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ONE-WAY HUMORAL IMMUNE CROSS-REACTIVITY BETWEEN BOVINE SPINAL CORD PROTEIN AND BOVINE MYELIN BASIC PROTEIN

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Abstract—Whether bovine myelin basic protein (BP) and bovine spinal cord protein (SCP) cross-react at the humoral immune level was assessed with a sensitive solid-phase enzyme immunoassay. We found that a hyperimmune anti-SCP serum reacted strongly with SCP and cross-reacted nearly as well with BP. A hyperimmune anti-BP serum reacted only with BP. Antigenic competition analysis revealed that SCP and BP both inhibited binding of the hyperimmune anti-SCP serum to solid-phase adsorbed SCP and BP, while only BP inhibited binding of the hyperimmune anti-BP serum to solid-phase adsorbed BP. Finally, BP cross-reactivity antibodies were present in early bleedings from rabbits immunized with SCP that had been passed through an anti-BP immunosorbent column. These results clearly show there is a one-way humoral immune cross-reactivity between SCP and BP which goes in the direction of SCP to BP.

INTRODUCTION

SCP‖ (Yo and MacPherson, 1972; MacPherson *et al.*, 1976) is important because of its ability to prevent or suppress EAE in outbred guinea pigs (MacPherson and Yo, 1973; MacPherson *et al.*, 1977; Montgomery and Rauch, 1980; MacPherson, 1980). EAE is an autoimmune inflammatory disease of the CNS induced by injecting myelin or purified BP in FCA (Kies *et al.*, 1965). The pathogenesis of EAE is known to involve T-cells (Waksman and Adams, 1962; Gonatas and Howard, 1974). Consequently, SCP modulation of EAE represents a paradox, because SCP is definitely not encephalitogenic and immunodiffusion and radioimmunoassay analyses have indicated that SCP is immunologically distinct from BP (MacPherson *et al.*, 1976; MacPherson and Armstrong, 1977; Deibler *et al.*, 1978).

More recent work by Ramshaw *et al.* (1982), however, suggests that SCP and BP are related immunologically. Lymph node cells from BP- and SCP-immunized guinea pigs responded well to the homologous antigen and partially to the heterologous antigen in an *in vitro* transformation assay. Since lymph node cells from control animals did not respond to either BP and SCP, these results can only be explained if BP and SCP share at least one common antigenic determinant.

If SCP and BP do share a common antigenic determinant recognized by T-cells it is reasonable to expect that a humoral immune relationship might also exist between these two proteins. Although BP and SCP show no cross-reactivity in immunodiffusion tests (MacPherson *et al.*, 1976), whether or not they induce cross-reactive antibodies can only be unequivocally answered when cross-reactive, but non-precipitating antibodies are detected or, alternatively, ruled out. This led us to re-examine whether antibodies to SCP react with BP and vice versa.

In our re-examination of the humoral immune relationship between SCP and BP, we used a solid-phase EIA. This choice was made because solid-phase EIAs are extremely sensitive and because they detect all antibodies which bind to the solid-phase adsorbed antigen. Using this approach, we ascertained that a one-way humoral immune cross-reactivity exists between SCP and BP. We found that a hyperimmune SCP antiserum reacted with both SCP and BP, while a hyperimmune BP antiserum reacted only with BP. Confirmation of this one-way humoral immune cross-reactivity was obtained by antigenic competition analysis and by showing that SCPc still induced antibodies which cross-react with BP.

MATERIALS AND METHODS

Antigens

SCP and trypsin-derivatized RNP were prepared as described by MacPherson *et al.* (1976) and Ziola *et al.* (1982), respectively.

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‖Abbreviations: BP, bovine myelin basic protein; EAE, experimental allergic encephalitis; EIA, enzyme immunoassay; FCA, Freund's complete adjuvant; FICA, Freund's incomplete adjuvant; PBS, 0.02 *M* sodium phosphate, pH 7.4, containing 0.14 *M* NaCl; RNP, measles virus ribonucleoprotein; SCP, bovine spinal cord protein; SCPc, SCP passed through an anti-BP immunosorbent column; SCP-PN, peripheral nervous system form of SCP.

