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Chronic Theiler's virus infection in mice: appearance of myelin basic protein in the cerebrospinal fluid and serum antibody directed against MBP

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Summary

Myelin basic protein (MBP) appears frequently in the cerebrospinal fluid (CSF) of mice with chronic demyelination following intracerebral infection with Theiler's murine encephalomyelitis virus (TMEV); antibody to MBP can frequently be found in the sera. The peaks of the immune responses to both MBP and TMEV coincide with the time course of the clinical signs of disease. Adsorption of mouse sera with TMEV or MBP indicate the non-identity of the antigens and the specificity of the antisera as measured by ELISA. Immunoblot analysis of sera confirmed the ELISA findings. The mechanism of induction of antibody directed against MBP and its role in TMEV-associated demyelination remain to be determined.

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Abbreviations: CNS, central nervous system; CSF, cerebrospinal fluid; EAE, experimental allergic encephalomyelitis; i.c., intracerebrally; LODD, late-onset demyelinating disease; LMND, lower motor neuron disease; MBP, myelin basic protein; RR, righting reflex; TMEV, Theiler's murine encephalomyelitis virus.

Introduction

Infection with the DA isolate of Theiler's murine encephalomyelitis virus (TMEV, a picornavirus) produces an excellent model of delayed onset of chronic demyelination that appears to be an immunopathologic consequence of acute infection or complications of persistent infection (Theiler 1937; Lipton 1975; Lipton and dal Canto 1979; Rauch and Montgomery 1986). Although TMEV is an enteric pathogen of mice, certain neurotropic strains when injected i.c. present a unique biphasic disease (Lipton 1975). The first phase, acute infection (2–3 weeks), is characterized by destruction of infected ventral horn neurons in the central nervous system (CNS) gray matter. This phase depends upon dose and strain of virus and thus is not always clinically apparent in all infected mice. If present, the paralysis associated with lower motor neuron disease (LMND) usually stabilizes. The second phase consists of chronic inflammatory demyelinating lesions which may persist for months (Lipton 1975). In this late-onset demyelinating disease that is associated with chronic TMEV infection, the clinical signs observed are a gait abnormality and loss of righting reflex (RR). Signs generally occur 9–12 weeks following infection. However, Lipton and Dal Canto (1976, 1977) have shown that demyelination is present as early as 3 weeks post-infection, even while signs of progressive LMND may be present.

Compromise of the immune system following treatment of hosts with antithymocyte sera or cyclophosphamide administration can forestall the demyelination process at an early stage following TMEV infection (Lipton and dal Canto 1976, 1977; Roos et al. 1982) even though virus is present (Rodriguez et al. 1983; Lipton et al. 1984). Further evidence that the immune response may be significant in producing the demyelinating pathology derives from our finding of lymphoproliferative and antibody responses to TMEV which correlate with the development of the clinical signs associated with demyelination (Rauch and Montgomery 1986).

In this report, we present data showing that an immune response is induced to an autoantigen, myelin basic protein (MBP) in the form of serum antibody directed against MBP (Ab[MBP]). MBP appears in the cerebrospinal fluid (CSF), presumably released as a consequence of myelin degradation following CNS inflammation, as it is in experimental allergic encephalomyelitis (EAE) (Rauch et al. 1986) and MS (Cohen et al. 1976). Specific Ab[MBP] may thus be elicited if MBP enters the vascular compartment in an immunogenic form. We have eliminated TMEV viral protein as a source of a common or cross-reacting epitope by both absorption and immunoblot analysis, but the mechanism of induction of Ab[MBP] and its role, if any, in the clinical and pathologic development of TMEV-associated demyelination remains to be determined.

