Antibody-Induced Restriction of Viral Gene Expression in Measles Encephalitis in Rats

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After infection with the neurotropic CAM/RBH measles virus (MV) strain, newborn Lewis rats succumb to an acute necrotizing encephalopathy. Passive transfer of neutralizing monoclonal antibodies directed against MV hemagglutinin prevented this disease process. Instead, either an antibody-induced acute or subacute measles encephalitis developed after a prolonged incubation period with a restricted expression of MV structural proteins. The molecular biological analysis of MV gene expression in brain tissue of rats treated with MV-neutralizing antibodies revealed a transcriptional restriction of viral mRNAs, particularly for the envelope proteins, leading to a steep expression gradient. Based on in situ hybridization, it was concluded that the efficiency of transcription of viral genes at the single-cell level is reduced compared with that of controls. Passive immunization with monoclonal antibodies directed against other MV structural proteins proved to be ineffective. Similar results were obtained in MV-infected weanling Brown Norway rats. These rats developed a clinically silent encephalitis in the presence of high titers of neutralizing antibodies. In such animals, a pronounced attenuation of the viral gene transcription was observed. These findings indicated that neutralizing antibodies directed against a restricted set of specific antigenic sites on the viral hemagglutinin protein expressed on cell membranes exert a modulating effect on the viral gene expression at the level of transcription. This phenomenon contributes to the switch from the acute cytopathic effect to a persistent infection in the central nervous system.

Persistent measles virus (MV) infections of the central nervous system (CNS) are associated with subacute or chronic disease processes (14, 22). In humans, subacute sclerosing panencephalitis and measles inclusion body encephalitis develop as rare complications months to years after the onset of acute measles. In these two diseases, the MV infection has been characterized and it can be shown that a restriction of MV gene expression enables the virus to persist (2, 3, 8, 9, 16). However, no information is available about factors contributing to the establishment and initial maintenance of a persistent MV infection in the CNS. So far, only in vitro studies have been done with tissue culture cells of neural or nonneural origin to characterize the events which lead to persistence (18, 25). These experiments suggest that possible determinants reside in specific virus-host cell interactions governed by highly specialized target cells and their stage of differentiation. Moreover, it was found that antibodies interacting with MV glycoproteins expressed on infected cell membranes may initiate and support the establishment of a persistent infection by interference with the productive replication of the viral genome and an alteration of the synthesis of MV structural proteins (4, 11, 12). This phenomenon has been called antibody-induced antigenic modulation.

A suitable model to analyze these factors is provided by inbred rats with a MV infection of the CNS. In this model, infected newborn Lewis and Brown Norway (BN) rats die from a necrotizing encephalopathy (NE), whereas weanling Lewis rats develop inflammatory CNS disease processes in the form of either an acute encephalitis (AE) or a subacute measles encephalitis (SAME). In contrast, infected weanling BN rats do not develop clinical signs, although a subacute encephalitis (clinically silent encephalitis [CSE]) can be

documented histopathologically (17). The basis for the development of SAME and CSE is a persistent MV infection without production of infectious virus in brain tissue. This defective replication is characterized by restricted synthesis of MV envelope proteins in infected brain cells which starts in rats with AE (17). Furthermore, in Lewis rats with SAME, an attenuation of the transcription of MV envelope genes and translational defects for the matrix (M)-specific mRNA (21) have been observed which resemble the situation in subacute sclerosing panencephalitis and measles inclusion body encephalitis (2, 3, 8, 9, 16).

In the present study, the effect of antiviral antibodies on the course of experimental MV infection of brain tissue in rats was analyzed. Passive transfer of neutralizing monoclonal antibodies directed against the MV hemagglutinin (H) to infected newborn Lewis and BN rats was found to prevent the occurrence of the NE. Instead, antibody-induced AE (AI-AE) and AI-SAME developed after prolonged incubation periods. Both disease types revealed a pronounced restriction of MV gene expression in comparison with control animals. In weanling BN rats, a similar effect on MV replication was observed, probably as the result of the naturally occurring humoral hyperimmune response developing against the viral infection in such rats. These experiments indicated that MV antibodies interfere with MV gene expression and support the establishment of virus persistence in brain tissue.

MATERIALS AND METHODS

Animals and inoculation. Pregnant SPF rats of the Lewis and BN inbred strains were obtained from the Zentralinstitut für Versuchstiere, Hannover, Federal Republic of Germany. Newborn or weanling (3 to 4 weeks old) rats were infected by intracerebral inoculation with 10^4 50% tissue culture infective doses in 25 µl of our neurotropic CAM/RBH strain

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of MV, a derivative of the CAM R/40 strain which we originally obtained from K. Yamanouchi, Measles Institute, Tokyo, Japan (15, 17). Newborn rats were injected intraperitoneally with monoclonal antibodies directed against measles structural proteins (50 to 250 μl of antibody-containing ascites) 5 to 8 h after infection. In weanling rats, no attempts were made to interfere with the natural course of the CNS infection by passive transfer of antiviral antibodies.

