Since January 2020 Elsevier has created a COVID-19 resource centre with free information in English and Mandarin on the novel coronavirus COVID-19. The COVID-19 resource centre is hosted on Elsevier Connect, the company's public news and information website.

Journal of Neuroimmunology, 17 (1988) 103–118
Elsevier

JNI 00543

Induction of autoimmune reactions to myelin basic protein in measles virus encephalitis in Lewis rats

Uwe G. Liebert, Christopher Linington [1,*] and Volker ter Meulen

Institut für Virologie und Immunbiologie, Universität Würzburg, Würzburg, and [1] Max-Planck-Gesellschaft, Klinische Forschungsgruppe für Multiple Sklerose, Würzburg, F.R.G.

(Received 24 June 1987)
(Accepted 3 July 1987)

Key words: Autoimmunity; Myelin basic protein; Measles virus encephalitis; Encephalomyelitis

Summary

Intracerebral inoculation of weanling Lewis rats with measles virus led to the development of subacute measles encephalomyelitis (SAME) 4–8 weeks after infection. The disease is characterized pathologically by an intense inflammatory infiltration within both the white and grey matter of the central nervous system (CNS) without apparent demyelination. Both during and after SAME splenic lymphocytes from these animals could be restimulated in vitro to proliferate in the presence of myelin basic protein (MBP). MBP-specific class II MHC-restricted T cell lines were isolated from this cell population. They were shown to exhibit no cross-reactivity with measles virus and to induce experimental allergic encephalitis (EAE) in naive syngeneic recipients following adoptive transfer. The clinical and histopathological signs of this T cell-mediated disease were identical to that seen in classical T cell-mediated EAE. A humoral immune response to MBP was only detected in a

Address for correspondence: V. ter Meulen, Institut für Virologie und Immunbiologie, Universität Würzburg, Versbacher Str. 7, 8700 Würzburg, F.R.G.

* Present address: Department of Medicine, University of Wales, College of Medicine, Heath Park, Cardiff CF4 4XN, U.K.

Abbreviations: SAME, subacute measles encephalomyelitis; CNS, central nervous system; MBP, myelin basic protein; MV, measles viral antigen; VC, Vero cell membrane antigen; PPD, purified protein derivative of tuberculin; EAE, experimental allergic encephalomyelitis; BM, basic culture medium; TCGF, T cell growth factor; APC, antigen presenting cells; CFA, complete Freund's adjuvant; KLH, keyhole limpet hemocyanin.

limited number of those rats with SAME. These results indicate that autoimmune reactions to brain antigen can arise during measles virus infection which may contribute to the pathogenesis of measles virus-associated encephalomyelitis.

Introduction

Measles, a highly contagious infectious disease of man caused by a paramyxovirus, is associated with a CNS complication known as post-infectious encephalitis. This disease occurs with a frequency of 1 in 1000–2000 patients and has a fatal outcome in 10–20% of the cases (Miller et al., 1956). The pathogenesis of post-infectious encephalitis is not understood. Histopathologically a perivenous demyelinating encephalomyelitis is found without detection of measles virus antigen. Most attempts to recover infectious virus from brain tissue have been unsuccessful, suggesting that measles virus probably does not replicate in the brain during post-infectious encephalitis (Gendelman et al., 1984). At present it is thought that an autoimmune reaction to CNS tissue may play a pathogenetic role since in some patients with this CNS disease peripheral blood lymphocytes proliferate in vitro in the presence of MBP (Johnson et al., 1984). However, definite proof of an existing immune-mediated process in post-infectious encephalitis is missing. In view of the medical importance of measles virus infection and its possible association with chronic inflammatory CNS diseases such as multiple sclerosis, it is desirable to study the aspect of measles virus-induced autoimmune reactions in an experimental animal model. In the following, experiments are presented which document that in Lewis rats humoral and cell-mediated immune reactions to MBP of potential pathological significance occur during the course of a non-demyelinating subacute measles virus encephalomyelitis.

Materials and methods

Animals and infection

Three- to 4-week-old inbred Lewis rats (Zentralinstitut für Versuchstiere, Hannover, F.R.G.) were intracerebrally inoculated with 5×10^4 plaque forming units in a volume of 25 μl of the rat brain-adapted measles virus (CAM) originally obtained from Dr. Yamanouchi (Measles Virus Institute, Tokyo, Japan). This virus was further passaged three times on Vero cells (monkey kidney fibroblasts, ATCC, Rockville, MD) before inoculation. Attempts to recover infectious virus either directly from brain homogenates or by cocultivating the brain tissue with Vero cells were performed as described previously (Liebert and ter Meulen, 1987).

Isolation and propagation of T cell lines

T cell lines were separated from bulk cultures of rat spleen cells employing similar methods which were used previously to isolate antigen-specific T cells from

rat lymph nodes (Ben-Nun et al., 1981; Linington et al., 1984). Single cell suspensions (10^7/ml) were cultured in medium to which indomethacin (2 μg/ml, Sigma, Munich, F.R.G.), and the relevant antigen were added. The basic medium (BM) for all T cell cultures was RPMI 1640 (Biochrom, West Berlin, Germany), supplemented with L-glutamine (2 mM), sodium pyruvate (1 mM), non-essential amino acids (1%), 2-mercaptoethanol (0.05 mM), antibiotics and 1% syngeneic rat serum. After 72 h viable cells were separated by centrifugation on a Ficoll density gradient (Pharmacia, Freiburg, F.R.G.) and expanded for 3–10 days in BM containing 10–15% TCGF and 10% FCS. T cell growth factor (TCGF) was obtained as follows. Spleen cells from Lewis rats were cultured for 24–36 h at 10^7 cells/ml in BM containing 2% fresh rat serum, 2 μg/ml indomethacin and 5 μg/ml concanavalin A (ConA, Pharmacia). The cell suspensions were centrifuged, and the conditioned supernatants pooled and stored at $-20\,^{\circ}$C. Their ability to maintain T cell growth was tested with a TCGF-dependent T cell line and concentrations of 15–20% were used in all experiments. After expansion the T cell lines ($2–4 \times 10^5$/ml) were mixed with irradiated (3000 R) syngeneic spleen and thymus cells serving as antigen presenting cells (APC, 8×10^6/ml) and cultured in presence of optimal concentrations of antigen. Alternating cycles of restimulation in presence of irradiated APC and antigen, and propagation in TCGF containing media were repeated at intervals of 10–14 days. At each restimulation the cells were tested in vitro for antigen specificity.