Materials and Methods

Infection of mice with TMEV

Male SJL/J mice are infected i.c. upon arrival from Jackson Laboratories; they are between 3 and 4 weeks of age at the time of infection. Each animal is given a

fourth passage isolate of the DA strain of TMEV in 0.02 ml of a 1 : 5000 dilution of a clarified homogenate of infected brain. The homogenate is obtained from i.c. infected Swiss-Webster suckling mice. The TMEV stock used to establish the fourth-passage isolate is a third-passage virus stock supplied by Dr. H. Lipton, Northwestern University. A small percentage (< 1%) of the i.c. infected animals develop an encephalitis and die within the first 2 weeks when the stock is injected neat. However, we have established an infective dose of virus that does not produce encephalitis, does produce some LMND coupled with a high incidence of the clinical signs associated with demyelination, particularly the loss of righting reflex. Control mice are injected i.c. with a clarified homogenate of uninfected brain. Infected mice are individually caged and are housed in a separate facility from the breeding colony and other experimental mice.

Clinical and histopathologic assessment of TMEV progression

Mice are observed weekly. Clinical signs of TMEV-associated demyelination are noted such as gait abnormality and loss of righting reflex (RR). RR is scored on a scale of (+) to (+ + +). The mouse's tail is held between the thumb and forefinger and gently twisted; if the animal can be turned on its back its RR is impaired. As soon as the animal is on its back, the tail is released. If the animal then rights itself immediately it is scored as (+). If it takes up to 20 s to right itself it is scored as (+ +), greater than 20 s is (+ + +).

When animals are sacrificed, CSF and CNS tissues are removed. Sections are prepared from tissue fixed in Bouin's, paraffin embedded and stained with hematoxylin and eosin. All slides are scored without the examiner knowing the clinical signs exhibited by the animal. The pathologic signs are graded on three or more sagittal sections of the entire brain or cord based on a scale of 1 to 4 as follows: 1 = meningitis; 2 = meningitis and one or two vessels cuffed by infiltrating mononuclear cells per section; 3 = several cuffed vessels per section; and 4 = heavily cuffed vessels in every section and widespread inflammation. Slides from a representative sampling of animals are screened; in general the histopathology reveals a relatively moderate inflammation resembling that of murine EAE at the light microscope level.

CSF collection

CSF is obtained from the mice as demonstrated to us by D. Griffin (Johns Hopkins University, Baltimore, MD). Briefly, animals are deeply anesthetized by ether, the nuchal skin and muscles are laid open, and a specially drawn capillary tube is inserted through the meninges into the foramen magnum. Usually 2–5 μl of CSF are collected prior to death. The volume of CSF is immediately measured and the CSF is transferred to a 1 ml plastic microfuge tube previously coated with a protease inhibitor to prevent rapid enzymatic breakdown of any MBP present. The CSF is stored at $-70°$C until assay. Only one assay is possible with each specimen.

MBP determination by ELISA

The assay is a modification of the inhibition-ELISA of Groome (Groome 1980, Rauch et al. 1986). MBP was isolated by the method of Nakao et al. (1966) and

Hinman et al. (1982). For coating plates, mouse MBP (1 mg/ml ddH_2O) is diluted in 0.05 M carbonate coating buffer, pH 9.6, with 0.2% (w/v) sodium azide; final concentration of MBP is 0.1 μg/ml. MBP is applied to the inner wells of polystyrene microtitration plates (Immulon, Dynatech, Alexandria, VA) and incubated overnight at 37°C. For the MBP standard curve, MBP is diluted in DPBS/T (Dulbecco's phosphate-buffered saline, pH 7.2, with 0.05% Tween 20) containing 1 mg/ml BSA such that MBP concentrations between 3200 ng/ml and 0.31 ng/ml are available for antibody binding in the standard curve; generally 12 points are selected between 200 and 0.31 ng/ml. 200 μl of each concentration is used per well, giving a sensitivity in the assay of from 40 to 0.0625 ng/well.

Diluted CSF samples and standards are then incubated with anti-MBP antisera. The resulting preabsorbed standards are added in triplicate to the plate. Each of the experimental mouse CSF samples is also added to a well. After incubation, alkaline phosphatase-conjugated second antibody (Sigma; goat anti-rabbit IgG) is added to each well and again incubated. The enzyme substrate 1 mg/ml of *p*-nitrophenylphosphate (Voller et al. 1977) is in 10% diethanolamine solution (pH 9.7) with 0.01% (w/v) $MgCl_2$ and 0.02% (w/v) NaN_3. The enzyme-substrate reaction is allowed to proceed at RT until the OD reading reaches 1.1 in the positive control well (20–30 min). Enzyme activity is inhibited by the addition of 50 μl of 3 M NaOH to each well. Substrate conversion is measured spectrophotometrically at 405 nm against the reference blank, substrate-NaOH, using a Plate Reader (Biotek, Burlington, VT, Model EL307).

