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MONOCLONAL ANTIBODIES REACTIVE WITH MYELIN BASIC PROTEIN

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Abstract—New monoclonal antibodies (MAbs) to myelin basic protein (BP) reveal epitopes to be in sequences 22–34, 75–82, 83–96, 118–131 and 125–131. Comparison of these results with those previously reported suggest that almost every sequence of about 10 amino acid residues may be sufficiently antigenic to make a single MA but that certain regions are immunodominant, strong enough to make practically the same MA repeatedly. One of these new MAbs (clone 3) has especially interesting reactivity, sharply limited to residues 75–82 in bovine and porcine BP. Lys-Ala-Gln-His-Gly-Arg-Pro. Whales presumably have the same sequence, since their BPs are fully reactive with clone 3 MA, but all other species of BP, with known sequences of BP, have at least two changes in this sequence. Deletion of Lys3 (as in a tryptic peptide of porcine BP) reduces reactivity with the MA about 10-fold, whereas substitution of Ala3 by Ser (as in all other species of BP) and a deletion of Gly7 (as in human, monkey and rabbit BP) or His8 (as in the guinea pig and rat BP) or substitution of Pro10 by Thr (as in human, monkey, rat and mouse BP) eliminates reactivity. We speculate that woodchuck and prairie dog BPs in this region closely resemble chicken BP, which has about 2% of the original reactivity. However, squirrel BP is unique, probably having only one of the changes in this region of BP, since it possesses 10–20 times the reactivity of chicken BP but still only 20–50% of the original reactivity with clone 3 MA, a degree of reactivity not seen with any other species of BP.

INTRODUCTION

This report concerns the fine specificities of certain new monoclonal antibodies (MAbs) to encephalitogenic myelin basic protein (BP). Using the ELISA inhibition assay with purified and well-defined proteolytic and synthetic fragments of BP, we have compared these new results to those obtained with other mouse and rat MAbs (HRUBY et al., 1985) that most closely resemble the new ones. We can localize the epitopes to several different sequences of about 10 amino acid residues in sequences 22–34, 75–82, 83–96, 118–131 and 125–131.

MATERIALS AND METHODS

Clones 2, 3, 12 and 17 were prepared by Groome et al. (1985, 1986). Rat clones 2.225.6 and 2.204.2 were prepared by Hickey et al. (1983), as previously reported by HRUBY et al. (1985). Mouse clone 2.242.22 was produced as previously reported by Sires et al. (1981, 1983). MAbs were used as hybridoma supernatants or were purified from ascites fluids by Sepharose–Protein A (Pharmacia), as described by EY et al. (1978). The clones, species and isotypes are summarized in Table I. Myelin BP preparations from different species and their fragments were the same as previously described by HRUBY et al. (1985). In addition, BPs of chinchilla (Chinchilla laniger), hamster (Mesocricetus auratus), prairie dog (Cynomys ludovicianus), woodchuck (Marmota monax), and squirrel (Sciurus carolinensis) were prepared from the pH 3.0 extracts of central nervous system tissues described previously byMartenson et al. (1971). The lyophilized extracts had been stored desiccated at 5°C. The BPs of the last four species were partially purified by repeated gel filtration through Sephadex G-100 in 0.01 M HCl at 5°C. All five proteins were subjected to a final purification on carboxymethylcellulose in NH4HCO3, pH 8.2, with elution carried out with a linear NH4HCO3 gradient. Sperm whale (Physeter catodon) and finback whale (Bal-}

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Table 1. Characteristics of monoclonal antibodies

<table>
<thead>
<tr>
<th>Clone No.</th>
<th>Species</th>
<th>Isotype</th>
<th>Immunizing antigen</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Mouse</td>
<td>lgGl</td>
<td>Bovine BP</td>
</tr>
<tr>
<td>3</td>
<td>Mouse</td>
<td>lgGl</td>
<td>Bovine BP</td>
</tr>
<tr>
<td>12</td>
<td>Rat</td>
<td>lgG</td>
<td>Bovine BP</td>
</tr>
<tr>
<td>17</td>
<td>Rat</td>
<td>lgG</td>
<td>Bovine BP</td>
</tr>
<tr>
<td>19242</td>
<td>Mouse</td>
<td>lgGl</td>
<td>Guinea pig BP</td>
</tr>
</tbody>
</table>

