as Antigens (Ciba Symposium 119), Wiley, Chichester, pp. 25–40.

Morrison, C.A., Fishleigh, R.V., Ward, D.J. and Robson, B. (1987) Computer aided design and physiological testing of a luteinising hormone-releasing hormone analogue for 'adjuvant free' immunocastration. FEBS Lett. 214, 65–70.

Palfreyman, J.W., Aitcheson, T.C. and Taylor, P. (1984) Guidelines for the production of polypeptide specific antisera using small oligopeptides as immunogens. J. Immunol. Methods 75, 383–393.

Price, J.O., Whitaker, J.N., Vasu, R.I. and Metzger, D.W. (1986) Multiple epitopes in a dodecapeptide of myelin basic protein determined by monoclonal antibodies. J. Immunol. 136, 2426–2431.

Tainer, J.A., Getzoff, E.D., Alexander, H., Houghton, R.A., Olson, A.J. and Lerner, R.A. (1984) The reactivity of anti-peptide antibodies is a function of atomic mobility. Nature 312, 127–134.

Whitaker, J.N. (1984) Indicators of disease activity in multiple sclerosis. Ann. N.Y. Acad. Sci. 436, 140–150.

Whitaker, J.N. (1987) The presence of immunoreactive myelin basic protein in urine of persons with multiple sclerosis. Ann. Neurol. 22, 648–655.

Whitaker, J.N., Bashir, R.M., Chou, C.H. and Kibler, R.F. (1980) Antigenic features of myelin basic protein-like material in cerebrospinal fluid. J. Immunol. 124, 1148–1153.

Whitaker, J.N., Gupta, M. and Smith, O.F. (1986) Epitopes of myelin basic protein in human cerebrospinal fluid. Ann. Neurol. 20, 329–336.