JOURNAL OF VIROLOGY, Feb. 1995, p. 734–740 Vol. 69, No. 2
0022-538X/95/$04.00+0

Suppression of Measles Virus Expression by Noncytolytic Antibody in an Immortalized Macrophage Cell Line

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Received 26 August 1994/Accepted 2 November 1994

Immune regulation of measles virus (MV) expression was studied in a persistently infected mouse macrophage cell line. Synthesis of both membrane-associated and internal MV antigens was suppressed when infected macrophages were treated with polyclonal rabbit anti-MV antibody that was specific for MV proteins. Persistently infected macrophages were treated for 3, 5, or 7 days with increasing doses of anti-MV antibody. All MV proteins were down-regulated 2 days after treatment was terminated. One week after treatment was terminated, down-regulation was still evident but to a lesser degree. MV protein synthesis was suppressed whether or not complement components were inactivated by heating all serum supplements and antibodies. However, when complement was active, cell lysis accounted for some of the reduced MV protein synthesis. When lytic destruction of infected cells by antibody and complement was prevented by inactivation of complement, antibody alone reduced the cellular synthesis of viral proteins by noncytolytic mechanisms. The absence of cell death in the absence of complement was confirmed by the lack of ^{51}Cr release from labeled cells, the lack of reduction in cell number, and the lack of a decrease in total protein synthesis when radiolabeled infected cells were treated with antibody. It is noteworthy that low doses of antibody were optimal for suppression in the longer-term experiments and did not cause lysis, even in the presence of active complement. Since infected macrophages disseminate virus in measles infection, noncytolytic regulation of these cells by antibody may supplement viral clearance by cytolytic T cells and other immune mechanisms.

Antibody directed against individual components of a cell, including viral antigens, may have a number of different effects. Antibody binding to antigen on the cell surface may lead to aggregation of the bound molecules at one pole of the cell in a process termed capping. Antibody may activate the complement system, leading to opsonization or cell lysis. Antibody may enable lysis of the infected cell by lymphocytes in a process termed antibody-dependent cell cytotoxicity. Antibody binding to surface antigens may lead to induction of transmembrane signaling pathways to alter cell functions. Antibody binding to surface antigens may lead to endocytosis of a surface component and, once inside the cell, it may modulate cellular processes or simply be degraded. Antibody binding to surface antigens may lead to down-regulation of synthesis of cellular or viral components with which the antibody reacts in a process we have termed antigenic suppression, to distinguish it from several other immunologic processes that are referred to as suppression.

Other laboratories have demonstrated that antibodies to different measles virus (MV) proteins suppress expression in vitro and are protective in vivo. Fujinami and Oldstone documented that when acutely infected HeLa cells infected with the Edmonston strain MV are treated briefly with polyclonal anti-MV serum (12) or monoclonal antibodies to the hemagglutinin (H) protein (11), expression of the membrane fusion (F) protein and the internal proteins, phosphoprotein (P) and matrix (M) protein, decreases. These studies have been extended by other investigators using persistently infected cell lines. Treatment of a rat glioma cell line persistently infected with a measles subacute sclerosing panencephalitis virus with polyclonal anti-H antibodies suppresses expression of H protein and, eventually, all MV antigens. In contrast, treatment with a monoclonal anti-H antibody has only a transient effect (5, 45). Monoclonal antibodies to H protein but not to other MV proteins protect newborn mice from an MV-induced encephalitis that is normally fatal (14). Administration of anti-H antibodies but not other antibodies also prevents fatal necrotizing encephalopathy in newborn Lewis rats injected with neurotropic CAM/RBH MV (29). Instead, these animals develop inflammatory central nervous system disease. Analysis of MV mRNA in the brains of these rats demonstrates an altered transcriptional gradient that leads to reduced transcription in individual cells rather than to fewer infected cells. Protection of rats and mice was also conferred by immunization with vaccinia virus recombinants expressing either the N antigen (4) or that of H or F (9). Treatment of MV-infected murine neuroblastoma cells with anti-H antibodies causes a decrease in transcription of all measles mRNAs followed by a decrease in transcription of genomic RNA (37, 38). Therefore, both in vivo treatment and in vitro treatment with antibody to MV restrict transcription and replication of the virus in neuronal cells. We wanted to determine if expression of virally encoded proteins could be controlled in effector cells of the immune system, such as macrophages, that are important for a normal host response to MV infection. The experiments presented in this paper demonstrated that synthesis of MV proteins in persistently infected macrophages is down-regulated by treatment with polyclonal antibodies directed against these proteins.

To carry out these experiments, we first established a persistent infection with the Edmonston strain of MV in IC-21 cells, a simian virus 40-immortalized mouse macrophage cell line (19, 34). At least 65% of these cells carry infectious par-

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ticles, as demonstrated by infectious center assays (19). Approximately 75% exhibit viral antigens, as assessed by fluorescence-activated cell sorting (19). Limiting dilution analysis followed by clonal expansion demonstrated that more than 90% of the cells are infected and that the persistent infection is passaged during mitosis (19). This persistent infection is characterized by the absence of syncytia, so that infected cells do not lyse and can multiply in culture indefinitely. Therefore, long-term in vitro studies that are not possible with lytically infected cells can be performed with these cells. This has allowed us to treat infected cells with antibody for as long as a week in culture and to study the subsequent effects on MV synthesis for any period that we choose. Here, we report that antibody leads to a reduction in expression of virally encoded proteins in a manner that down-regulates viral protein synthesis but does not destroy the infected cell.

MATERIALS AND METHODS

MV. Plaque-purified MV of the Edmonston strain (ATCC VR-24) was passaged through Vero cells at a low multiplicity of infection (less than 0.1). Infected Vero cell monolayers exhibiting widespread syncytium formation were lysed with three freeze (−70°C)-thaw cycles. Lysates were clarified by centrifugation at 700 × *g* for 10 min and stored in 1-ml aliquots at −70°C. When grown in Vero cells, the virus stocks typically had titers between 2×10^6 and 2×10^7 PFU/ml.

