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Protective Effects of Glycoprotein-Specific Monoclonal Antibodies on the Course of Experimental Mumps Virus Meningoencephalitis

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Newborn Syrian hamsters were challanged with an intracerebral inoculum containing 128 50% lethal doses of the Kilham strain of mumps virus and treated 24 h later with a single intraperitoneal injection of mouse monoclonal antibody. Monoclonal antibodies reactive with epitopes on the fusion glycoprotein of mumps virus could not inhibit hemagglutination or neutralize infectivity in vitro and failed to provide biologically important protection against the in vivo infection. In contrast, monoclonal antibodies reactive with epitopes on the hemagglutinin-neuraminidase glycoprotein of mumps virus inhibited hemagglutination and neutralized infectivity in vitro and protected infected animals from the otherwise lethal central nervous system virus infection. Similar protection was provided by both purified immunoglobulin and $F(ab')_2$ fragments. Immunocytochemical and virological studies showed diminished virus antigen and virus titers in the brains of successfully treated animals. It appears that a topographically restricted region of the hemagglutinin-neuraminidase molecule of the Kilham strain of mumps virus is of critical importance for immune recognition by the infected host.

Mumps virus, a member of the paramyxovirus family, is an enveloped, negative-strand RNA virus which contains six major structural polypeptides (11, 12, 24, 27, 28). The hemagglutinin-neuraminidase (HN) and fusion (F) glycoprotein molecules project from the surfaces of mumps virions and mumps virus-infected cells. The HN glycoprotein binds to sialyl groups on the host cell membrane during the adsorption phase of the infection process. The F_1 portion of the F glycoprotein, by analogy with other paramyxoviruses, initiates virion membrane fusion with the host cell plasma membrane to allow penetration of the viral nucleocapsid into the host cell. The remaining structural proteins are the major nucleocapsid-associated protein (NP), two less prominent nucleocapsid-associated proteins (P and L), and the membrane-associated or matrix (M) protein. These nonglycosylated proteins have an internal location in the virion, are found within infected cells, and are unlikely to be important targets of a protective immune response.

Monoclonal antibodies (Mabs) specific for the HN glycoprotein of mumps virus have been used to show that sites on this molecule are important for hemadsorption of virions to erythrocytes and for the neutralization of infectivity in vitro (30, 36). However, Mabs specific for the F glycoprotein of mumps virus, although sometimes inhibiting the lysis of erythrocytes, do not seem to affect neutralization of infectivity in vitro (30). A polyvalent antibody generated to purified mumps virus F glycoprotein also inhibits hemolysis but fails to neutralize virus activity in vitro (29). The HN and F glycoproteins are likely candidate targets of protective humoral and cellular immune responses.

After intracerebral (i.c.) inoculation, selected strains of mumps virus cause a widespread neuronal infection of newborn hamsters which is lethal (16, 25, 40). We chose to use this model infection to explore the protective effects of selected mouse Mabs specific for the glycoproteins of mumps virus. In this manner we hoped to deduce the relative importance of the host immune response to these surface molecules for protection in vivo. None of the Mabs used in this study, specific for a number of distinct epitopes on the F glycoprotein, could confer protection. In contrast, dramatic protective effects were observed after passive administration of HN-specific Mabs.

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MATERIALS AND METHODS

Cells and virus. The Kilham strain of mumps virus was used for all of the in vitro studies. This virus underwent three cycles of plaque purification in CV-1 cells before its expansion to form a stock pool. Virus was stored in samples at −70°C before use and contained $10^{7.4}$ PFU/ml when assayed as previously detailed (26).

Six additional strains of mumps virus which have been previously characterized (24, 26, 36) were used in the study of the reactivity patterns of the Mabs. These include the Enders, Jeryl-Lynn B, O'Take, MJ, and RW strains and a syncitia-forming variant of RW (RW_s). All of these strains were propagated in CV-1 cells, with the exception of the Enders strain which was grown in embryonated chicken eggs.

Homogenates of hamster brain were prepared in Hanks balanced salt solution (HBSS) as 10% (wt/wt) suspensions and then clarified as previously detailed (41). They were stored at −70°C before assay on CV-1 cell monolayers.

Animals and statistical treatment of survival data. Pregnant multiparous Syrian hamsters were obtained from Charles River Breeding Laboratories, Inc., Wilmington, Mass. Animals were received during week 2 of gestation and housed separately. Pups were inoculated within 24 h of birth with a 25-μl volume of virus delivered via a 25-gauge needle into the parietal region of the left brain hemisphere. Treatment consisted of 0.1-ml volumes of either ascites fluid diluted in HBSS or HBSS alone administered via a 25-gauge needle into the abdominal cavity at the indicated times postinfection.

Treatment was not varied within a given litter, and the litters were randomly selected for treatment. Census was taken daily. All deaths occurring within 72 h of birth were attributed to nonspecific causes and were excluded from both the experimental and control groups and from further

consideration. Animals removed from litters for histological or virological analysis were handled as censored data at the time of harvest. All such censored animals were randomly selected from available litters at preselected time intervals appropriate to a given protocol. Observations in a given experiment were terminated either when all animals had died or arbitrarily at or beyond 40 days after infection at which time all surviving animals were censored (21). Survival curves were constructed by the method of Kaplan and Meier (15). Evaluation of treatment-dependent differences between the survival curves was by published FORTRAN programs of a nonparametric comparison test for multiple samples containing censored observations (18). Comparisons within groups reflect two-tailed analysis.

