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Characterization of measles virus-induced cellular autoimmune reactions against myelin basic protein in Lewis rats

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Summary

Subacute encephalomyelitis (SAME) in Lewis rats following infection with a neurotropic measles virus (MV) is associated with a cell-mediated autoimmune response (CMAI) to myelin basic protein (MBP). MBP-selected CD4⁺ T cell lines both from measles-infected animals as well as from rats challenged with guinea pig MBP (Gp-MBP) had a similar pattern of response in the presence of synthetic peptides to Gp-MBP and specifically responded *in vitro* only to the encephalitogenic and not the non-encephalitogenic or other control peptides. In primary splenic lymphocyte cultures from SAME animals, however, a low but significant T-cell response was obtained against the non-encephalitogenic peptide S67 (residues 69–81) of the Gp-MBP. Moreover, immunization of MV-infected rats with this peptide induced clinical and histological experimental allergic encephalomyelitis (EAE) in 38% of the animals. The results of the study show that the non-encephalitogenic peptide S67 can be rendered encephalitogenic in rats when an additional stimulus is given in the form of MV infection. The data indicate further that MV infection of the central nervous system (CNS) enhances the susceptibility of the CNS to autoimmune T cell aggression.

Introduction

Autoimmune reactions have been described in acute and chronic viral infections both with RNA and DNA viruses in animal and man (Notkins et al., 1984). Autoantibodies seen in the course of such viral infections are usually of low titer and

probably not involved in the development of a disease process since these antibodies disappear when the causative viral agent is cleared by the host defence mechanisms. In contrast, cell-mediated immune reactions against autoantigens (CMAI) persist longer, for example, in disseminated post-infectious encephalomyelitis (Behan et al., 1968; Lisak et al., 1974), in measles encephalitis (Gendelman et al., 1984; Johnson et al., 1984) or after rabies vaccination (Hemachudha et al., 1987a, b). While the pathologic role of this CMAI is largely unknown, it is nevertheless clear that clini-

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cal disease and neuropathological changes may be mediated by autoantigen-specific immune reactions such as is seen following the adoptive transfer of myelin basic protein (MBP)-specific CD4⁺ T cells and subsequent induction of experimental allergic encephalomyelitis (EAE) in rats and mice (Sedgwick et al., 1987; Zamvil et al., 1985).

Recently, we have observed that measles virus infection in Lewis rats leads to acute and subacute disease processes of the central nervous system (CNS) (Liebert and ter Meulen, 1987). Moreover, animals developing a subacute measles encephalomyelitis (SAME) contain T cells primed for MBP which may be isolated as MBP-specific CD4⁺ T cell lines capable of adoptively transferring EAE into naive syngeneic recipients (Liebert et al., 1988). Similar observations have been made in the JHM coronavirus-induced subacute demyelinating encephalitis (Watanabe et al., 1983).

In the present study we have characterized the CD4⁺ MBP-specific T cell lines and show that the amino acid sequence of the MBP region recognized by these lines is comprised within the major encephalitogenic region of guinea pig (Gp)-MBP which induces EAE in Lewis rats. By using a panel of synthetic peptides covering this Gp-MBP encephalitogenic region it was found that the fine specificity was identical in SAME and EAE-derived MBP-specific T cell lines. We further observed that measles virus (MV)-infected rats immunized with a non-encephalitogenic peptide of Gp-MBP developed EAE. These experiments indicate that MV infection of the CNS may not only lead to the generation and/or expansion of encephalitogenic MBP-specific CD4⁺ lymphocytes, but also may convert a non-pathologic CMAI to an encephalitogenic one. A combination of these two processes may contribute to a virus-induced immunopathological process within the CNS.

