Role of Immune Cells in Protection against and Control of Reovirus Infection in Neonatal Mice

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We studied the role of T cells in resistance to reovirus intestinal and central nervous system infection. Transfer of reovirus-immune adult spleen cells protected neonatal mice from (i) lethal infection with reovirus serotype 3 Dearing (T3D, footpad inoculation) and serotype 3 clone 9 (T3C9, oral inoculation) and (ii) hydrocephalus caused by serotype 1 Lang (T1L, intracranial [i.c.] inoculation). Cell-mediated protection was not serotype specific. While immune cells protected against T1L i.c., they failed to protect against 1/5,000 of the dose of T3D i.c. Two types of experiments showed that both CD4 and CD8 T cells are involved in reovirus resistance. First, immune cell-mediated protection against T3D was abrogated by in vivo treatment with anti-CD4 monoclonal antibody (MAb) and significantly inhibited by in vivo treatment with anti-CD8 MAb. Second, T3C9-infected neonatal mice treated with anti-CD4 and/or anti-CD8 developed a novel disease phenotype, an oily hair syndrome, associated with severe hepatobiliary pathology and increased viral titer in heart and liver. Immune cells and an MAb to the cell attachment protein σ 1 (MAb G5) protected by different mechanisms. Immune cells were more effective than σ 1 MAb G5 at controlling primary replication, while σ 1 MAb G5 was more effective than immune cells at inhibiting neural spread of virus. We conclude that both CD4 and CD8 T cells are important for reovirus resistance, that cells and antibody act preferentially at different stages in pathogenesis in vivo, and that adoptively transferred immune cells can protect both the central nervous system and intestine.

Experimental murine infection with reoviruses provides an excellent model for studying how components of the immune system act at specific stages in viral pathogenesis. We studied the role of T cells in protection against three different reoviruses to understand which T cells are involved in reovirus resistance and how immune cells and antibody act in vivo.

Reoviruses are nonenveloped double-stranded RNA viruses with a protein-RNA core surrounded by an outer capsid (30). Reoviruses are classified into three serotypes (28), which differ strikingly in pathogenetic properties (37). After footpad (FP) inoculation into newborn mice, serotype 3 Dearing (T3D) replicates in skeletal muscle and spreads neurally to the central nervous system (CNS), where it infects neurons and causes lethal encephalitis (38, 44). T3D's capacity for neural spread and neuronal tropism is conferred by the T3D S1 gene segment (38, 42, 44) encoding the cell attachment protein σ 1 (19). Reovirus serotype 3 clone 9 (T3C9) also causes lethal encephalitis but, in contrast to T3D, is lethal after oral (p.o.) inoculation (14a, 39). Reovirus serotype 1 Lang (T1L) causes hydrocephalus after intracranial (i.c.) inoculation and is trophic for ependymal cells (16, 41, 42, 44).

While the pathogenesis of reovirus infection has been extensively studied, less is known about reovirus immunity. Immunity is important, since mice with severe combined immunodeficiency (12) die from reovirus infection while

normal adult mice are resistant (36). Passively transferred polyclonal antibody protects mice from reovirus infection with T3D and T3C9 (5, 11, 39, 40). Anti- σ 1 monoclonal antibody (MAb) G5 protects mice by blocking neural spread to and within the CNS without significant effects on primary infection in muscle or intestine (39, 40). The present studies used adoptive transfer of immune spleen cells and in vivo treatment with anti-T cell MAbs to define the role of T cells in protecting against reoviruses with different pathogenetic properties. We also compared mechanisms of humoral and cellular immunity.

MATERIALS AND METHODS

Virus isolation, assay, purification, and storage. Reoviruses T3D, T3C9, and T1L were prepared and titered by plaque assay with L929 cells (40). Viral titers are reported as PFU per milliliter (organs were harvested into 1 ml of gel saline). Purification of virus on CsCl gradients was as described previously except that 0.25 mM phenylmethylsulfonyl fluoride, 1% (vol/vol) Trasylol, and 0.5 μ g each of pepstatin and leupeptin A per ml (all from Sigma Chemical Co., St. Louis, Mo.) were added before sonication of virus-containing cell pellets. Virus concentration was determined assuming 1.0 U of optical density at 260 nm = 2.1×10^{12} particles per ml (32). Some in vivo experiments used second-passage virus without purification, while others used purified virus frozen at -70°C as aliquots in Hanks balanced salt solution (Biofluids, Rockville, Md.) containing 5% fetal calf serum, 1% (vol/vol) of 1 M HEPES (*N*-2-hydroxyethylpiperazine-*N'*-2-ethanesulfonic acid) (Biofluids), 1 U of penicillin per ml, and 1 μ g of streptomycin per ml (Irvine Scientific, Santa Ana, Calif.). Frozen stocks maintained stable titers over many months (data not shown) and were used within 2 weeks of thawing.

