

Antibody-Dependent Transcriptional Regulation of Measles Virus in Persistently Infected Neural Cells

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Application of neutralizing anti-hemagglutinin antibodies to mouse neuroblastoma cells (NS20Y/MS) persistently infected with measles virus (MV) leads to a significant reduction of viral structural proteins within 6 days. While the transcriptional gradient for MV-specific mRNAs remained unaffected upon antibody treatment, the total amount of MV-specific transcripts dropped by 80% after 24 h. The expression of genomic RNA was affected similarly, with slightly slower time kinetics. Both transcription and expression of the viral structural proteins could be completely reactivated when viral antibodies were removed from the tissue culture. The same findings could be obtained in rat glioma cells persistently infected with subacute sclerosing panencephalitis virus (C6/SSPE) but not in cells of nonneural origin. The data indicate that antibody-induced antigenic modulation affects the early stages of viral transcription within a few hours after the addition of antibodies and leads to an almost complete repression of viral gene expression in cells of neural origin.

Measles virus (MV) is the etiological agent of subacute sclerosing panencephalitis (SSPE) and measles inclusion body encephalitis, which develop after incubation periods of months to years on the basis of a persistent infection in neurons and ganglion cells (41). Intense molecular analyses performed on autopsy brain material revealed that MV persistence is characterized by alterations of viral gene expression at the levels of transcription and translation (2, 6, 7, 8, 13). The mechanisms governing the establishment and maintenance of a persistent MV infection in brain cells are still largely unknown.

Recently, it has been shown that the transcriptional down regulation of MV-specific monocistronic mRNAs 3' of the N gene in brain material of patients with SSPE seems to represent the action of cell-type-specific factors rather than a consequence of viral persistence (39, 40). Furthermore, a cellular double-stranded RNA-specific unwinding-modification activity has been suggested to act on MV gene transcripts in brain cells, leading to hypermutation events that in turn could lead to down regulation of MV gene expression (5, 14, 34). In addition, MV transcription may also be influenced by the state of differentiation of the infected cells (25, 37, 44).

Besides intracellular mechanisms, virus-neutralizing antibodies are also effective in regulating MV gene expression as described in tissue cultures as well as in *in vivo* experiments (4, 17, 18, 24, 32, 38). Antibody-dependent down regulation of intracellular MV-specific structural proteins has been observed after prolonged passages in rat glioma C6/SSPE and mouse neuroblastoma NS20Y/MS cells (4, 32) but not in cells of nonneural origin (4, 38). *In vivo*, passive transfer of virus-neutralizing antibodies leads to a prolongation of experimentally induced infections of the central nervous system in rodents accompanied by a highly restricted expression of viral structural proteins and mRNAs (24, 33).

In order to investigate the early effects of antibody-

dependent antigenic modulation on MV gene expression in cells of neural origin, we analyzed viral replication and transcription during the first few days after the application of virus-neutralizing antibodies to mouse neuroblastoma NS20Y/MS and rat glioma C6/SSPE cells.

The data reveal a significant reduction of the intracellular viral structural proteins after antibody treatment as well as a strong decrease in virus-specific transcription to 20% of the original expression frequency within 24 h. Since transcription of genomic RNA was equally affected and the slope of the gradient of the monocistronic mRNAs was similar to that of untreated cells, the down regulation of viral transcription in our system is not specific for individual RNAs but rather reflects a general inhibition of MV transcription during persistent infection.

MATERIALS AND METHODS

Cell lines and viruses. The C1300 neuroblastoma clone NS20Y of the A/J mouse strain was used. The establishment of MV persistently infected (PI) NS20Y cells is described in detail elsewhere (32). For our experiments, subclone NS20Y/MS was used, which did not produce infectious virus at any temperature. In addition, rat glioma C6 cells PI with SSPE LEC virus (19), human lung fibroblast cells Car Lu PI with the Edmonston strain of MV (28), and Vero cells were used. MV/CAM was propagated in Vero cells (22). For the lytic infection, Vero cells were infected at a multiplicity of infection of 0.5 PFU per cell and were harvested after 72 h.

Antibodies and antibody treatment. Polyclonal anti-MV hyperimmune serum was obtained after immunization of rabbits with purified MV Edmonston grown on Vero cells (21). The preparation and characterization of monoclonal antibodies against MV N and H proteins were described elsewhere (8, 10, 29). For treatment with antibodies, the medium of semiconfluent cultures was replaced with fresh medium containing anti-MV hyperimmune serum (sufficient to neutralize 10⁴ PFU of MV) or, when indicated, a mixture of neutralizing anti-H antibodies (L77, NC 32, and K83) as a

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stock solution with a titer in the hemagglutinin inhibition (HI) assay of 16,000 HI U/ml; the mixture was prediluted in minimum essential medium before application on tissue culture cells (40 HI U/ml). For the time kinetics, antibody-treated or untreated cells were harvested at the times indicated after antibody treatment. After 3 days, the cells were split and fresh antibodies were applied to the medium. For reactivation, antibodies were removed from the culture supernatant and cells were harvested 144 h, 216 h, and 14 days later.