The standard curve is plotted using a least-squares cubic spline fit. For a standard curve to be acceptable, the standard deviation of the triplicate values of each of the curve points must be less than 5–10% of the mean value of the triplicates. Standard curves run on different days can be superimposed, indicating that the slope of the curve is relatively constant between assays. However, each plate has its own standard curve and positive control to allow for differing times of color development. Positive control samples are normal CSF pools which have tested negative in the assay and to which we add known amounts of MBP. The amount of MBP present in each sample is interpolated from the spline fitted curve, and allows for measurement of values as low as 0.0625 ng/well. This program, written for an Apple II^+ or IIe, is available upon request.

MBP values are reported as ng/μl. In those samples which contain less than 0.0625 ng of MBP, a value must be assigned to allow for calculation of sample population means. To determine the maximum possible value that could be present and not measured (i.e., below the sensitivity of the assay), the following calculation is made. 0.06 ng is divided by the sample volume and the sample is then assigned that value/μl for calculation purposes. Therefore reported mean values may be slightly higher than the actual value.

The range of MBP values in control animals was established using CSF from uninjected normal SJL and other strains of mice. The mean value for uninjected mice was 0.024 ± 0.022 ng/μl. Specimens are considered positive for MBP when they have more than 0.069 ng/μl, the mean of the uninjected control mice plus 2 SD.

We monitored for the immediate release of MBP by assaying for MBP in the CSF 24 h after intracerebral injection. No significant levels of MBP were detected.

To further assure that the presence of MBP in the CSF was not due to non-specific inflammatory response to injury caused by intracerebral injection, we assayed CSF from 14 mice within 2 weeks of injection. These animals were injected either with the virus stock or uninfected brain homogenate. Thirteen did not show any MBP in their CSF (< 0.31 ng/ml or 0.0625 ng/well, the level of sensitivity of the assay). One sample obtained 7 days after injection had > 50 ng of MBP in the sample. We presume this was due to injury during injection. Therefore as a further control for injury-related release, MBP values given in this paper are from animals sampled 2 months or later after infection.

MBP antibody determination

The Ab[MBP] in mouse sera is also determined by an ELISA (Groome 1980). Plates are coated with mouse MBP as previously described. The mouse sera are diluted for testing by adding 5 μl of sera to 300 μl of DPBS/T plus 100 μl of BSA (2 mg/ml). The diluted sera are added to wells and incubated at 37°C for 90 min. After incubation the plate is washed 3 times with PBS/T, and a 1 : 1000 dilution of goat anti-mouse IgG alkaline phosphatase conjugate (Sigma) is added. The plate is again incubated (90 min, 37°C) and washed; the enzyme substrate is added and the procedure continued as described for the ELISA determination of MBP. Presence of Ab[MBP] is determined with reference to positive control sera (from mice injected with MBP). Levels are reported as presence (+), absence (−) or equivocal (±) for Ab[MBP]. Positive sera are not further diluted to determine titer.

TMEV antibody determination

Antibody directed against TMEV is determined using an ELISA kit from Litton Bionetics (Charleston, SC). In this procedure the TMEV protein is coated on ferrous metallic beads. The beads are placed in the sera to be tested. They are removed with a magnet and transferred to another well containing the second antibody, a goat anti-mouse IgG peroxidase conjugate. After incubation the bead is again transferred using the magnet, to a new well which contains the enzyme substrate. Beads are thoroughly washed between exposures to reagents. The extent of substrate conversion is measured spectrophotometrically at 405 nm with a plate reader and this reading is interpreted as presence (+), absence (−) of antibody or an equivocal reading (±) based on standards (+ and −) provided by the company.

