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Evidence for CD8$^+$ T-Cell Immunity to Murine Rotavirus in the Absence of Perforin, Fas, and Gamma Interferon

MANUEL A. FRANCO,[1,2]* CRAIG TIN,[1,2] LUSIJAH S. ROTT,[1,3] JOHN L. VANCOTT,[4] JERRY R. MCGHEE,[4] AND HARRY B. GREENBERG[1,2]

Departments of Medicine, Microbiology, and Immunology,[1] and Pathology,[3] Stanford University School of Medicine, Stanford, California 94305; Palo Alto Veterans Affairs Medical Center, Palo Alto, California 94304[2]; and Department of Microbiology, Immunobiology Vaccine Center, University of Alabama at Birmingham, Birmingham Alabama 35294[4]

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We recently showed that class I-restricted CD8$^+$ T cells mediate clearance of primary rotavirus infection in mice: J_HD knockout (J_HD −/−) (B-cell-deficient) mice depleted of CD8$^+$ T cells become chronically infected with murine rotavirus, and β_2 microglobulin knockout (β_2 m −/−) mice have delayed but complete clearance of primary rotavirus infection. In the present work we have analyzed the mechanism used by CD8$^+$ T cells to clear rotavirus infection. We first determined that perforin knockout (perforin −/−) mice and *lpr* (fas-deficient) mice clear rotavirus infection with the same kinetics as control mice. When perforin −/− or perforin +/+ mice were depleted of CD8$^+$ T cells by administration of an anti-CD8 monoclonal antibody, they showed a delay of 1 to 2 days in the clearance of rotavirus infection compared to the clearance time for untreated control mice, indicating that CD8$^+$ T cells in both groups of mice participate in the resolution of primary rotavirus infection. In addition, passively transferred CD8$^+$ T cells from rotavirus-infected perforin +/+ and perforin −/− mice were able to mediate viral clearance in Rag 2 knockout (Rag 2 −/−) mice chronically infected with rotavirus with similar kinetics, suggesting that CD8$^+$ T cells from perforin −/− mice are as efficient as CD8$^+$ T cells from perforin +/+ mice in clearing a rotavirus infection. Gamma interferon (IFN-γ) was also shown to be unnecessary for the antirotavirus effect of CD8$^+$ T cells: IFN-γ knockout (IFN-γ −/−) mice and J_HD −/−, perforin −/−, and perforin +/+ mice depleted of IFN-γ by administration of an anti-IFN-γ monoclonal antibody cleared rotavirus infection with the same kinetics as those for control mice. Hence, CD8$^+$ T cells have an antirotaviral effect that is not mediated by perforin and appears to be independent of fas and the release of IFN-γ.

Rotaviruses are the main cause of severe diarrhea in children (12). A rotavirus vaccine of considerable efficacy has been recently developed, but its utility in less-developed countries and in prevention of mild to moderate disease has not been demonstrated (2). With the aim of developing more broadly active and efficient strategies for prevention and treatment of rotaviral disease our laboratory has investigated a mouse model of rotavirus infection (4, 9). Rotaviruses infect the enterocytes of both adult and suckling mice but only induce diarrhea in mouse pups (4). Immune responses and viral shedding following primary rotavirus infection are comparable in suckling and adult mice (4). In order to study factors that regulate protection from viral reinfection, many of our studies have been done with adult mice (4).