The preparations of guinea pig, bovine, porcine, rabbit and rat BP have been described by Hruby et al. (1985). Bovine peptides 1-91, 45-91, 30-91, and 1-44 were produced as described by Martenson et al. (1975). Porcine peptides 1-99, 66-75, and 76-93 were prepared as described by Kira et al. (1985). Mouse 14K BP was provided by Chou et al. (1983). Groome provided synthetic peptide Cys 82-99-Gly, corresponding to the human sequence with the addition of Gly and Cys at either end. The numbering of the residues is as summarized by Martenson (1984).

The antigenic specificities of the MAbs were determined by the ELISA inhibition method, as described by Hruby et al. (1985). Briefly, the relative binding of each MAb to various BP fragments was determined by comparison with the degree of inhibition of a standard BP solution (in each case, the reference standard was the antigen used in the initial immunization). From the family of approximately parallel inhibition curves we could calculate the amount of each putative antigen required for 50% inhibition. Reactivity in % was calculated as mols of standard antigen at 50% inhibition divided by mols of putative antigen at 50% inhibition multiplied by 100. The relative bindings must differ by a factor of 2 to be considered significantly different (Hruby et al., 1985).

RESULTS

Tables 2, 3, 4 and 5 show the relative bindings of each of the MAbs to various species of BP and their proteolytic fragments or synthetic analogues. Some of the previously published results of Hruby et al. (1985) are also included for comparison, especially to demonstrate the similarities and differences in some of the specificities of the new MAbs. The fact that all of the tests were performed with the same preparations of the putative antigens, frequently simultaneously, makes the comparisons and interpretations easier and more relevant.

As seen in Table 2, antibodies of clones 2 and 17 react fully with the C-terminal half of BP (peptide 92-128). Major differences can be seen in the reactions with human BP and especially with the two synthetic peptides with human BP sequences,
Monoclonal antibodies to myelin BP

Table 3. Results of ELISA inhibition tests for two different clones

<table>
<thead>
<tr>
<th>Antigens</th>
<th>Anti-bovine BP</th>
<th>Anti-guinea pig BP</th>
</tr>
</thead>
<tbody>
<tr>
<td>Bovine BP</td>
<td>100</td>
<td>117</td>
</tr>
<tr>
<td>Guinea pig BP</td>
<td>76</td>
<td>100</td>
</tr>
<tr>
<td>Rabbit BP</td>
<td>92</td>
<td>96</td>
</tr>
<tr>
<td>Chicken BP</td>
<td>100</td>
<td>0.2</td>
</tr>
<tr>
<td>Guinea pig 92-178</td>
<td>N.D.</td>
<td>0.3</td>
</tr>
<tr>
<td>Guinea pig 1-91</td>
<td>N.D.</td>
<td>0.16</td>
</tr>
<tr>
<td>Bovine 1-91</td>
<td>0.16</td>
<td>N.D.</td>
</tr>
<tr>
<td>Bovine 42-91</td>
<td>0.08</td>
<td>N.D.</td>
</tr>
<tr>
<td>Bovine 117 oxidized</td>
<td>320</td>
<td>N.D.</td>
</tr>
<tr>
<td>Rabbit 1-99</td>
<td>184</td>
<td>70</td>
</tr>
<tr>
<td>Rabbit 32-99</td>
<td>73</td>
<td>73</td>
</tr>
<tr>
<td>Rabbit 66-99</td>
<td>N.D.</td>
<td>83</td>
</tr>
<tr>
<td>Rabbit 1-93</td>
<td>7</td>
<td>N.D.</td>
</tr>
</tbody>
</table>

Synthetic peptides:

Cys-82-99-Gly

100, 600

Tyr-85-98

90-100

85-100

Possible epitope: Gln<sup>11</sup>-Val<sup>10</sup> His<sup>19</sup>-Arg<sup>20</sup>