T cell proliferation test

For primary in vitro restimulation, single cell suspensions ($3–10 \times 10^5$) from the spleens of measles virus-infected or control rats were incubated in 200 μl BM in round-bottomed microtiter plates in presence of antigen in optimal concentrations. T line cells ($1–6 \times 10^4$/well) were cultured together with X-irradiated APC ($5–10 \times 10^5$/well) and antigen. After 48 h tritiated thymidine (0.2 μCi/well, spec. act. 43 Ci/mM, Amersham, Braunschweig, F.R.G.) was added. The cultures were harvested 18–24 h later with a Titertek multiple harvester (Flow Laboratories, Irvine, U.K.) and incorporation of tritiated thymidine was determined with a liquid scintillation counter (Beckman, Munich, F.R.G.).

Antigens

Rat MBP was prepared from spinal cord and brain according to standard techniques (Eylar et al., 1979). Measles virus antigen (MV) was prepared as described (Liebert and ter Meulen, 1987). Briefly, Vero cells were infected with measles virus CAM. Cultures exhibiting 90–95% measles virus cytopathic effect were harvested and cells were disrupted by Dounce homogenization in PBS. After centrifugation at $17\,000 \times g$ for 20 min, the supernatant was further centrifuged at $85\,000 \times g$ for 90 min. The virus-enriched material in the pellet was resuspended in buffer and banded on a 60% sucrose cushion ($85\,000 \times g$ for 90 min). The band containing the virus was further purified by centrifugation on a potassium tartrate gradient (15–40%, $85\,000 \times g$ for 8–12 h). The pellets of the virus containing

fractions were resuspended in PBS and the measles viral antigen (MV) was UV-inactivated (5 min) and X-irradiated (10000 R). Total Vero cell (VC) membranes were obtained by homogenisation of cells in PBS followed by centrifugation (85000 × *g*/90 min). The pellet was resuspended to a concentration of 10 mg/ml protein as determined by the Bradford method (Bio-Rad kit, Bio-Rad, Munich, F.R.G.). Tuberculin (PPD, Statens Seruminstitut, Copenhagen, Denmark) and hemocyanin (KLH, Sigma) were purchased.

Analysis of the membrane phenotype of antigen-specific T line cells

The cell surface phenotype of antigen-specific T line cells from SAME animals was determined by indirect immunofluorescence using mouse monoclonal antibodies to rat pan-T cell (W3/13), T helper cells (W3/25), T cytotoxic/suppressor cells (OX8), rat Ia (OX6, OX17) (Seralab Biochrom, West Berlin, Germany). Immunofluorescence was measured with a cytofluorograph system 30-50 (Ortho Instruments, Westwood, CA, U.S.A.).

Determination of the humoral immune response

The humoral immune response to measles virus and myelin basic protein was determined with an enzyme-linked immunoassay (EIA) as described (Wege et al., 1984). Measles virus antigen, control Vero cell membrane antigen and MBP were calibrated by block titrations for reproducible sensitivity. Flat-bottomed microtiter plates (Nunc, Wiesbaden, F.R.G.) were coated in duplicates with 0.5 or 1 μg per well of antigens. Subsequently, the plates were incubated with serum dilutions and then with a peroxidase conjugate of anti-rat immunoglobulin (Dakopatts, Hamburg, F.R.G.). Orthophenylene diamine served as substrate. The optical densities were determined with a multichannel spectrophotometer at 492 nm. The EIA titer was defined by the serum dilution giving an optical density (OD measles − OD Vero) greater than 0.2. Serum samples giving positive titers with either antigen were absorbed for 2 h at 37 °C under constant stirring with MBP or measles virus, respectively and retested in the same experiment with non-absorbed serum samples.

Clinical assessment of EAE after adoptive transfer

All animals were weighed and examined daily for neurological signs. The clinical severity of the disease was graded on a scale of 0–5 (Lassmann, 1983).

Histological procedures

Brain and spinal cord were fixed in freshly prepared 4% paraformaldehyde in 0.1 M phosphate buffer, pH 7.4 (Liebert and ter Meulen, 1987). Sections were cut from paraffin blocks and stained with hematoxylin and eosin. Immunohistological investigation for presence of measles viral antigen was done on sections, 20–40 μm thick, cut from paraformaldehyde-fixed material with a Vibratome 1000 (TPI, St. Louis, MO, U.S.A.). In a modified PAP technique (Liebert et al., 1985) rabbit hyperimmune serum against measles viral structural proteins was used as first antibody.

Results

Subacute measles encephalomyelitis

The intracerebral inoculation of weanling Lewis rats with CAM measles virus led to the occurrence of an acute encephalitis in about 70% of infected animals. The acute disease was invariably fatal. About 50% of those rats which survived the infection developed a subacute measles encephalomyelitis (SAME) after incubation periods ranging from 4 to 9 weeks following the intracerebral infection. The disease was characterized by weight loss, unsteadiness, and abnormal posturing of the limbs with hindleg paresis. Approximately 40% of the diseased animals died during the course of this monophasic disease and no relapses have been noted in survivors. The neuropathological changes of the SAME animals consisted of extensive perivascular infiltrations of mononuclear cells throughout the entire central nervous system (Fig. 1*A*). Infectious virus could not be recovered either directly from brain homogenates or by cocultivation techniques, and viral antigen was only occasionally detected in sporadic cells (Fig. 1*B*). Animals which were killed at various intervals after recovery from clinical disease had neuropathologically either persisting inflammatory lesions or residual changes typical of a previous encephalitic attack in the absence of immunohistologically demonstrable viral antigen. A detailed description of the virological aspects of measles virus-induced encephalitis in rats has been presented elsewhere (Liebert and ter Meulen, 1987).