Antibodies. The monoclonal antibodies used in this study are directed against the following structural proteins of MV: the nucleocapsid (N) protein (antibodies F227 and L396), the phospho (P) protein (16AF10, a kind gift from E. Norrby, Karolinska Institutet, Stockholm, Sweden [19]), the matrix (M) protein (F266, K121, B117), the fusion (F) protein (A351, A352, A5048), and the H protein (L77, K17, NC32; referred to in this report as H1 to H3). The antigen specificity of these antibodies was tested by radioimmunoprecipitation of [^{35}S]methionine-labeled MV-infected Vero cell lysates (6, 7). All antibodies are of the immunoglobulin G (IgG) isotype (IgG1, IgG2a, IgG3) except L396, which is an IgM antibody. The anti-H antibodies used neutralize infectious MV. Approximately 5,000 neutralizing units (NU) (as 0.15 to 0.3 mg of immunoglobulin as determined after protein A column purification) were injected into each rat. One NU was defined as the concentration of antibody that neutralizes 50% of 100 PFU. The nonneutralizing N-, P-, M-, and F-specific antibodies were used at a concentration of 0.25 mg of immunoglobulin. As a control, we used a monoclonal antibody directed against the E2-spike glycoprotein of JHM coronavirus that was kindly provided by Helmut Wege, Institut für Virologie und Immunbiologie, Universität Würzburg.

Determination of antiviral humoral immune response. Paired samples of serum and cerebrospinal fluid (CSF) of rats of both inbred strains with histologically verified AE and SAME and CSE, respectively, were analyzed by an enzyme-linked immunoassay as described previously (17). The titer of neutralizing antibodies was determined by a neutralization assay. Briefly, 100 PFU of the Edmonston strain of MV were incubated at 37°C for 1 h with serial dilutions of rat serum or CSF. Subsequently, these mixtures were seeded in quadruplicate on just confluent monolayers of Vero cells in microdilution plates. The occurrence of the viral cytopathic effect was monitored after 4 days, and the neutralization titer was determined by standard procedures.

Virus isolation. A modification of a previously published procedure was used for virus isolation (17). Briefly, MV was reisolated from brain tissue of infected animals by incubation of diluted brain homogenate (Dounce homogenized and clarified by centrifugation) on monolayers of Vero cells, and the titer of the isolated virus was determined.

Histology and immunohistology. Brain and spinal cord were fixed in buffered paraformaldehyde, and sections were cut from paraffin blocks and stained with hematoxylin. The in vivo expression of MV structural proteins in the CNS was analyzed on snap-frozen brain material by using indirect immunofluorescence as described previously (16). As the first reagent, pooled monoclonal antibodies against the individual proteins were used in the appropriate dilution followed by incubation with a fluorescein isothiocyanate conjugate directed against the F(ab')$_2$ fragment of mouse IgG. On serial acetone-fixed sections, the number of cells expressing the individual MV proteins was determined and the frequency was expressed relative to the cells labeled with the N-specific antibodies. Cells efficiently synthesizing the MV N protein were defined as infected.

Extraction of RNA and Northern (RNA) blot experiments. RNA was extracted from snap-frozen brain material as described previously (21). Briefly, brain material was homogenized in guanidinium isothiocyanate buffer, and RNA was purified by centrifugation through a CsCl cushion (1.7 g/cm^3). Polyadenylated RNA was purified by one cycle of oligo(dT) selection, separated on a 1.5% agarose gel containing formaldehyde, and transferred to nitrocellulose filters. Hybridization was performed with strand-specific [^{32}P]CTP-labeled RNA probes transcribed in vitro from Gem-1-cloned MV-specific cDNAs for the following genes: 851-base-pair (bp) *EcoRV-XbaI* fragment of the N gene, 531-bp *BalI-HindIII* fragment of the P gene, 526-bp *BglIII-EcoRI* fragment of the M gene, 320-bp *TaqI-DdeI* fragment of the F gene, and 787-bp *BglIII-AvaI* fragment of the H gene. The cDNA clones were kindly provided by M. A. Billeter, Institut für Molekularbiologie II, Universität Zürich, and were described previously (8). To each RNA sample analyzed, 2 fmol of a mixture of standard RNA transcripts was added before electrophoresis. Standard RNAs were synthesized in vitro from the cDNA clones described above in opposite orientation to the hybridization probes and therefore migrated corresponding to the length of the individual cDNA. After autoradiography, bands for the standard RNA transcripts and the monocistronic MV-specific mRNAs were excised from the nitrocellulose filters and the radioactivity retained was determined by scintillation counting.

In situ hybridization. In situ hybridization was performed as described previously with slight modifications (21). Briefly, paraffin-embedded tissues were cut in 5- to 8-μm-thick sections and applied to pretreated slides. Sections were deparaffinized and deproteinized. MV-specific cDNA clones described above were transcribed in vitro in the presence of [^{35}S]UTP and hybridized to the brain sections at 48°C for 16 h. After hybridization, slides were washed in three changes of HWM (50% formamide, 0.6 M NaCl, 20 mM Tris hydrochloride [pH 7.2], 2 mM EDTA) at 48 to 50°C for 45 min. After dehydration, slides were immersed in Kodak NTB2 emulsion, exposed for 4 days at 4°C, developed in D19 developer (Kodak), fixed in 30% sodium thiosulfate, and counterstained with either hematoxylin and eosin or Giemsa stain.