Fluorescence analysis. The percentage of MV-positive cells in the cultures was determined by using either indirect immunofluorescence or fluorescence-activated cell sorter (FACS) analysis. After extensive washing, cells were fixed for FACS analysis in 10% paraformaldehyde in phosphate-buffered saline (PBS) for 15 min followed by two rinses in PBS and subsequent permeabilization in 0.25% Triton X-100 for 20 min at room temperature. After a further rinse in PBS, the primary antibody (either monoclonal antibodies directed against N, M, F, or H or a polyclonal measles serum; each as a dilution of 1:50) was applied for 3 h at room temperature. Cells were rinsed in PBS and incubated with the second antibody (goat anti-mouse or goat anti-human immunoglobulin, respectively, diluted 1:50 in PBS containing 5% newborn rat serum) at 4°C for 150 min. After a final rinse in PBS, cells were analyzed by FACScan.

Preparation of cell extracts and Western immunoblot analysis. For protein analysis, cells were washed with PBS, resuspended, and lysed in ice-cold buffer containing 50 mM Tris HCl (pH 7.5), 150 mM NaCl, 0.05% Nonidet P-40, 0.1% SDS, and 0.25% sodium deoxycholate. Extracts were clarified by centrifugation for 30 min at 4°C at 12,000 × *g*. After determination of the protein concentration, samples containing 150 µg of protein were separated on 10% polyacrylamide gels and transferred to nitrocellulose filters. After being blocked for 1 h in 10% milk (1.5% fat) in PBS at 37°C, the nitrocellulose filters were incubated overnight at 4°C with MV antibodies. Filters were washed three times with PBS at room temperature and incubated with peroxidase-conjugated anti-rabbit immunoglobulin in 1% milk for 1 h. After being washed as indicated above, filters were developed with 4-chloronaphthol (0.3 mg/ml) and H₂O₂.

RNA extraction and quantitative Northern (RNA) blot analyses. RNA extraction, selection of poly(A)⁺ RNA, and quantitative Northern blot analyses were performed as described before (39). Briefly, after cell lysis in 4 M guanidinium isothiocyanate buffer, the RNA was separated by cesium chloride centrifugation and used either directly as total RNA or enriched for poly(A)⁺ RNA by a single selection over an oligo(dT) column. Each 2 µg of poly(A)⁺ RNA together with a mixture of synthetic standard RNA transcripts (11, 12) was separated on 1.5% formaldehyde-containing agarose gels, blotted onto nitrocellulose, and hybridized to [³²P]CTP-labelled single-stranded RNA transcripts specific for each individual MV structural gene except L. After exposition, signals specific for the monocistronic MV-specific and standard RNA transcripts were excised and the radioactivity retained was determined by scintillation counting. Rat GAPDH-specific cDNA (16) and the MV N-specific 851-bp cDNA (12) were used as gel-purified fragments for random priming in the presence of [³²P]dCTP, following the manufacturer's protocol (Boehringer GmbH, Mannheim, Germany).

In vitro translation. Each 1 µg of poly(A)⁺-enriched RNA was translated in vitro in the presence of 30 µCi of [³⁵S]methionine in rabbit reticulocyte lysate for 90 min, following

the manufacturer's protocol (Promega). MV-specific translation products were immunoprecipitated by using a polyclonal rabbit anti-MV hyperimmune serum and subsequently were separated by 10% polyacrylamide gel electrophoresis as described previously (39).

RESULTS

Characterization of untreated NS20Y/MS and C6/SSPE cells PI with MV. By immunofluorescence with a polyclonal anti-MV hyperimmune serum, 100% of C6/SSPE cells exhibited positive staining and 35 to 40% of NS20Y/MS cells exhibited positive staining. Using monoclonal antibodies, we could show that in both cell lines, every infected cell stained positive for the individual structural proteins N, M, F, and H (not shown). Transcription of MV-specific monocistronic mRNAs was analyzed by quantitative Northern blot experiments using poly(A)⁺-enriched RNA from NS20Y/MS, C6/SSPE, and lytically infected Vero cells (Fig. 1). Compared with the lytic control in Vero cells (Fig. 1a), the transcriptional activity in NS20Y/MS cells was reduced to about 5%, as determined by measuring the expression of the N mRNA-specific transcript per 10 pg of RNA (Fig. 1b). The frequency of expression of the monocistronic MV-specific transcripts decreased markedly along the gene order, with the P mRNA being expressed to 52.4% of the N mRNA, the M mRNA being expressed to 26%, the F mRNA being expressed to 10%, and the H mRNA being expressed to 5.5% as shown for NS20Y/MS cells (Table 1). In addition to the quantitative alterations, the M-specific mRNA appeared as a double band in these cells (Fig. 1b, lane 3).

This particular down regulation of transcripts specific for the envelope genes was described previously in brain material of patients with SSPE and experimentally MV-infected rats (indicated for comparison in Fig. 1d) and could also be found in C6/SSPE cells (Fig. 1c).