Determination of cellular immune reaction

The discrepancy of persisting mononuclear cell infiltration and absence of viral antigen within the sensitivity of the test employed, directed us to investigate the cellular immune reaction of infected animals. Single cell suspensions from spleen cells were restimulated with antigen or mitogen. As summarized in Table 1 the in vitro proliferative response of spleen cells to MBP was significantly elevated in five from eight clinically ill SAME animals (group 1) and in nine out of 14 animals which had recovered from clinical disease but still had histologically active encephalitis (group 2). Although the observed stimulation indices (SI) for MBP are low in SAME when compared to those animals with EAE (group 4) the mean SI (2.3, groups 1 + 2) is still higher than that of those controls not exposed to MBP (groups 5, 6 and 7, mean = 0.97). This cellular response to MBP was detected for up to 270 days post-infection. However, in those animals which had recovered from SAME and histologically had no signs of an active encephalitis no significant in vitro proliferation to MBP was observed (group 3). Furthermore, positive proliferative responses to MBP in the various control groups were only seen in animals in which EAE was actively induced (group 4). Moreover, all measles-infected (groups 1–3) or immunized (group 7) animals exhibited a significant proliferative response to measles virus independent of their clinical and neuropathological status. In the control groups 4, 5 and 6 which had not been exposed to the virus no proliferative response to MV could be elicited. Significant in vitro proliferation to the other antigens, VC, KLH and PPD, was not observed in any group.

TABLE 1

PROLIFERATIVE RESPONSES OF SPLEEN LYMPHOCYTES FROM RATS WITH SAME

Spleen cells were incubated in BM (control) or in presence of inactivated MV (10 μg/ml), rat MBP (20 μg/ml), KLH (20 μg/ml), VC (40 μg/ml), PPD (30 μg/ml), or ConA (2.5 μg/ml). The results represent the mean of triplicate determinations with standard deviations not exceeding 20% except in isolated instances. Stimulation indices listed in parentheses were calculated as ratio of cpm antigen/cpm control. Significant responses are underlined.

	Animal No.	Days p.i. [a]	[^{3}H]Thymidine incorporation (cpm)					
			Control	ConA	MV	MBP	VC	KLH or PPD [b]
Group 1								
Diseased rats	1	27	1593	63769	3206 (2.0)	3241 (2.0)	ND	ND
with SAME [c]	2	27	843	58711	2631 (3.1)	1298 (1.5)	ND	1208 * (1.4)
	3	27	1481	90632	5182 (3.5)	2315 (1.6)	1086 (0.7)	1866 * (1.3)
	4	29	1344	85928	9249 (6.9)	3832 (2.9)	ND	1425 * (1.1)
	5	33	312	53018	780 (2.5)	456 (1.5)	452 (1.4)	599 * (1.9)
	6	34	784	73410	3011 (3.8)	1832 (2.3)	1114 (1.4)	1063 (1.4)
	7	48	615	58418	2318 (3.8)	2004 (3.3)	1001 (1.6)	724 (1.2)
	8	65	836	56671	2783 (3.3)	2292 (2.7)	993 (1.2)	928 (1.1)
	Average		876	67570	3645 (3.6)	2159 (2.2)	929 (1.3)	–
Group 2								
Recovered SAME	1	44	1390	74803	8618 (6.2)	3614 (2.6)	1648 (1.2)	1299 * (0.9)
rats with	2	50	588	94643	1549 (2.6)	1034 (1.8)	ND	ND
inflammatory	3	53	386	47588	1863 (4.8)	1267 (3.3)	ND	626 * (1.6)
lesions [d]	4	60	1380	90267	3851 (2.8)	4186 (3.0)	1839 (1.3)	1715 (1.2)
	5	66	2384	64727	14685 (6.2)	3569 (1.5)	ND	1478 * (0.6)
	6	80	659	43504	2370 (3.6)	1323 (2.0)	1139 (1.7)	919 * (1.4)
	7	98	995	95210	2285 (2.3)	2457 (2.5)	ND	ND
	8	100	1150	89411	3486 (3.0)	2080 (1.8)	866 (0.8)	915 (0.8)
	9	132	456	47915	1884 (4.1)	1046 (2.3)	483 (1.1)	706 (1.5)
	10	136	843	61426	3053 (3.6)	1366 (1.6)	615 (0.7)	886 (1.1)
	11	158	711	53014	2601 (3.7)	1975 (2.8)	496 (0.7)	1092 (1.5)
	12	172	1226	51269	3721 (3.0)	4148 (3.4)	873 (0.7)	1386 (1.1)
	13	272	436	83560	2108 (4.8)	1026 (2.4)	ND	ND
	14	274	693	88006	2093 (3.0)	1275 (1.9)	ND	ND
	Average		950	70382	3869 (3.8)	2169 (2.4)	920 (1.0)	–

(continued)

TABLE 1 (continued)

	Animal No.	Days p.i. [a]	[^{3}H]Thymidine incorporation (cpm)					
			Control	ConA	MV	MBP	VC	KLH or PPD [b]
Group 3								
Recovered SAME rats without inflammation [d]	1–26							
	Range	29–307	383–2692	38989–104568	890–10470 (2.0–9.5)	292–2310 (0.4–1.8)	512–1956 (0.6–1.5)	712–2481 (0.7–1.9)
	Average		990	72404	3397 (3.8)	863 (0.9)	1102 (1.1)	1241 (1.3)
Group 4								
EAE rats	1–7							
	Range	11–13	823–4950	39220–81353	481–2819 (0.5–0.6)	4779–30944 (5.8–20.8)	ND	2259–5866 (1.1–3.0)
	Average		2054	61378	1362 (0.6)	16421 (9.8)		3198 * (1.6)
Group 5								
Normal rats	1–8							
	Range	–	418–1882	32317–79935	470–1786 (0.8–1.2)	387–1812 (0.6–1.1)	ND	ND
	Average		692	50913	729 (1.1)	603 (0.9)		
Group 6								
Mock infected rats [c]	1–8							
	Range	20–48	843–1954	36445–73812	962–1639 (0.8–1.2)	1109–2026 (0.9–1.3)	1037–1792 (0.8–1.7)	911–1748 (0.6–1.3)
	Average		1401	61716	1238 (0.9)	1325 (1.1)	1468 (1.0)	1170 (0.8)
Group 7								
Rats immunized with MV [f]	1–6							
	Range	11–13	926–2104	49811–66415	5794–9934 (4.5–9.8)	677–1798 (0.7–1.1)	784–2815 (0.7–1.8)	ND
	Average		1426	58771	8156 (6.1)	1225 (0.9)	1513 (1.1)	