BP was prepared from bovine brain by the batch procedure of Deibler *et al.* (1972), modified to ensure complete removal of SCP. Beginning with the pH-3.0 extract, steps in the modified BP preparation were as follows: (1) 50 ml of the pH-3.0 extract were neutralized with 1 *N* NaOH, concentrated 50 times by flash evaporation and an aliquot was stored frozen at −20°C until analyzed for SCP content; (2) appropriate amounts of urea and CM-52, equilibrated to pH 3.0, were added to the remaining pH-3.0 extract; (3) the mixture was stirred gently for 15 min and then adjusted to pH 9.0 by dropwise addition of 1 *N* NaOH; (4) the CM-52 was collected by vacuum filtration and an aliquot of the filtrate (No. 1) was retained for SCP analysis; (5) the CM-52 was resuspended twice in 200 ml of wash solution (2 *M* urea adjusted to pH 9.0 with 1 *N* NaOH) and aliquots of filtrates No. 2 and 3 were retained for SCP analysis; (6) the CM-52 was then suspended in 1 l of 2 *M* urea, pH 9.0, and adjusted to pH 11.6 with 1 *N* NaOH; and (7) an aliquot of the pH-11.6 filtrate (No. 4) was saved for SCP analysis. All subsequent steps for recovery of BP were as described by Deibler *et al.* (1972). This modification for preparing BP takes advantage of SCP elution from CM-52 in solutions of low ionic strength at pH 7.0 or greater. SCP concns in the original pH-3.0 extract and in filtrates Nos 1–4 (freed of urea by dialysis) were measured by radial immunodiffusion after suitable concentration by flash evaporation. SCP in the original pH-3.0 extract was subsequently found in filtrates Nos 1 and 2.

Preparation of antisera

Hyperimmune rabbit anti-SCP and anti-BP antisera were raised as described earlier (MacPherson *et al.*, 1976). The IgG fraction of the hyperimmune anti-BP serum was used for preparation of the anti-BP immunosorbent column. The IgG was first precipitated with 18% Na_2SO_4 and then purified by chromatography on DEAE-Sepharose (Pharmacia Fine Chemicals, Montreal, Quebec) equilibrated in 0.1 *M* Tris–HCl, pH 8.0.

Early immune rabbit antisera were raised against BP, SCP and SCPc. Prior to immunization, all rabbits were screened for antibodies to SCP and BP by the EIA run in plates. The rabbits were then immunized with 1 ml of an emulsion containing 100 μg of antigen and FICA. Injections were given intradermally at four sites: one in the skin over each thigh and two along the back. Immunizations were carried out on days 0, 7, 35 and 65, while sample bleedings were taken on days 0, 7, 14, 21, 28, 49, 70 and 77. This immunization and bleeding schedule permitted close monitoring of specific and cross-reactive antibody induction.

The hyperimmune RNP antiserum used as a control in the antigenic competition studies was prepared by intradermally injecting a rabbit at bi-weekly intervals with 100 μg of RNP. The first emulsion contained FCA, while subsequent emulsions contained FICA. Four weeks after the fourth immunization, 400 μg of RNP in PBS was administered intravenously. Four days later, the rabbit was anesthetized, exsanguinated and the hyperimmune anti-RNP serum was prepared.

EIA of hyperimmune antisera

Specular surface polystyrene beads [6.4 mm dia (Precision Plastic Ball Co., Chicago, IL)] and antigen (1 μg/bead) were combined in PBS. After successive 1-hr incubations at 37°C and room temp, the buffer containing unadsorbed antigen was aspirated. The beads were washed twice with PBS and dried in a 37°C air stream. Each bead was then rolled into a 12 × 70 mm disposable tube containing 0.2 ml of antisera diluted in PBS containing 5% heat-inactivated normal pig serum, 0.5% Tween 20 and 10^{-4} *M* merthiolate (assay buffer). After a 2-hr incubation at room temp, the antiserum dilutions were aspirated and each bead was washed twice with 4 ml of tap water.