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Virus inoculation. Neonatal mice were inoculated with T3D either in the left hind FP or i.c. in 10 μ l with a 30-gauge needle (40). Mice were infected p.o. with 50 μ l of T3C9 as described previously (29, 39). Unless otherwise noted, mortality was assessed for 21 days, or 5 days after the last death, whichever was longer. T1L was inoculated i.c. (10 μ l), and mice were observed for 28 days for mortality. On day 28, mice were sacrificed, and one or more coronal sections of brain were examined for gross hydrocephalus, defined as the presence of dilatation of one or both lateral ventricles. Tissue samples were taken and stored at -70°C before titers were determined (39, 40).

Mice, immunizations, and spleen cell preparation. BALB/c AnNCr, A/JCr, and NIH Swiss mice obtained from the National Cancer Institute (Frederick, Md.) were maintained in an animal biosafety level 2 facility accredited by the American Association for Laboratory Animal Care in accordance with the standards outlined in Department of Health and Human Service publication NIH 86-23 (5a) and "PHS Policy on Humane Care and Use of Laboratory Animals" (5b). Male BALB/c AnNCr mice were bred with female A/JCr mice, and the progeny BALB/c AnNCr $\times$ A/JCr F_1 (CAF₁) mice were used for immunizations and adoptive transfer studies. Adult CAF₁ mice were immunized and boosted intraperitoneally (i.p.) with 50 to 100 μ g of T3D, T3C9, or T1L either as purified virus or as viral top component in complete Freund's adjuvant (Colorado Serum Co., Denver, Colo.).

Lymphocytes were prepared in either RPMI 1640 medium or Iscove's modification of Dulbecco's modified Eagle's medium (Biofluids) containing 10% fetal calf serum, 2 mM L-glutamine, 1 U of penicillin per ml, 1 μ g of streptomycin per ml, and 1% (vol/vol) of 1.0 M HEPES buffer. Spleen cells were harvested from mice within 2 months of a single immunization or 3 to 4 months of a booster immunization. Spleen cells from mice immunized with complete Freund's adjuvant alone were used as controls. Spleens were removed, minced with scissors on a sterile glass slide, passed ten times through an 18-gauge needle, filtered through Nytex (Telko, Elmsford, N.Y.), and collected by centrifugation. Erythrocytes were lysed in Tris-buffered ammonium chloride at 37°C for 5 min. Spleen cells were $>95\%$ viable by trypan blue exclusion. For proliferation assays, 10^6 spleen cells per well were added to 96-well tissue culture plates (Costar, Cambridge, Mass.) containing serial dilutions of heat-inactivated (80°C for 1 h), gradient-purified viral top component as the antigen. After 48 h, 0.4 μCi of [^3H]thymidine (NEN Dupont, Wilmington, Del.) per well was added. Samples were collected 16 to 20 h later onto glass filter strips by using a multichannel cell harvester (PHD Harvester; Cambridge Technologies, Cambridge, Mass.) and counted. While data from a single concentration of top component are presented (see Fig. 3), similar data were obtained over a range of viral antigen concentrations. Cells for adoptive transfer were collected by centrifugation, resuspended at 2×10^6 cells per ml (unless otherwise noted) in RPMI 1640 medium, and injected into neonatal mice (50 μ l i.p.) with a 30-gauge needle and a glass syringe. Spleen cells harvested from mice immunized with reovirus did not contain live virus (data not shown).