[a] Days p.i. = days post-infection/immunization, indicating time period between inoculation and sampling of spleen cells.
[b] Proliferative response to either KLH or PPD measured, the latter are marked with an asterisk.
[c] Spleen cells were obtained at days 1 or 2 of clinical disease.
[d] Spleen cells were obtained between 2 and 34 weeks after recovery from SAME.
[e] Animals were injected with supernatant from Vero cell cultures.
[f] Animals received 100 μg of purified MV in complete adjuvant.

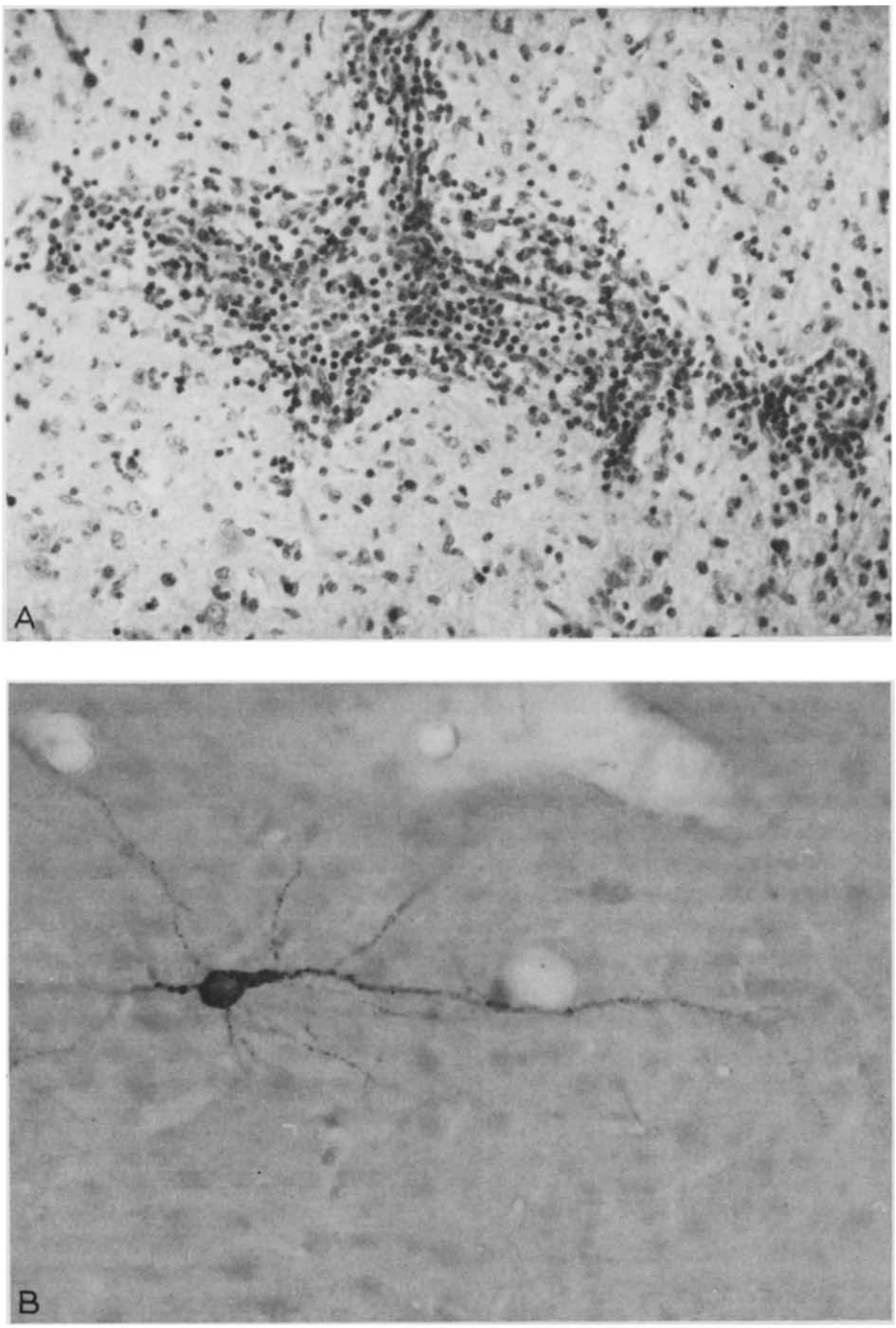

Fig. 1. (*A*) Subacute measles encephalomyelitis, 34 days, p.i. (animal No. 6, group 1, Table 1). Perivascular lymphomonocytic infiltration in the periventricular region of the parietal cortex. Hematoxylin and eosin, ×350. (*B*) Subacute measles encephalomyelitis, 29 days p.i. (animal No. 4, group 1, Table 1). Cortical neurone stained with hyperimmune anti-measles serum in a PAP technique with diaminobenzidine (Sigma) serving as substrate. Vibratome section, 25 μm thick, no counterstaining, ×400.