Rabbit anti-MV antisera. MV stock prepared in 50 mM sodium phosphate buffer, pH 7.5, was purified over a 30-ml Cellufine sulfate column (Amicon, Danvers, Mass.) according to the manufacturer's suggestions, except that the virus was eluted with a 225-ml NaCl gradient (0 to 3 M) in 0.02 M sodium phosphate buffer, pH 7.0. New Zealand White rabbits (Hazelton Research Animals, Hazelton, Pa.) were immunized with 2 ml of purified virus in complete Freund's adjuvant (1:1) and with five subsequent immunizations of purified virus in incomplete Freund's adjuvant. An immunoglobulin G fraction of the antiserum was prepared by passing 5 ml of serum over a 5-ml recombinant protein A column (Pierce, Rockford, Ill.) with the manufacturer's binding and elution buffers.

Cell culture. IC-21 cells, a simian virus 40-immortalized cell line (ATCC TIB 186), were infected with MV and maintained in RPMI 1640 medium containing 25 mM HEPES (*N*-2-hydroxyethylpiperazine-*N*′-2-ethanesulfonic acid) (GIBCO-BRL, Gaithersburg, Md.). All media were supplemented with 10% heat-inactivated fetal bovine serum (HyClone, Logan, Utah), 2 mM glutamine (GIBCO-BRL), 100 U of penicillin (Squibb-Marsam, Cherry Hill, N.J.) per ml, and 10 μg of gentamicin (GIBCO-BRL) per ml. For individual experiments, 1-ml aliquots of cells (1×10^5/ml) were distributed into tubes (12 by 75 mm, Falcon 2054; Becton Dickinson, Mountain View, Calif.). Cells were centrifuged at 180 × *g* for 5 min, media were removed, and media containing 3% rabbit serum and 7% fetal bovine serum were added. The 3% rabbit serum was either nonimmune serum or a dilution of antiserum with nonimmune serum added to maintain a total rabbit serum concentration of 3%. Throughout the experiment, culture tubes were rocked gently to ensure sufficient mixing of media. Cells were centrifuged for 5 min at 180 × *g* prior to each medium change. Depending on the experiment, rabbit serum remained in the cultures from several hours to several days. At appropriate time points after treatment was terminated, cultures were radiolabeled with [^{35}S]methionine to study nascent protein synthesis. After being rinsed three times with high-glucose D-MEM lacking methionine (GIBCO-BRL), cells were radiolabeled with 30 μCi of [^{35}S]methionine (DuPont NEN, Boston, Mass.) in 0.5 ml of this same medium. After 4 h, culture supernatants were collected and the cells were lysed and stored as previously described (23).

Determination of total protein synthesis. Analysis of total protein synthesis was carried out by a modification of the Mans-Novelli procedure (30). Aliquots of cell lysates containing ^{35}S-proteins were diluted in 1% bovine serum albumin and dried onto 2.1-cm glass microfiber filters (Whatman, Inc., Clifton, N.J.) in 12-well tissue culture plates (Elkay Products, Shrewsbury, Mass.). Protein was precipitated with cold 10% trichloroacetic acid containing 10 mM L-methionine. The filters were washed three times with 10% trichloroacetic acid and twice with 95% ethanol and then air dried and counted in a Beckman LS9000 liquid scintillation counter (Beckman Instruments, Fullerton, Calif.).

Chromium release assay. MV-infected IC-21 cells (10^5/ml), as well as uninfected cells, were incubated with 150 μCi of sodium [^{51}Cr]chromate (Amersham Corp., Arlington Heights, Ill.) for 1 h at 37°C with rocking (7). Labeled cells were incubated with dilutions of MV-immune or nonimmune rabbit serum that were either heated to inactivate complement or untreated to retain complement activity. Aliquots of cell supernatants and control samples were counted in a Beckman model 8500 gamma counter to determine the extent of ^{51}Cr release.

Immunoprecipitation of radiolabeled MV proteins. Cell lysates were immunoprecipitated according to procedures previously described (23) with the following modifications: EDTA was added to 25 mM to each lysate before the immunoprecipitations were begun; lysates were cleared three times with 10^9 heat-killed, formalinized *Staphylococcus aureus* organisms (Boehringer Mannheim, Indianapolis, Ind.); the factor B clearance was omitted; incubation with 25 μl of guinea pig anti-MV antiserum (Whittaker Bioproducts, Walkersville, Md.) 1:10 in phosphate-buffered saline was for 1 h at room temperature instead of 37°C; and finally, 10^9 instead of 5×10^8 *S. aureus* organisms were used to adsorb the antigen-antibody complex. *S. aureus* pellet washing and sample digestion prior to sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) were carried out as described (23).

SDS-PAGE. SDS-PAGE with discontinuous buffers was performed according to procedures that are standard for our laboratory (23). Gels with 9% acrylamide and 2.6% bisacrylamide were prepared in 0.375 M Tris (pH 8.8)–0.1% SDS. Samples were loaded onto a 4% acrylamide stacking gel prepared in 0.125 M Tris (pH 6.8)–0.1% SDS. Electrophoresis in 0.025 M Tris–0.192 M glycine–0.1% SDS (pH 8.3) was carried out at room temperature in an LKB2001 vertical electrophoresis unit (Pharmacia Biotech Inc., Piscataway, N.J.) at 25 mA per gel for the first hour and at 50 mA per gel for the remaining 2 to 3 h. For autoradiography, the gels were fixed for 1 h in 25% methanol-10% acetic acid; this step was followed by soaking for 1 h in En^{3}Hance (DuPont NEN) and for 10 min in water. The gels were dried overnight on filter paper with BioGel wrap (BioDesign, Carmel, N.Y.) and exposed to Kodak XAR-5 X-ray film at −70°C. On autoradiograms, the H, P, nucleocapsid (N), F, and M proteins were readily identifiable. The large (L) protein was difficult to distinguish in most cases. MV proteins were quantified by analyzing autoradiograms on a laser densitometer (model 100A; Molecular Dynamics, Sunnyvale, Calif.) with PDQuest analysis software (Protein Database, Inc., Huntington Station, N.Y.). To determine the magnitude of suppression of each MV protein, the density of the autoradiography bands obtained by SDS-PAGE analysis of MV protein synthesis in radiolabeled cultures treated with antibody was compared with those from cultures treated with identical concentrations of nonimmune serum. Percent suppression was calculated as follows: [1 − (OD antibody treated/OD nonimmune serum treated)] × 100, where OD is the optical density.