Ph<sup>116</sup>-Arg<sup>20</sup> and Ser<sup>114</sup>-Gly<sup>117</sup>. The weak but equal reactions of clone 2 MAb with human BP and its synthetic peptide 114-131 contrast markedly with the 10 times stronger reaction with the immunizing antigen, bovine BP. Since the only difference between bovine and human BPs in this region is the Lys<sup>116</sup>-Arg<sup>117</sup> substitution at residue 124 (Martenson, 1984), one can conclude that Lys<sup>116</sup> is especially important for clone 2 MAb since its substitution by Arg in human BP decreases the binding 10 times. Trp<sup>118</sup> is of lesser significance in this epitope since its blockage, as in the hydroxynitrobenzylated derivative (BP·HNB), decreases the binding to clone 2 MAb only by about half. The non-reactivity of a short human peptide, residues 116-124, indicates that the epitope is larger than this nonapeptide, probably not in the amino direction in view of the weakness of Trp<sup>118</sup> but probably in the carboxyl direction. By contrast, clone 17 is fully reactive with peptide 114-131, human BP and HNB·BP. Therefore, its epitope does not include Trp<sup>118</sup> or Lys<sup>116</sup> and is probably included in the seaptapeptide Pro<sup>120</sup>-Gly<sup>123</sup>. The results previously obtained by Hruby et al. (1985) with rat clone 2.204.2 MAb indicate its reactive epitope to be between residues 114 and 121, thus very close to the epitope of clone 2 MAb.

The results in Table 2 also indicate one or more other reactive sites in BP for clone 17 and 2.204.2 MAb. One of these is only weakly cross-reacting, an epitope between positions 45 and 91 for clone 17 MAb but not for clone 2 MAb. By contrast, clone 2.204.2 MAb has a strong cross-reactive site in this area (residues 39-91).

Table 3 illustrates another pair of MAbB's with closely overlapping specificities. Major differences between the two rat MAbB's, clone 12 and clone 2.225.6, are shown by the reactions of chicken BP and two synthetic peptides, 90-99 and 88-99. Clone 12 MAb reacts fully with chicken BP, whereas MAb 2.225.6 is 500 times less reactive. This difference strongly suggests that Thr<sup>99</sup>, which is substituted by Ser in chicken BP (Martenson, 1984), is in the epitope for MAb 2.225.6 but not in the epitope for clone 12 MAb. The complete reactivity of MAb 2.225.6 with the synthetic peptide 90-99 and the 50-fold reduction with synthetic peptide Tyr-85-98 indicates the importance of Arg<sup>98</sup> and the unimportance of residues 1-89 as previously reported by Hruby et al. (1985). By contrast, the failure of clone 12 to react with the synthetic peptides 90-99 and 88-99, coupled with the full reactivity with peptides 33-99 and 82-99 and with chicken BP, suggest that the epitope extends maximally from Gln<sup>11</sup> to Val<sup>10</sup>.

Owing to the failure of the epitope to be extended beyond Arg<sup>98</sup>, it is possible that the epitope ends at this position. Whether positions 83-85 are important remains to be proved, since the failure of Whitaker's synthetic peptide Tyr-85-98 may be attributed either to its shorter length or to the bulky Thr which was added to the amino terminal.

Table 4 shows the reactivities of MAb 1924.22, a mouse IgGl, evoked by guinea pig BP. This MAb reacts fully with BPs of most species used in these assays: guinea pig, bovine, pig, rabbit, rat, mouse and monkey but not at all with chicken BP. Major C-terminal portions of the molecule (peptides 45-178 and 92-178) are completely non-reactive, as are the long fragments in the middle, peptides 39-91 and 32-98. Since bovine peptides 22-178 and 1-44 bind fully, whereas peptides 1-21 and 32-98 do not react, we can conclude that the determinant certainly includes residues between positions 22 and 31 possibly...
Extending as far as residue 44. From the known sequences of BP from different species (Martenson, 1984) we observe that there is a complete homology in sequence 22-34 in all species except chicken, in which a substitution and a deletion occur at residues 28 and 29. These two changes could easily account for the non-reactivity of chicken BP. We can then speculate that the epitope extends between residues 22 and 34 (DHARHGFLPRHRD), a part of the BP molecule which is highly conserved throughout evolution (Martenson, 1984).