Immunoblot procedure

Aliquots of protein are separated by SDS gel electrophoresis, using a 5% acrylamide stacking gel and 12% running gel (Greenfield at al. 1971). Proteins are transferred to nitrocellulose by electrophoresis at 30 volts for 18 h; a filter paper soaked in 5% SDS is laid on the cathodal side of the gel (Macklin et al. 1982). After transfer, the blot is dried and proteins are stained using either (a) acid fast green to locate all proteins on the strip or (b) rabbit or mouse sera containing antibody directed against the proteins on the blot as described in the text. When the antisera

are used, the blot is blocked with normal goat serum and 1% gelatin, then incubated with the antiserum, followed by goat anti-rabbit-PAP or rabbit anti-mouse-PAP; color is developed with 4-chloro-naphthol as substrate. Protein standards are stained and compared with the results obtained with the experimental preparation to identify the component binding the immune serum.

Results

Clinical appearance of TMEV-associated demyelination coincides with the appearance of MBP in the CSF, and serum antibody directed against both TMEV and MBP (Table 1, Fig. 1)

Antibodies directed against the virus (Ab[TMEV]) and against MBP (Ab[MBP]) appear in the sera of infected mice (Table 1) between 1 and 12 weeks after i.c. injection of TMEV. The incidence at 12 weeks coincides with the mean onset of clinical signs of TMEV-associated demyelination and the presence of MBP in the CSF of these animals. Of the 80 samples assayed, 42/80 (52%) were considered positive for MBP (0.069 ng/μl, the mean of the controls plus 2 SD). The average amount of MBP in the CSF of all TMEV-infected mice assayed was 0.144 ± 0.16. This value is higher than the mean for uninfected mice, 0.025 ± 0.022. This mean is for animals autopsied at least 2 months post-infection.

The mean value of MBP in CSF of mice exhibiting severe clinical signs (RR^{++}, RR^{+++}) at autopsy is 0.178 ± 0.216, with 12/19 (63%) of the specimens in the positive range (Fig. 1, shaded squares). The mean value for TMEV-infected mice with minimal clinical signs (RR^{+}) at autopsy is 0.138 ± 0.134, with 9/18 (50%) in the positive range. TMEV-infected mice which were asymptomatic and autopsied 2 months post-infection had a mean value of MBP in the CSF of 0.13 ± 0.14, with 22/43 (51.2%) of the specimens in the positive range.

TABLE 1

OCCURRENCE OF Ab[MBP] IN TMEV-INFECTED MICE

Autopsy Time post-infection	Number	Clinical signs		MBP in CSF [a]	Sera at autopsy	
		LMND	RR^{++}/RR^{+++}		Ab[MBP] [b]	Ab[TMEV]
1 week	4	1	0	0/4	1/4	0/1
2 weeks	5	2	0	0/5	1/5	2/4
4 weeks	3	1	0	2/3	0/3	0/2
6 weeks	2	1	0	1/2	0/2	0/1
8 weeks	4	3	0	0/4	0/3	nd
12 weeks [c]	20	1	9	14/19	10/14	7/11

[a] Positive level of MBP is 0.069 ng/μl (mean of control CSF + 2 SD).

[b] All animals were bled prior to infection and all such sera were negative for Ab[MBP] and Ab[TMEV].

[c] Six of the ten animals that were positive for Ab[MBP] at autopsy, had been checked for Ab levels one month prior to autopsy and were negative at that time.

Abbreviations: LMND, lower motor neuron disease: RR, loss of righting reflex; Ab[MBP], antibody to myelin basic protein; Ab[TMEV], antibody to Theiler's virus; nd, not determined.