MAbs and immune cell depletions. The anti-T3D-T3C9 $\sigma 1$ MAb G5 (3) and anti-T1L $\sigma 1$ MAb 5C6 (40a) were prepared as described previously (40). Cell lines producing the rat immunoglobulin G2b (IgG2b) anti-CD4 MAb GK1.5 (ATCC TIB 207 [6]), the rat IgG2a anti-CD8 MAb 53-6.72 (ATCC TIB 105 [18]), and the rat IgG2b anti-B/T cell MAb 14.8

(ATCC TIB 164 [17]) were obtained from the American Type Culture Collection (Rockville, Md.). MAbs were produced as described previously (40), except that protein G 4 Fast Flow (Pharmacia Fine Chemicals, Piscataway, N.J.) replaced protein A-Sepharose for rat MAbs. Purified MAbs were dialyzed against phosphate-buffered saline (PBS), concentrated to 1 mg/ml (PM30 filter; Amicon WR Grace, Danvers, Mass.), sterilized by using a 0.22- μm -pore-size Millex-GV filter (Millipore, Bedford, Mass.), and frozen at -70°C . Antibody concentrations were determined assuming 1.4 U of optical density at 280 nm = 1.0 mg/ml. MAbs were adoptively transferred in 100- μ l volumes i.p. with a 30-gauge needle and a tuberculin syringe.

For in vivo depletion of T-cell subsets, 25 μ g of antilymphocyte MAbs per mouse was injected i.p. on each of the first 4 days of life and again on day 8 of life. The protocol for MAb treatment is modified from that of Goronzy et al. (13) and was chosen when preliminary experiments indicated that treating neonatal mice multiple times over the first few days of life was important for maximizing the effect of anti-CD4 MAb on the humoral response to T3D and T3C9 (data not shown).

ELISA. Samples of blood were collected for assay of antireovirus antibody as 1 drop in 1 ml of gel saline (40). To correct for variation in volume collected, samples were diluted in an enzyme-linked immunosorbent assay (ELISA) plate and the optical density at 570 nm was determined as an index of hemoglobin concentration. Sample volumes were adjusted to normalize the hemoglobin concentration. Antireovirus antibody concentration was determined by ELISA (40). Briefly, plates were coated with purified virus (1 μ g per well) in 0.1 M Na_2CO_3 (pH 9.6) (coating buffer) and then blocked with 3% (wt/vol) bovine serum albumin (Sigma) in coating buffer. Serum samples were serially diluted in duplicate on virus-coated plates, and antireovirus antibody was detected with either peroxidase-conjugated goat anti-mouse IgG (heavy- and light-chain specific; Cappel Organon Teknika, Malvern, Pa.), or protein A-peroxidase (Zymed, San Francisco, Calif.) (1:200 to 1:400), using 2,2'-azino-bis(3-ethylbenz-thiazoline sulfonic acid) (ABTS) as the chromogen. Optical density at 570 nm was determined in an MR700 ELISA reader (Dynatech Laboratories, Inc., Chantilly, Va.).

RESULTS

Protection against reovirus by immune cells. Mice were given reovirus-immune or control cells on the day of birth (T3C9) or day 1 of life (T3D, T1L). Twenty-four hours later, mice were inoculated with T3D either via FP or i.c., T1L i.c., or T3C9 p.o. (Fig. 1A to D). Doses of virus were chosen to ensure $>90\%$ mortality in the absence of experimental treatment for T3D and T3C9 (39, 40) and to generate hydrocephalus in greater than 70% of surviving mice (T1L). Immune, but not control, cells protected mice from lethal infection with T3D via FP (Fig. 1A). Protection against T3D via FP was essentially complete with transfer of 1×10^7 immune cells, was partial with 5×10^6 cells, and was not observed with 1×10^6 cells (data not shown). Cells were protective when administered before viral infection, partially protective when administered on the day of viral challenge, and not protective if administered 1 to 2 days after infection (Fig. 2). Immune cells also protected neonatal mice against lethal challenge with T3C9 p.o. (Fig. 1B). Immune cells did not protect against T3D when administered i.c. (Fig. 1C) but did protect mice against T1L-induced hydrocephalus after

FIG. 1. Mice received 10^7 immune or control cells on the first day of life (A, C, and D) or the day of birth (B). CFAI, complete Freund's adjuvant immune (control) cells; T3DI, T3D immune cells; T1LI, T1L immune cells; T3C9I, T3C9 immune cells. (A) Protection against FP inoculation of T3D (4×10^6 to 8×10^6 PFU per mouse). (B) Protection against p.o. inoculation with T3C9 (1.5×10^7 PFU per mouse). (C) Lack of protection against i.c. inoculation of T3D (1×10^3 PFU per mouse). (D) Protection against hydrocephalus after i.c. inoculation of T1L (5×10^6 PFU per mouse).

i.c. challenge (Fig. 1D). Protection was seen against T1L-induced hydrocephalus but not T3D-induced meningoencephalitis despite the fact that 5,000-fold more T1L was administered than T3D.