The use of mutant strains of mice has already been shown to be a useful strategy to study the roles of the different arms of the immune system in clearing primary rotavirus infection and protecting from reinfection (7, 10, 26). T and/or B cells are necessary for clearing primary rotavirus infection in mice since SCID and Rag 2 knockout (Rag 2 −/−) mice become chronically infected with murine rotavirus (10, 31). Passive transfer experiments have shown that both rotavirus-specific CD8$^+$ T cells and antibody can clear the chronic rotavirus infection of SCID mice (5, 7). The use of mice deficient in B cells or in class I-restricted CD8$^+$ T cells has clarified the role of B cells and CD8$^+$ T cells in clearing primary rotavirus infection. Most B-cell-deficient J_HD knockout (J_HD −/−) mice clear primary rotavirus infection with the same kinetics as those for immunocompetent mice (10, 26). The μMT knockout mouse strain (another B-cell-deficient strain of mice) chronically shed low levels of rotavirus for up to 93 days following primary infection (26). In contrast, β_2 microglobulin knockout (β_2m −/−) mice (deficient in class I-restricted CD8$^+$ T cells) clear primary rotavirus infection with a delay of 1 to 3 days compared to immunocompetent mice (10). J_HD −/− mice depleted of CD8$^+$ T cells by administration of an anti-CD8 monoclonal antibody become chronically infected with murine rotavirus (10, 26). Thus, in the presence of antirotavirus antibody, CD8$^+$ T cells are necessary for timely but not for complete clearance of primary rotavirus infection. In the B-cell-deficient mice, they are necessary for the resolution of primary infection. Although passively transferred CD8$^+$ T cells have been shown to protect against rotavirus-induced diarrhea (28), B cells seem to be the primary effector cells in prevention of rotavirus reinfection: β_2m −/− mice are completely resistant to reinfection while J_HD −/− mice are susceptible to reinfection, although to a lesser degree than naive mice (10, 26).

CD8$^+$ T cells have been postulated to mediate an in vivo antiviral effect either by direct lysis of the virus-infected host cell or by the release of cytokines that induce an antiviral effect (21, 30). At least two mechanisms (mediated by perforin or fas) have been described by which CD8$^+$ T cells kill virus-infected host cells (1a). Fas-deficient *lpr* mice efficiently clear infection with lymphocytic choriomeningitis virus (LCMV) (18). In contrast, perforin knockout (perforin −/−) mice are unable to clear clear LCMV (17, 38) and adenovirus infections (39). Since clearance of these viral infections is dependent on CD8$^+$

* Corresponding author. Mailing address: Stanford University, School of Medicine, Lab Surge, P304, Stanford, CA 94305. Phone: (415) 493-5000, ext. 63124. Fax: (415) 852-3259. E-mail: francoma@leland.stanford.edu.

T cells, these results imply that perforin-mediated killing of the infected target cell by the $CD8^+$ T cell is crucial and that the cytotoxic effect mediated by fas or cytokines is not significant as an antiviral mechanism in vivo against these viruses. On the other hand, perforin −/− mice and *lpr* mice have been reported to develop efficient immunity to vaccinia virus (VV) (18). Since $CD8^+$ T cells mediate immunity to VV, $CD8^+$ T cells can also have an antiviral effect independent of perforin or fas (18).

Gamma interferon (IFN-γ) is a cytokine produced by T and NK cells (41). IFN-γ was discovered because of its antiviral effect and has been shown to have a role in immunoregulation (41). IFN-γ has been implicated as a cytokine that mediates a $CD8^+$ T-cell-dependent antiviral effect in vivo against VV and herpes simplex virus (20, 32, 36). IFN-γ and tumor necrosis factor (TNF) were proposed as mediators released by $CD8^+$ T cells that inhibit hepatitis B virus gene expression in hepatitis B virus transgenic mice (13). Recently this group showed that a hepatitis B-specific $CD8^+$ T-cell clone derived from a perforin −/− mouse can abolish hepatitis B virus gene expression in transgenic mice through an IFN-γ- and TNF-dependent pathway (14).

In the present work we studied rotavirus infection in perforin −/−, IFN-γ knockout (IFN-γ −/−), and *lpr* (fas-deficient) mice and in J_HD −/−, perforin −/−, and perforin +/+ mice depleted of IFN-γ by administration of an anti-IFN-γ monoclonal antibody (MAb) to better understand the mechanism used by $CD8^+$ T cells in clearing primary rotavirus infection in mice.

MATERIALS AND METHODS

Cells. MA104 cells were grown as previously described (15). Plastic adherent MC57 cells (*H-2*^{b}) were grown in Dulbecco's modified Eagle's medium (Bio Whittaker, Walkersville, Md.) containing 10% heat-inactivated fetal bovine serum (FBS), 100 μg of streptomycin sulfate per ml and 100 U of penicillin per ml.