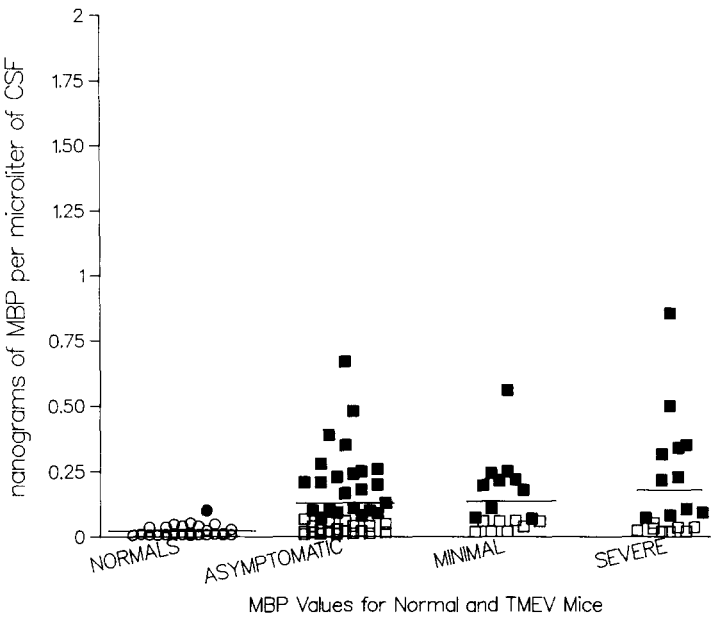

Fig. 1. Levels of MBP (ng/μl) in the CSF uninfected mice (○) and TMEV-infected mice presenting with severe (RR^{++} or RR^{+++}) clinical signs, minimal signs (RR^{+}) and asymptomatic infected mice. Those TMEV-infected mice with MBP levels considered positive are indicated by ■, those with a negative value are indicated by □. Uninfected mice with an elevated level of MBP are designated by ●. The mean of each sample population is indicated (———). The means of the three TMEV-infected groups differ significantly from the mean of the uninfected control group (one-tailed Student's *t*-test, $P < 0.01$).

By 12 weeks post-infection, more than half of the animals tested were positive for both Ab[TMEV] and Ab[MBP]. All the animals were bled before being infected and all these sera were negative for both antibodies. Some of the animals were bled repetitively during the course of the infection. Of the ten animals positive for Ab[MBP] at 12 weeks post-infection, six had been bled one month previously. These six animals did not have measurable Ab[MBP] at that time.

A low incidence of Ab[TMEV] occurs spontaneously due to the presence of wild-type virus in mouse colonies (Table 2)

Uninoculated mice held in the main animal quarters (TMEV-infected mice are held in a separate facility) rarely possess Ab[TMEV]; when present it is due to the occurrence of wild-type TMEV infection. Sera pools are accumulated from uninfected animals (using groups of ten animals). These are often littermates and range in age from 8 to 20 weeks. No attempt is made to pool by age or sex; however, retired breeders, representing the oldest mice, are generally not used in these pools. Pools of sera were screened in a random order until a positive pool was found. From this screening, and the quarterly viral screening of the colony (Charles River Biotechnology Service), we estimate the occurrence of natural infection at 10–20% of the mice in the colony.

TABLE 2

SERUM POOLS FROM CONTROL MICE NOT INOCULATED i.c. WITH TMEV

Serum pool	Ab[MBP]	Ab[TMEV]	Absorbed with	After absorption	
				Ab[MBP] [a]	Ab[TMEV] [b]
Control SJL	−	−	TMEV	−	−
			gpMBP	±	−
			TMEV-BHK	−	−
			BHK	−	−
Control A	−	±	TMEV	−	−
			gpMBP	−	±
			TMEV-BHK	−	−
			BHK	−	+
CNS injected mice [c]					
Control B	+ + +	−	gpMBP	+ +	−
			TMEV-BHK	−	nd
Control C	+ + + +	+	TMEV	+ + + +	−
			gpMBP	+ +	+
			TMEV-BHK	−	−
			BHK	+ + +	−

[a] The Ab[MBP] was not completely absorbed out. Some antibodies present in the serum may be directed to an epitope on mouse MBP that is absent in gpMBP.

[b] The increasing number of (+) reflects a relative increase or decrease in amount of antibody present compared to positive control sera. A change of one (+) is not always significant and is within the variation of the test.

[c] Mice are injected intradermally in the footpads with an emulsion of murine CNS tissue emulsified in complete Freund's adjuvant followed immediately and 72 h later by an i.v. injection of pertussis vaccine.

Abbreviations: BHK, baby hamster kidney cell lysate; TMEV-BHK, lysate of TMEV-infected BHK cells; Ab[MBP], antibody to myelin basic protein; Ab[TMEV], antibody to Theiler's virus; nd, not determined.