TABLE 2

HUMORAL IMMUNE RESPONSE TO MEASLES VIRUS AND MBP IN SAME RATS

Animal group No. [a]		MV	MBP	MV	MBP	MV	MBP
		Before absorption		After absorption with MV		After absorption with MBP	
2.	6	128 [b]	128	< 4	64	128	< 2
	8	16	4	< 4	4	32	< 2
	11	64	128	< 4	128	32	< 2
	13	64	500	< 4	256	32	< 2
4.	1	4	2000	ND	2000	ND	< 2
	2	4	8000	ND	4000	ND	< 2
	3	4	2000	ND	2000	ND	< 2
	4	4	1000	ND	1000	ND	< 2
	5	4	8000	ND	8000	ND	< 2
	6	4	4000	ND	4000	ND	< 2
	7	4	8000	ND	4000	ND	< 2
7.	1	2000	< 2	< 4	ND	500	ND
	2	1000	< 2	< 4	ND	500	ND
	3	4000	< 2	< 4	ND	2000	ND
	4	4000	< 2	< 4	ND	4000	ND
	5	2000	< 2	< 4	ND	2000	ND
	6	500	< 2	< 4	ND	500	ND

[a] Refers to group and animal number in Table 1.

[b] Reciprocal titers are listed as obtained in EIA. All sera from animals of the respective groups 1, 2, 3, 5 and 6 not listed were negative for anti-MBP antibodies.

Determination of humoral immune response

The humoral immune response to measles virus in serum of infected rats was determined by an enzyme immunoassay (EIA). During the clinical disease and in recovered SAME animals both with and without active encephalitis titers ranged from 1 : 16 to 1 : 256, but rose to high levels in animals actively immunized with MV (Table 2, groups 2 and 7). Low titers of circulating antibodies against MBP were detected only in some animals in group 2, which contrasted to the far higher titers found in animals actively immunized with MBP (Table 2). After absorption of the sera with measles virus antigen they still reacted with MBP but not with measles virus, while absorption with MBP led to the abolition of MBP reactivity.

In vitro and in vivo studies with T cell lines

T cell lines specific for either MBP or MV were established from SAME animals employing similar methods as previously used to generate antigen-specific T cell lines from rat lymph nodes (Ben-Nun et al., 1981; Linington et al., 1984). The antigen specificity of these T cell lines was tested with a panel of antigens including rat MBP, MV, Vero cell membrane antigen (VC), tuberculin (PPD) and hemocyanin (KLH). All of the lines established exhibited no reactivity with the inappropriate antigen (Table 3). This demonstrates that there was no cross-reactivity between the

TABLE 3

SPECIFICITY OF MBP- AND MV-SPECIFIC T CELL LINES DERIVED FROM SAME ANIMALS

The results represent the mean of triplicates with standard deviations below 15%.

	[^{3}H]Thymidine incorporation (cpm)						
	Control	ConA	MBP	MV	PPD	VC	KLH
MBP-SAME1	5833	63980	30009	6929	5270	3549	ND [a]
MBP-SAME2	3108	88215	28300	2748	3044	2356	3218
MBP-SAME3	1975	69091	39653	2615	1837	1988	1453
MBP-SAME4	18216	68134	67243	12576	ND	2031	ND
MPB-SAME4	2135	62614	3064	47060	2639	1891	1908
MPB-SAME5	9634	73357	6812	48849	8120	7705	9008
MPB-EAE [b]	4632	57963	62441	3811	4163	ND	5767

[a] ND = not determined.

[b] Derived from a Lewis rat in which EAE was actively induced (100 μg guinea pig MBP, 100 μg *Mycobacterium tuberculosis* H37Ra (Difco, Detroit, MI, U.S.A.) in 100 μl complete Freund's adjuvant).

MBP and MV epitopes recognized by the T cell lines established in these experiments. The phenotype of the lines was W3/13$^+$, W3/25$^+$, OX8$^-$ as determined using a panel of monoclonal antibodies specific for lymphocyte differentiation markers (Table 4). The lines are therefore predominantly of the class II MHC-restricted 'helper' subset.

To investigate the possible pathological effects of the T cell lines in vivo, activated T cell blasts from each of the MBP-specific and MV-specific T cell lines were adoptively transferred into naive Lewis rats via the tail vein. Each of the MBP-specific T cell lines was effective at a dose of 6×10^6 cells and induced some clinical and histological signs typical for EAE in the recipients (Table 5). In addition, graded doses of cells of the line MBP-SAME3 were adoptively transferred

TABLE 4

CHARACTERIZATION OF THE SURFACE PHENOTYPE OF MBP- AND MV-SPECIFIC T CELL LINES DERIVED FROM SAME RATS

T cell line	Percentage of cells demonstrating fluorescence [a]				
	W3/13	W3/25	OX8	OX6	OX17
MBP-SAME1	94	78	7	3	3
MBP-SAME2	81	75	2	2	3
MBP-SAME3	97	88	9	4	2
MBP-SAME4	86	76	7	1	3
MV-SAME5	95	88	4	3	1
MV-SAME4	96	84	3	2	1

[a] The monoclonal antibodies define all T lymphocytes (W3/13); helper T lymphocytes (W3/25); cytotoxic/suppressor T lymphocytes (OX8). OX6 binds to a polymorphic determinant of I-A region-encoded Ia antigen; and OX17 is specific for I-E region-encoded Ia antigens.

TABLE 5

IN VIVO EFFECT OF MBP- AND MV-SPECIFIC T CELL LINES DERIVED FROM SAME RATS

After harvesting cells were washed twice and injected into the tail vein in a volume of 0.5–1 ml. Animals were weighed and observed daily for clinical signs of EAE, and disease severity was graded on a scale from 0 to 5 (Lassmann, 1983).