Mice. Heterozygote perforin knockout (perforin +/−) mice (derived from [129 × B6]F_2 founders [*H-2*^{b}], a gift from W. Clark, UCLA) were bred in our facility (38). From these mice, perforin +/+ and perforin −/− mice were selected by using Northern blotting that employed the strategy and probe (also a gift from W. Clark) described previously (38). In addition, breeding couples of both perforin −/− and perforin +/+ mice and mice used as donors for the passive transfer experiments (see below) were confirmed as either having or not having the perforin gene with a PCR assay developed by H. Aguila, Stanford University, Stanford, Calif. This PCR assay uses the following primers: 5′-CAC TCGGTCAGAATGCAAGC-3′ and 5′-CTCTTACGTGTACATGCGACA-3′. DNA from perforin −/− and perforin +/+ mice amplified with these primers gives bands of approximately 500 and 1,400 bp, respectively. Adult 6- to 8-week-old B6.MRL-*Fas*^{lpr} ([*H-2*^{b}] *lpr* mice), C57BL/6J (*H-2*^{b}), and 129/SvJ (*H-2*^{b}) mice were obtained from the Jackson Laboratory, Bar Harbor, Maine. J_HD mice derived from (129 × B6)F_2 founder (*H-2*^{b}) mice were obtained from D. Huszar (Genpharm International, Mountain View, Calif.). Rag 2 −/− (derived from [129 × B6]F2 founders [*H-2*^{b}]) mice were obtained from Genpharm International. BALB/c (*H-2*^{b}) mice were obtained from Simonsen Inc. (Gilroy, Calif.) and bred in our facility. IFN-γ −/− (*H-2*^{b}) mice originally described by Dalton et al. (6a) were bred in the University of Alabama at Birmingham and shipped to our facility in California where the experiments described were performed. Mice bred in both facilities were weaned at 4 weeks of age and segregated according to sex into separate microisolator cages (five or less mice in each cage). Mice used for the experiments were between 6 and 8 weeks of age. All naive mice studied were negative for rotavirus-specific immunoglobulin A (IgA) in their stools before use. Periodic health screens were performed on sentinel mice to ensure that all mice were free of rotavirus and other mouse pathogens.

Viruses and virus inoculation. The tissue culture-adapted RRV strain of simian rotavirus (G3, P3) was grown and the titers of the virus in MA104 cells were determined in the presence of trypsin as previously described (15). The titer of the stock used was 2.2×10^8 PFU/ml. The recombinant VV expressing RF bovine rotavirus strain VP7 (rVV7) and the nonrecombinant VV strain VVD4 were grown and the titers of the viruses were determined as previously described (11). Stocks of wild-type murine rotavirus strains EWw (G3, P16) and ECw (G3, P16) were prepared as intestinal homogenates, and the titers of the viruses in mice were determined as previously described with slight modifications (4). Pups were inoculated with virus orally at 4 days of age. Adult mice were orally gavaged with virus following oral administration of 100 μl of 1.33% sodium bicarbonate to neutralize stomach acid. For virus titration, groups of four to six mice were given serial 10-fold dilutions of intestinal homogenates and monitored daily; the pups were monitored for diarrhea and the adult mice were monitored for viral antigen in stool samples. To determine the virus titer, mice were scored as infected by the virus administered (not by virus shed by mice in the same cage) if they developed diarrhea or shed viral antigen up to 3 days after primary inoculation. C57BL/6 adult mice administered low doses of ECw virus started shedding viral antigen 4 days after inoculation without other mice in the cage being infected. These mice were also considered to be infected by the initial virus administered for purposes of calculating shedding dose 50 (SD_{50}). Virus diarrhea dose 50 and SD_{50} were calculated using the Reed and Muench method (8).