As shown in Table 5, clone 3 reacts well only with bovine, porcine and whale BPs and with the aminoterminal half of bovine and porcine BP. In the known sequences of BPs of many species (Martenson, 1984) there is only one place where bovine and porcine BPs are identical and different from all other species of BP, whale BP being not yet sequenced: Lys-Ala-Gln-His-Gly-Arg-Pro (positions 75-82; see footnote on page 1360). All other species so far analyzed have at least two changes in the sequence, a substitution of Ser for Ala at position 76 and something else: most species have deletions of either Gln77 (human, monkey and rabbit) or His79 (rat and guinea pig) and have no reaction with clone 3. Only the chicken and mouse possess the Gln-His dipeptide which is present in bovine and porcine BPs; chicken BP has a deletion of Gly79 and a slight reaction with clone 3, whereas mouse BP has a substitution of Thr for Pro82 and is completely non-reactive with clone 3. Therefore, we place the epitope of clone MAb 3 at residues 75-82 in bovine BP, KAQHGRP, with Ala, Gln and Pro as immunodominant residues.

Reactions with BPs of different rodents contribute little to the definition of this epitope because their amino acid sequences are not known but they permit some speculations, which will be discussed later.

**DISCUSSION**

The epitopes discovered with the MAbs reported here are representative of a group of epitopes in which a short sequence of amino acids has the same

### Table 5. Reactivities of clone 3 with peptides and BPs from various species

<table>
<thead>
<tr>
<th>Species</th>
<th>Reactivity (%)</th>
<th>75-82</th>
</tr>
</thead>
<tbody>
<tr>
<td>Bovine BP</td>
<td>100</td>
<td>KAQHGRP</td>
</tr>
<tr>
<td>Pig BP</td>
<td>160, 311</td>
<td>KAQHGRP</td>
</tr>
<tr>
<td>Bovine 1-91</td>
<td>79</td>
<td>KAQHGRP</td>
</tr>
<tr>
<td>Pig 45-91</td>
<td>94</td>
<td>KAQHGRP</td>
</tr>
<tr>
<td>Bovine 39-91</td>
<td>94</td>
<td>KAQHGRP</td>
</tr>
<tr>
<td>Bovine 1-44</td>
<td>9</td>
<td></td>
</tr>
<tr>
<td>Pig 1-90</td>
<td>51</td>
<td>KAQHGRP</td>
</tr>
<tr>
<td>Pig 71 peptide</td>
<td>7.5</td>
<td>-AQHGRP</td>
</tr>
<tr>
<td>Fishhook white BP</td>
<td>100</td>
<td></td>
</tr>
<tr>
<td>Human BP</td>
<td>0</td>
<td>KS-HGRT</td>
</tr>
<tr>
<td>Mouse BP</td>
<td>0</td>
<td>KS-HGRT</td>
</tr>
<tr>
<td>Rabbit BP</td>
<td>0</td>
<td>KS-HGRT</td>
</tr>
<tr>
<td>Chicken BP</td>
<td>1.3, 2.5</td>
<td>RSQHBP</td>
</tr>
<tr>
<td>Myomorpha Rat 18 K BP</td>
<td>0</td>
<td>KSO-RT</td>
</tr>
<tr>
<td>Myomorpha Mouse 14 K</td>
<td>0</td>
<td>KSOHGR</td>
</tr>
<tr>
<td>Myomorpha Hamster BP</td>
<td>0</td>
<td></td>
</tr>
<tr>
<td>Myomorpha Hamster (mostly 14 K)</td>
<td>0</td>
<td></td>
</tr>
<tr>
<td>Sciuromorpha Woodchuck 14 K BP</td>
<td>3.2</td>
<td></td>
</tr>
<tr>
<td>Sciuromorpha Woodchuck 14 K BP purified</td>
<td>3.2</td>
<td></td>
</tr>
<tr>
<td>Sciuromorpha Prairie Dog 14 K BP</td>
<td>2.6</td>
<td></td>
</tr>
<tr>
<td>Sciuromorpha Prairie Dog 14 K BP purified</td>
<td>2.2</td>
<td></td>
</tr>
<tr>
<td>Hysticomorpha Guinea pig</td>
<td>0</td>
<td>KSO-RS</td>
</tr>
<tr>
<td>Hysticomorpha Squirrel BP</td>
<td>20, 32</td>
<td></td>
</tr>
<tr>
<td>Hysticomorpha Squirrel (mostly 14 K)</td>
<td>46</td>
<td></td>
</tr>
<tr>
<td>Hysticomorpha Hamster BP</td>
<td>0</td>
<td></td>
</tr>
<tr>
<td>Hysticomorpha Hamster (mostly 14 K)</td>
<td>0</td>
<td></td>
</tr>
<tr>
<td>Hysticomorpha Chipmunk BP</td>
<td>0</td>
<td></td>
</tr>
<tr>
<td>Hysticomorpha Chipmunk 18 K purified</td>
<td>0</td>
<td></td>
</tr>
</tbody>
</table>