Effects of antibody treatment on the synthesis of MV structural proteins. To investigate the effect of anti-MV treatment on the synthesis of MV structural proteins that normally accumulate to high quantities in PI NS20Y/MS cells, a polyclonal antiserum was applied for different time periods. Cells were harvested at different time intervals as indicated and stained for the presence of MV structural proteins N, M, F, and H (Table 2). Whereas the percentage of positive cells and relative fluorescence intensities for M, F, and H had decreased significantly by 48 or 72 h after treatment, the percentage of cells expressing N protein (in total 45%) was not changed at that time. After day 4, the number of N-expressing cells in the culture and the relative fluorescence intensity was also drastically reduced. To monitor the further disappearance of this protein, cells were harvested on days 6, 16, and 23 and analyzed for the expression of MV N protein by Western blot analysis, using a monoclonal anti-N antibody (Fig. 2a). Although N protein could still be detected after 6 days, the total amount of this protein was markedly reduced (Fig. 2a, lane 2). After 16 and 23 days of treatment, no N protein synthesis could be detected (Fig. 2a, lanes 3 and 4). The down regulation of MV structural proteins by anti-MV antibodies seems to be specific since treatment with a nonspecific polyclonal rabbit antiserum for the same time period had no effect (Fig. 2b). Upon removal of the antibodies and analysis after 14 days, N protein was reexpressed (Fig. 2a, lane 5). Thus, modulation of MV protein synthesis in PI cells is reversible and depends on the presence of MV-specific antibodies. No influence on intracellular MV protein expression could be observed with