To insure that the Ab[MBP] found in the infected animals did not develop spontaneously due to an aging phenomenon, we screened 40 SJL breeders in our colony, ranging in age from 3 months to 9 months. Of these 40, 37 were negative, one had a score of (+) in our ELISA assay and two had a score of (±).

The sera of experimental mice subjected to an encephalitogenic CNS tissue challenge (no viral inoculum) and which developed paralysis indicative of EAE were used as the source of sera to be used as a positive control for Ab[MBP]. Ab[TMEV] was not usually encountered in these EAE mice; the frequency of its occurrence was as in the entire colony.

The Ab[TMEV] and Ab[MBP] are specific: Ab[MBP] does not react with purified TMEV. Ab[TMEV] does not react with MBP (Table 3)

A question as to the specificity of these antibodies arose inasmuch as appearance of both Ab[TMEV] and Ab[MBP] coincided in time. Absorption of antibody by one antigen indicated specificity by a decrease in titer to only that absorbent antigen

TABLE 3

ABSORPTION STUDIES WITH MBP, TMEV AND LYSATES OF TMEV-INFECTED AND UNINFECTED BHK CELLS

Serum pool [a]	Clinical signs	Ab[MBP] [b]	Ab[TMEV]	Absorbed with	After absorption	
					Ab[MBP]	Ab[TMEV]
160A	RR^{+}	+++	+	gpMBP	−	nd
				TMEV-BHK	−	−
160B	RR^{+}	++	−	gpMBP	−	−
				TMEV-BHK	−	−
240E/G	RR^{++}	+	+	gpMBP	−	nd
				TMEV-BHK	−	nd
210A/C	RR^{+++}	−	+	gpMBP	−	nd
				TMEV-BHK	−	nd
410	RR^{+++}	+	+++	TMEV	+	−
				gpMBP	+	+++
				TMEV-BHK	±	+
				BHK	−	+++
440	RR^{++}	±	+++	TMEV	+	−
				gpMBP	−	++
				TMEV-BHK	+	+
				BHK	−	++

[a] Not every test could be done on every pool due to limited quantities.

[b] The increasing number of (+) reflects a relative increase or decrease in amount of antibody present compared to positive control sera. A change of one (+) is not always significant and is within the variation of the test.

Abbreviations: BHK, baby hamster kidney cell lysate; TMEV-BHK, lysate of TMEV-infected BHK cells; RR, loss of righting reflex; Ab[MBP], antibody to myelin basic protein; Ab[TMEV], antibody to Theiler's virus; nd, not determined.

and not to the other antigen. Specificity was also indicated by specific absorption of the control sera (Table 2).

Initially the viral protein preparation used for absorption was an infected BHK cell lysate containing virus and viral proteins (TMEV-BHK) among other cell components (Microbiological Associates). A control of uninfected BHK cell lysate was subsequently tested as an absorbent when it was noted that some TMEV-BHK preparations removed Ab[MBP]. When the clarified lysate of TMEV-infected BHK cells was used as the source of virus for absorption, the titer to both TMEV and MBP was reduced, indicating cross-reaction between the BHK lysate and MBP. When uninfected BHK cell lysate was used as an absorbent, Ab[MBP] titer was reduced but Ab[TMEV] remained relatively unchanged. Absorption with purified MBP did not significantly reduce the Ab[TMEV]. Inasmuch as uninfected BHK cell lysates absorbed Ab[MBP], we repeated the absorptions with purified TMEV (a gift of Dr. H. Lipton, Northwestern University). The antisera were highly specific in that Ab[TMEV] titer was reduced following absorption with TMEV protein but not with MBP. Conversely the Ab[MBP] titer was reduced following absorption with MBP and not following absorption with TMEV. Guinea pig MBP (gpMBP) was used to absorb Ab[MBP]. Since the induced antibody was presumably elicited

against degraded mouse myelin, the difference between the absorbent antigen and the immunogen could account for the occasionally inadequate adsorptions of Ab[MBP] by gpMBP. The BHK cell lysate may contain a protein which has a partial analogy with MBP; this could account for the observed cross-reactivity.