RESULTS

Short-term treatment of infected IC-21 cells with anti-MV antibody suppresses MV protein synthesis with or without cytotoxicity. Antibody may have different effector functions in the presence and absence of complement. In the presence of antibody and complement, virally infected cells may be lysed. In the absence of complement, the effects of antibody are less destructive. It is noteworthy that antibody has been shown to clear alphavirus, a positive-sense RNA virus, independent of complement lysis, neutralization, and antibody-dependent cytotoxicity (28). The following experiments were carried out to determine if antibody could modulate MV expression in an infected macrophage cell line when cell lysis is prevented.

The experiment whose results are shown in Fig. 1 compared the effects of different doses of a polyclonal rabbit anti-MV antibody on MV protein synthesis, total protein synthesis, and cell viability. This antibody immunoprecipitated N, P, M, F, and H proteins from radiolabeled lysates of infected macrophages. When these immunoprecipitates were analyzed by SDS-PAGE and autoradiography, their patterns and molecular sizes were identical to those of proteins immunoprecipitated by polyclonal guinea pig anti-MV antibody (Whittaker Bioproducts). Neither antibody clearly identified the L protein. As shown in Fig. 1A, after a 2-h treatment of MV-infected macrophages with 1% (a 1:100 dilution) antibody, synthesis of the N, P, M, and H proteins was reduced by 35 to 55% when complement was inactivated. Treatment with 3% (a 1:33 dilution) antibody produced a distinct prozone effect on MV synthesis (i.e., less of an effect at higher doses), and suppression of only 5 to 15% was achieved. Nonimmune serum had no effect on MV protein synthesis. As shown in Fig. 1B, in the presence of an active complement system suppression was increased, especially at the 3% dose of antibody. As described below, however, the apparent increase in suppression was probably the result of disruption of total cellular protein synthesis caused by complement-mediated cell lysis.

Lysates of the cells from the experiment whose results are shown in Fig. 1A and B were analyzed for total protein synthesis to verify that cell lysis occurred in the presence of com-

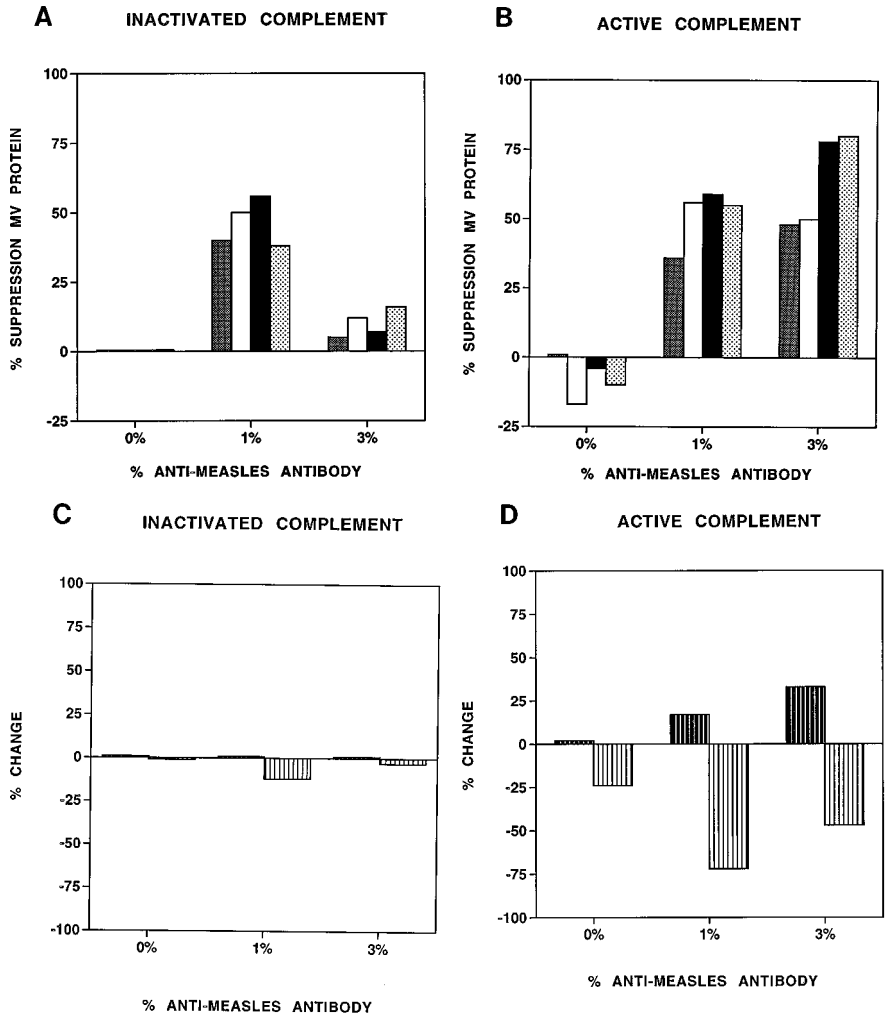

FIG. 1. The degree of suppression of MV protein synthesis achieved by a 2-h treatment with rabbit anti-MV antibody was dependent upon the dose of antibody and occurred in the absence of cell lysis when complement was inactivated. MV-infected cells (10^5) were incubated for 1, 2, or 4 h with nonimmune serum or with anti-MV antibody, after which the cells were washed to remove antibody and then incubated for 2 h with 100 μCi of [^{35}S]methionine. All serum components were either unheated (B and D) or heated to 56°C for 30 min to inactivate complement activity (A and C). MV proteins were immunoprecipitated from cell lysates and analyzed by SDS-PAGE, and the percent suppression was determined as described in Materials and Methods. The individual MV proteins shown after 2 h of treatment are N protein (▨), P protein (□), M protein (■), and H protein (▤). Total protein synthesis (▥) for the lysates was determined by trichloroacetic acid precipitation as described in Materials and Methods. A portion of the persistently infected cells were radiolabeled with ^{51}Cr and simultaneously treated in parallel with the other samples depicted, and the amount of ^{51}Cr release (▥) was determined. The percent change in trichloroacetic acid incorporation into protein and in ^{51}Cr release in the absence (C) or presence (D) of active complement was determined for samples treated with immune or nonimmune serum. This experiment is representative of four similar experiments.

plement and that heat inactivation of complement prevented lysis. In addition, aliquots of ^{51}Cr-labeled cells that had been treated simultaneously with identical reagents were analyzed for ^{51}Cr release. We had previously demonstrated that total protein synthesis by these cells correlates well with viable cell number, and ^{51}Cr release assays are among the many standard assays for cell lysis. As shown in Fig. 1C, when complement had been inactivated, no cytolysis occurred at any dose of antibody. Fig. 1D demonstrates, however, that as the antibody dose increased, total cellular protein synthesis decreased and ^{51}Cr release increased. This documented active complement-mediated cell lysis. It is not clear how much of the decrease in MV protein synthesis in the presence of active complement was due to suppression of synthesis and how much was due to loss of cells.