Lack of serotype specificity for protection. We tested the

FIG. 2. Effect of time of immune cell transfer on their capacity to protect neonatal mice against lethal FP inoculation with T3D (4×10^6 to 8×10^6 PFU per mouse). All mice received virus on day 2 of life. Timing of cell transfer was varied in relation to the day of virus challenge (day 0). The number of mice in each group is indicated above each bar.

capacity of immune cells to protect across serotypes. T1L-immune spleen cells protected against T3D (Fig. 1A), and T3D-immune cells protected against T1L-induced hydrocephalus (Fig. 1D). Both T1L- and T3D-immune spleen cells proliferated in response to either purified T1L or T3D top component (virions lacking double-stranded RNA) (Fig. 3). Thus, cell-mediated protection occurred across serotypic boundaries, and reovirus-immune T cells recognized non-type-specific determinants on virions (21).

Effect of immune cells on viral titer at primary and secondary sites of infection. We assessed the mechanism of immune cell-mediated protection by measuring viral titer at primary and secondary sites of infection early (day 3) and late (day 9) after FP challenge with T3D. After FP inoculation, T3D spreads from muscle to CNS via the sciatic nerve (38). Mice were given antibody or immune cells 1 day before T3D challenge (Fig. 4 and 5A). By day 3, $\sigma 1$ MAb G5 inhibited neural spread of T3D from muscle to spinal cord (Fig. 4) (39). In contrast, immune cells did not alter either viral titer in muscle or spread to CNS on day 3. Thus, $\sigma 1$ MAb G5 acted more rapidly than immune cells to alter this step in reovirus pathogenesis. By day 9, immune cells completely eliminated virus from skeletal muscle and produced a striking decrease in the amount of T3D in the CNS (Fig. 5A). $\sigma 1$ MAb G5 also

FIG. 3. Proliferation of T1L and T3D immune cells in response to reovirus antigen. Immune cells were challenged with 2.5 μ g of top component (empty viral capsids) per ml from either T3D or T1L in order to assess serotype-specific proliferation. Results are shown as mean counts per minute $\pm$ standard deviation derived from four wells per condition.

decreased T3D titer in skeletal muscle on day 9 but was less effective than immune cells.

Similar results were found when immune cells and σ 1 MAb G5 were compared at a late time point after T3C9 p.o. inoculation (Fig. 5B). Immune cells were more effective than σ 1 MAb G5 at decreasing viral titer in intestine. σ 1 MAb G5 had minimal effects on viral titer in intestine even late in infection (Fig. 5B) (39), whereas immune cells reduced viral titer more than 1,000-fold. Immune cells also decreased viral titer in secondarily infected organs such as the heart. Immune cells were less effective than σ 1 MAb G5 in reducing titer of virus in the brain.

Cell types involved in protection. To determine the pheno-

FIG. 4. Effect of cells (10^7 cells per mouse) or σ 1 MAbs (100 μ g per mouse) on viral titer in muscle and spinal cord early after FP inoculation with T3D (7.5×10^6 PFU per mouse). Animals received cells or σ 1 MAb 24 h before viral inoculation. Tissues were harvested from six to eight mice per group 3 days after viral inoculation and assayed for virus. 5C6 is a control σ 1 MAb which binds T1L but not T3D. G5 is a T3-specific σ 1 MAb. CFAI, complete Freund's adjuvant immune (control) cells; T3DI, T3D immune cells. Values shown are virus titer (mean log₁₀ PFU per milliliter) $\pm$ standard error of the mean.