Cells adoptively transferred	Dose	Mean clinical score	Incidence of clinical disease	Incidence of histologic lesions of EAE
MBP-SAME3 'resting'	6×10^7	–	0/4	0/4
MBP-SAME3 ConA-activated	2×10^6	1.3	2/3	3/3
MBP-SAME3 ConA-activated	1×10^7	2.3	3/3	3/3
MBP-SAME3 MBP-activated	1×10^6	–	0/4	1/4
MBP-SAME3 MBP-activated	2×10^6	1.0	2/4	3/4
MBP-SAME3 MBP-activated	6×10^6	2.0	4/4	4/4
MBP-SAME3 MBP-activated	1×10^7	2.7	3/3	3/3
MBP-SAME3 MBP-activated	2×10^7	3.0	3/3	3/3
MBP-SAME1 MBP-activated	6×10^6	2.2	6/6	6/6
MBP-SAME2 MBP-activated	6×10^6	1.5	4/5	5/5
MBP-SAME4 MBP-activated	6×10^6	0.7	1/3	3/3
MBP-EAE MBP-activated	2×10^5	0.7	1/3	3/3
MBP-EAE MBP-activated	1×10^6	3.0	3/3	3/3
MBP-EAE MBP-activated	6×10^6	3.0	3/3	3/3
MV-SAME4 MV-activated	6×10^7	–	0/6	0/6
MV-SAME5 MV-activated	6×10^7	–	0/4	0/4

into recipients. Cells injected were either freshly activated with MBP or ConA, or 'resting T cells' were injected following expansion in TCGF for 10 days. As can be seen in Table 5, activation with either antigen or mitogen was effective to a similar degree. A dose above 1×10^6 of these cells was able to produce clinical signs of EAE and there was a marked increase in the clinical severity of the disease when more cells were transferred. Within 7 days 85% of the recipients exhibited clinical signs of EAE. Adoptive transfer of 6×10^7 resting T cells into naive recipients was completely ineffective. The recipients developed neither clinical nor histological signs of EAE. Compared to a MBP-specific T cell line, MBP-EAE, derived from the spleen of an animal actively immunized (100 μg rat MBP, 100 μg heat-inactivated *Mycobacterium tuberculosis* H37Ra (Difco, Detroit, MI, U.S.A.) in 100 μl CFA) the MBP-SAME3 line was less effective at inducing EAE. Transfer of as little as 2×10^5 activated MBP-EAE cells produced some signs of EAE in recipients and with a dose of 10^6 cells induced paraparesis. The mean onset of clinical symptoms was 4 days post-cell transfer (6×10^6 cells) with all MBP-specific T cell lines. In contrast, the two MV-specific T cell lines (MV-SAME4, MV-SAME5, 6×10^7 cells) induced neither clinical nor histological abnormalities in naive recipient rats.

Animals having received T cell lines were killed and the CNS was examined for neuropathological changes and for evidence of measles virus replication. Histologi-

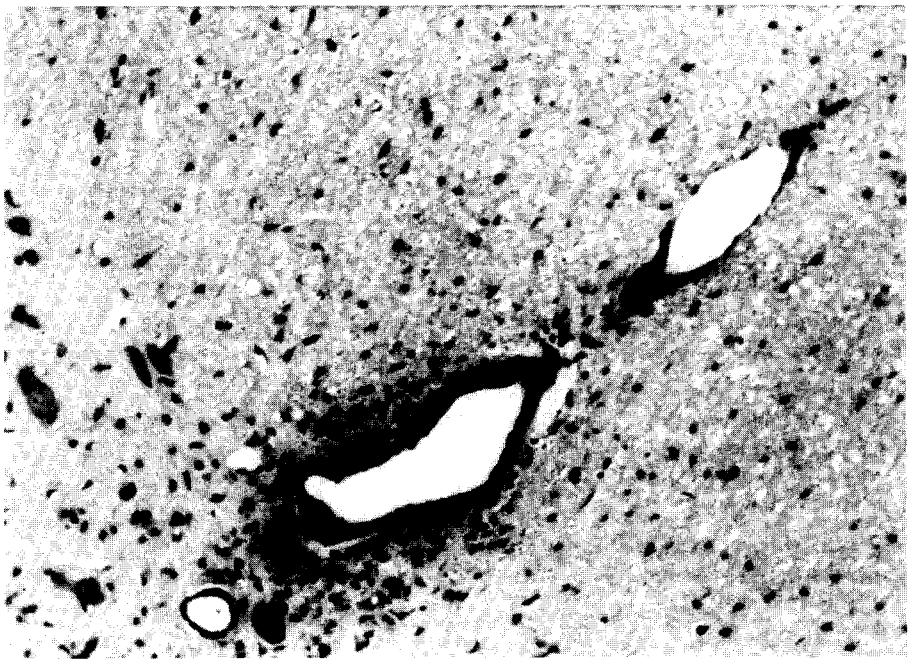

Fig. 2. Perivascular inflammation consisting of mononuclear cells 7 days after adoptive transfer of MBP-specific T cells (MBP-SAME3, 6×10^6). Transverse section of lumbar spinal cord, hematoxylin and eosin, ×150.

cal lesions consisting of mononuclear inflammatory infiltrates and perivascular cuffs typical for EAE were present at the sites of predeliction (Fig. 2) (Levine and Wenk, 1961; Levine, 1974; Willenborg, 1979) in all those animals receiving effective doses of MBP-specific T cell lines. However, no viral antigen or infectious virus could be detected in brain tissue of any of the animals injected with T cell lines derived from SAME animals.

Discussion

The subacute measles encephalomyelitis (SAME) of Lewis rats developing 4–8 weeks after intracerebral infection with a neurotropic measles virus has characteristic histological and immunological features which resemble to some extent those observed in EAE. From animals that develop SAME, MBP-specific lymphocytes can be isolated, in particular when CNS lesions of an active encephalitis (Table 1, groups 1 + 2) were present either during the clinical disease or after recovery. These lymphocytes not only proliferate in the presence of MBP, but in adoptive transfer experiments proved to be encephalitogenic in naive syngeneic recipients. The histopathology of the resulting CNS lesions was similar to the one observed after adoptive transfer of MBP-specific T cells derived from EAE animals. However, the

lower stimulation indices found with lymphocytes from SAME animals as compared to those with EAE (group 4) indicate that expansion of pre-existing MBP specific clones (Schluesener and Wekerle, 1985) is less efficient in measles virus-infected rats.

The CNS changes induced in the transfer experiments are probably the result of an immune pathological reaction mediated by MBP-specific T cells derived from SAME animals. It is unlikely that these lesions are caused by transfer of measles virus for the following two reasons. First, we were unable to either re-isolate infectious virus or detect viral antigen in the T cell lines or in brain tissue of recipient rats. Second, no clinical or neuropathological changes were seen in normal rats receiving syngeneic spleen lymphocytes either incubated with measles virus in vitro, or directly isolated from SAME rats without in vitro restimulation. Furthermore, MV-specific T cell lines themselves were unable to induce either clinical or histological evidence of a disease in naive recipient animals.