Detection of viral antigen and virus-specific IgA by ELISA. Viral antigen detection was performed by using the previously described enzyme-linked immunosorbent assay (ELISA) (4). Microtiter plates (Dynatech, McLean, Va.) were coated with diluted hyperimmune rabbit anti-rhesus rotavirus serum and blocked, and then the 5% stool sample suspensions were added to individual wells and incubated overnight at 4°C. Plates were washed, and diluted guinea pig anti-rhesus rotavirus hyperimmune serum was added to the plates for 1 h at 37°C. After two washes, diluted horseradish peroxidase-conjugated goat anti-guinea pig IgG serum (Kirkegaard and Perry Labs., Gaithersburg, Md.) was added to the plates and the plates were incubated for 1 h at 37°C. After two washes ABTS (2,2′-azino-di-[3-ethylbenzthiazoline sulfonate (6)] substrate (Kirkegaard and Perry Labs.), was added, the plates were developed for 10 min at room temperature, and the reaction was stopped by the addition of 10% sodium dodecyl sulfate. The absorbance at 405 nm was read with a plate reader (BIO-TEK Instruments, Burlington, Vt.). A sample was considered positive for viral antigen if its optical density (OD) was at least 0.1 greater than the ODs of wells from naive mice on the day of infection. The mean OD of naive mice on the day of infection for the experiments shown ($n = 30$) was 0.019 ± 0.005 (standard error of the mean [SEM]).

For the detection of virus-specific intestinal and serum antibodies, plates were coated and blocked as for the antigen detection ELISA and then incubated with a 1:8 dilution of RRV stock virus overnight at 4°C. After two washes, 5% stool samples or dilutions of serum were added to the plates. After 2 h of incubation at 37°C, the plates were washed three times and either diluted peroxidase-conjugated anti-mouse IgA (0.25 μg/ml), IgG (0.25 μg/ml), or IgM (0.25 μg/ml) antiserum (Kirkegaard and Perry Labs.) or diluted anti-IgG1 (1 μg/ml), -IgG2a (0.5 μg/ml), -IgG2b (0.125 μg/ml) or -IgG3 (0.5 μg/ml) biotin-labeled MAbs (all MAbs were obtained from Pharmingen, San Diego, Calif.) was added to the plates. After 1 h of incubation at 37°C the plates were washed and developed as described above for the antigen ELISA. The plates with biotin-labeled MAbs were further incubated with streptavidin peroxidase (1:1,000 dilution; Kirkegaard and Perry Labs.) for 1 h at 37°C and then washed and developed as for the antigen ELISA.

The results from the rotavirus-specific stool IgA determination were expressed as net OD. The net OD was calculated by subtracting the OD obtained with a stool sample in the assay described above from the OD obtained by the same stool sample in a well on the same plate in which RRV antigen had been omitted. To obtain antirotavirus serum titers, serial threefold dilutions of serum were performed (from 1:50 to 1:109,350). The titer for the serum for each antibody isotype or subclass was defined as the highest dilution of serum that had a net OD reading of greater than 0.1. The net OD reading was calculated as the OD of a sample minus the OD reading of a normal control serum at the same dilution. The serum titers for individual mice were $\log_3$ transformed for geometric mean titer calculation and statistical analysis. Negative samples were arbitrarily assigned a titer of 1:16.7 (threefold below 1:50) for statistical calculations.

In vivo depletion of $CD8^+$ T cells and IFN-γ. Mice were depleted of $CD8^+$ T cells by administration of ascites fluid containing the rat anti-mouse CD8 MAb 2.43 as previously described (10). Each mouse received 0.5 ml of ascites fluid 5, 4, and 3 days before rotavirus infection, on the day of rotavirus infection, and on days 3, 6, and 9 after infection. On the day of rotavirus infection, depleted and nondepleted control mice were killed to verify depletion of $CD8^+$ T cells in the spleen and among intraepithelial lymphocytes (IELs) by fluorescence-activated cell sorter analysis. Spleen cells and IELs were stained with fluorescein isothiocyanate-labeled anti-CD8 MAb 53 6.7 and either anti-γδ T-cell receptor (MAb GL3) or anti-αβ T-cell receptor (MAb H57-597) MAbs, both labeled with phycoerythrin (all labeled MAbs were obtained from Pharmingen). For depletion of IFN-γ, mice were administered 2 mg of anti-IFN-γ MAb (hybridoma clone XmG1.2, originally obtained from DNAX Corp. Palo Alto, Calif.) on the day before infection and on days 1, 4, and 7 after infection (33). Large quantities of anti-IFN-γ antibody were generated by growth in a cell bioreactor (Unisyn Technologies, San Diego, Calif.). Administration of the same batch of this antibody to BALB/c mice rendered them more susceptible to salmonella infection (37a).