FIG. 5. Effect of cells or σ 1 MAb G5 on viral titer in primarily or secondarily infected tissues late after infection. Mice were given 10^7 immune or control cells or 100 μ g of σ 1 MAb G5 and then inoculated with T3D in the FP (3×10^7 PFU per mouse) (A) or with T3C9 p.o. (1×10^7 PFU per mouse) (B). Either 10 (A) or 9 to 10 (B) days after virus inoculation, tissues (5 to 10 mice per group) were harvested and assayed for virus. Values shown are virus titer (mean log₁₀ PFU per milliliter) $\pm$ standard error of the mean. Abbreviations used are defined in the legend to Fig. 1 and 4. ISC, inferior spinal cord.

type of protective cells, we used anti-T cell MAbs in vivo to deplete specific subpopulations of transferred cells (13). Anti-CD4 abrogated protection by transferred immune cells, while anti-CD8 had a less dramatic but reproducible inhibitory effect (Fig. 6A). MAb 14.8, specific for an antigen on B-cell precursors and some peripheral T cells (17), was used as a control and had no effect on immune cell-mediated protection. This experiment showed that both CD4 and CD8 T cells are involved in immune cell-mediated protection against T3D.

We next assessed whether blockade of immune cell-mediated protection by anti-T cell MAbs was due to inhibition of humoral immune responses to reovirus. Neonatal mice treated with PBS after receiving immune cells made a significant antireovirus antibody response (Fig. 6B). Mice surviving treatment with either anti-B/T MAb 14.8 or anti-CD8 made antireovirus antibody, but the amount of antibody was decreased compared with that made by PBS-injected control mice. Inhibition of cell-mediated protection by anti-CD8 (Fig. 6A) is not explained by reduced antibody responses since mice treated with anti-B/T MAb 14.8 had almost identical antibody levels and were fully protected. As

FIG. 6. Effect of in vivo depletion of T-cell subsets on the capacity of immune cells (10^7 cells per mouse) to protect against lethal FP challenge with T3D (2×10^7 PFU per mouse) (A) and on the antibody response of neonatal mice receiving transferred cells (B). Mice were treated with PBS or anti-T cell MAB (25 µg per mouse) on each of the first 4 days of life and again on day 8. Antireovirus antibody levels were determined by ELISA on the serum of mice surviving 21 days after cell transfer in each treatment group. Protection experiments were done with 10 to 20 mice in two separate experiments per experimental condition. Antibody levels were measured on 8 to 15 mice per group except for the anti-CD4 group, from which there was only one survivor. OD₅₇₀, optical density at 570 nm.

expected, treatment with anti-CD4 inhibited antireovirus antibody responses (13; data not shown). Only a single mouse survived treatment with anti-CD4, and its antibody response was markedly lower than that seen in any other animal. Blockade of immune cell-mediated protection by anti-CD4 may be due to inhibition of humoral immune responses to the virus.

Effect of antilymphocyte MABs in the absence of adoptively transferred immune cells. The above experiment showed that CD4 and CD8 T cells are involved in protection of neonates by adoptively transferred adult immune cells. This does not show that these cells are part of the normal neonatal response to reovirus. We assessed this by studying the effects of anti-CD4 and anti-CD8 on the course of reovirus infection in neonatal mice. Treatment of mice with anti-CD4 or anti-CD8 did not increase the lethality of T3D, T3C9, or T1L (data not shown). However, treatment of CAF₁ mice with anti-T cell MABs resulted in a profound alteration in T3C9-induced clinical disease. CAF₁ mice treated with anti-CD4 plus anti-CD8 developed severe diarrhea and oily-appearing fur 9 to 21 days after T3C9 p.o. challenge. Livers of T3C9-infected mice surviving treatment with anti-CD4 plus

FIG. 7. Effect of in vivo treatment with anti-T cell MABs on viral titer in various organs after p.o. inoculation of T3C9 (5×10^4 PFU per mouse). Mice were treated with PBS or 25 µg of anti-T cell MAB (or control B/T MAB) per mouse on each of the first 4 days of life and again on day 8. Nine days after viral inoculation (day 11 of life), survivors (8 to 10 mice per group) were sacrificed and tissues were harvested and assayed for virus. Before tissue harvest, there were one to two deaths in all but the MAB 14.8-treated group. Values shown are virus titers (mean log₁₀ PFU per milliliter) $\pm$ standard error of the mean.

anti-CD8 showed bile duct proliferation, inflammation at the borders of hepatic lobules, areas of hepatocellular necrosis adjacent to regions of bile duct proliferation and inflammation, and lack of centrilobular necrosis or inflammation (data not shown). None of these changes were noted in mice surviving T3C9 infection in the absence of treatment with anti-T cell MABs. Less extensive increases in biliary pathology were found after treatment with either anti-CD4 or anti-CD8 MAB alone.