Similar observations were made in Lewis rats infected with murine coronavirus of JHM strain (Watanabe et al., 1983). In this model JHM virus induces in weanling rats a subacute demyelinating encephalomyelitis (SDE) which is characterized by an inflammatory reaction with marked demyelination. In the course of a persistent JHM virus infection in brain tissue a T cell-mediated immune reaction against MBP develops. The MBP-specific T cells led in adoptive transfer experiments to the induction of EAE. There are, however, important differences to the SAME animal model. In JHM virus-induced SDE the target cells for the virus are glial cells (Nagashima et al., 1978; Massa et al., 1986a, b), demyelinating plaques reveal infected cells, and infectious JHM virus can always be recovered from SDE brain tissue (Wege et al., 1984). Contrary to this, the principle target cells for measles virus in SAME are neurons, the disease is inflammatory and demyelinated plaques are not detected. Infectious measles virus cannot be isolated from SAME rats and viral antigen is only occasionally found (Liebert and ter Meulen, 1987). The different target cells and virus–cell interactions in the two models conceivably influence pathology and disease course. Yet, a T cell-mediated autoimmune reaction to MBP of unknown pathogenetic role develops in the course of both JHM and measles virus infections in brain tissue. In the case of SAME MBP-specific T cells possibly maintain the inflammatory CNS reaction in those animals with a persisting encephalitis in the absence of measles virus, whereas in JHM-SDE the autoimmune reaction to MBP is thought to be involved in the development of the relapsing SDE occurring in some of these animals (Wege et al., 1984).

How the measles virus infection in Lewis rats induces such an immune response against MBP is unknown. There are several possibilities which could lead to an autoimmune reaction. It has been shown that viral infections may trigger the production of T and B cell responses that cross-react with host cell proteins by a mechanism termed molecular mimicry (Notkins et al., 1984). In addition, monoclonal antibodies against viral structural proteins including those of measles virus, have been obtained which also recognized cellular antigens (Fujinami et al., 1983; Sheshberadaran and Norrby, 1984). In few instances such cellular antigens have been identified by sequence analysis. For example, amino acid sequence homologies

have been found between the encephalitogenic portion of rabbit MBP and the hepatitis B virus polymerase (Fujinami and Oldstone, 1985), and between oligopeptides of human myelin proteins and measles virus nucleocapsid and C proteins (Jahnke et al., 1985). However, these reported sequence homologies are not identical to the peptides which represent either the major or the minor encephalitogenic determinants of MBP for the Lewis rat (Alvord, 1984; Martenson, 1984). In SAME animals, no cross-reactivity between antibodies directed against measles virus or MBP was observed. Furthermore, MBP-specific T cell blasts from SAME animals did not react with inactivated measles virus and conversely the MV-specific line failed to proliferate in the presence of MBP suggesting that molecular mimicry between measles virus proteins and rat MBP is unlikely. However, it cannot be ruled out that cross-reactivity exists between measles virus and other potentially encephalitogenic proteins such as the proteolipid protein (Williams et al., 1982; Yoshimura et al., 1985) which do not have any sequences homologous to MBP.

Other ways by which an autoimmune reaction could develop would include the enhanced release of host antigens from infected cells leading to a partial breakdown of tolerance and expansion of the irrelevant T cell clones. Additionally, viral infection may result in a general polyclonal activation of the immune system including those clones which recognize self antigens. Such mechanisms acting in conjunction with the induction of Ia antigen on astrocytes (Fontana et al., 1984) could be a determining factor for the development of an immune pathological reaction in the course of a CNS viral infection (Massa et al., 1986a, b). In the case of a high level of Ia expression on astrocytes, inappropriate presentation of self antigens may occur as has been suggested in autoimmune processes (Londei et al., 1984).

None of the above mechanisms has been proven in any of the various models for virus-induced encephalitis. However, it must be noted that not only do SAME animals have a high MV-specific T cell response, but MV antigens are also detected in some animals. This observation indicates that the cell-mediated immune response to measles virus could also play some role in the development of the CNS lesions. Future experiments will clarify this point, but it is now apparent that CNS measles virus infection can lead to a putatively pathogenic autoimmune response by triggering or potentiating the proliferation of pre-existing MBP-specific T cell clones in SAME animals. In addition, there is evidence that a persistent measles virus infection in hamsters supports the development of EAE when such animals are challenged with MBP in complete adjuvant (Massanari et al., 1979). Whether the measles virus renders the CNS more vulnerable to immunological injury or potentiates a cell-mediated immune response to myelin in this model has not yet been elucidated.

In conclusion, our model of measles encephalomyelitis in the Lewis rat may allow the analysis of the mechanisms responsible for the induction of autoimmune responses to brain antigen in the course of measles virus infection. This would provide information important not only to our understanding of the pathogenesis of measles encephalitis in man, but also for other autoimmune diseases associated with viral infections.

Acknowledgements

We thank Ms. S. Kotrla for technical assistance and H. Kriesinger for typing the manuscript. This work was supported by the Deutsche Forschungsgemeinschaft and the Hermann and Lilly Schilling Stiftung.

References

Alvord, E.C. (1984) In: E.C. Alvord, Jr., M.W. Kies and A.J. Suckling (Eds.), Experimental Allergic Encephalomyelitis, A Useful Model for Multiple Sclerosis, Alan R. Liss, New York, pp. 523–537.

Ben-Nun, A., Wekerle, H. and Cohen, I.R. (1981) The rapid isolation of clonable antigen-specific T lymphocyte lines capable of mediating autoimmune encephalomyelitis. Eur. J. Immunol. 11, 195–197.

Eylar, E.H., Kniskern, P.J. and Jackson, J.J. (1979) Myelin basic protein. Methods Enzymol. 32B, 323.