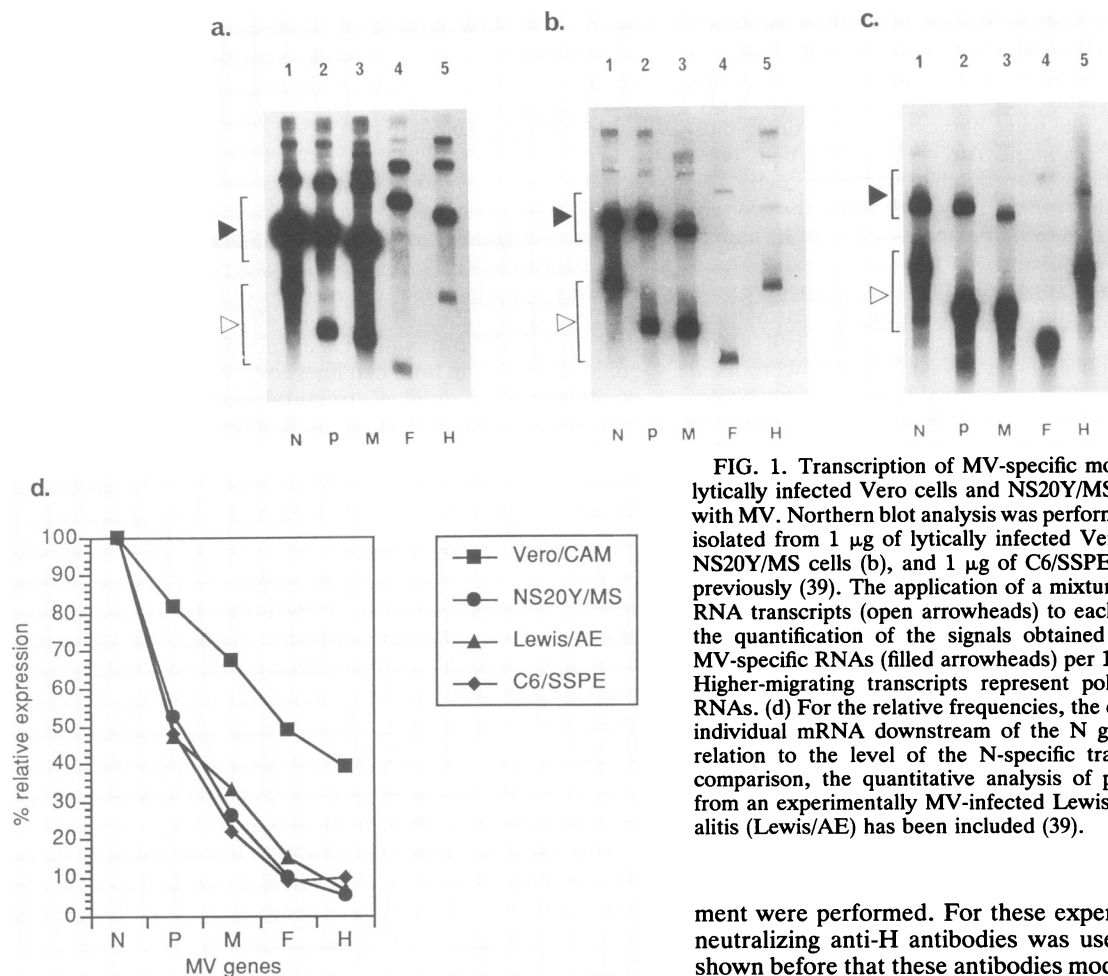


FIG. 1. Transcription of MV-specific monocistronic mRNAs in lytically infected Vero cells and NS20Y/MS and C6/SSPE cells PI with MV. Northern blot analysis was performed with poly(A)⁺ RNA isolated from 1 μ g of lytically infected Vero cells (a), 2 μ g of PI NS20Y/MS cells (b), and 1 μ g of C6/SSPE cells (c), as described previously (39). The application of a mixture of synthetic standard RNA transcripts (open arrowheads) to each RNA sample allowed the quantification of the signals obtained for the monocistronic MV-specific RNAs (filled arrowheads) per 10 pg of total RNA (12). Higher-migrating transcripts represent polycistronic MV-specific RNAs. (d) For the relative frequencies, the expression rate for each individual mRNA downstream of the N gene was determined in relation to the level of the N-specific transcript (Table 1). For comparison, the quantitative analysis of poly(A)⁺ RNA isolated from an experimentally MV-infected Lewis rat with acute encephalitis (Lewis/AE) has been included (39).

Car Lu cells when neutralizing anti-H antibodies or anti-MV hyperimmune serum was used (not shown).

Regulation of MV-specific transcription in antibody-treated NS20Y/MS cells. To test for the biologic activity of MV-specific mRNAs in antibody-treated cells, *in vitro* translation experiments with poly(A)⁺ RNA from NS20Y/MS, C6/SSPE, and Car Lu cells obtained 72 h after antibody treat-

ment were performed. For these experiments, a mixture of neutralizing anti-H antibodies was used, since it has been shown before that these antibodies modulate as efficiently as complete antiserum (4, 24). Corresponding to the data obtained *in vivo*, antibody treatment revealed only a slight effect on MV-specific protein expression *in vitro* in Car Lu cells (Fig. 3, lanes 2 and 3), whereas in NS20Y/MS (not shown) and C6/SSPE cells a significant reduction of MV-specific protein synthesis could be observed compared with that for the untreated control (C6/SSPE; Fig. 3, lanes 4 and 5). The differences in the electrophoretic mobilities of MV N and P proteins between lytic and persistent infections have been described earlier (Fig. 3, lanes 1, 4, and 5) (35).

TABLE 1. Relative expression frequencies of the monocistronic MV-specific transcripts in lytically infected Vero, NS20Y/MS, and C6/SSPE cells and in brain material of an experimentally infected Lewis rat (39)^a

MV gene	Expression frequency (%) ^b			
	Vero/CAM	NS20Y/MS	C6/SSPE	Lewis/AE ^c
N	100	100	100	100
P	82	52.4	48	47
M	67.5	26	22	33
F	46	10	9	15
H	33	5.5	10	7

^a Signals specific for the monocistronic MV-specific transcripts in lytically infected Vero cells (Fig. 1a) and NS20Y/MS (Fig. 1b), and C6/SSPE cells (Fig. 1c) and for the standard RNA transcripts were excised, and the retained radioactivity was determined by scintillation counting.

^b Expression frequencies for the individual transcripts were calculated relative to the N mRNA expression.

^c Brain material from a Lewis rat with acute MV encephalitis.

TABLE 2. Expression of MV structural proteins in antibody-treated NS20Y/MS cells^a

Treatment	MV structural protein expression ^b			
	N	M	F	H
Untreated	+++	+++	+++	+++
Day 1	+++	+++	+++	+
Day 2	+++	++	+	(+)
Day 3	++	+	+	(+)
Day 4	++	+	(+)	(+)
Day 5	+	(+)	(+)	ND
Day 6	+	(+)	ND	ND

^a Cells were incubated with a polyclonal rabbit anti-MV hyperimmune serum for the intervals indicated, harvested, fixed, and analyzed for the expression of MV N, M, F, and H proteins by immunofluorescence, using monoclonal antibodies.

^b +++, strong; ++, intermediate; +, low; and (+), very low relative fluorescence intensities. ND, not detectable.

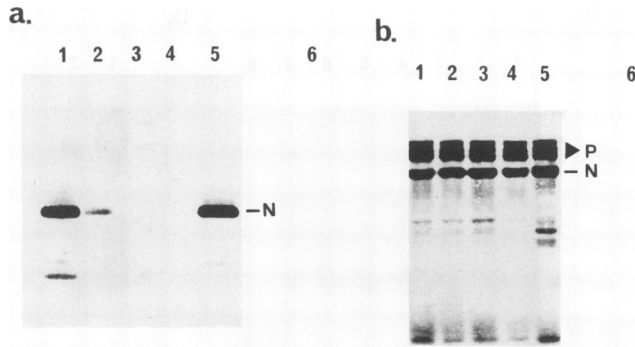


FIG. 2. Modulation of MV protein synthesis by polyclonal MV antiserum. NS20Y/MS cells were cultured in the presence of rabbit polyclonal MV antiserum (a) or nonspecific rabbit antibodies (b), and extracts were prepared and Western blot analysis was performed by using a monoclonal mouse anti-N antibody (a) and a polyclonal anti-MV antiserum (b). Lanes: 1, untreated NS20Y/MS cells; 2, 6 days after antibody treatment; 3, 16 days after treatment; 4, 23 days after antibody treatment; 5, 23 days after antibody treatment plus 14 days without antibodies.