RESULTS

Antibody effect on course of MV encephalitis. Newborn Lewis and BN rats succumb to infection with the neurotropic MV within 5 to 6 days. The histological changes are of a degenerative and necrotic type without accompanying cellular infiltrates, revealing an acute NE. Weanling Lewis rats (3 to 4 weeks old) developed AE and SAME. The latter is characterized by dense perivascular infiltration with lymphomonocytic cells in the grey and white matter of the CNS with little involvement of the parenchyma. In contrast, the salient features of the AE are scant disseminated lymphomonocytic cell infiltration of the parenchyma with neuronophagia and glial nodules. The large majority of infected weanling BN rats does not develop a clinical disease, but in infected animals, a subacute CSE can be documented histopathologically, with gliosis and plasma cell infiltration in brain tissue and perivascular spaces (17). Monoclonal antibodies directed against the different structural proteins of MV were tested for their ability to protect the newborn Lewis rats from the acute fatal NE (Table 1). None of the nonneutralizing antibodies (directed against N, P, M, and F) at any dose (up to 2 mg of purified immunoglobulin) given

TABLE 1. In vivo effect of virus-specific monoclonal antibodies on the course of experimental measles encephalitis in Lewis rats

Treatment with antibody[a]	No. of animals infected	Incubation period (days)	Neuro-pathology	Incidence (%)	Virus isolation (log PFU)[b]
Control[c]	8	5–6	NE	100	4.5
Anti-N	12	5–7	NE	100	4.1
Anti-P	4	6–7	NE	100	5.0
Anti-M	12	5–7	NE	100	4.7
Anti-F	20	5–10	NE	100	4.2
Anti-H1	10	8–12	AI-AE	63	2.1
		18–27	AI-SAME	27	0
Anti-H2	5	11–16	AI-AE	80	2.9
		36	AI-SAME	20	0
Anti-H3	8	8–13	AI-AE	75	1.7
		27–31	AI-SAME	25	0

[a] A 250-μg portion of purified immunoglobulin of the control or individual anti-N, -P, -M, and -F antibodies or 5,000 NU of the anti-H antibody (150 to 300 μg) was injected into each animal. Groups of six animals received anti-N antibody F227 or L396 or anti-F antibody A352 or A5084; groups of four rats were treated with anti-M antibody F266 or K121 or B117; and eight rats were injected with the anti-F A351 antibody.

[b] Mean titer per gram of brain weight.

[c] Includes four untreated animals and four animals treated with a mouse monoclonal antibody directed against JHM coronavirus.

TABLE 2. Expression of MV structural proteins in infected brain cells

Antibody treatment	No. of animals studied	Disease type	Structural protein (%)[a]				
			N	P	M	F	H
Nonneutralizing antibodies							
Anti-JHM	4	NE	100	93	36	35	33
Anti-N	6	NE	100	98	33	26	30
Anti-P	4	NE	100	93	38	30	32
Anti-M	5	NE	100	97	42	26	34
Anti-F	9	NE	100	96	31	25	29
Neutralizing antibodies							
Anti-H1 to H3	8	AI-AE	100	91	28	21	12
Anti-H1 to H3	7	AI-SAME	100	89	2	4.5	1

[a] Mean expression of MV structural proteins calculated in relation to N protein-expressing cells.

either as mixtures or as individual monoclonal antibodies had an effect on the clinical course of the disease except for a slight prolongation of the incubation period in anti-F-treated rats. Titers of MV isolated from brain tissue homogenate were in the same range as in animals that were passively immunized with a monoclonal antibody to JHM coronavirus (Table 1). The histological examination revealed no significant differences in the pattern and distribution of the neuropathological lesions in these animals. In contrast, the monoclonal antibodies against MV H prevented the development of the NE in infected animals. The passive transfer of more than 10,000 NU (approximately 0.5 mg of immunoglobulin) resulted in an increasing percentage of animals in which no evidence for viral replication in the brain tissue could be obtained, and less than 500 NU were ineffective. A dose of 5,000 NU prevented the NE but allowed viral replication in almost all animals. This dose was therefore used for further experiments reported in this study.

After passive transfer of anti-H antibodies, 74% of MV-infected newborn Lewis rats (17 of 23) developed an AI-AE after an incubation period of 8 to 15 days and 26% (6 of 23) developed an AI-SAME after incubation periods of 3 to 5 weeks. The histological changes observed in these rats were similar to those described for AE and SAME in weanling Lewis rats (17). Thus, AI-AE is characterized by neuronophagia and glial nodules, while the lesions in AI-SAME consisted mainly of lymphocytic infiltrations, predominantly located perivascularly in the white matter. The effect on the disease course of antibody treatment of newborn BN rats was essentially identical (data not shown). Only the anti-H antibodies protected the newborn rats from the fatal encephalopathy. Histopathologically, an AI-CSE was verified in 40% of the infected BN rats after passive transfer of 5,000 NU of anti-H antibodies.

Expression of viral proteins in brain tissue in vivo. Measles antigen was detected in the brain sections of all animals examined regardless of the antibodies transferred (Table 2). In animals treated with virus-specific nonneutralizing antibodies, the viral P protein was present in 93 to 98% of the infected cells, whereas the envelope proteins were detected at a lower percentage: the M protein in 31 to 42%, the F protein in 25 to 35%, and the H protein in 29 to 34% of the infected cells. In animals treated by passive transfer of neutralizing antibodies directed against the H protein, however, an attenuation of the envelope protein expression was observed. Already after a short incubation period (8 to 13 days), a restriction of envelope protein synthesis could be seen which was amplified in animals that developed AI-SAME with a survival period of 3 and more weeks.