Since treatment with anti-T cell MABs altered both the clinical disease and pathologic features of oral infection with T3C9, we examined the effect of anti-T cell MABs at earlier time points. NIH Swiss mice were used for this experiment because of the difficulty in obtaining sufficient numbers of synchronized births from our CAF₁ breeding colony. Treatment of NIH Swiss mice with either anti-CD4 or anti-CD8 alone led to the oily hair and diarrhea phenotype of T3C9 disease, and treatment with anti-CD4 decreased the antireovirus antibody response (data not shown). A smaller reduction in antireovirus antibody titer was seen in mice treated with either anti-CD8 or control MAB. MAB treatment had minimal effects on viral titer in intestine and almost no effect on viral titer in brain or blood (Fig. 7; data not shown). However, viral titer in both liver and heart was increased 10- to 100-fold by anti-CD8 and anti-CD8 plus anti-CD4. Both anti-CD4 and anti-CD8 alone increased hepatic pathology, with the combination of anti-CD4 plus anti-CD8 causing the most dramatic increases in pathology (data not shown).

DISCUSSION

In this study, we used infections with reoviruses T3D, T3C9, and T1L as models for analyzing cellular and humoral immunity. These viruses utilize different pathogenetic strategies to cause disease despite being very closely related antigenically. Our primary findings were that (i) both CD4 and CD8 T cells play a role in reovirus resistance, (ii)

protective immunity to reovirus is not serotype specific, (iii) antibody and immune cells have different actions at primary and secondary sites of infection, (iv) adoptively transferred immune cells can act in the intestine and CNS to combat reovirus infection, and (v) immune cells differed in the effectiveness with which they controlled infection by viruses using different pathogenetic strategies.

Serotype specificity. Reovirus induces both serotype-specific and nonspecific humoral and cellular immune responses (7, 8, 21). Serotype-specific humoral and cellular immunity is directed primarily against the $\sigma 1$ protein (7, 8, 43, 45), which differs more between prototype strains than any other protein (10). Both antibody and cells can recognize non-serotype-specific determinants (Fig. 3) (21, 40). Passively transferred immune cells protected mice against reovirus in a non-serotype-specific manner (Fig. 1). Polyclonal antibody-mediated protection is also not serotype specific (5, 40). The presence of serotype-specific humoral (11, 43, 45, 46) and cell-mediated (7, 8, 31) immune responses to reovirus has been repeatedly emphasized. The physiologic importance of serotype specificity is unclear since both cells and antibodies recognizing non-serotype-specific determinants are protective *in vivo*.

CD4 and CD8 cells in reovirus resistance. Two lines of evidence showed that both CD4 and CD8 T cells are involved in reovirus resistance. First, treatment with anti-CD4 abrogated, and treatment with anti-CD8 inhibited, immune cell-mediated protection against T3D (Fig. 6). Second, treatment with anti-CD4 and/or anti-CD8 altered T3C9-induced disease in neonatal mice (Fig. 7). In the latter system, anti-CD8 had the most impressive effects on viral titer in different organs (Fig. 7), while anti-CD4 was most effective at blocking adoptive transfer of protection (Fig. 6). A role for both CD4 and CD8 T cells in control of viral infection has also been reported with herpes simplex virus (14), influenza virus (20, 22, 23), and Sendai virus (15). Control of other viral infections is thought to depend primarily on a particular T-cell subset (1, 24, 35).

CD4 T cells may have contributed to protection in these experiments via provision of help to B cells. However, T-cell help for B cells does not explain all the observed actions of T cells. Antireovirus antibody titers were identical in mice receiving anti-B/T MAb 14.8 and anti-CD8, yet only anti-CD8 treatment inhibited adoptive transfer of protection (Fig. 6). Antireovirus antibody levels were also identical in neonatal mice treated with anti-B/T MAb and anti-CD8 in the absence of cell transfer (data not shown). Anti-CD8, but not control anti-B/T MAb, altered T3C9 disease expression and significantly increased viral titer in these mice (Fig. 7). Last, immune cells and antibody acted by different mechanisms at defined stages in pathogenesis (see below).