Materials and methods

Animals and experimental protocol

Inbred Lewis rats were obtained from the Zentralinstitut für Versuchstiere, Hannover, F.R.G. Three- to 4-week-old rats were intracerebrally inoculated with the neuro-adapted CAM/RBH measles virus (MV) strain or were

mock-infected with a rat brain homogenate containing no MV antigens as described (Kobune et al., 1983; Liebert and ter Meulen, 1987). From rats developing a SAME 4–8 weeks after infection, splenic lymphocytes were cultured during the disease period or within 3 weeks after clinical recovery. EAE was actively induced in 8-week-old Lewis rats by injection into the footpad of 100 µg guinea pig MBP and 100 µg *Mycobacterium tuberculosis* H37Ra (Difco) emulsified in 100 µl complete Freund's adjuvant. The passive transfer of EAE into naive syngeneic recipients aged 8–14 weeks required the administration via the tail vein of freshly activated antigen-specific T cell lines ($2-5 \times 10^6$). The clinical severity of actively or passively induced EAE was assessed using a scale of 0–5 (Lassmann, 1983). Histological examination was done on hematoxylin and eosin (H&E) and Luxol fast blue (LFB)-stained paraformaldehyde-fixed and paraffin-embedded brain and spinal cord sections (Liebert and ter Meulen, 1987).

Propagation and analysis of T cell lines

Spleen cell cultures were established and the cell-mediated immune response assessed with a T cell proliferation assay as described (Liebert et al., 1988). The lymphoproliferative response (LPR) of T cell lines was measured in counts per minute (cpm) of [³H]thymidine uptake and expressed as a stimulation index representing the ratio of the ³H uptake in stimulated compared with unstimulated cells according to the formula:

$$\frac{{}^3\text{H uptake (MV stimulated)}}{{}^3\text{H uptake (mock stimulated)}} \cdot \frac{{}^3\text{H uptake (mock unstimulated)}}{{}^3\text{H uptake (MV unstimulated)}}$$

T cell lines were separated from bulk polyclonal splenocyte cultures by alternating cycles of antigen restimulation in the presence of irradiated antigen-presenting cells (APC) and propagation in T cell growth factor (TCGF)-containing media as described (Liebert et al., 1988).

Antigens and peptides

Rat and guinea pig MBP were prepared from spinal cord and brain according to standard tech-

niques (Eylar et al., 1979). Proteolipid apoprotein (PLP) was a kind gift from Dr. Tabira, National Institute of Science, Tokyo, Japan, and MV antigen was prepared from infected Vero cells and purified as described previously (Liebert and ter Meulen, 1987). MV was inactivated by ultraviolet irradiation for 5 min and incubation at 56°C for 30 min. The MBP peptides were synthesized by the solid-phase method and purified to homogeneity as described (Hashim et al., 1986).

Results

Delineation of the fine specificity of MBP-specific T cell lines selected from animals with SAME and EAE

Four MBP-specific T cell lines were isolated from different Lewis rats with SAME. Details of their phenotypic and functional characteristics have been published (Liebert et al., 1988). The membrane phenotype was CD4⁺, CD8⁻, and activated line lymphoblasts induced EAE-like lesions when adoptively transferred into naive syngeneic recipients. In addition, five MBP-specific T cell lines were obtained from Lewis rats with EAE which revealed the same properties as those derived from SAME rats. To determine the fine specificity of these autoreactive T cell lines a panel of synthetic peptides summarized in Table 1 was used in lymphocyte proliferation assays. As shown in previous studies, the peptides of the Gp-MBP designated S49 and S55 are encephalitogenic in Lewis rats whereas the peptides S53 and S67 are non-encephalitogenic (Offner et al., 1987).

After the third or fourth in vitro restimulation, the T cell lines included in this study were highly MBP-specific and exhibited a significant lymphoproliferation (LPR) when purified MBP or peptides S49 and S55 were added to the cultures (Tables 2 and 3). The positive stimulation was obtained with MBP-selected T cells of SAME as well as EAE origin, and the pattern of reactivity against the different peptides was almost identical for the cell lines of both diseases. The stimulation indices (SI) varied to some extent between the individual cell lines. Higher SI were generally obtained with cells of EAE origin, but the range of the SI was overlapping with that seen with cell lines isolated from SAME animals. No significant LPR was observed upon the addition of the non-encephalitogenic peptides S53 and S67, and the cell lines did not proliferate in the presence of irrelevant antigens such as PLP, purified protein derivative (PPD) and MV and were thus antigen-specific. Furthermore, T cell lines with specificity for MV (SAME4-MV and SAME5-MV, Table 2) did not proliferate in the presence of Gp-MBP or its peptides.