Mabs. Mabs were generated and characterized by modification of previously detailed methods (36). In brief, spleen donors were prepared by infecting Balb/c mice (Charles River Breeding Laboratories) with a single intraperitoneal (i.p.) inoculation of $10^{6.5}$ PFU of the Kilham strain of mumps virus, followed at least 2 weeks later by a single intravenous challenge with $10^{6.5}$ PFU of live virus 4 days before spleen harvest. Fusions were carried out with both the NS-1 and X 63-Ag 8.653 mouse myeloma cell lines. Secreting hybrids were selected on the basis of an enzyme-linked immunosorbent assay (ELISA), with a horseradish peroxidase-conjugated goat anti-mouse immunoglobulin G (IgG) heavy- and light-chain specific antibody (Tago, Inc., Burlingame, Calif.) (38). This assay used purified Kilham strain mumps virions as the target antigen. Secreting hybrids were cloned either by limiting dilution as previously described (36) or by two passages in soft agar (7). Ascites fluids were produced in Balb/c mice that had been pretreated with 2,6,10,14-tetramethylpentadecane (Aldrich Chemical Co., Inc., Milwaukee, Wis.) before the i.p. injection of ca. 10^7 hybridoma cells. The Mabs were characterized according to the virus polypeptide that they precipitated from [^{35}S]methionine-labeled Kilham strain mumps virus-infected cell lysates as previously detailed (36). Isotypes were determined by a four-step ELISA in which mouse IgG1, IgG2a, IgG2b, and IgG3 isotype-specific rabbit immunoglobulins (Miles Scientific, Naperville, Ill.) were used to seek Mab bound to the plates and in turn localized with biotinylated goat anti-rabbit IgG (Bethesda Research Laboratories, Gaithersburg, Md.), followed by a streptavidin-horseradish peroxidase conjugate. Hemagglutination inhibition and neutralization assays were performed as previously detailed (36).

Purification of Mab, biotinylation, and production of F(ab')$_2$ fragments. Selected Mabs were purified from ascites fluids by two cycles of precipitation with $(NH_4)_2SO_4$ at 50% saturation (17) and exhaustively dialyzed against phosphate-buffered saline. Protein levels of purified immunoglobulin preparations were adjusted to contain ca. 20 mg of protein per ml, made alkaline with 0.5 M $NaHCO_3$ (pH 9.0), and biotinylated (b-) by the addition of biotin *N*-hydroxysuccinimide ester solubilized in anhydrous dimethyl formamide according to the directions of the manufacturer (Bethesda Research Laboratories). The reaction was stopped by the addition of 1 M NH_4Cl, and unreacted biotin was removed by dialysis against phosphate-buffered saline. Divalent fragments were generated by a 48-h (32) pepsin digestion (Sigma Chemical Co., St. Louis, Mo.) at pH 4.5 at a pepsin-to-IgG ratio of 1:50. The purity of IgG and F(ab')$_2$ fragments was ascertained by analysis of Coomassie stain patterns of proteins separated by sodium dodecyl sulfate-polyacrylamide gel electrophoresis under unreduced and reduced conditions by previously detailed methods (38).

Competitive ELISA and reactivity profiles. Gradient-purified Kilham strain mumps virus grown in CV-1 cells was used as antigen in the competitive ELISA. Viral antigen protein (50 ng per well in 0.1 ml of 0.05 M $NaHCO_3$ [pH 9.0]) was allowed to bind to the bottom of 96-well microtiter plates (Immulon 2; Dynatech Laboratories, Inc., Alexandria, Va.) overnight at 4°C. End-dilution titers of the b-Mabs were determined by applying increasing doubling dilutions of the b-Mabs in ELISA buffer (phosphate-buffered saline with 0.1% Tween-20 and 0.5% bovine serum albumin) to wells of the plate which were then incubated for 90 min at room temperature. Bound b-Mab was sought by a 30-min incubation with a 1:500 dilution of streptavidin-horseradish peroxidase (Bethesda Research Laboratories), and a reaction product was developed with orthophenylenediamine and quantitated in a DU-8 spectrophotometer (Beckman Instruments, Inc., Palo Alto, Calif.). All wash steps were as previously detailed (42). From these initial titration curves, an appropriate dilution of a given b-Mab was chosen that would generate 0.3 optical density units in the ELISA. A fixed amount of a given b-Mab then was mixed with appropriate dilutions of unlabeled Mab, and these mixtures were assayed as above. In all instances, identical b-Mab–Mab pairs showed competitive inhibition curves with increasing amounts of unlabeled Mab.