*The deletion of residue 80 in the sequence originally proposed by Martenson (1984) has been found to be unnecessary (Dembler et al., 1985) and, for simplicity in referring to the published sequences of Martenson (1984), we have retained the number 90 even though there is no corresponding residue or deletion.*
Monoclonal antibodies to myelin BP

conformation as in the whole protein molecule; such
epitopes are usually called sequential epitopes. They
differ from other epitopes reported by Alvord et al.
(1986) and Boggs et al. (1985) in BP, in which
conformational or 3-dimensional differences are of
critical importance, and which are usually called
conformational epitopes.

The reactions of MAb from clone 3 deserve a more
detailed discussion. At first glance a Ser-Asp substi-
tution at position 76 appears to be the dominant
factor in destroying the binding to clone 3, but the
additional changes in known sequences of BP make
the analysis somewhat more complicated. Two of
these additional changes are probably most im-
portant: the Gin 75-His 76 preservation may (as in
chicken BP) or may not (as in mouse BP) restore
some slight reactivity with clone 3. From the com-
plete sequence of mouse BP in this region recently
reported by Zeller et al. (1984) and de Ferra et al.
(1985), we suggest that the non-reactivity of mouse
BP could be explained by another substitution (Thr
for Pro 75), thereby extending the epitope to that
position.

Species of the suborders Myomorpha (rat, mouse
and hamster) and Hystrixomorpha (guinea pig and
chinchilla) do not bind at all with clone 3, whereas
some of those of the suborder Sciuromorpha (wood-
chuck and prairie dog) bind to the same slight degree
as chicken BP. Although any number of as yet
unidentified changes in the sequence of this epitope
could account for this slight reactivity, comparison of
the available and, therefore, genetically acceptable
sequences suggests that the most likely would involve
Lys 75 (either delete or substitute Arg), Ala 76 (sub-
stitute Ser) or Gly 78 (delete). These are the changes
that occur in chicken BP. All other known changes,
admittedly not all singular, abolish all reactivity. The
still-better reaction of another member of the Sci-
romorpha (squirrel) suggests an even closer, as yet
unobserved chemical similarity to the bovine and
porcine sequence, perhaps with only one change, such
as the Arg-Lys substitution at position 75, the Ser-Asp
substitution at position 76 or the deletion of Gly 78,
rather than the two or more changes that characterize
all other species and that abolish all reactivity.

From our studies (Alvord et al., 1986; Groome
et al., 1985, 1986; Hruby et al., 1985; Sires et al.,
1981, 1983) and from the studies of other inves-
tigators (Bansal et al., 1985; Carnegie et al., 1983;
Chou et al., 1985, 1986; Elfman et al., 1986; Fritz and
Chou, 1983; Lazarus et al., 1983; Potter et al., 1986)
it is becoming increasingly clear that myelin BP has
many antigenic determinants on its molecule, many
more than the ones even quite recently summarized
by Alvord (1984) and Day and Potter (1986). The
results with this many monoclonal antibodies suggest
that epitopes may extend almost continuously along
the entire length of the molecule with several immu-
nodominant regions occurring in sequences 76-82,
112-131 and 130-157 and evoking similar MAb's in
several different laboratories (Boggs et al., 1985;
Chou et al., 1985, 1986; Fritz and Chou, 1983;
Whiluker, 1982; Carnegie et al., 1983; Sires et al.,
1981). Monoclonal antibodies have proven again to
be an excellent tool to study the immunological
properties of this important constituent of the central
nervous system.

Acknowledgement—We thank Terry Woolley for technical
assistance.

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