Immunoblot discloses specific protein staining patterns with Ab[TMEV] and Ab[MBP] (Fig. 2)

When the TMEV proteins were separated on SDS gels, three bands were seen following silver staining of the gel. Sera from TMEV-inoculated mice (strips B–D) that contained Ab[TMEV] and Ab[TMEV] serum from a hyperimmunized rabbit (gift of Dr. Lipton) bound to the three bands following transfer of the proteins to nitrocellulose. These sera did not bind to any bands in the blots of either mouse spinal cord myelin extract or mouse spinal cord homogenate. Strip D was incubated with a serum pool, from TMEV- infected mice, that did not test positive for Ab[TMEV] using the commercial ELISA kit. We noted faint bands on the immunoblot in the region of the TMEV proteins.

The hyperimmune rabbit Ab[MBP] serum bound to the mouse MBP bands on a separate blot and to the corresponding bands of mouse spinal cord homogenate; this serum did not bind to TMEV proteins (blot not shown).

Blots of the TMEV-BHK lysate bound rabbit Ab[TMEV] and Ab[MBP] as well as Ab[TMEV] from several of the mouse serum pools from infected animals,

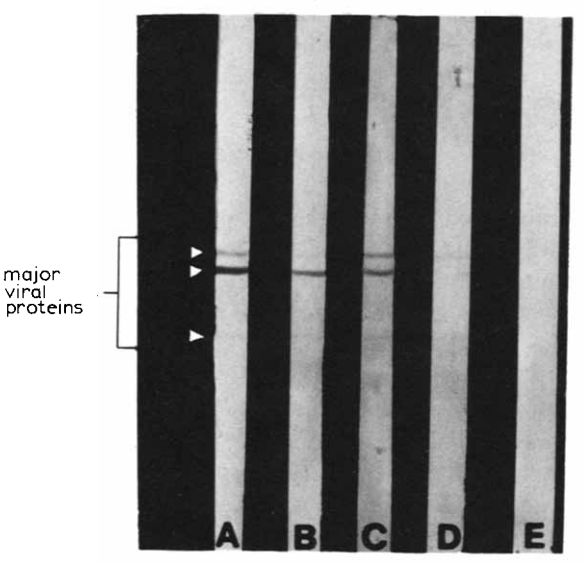

Fig. 2. Purified TMEV protein binds mouse Ab[TMEV]. TMEV protein was separated on SDS gels and transferred to nitrocellulose strips. Lane A: strip incubated with mouse Ab[TMEV] (Microbiological Associates); lanes B–D: strips incubated with sera from TMEV infected mice (lane B: pool 440: Ab[TMEV] by ELISA; lane C: pool 410: Ab[TMEV] by ELISA; lane D: pool 160B: no Ab[TMEV] by ELISA); lane E: strip incubated with uninoculated negative control serum pool.

corresponding to the results obtained by ELISA. As expected, the rabbit Ab[TMEV] sera bound to the TMEV bands only. However, rabbit Ab[MBP] sera bound to three bands in the 100–150 kDa molecular weight region, which corroborated the data on the absorption of Ab[MBP] by BHK lysate alone (blots not shown).

Discussion

We report here the appearance of MBP in the CSF of mice 12 weeks after CNS infection with TMEV. Half of the infected animals had measurable levels of MBP, even those that were asymptomatic. This suggests that the quantity of MBP in the CSF reflects the intensity of the chronic inflammatory response to the persisting infection rather than clinical signs.

The Ab[MBP] and Ab[TMEV] appear at the same time, particularly in animals with clinical signs, suggesting a relationship between immune responsiveness and these clinical signs. Although the incidence of autoantibodies in general may be increased with age in animals, the appearance of Ab[MBP] in uninoculated mice in our colony is a rare observation, and is not associated with age. Ab[TMEV] is seen at a frequency of 10–20% in our uninfected mouse colony, presumably due to the occurrence of sporadic natural infection with wild-type virus. However, the experimental mice arrive from Jackson Laboratories at 3 weeks of age, are immediately placed in isolation, and within 24 h after arrival are inoculated i.c. with the DA strain of the virus, thereby assuring that the observed Ab[TMEV] is due specifically to the i.c. infection of these animals.