Sucrose gradient-purified viruses either from supernatants of infected CV-1 cells or, in the case of the Enders strain of mumps virus, from allantoic fluids of infected eggs were used as antigens to determine the reactivity profiles of the Mabs to the various mumps virus strains. The amount of virus antigen protein for each of the virus strains was adjusted to between 160 and 580 ng per well to provide an optimal amount of antigen for each strain and adsorbed to the wells of microtiter plates as described above. The Mabs were then added to duplicate wells of these plates and incubated for 90 min at room temperature. Bound Mab was sought with a fixed amount of a horseradish peroxidase-conjugated goat anti-mouse heavy- and light-chain specific antibody (Tago, Inc.), and a reaction product was generated and quantitated as described above. The reaction product generated against the Kilham strain of mumps virus antigen was set at 100% activity, and the reactivity of a specific Mab with the other virus strain antigens was calculated relative to the Kilham strain virus antigen result.

Immunocytochemistry. The distribution of virus-infected cells in brains from selected infected and HN8 Mab-treated, or infected and HBSS-treated (control), hamsters was determined as follows. Animals were killed at 4, 6, and 10 days after infection by intracardiac perfusion-fixation with phosphate-buffered 4% paraformaldehyde solution by previously described procedures (40). The brains were embedded in paraffin, sectioned, deparaffinized, and processed for standard histology and immunohistochemical localization of mumps virus nucleocapsid-associated antigen. The latter used mouse ascites fluid from the NP4 monoclone at a 10^{-5} dilution and horseradish peroxidase conjugated to goat anti-mouse IgG in an indirect technique. An insoluble reaction product was then generated with diaminobenzidine (43).

RESULTS

Characterization of the untreated infection. Survival curves were generated for newborn hamsters i.c. inoculated within the first 24 h of life with $10^{0.8}$ to $10^{4.8}$ PFU of the Kilham strain of mumps virus (Fig. 1). Survivors of acute infection were only observed at inocula below $10^{3.8}$ PFU, and long-term survivors, beyond 3 weeks of age, were seen only at

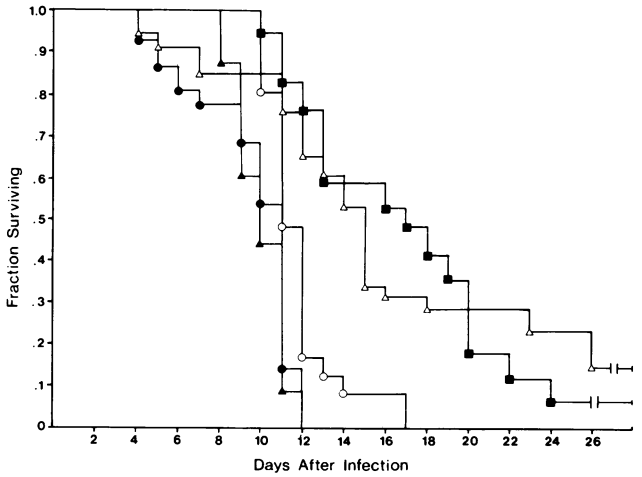

FIG. 1. Survival curves for newborn hamsters receiving an i.c. inoculation of various doses of the Kilham strain of mumps virus within 24 h of birth. The animals were inoculated with $10^{4.8}$ (▲), $10^{3.8}$ (●), $10^{2.8}$ (○), $10^{1.8}$ (△), or $10^{0.8}$ (■) PFU of virus. The total number of animals observed in each group was 16, 55, 27, 32, and 17, respectively. Fifty PFU is equivalent to 1 50% lethal dosage as determined from survival data 14 days after the infection. Survival was prolonged as the inocula was reduced from $10^{3.8}$ to $10^{2.8}$ PFU ($P = 0.002$) and from $10^{2.8}$ to $10^{1.8}$ PFU ($P = 0.009$).

inocula of $10^{1.8}$ PFU or lower. At the lower inocula, deaths which occurred within the first 2 weeks of infection appeared to be attributable to acute encephalitis; later deaths were more likely related to evolving obstructive hydrocephalus. When a conventional 50% lethal dosage was calculated from survival data at day 14 of the infection, stock virus contained $10^{5.7}$ 50% lethal dosages per ml.

After i.c. infection with $10^{3.8}$ PFU virus (128 50% lethal dosages), animals showed substantial virus in the brain 1 day after the infection ($10^{4.4 \pm 0.4}$ PFU/g of tissue), developed a peak virus titer ($10^{6.0 \pm 0.1}$ PFU/g) in the brain on day 4 of the infection, and continued to have significant amounts of recoverable virus ($10^{5.0 \pm 0.6}$ PFU/g) before death (Fig. 2). Infection of intraparanchymal neurons was widespread, as assessed immunohistochemically (Fig. 3A). Clinically, these animals appeared normal when compared with uninfected controls for the first 5 days of the infection. Subtle abnormalities were first detected on day 6 when the animals evidenced weight loss, ruffled fur, and reduced spontaneous activity. By day 7 the majority of infected animals were obviously sick or moribund. In a cumulative control group of 127 uncensored animals infected with $10^{3.8}$ PFU of virus and receiving a single 0.1-ml treatment of saline by i.p. inoculation 24 h after the i.c. inoculation, no animal survived beyond 13 days of the infection.

Characteristics of the Mabs. Fifty-two cloned hybridoma cell line-secreting Mabs specific for the major structural polypeptides of Kilham strain mumps virus have been generated (specificities: 14 HN, 18 NP, 10 F, 4 P, and 7 M) of which 6 HN-, 6 F-, and 2 NP-specific Mabs were chosen for this study (Table 1). All of these HN Mabs showed hemagglutination inhibition and neutralization activity when assayed in vitro; none of the F or NP Mabs showed such activity. Only IgG class antibodies have been generated with representatives of most isotypes used in the present study.