Rabbit IgG bound to the solid-phase adsorbed antigens was detected by incubating each bead in 0.2 ml of assay buffer containing horseradish peroxidase conjugated anti-rabbit IgG swine IgG. The enzyme (type VI) was obtained from Sigma Chemical Co. (St. Louis, MO) and the swine anti-rabbit IgG was obtained from Dako Immunoglobulins (Cederlane Laboratories Ltd, Hornby, Ontario). Enzyme was conjugated to the indicator antibodies according to the method of Nakane and Kawaoi (1974), except for the omission of the fluorodinitrobenzene blocking and Sephadex separation steps. The enzyme conjugate was used at a dilution (1:2500) which gave a final $O.D._{492}$ of 3.0 with beads coated with rabbit IgG instead of BP or SCP.

Incubation of the beads with the enzyme-conjugated indicator antibodies was at room temp. After 1 hr, the enzyme–conjugate solution was aspirated and each bead washed twice with 4 ml of tap water. Each bead was then immediately rolled into a clean tube containing 0.5 ml of enzyme substrate solution (0.1 *M* citrate/phosphate, pH 5.5, containing freshly dissolved 3 mg/ml *o*-phenylenediamine and 0.2% H_2O_2). After 30 min in the dark at room temp, the enzyme reaction was stopped with 0.75 ml of 2 *N* HCl. The $O.D._{492}$ of each sample was finally read in a Gilford Instrument model 250 spectrophotometer fitted with a model 2443A rapid sampler and a model 4019 thermal printer. Assays were run in quadruplicate and adsorption values were always corrected for non-specific binding of the enzyme-conjugated indicator antibodies to the solid-phase adsorbed antigen.

The ability of SCP and BP to inhibit binding of the hyperimmune SCP antiserum to solid-phase adsorbed SCP and BP and binding of the hyperimmune BP antiserum to solid-phase adsorbed BP was evaluated. The ability of RNP, SCP and BP to inhibit binding of hyperimmune RNP antiserum to

solid-phase adsorbed RNP was used as the control in this antigen competition analysis. The antisera were each diluted to 1:1500 and 0.45-ml aliquots were mixed with 0.45 ml of a solution containing competing antigen. Antigen levels of 0, 0.6, 2.5, 10 and 40 μg per tube were used. After an overnight incubation at room temp, 0.2-ml aliquots were placed into four tubes containing homologous antigen-coated beads. Inhibition of antibody binding to heterologous antigen-coated beads was assessed the same way. Preparation of the antigen-coated beads and running of the EIA were described for titration of the hyperimmune antisera, except that the assay buffer used was PBS containing 2% bovine serum albumin, 2% Tween 20 and 10^{-4} *M* merthiolate.

EIA of early immune antisera

The appearance of SCP, BP and cross-reactive antibodies following immunization or rabbits with SCP, BP and SCPc was assessed using EIAs run in 96-well flat-bottomed polystyrene plates [substrate plates (Dynatech Labs Inc., Alexandria, VA)]. Using 96-well plates as the EIA solid-phase increased the assay sensitivity and facilitated testing of many sera compared to running the EIAs with a bead as the solid phase. Each well was sensitized with 1 μg of antigen in 0.2 ml of PBS for the same length of time as in the bead-based assay. Assay conditions and vols used per well were identical to those used with the bead-based EIAs, except that the enzyme-conjugated indicator antibodies were used at a dilution of 1:5000 and enzyme substrate solution and 2 *N* HCl were used at 0.225 and 0.1 ml per well, respectively.

Preparation of SCPc

Coupling of the purified hyperimmune anti-BP IgG to AH-Sepharose 4B (Pharmacia Fine Chemicals) was done according to the method of Cambiaso *et al.* (1975). Each millilitre of settled gel was assessed to contain 1.5 mg of anti-BP IgG. Four milligrams of SCP in 0.1 *M* Na_2CO_3, pH 9.5, were run into a column which had a settled bed vol of 4.5 ml. After 30 min at 4°C, unbound material was eluted from the column by washing with the same buffer. The unbound protein was dialyzed against PBS (Spectraphor No. 3 tubing) and concentrated by Amicon ultrafiltration (UM2 membrane). The anti-BP immunosorbent column was eluted with 0.05 *M* glycine–HCl, pH 2.8, and re-equilibrated with 0.1 *M* Na_2CO_3, pH 9.5, between runs.