Expression of MV-specific transcripts in brain material. To examine the expression of virus-specific mRNAs in brain material from antibody-treated and untreated control animals, polyadenylated RNA was extracted from the brains of MV-infected animals and subjected to Northern blotting. In Fig. 1, typical expression patterns for MV transcription in brain material from untreated control animals (Fig. 1A), anti-N-treated animals (Fig. 1B), and anti-F-treated animals (Fig. 1C) are shown. The pattern of hybridization was similar in these animals, and signals for all MV-specific monocistronic polyadenylated transcripts (N, P, M, F, and H) could be seen as well as signals for bicistronic and polycistronic transcripts. The same results were obtained when infected animals were treated with anti-M antibodies (data not shown). However, the Northern blots obtained for +pA RNA derived from the brains of animals treated with anti-H antibodies were different, revealing a generally restricted transcriptional efficiency and a reduced expression of MV-specific F and H transcripts (Fig. 1D).

To quantify the expression of MV-specific transcripts in brain material seen in the Northern blots, the bands corresponding to the moncistronic mRNAs were excised and the individual copy number per 10 pg of RNA was determined in relation to the standard RNA transcripts added before electrophoresis. In addition, the absolute expression rates were determined as relative expression values in relation to the signal obtained for the corresponding N mRNA (100% expression) (Table 3). No significant differences in the transcription pattern were found between control animals and those treated with nonneutralizing antibodies (anti-N, anti-M, and anti-F). An almost identical expression gradient could be seen according to the gene order which is similar to the one observed previously in weanling rats (21).
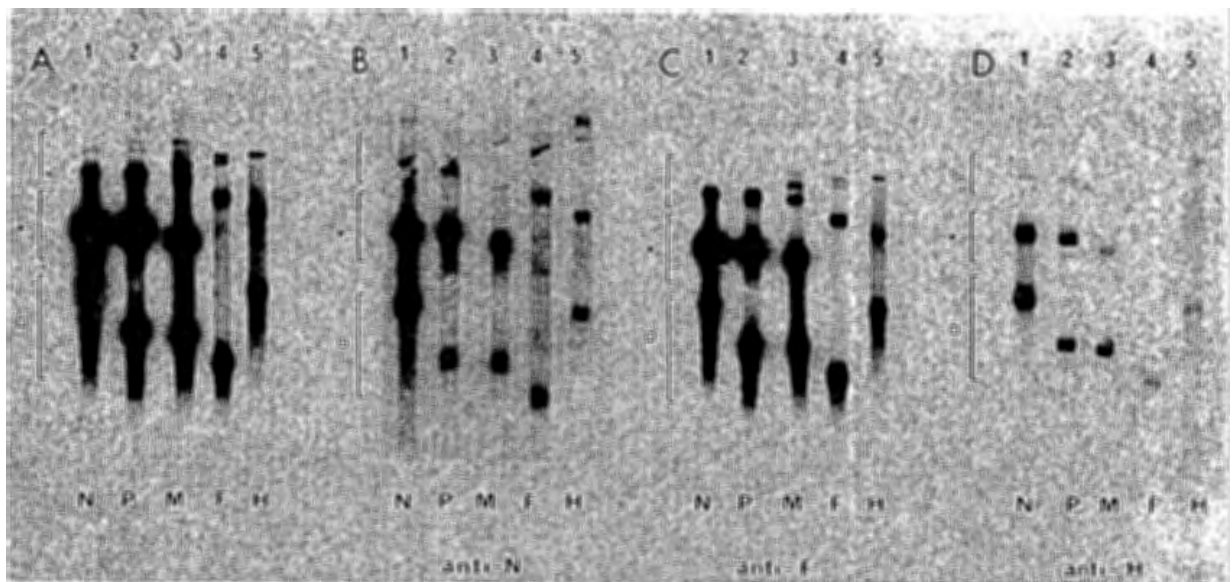
FIG. 1. Northern blot analysis of 2 μg of each +pA RNA derived from brain material of newborn Lewis rats with NE (A to C) and AI-SAME (D). (A) Untreated animal (5 days p.i.); (B) anti-N-treated animal (6 days p.i.); (C) anti-F-treated animal (8 days p.i.); (D) anti-H-treated animal (36 days p.i.). RNAs were hybridized as described in the text to RNA probes specific for the N gene (lanes 1), the P gene (lanes 2), the M gene (lanes 3), the F gene (lanes 4), and the H gene (lanes 5). Symbols: ▷, position of the polycistronic polyadenylated RNAs; ▶, position of the monocistronic polyadenylated RNAs; □, position of the standard RNA transcripts migrating according to the length of the individual probe. Total amounts of the standard RNAs were 2 fmol, except in panel D, lane 5, where only 1 fmol of the H standard was applied. In panel B, an artifact due to the transfer procedures is visible at the position of the polycistronic RNAs of M, F, and H.

A significantly different effect on MV transcription was achieved in animals treated with neutralizing anti-H antibodies. Both groups of animals with either AI-AE or AI-SAME revealed a restriction of MV gene expression. The data obtained on transcriptional efficiencies for MV-specific mRNAs did not show significant differences for animals with AI-AE (10 to 14 days postinfection [p.i.]) and animals with AI-SAME with longer incubation periods (27 and 36 days p.i.). The intensity of the hybridization signals of the N mRNA was reduced to about 40 to 57% in comparison with the control animals, and the transcription of P, M, F, and H genes was drastically restricted. The expression of P, M, and F mRNAs of these two groups treated with anti-H antibodies was reduced 47 to 44, 26 to 20, and 10 to 6%, respectively. The H mRNA was not detectable in four of the five experiments. Similar results were obtained in experiments with newborn BN rats with the same set of monoclonal antibodies

(data not shown). In vitro translation experiments performed with polyadenylated RNA from brain material of animals with antibody-induced encephalitis confirmed these results since only N- and P-specific translation products could be detected (data not shown). Because of the low copy number of the mRNA for M protein, we could not determine whether there are additional translational defects, as shown for SAME in weanling Lewis rats (21).