All of the anti-HN Mabs showed similar competitive inhibition curves, and as anticipated none could be competed with excess amounts of unlabeled anti-F Mabs (e.g., Fig. 4). In contrast, the six anti-F Mabs could be grouped on the basis of four distinct displacement curve patterns, some examples of which are shown in Fig. 5. None of the anti-F Mabs were displaced by excess unlabeled anti-HN Mabs (data not shown).

The reactivity profiles of the anti-HN Mabs with the seven mumps virus strain antigens delineated at least five distinct patterns (Fig. 6). In contrast, the reactivity profiles for the anti-F Mabs were quite similar, with only Mab F5 showing a suggestively distinct pattern.

A number of assumptions must be made which limit interpretation of these data. Yet, it appears reasonable to infer from these data that the HN-specific Mabs operationally define at least five distinct epitopes on the HN molecule of the Kilham strain of mumps virus which are topographically arranged in such a manner that they are not distinguished in the competitive inhibition studies (Table 1). The F-specific Mabs define at least four topographically distinct epitopes on the Kilham strain F glycoprotein by competitive inhibition analysis. However, the reactivity profiles of these Mabs suggest that the epitopes on the F molecule are relatively conserved amongst the mumps virus strain antigens used.

Effects of Mab treatment on the course of the infection. Ascites fluids derived from all of the hybridomas secreting

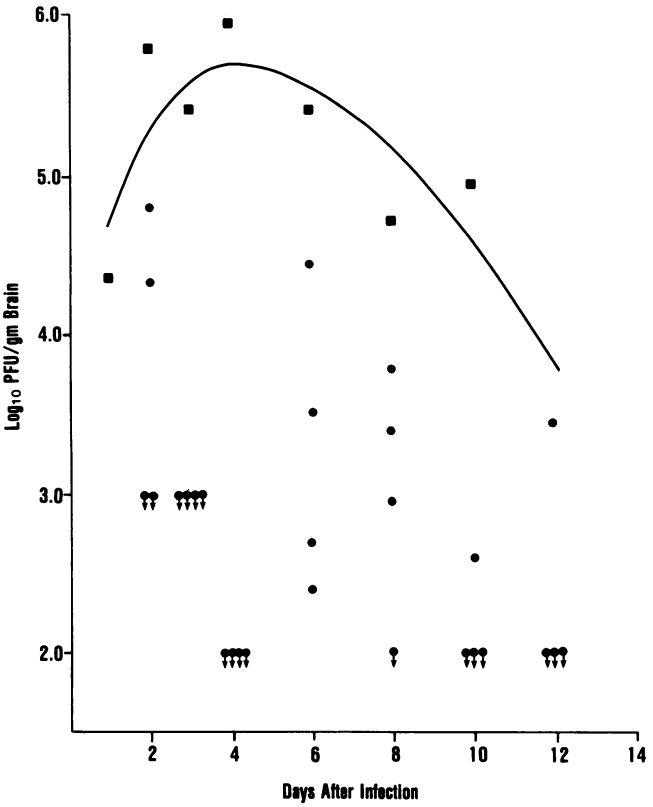

FIG. 2. Virus titers in homogenates of hamster brains at various times after infection. All animals were infected with $10^{3.8}$ PFU mumps virus within 1 day of birth and subsequently treated at 24 h after infection with either HBSS (control [■]) or ascites fluid containing Mab HN8 (●). Each data point for the control animals represents the mean from four individually titered brain homogenates. Data points for treated animals are from homogenates of individual animal brains. The solid line is a best fit curve for the control data. The arrows represent no detectable virus at the lowest limits of the assay.