RESULTS

EIA development

During development of the solid-phase EIA used in this study, a number of different assay buffers were evaluated for their effect on the binding of anti-SCP and anti-BP antibodies to solid-phase adsorbed homologous antigen. Two were found to allow high binding of specific antibodies, while keeping non-specific binding of antibodies as well as enzyme-conjugated indicator antibodies at low levels. The assay buffers found acceptable were: (1) PBS containing 5% heat-inactivated normal pig serum, 0.5% Tween 20 and 10^{-4} *M* merthiolate; and (2) PBS containing 2% bovine serum albumin, 2% Tween 20 and 10^{-4} *M* merthiolate. The latter solution is very similar to the PBS containing 2% bovine serum albumin and 0.05% Tween 20 used by Irie *et al.* (1981) in their recently described EIA for antibodies to BP. In our hands, PBS containing solely Tween 20 at levels as low as 0.05% was not suitable as it inhibited binding of anti-SCP and anti-BP antibodies to the solid-phase adsorbed homologous antigen.

Assay buffer (1) was used when we determined the homologous and cross-reactive antibody titers of both the hyperimmune and the early immune antisera. This choice was made because it gave slightly better positive to negative serum antibody binding ratios than did buffer (2). We utilized buffer (2), which contained no IgG, for the antigenic competition analysis. This avoided possible non-specific binding of the soluble BP to pig IgG which might have occurred during the initial overnight incubation had buffer (1) been used.

Cross-reactivity of rabbit hyperimmune antisera

EIA evaluation of SCP and BP hyperimmune antisera binding to solid-phase adsorbed homologous and heterologous antigen is shown in Fig. 1. The BP antiserum reacted well with BP (Fig. 1B), but very weakly with SCP (Fig. 1A). The SCP antiserum reacted well with SCP (Fig. 1A), and nearly as well with BP (Fig. 1B). The negative control rabbit serum did not bind to either of the antigens. End-point titers were assigned to the two hyperimmune antisera

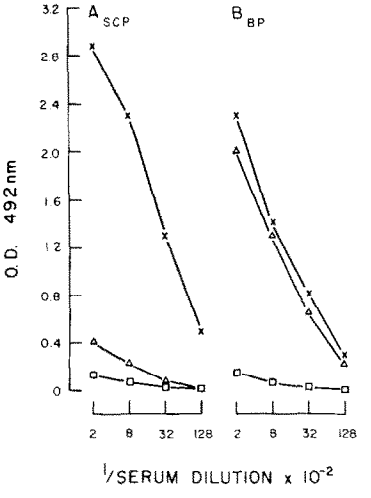

Fig. 1. EIA detection of antibody binding to solid-phase adsorbed SCP (panel A) and BP (panel B). Hyperimmune rabbit anti-SCP (×——×) and anti-BP (△——△) antisera as well as a negative control rabbit serum (□——□) were tested.

Table 1. Titration of hyperimmune anti-SCP and anti-BP sera against homologous and heterologous antigen[a]

EIA solid-phase antigen[b]	Antiserum to SCP	Antiserum to BP	Antiserum to Control
SCP	12,800	200	<200
BP	9600	6400	<200

[a]The serum dilution giving an EIA O.D.$_{492}$ value of 0.4 was taken as the end-point titer (see Fig. 1).
[b]Beads were used as the EIA solid phase.

(Table 1). The BP antiserum titer against BP was 32-fold higher than to the heterologous antigen SCP (1:6400 vs 1:200). In contrast, the SCP antiserum titer to BP (1:9600) was almost equal to its titer to SCP (1:12,800).

Antigenic competition analysis

Liquid-phase antigenic competition studies were undertaken to confirm the specificity of the one-way antibody cross-reactivity between SCP and BP. Binding of antibodies in the SCP hyperimmune antiserum to solid-phase adsorbed SCP and BP was inhibited by both soluble SCP (Fig. 2A) and soluble BP (Fig. 2C). Binding of antibodies in the BP hyperimmune antiserum to solid-phase adsorbed BP was inhibited by soluble BP, but not by soluble SCP (Fig. 2B). Liquid-phase antigenic competition by BP occurred only at higher antigen concns irrespective of whether anti-SCP or anti-BP antibody binding to solid-phase adsorbed SCP or BP was being evaluated (Fig. 2A–C). On the other hand, inhibition of binding by soluble SCP began at low antigen concns regardless of whether anti-SCP binding to solid-phase adsorbed SCP or BP was being evaluated (Fig. 2A and C).