Proliferative response of primary splenic lymphocyte cultures of animals with SAME

It cannot be excluded that the procedures used to obtain T cell lines resulted in the preferential selection of clones of restricted specificity. Therefore, the above results obtained with the lines may reflect only part of the in vivo situation since it is conceivable that the range of proliferative responses potentially present in primary lymphocyte cultures from MV-infected animals is much

TABLE 1
SYNTHETIC PEPTIDES FROM REGION 69-84 OF GUINEA PIG MBP

Region 69-84 of the guinea pig MBP (Gp-MBP) is shown together with peptides derived from it.

Code name	Peptide sequence	EAE activity in Lewis rats
Gp-MBP	*** G S L P Q K S Q - - R S Q D E N ***	+
S49	G S L P Q K S Q - - R S Q D E N G	+
S55	P Q K S Q - - R S Q D E N G	+
S53	S Q - - R S Q D E N G	-
Leu4-S53	L L L L S Q - - R S Q D E N G	-
S67	G S L P Q K S Q - - R S Q - - - G	-

broader. To test this possibility polyclonal splenic lymphocyte cultures from six randomly selected Lewis rats, that developed SAME 26–48 days after infection, were investigated for MBP specificities. As shown in Table 4, two of the six cultures from SAME rats responded to MBP with SI above 2.0. Positive LPR in the presence of encephalitogenic peptides was detected in these and two other cell populations (Nos. B732 and B745) that exhibited $SI \leq 2.0$ in the presence of Gp-MBP. However, two animals with incubation periods of 39 and 48 days respectively (Nos. B733 and B745) exhibited additionally a positive SI when the non-encephalitogenic peptide S67 was added to the cultures. SI obtained from naive control rats were consistently below 1.5.

Induction of EAE in MV-infected rats by immunization with non-encephalitogenic peptides

Previously it has been shown that only the immunization of rats with both peptides, S67 and S53, leads to EAE but given alone neither is effective (Offner et al., 1987; Hashim and Day,

1988). Therefore, the observation in SAME rats of a reactivity with peptide S67 led us to a set of experiments to define the role of MV infection in inducing the autoimmune response against this non-encephalitogenic peptide. For these experiments a dose of MV (10^3 TCID₅₀ (tissue culture infectious dose) of CAM/RBH) was chosen that failed to induce a clinically recognizable subacute CNS disease in animals. Histologically, these rats did not reveal changes indicative of an encephalitic disease process when examined 28–42 days post-infection. Moreover, neither infectious virus could be isolated from nor MV antigen was demonstrable in brain tissue of these animals. However, in serum and cerebrospinal fluid (CSF), low titers of neutralizing anti-measles antibodies were detected (in the range of 1:50 in serum and 1:10 in CSF) documenting the interaction of the host immune system with the infecting virus.

Such animals were immunized 4–8 weeks post-infection with either of the two non-encephalitogenic peptides Leu4-S53 or S67 emulsified in complete Freund's adjuvant (CFA). While the Leu4-

TABLE 2

PROLIFERATIVE RESPONSE OF MBP- AND MV-SPECIFIC T CELL LINES SELECTED FROM RATS WITH SAME

T cell lines with specificity for MBP (SAME-1 to SAME-4) or MV (SAME4-MV, SAME5-MV) were isolated from polyclonal splenocyte cultures from different rats with SAME. 2×10^4 line cells were incubated with 1×10^6 irradiated (2000 R gamma) thymocytes and 5–30 $\mu\text{g}/\text{ml}$ of purified antigens or peptides. The LPR was determined in triplicates (SD < 15%) and expressed as stimulation indices representing the ratio of the [³H]thymidine uptake in stimulated to unstimulated cells. ³H incorporation in cpm without antigen was 722 (SAME-1), 647 (SAME-2), 516 (SAME-3), 974 (SAME-4), 524 (SAME4-MV), and 2168 (SAME5-MV).