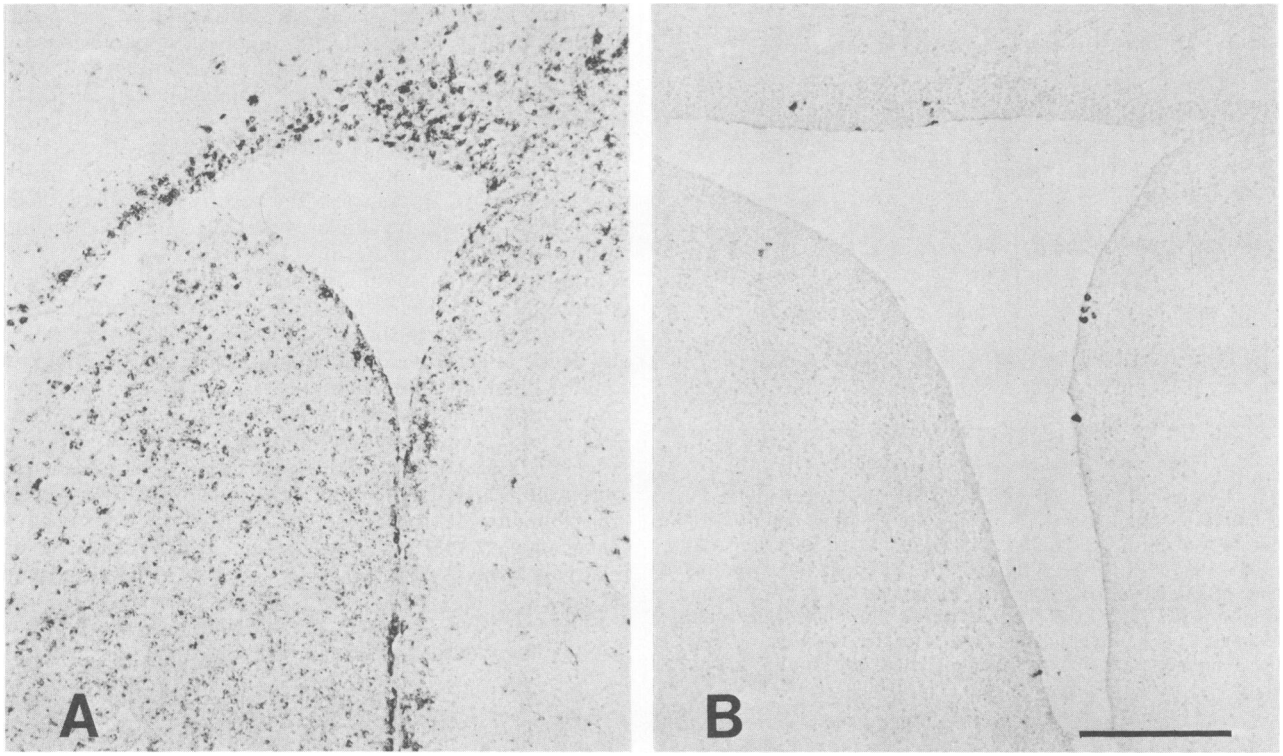

FIG. 3. Distribution of viral antigens in the brain 6 days after infection with $10^{3.8}$ PFU of the Kilham strain of mumps virus. The black reaction product in these photomicrographs delineates virus-infected cells expressing nucleocapsid protein antigen. (A) Numerous antigen-bearing ependyma cells of the lateral ventricle and parenchymal neurons are seen in this coronal section at the level of the caudate-putamen from an infected animal treated with HBSS. (B) Only a limited number of ependymal cells are seen to express viral antigen in this coronal section at a level similar to that in A from an infected animal treated with Mab HN8. Originals: both ×100; bar equals 200 μm.

Mabs reactive with the HN glycoprotein of mumps virus exerted statistically significant protective effects when diluted in an equal volume of HBSS and administered as a single 0.1-ml i.p. injection 24 h after establishing the infection (Fig. 7). The proportion of animals surviving to 40 days ranged from 67 to 96%. In selected experiments animals were maintained for up to 3 months with little additional late mortality. Late survivors were often small for their age but otherwise were behaviorally normal. However, various degrees of obstructive hydrocephalus were found at autopsy in 50% of such animals. Hydrocephalus secondary to aqueductal stenosis is a well-recognized static sequalae of the ependymitis that accompanies acute mumps infection of the newborn hamster brain (14, 39). Immunoprecipitation studies with serum and ventricular fluid from selected survivors showed that immunoglobulins recognizing all of the major structural polypeptides of mumps virus were present (unpublished data). Therefore, the administration of protective Mab did not qualitatively affect the normal humoral immune response in these animals.

Virus titers in brains of individual animals treated with HN8 Mab and killed at various times after infection varied considerably (Fig. 2). However, in all instances virus titers from individual brains of treated animals were lower than those of untreated controls, and virus could not be titered from 17 of 28 treated animals.

Similarly, the distribution of virus antigen in successfully treated animals was markedly reduced compared with saline-treated controls. Most treated animals had no or only limited mumps virus NP antigen detected in the brain (13/18), and when present, antigen was limited to scattered ependymal cells (Fig. 3B). Only two of the five remaining treated animals had amounts of NP antigen in the brain comparable to the untreated animals.

In contrast to the results obtained with the HN Mabs, an identical treatment protocol with ascites fluid containing Mabs to the F glycoprotein rarely showed protective effects (Fig. 8). The only statistically significant protective effect was observed with Mab F4; this was due to a modest shift of the survival curve to the right, and no animals survived infection. As expected, the Mabs NP3 and NP4 with specificity for an internal virus protein showed survival curves comparable to those for saline-treated controls (data not shown).

Effects of delayed treatment with protective Mabs. We had previously shown that Mab administered as late as 48 h after infection could prolong survival but with diminished proportions of long-lived animals (43). The Mab HN10 which showed protection when administered at 24 h postinfection was selected for use in a therapeutic protocol. Intraperitoneal administration of 0.1 ml of HN10 ascites fluid diluted in an equal volume of HBSS was begun at 5, 6, 7, or 9 days after infection and then repeated every other day over the course of the infection. Survival was not significantly prolonged with treatment initiated at or just before clinical disease onset (data not shown).