Ability of liquid-phase BP and SCP to inhibit binding of RNP antibodies to solid-phase adsorbed RNP was arbitrarily used as a negative control in these experiments. Neither soluble BP or SCP caused inhibition, while soluble RNP did cause inhibition of RNP antibody binding (Fig. 2D). As expected, antibodies present in the RNP antiserum did not bind to solid-phase adsorbed BP or SCP (data not shown).

Cross-reactivity of rabbit early immune antisera

Preimmune sera were not available from the rabbits in which the hyperimmune antisera to SCP and BP had been raised. Therefore, early immune rabbit antisera to SCP, BP and SCPc were prepared to rectify this deficiency and to reaffirm the one-way antibody cross-reactivity demonstrated between SCP and BP in Fig. 1. In raising these antisera, it is important to note that only small doses of antigen were given ($4 \times 100\,\mu g$) and that the antigen was emulsified in FICA. Sera prepared at various times of the immunization schedule were then tested for antibodies to SCP and BP.

Low levels of antibodies specific for the homologous antigen appeared at day 28 (after two immunizations). Levels of antibodies to the homologous antigen were higher at day 49 (after three immunizations) and low levels of antibodies binding to BP were now detected in the anti-SCP and anti-SCPc sera. Antibody titers to the homologous and heterologous antigen in day 77 sera (after four immunizations) are shown in Table 2. Antibodies raised against SCP and SCPc reacted with both SCP and BP as expected based on the results with the hyperimmune anti-SCP rabbit serum (Fig. 1). Also, as expected, the day 77 serum raised against BP reacted with BP, but not with SCP.

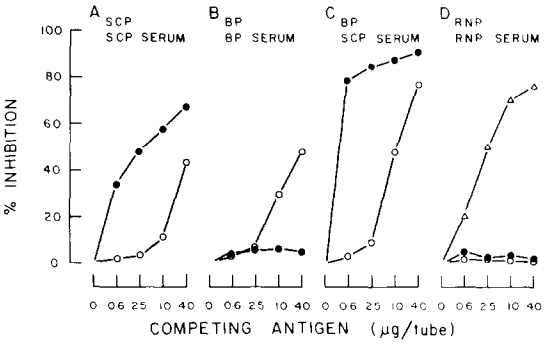

Fig. 2. Ability of soluble SCP (●——●), BP (○——○) and RNP (△——△) to inhibit binding of: anti-SCP antibodies to solid-phase adsorbed SCP (panel A) and BP (panel C), anti-BP antibodies to solid phase adsorbed BP (panel B) and anti-RNP antibodies to solid-phase adsorbed RNP (panel D). The hyperimmune rabbit antisera were used at a final dilution of 1:3000 (see Fig. 1). In the absence of soluble competing antigen, this gave EIA O.D.$_{492}$ values of 1.3, 0.8 and 1.6 for antibody binding to the homologous solid-phase adsorbed antigens SCP, BP and RNP, respectively. Similarly, this dilution of the anti-SCP serum gave an EIA O.D.$_{492}$ value of approximately 0.9 for antibody binding to solid-phase adsorbed BP. Abilities of the various soluble antigens to inhibit these starting O.D.$_{492}$ values were then expressed as % inhibition.

DISCUSSION

To demonstrate humoral immune cross-reactivity between two antigens, it is imperative that the antigens are not contaminated with each other. This criterion, therefore, had to be fulfilled for both BP and SCP before the unequivocal assignment of one-way humoral immune cross-reactivity between these two antigens could be made.