Long-term treatment of infected IC-21 cells with anti-MV antibody induces suppression of MV protein synthesis that continues for days after treatment is terminated. Having demonstrated that a 2-h exposure to anti-MV serum suppressed viral protein synthesis in infected cells, we next determined whether extended treatment periods prolonged the suppression in a manner that was similar to what had been observed for intrinsic cell products (15–24). In the experiment whose results are shown in Fig. 2, MV-infected IC-21 cells were exposed to anti-MV antibody for 5 days. Control cultures received 0.3% nonimmune rabbit serum, whereas treated cultures received the indicated dilutions of rabbit hyperimmune anti-MV serum with sufficient nonimmune serum added to make the total concentration of rabbit serum 0.3%. At the end of the treatment period, the cells were washed three times and maintained without further antibody treatment. MV protein synthesis was assayed 2 and 7 days after antibody was removed. At these time points, cultures were radiolabeled with [^{35}S]methionine and MV proteins were immunoprecipitated and ana-

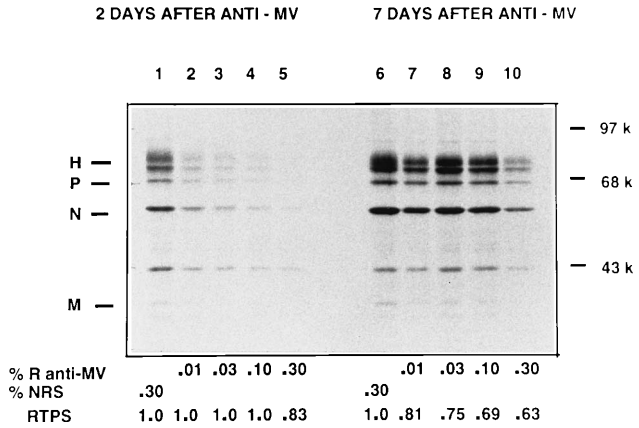

FIG. 2. IC-21 cells persistently infected with MV were treated for 5 days with different doses of rabbit anti-MV antibody, resulting in suppressed synthesis of MV proteins. IC-21 cells (10^5) were treated with 0.3% nonimmune rabbit serum or with 0.01, 0.03, 0.10, or 0.30% rabbit hyperimmune anti-MV serum to which was added sufficient nonimmune rabbit serum to achieve a total rabbit serum concentration of 0.3%. Shown is the autoradiogram obtained from the SDS-PAGE analysis of newly synthesized [^{35}S]methionine-labeled MV proteins immunoprecipitated from cell lysates 2 and 7 days after antibody treatment was terminated. The two largest bands are H protein. Pulse-chase experiments have demonstrated a shift with time from the faster-migrating to the more slowly migrating form. This experiment is representative of four similar experiments. The numbers on the right are molecular mass markers, in kilodaltons.

lyzed by SDS-PAGE. The autoradiograms for this experiment demonstrated the pattern typical of MV proteins when cultures were treated with normal rabbit serum (Fig. 2, lanes 1 and 6). For cultures treated with as little as 0.01% antibody, there was significant suppression of MV protein synthesis (lane 2) 2 days after the removal of anti-MV antibodies. An increased antibody dose correlated with increased suppression (lanes 2 through 5). Seven days after the removal of antibody (lanes 7 through 10), MV protein synthesis was still suppressed but to a lesser extent.

Although lysis of cells by antibody and complement was blocked, we needed to take into account any cell loss or decrease in total protein synthesis that might occur for other reasons. Antibody might have had effects on the overall cell metabolism, and overcrowding might have occurred near the end of the experiment since the cell cultures were not split. Therefore, total protein synthesis in each antibody-treated culture was compared with that in the control cultures to determine the relative total protein synthesis (RTPS). As shown in Fig. 2, 2 days after treatment was terminated (day 7 of the experiment), only one of five samples showed a decrease in RTPS (83% of the control value). Seven days after treatment was terminated (day 12 of the experiment), four of five antibody-treated samples showed a decrease in RTPS that ranged between 81 and 63% of the control value. This implied that total cellular protein synthesis was reduced and/or that cell death had occurred. The RTPS was used to normalize all data to compensate for cell death or reduced total cellular protein synthesis that, if not considered, could cause the percent suppression to be overestimated.

The dose of antibody and duration of exposure determine the extent and duration of suppression after antibody removal. MV-infected cells were treated with different concentrations of antibody ranging from 0.01 to 0.3% for 3, 5, or 7 days. Two and seven days after the antibody treatment was terminated, aliquots were radiolabeled with [^{35}S]methionine for 4 h. After immunoprecipitation and SDS-PAGE analysis, autoradiograms were scanned on a laser densitometer to quantitate the density of each MV protein band. Shown in Fig. 3 are the values for percent suppression of H, P, N, and M protein synthesis 2 days after the removal of antibody. Although the magnitude of suppression of the individual MV proteins varied with the duration of antibody treatment and the dose of anti-MV serum, suppression was always significant ($P < 0.05$) 2 days after antibody was removed. The percent suppression ranged from 45 to 98% for the H protein, 45 to 94% for the P protein, and 36 to 94% for the N protein. As shown in Fig. 2, the band for the M protein is always the least dense and its quantitation is therefore less reliable. Nevertheless, suppression ranged from 63 to 99%.

In another experiment (data not shown), an autoradiogram of 12 replicate control samples maintained under identical culture conditions was scanned to determine the expected statistical variation in the concentration of each MV protein that was due to random sample fluctuations. Two days after the removal of nonimmune serum, variations of $\leq$21% for H protein, $\leq$24% for P protein, and $\leq$30% for N protein represented $\pm$2 standard deviations.