Antigen	T cell lines					
	MBP-specific				MV-specific	
	SAME-1	SAME-2	SAME-3	SAME-4	SAME4-MV	SAME5-MV
<i>Encephalitogenic</i>						
MBP	28.8	12.1	20.3	12.7	1.4	0.7
S49	31.5	6.9	17.6	7.7	0.9	1.1
S55	20.1	4.4	18.6	13.1	0.7	0.7
<i>Non-encephalitogenic</i>						
S53	1.2	0.8	0.6	1.0	0.6	0.9
Leu4-S53	1.1	0.7	0.4	0.8	1.0	0.9
S67	1.3	0.6	0.6	1.1	1.0	1.2
<i>Others</i>						
PPD	1.2	0.9	0.9	1.3	1.0	0.8
PLP	1.4	1.1	0.7	1.3	0.9	0.9
MV	1.5	1.1	1.1	1.3	29.3	15.1
<i>EAE-inducing capacity</i>						
	++	++	++	+	-	-

TABLE 3

PROLIFERATIVE RESPONSE OF MBP-SPECIFIC T CELL LINES SELECTED FROM RATS WITH EAE

T cell lines were isolated from Lewis rats 11–14 days after immunization with MBP when the classical signs of EAE developed both clinically and histologically. SI are shown in the table. The ^3H incorporation in cpm of unstimulated cells was 1654 (EAE-1), 3431 (EAE-2), 613 (EAE-3), 489 (EAE-4), and 785 (EAE-5).

Antigen	T cell lines				
	EAE-1	EAE-2	EAE-3	EAE-4	EAE-5
<i>Encephalitogenic</i>					
MBP	23.2	12.8	59.0	38.3	20.8
S49	19.2	4.0	51.3	25.7	22.5
S55	26.1	4.8	54.8	29.1	19.6
<i>Non-encephalitogenic</i>					
S53	0.5	1.0	0.8	0.6	0.7
Leu4-S53	0.4	1.0	1.2	0.7	0.6
S67	0.7	1.6	1.4	1.9	0.6
<i>Others</i>					
PPD	1.2	1.5	1.0	1.4	1.2
PLP	1.2	1.0	1.1	0.9	1.2
MV	0.6	1.3	0.8	0.7	1.5
<i>EAE inducing capacity</i>					
	+++	+	++	+++	+++

S53 peptide had no effect, immunization with S67 peptide led to clinical and histological changes. As shown in Table 5, two rats developed clinical disease consisting of limp tail, ataxia and paresis

TABLE 4

PROLIFERATIVE RESPONSE OF PRIMARY SPLEEN CELL CULTURES SELECTED FROM RATS WITH SAME

The SI (representing the ratio of thymidine uptake in stimulated cells from MV-infected rats multiplied by the uptake in unstimulated cells from mock-infected rats compared to stimulated cells from controls multiplied by unstimulated cells from MV-infected rats, as outlined in the Materials and Methods section) of six randomly selected rats (B709 through B745) that developed SAME 26–48 days after infection with MV are shown. $1-3 \times 10^5$ splenic lymphocytes were incubated with and without antigens (5–50 $\mu\text{g}/\text{ml}$).

Antigen	Number of animals						
	B709	B720	B721	B732	B733	B745	Control ^a
None ^b	1483	786	1055	990	1461	1336	1217 \pm 367
MV	2.5	2.9	3.5	3.9	5.1	2.4	0.9 \pm 0.4 (1040)
MBP	2.6	1.0	0.9	2.0	2.3	1.7	0.9 \pm 0.1 (1119)
S49	3.6	1.4	1.4	2.2	2.4	2.7	0.9 \pm 0.2 (1144)
S55	n.d.	1.3	1.4	1.7	1.9	2.2	1.1 \pm 0.3 (1391)
S53	0.5	0.9	1.2	1.1	0.7	1.1	1.1 \pm 0.3 (1375)
S67	0.7	1.1	1.6	1.6	2.1	2.3	1.1 \pm 0.1 (1283)

^a The control values of six mock-infected rats represent the mean \pm SD of SI (thymidine uptake expressed as cpm).

^b The [^3H]thymidine uptake in cpm in unstimulated cells is shown.