In order to determine the basis of the down regulation of MV-specific structural protein synthesis after treatment with antibodies *in vivo* and *in vitro*, poly(A)⁺ RNA was isolated from antibody-treated and untreated NS20Y/MS cells at different time points and subjected to quantitative Northern blot analysis. To determine the immediate effects on MV transcription, we decided to choose early time points (12 to 72 h after antibody treatment) for our analysis. Whereas at 12 h after treatment no effect on MV transcription could be observed (Fig. 4a; Table 3), total transcription of MV-specific monocistronic mRNAs as measured by the expression of the N-specific mRNA was attenuated significantly to 19 and 10% after 24, 48, and 72 h, respectively (Fig. 4b and

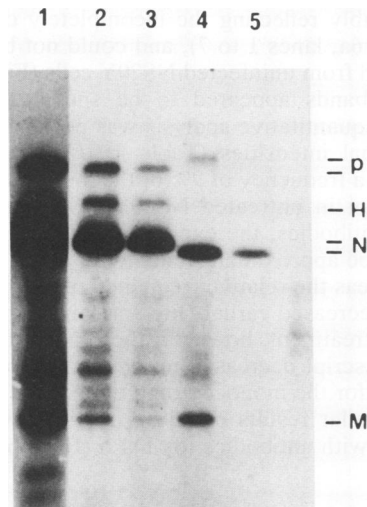


FIG. 3. *In vitro* translation of poly(A)⁺ RNA from antibody-treated and untreated C6/SSPE and Car Lu cells. Each 1 μ g of poly(A)⁺ RNA from lytically infected Vero cells (lane 1), Car Lu cells (lane 2), Car Lu cells treated with antibodies for 3 weeks (lane 3), C6/SSPE cells (lane 4), and C6/SSPE cells treated with antibodies for 72 h (lane 5) was translated *in vitro* and immunoprecipitated with anti-MV hyperimmune serum. Samples were separated on 10% polyacrylamide gels.

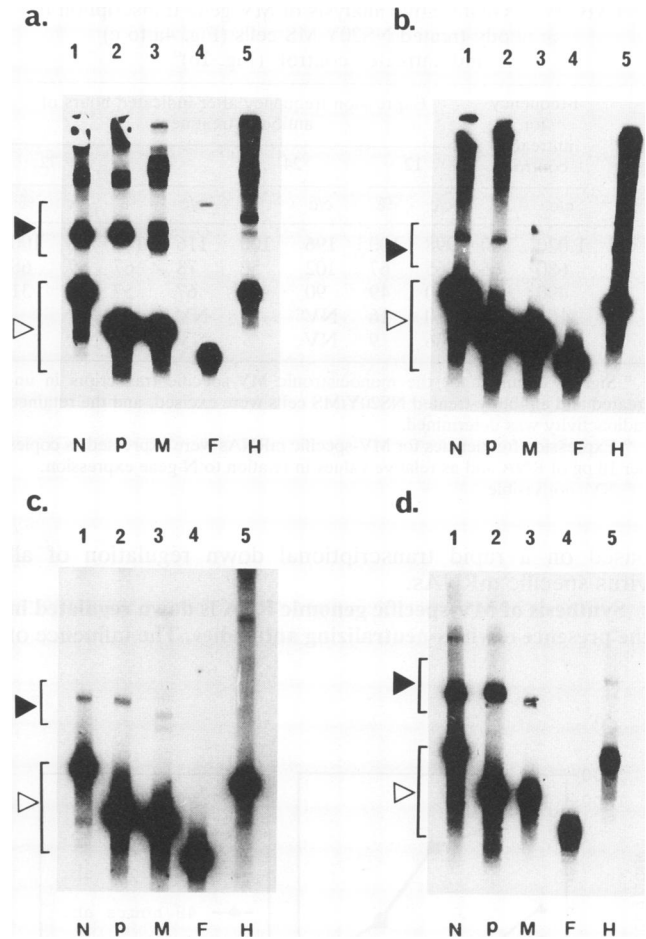


FIG. 4. Expression of monocistronic MV-specific mRNAs in antibody-treated NS20Y/MS cells. Northern blot analysis of each 2 μ g of poly(A)⁺ RNA isolated from NS20Y/MS cells incubated with a mixture of virus-neutralizing monoclonal anti-H antibodies for 12 (a), 48 (b), 72 (c), and 144 h after antibody removal from cells treated previously with antibodies for 144 h (d). Signals corresponding to the monocistronic MV-specific transcripts are indicated by filled arrowheads, and those for the standard RNA transcripts are indicated by open arrowheads.