Seven days after antibody removal, MV protein synthesis was still suppressed but not to the extent seen at the earlier time point. The magnitude of down-regulation of MV expression was related to the dose and duration of antibody treatment. Those cultures treated with the highest dose of antiserum were suppressed at 7 days almost as much as at 2 days. In contrast, some of the cultures treated with the lowest dose of antiserum not only broke suppression but had enhanced levels of MV protein synthesis. The percent change ranged from 16% stimulation to 88% suppression for H protein, 13 to 89% suppression for P protein, 0 to 87% suppression for N protein, and 20% stimulation to 94% suppression for M protein. Although there was less suppression at this second time point, it was still significant for all samples, except those treated with 0.01% anti-MV serum for 7 days. The RTPS was used to correct for cell death that might have occurred. Suppression of MV synthesis cannot be explained by neutralization or masking by residual antibody, since there were at least 10 medium changes after antibody was removed.

DISCUSSION

In subacute sclerosing panencephalitis, MV persists in the brain and neurological disease develops. Probably for this reason, laboratory studies have focused on persistent MV infections in neuronal cell lines. However, neuronal cells lack major histocompatibility complex class I molecules (26), and this may help virally infected neuronal cells escape immune surveillance. In natural MV infection, monocytes and macrophages are important targets of the virus (10). They become infected and then disseminate the virus. They also produce cytokines that are important in the immune response to the infection. Our studies were undertaken to understand better how antibody might control MV infection of macrophages. Noncytolytic antibody is important in the control of infection before cytotoxic T cells appear and may augment viral clearance once cytotoxic T cells are generated.

All four MV proteins assessed were suppressed by treatment with anti-MV antibody. H and F proteins are membrane associated and readily accessible to exogenous antibody. For antibody to interact directly with N, P, or M (or L) proteins, it would presumably have to be internalized and be resistant to degradation by cytoplasmic proteases. Alternatively, suppression of the internal proteins could be a secondary effect. H, M, and F proteins are proteins that complex with the viral enve-

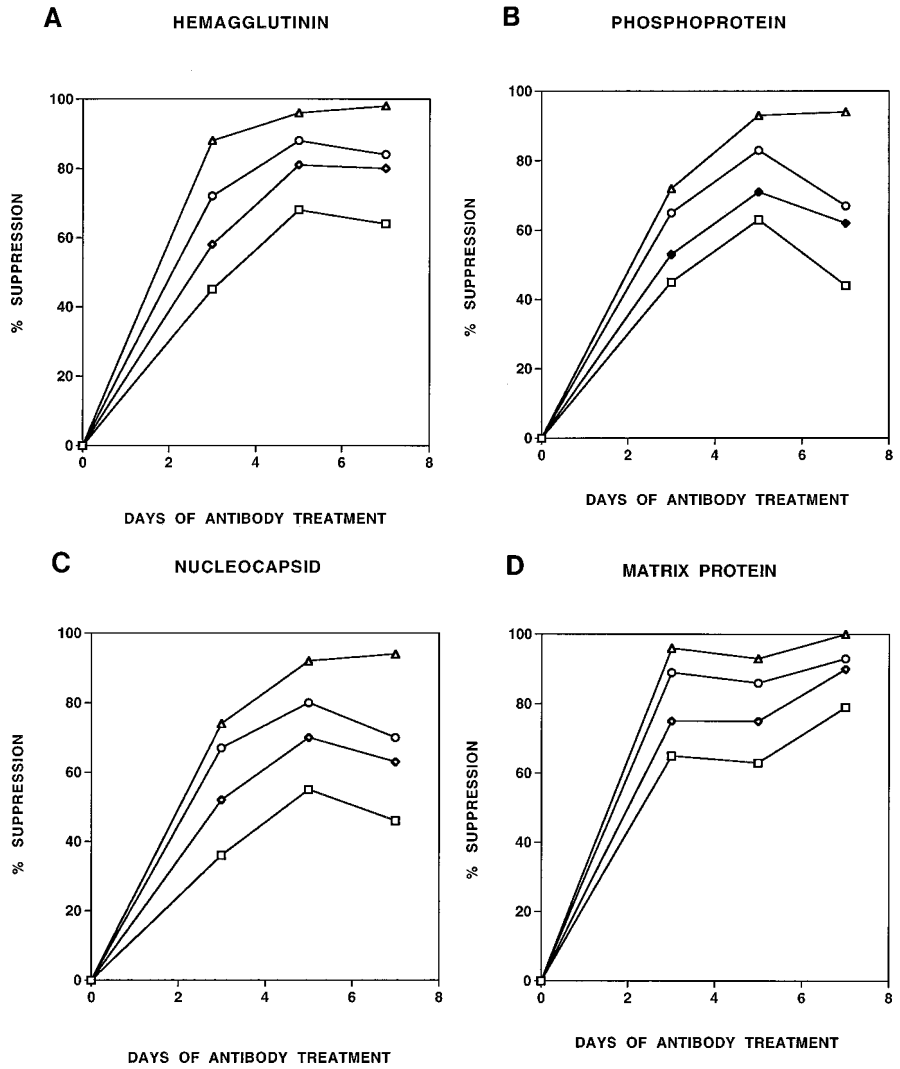

FIG. 3. The extent of suppression of MV protein synthesis 2 days after treatment was terminated is dependent upon antibody dose and duration of exposure. The results are shown for H protein (A), P protein (B), N protein (C), and M protein (D) after treatment for 3, 5, or 7 days with the following concentrations of anti-MV antibody: 0.01% (□), 0.03% (◇), 0.1% (○), and 0.3% (△). The percent suppression is calculated as described in Materials and Methods. All data have been corrected for RTPS. This experiment is representative of four similar experiments.

lope and that are important in the formation of mature, functional virions. Suppression of these proteins would be expected to affect virion formation. If assembly of the envelope is impaired and structural proteins accumulate, their transcription may be inhibited. Others have shown that the accumulation of abnormally large amounts of M protein of vesicular stomatitis virus inhibits transcription (3). Similar results have now been obtained with MV (41). N, P, and L proteins are part of the encapsidated ribonucleoprotein complex, formed with the negative-sense genome in which primary transcription occurs. Without P or L proteins, the polymerase complex cannot form. Without N protein, the negative-sense viral RNA is not encapsidated and the polymerase complex cannot function. Therefore, suppression of any MV protein could have secondary effects on the transcription and translation of all other MV proteins as well as on replication of the genome.