Table 2. Titration of early immune anti-SCP, anti-SCPc and anti-BP sera against SCP and BP[a]

EIA solid-phase antigen[b]	Antiserum[c] to SCP	SCPc rabbit A	SCPc rabbit B	BP
SCP	800	1600	800	<25
BP	100	600	800	200

[a]The serum dilution giving an EIA O.D.$_{492}$ value of 0.2 was taken as the end-point titer.
[b]Flat-bottomed microtiter plate wells were used as the EIA solid phase.
[c]Titers of day 77 sera are shown. Preimmune sera from all four rabbits had titers of <25 against both SCP and BP.

Two lines of evidence indicate the BP we used was free from SCP. First, bovine brain contains little SCP (Yo and MacPherson, 1972). This fact, coupled with SCP elution from CM-52 at pH 7.0 with solutions of low ionic strength, ensures that BP prepared from bovine brain by our modification of the Deibler *et al.* (1978) method contains little, if any, SCP. At the resolution limits of polyacrylamide gel electrophoresis, BP so prepared is free from SCP. Second, and more important, early immune and hyperimmune rabbit antisera raised against BP prepared by the modified method did not react with solid-phase adsorbed SCP (Tables 1 and 2) or with SCP in solution (Fig. 2B). Since SCP is highly immunogenic compared to BP, even traces of SCP in the BP preparations used to raise at least the hyperimmune anti-BP serum would have induced concurrent production of a large proportion of anti-SCP antibodies. For example, the presence of anti-SCP antibodies in antisera raised against batch-purified BP is well illustrated in Deibler *et al.* (1978).

Several lines of evidence also indicate our SCP was free from BP. First, SCP is routinely prepared by extracting fresh or frozen undefatted bovine spinal cord tissue with 0.1 *M* NaCl. BP is not extracted under these conditions (Yo and MacPherson, 1972; Weir and MacPherson, 1980). Second, any BP remaining in the SCP is likely retained on the CM-52 at pH 5.8, when the SCP is eluted with 0.05–0.2 *M* NaCl. Polyacrylamide gel electrophoresis analysis of SCP so prepared reveal it to be free of BP (Yo and MacPherson, 1972; MacPherson *et al.*, 1976). Third, since BP is a poor immunogen compared to SCP, BP contamination of SCP would have to be high to account for the observed proportion of anti-SCP antibodies which bind to solid-phase adsorbed BP (Tables 1 and 2). Such high BP contamination of our SCP certainly is not the case. Fourth, the data presented in Fig. 2C show that 40 μg of soluble BP are required to effect the same inhibition of anti-SCP antibody binding to solid-phase adsorbed BP caused by 0.6 μg of soluble SCP. In other words, there would have to be 40 μg of BP in the 0.6 μg of SCP antigen to obtain the inhibition caused by this small amount of SCP. Similar conclusions can be drawn from the ability of soluble SCP and BP to inhibit binding of anti-SCP antibodies to solid-phase adsorbed SCP (Fig. 2A).

In actual fact, the second, third and fourth lines of evidence just given for SCP purity are strongly supported by the results of an independent assessment of SCP purity. Radioimmunoassay analysis of BP contamination of SCP has revealed that SCP contains $<0.002\%$ BP (Lennon V., personal communication) [see MacPherson and Armstrong (1977)]. Such a minute level of BP in purified SCP would not be detected in polyacrylamide gels, would not induce the level of BP-reactive antibodies found in SCP antisera and clearly could not account for the data presented in Fig. 2A and C.

Although we were certain our SCP was free of BP, this fact was so crucial to demonstrating a humoral immune relationship between SCP and BP that we took purification of SCP one step further. SCP was passed through an anti-BP immunosorbent column and then retested for the ability to induce BP cross-reactive antibodies. Because SCPc induced BP cross-reactive antibodies (Table 2), this confirmed that the BP cross-reactive antibodies seen in the hyperimmune anti-SCP serum (Table 1) *could not be due* to BP in the SCP preparations used for immunization.

Based on all these considerations, we are convinced our BP and SCP preparations were not cross-contaminated to an extent which could account for the results we have presented. Perhaps the best way to underscore this fact is to emphasize that BP antibodies do not bind to solid- (Tables 1 and 2) or liquid-phase (Fig. 2B) SCP, yet BP can inhibit the binding of SCP antibodies to SCP (Fig. 2A). If SCP contained enough BP to induce the level of BP-reactive antibodies found in SCP antisera, then surely BP-induced BP antibodies would bind to SCP. That this is not the case logically leads to the conclusion that a one-way humoral immune relationship exists between SCP and BP.