TABLE 5

IN VIVO EFFECT OF IMMUNIZATION WITH MBP PEPTIDES AFTER SUBCLINICAL MV INFECTION

Treatment of animals ^a		Clinical disease	Histology of EAE
Infection	Challenge		
MV i.c.	None	0/16	0/16
mock-infected	Leu4-S53	0/6	0/6
mock-infected	S67	0/9	0/9
MV i.c.	Leu4-S53	0/6	0/6
MV i.c.	S67	4/14	5/14
MV i.c. (inactivated)	S67	0/8	0/8
MV i.p.	S67	0/9	0/9
None	MV + S67	0/8	0/8

^a Three-week-old rats were infected with CAM/RBH (10^3 TCID₅₀ i.c. or 10^6 TCID₅₀ i.p.). No clinical disease developed and histological lesions indicative for an active encephalitic process were not detected. Four to 8 weeks post-infection the animals were immunized with antigen (100 μg of peptide or MV antigen) in complete Freund's adjuvant. The statistical evaluation by means of the Fisher test revealed a > 99% probability that the results are not randomly distributed.

of hind legs whereas three of the eight animals revealed histological EAE lesions with perivascular cuffing of lymphomonocytic cells in the lumbar spinal cord (Fig. 1). The distribution of these pathological changes is distinctly different from the MV-induced CNS lesions which are chiefly

located in the grey and white matter of the cerebral hemispheres, midbrain and upper spinal cord. The peptide S67-induced disease is not due to activa-

tion of MV in the brain of immunized Lewis rats because MV could not be isolated from brain material of these three rats and no measles antigen