Our results from experiments on the immune regulation of viral proteins are consistent with our results from experiments on the immune regulation of proteins normally expressed by macrophages, e.g., complement components. For several years our laboratory has been studying the mechanisms whereby synthesis of individual complement components can be suppressed by in vivo or in vitro treatment of primary macrophages or hepatocytes with specific antibody or hyperimmune lymphoid cells directed against that component (15–24, 32). Experiments with in vivo and in vitro complement suppression have demonstrated the following characteristics and requirements for antigenic suppression. (i) Antigenic suppression is specific for the protein to which antibody is directed. (ii) Antigenic suppression is mediated by noncytotoxic mechanisms. (iii) Other macrophage or hepatocyte products may be nonspecifically stimulated during and after recovery from suppression, but these other products are never suppressed. (iv) Antigenic suppression requires an intact antibody molecule; the antigen-combining regions alone are not sufficient. (v) Increased levels of intracellular cyclic nucleotides augment suppression. (vi) If there is recovery of synthesis, intracellular levels of the suppressed component are markedly diminished

because of a shortened interval from synthesis to secretion from the cell. (vii) There are no detectable qualitative changes in the suppressed molecules after recovery from suppression. (viii) Antigenic suppression is mediated by at least one genetically unrestricted soluble specific suppressor factor. (ix) There is an absolute requirement for T cells of both $CD4^+$ and $CD8^+$ subsets. (x) Antigenic suppression requires a high cell density and probably requires contact between effector cells and target cells in the early stages of suppression. (xi) The rate-determining step in antigenic suppression involves translation but not transcription.

Antibody-mediated effects have been documented with numerous viruses in several species. Passively administered neutralizing antibodies to feline immunodeficiency virus from vaccinated cats or experimentally infected cats prevent a usually fatal infection (25). Neutralizing polyclonal or monoclonal antibodies to simian immunodeficiency virus restrict the spread of the virus from cell to cell in macaque macrophages and prevent full viral expression within individual cells so that minimal amounts of viral protein are present (31). These studies suggest that neutralizing antibodies prevent macrophages from reaching the critical level of viral replication necessary for maximum infection. In contrast, other laboratories have demonstrated that plasma from simian immunodeficiency virus-infected monkeys enhances infection of a human lymphoblastoid cell line by a complement-dependent process that utilizes the third component of complement, C3, and one of its receptors (CR2) (33). Neutralizing antibodies protect mice against the neurotropic Armstrong strain of lymphocytic choriomeningitis virus (LCMV) but enhance lethality against a viscerotropic and lymphocyto-viscerotropic isolate (6). Therefore, antibodies are often protective in controlling the spread of viral infections, but they may work to the advantage of the virus rather than the host in some circumstances.

There are many examples for which the protective effects of antibody cannot be explained by viral neutralization. Treatment of human neuroblastoma cells with antiserum to poliovirus converts a virulent cytopathic infection into a persistent one (42). The effectiveness of the antibodies relates to their specificity for the assembled capsid of the virus and is unrelated to their neutralizing capacity. Studies with monoclonal antibodies to reoviruses with different levels of virulence suggest that antibody-mediated protection requires recognition of conserved epitopes involved in critical functions common to all reoviruses (43). Protection in vivo correlates with specificity for a cross-reactive determinant of the capsid protein and is unrelated to isotype, neutralizing capacity, and binding avidity. All protective antibodies for reovirus inhibit viremia, but not all antibodies inhibit primary viral replication.

Antiviral antibodies can have an important protective role even in the presence of cytolytic T cells, and the effects of those T cells can mask the auxiliary or enhancing effects of antibody. Mice that are B-cell deficient (immunoglobulin μ chain suppressed, i.e., cannot produce antibodies but have normal T-cell function) exhibit significantly delayed clearance of LCMV. Therefore, antibody is important for accelerating clearance of LCMV, even in the presence of cytolytic activity (8). When antibody is administered to adult mice that have been injected intracranially with LCMV, viral replication is suppressed and the acute infection is attenuated. Reduced viral replication leads to a diminished cytolytic T-cell response, which in turn leads to diminished immunopathology caused by the T cells (2, 44). Protection is conferred only by intact antibody of a specific subclass that recognizes a specific glycoprotein. Protection is unrelated to the ability to neutralize viral antigens or to activate complement.

Several laboratories have demonstrated control of viral infections with antibody in the absence of cytolytic responses. In the experiments just described for LCMV, antibody was also protective in nude mice (which lack mature T cells) (2). Passive antibody against Theiler's encephalomyelitis virus also is protective in nude mice (13). Administration of neutralizing monoclonal antibody to the LCMV VP-1 structural protein reduces the amount of infectious virus in the brain, increases survival, prevents demyelination in approximately half of a population of infected nude mice, and facilitates remyelination (13). Sindbis virus, a single-stranded positive-sense RNA virus that causes both fatal and persistent encephalitis in mice with severe combined immunodeficiency, is cleared from severe combined immunodeficiency mice by adoptive transfer of hyperimmune serum but not by adoptive transfer of sensitized lymphocytes (28). The mechanism responsible is unrelated to antibody-dependent cell cytotoxicity, antibody-dependent complement-mediated lysis, and viral neutralization. Diabetes-prone BioBreeding Worcester rats, which fail to generate virus-specific major histocompatibility complex-restricted cytotoxic T cells, effectively clear LCMV and maintain immunity with neutralizing antibodies alone (35). The depletion of $CD4^+$ T cells converts mice that are resistant to street rabies virus into mice that are susceptible to this virus because the mice no longer produce antibodies (36). The depletion of $CD8^+$ cells (which includes the cytolytic population for this virus) has no effect. The passive transfer of monoclonal antibodies reactive against glycoprotein D of herpes simplex virus type 1 prevents the spread of this virus to the central nervous system in mice depleted of either $CD4^+$, $CD8^+$, or NK cells but is ineffective in mice depleted of both $CD4^+$ and $CD8^+$ cells (40). Therefore, in some circumstances antibody can limit viral spread in vivo in the absence of a functioning T-cell response or in the absence of functioning individual T-cell subsets.