Our finding of one-way humoral immune cross-reactivity between SCP and BP is not unique, since similar results have been reported several times for antibodies against strains of a given virus. Examples of one-way antibody cross-reactivity between two coronaviruses (strains 229E and OC43) and two adenoviruses (strains ICH and ICL) are found in Bradburne and Tyrrell (1971) and Marusyk (1972), respectively. That two structurally unrelated proteins such as SCP and BP should be immunologically related also is not unique. Recently, Nagy *et al.* (1983) reported that T-cell cross-reactivity exists between lactate dehydrogenase B and IgG2a myeloma protein. These authors suggests, and we concur, such immune cross-reactivity between two structurally unrelated proteins could be induced by a conformational determinant that is dependent on the tertiary structure of the two proteins involved.

The significance of the one-way antibody cross-reactivity between SCP and BP is related to the relevance of SCP to BP-induced EAE. Our earlier finding of T-cell cross-reactivity between BP and SCP (Ramshaw *et al.*, 1982) is now complemented by our present finding that SCP antibodies cross-react with BP. Together these observations provide the basis for explaining how SCP can protect animals from BP-induced EAE. SCP and BP must share at least one antigenic (conformational?) determinant to account for these cross-reactivity data. Consequently, pretreatment of animals with SCP induces immune unresponsiveness to BP. This SCP-induced immune unresponsiveness then effectively modulates EAE induced by BP.

The revelation of the one-way humoral immune

cross-reactivity between SCP and BP will also be of crucial importance in resolving the controversy over whether SCP is localized in neurons or myelin. This controversy is derived in part from the report of Brostoff and Eylar (1972) which claimed that P2, SCP-PN, is an integral component of peripheral nerve myelin. In contrast, experiments by Weir and MacPherson (1980) indicated that the SCP-PN found in purified peripheral myelin preparations is an artifact. During the isolation of myelin by density centrifugation of 0.8 *M* sucrose homegenates of peripheral nerve, the basic SCP-PN released from the cytoplasm of neurons binds to the acidic myelin by electrostatic interactions. This binding of SCP-PN to myelin is not specific as lysozyme will bind equally well and the SCP-PN can be washed out of the purified myelin with 0.1 *M* NaCl.

Immunohistological reports on the cellular and subcellular location of SCP likewise have not agreed. When the indirect immunofluorescence method and an SCP antiserum were used to localize SCP-PN in unfixed or acetone-fixed cryostat sections of bone spinal root, SCP-PN was found in the axons and was clearly absent from myelin (MacPherson and Wallace, 1980). However, if such cryostat sections are fixed in alcohol (a fixative required for exposure of BP antigenic determinants so that anti-BP antibodies can bind to BP), then the SCP antiserum will stain myelin (MacPherson C. F. C., unpublished observations; Eylar *et al.*, 1980; Winter *et al.*, 1982). We believe that such results arise because the BP cross-reactive antibodies in the SCP antisera can bind immunologically to exposed antigenic determinants of BP in alcohol-fixed tissue sections.

It thus appears that many immunohistological studies of myelinogenesis in which SCP-PN (= P2) antiserum has been used in determining the location of this protein in tissues will have to be reassessed in light of the one-way antibody cross-reactivity between SCP and BP. In support of this contention are our immunohistological findings which indicate that one-way antibody cross-reactivity also exists between SCP-PN and the BP found in both the CNS and the peripheral nervous system. A word of caution is necessary here. Because the CNS form of BP must be slightly different antigenically from the peripheral nervous system form of BP (i.e. the latter does not usually induce EAE) and because the CNS form of SCP and SCP-PN may also be slightly different antigenically (i.e. SCP-PN lacks the anti-encephalitogenic activity of SCP) means that antibodies against SCP and SCP-PN may cross-react to different degrees with the two forms of BP. Only careful purification of these four proteins, followed by production of polyclonal and monoclonal antibodies to each and utilization of these antibodies in sensitive immunoassay and immunoprecipitation tests will allow full description of the humoral immune relationships which exist among these four proteins.

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