Effects of purified monoclonal IgG and F(ab')$_2$ fragments on survival. The Mab HN8 is an IgG1 mouse immunoglobulin which should not bind complement (37). However, to be certain that its protective effect was complement independent and to determine whether antibody-dependent cell-mediated cytotoxicity responses might contribute to its

TABLE 1. Properties of the Mabs

Designation	Isotype	Epitope[a]	HAI[b]	Nt[c]	Rx[d]	Titer[e]
HN4	IgG1	A_1	+	+	++	4.8
HN5	IgG1	A_2	+	+	++	5.4
HN7	IgG2b	A_3	+	+	++	5.3
HN8	IgG1	A_3	+	+	++	3.8
HN10	IgG2a	A_4	+	+	++	5.2
HN12	IgG2a	A_5	+	+	++	6.1
F1	IgG2a	A_1	0	0	0	3.6
F2	IgG3	B_1	0	0	0	4.8
F4	IgG2a	B_1	0	0	+	5.0
F5	IgG1	C_1	0	0	0	2.3
F6	IgG2a	B_1	0	0	0	5.8
F10	IgG2a	D_1	0	0	0	4.0
NP3	IgG2a	ND[f]	0	ND	0	7.2
NP4	IgG2a	ND	0	0	0	ND

[a] Capital-letter designations refer to the results of reciprocal competitive inhibition studies; numerical subscripts refer to the results of reactivity profiles in ELISA with the seven mumps virus strain antigens.
[b] HAI, Hemagglutination inhibition; +, positive; 0, negative.
[c] Nt, Neutralization activity; +, positive, 0, negative.
[d] Rx, Improved survival of animals treated with ascitic fluid at 24 h after i.c. infection; ++, long-term survivors; +, statistically significant protection ($P \leq 0.001$) but no long-term survivors; 0, no significant effect.
[e] Titer, Log_{10} end-dilution ELISA titer of 0.1 ml of ascites fluid administered in protection studies.
[f] ND, Not done.

protective effect, $F(ab')_2$ fragments were generated. Infected animals were inoculated i.p. with 2,000 end-dilution ELISA units of HN8 $F(ab')_2$ fragments (470 μg of protein) at 24 h after infection (Fig. 9). This increased survival to an extent comparable to that of treatment with an equivalent amount of HN8 IgG (2,000 end-dilution ELISA units; 360 μg of protein), suggesting that the observed protective effect was independent of F_c-related biological activities of the immunoglobulin molecule.

DISCUSSION

The ability of selected Mabs to passively transfer protection from challange with an otherwise fatal virus inoculum has recently been well demonstrated for representative members of several diverse virus families in model murine infections (1–3, 5, 6, 10, 19, 20, 22, 23, 33–35). The present study demonstrates that murine Mabs directed at the HN glycoprotein of mumps virus which neutralize infectivity in vitro can protect newborn hamsters from otherwise fatal experimental mumps meningoencephalitis. The protection appears to be the result of the Mab limiting the spread of infection in the brain. Since $F(ab')_2$ fragments of Mab are as

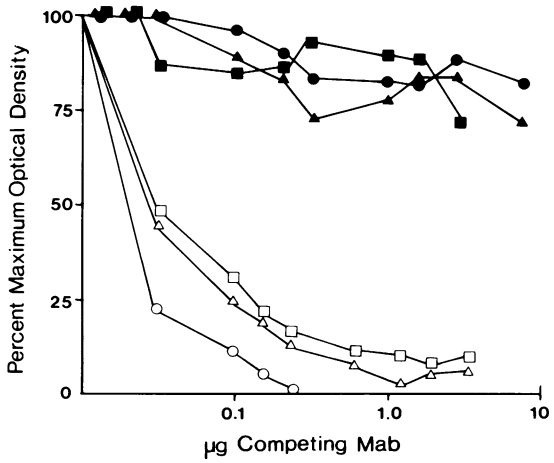

FIG. 4. Representative competitive inhibition curves for determination of HN glycoprotein epitope specificities of the Mabs. The b- and competing unconjugated monoclonal immunoglobulin pairs are: b-HN12 and HN12 (○); b-HN12 and F1 (●); b-HN4 and HN12 (△); b-HN4 and F1 (▲); b-HN10 and HN12 (□); b-HN10 and F1 (■).

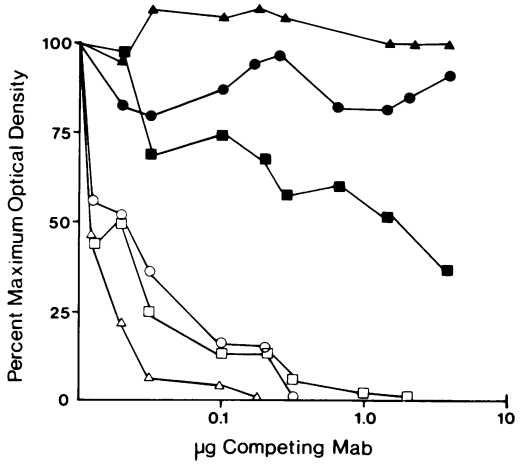

FIG. 5. Representative competitive inhibition curves for determination of fusion glycoprotein epitope specificities of the Mabs. The b- and competing unconjugated monoclonal immunoglobulin pairs are: b-F1 and F1 (○); b-F1 and F4 (●); b-F5 and F5 (△); b-F5 and F4 (▲); b-F6 and F4 (□); b-F5 and F10 (■). F1, F4, F5, F6, and F10 are representative of operationally defined fusion glycoprotein epitopes A, B, C, B, and D, respectively.