One of the mechanisms that is emerging for the role of antibody in viral clearance is its ability to restrict replication in some circumstances. On the basis of our studies of immune regulation of complement biosynthesis in uninfected macrophages, we speculate that antibody treatment of infected macrophages interferes with MV protein synthesis by blocking translation. Reduced synthesis of MV proteins necessary for polymerase formation and encapsidation could then have secondary effects on transcription and replication. In the experiments described above for Sindbis virus, animals that were free of histological abnormalities in the brain still had viral RNA detectable by PCR (27). Therefore, antibody mediated the clearance of Sindbis virus by restricting its replication and allowing persistence of the viral genome in a nonproductive form. The antibody dose determines if reactivation occurs or if clearance is permanent. When kits are administered passive antibodies to Aleutian mink disease parvovirus, they survive the acute viral pneumonia but later develop chronic immune complex disease. The number of infected cells is the same for treated and untreated kits, but in treated kits viral replication is restricted on a per-cell basis (1). The persistent virus still causes fatal disease upon transfer into previously uninfected animals. Both in vivo treatment and in vitro treatment with antibody to MV lead to restricted replication of the virus in neuronal cells. Treatment of MV-infected murine neuroblastoma cell lines with anti-H antibodies decreases transcription of all measles mRNAs. A decrease in the transcription of genomic RNA follows (37). Changes in phosphorylating activity also occur. Total phosphorylation and protein kinase C activity decrease to levels comparable to those in uninfected cells and gradually return to elevated levels characteristic of

infected cells when antibody is removed (39). Therefore, signal transduction pathways are likely to be important in the noncytolytic control of viral infection by antibody. Future investigations are needed to define more precisely the mechanisms by which antibody regulates MV gene expression in these in vivo and in vitro models.

ACKNOWLEDGMENTS

We are grateful to David Spector for his thoughtful suggestions and comments.

Portions of this work were supported by a grant from the Eleanor Naylor Dana Charitable Trust.