In situ hybridization. In situ hybridization experiments were performed on brain sections from animals with AI-SAME to gain more information on the observed restricted transcription. Brain sections of animals treated with the neutralizing anti-H antibodies were hybridized with the same strand-specific RNA probes as for the Northern blot experiments. Since the age and the maturation of the developing brain may influence MV gene expression in brain cells, sections from age-matched treated and untreated animals

TABLE 3. Quantitative analysis of MV-specific gene expression in brains of antibody-treated and control Lewis rats

Animal group	CNS disease (no. of rats)	Incubation period (days p.i.)	No. of copies (%) of MV-specific mRNA[a]				
			N	P	M	F	H
Untreated control animals	NE (4)	5–7	3,600 (100)	2,100 (58.3)	1,600 (44.4)	600 (17.0)	350 (9.7)
Nonneutralizing antibodies							
Anti-N	NE (4)	5–7	4,600 (100)	3,100 (67.4)	2,200 (47.8)	1,000 (21.7)	510 (11.1)
Anti-M	NE (4)	5–7	5,500 (100)	3,200 (58.2)	2,500 (45.4)	1,200 (21.8)	720 (13.1)
Anti-F	NE (5)	5–8	4,000 (100)	2,450 (61.3)	1,820 (45.5)	840 (21.0)	480 (12.)
Neutralizing anti-H	AI-AE (3)	10, 12, 14	1,900 (100)	900 (47.3)	500 (26.3)	200 (10.5)	ND[b]
antibodies	AI-SAME (2)	27, 36	2,050 (100)	900 (44.0)	400 (19.5)	130 (6.3)	(50) (2.4)[c]