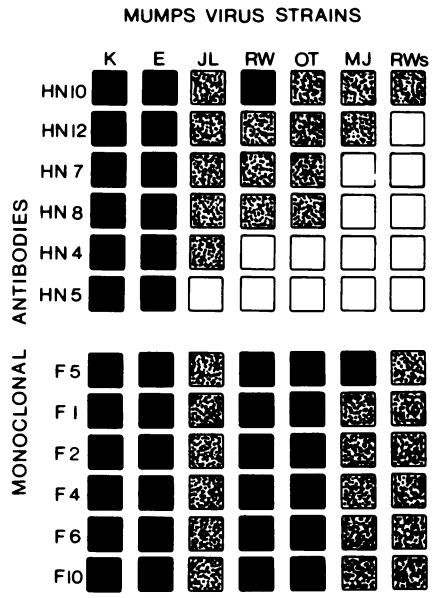

FIG. 6. Reactivity profiles of the HN and F Mabs with the seven mumps virus strain antigens. The amount of reaction product generated in the virus strain-specific ELISA is shown relative to the Kilham strain antigen for the ranges less than 10% (□), 10 to 90% (▨), and more than 90% (■). The mumps virus strains used were Kilham (K), Enders (E), Jeryl-Lynn (JL), RW, O'Take (OT), MK, and RW_s.

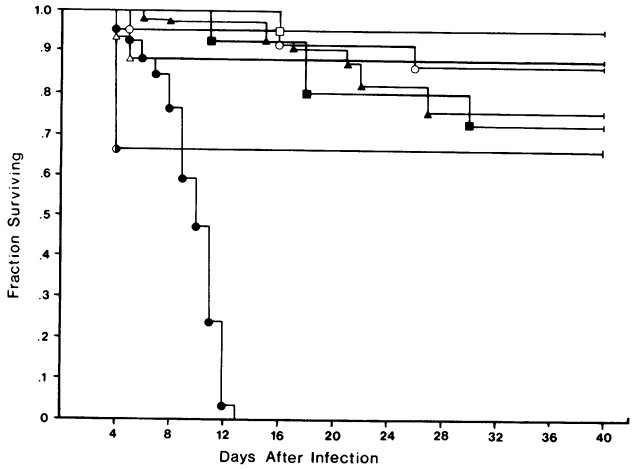

FIG. 7. Survival curves for newborn hamsters infected with mumps virus and treated with a single i.p. injection of ascites fluid containing HN-specific Mabs at 24 h after infection. All animals were infected i.c. with $10^{3.8}$ PFU of the Kilham strain of mumps virus within 24 h of birth. Groups of animals were treated with HBSS (●) or Mab HN4 (○), HN5 (△), HN7 (□), HN8 (▲), HN10 (■), or HN12 (◑). The total number of animals observed in each group was 185, 23, 17, 22, 86, 15, and 6, respectively. All of the groups receiving HN series Mab treatment showed prolonged survival compared with the mock-treated controls with an overall significance level of $P < 0.00001$. The extent of protection with the various HN series Mabs was nearly comparable as determined by survival curve analysis ($P = 0.08$).

effective as whole IgG, the protection can occur independent of the participation of complement or antibody-dependent cell-mediated cytotoxicity mechanisms. In contrast, none of the Mabs with specificity for the F glycoprotein of mumps virus conferred biologically significant protection in this model system. The administration of either protective HN or nonprotective F Mab does not result in the emergence of chronic progressive disease or an atypical neuropathological response as has been reported with measles virus (33) and mouse hepatitis virus (3) model infections.

All of the HN Mabs used in this study appeared to react with a topographically restricted region of the mumps virus HN molecule as determined by reciprocal competitive inhibition, hemagglutination inhibition, and in vitro neutralization assays (Table 1). However, the specificity of these Mabs for closely spaced epitopes within this topographically restricted region of the HN molecule is likely. This later conclusion is based on the observed rectivity profiles for the HN Mabs in ELISA in which the antigens used were prepared from six mumps virus strains distinct from the Kilham strain (Table 1, Fig. 6). It is reasonable to suspect that this region of the HN glycoprotein of mumps virus is a critical site for successful immune recognition by an infected host.

The HN Mabs must exert their protective effect in the newborn hamster in part by limiting the spread of infection within the central nervous system (CNS). Virus titers in the brain are already high within 24 h of infection at the time of inoculation of the Mabs (Fig. 2) and probably continue to rise further until the Mabs are adsorbed from the peritoneal cavity to enter the blood stream before their initial entry into the brain across the blood brain barrier. Transfer of mouse Mab into the hamster brain is undoubtedly facilitated by local alterations in the blood brain barrier related to trauma at the injection site, and the rate of transfer can be expected to increase further as the infection proceeds and causes additional barrier impairment (6, 9). The ability to alter the outcome of an experimental viral infection of the brain by the administration of protective Mabs as late as 1 to 3 days after initiating the brain infection also has been reported for experimental measles (33), influenza (6), mouse hepatitis (3), herpes simplex (5, 34), and Saint Louis encephalitis (23) virus infections.