Fontana, A., Fierz, W. and Wekerle, H., (1984) Astrocytes present myelin basic protein to encephalitogenic T-cell lines. Nature 307, 273–276.

Fujinami, R.S. and Oldstone, M.B.A. (1985) Amino acid homology between the encephalitogenic site of myelin basic protein and virus: mechanism for autoimmunity. Science 230, 1043–1045.

Fujinami, R.S., Oldstone, M.B.A., Wroblewska, Z., Frankel, M.E. and Koprowski, H. (1983) Molecular mimicry in virus infection: crossreaction of measles virus phosphoprotein or of herpes simplex virus protein with human intermediate filaments. Proc. Natl. Acad. Sci. U.S.A. 80, 2346–2350.

Gendelman, H.E., Wolinsky, J.S., Johnson, R.T., Pressman, N.J., Pezeshkpour, G.H. and Boisset, G.F. (1984) Measles encephalomyelitis: lack of evidence of viral invasion of the central nervous system and quantitative study of the nature of demyelination. Ann. Neurol. 15, 353–360.

Jahnke, U., Fischer, E.H. and Alvord, Jr., E.C. (1985) Sequence homology between certain viral proteins and proteins related to encephalomyelitis and neuritis. Science, 229, 282–284.

Johnson, R.T., Griffin, D.E., Hirsch, R.L., Wolinsky, J.S., Roedenbeck, S. and Soriano, I.L. (1984) Measles encephalomyelitis: clinical and immunological studies. N. Engl. J. Med. 310, 137–142.

Lassmann, H. (1983) Comparative Neuropathology of Chronic Experimental Allergic Encephalomyelitis and Multiple Sclerosis, Springer Verlag, Berlin – Heidelberg – New York – Tokyo.

Levine, S. (1974) Hyperacute, neutrophilic, and localized forms of experimental allergic encephalomyelitis: a review. Acta Neuropathol. 28, 179–189.

Levine, S. and Wenk, E.J. (1961) Studies on the mechanism of altered susceptibility to experimental allergic encephalomyelitis. Am. J. Pathol. 39, 419–441.

Liebert, U.G. and ter Meulen, V. (1987) Virological aspects of measles virus induced encephalomyelitis in Lewis and BN rats. J. Gen. Virol. 68, 1715–1722.

Liebert, U.G., Seitz, R.J., Weber, T. and Wechsler, W. (1985) Immunocytochemical studies of serum proteins and immunoglobulins in human sural nerve biopsies. Acta Neuropathol. 68, 39–51.

Linington, C., Izumo, S., Suzuki, M., Uyemura, K., Meyermann, R. and Wekerle, H. (1984) A permanent rat T cell line that mediates experimental allergic neuritis in the Lewis rat in vivo. J. Immunol. 133, 1946–1950.

Londei, M., Lamb, J.R., Bottazzo, G.F. and Feldmann, M. (1984) Epithelial cells expressing aberrant MHC class II determinants can present antigen to cloned human T cells. Nature 312, 639–641.

Martenson, R.E. (1984) In: E.C. Alvord, Jr., M.W. Kies and A.J. Suckling (Eds.), Experimental Allergic Encephalomyelitis, A Useful Model for Multiple Sclerosis, Alan R. Liss, New York, pp. 511–521.

Massa, P.T., Wege, H. and ter Meulen, V. (1986a) Analysis of murine hepatitis virus (JHM strain) tropism toward Lewis rat glial cells in vitro. Lab. Invest. 55, 318–327.

Massa, P.T., Dörries, R. and ter Meulen, V. (1986b) Viral particles induce Ia antigen expression on astrocytes. Nature 320, 543–546.

Massanari, R.M., Paterson, P.Y. and Lipton, H.L. (1979) Potentiation of experimental allergic encephalomyelitis in hamsters with persistent encephalitis due to measles virus. J. Infect. Dis. 139, 297–303.

Miller, H.G., Stanton, J.B. and Gibbons, J.L. (1956) Parainfectious encephalomyelitis and related syndromes. Q. J. Med. 25, 427–505.

Nagashima, K., Wege, H., Meyermann, R. and ter Meulen, V. (1978) Coronavirus induced subacute demyelinating encephalomyelitis in rat. A morphological analysis. Acta Neuropathol. 44, 63–70.

Notkins, A.L., Onodera, T. and Prabhakar, E.L. (1984) In: A.L. Notkins and M.B.A. Oldstone (Eds.), Concepts in Viral Pathogenesis, Springer, New York – Berlin – Heidelberg – Tokyo, pp. 210–215.

Schluesener, H.J. and Wekerle, H. (1985) Autoaggressive T lymphocyte lines recognize the encephalitogenic region of myelin basic protein: in vitro selection from unprimed rat T lymphocyte populations. J. Immunol. 135, 3128–3133.

Sheshberadaran, H. and Norrby, E. (1984) Three monoclonal antibodies against measles virus F protein cross-react with cellular stress proteins. J. Virol. 52, 995–999.

Watanabe, R., Wege, H. and ter Meulen, V. (1983) Adoptive transfer of EAE-like lesions from rats with coronavirus-induced demyelinating encephalomyelitis. Nature 305, 150–153.

Wege, H., Watanabe, R. and ter Meulen, V. (1984) Relapsing subacute demyelinating encephalomyelitis in rats during the course of coronavirus JHM infection. J. Neuroimmunol. 6, 325–336.

Willenborg, D.O. (1979) Experimental allergic encephalomyelitis in the Lewis rat: studies on the mechanisms of recovery from disease and acquired resistance reinduction. J. Immunol. 123, 1145–1150.

Williams, R.M., Lees, M.B., Cambi, F. and Macklin, W.B. (1982) Chronic experimental allergic encephalomyelitis induced in rabbits with bovine white matter proteolipid apoprotein, J. Neuropathol. Exp. Neurol. 41, 508–521.

Yoshimura, T., Kunishiha, T., Sakai, K., Endoh, M., Namikawa, T. and Tabira, T. (1985) Chronic experimental allergic encephalomyelitis in guinea pigs induced by proteolipid protein. J. Neurol. Sci. 69, 47–58.