[a] The absolute number of the individual transcripts was determined as copies per 10 pg of RNA (copies), and the relative expression rates in relation to the expression of the corresponding N mRNA (N = 100%) are given as mean percent values for each experimental group.
[b] ND, No signal detectable.
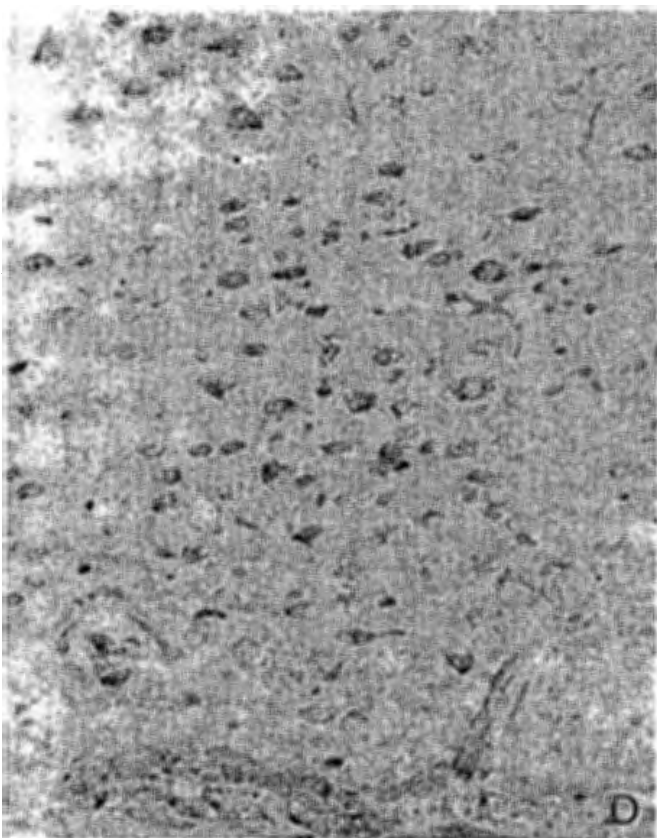[c] In one animal, 50 copies of H mRNA were detected; the other animal did not show an H-specific signal.
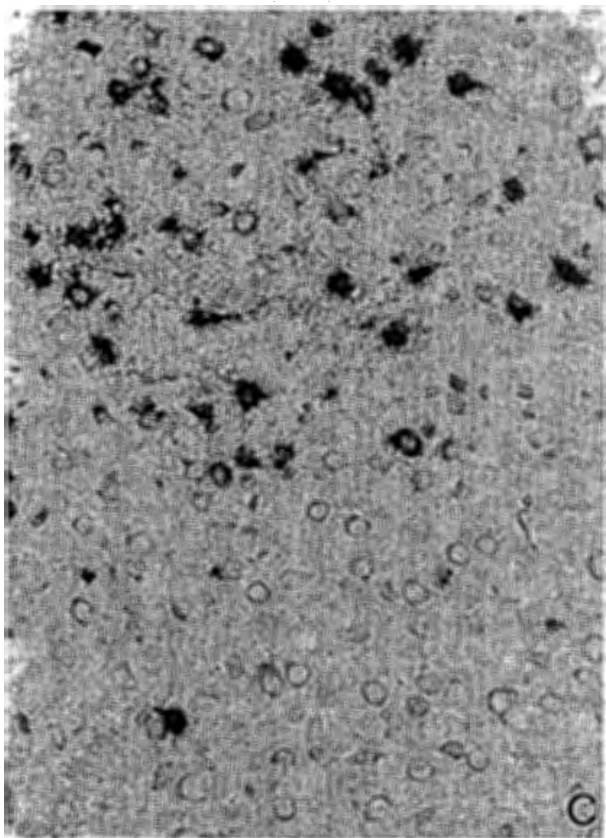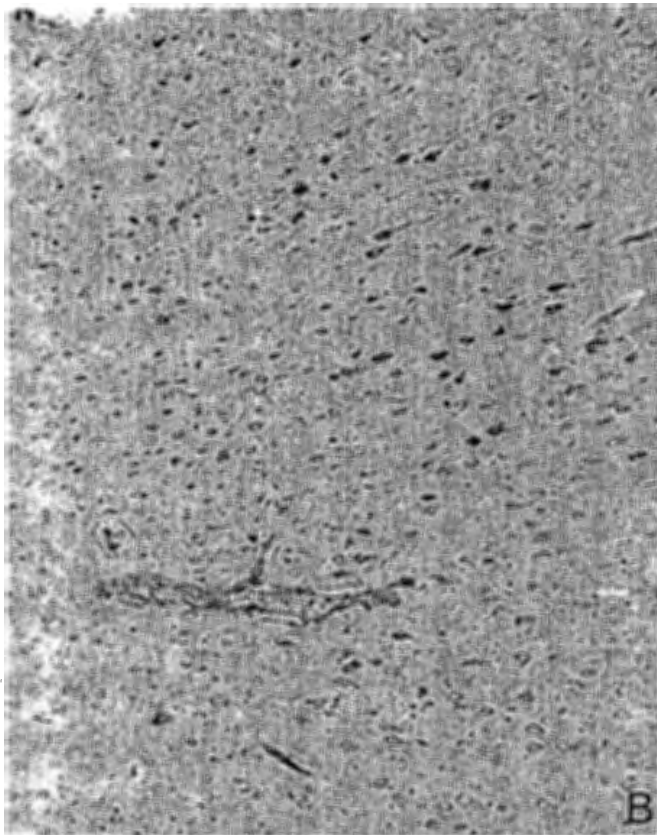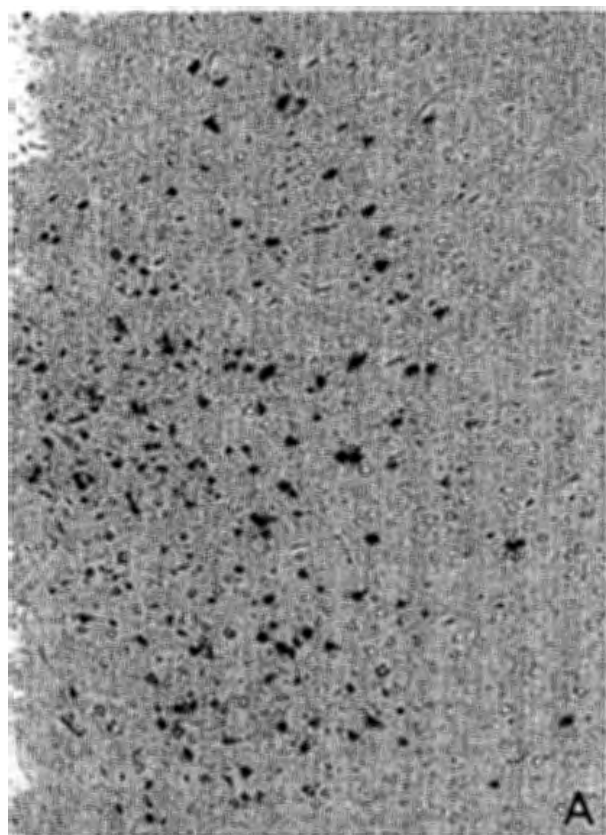
TABLE 4. Antiviral humoral immune response in weanling
Lewis and BN rats

Disease type	Strain	No. of animals analyzed	Antibody titer[a] (mean [range])	
			EIA	NT
			Serum	Serum
AE	Lewis	16	<25 (NA[b])	<10 (NA)
AE	BN	13	<25 (NA)	<10 (NA)
SAME	Lewis	12	400 (<25–1,600)	20 (<10–80)
CSE	BN	15	1,600 (200–6,400)	320 (80–640)
			CSF	CSF
AE	Lewis	16	<25 (NA)	<10 (NA)
AE	BN	13	<25 (NA)	<10 (NA)
SAME	Lewis	12	50 (<25–100)	<10 (<10–10)
CSE	BN	15	200 (50–800)	40 (10–80)

[a] Reciprocal of endpoint dilutions of MV-specific antibodies in enzyme immunoassay (EIA) and neutralization test (NT). Serum and CSF samples were obtained from the same group of animals.
[b] NA, Not applicable.

were used in parallel. Hybridization with the N gene-specific probe revealed no significant difference in the number of infected cells between the two groups of animals (Fig. 2A and B). This finding was confirmed by using the hybridization probe of the opposite sense detecting the negative-strand genomic RNA (data not shown). The hybridization signal in the infected brain cells of the antibody-treated animals, however, revealed a much lower intensity for the N mRNA as evident at higher magnification (Fig. 2C and D). Thus, the overall restriction of MV gene expression obviously results from restricted MV gene expression in the majority of infected cells rather than from a reduced number of infected cells. The number of cells showing a positive signal for the P mRNA was almost identical to the number of N mRNA-expressing cells, whereas M, F, and H mRNA-positive cells were barely visible in the antibody-modulated animals (data not shown).