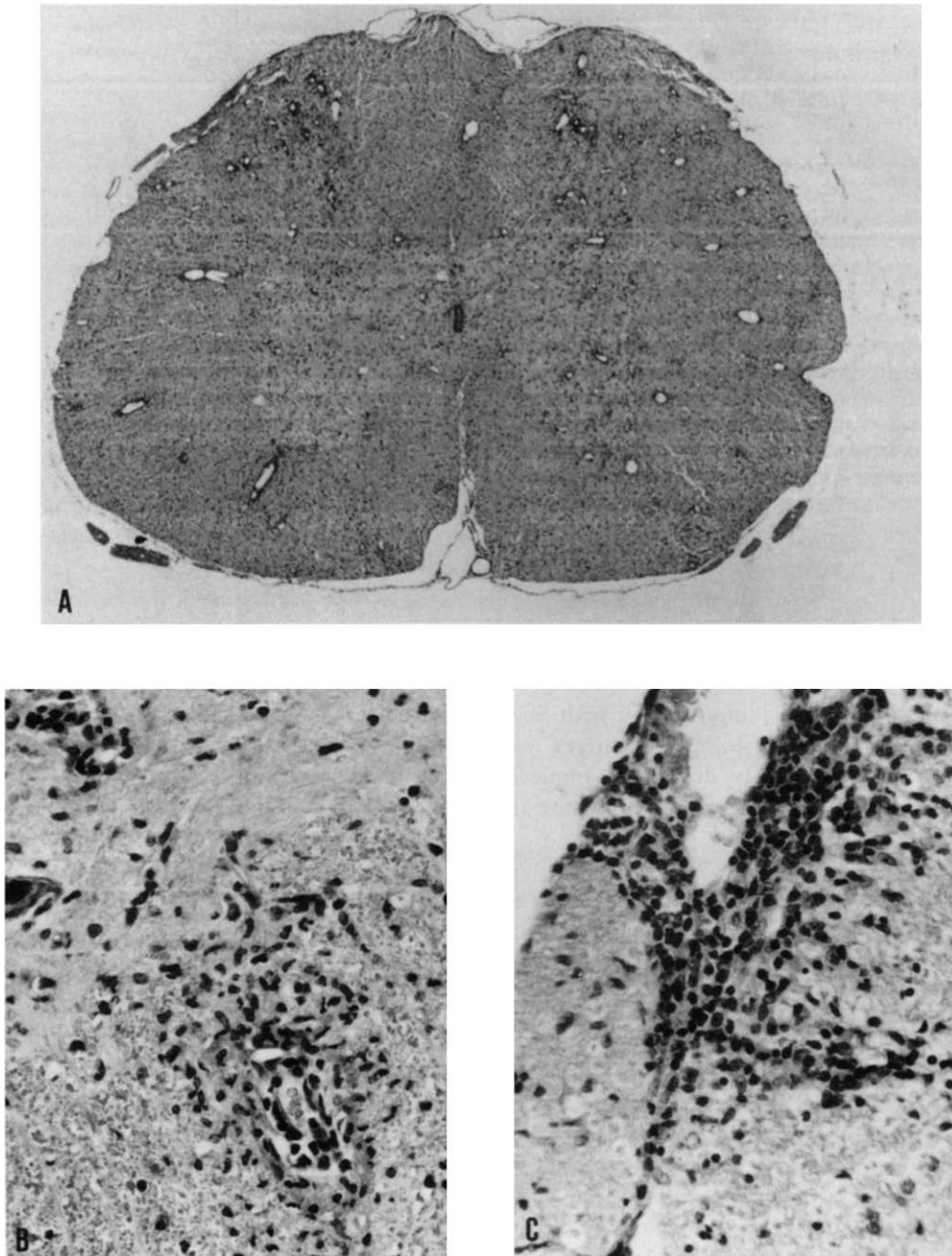


Fig. 1. H&E-stained cross-section through lower spinal cord of formalin-fixed, paraffin-embedded material obtained 16 days after immunization with peptide S67. The Lewis rat was infected intracerebrally with a subclinical dose of MV 71 days previously. The histology shows lesions typical for actively induced EAE with perivascular lymphomonocytic cuffing. (A) Overview, (B) perivascular infiltrate in anterolateral white matter, (C) subpial infiltrate at root-entry zone. Magnification $\times 80$ (A), and $\times 320$ (B and C).

was detected immunohistologically. However, the occurrence of EAE after immunization with the non-encephalitogenic S67 peptide appeared to depend initially on MV replication in brain tissue since neither intraperitoneal injection of infectious virus nor the intracerebral inoculation of heat-inactivated MV or the simultaneous immunization with S67 peptide and purified MV antigen resulted in EAE or histological lesions in brain and spinal cord.

Discussion

The results of this study demonstrate that the MBP-selected T cell lines from MV-infected Lewis rats specifically recognize epitopes within the sequence of the Gp-MBP region (amino acid residues 69–84) that comprise the major encephalitogenic sequence for Lewis rats. The MBP-specific T cell lines from both measles-infected and MBP-challenged rats share the same fine specificity and they respond to *in vitro* stimulation with the encephalitogenic peptides S49 and S55 of Gp-MBP, but not to the non-encephalitogenic peptides S53 and S67. This high degree of antigenic specificity is further supported by the failure of all these T cell lines to proliferate in the presence of MV particles, MV antigens and other control antigens or peptide sequences. In contrast, MV-specific T cell lines only respond to MV proteins but not when MBP or synthetic peptides were added to the cultures (Table 2).

We also looked for possible additional epitope specificities in the polyclonal spleen cell cultures from MV-infected rats. An additional reaction to the non-encephalitogenic S67 peptide was found in some rats but not to the other peptides included in this study. This observation is in line with perviously published results (Mannie et al., 1985). Although the SI appear low compared to the reaction obtained from regional lymph nodes after adjuvant-aided immunization with autoantigens, the values for thymidine incorporation in presence of the S67 peptide of animals Nos. B733 and B745 clearly differ from the naive controls (Table 4). It has also to be remembered that we looked at a non-preselected population of splenic lymphocytes with many specificities and that no stimulus to the immune system other than the viral infection was

given. In such a situation it is known that antigen-specific lymphoproliferation is always low also in viral antigens (B. Askonas, personal communication). Nonetheless, from two spleen cell cultures from SAME rats, CD4⁺ T cell lines with specificity for the S67 peptide could be isolated (data not shown). Although autoreactive T cell lines can be isolated occasionally from naive rats this requires sophisticated methods that were not used in the present study (Schluesener and Wekerle, 1985). Unlike S49, the peptide S67 is not sufficient to induce EAE unless a concomitant immune response occurs against non-encephalitogenic peptide S53. As recently shown, this response is of a humoral nature whereas peptide S67 predominantly induces a T cell reaction (Hashim et al., 1988). Surprisingly, the immune responses to S67 peptide immunization and MV infection act synergistically in the induction of EAE similarly as immunization with both peptides S67 and S53. This phenomenon was not observed when rats were infected intraperitoneally or were preimmunized with inactivated measles virus, indicating that MV replication in brain cells is probably a prerequisite for a CNS autoimmune reaction to occur in this system.