REFERENCES

1. **Alexandersen, S., S. Larsen, A. Cohn, A. Uttenthal, R. E. Race, B. Aasted, M. Hansen, and M. E. Bloom.** 1989. Passive transfer of antiviral antibodies restricts replication of Aleutian mink disease parvovirus in vivo. J. Virol. **63:**9–17.
2. **Baldridge, J. R., and M. J. Buchmeier.** 1992. Mechanisms of antibody-mediated protection against lymphocytic choriomeningitis virus infection: mother-to-baby transfer of humoral protection. J. Virol. **66:**4252–4257.
3. **Banerjee, A. K.** 1987. Transcription and replication of rhabdoviruses. Microbiol. Rev. **51:**66–87.
4. **Bankamp, B., U. G. Brinckmann, A. Reich, S. Niewiesk, V. ter Meulen, and U. G. Liebert.** 1991. Measles virus nucleocapsid protein protects rats from encephalitis. J. Virol. **65:**1695–1700.
5. **Barrett, P. N., K. Koschel, M. Carter, and V. ter Meulen.** 1985. Effect of measles virus antibodies on a measles SSPE virus persistently infected C6 rat glioma cell line. J. Gen. Virol. **66:**1411–1421.
6. **Battegay, M., D. Kyburz, H. Hengartner, and R. M. Zinkernagel.** 1993. Enhancement of disease by neutralizing antiviral antibodies in the absence of primed antiviral cytotoxic T cells. Eur. J. Immunol. **23:**3236–3241.
7. **Brunner, K. T., J. Manuel, J.-C. Cerrottini, and B. Chapuis.** 1968. Quantitative assay of the lytic action of immune lymphoid cells on ^{51}Cr-labelled allogeneic target cells *in vitro*; inhibition by isoantibody and by drugs. Immunology **14:**181–196.
8. **Cerny, A., S. Sutter, H. Bazin, H. Hengartner, and R. M. Zinkernagel.** 1988. Clearance of lymphocytic choriomeningitis virus in antibody- and B-cell-deprived mice. J. Virol. **62:**1803–1807.
9. **Drillien, R., D. Spehner, A. Kirn, P. Giraudon, R. Buckland, F. Wild, and J. P. Lecocq.** 1988. Protection of mice from fatal measles encephalitis by vaccination with vaccinia virus recombinants encoding either the hemagglutinin or fusion protein. Proc. Natl. Acad. Sci. USA **85:**1252–1256.
10. **Esolen, L. M., B. J. Ward, T. R. Moench, and D. E. Griffin.** 1993. Infection of monocytes during measles. J. Infect. Dis. **168:**47–52.
11. **Fujinami, R. S., E. Norrby, and M. B. A. Oldstone.** 1984. Antigenic modulation induced by monoclonal antibodies: antibodies to measles virus hemagglutinin alters expression of other viral polypeptides in infected cells. J. Immunol. **132:**2618–2621.
12. **Fujinami, R. S., and M. B. A. Oldstone.** 1980. Alternations in expression of measles virus polypeptides by antibody: molecular events in antibody-induced antigenic modulation. J. Immunol. **125:**78–85.
13. **Fujinami, R. S., A. Rosenthal, P. W. Lampert, A. Zurbriggen, and M. Yamada.** 1989. Survival of athymic (*nu/nu*) mice after Theiler's murine encephalomyelitis virus infection by passive administration of neutralizing monoclonal antibody. J. Virol. **63:**2081–2087.
14. **Giraudon, P., and T. F. Wild.** 1985. Correlation between epitopes on hemagglutinin of measles virus and biological activities: passive protection by monoclonal antibodies is related to their hemagglutination inhibiting activity. Virology **144:**46–58.
15. **Goldman, J. N., and M. B. Goldman.** 1978. Antibody-induced suppression of the fifth component of complement in mice. J. Immunol. **120:**400–407.
16. **Goldman, J. N., K. S. O'Rourke, and M. B. Goldman.** 1985. Antibody-induced suppression and postsuppression stimulation *in vitro*. III. Long-term C4 suppression is actively maintained by a soluble suppressor factor (F_SC4). Cell. Immunol. **96:**26–37.
17. **Goldman, J. N., K. S. O'Rourke, J. D. McMannis, and M. B. Goldman.** 1988. Effects of anti-C4 antibody on complement production by splenic and peritoneal macrophages. Complement **5:**13–26.
18. **Goldman, M. B., D. S. Becker, K. S. O'Rourke, and J. N. Goldman.** 1985. Enhancement by cyclic AMP of antibody-induced suppression of the fourth component of complement. J. Immunol. **135:**2702–2706.
19. **Goldman, M. B., D. J. Buckthal, S. Picciotto, T. A. O'Bryan, and J. N. Goldman.** Measles virus persistence in an immortalized murine macrophage cell line. Virology, in press.
20. **Goldman, M. B., D. L. French, and J. N. Goldman.** 1979. Cell-mediated suppression of the fifth component of complement in mice. J. Immunol. **122:**246–252.
21. **Goldman, M. B., M. A. Knovich, and J. N. Goldman.** 1991. Immunologic control of C3 gene expression in tissue macrophages. J. Immunol. **147:**4248–4255.
22. **Goldman, M. B., M. A. Knovich, and J. N. Goldman.** 1992. T lymphocytes mediate immunologic control of C3 gene expression. Eur. J. Immunol. **22:**3103–3109.
23. **Goldman, M. B., K. S. O'Rourke, D. S. Becker, and J. N. Goldman.** 1985. Antibody-induced suppression and postsuppression stimulation of complement *in vitro*. II. Intracellular and extracellular changes in C4 during long-term C4 suppression in guinea pig splenic fragments. J. Immunol. **134:**3298–3306.
24. **Goldman, M. B., K. S. O'Rourke, and J. N. Goldman.** 1982. Antibody-induced suppression and postsuppression stimulation of complement *in vitro*. I. Effects of anti-C4 on cultured guinea pig peritoneal cells. Cell. Immunol. **70:**118–131.
25. **Hohdatsu, T., R. Pu, B. A. Torres, S. Trujillo, M. B. Gardner, and J. K. Yamamoto.** 1993. Passive antibody protection of cats against feline immunodeficiency virus infection. J. Virol. **67:**2344–2348.
26. **Joly, E., L. Mucke, and M. B. A. Oldstone.** 1991. Viral persistence in neurons explained by lack of major histocompatibility class I expression. Science **253:**1283–1285.
27. **Levine, B., and D. E. Griffin.** 1992. Persistance of viral RNA in mouse brains after recovery from acute alphavirus encephalitis. J. Virol. **66:**6429–6435.
28. **Levine, B., J. M. Hardwick, B. D. Trapp, T. O. Crawford, R. C. Bollinger, and D. E. Griffin.** 1991. Antibody-mediated clearance of alphavirus infection from neurons. Science **254:**856–859.
29. **Liebert, U. G., S. Schneider-Schaulies, K. Baczko, and V. ter Meulen.** 1990. Antibody-induced restriction of viral gene expression in measles encephalitis in rats. J. Virol. **64:**706–713.
30. **Mans, R. J., and G. D. Novelli.** 1961. Measurement of incorporation of radioactive amino acids into protein by a filter-paper disc method. Arch. Biochem. Biophys. **94:**48–53.
31. **McEntee, M. F., M. C. Zink, M. G. Anderson, H. Farzadegan, R. J. Adams, K. A. Kent, E. J. Stott, J. E. Clements, and O. Narayan.** 1992. Neutralizing antibodies modulate replication of simian immunodeficiency virus SIV_{mac} in primary macaque macrophages. J. Virol. **66:**6200–6203.
32. **McMannis, J. D., M. B. Goldman, and J. N. Goldman.** 1987. The role of lymphoid cells in antibody-induced suppression of the fourth component of guinea pig complement. Cell. Immunol. **106:**22–32.
33. **Montefiori, D. C., W. E. Robinson, Jr., V. M. Hirsch, A. Modliszewski, W. M. Mitchell, and P. R. Johnson.** 1990. Antibody-dependent enhancement of simian immunodeficiency virus (SIV) infection in vitro by plasma from SIV-infected rhesus macaques. J. Virol. **64:**113–119.
34. **O'Bryan, T. A., M. B. Goldman, and J. N. Goldman.** 1993. A murine model for examining immunologic control of measles virus gene expression. J. Cell. Biochem. Suppl. **17D:**59.
35. **Oldstone, M. B. A., A. Tishon, P. L. Schwimmbeck, S. Shyp, H. Lewicki, and T. Dryberg.** 1990. Cytotoxic lymphocytes do not control lymphocytic choriomeningitis virus infection of BB diabetes prone rats. J. Gen. Virol. **71:**785–791.
36. **Perry, L. L., and D. L. Lodmell.** 1991. Role of $CD4^+$ and $CD8^+$ T cells in murine resistance to street rabies virus. J. Virol. **65:**3429–3434.
37. **Schneider-Schaulies, S., U. G. Liebert, K. Baczko, and V. ter Meulen.** 1990. Restricted expression of measles virus in primary rat astroglial cells. Virology **177:**802–806.
38. **Schneider-Schaulies, S., U. G. Liebert, Y. Segev, B. Rager-Zisman, M. Wolfson, and V. Ter Meulen.** 1992. Antibody-dependent transcriptional regulation of measles virus in persistently infected neural cells. J. Virol. **66:**5534–5541.
39. **Segev, Y., B. Rager-Zisman, N. Isakov, S. Schneider-Schaulies, V. ter Meulen, S. Udem, S. Segal, and M. Wolfson.** 1994. Reversal of the measles virus-mediated increase of phosphorylating activity in persistently infected mouse neuroblastoma cells by anti-measles virus antibodies. J. Gen. Virol. **75:**819–827.
40. **Statts, H. F., J. E. Oakes, and R. N. Lausch.** 1991. Anti-glycoprotein D monoclonal antibody protects against herpes simplex virus type 1-induced diseases in mice functionally depleted of selected T-cell subsets or asialo $GM1^+$ cells. J. Virol. **65:**6008–6014.
41. **Suryanarayana, K., K. Baczko, V. ter Meulen, and R. R. Wagner.** 1994. Transcription inhibition and other properties of matrix proteins expressed by M genes cloned from measles viruses and diseased human brain tissue. J. Virol. **68:**1532–1543.
42. **Tolskaya, E. A., T. A. Ivannikova, M. S. Kolesnikova, S. G. Drozdov, and V. I. Agol.** 1992. Postinfection treatment with antiviral serum results in survival of neural cells productively infected with virulent poliovirus. J. Virol. **66:**5152–5156.
43. **Tyler, K. L., M. A. Mann, B. N. Fields, and H. W. Virgin IV.** 1993. Protective anti-reovirus monoclonal antibodies and their effects on viral pathogenesis. J. Virol. **67:**3446–3453.
44. **Wright, K. E., and M. J. Buchmeier.** 1991. Antiviral antibodies attentuate T-cell-mediated immunopathology following acute lymphocytic choriomeningitis virus infection. J. Virol. **65:**3001–3006.
45. **Zinnheimer-Dreikorn, J., and K. P. Koschel.** 1990. Antigenic modulation of measles subacute sclerosing panencephalitis virus in a persistently infected rat glioma cell line by monoclonal anti-hemagglutinin antibodies. J. Gen. Virol. **71:**1391–1394.