In many systems, immune responses (especially CD8 T cells) have been thought to contribute to virus-induced pathology. An oily hair syndrome hypothesized to be due to immune-mediated hepatobiliary pathology has been described in reovirus-infected mice (33, 34). This syndrome is clinically similar to the syndrome generated by treating T3C9-infected mice with anti-CD8 and anti-CD4. Our data suggest that, rather than enhancing pathology, the immune response prevents T3C9 from causing injury. This finding is consistent with previous studies (12) and other experiments reported here which have consistently shown only protective effects of the cellular immune response during reovirus infection.

Differences between the effects of cellular and humoral immunity. We found a number of differences between the

actions of immune cells and those of $\sigma 1$ MAb G5. Immune cells were effective in preventing hydrocephalus after i.c. inoculation with T1L but not in protecting against meningoencephalitis after inoculation with 1/5,000 as much T3D (Fig. 1). In contrast, $\sigma 1$ MABs prevent both T3D-induced meningoencephalitis (39, 40) and T1L-induced hydrocephalus (38a). Immune cells and $\sigma 1$ MAb G5 also differed in their actions in different organs and at different pathogenetic stages of infection. $\sigma 1$ MAb G5, but not immune cells, inhibited neural spread to the CNS (Fig. 4). Later in infection, immune cells completely eliminated detectable virus from muscle after T3D inoculation (Fig. 5A) and dramatically reduced the amount of virus present in the spinal cord and brain. Immune cells also reduced viral titer in intestine 1,000- to 10,000-fold later (9 to 10 days) after p.o. inoculation of T3C9 (Fig. 5B). In both pathogenetic models (T3C9 p.o. and T3D FP), immune cells were more effective than anti- $\sigma 1$ MAb G5 in clearing virus from primary sites of infection late in infection. Organ-specific differences in the capacity of T cells to clear viral infection from the CNS compared with other organs have been described for herpes simplex virus (25) and lymphocytic choriomeningitis virus (2, 27) infection.

The role of cells in controlling primary intestinal infection is worthy of special note. We confirmed the fact that systemic IgG does not alter intestinal reovirus infection with a panel of newly isolated MABs to outer capsid proteins (38a, 39). This is reminiscent of the inefficiency of systemic IgG responses generated by killed poliovirus vaccines in controlling enteric poliovirus infection (4, 9, 26). In contrast, adoptively transferred immune spleen cells generated by i.p. immunization reduced reovirus titers in the intestine after oral inoculation (Fig. 5B). Thus, systemic immunization can provide protection of mucosal surfaces. This system provides a model for analysis of how systemic immunization contributes to mucosal immunity.

Immunity and virulence of viruses. Two findings point to important relationships between viral virulence and the immune system. First, in contrast to immune cells (Fig. 1), anti- $\sigma 1$ MABs protect against both T1L- and T3D-induced CNS disease after i.c. inoculation (38a, 39, 40). Following i.c. inoculation, T1L predominantly infects ependymal cells lining the cerebral ventricles, while T3D infects neurons (42, 44). One explanation for the failure of cells to protect against T3D i.c. would be that immune cells can clear infection from certain cells in the CNS better than others. This could reflect differences in the nature or accessibility of the cell populations involved (neurons versus ependymal cells). An alternate explanation would be that T3D is more virulent than T1L in the face of active immunity, by virtue of currently undefined genetic factors.

A second observation underlining the importance of host immunity in determining virulence is the fact that treatment of neonatal mice with anti-T cell MABs resulted in the expression of a previously unrecognized pathogenetic capacity of T3C9. Liver pathology in mice recovered from T3C9 infection is minimal under normal circumstances. After treatment with anti-T cell MABs, new patterns of hepatobiliary pathology were revealed. Thus, a pathogenetic capacity of T3C9 was unmasked by inhibiting a specific component of the immune system. This suggests that mapping of virulence factors by genetic techniques will yield different results depending on the immunocompetence of the host.

We showed that non-serotype-specific cell-mediated immunity can play a critical role in controlling reovirus infection in neonatal mice. Cells and antibody have different actions at defined stages in pathogenesis. Defining the mech-

anisms underlying these differences may lead to improved strategies for both the prevention and treatment of viral diseases. The increasingly well understood pathogenesis of reovirus infection, combined with the fact that reoviruses with different pathogenetic properties are closely related antigenically, makes this a useful model for studying these issues.

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