c; Fig. 5a; Table 3), compared with that of the untreated control. Again, the M-specific probe hybridized with two M-specific poly(A)⁺ transcripts migrating at approximately the same rate (Fig. 4a to c, lanes 3). After removal of the antibodies 6 days after treatment, transcription could be completely restored within 144 h (Fig. 4d). Control hybridizations using a rat GAPDH cDNA were performed to confirm that equal amounts of poly(A)⁺ RNA had been analyzed (not shown). The quantitative analysis for the first three genes, N, P, and M, revealed no obvious differences in their relative expression frequencies in antibody-treated cells compared with those of the untreated controls (Fig. 1b; Fig. 5b; Table 3). Quantification of the signals obtained for the monocistronic RNAs downstream of the N gene, in particular for envelope gene-specific mRNAs, was not possible due to their low frequencies (Fig. 4b and c). The same results were obtained with C6/SSPE cells (as exemplified in Fig. 6b). These data suggest that antibody-dependent restriction of MV-specific protein expression in neural cells is

TABLE 3. Quantitative analysis of MV gene transcription in antibody-treated NS20Y/MS cells (Fig. 4a to c) and untreated controls (Fig. 1b)^a

MV gene	Frequency for untreated control ^b		Expression frequency after indicated hours of antibody treatment							
	c/c	%	12		24		48		72	
			c/c	%	c/c	%	c/c	%	c/c	%
N	1,020	100	998	100	196	100	116	100	98	100
P	680	66	665	67	102	52	78	67	67	68
M	490	48	490	49	90	46	67	57	30	31
F	155	15	160	16	NV ^c		NV		NV	
H	100	9	86	9	NV		NV		NV	

^a Signals obtained for the monocistronic MV-specific transcripts in untreated and antibody-treated NS20Y/MS cells were excised, and the retained radioactivity was determined.

^b Expression frequencies for MV-specific mRNAs were expressed as copies per 10 pg of RNA and as relative values in relation to N-gene expression.

^c NV, not visible.

based on a rapid transcriptional down regulation of all virus-specific mRNAs.

Synthesis of MV-specific genomic RNA is down regulated in the presence of virus-neutralizing antibodies. The influence of

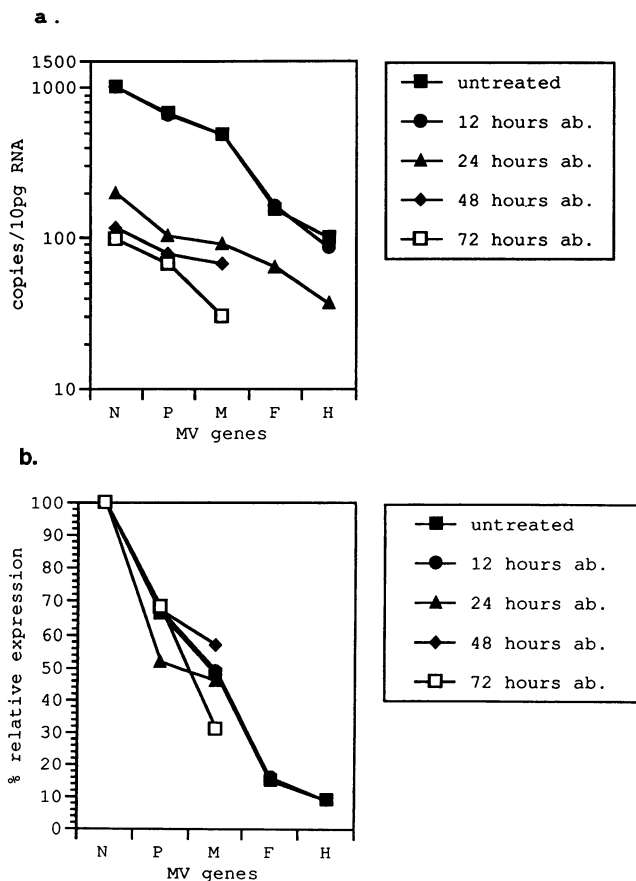


FIG. 5. Quantitative analysis of MV transcription in antibody-treated NS20Y/MS cells. Signals obtained for the monocistronic MV-specific transcripts and for their corresponding standard RNA transcripts were excised from the Northern blots shown in Fig. 3 and are shown as copy numbers per 10 pg of RNA (a) or as relative expression frequencies in relation to N gene expression (b).