A number of Mabs which are nonneutralizing in vitro have been found to protect in vivo (1, 2, 19, 22, 34, 35). Generally, these reagents are characterized by high avidity interaction with intact virions or infected cell surfaces and by the ability to bind complement or to participate in in vitro antibody-dependent cell-mediated cytotoxicity. Neutralizing HN and F and nonneutralizing HN Mabs to Sendai virus have been shown to substantially reduce virus titers in lungs when passively administered to mice with active Sendai virus infections (31). Presumably, antibody-dependent cell-mediated cytotoxicity mechanisms contributed to the protection seen with the nonneutralizing Mabs in that model. It is perhaps surprising that none of our F Mabs showed such an in vivo protective effect. One might have anticipated similar immune mechanisms to be active in the mumps meningoencephalitis system even though the passively administered F series Mabs were derived from a different heterologous donor.

Mumps virus is a common human pathogen. Before the widespread use of successful attenuated live-virus vaccines for mumps, mumps virus was probably the single most frequent virus to invade the CNS of humans (13). Up to 40% of all patients with uncomplicated mumps show a cerebrospinal fluid pleocytosis, indicative of the high rate of CNS invasion. Usually the CNS infection is benign, but fatal encephalitis can develop. Immunotherapy with hyperimmune antisera has been shown to favorably affect the course

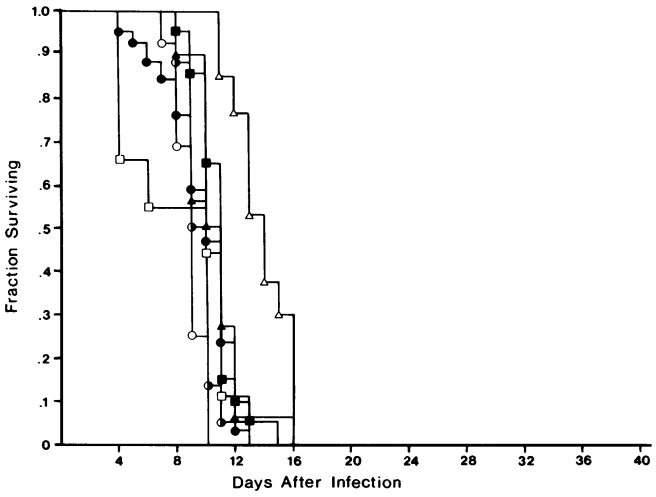

FIG. 8. Survival curves for newborn hamsters infected with mumps virus and treated with a single i.p. injection of ascites fluid containing fusion glycoprotein specific Mabs at 24 h after infection. All animals were infected i.c. with $10^{3.8}$ PFU of the Kilham strain of mumps virus within 24 h of birth. Groups of animals were treated wtih HBSS (●) or Mab F1 (○), F2 (▲), F4 (△), F5 (◑), F6 (□), or F10 (■). The total number of animals observed in each group was 185, 16, 18, 13, 25, 9, and 20, respectively. Only the group treated with F4 showed a survival curve statistically different ($P = 0.001$) from that of the mock-treated control group. None of the groups receiving F-specific Mab treatment survived beyond day 16 of the infection.

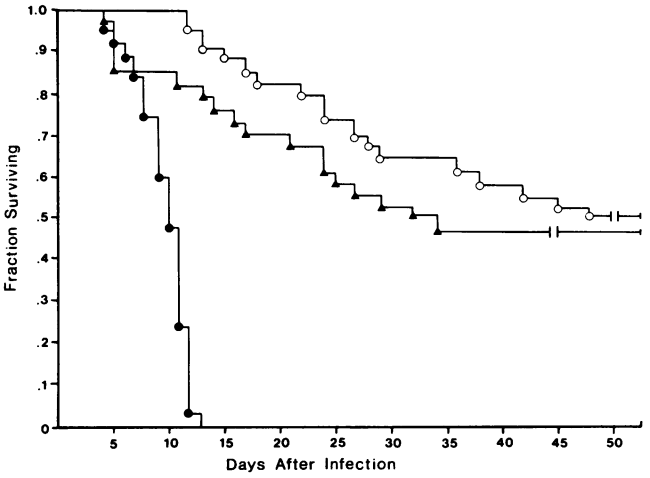

FIG. 9. Effects of purified HN8 monoclonal IgG and F(ab')$_2$ fragments on survival of newborn hamsters with mumps virus encephalitis. All animals were infected i.c. with $10^{3.8}$ PFU of the Kilham strain of mumps virus within 24 h of birth. Groups of animals were treated at 24 h after infection with HBSS (●), HN8 IgG (○), or HN8 F(ab')$_2$ (△). The total number of animals observed in each group was 185, 41, and 39, respectively. A statistically significant overall effect was noted with both HN8 IgG- and HN8 F(ab')$_2$-treated groups, showing improved survival compared to the mock-treated control group (P = 0.002). The HN8 IgG and HN8 F(ab')$_2$ survival curves are similar (P = 0.24).

of mumps when used early in the illness (8), and its use also has been reported to stem the spread of epidemics (4). The present study lends some additional credence to those observations of the potential efficacy of immunotherapy in mumps virus infections. In the hamster model, treatment at the onset of clinical disease proved ineffective. However, our ability to protect animals that already express high CNS titers of virus with a single i.p. inoculation of Mab suggests that adjuvant immunotherapy might be of clinical benefit in otherwise overwhelming CNS infections of humans.

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