The Ab[MBP] and Ab[TMEV] are specific and not cross-reactive as indicated by the cross-absorptions and immunoblot studies using purified MBP or TMEV protein preparations. These studies would appear to rule out generation of Ab[MBP] by TMEV protein determinants. An interesting cross-reaction was noted, however, with the clarified freeze-thaw cell lysate from BHK cells. When the TMEV-BHK and BHK cell lysate were both discovered to absorb Ab[MBP], immunoblot analysis was undertaken using the TMEV-BHK lysate. All Ab[TMEV] sera tested reacted with the three major TMEV protein bands on the blot and not with bands in blots of mouse spinal cord homogenate or myelin extract. Ab[MBP] sera from rabbits hyperimmunized with bovine or human MBP bound to three bands in the 100–150 kDa area of the blot of TMEV-BHK or control BHK lysate. It appears that the BHK cell lysate contains cross-reactive protein(s), the nature of which needs further clarification.

Hypotheses as to the induction of autoantibodies, as we observed in mice presenting signs of Theiler's associated demyelination, often include a role for viruses. It is challenging to determine how such a virus infection in fact triggers an autoimmune response. In the target cell or tissue, infection may release or expose new antigens. This could be the case in the coronavirus infection or in the TMEV infection as reported here. In other situations, extensive and chronic injury of a tissue or organ results in autoantibody production as in the case of severe chronic hepatitis (Meliconi et al. 1983). This may be one explanation for the appearance of

Ab[MBP] following inflammatory demyelination in Theiler's virus infection. Myelin degradation may lead to immunogenic forms of MBP that could enter the vascular compartment to induce an immune response. In fact, when MBP is injected into the CSF, it is transported through the blood-brain barrier and presented on the lumenal surface of endothelial cells of the cerebral and meningeal veins (Vass et al. 1984). This indicates a means by which MBP from inflammatory demyelinating lesions may enter the vascular compartment and elicit an immune response.

A virus infection could also result in an immunologically enhanced response due to presentation of normal brain constituents by antigen presenting cells, e.g. astrocytes. Interferons (IFN) are produced following some viral infections by activated T cells shown to be present in certain inflammatory brain lesions. Ordinarily brain cells express low levels of antigens encoded by the major histocompatibility complex (H-2), but in the presence of IFN_γ, MHC gene expression was dramatically enhanced: most astrocytes, oligodendrocytes, microglia and some neurons expressed H-2 antigens. IFN_γ, also induced Ia antigens on a subpopulation of astrocytes (Nakamura et al. 1984; Wong et al. 1984). The capacity of IFN, produced as a result in viral infection, to induce H-2 or Ia antigens may account for the findings of Fontana et al. (1984), who observed that astrocytes which displayed these antigens following contact with T cells could successfully present MBP to immunologically active encephalitogenic T cell lines.

In fact there is precedent for finding an autoimmune response directed against MBP following a viral infection. Watanabe et al. (1983) adoptively transferred EAE-like lesions using lymphocytes obtained from Lewis rats infected with murine coronavirus JHM and restimulated by MBP in culture.

A virus infection could trigger an autoimmune response by not only infecting the target cell but could do so by infecting the immunologically active cell as well. Epstein-Barr virus infection appears to trigger B cells to express autoantibodies against an array of tissue antigens; the target cell is not infected (Fong et al. 1981, 1982; Garzelli et al. 1984; Robinson and Stevens 1984). Infection of immunologically active cells outside the CNS is a distinct possibility following i.c. inoculation of TMEV (Clatch et al. 1986; Rauch and Montgomery 1986).

A common epitope, i.e. molecular mimicry, shared by the CNS and TMEV has been ruled out thus far by our studies, but is still feasible in other virus infections as suggested by apparent sequence homology between some viral proteins and nervous system proteins (Jahnke et al. 1985). Inasmuch as there are scattered reports of Ab[MBP] among MS patients (Gorny et al. 1983; Rabinowitz et al. 1983; Richert et al. 1983; Wajgt and Gorny 1983; Johnson et al. 1984), these mechanisms warrant further consideration.

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