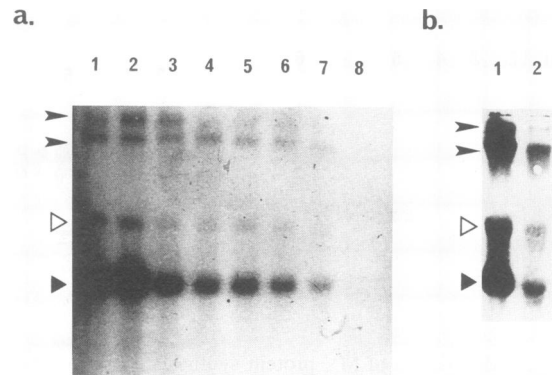


FIG. 6. Northern blot analysis of total RNA from NS20Y/MS, C6/SSPE, and uninfected NS20Y cells treated or untreated with antibodies. Each 15 μ g of total RNA was separated on an agarose gel, blotted onto nitrocellulose, and hybridized to an N-specific [³²P]dCTP-labelled DNA probe. The signal intensities were determined by scanning densitometry of the autoradiograph. (a) NS20Y/MS cells untreated (lane 1), treated with antibodies for 12 h (lane 2), 24 h (lane 3), 48 h (lane 4), 72 h (lane 5), 144 h (lane 6), and 196 h (lane 7) and uninfected NS20Y cells (lane 8). (b) C6/SSPE cells untreated (lane 1) and treated with antibodies for 144 h (lane 2).

virus-neutralizing antibodies on the expression of MV-specific bicistronic and genomic RNA was assayed by Northern blot analysis of total RNA isolated from antibody-treated cells. Total cellular RNAs were separated and hybridized to an N-specific DNA probe, and MV-specific signals were analyzed by scanning densitometry. As determined earlier for NS20Y/MS cells (Fig. 4a to c), the relative frequency of the monocistronic N gene-specific transcripts appeared stable at 12 h after treatment and decreased about fivefold after 24 h (Fig. 6a, lanes 1 to 7; Table 3). The intermediate-sized bicistronic NP transcript disappeared with approximately the same time kinetics (Fig. 6a, lanes 1 to 7; Table 3). Signals specific for the genomic RNA appeared to migrate as double bands, probably reflecting the incompletely denaturing gel system (Fig. 6a, lanes 1 to 7), and could not be detected in RNA isolated from uninfected NS20Y cells (Fig. 6a, lane 8). Since both bands appeared to be specific for the viral genome, the quantitative analysis was performed by adding up both signal intensities (Table 4). Genomic RNA was expressed at a frequency of 9% of the monocistronic N-specific transcript in untreated NS20Y/MS cells. After treatment with antibodies, the expression of the genomic RNA appeared to be approximately stable for 72 h (Fig. 6a, lanes 1 to 4), whereas the relative frequency of the monocistronic transcripts decreased earlier (Fig. 6a, lanes 1 to 5; Table 4). With longer treatment, however, the signal intensity for the genomic transcript decreased to almost undetectable levels as described for the monocistronic transcript (Fig. 6a, lanes 6 and 7). Similar results could be obtained with C6/SSPE cells treated with antibodies for 144 h (Fig. 6b, lane 2).

DISCUSSION

Persistent MV infection in cells of the central nervous system has been studied in detail at the molecular level, providing experimental evidence for sequence alterations of the viral genome that inhibit or impair the expression of viral structural proteins (6). However, little is known about the mechanisms underlying the establishment of a persistent

TABLE 4. Quantitative analysis of the expression of genomic, bicistronic, and monocistronic NS20Y/MS cells^a

Time (h) of antibody treatment	Expression (%) of indicated N-specific transcript			Ratio of expression of monocistronic/genomic transcripts
	Genomic	Bicistronic	Monocistronic	
0	100	100	100	11:1
12	93	100	100	11:1
24	100	80	38	4:1
48	72	27	22	3.5:1
72	51	10	23	7:1
96	19	NV ^b	11	7:1
144	(3.5) ^c	NV	NV	

^a Signal intensities for the genomic, bicistronic, and monocistronic N-specific transcripts shown in Fig. 6 were determined by scanning densitometry and expressed as percent of the untreated control (each 100% for the genomic, the bicistronic, and the monocistronic transcript).

^b NV, not clearly visible.

^c Value in parentheses was too low to be discerned clearly from the background.

infection in neural cells and what mechanisms support this particular virus-host interaction besides the action of cell-type-specific factors (39, 40).

Viral antibodies have been shown to modulate virus-induced disease processes in a variety of systems, including MV infections (1, 9, 23, 24, 33). In this context, a pathogenic role has been discussed earlier for virus-neutralizing antibodies that are present in extremely high amounts in serum and cerebrospinal fluid of patients with SSPE (17, 18). Although the H protein is only expressed in trace amounts in brain material of patients with SSPE, sequence analyses have revealed that the corresponding gene, in contrast to most of the other open reading frames of MV genes, is highly conserved (6, 7). Therefore, it seems as if there are no structural or functional changes in this particular protein.

To study the effect of antibody-induced antigenic modulation on MV gene expression, we investigated MV-specific RNA expression in mouse neuroblastoma NS20Y/MS and C6/SSPE cells PI with MV. It has been shown previously that nonneutralizing antibodies directed against the N, M, F, and H proteins do not exert any modulating effects in tissue culture or in vivo experiments (4, 24). Therefore, in part of our study, a mixture of three neutralizing anti-H antibodies was used. Either of the antibodies chosen alone has been shown to modulate MV gene expression in tissue culture and in vivo (4, 24). Confirming the results obtained with C6/SSPE cells, a significant reduction in the expression of all MV structural proteins upon antibody treatment was observed within a few days; that expression could be reactivated completely after removal of the antibodies (Fig. 2a) (4). This particular effect proved to be specific for persistently MV-infected cells of neural origin since infected lung fibroblasts did not show the antibody-dependent down regulation of MV protein synthesis (not shown). In addition, this effect appeared to be specific for MV antibodies (Fig. 2b).