Effect of naturally occurring antiviral humoral immune response on expression of viral transcripts in brain tissue. The phenomenon of antibody-induced restriction of MV gene expression in newborn rats depends on passive transfer of virus-specific monoclonal antibodies. To determine whether antibodies developing during MV infection in older animals influence viral replication, we included MV-infected weanling Lewis and BN rats with different types of encephalitis in our study. Rats with AE did not reveal viral antibodies in sera and CSF (Table 4), whereas those with SAME or CSE developed an MV-specific humoral immune response that was not detectable at incubation periods when AE occurred in other animals (data not shown). In particular, BN rats with CSE developed high titers of MV-specific neutralizing antibodies. The antibody titers were significantly lower in Lewis rats with SAME ($P > 99.5\%$, Mann-Whitney U test).

The expression gradient obtained by Northern blot analysis for MV-specific transcripts in weanling BN rats with AE was almost identical to that of Lewis rats with AE, and no differences were observed either in the overall expression rate of viral transcription or in the slope of the gradient (Table 5). Northern blot analysis of RNA derived from BN

rats with CSE, however, revealed differences. Whereas in Lewis rats with SAME the transcriptional efficiency of the MV genes was similar to the rate in rats with AE (Fig. 3A), the expression gradient of the viral mRNAs in BN rats with CSE was significantly steeper (Fig. 3B). The signal intensity for the P-, M-, and F-specific mRNAs was drastically decreased, and a signal specific for the H-specific mRNA was not detectable. This expression gradient was confirmed in five individual animals analyzed. By quantification of the signals obtained from BN rats with CSE, an overall reduction of the expression of the N gene by 65% compared with the Lewis rats with SAME was apparent (Table 5). The mean values for the expression of individual mRNAs relative to the N gene revealed a decrease in transcription of the P mRNA to 34.5% in animals with CSE (46% in rats with SAME), of the M mRNA to 15% (36% in rats with SAME), and of the F mRNA to 4.5% (12% in rats with SAME). H-specific mRNA was not detected in four of the five animals with CSE that were analyzed.

DISCUSSION

The infection of newborn Lewis rats with the neurotropic CAM/RBH strain of MV invariably leads to a fatal acute NE. By administration of neutralizing anti-H antibodies after the infection, this CNS disease process can be prevented. Instead, AI-AE or AI-SAME develops after prolonged incubation periods which are characterized by a transcriptional restriction of viral gene expression. This phenomenon does not occur when nonneutralizing antibodies are passively transferred. On the level of mRNA synthesis, the P and envelope genes are affected. While the reduced amount of P mRNA still directs the synthesis of sufficient protein to be demonstrated in almost all infected cells, the envelope proteins are not synthesized in detectable concentrations in the large majority of infected cells. This is accompanied by the failure to rescue infectious virus from the brains of animals with AI-SAME.

The same modulation of the CNS disease process with reduced efficiency of MV gene transcription occurs naturally in weanling BN rats. These rats develop CSE in association with high titers of virus-specific antibodies.

In our model, the development of a clinically overt CNS disease process seems to be linked to a low or absent humoral immune response against MV as seen in SAME or AE in Lewis rats (10). In contrast, the development of CSE in BN rats is correlated with high titers of MV-specific antibodies in serum and CSF and lower levels of virus replication compared with those in Lewis rats (17). Similar observations were made for MV or coronavirus infections in mice (5, 20). It was found that the administration of virus-specific antibodies can result in the emergence of chronic progressive CNS diseases or an atypical neuropathological response. Moreover, in mumps virus-induced meningoencephalitis in hamsters, a differentiated effect of the glycoprotein-specific antibodies on the course of the disease has been reported. Neutralizing hemagglutinin/neuraminidase-specific antibodies protected the animals from disease, while nonneutralizing F antibodies were ineffective (24). A correlation between an antiviral immune response and viral gene expression similar to our findings in the CNS of MV-infected rats

FIG. 2. In situ hybridization of paraffin-embedded brain sections with the [^{35}S]UTP-labeled N mRNA-specific RNA probe. (A) Weanling Lewis rat with AE (30 days old, 7 days p.i.); (B) newborn Lewis rat with AI-SAME (36 days p.i., anti-H); (C) detail panel of A; (D) detail of panel B; magnifications, ×250 (A and B) and ×600 (C and D).

TABLE 5. Quantitative analysis of MV-specific gene expression in brain material from weanling Lewis and BN rats[a]

Rat strain	Disease type	CSF antibodies against MV[b]	No. of copies (%) of MV-specific mRNA				
			N	P	M	F	H
Lewis	AE		4,400 (100)	2,400 (54.5)	1,850 (42)	650 (15)	400 (8)
BN	AE		3,900 (100)	2,400 (62)	1,550 (40)	650 (16.5)	300 (8)
Lewis	SAME	Low	3,850 (100)	1,770 (46)	1,390 (36)	460 (12)	180 (4.5)
BN	CSE	High	1,300 (100)	450 (34.5)	250 (15)	60 (4.5)	ND[c]

[a] Summarized are the mean values of the expression rates for groups of five animals each with AE (10 to 14 days p.i.), SAME (28 to 56 days p.i.), and CSE (30 to 60 days p.i.).
[b] The anti-MV antibody titers in CSF of all specimens are shown in Table 4.
[c] ND, No signal detectable.

has been observed in a nonneurological disease. In minks infected with Aleutian disease virus, it was found that a restriction of viral replication and transcription develops in lung tissue in the presence of antiviral antibodies (1). The alterations on the molecular level were accompanied by a chronic immune complex disease instead of an acute interstitial pneumonia that usually follows Aleutian disease virus infection in the absence of virus-specific antibodies. Although the specificity of the viral antibodies applied was not determined, the data show that antiviral antibodies contribute to chronic disease processes by downregulating viral gene expression.