The antigenic specificity of T cell lines for the 69–84 encephalitogenic sequence of Gp-MBP together with the lack of responses to viral antigens suggest that MV particles do not apparently directly contribute antigenic sequences for the development of MBP responses. While sequence homologies have been found between the N and C proteins of MV and human MBP (Jahnke et al., 1985), direct immunization of Lewis rats with inactivated MV fails to induce EAE-like activity or the expansion of autoreactive MBP-specific T cells, probably because pathologically relevant homologous sequences between Lewis rat MBP and MV do not exist (data not shown). Therefore, it does not appear that the phenomenon of 'molecular mimicry' plays a role in the establishment of the autoimmune reaction against MBP.

An alternative explanation may be related to the recent demonstration that the encephalitogenic region of Gp-MBP (residues 69–84) houses different epitopes inducing either predominant cellular or humoral immunity and that the immunological expression of either epitope alone does not lead to

the development of EAE in the Lewis rat (Hashim et al., 1986, 1988; Offner 1987). In the case of the experiments reported here where MV-infected Lewis rats were immunized with peptide S67, the MV infection could have generated a humoral immune response that leads to antibodies which interact with MBP. So far, preliminary attempts to detect antibodies against peptide S53 in the animal model have been unsuccessful. However, one also has to keep in mind that more than one encephalitogenic sequence may be expressed on the MBP molecule. For human MBP-specific T cells, for example, as many as ten potential T cell epitopes have been found by comparative proliferation with MBP and its fragments from different species that show limited amino acid sequence differences (Richert et al., 1989). Furthermore, it has been shown that the T cell repertoire for rat MBP includes encephalitogenic clones with reactivity to non-69-84 determinants (Happ and Heber-Katz, 1988). A minor I-E-restricted sequence encephalitogenic for the Lewis rat was defined recently as peptide S87-99 of Gp-MBP (Offner et al., 1989).

The observation that viral infections may enhance the susceptibility to EAE has been made in other studies showing that a preceding MV infection potentiates the development and severity of EAE in guinea pigs (Massanari et al., 1979) or that a Semliki Forest virus infection helps to induce EAE in resistant B6 mice (Mokhtarian and Swoveland, 1987). To explain this phenomenon several possibilities exist. It is conceivable that virus-induced damage to CNS tissue facilitates the subsequent priming or clonal expansion of pre-existing myelin-reactive T cells for example, via the induction of major histocompatibility complex (MHC) class II on astrocytes (Massa et al., 1987) and efficient presentation of neural antigen by these cells (Fontana et al., 1984; Sun and Wekerle, 1986). Additionally, in measles viral infection, alterations in the surface of infected cells occur potentially resulting in the exposure of cellular components together with the viral envelope proteins (Notkins et al., 1984). Such exposure taking place in infected brain cells may lead to the development of immunity against fragments of MBP that usually do not elicit immunogenic responses. Moreover, changes in the integrity of the blood-

brain barrier as a consequence of a viral infection of the brain may facilitate the entry of antigen-specific CD4⁺ T cells to the CNS.

It will be important to further investigate and search for measles virus-induced immune reactions that recognize MBP since our findings indicate that in MV-infected Lewis rats the immunological response to viral antigen complements the T cell responsiveness to a normally non-encephalitogenic MBP epitope. Obviously, the development of virus-induced autoimmune reactions against CNS tissue depends on several factors which have to be analyzed before one may fully understand how a virus infection leads to immune pathological reactions. It is tempting to speculate that a similar phenomenon occurring in a susceptible host may lead to chronic inflammatory CNS diseases such as multiple sclerosis.

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