The transcription pattern observed in the PI NS20Y/MS and C6/SSPE cells revealed restrictions of, in particular, the envelope gene-specific mRNAs identical to those observed in brain material of patients with SSPE and in experimentally infected rats (11, 12, 39) (Fig. 1b to d). This particular transcriptional regulation has not been described in PI cell lines of nonneural origin (11, 12) and may reflect a prerequisite for MV persistence in brain cells.

The analysis of MV-specific gene expression in antibody-

treated NS20Y/MS cells revealed that down regulation of MV-specific transcription, as monitored by the expression of the N-specific mRNA, occurs as early as 24 h after application of neutralizing antibodies to the tissue culture (Fig. 3). A complete restoration of transcription was observed by 144 h after removal of the antibodies. This increase in transcription most likely represents an activation of the viral genomes still present in the majority of cells rather than a clonal expansion. Barrett and coworkers (4) could show by single-cell cloning of antibody-treated, MV protein-negative C6/SSPE cells that MV expression could be reactivated after antibody removal in about 60% of all clones. In addition, in situ hybridization experiments performed on brain material of antibody-treated experimentally MV-infected Lewis rats revealed that, although a general down regulation of viral protein and RNA synthesis was obvious, the total number of cells containing MV-specific RNA was not significantly reduced (24).

In contrast to the results on down regulation of transcription in newborn experimentally infected Lewis rats with passive transfer of antibodies (24), we could not find an impact of the antibodies on the slope of the transcription gradient in our tissue culture experiments (Fig. 3 and 4). This discrepancy may be due to the fact that the experiments performed in vivo definitely reflect a more complex experimental system than our tissue culture experiment. We could, however, confirm that MV transcription is generally restricted by 80%, as monitored by expression of the N-specific mRNA (Table 3). Although the decrease of mono- and bicistronic N-specific transcripts seems to occur after 24 h, expression of the genomic RNA is down regulated later than 72 h after antibody treatment (Fig. 6, lanes 5 to 7). The data obtained in our study are consistent with the model that initiation of the polymerase complex for transcription and replication occurs at one polymerase entry site (20) and that replication of the viral genome is dependent on de novo synthesis of viral protein synthesis (6). Since we found a down regulation of MV-specific RNA and protein synthesis, it is likely that replication of the viral genome has to be affected. Moreover, it has been described recently that defective nonreplicating MV particles reveal a maximum intracellular stability of 3 days (26), so that in the absence of transcription of monocistronic mRNAs a persistent infection could not be maintained.

Experimental evidence for the inactivation of polymerase function after treatment with neutralizing antibodies has been obtained for influenza virus in vitro (31). Since the general transcription pattern of MV did not seem to be affected, our interpretation of the data suggests that the regulation provided by antibody-induced antigenic modulation is directed at the interaction between the polymerase complex and the promoter region of MV. Until now, the function of *cis*-acting sequences and their interaction with viral and/or cellular proteins could not be addressed experimentally. Recently, experiments have been designed and successfully applied for analyzing those particular problems (15, 27, 30). Since those systems, however, depend on the functional expression of the viral polymerase complex, their application to the mechanisms of antibody-induced antigenic modulation appears difficult.

It remains unknown how an external signal provided by the neutralizing anti-H antibodies is transmitted and leads to the regulation of MV transcription. Experimental evidence for a function of MV H protein as a signal-transducing receptor on C6/SSPE cells has been obtained by Weinmann-Dorsch and Koschel (43). After binding of neutralizing

anti-H antibodies, an increase in phosphoinositide breakdown followed by elevated levels of 1,2-diacylglycerol and inositol triphosphate could be measured. These substances act as second messengers in the activation of protein kinase C and the release of Ca^{2+} from intracellular stores. Whether this activation takes part in the intracellular down regulation of MV transcription remains speculative, since it became evident recently that an increase in intracellular Ca^{2+} and the activation of protein kinase C up regulates MV transcription in lytically MV-infected peripheral blood lymphocytes (42). It remains to be investigated whether the latter findings obtained with a different cell type from a lytic infection also hold true for PI neural cells. Cellular protein kinases are likely to contribute to the activity of the viral polymerase complex since at least N and P proteins have been shown to be phosphorylated (36). As shown for vesicular stomatitis virus, phosphorylation of the P protein seems to be essential for its transactivating capacity and is, in part, dependent on the action of cellular kinases (3).

Detailed analyses of the modifications and functions of the MV polymerase complex will be performed in order to understand its regulation by endogenous and exogenous factors present in cells of the central nervous system that enable the establishment and maintenance of persistent rather than lytic infections.

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