Besides the antibody-induced restriction on MV gene expression in brain tissue, cellular factors are also capable of inhibiting MV replication. In a recent study, we observed in newborn as well as in weanling Lewis rats with CNS infections an MV transcriptional restriction of the glycoprotein-specific mRNAs as soon as MV-specific transcripts were detectable in brain tissue (21). This phenomenon was independent of the age of the animals, the incubation period of disease, and the humoral immune response since MV antibodies were not detectable in infected newborn rats. These cellular factors suppress a lytic cycle of viral replication in many brain cells immediately after infection. However, alone they are not capable of preventing an acute CNS disease since still too many brain cells are destroyed or functionally impaired by the infection. Only in concert with

FIG. 3. Northern blot analysis of 1 μg of each +pA RNA from a Lewis rat with SAME (A) and a BN rat with CSE (B); both animals were at 30 days p.i. Symbols: ▷, Position of the polycistronic RNA transcripts; ▶, position of the monocistronic RNA transcripts; ☐, position of the standard RNA transcripts.

antiviral antibodies is the inhibition of MV replication in brain cells so effective that destruction of infected cells is either prevented or slowed down and an acute disease process avoided. This combined effect of host factors and antibodies on MV replication is documented by the reduced overall viral transcription rate of about 50% and an enhanced restriction of MV envelope-specific mRNAs in animals with AI-SAME in comparison with untreated animals. This interpretation is further supported by our in situ hybridization experiments. The analysis of the N gene-specific expression at the single-cell level revealed that the observed enhanced restriction in antibody-treated animals does not result from a reduced number of infected cells but rather from a lower gene expression per infected cell. This finding indicates a general effect of antiviral antibodies on the viral transcription machinery and does not suggest an elimination of infected brain cells by host defense mechanisms or a lytic MV infection.

It remains speculative how the intracellular effect of antibody-induced antigenic modulation is achieved. In tissue culture experiments, capping of viral surface proteins after the binding of the appropriate antibody has been described (13). These immune complexes are either internalized or shed from the cell surface, leading to a temporary disappearance of viral antigens. By this means, virus is able to persist since antibody- and complement-mediated cell lysis cannot occur. However, besides removing viral glycoproteins from cell surfaces, antibodies also change the expression of some intracellular viral polypeptides. It was observed that application of polyclonal antibodies against MV or monoclonal antibodies against MV H protein to infected cells led to a reduced amount of total MV P and M proteins (11). In another study, application of anti-H monoclonal antibodies downregulated intracellular viral protein synthesis only when rat glioma cells persistently infected with MV and not infected Vero cells or lung fibroblasts were used (4). These findings suggested that signal transmission leading to transcriptional alterations of the viral genome is cell type specific. In this context, an interesting observation has been made recently (23). The addition of antimeasles IgG to the culture medium of rat glioma cells persistently infected with MV led to the activation of a G protein-mediated induction of inositol polyphosphate production. In turn, phosphokinase C was activated, which could lead to differential phosphorylation and affect the viral transcriptase complex itself. Such alterations in phosphorylation of MV M and P proteins, which are components necessary for the viral transcription and maturation process, have been described in the presence of antiviral antibodies (11). Alternatively, the activity or synthesis of cellular cofactors for viral transcription could be altered by phosphorylation.

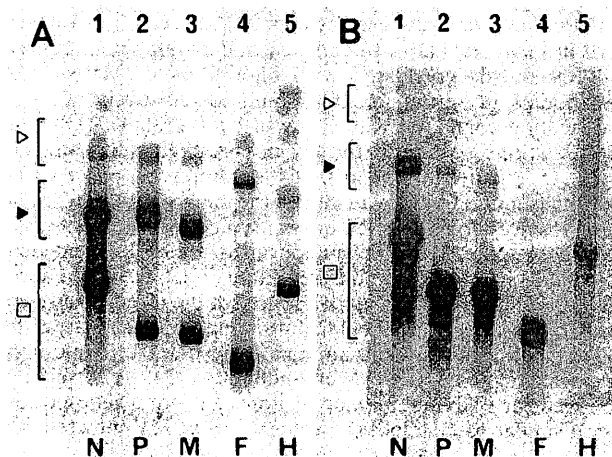At present, it is still unclear how the observed differential

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downregulation of the viral gene transcription in the brains of the two rat strains is achieved. The presence of antiviral antibodies certainly enhances host cell-specific restriction of the MV gene expression, thereby preventing an acute disease process and supporting the establishment of a persistent infection of the CNS. Without doubt, the phenomenon of antigenic modulation in vivo plays a pathogenetic role in the development of subacute or chronic diseases. It will be important to unravel the molecular mechanism by which viral antibodies interfere with intracellular events of viral replication.

ACKNOWLEDGMENTS

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