Transfer of $CD8^+$ T cells to chronically infected Rag 2 −/−. Single cell suspensions of spleen cells were prepared from donor mice that had been inoculated intraperitoneally (IP) with 10^7 PFU of RRV 18 and 4 days before the transfer (7). To avoid cell aggregates, spleen cells were incubated in low-ionic-strength isosmotic medium (77 mosM Hanks buffered salt solution, 16.8 mM HEPES, 0.246 M sorbitol, 38.5 mM glucose) for 10 min at 0°C, then washed and resuspended in RPMI 1640 containing 10% heat inactivated FBS at 4×10^7 cells/ml. The $CD8^+$ T cells were then enriched by panning twice for 20 min at room temperature on 100-mm-diameter polystyrene plates coated overnight with

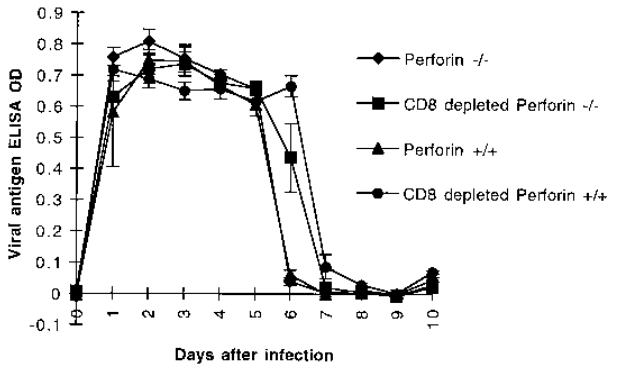

FIG. 1. Fecal viral-antigen-shedding curves for perforin +/+ and perforin −/− mice depleted of $CD8^+$ cells by administration of 2.43 anti-CD8 MAb or not depleted. Mice were challenged at 6 to 8 weeks of age with 10^5 SD_{50} of the ECw strain of murine rotavirus. Fecal rotavirus was measured by ELISA and results are expressed as OD readings. Each data point represents the mean OD for three mice ± the SEM. This experiment was repeated three times with the perforin −/− mice and twice with the perforin +/+ mice with similar results.

100 μg of rabbit anti-mouse IgG serum (Sigma Chemical Company, St. Louis, Mo.) per ml and ascites fluid (1/8 dilution) from the anti-CD4 MAb GK 1.5. The cells were stained with fluorescein isothiocyanate-labeled anti-CD8 MAb 53 6.7 and double sorted on a Facstar (Becton Dickinson, San Jose, Calif.). A total of 10^6 $CD8^+$ T cells in 0.5 ml of RPMI 1640 containing 10% heat inactivated FBS was transferred to chronically infected Rag 2 −/− mice by IP injection. The transferred $CD8^+$ T cells were at least 99.7% pure as assessed by fluorescence-activated cell sorter analysis. Stool samples from treated Rag 2 −/− mice were collected on the day of transfer and from day 7 to day 17 after transfer and were analyzed for rotavirus antigen and rotavirus-specific IgA by ELISA. Three to four weeks after transfer the Rag 2 −/− mice were bled and their serum was tested for rotavirus-specific antibody by ELISA.

Cytotoxicity assay. The assay has been previously described (10). For preparation of effector cells, mice were sacrificed 10 days after virus inoculation, and single-cell suspensions of spleen cells were prepared with RPMI 1640 containing 10% heat inactivated FBS, 100 μg of streptomycin sulfate per ml, 100 U of penicillin per ml, 2 mM L-glutamine, 2.5 mM sodium pyruvate, 20 mM HEPES, and 30 mM 2-mercaptoethanol. RRV (PFU/cell ratio of 0.3) was added to the cell suspension (5×10^6 cells/ml) which was then distributed to 24-well plates and cultured with 5% CO_2 at 37°C for 5 days. MC57 target cells were then infected with RRV or VVs, labeled with ^{51}Cr (Amersham, Arlington Heights, Ill.), and added to effector cells at the appropriate concentration to obtain the desired effector-to-target cell ratios in a total volume of 200 μl. After 5 h of incubation at 37°C, 50-μl samples were taken from each well and counted in a gamma counter. For calculating the spontaneous and maximum releases of ^{51}Cr, target cells were incubated with medium only and with 10% Triton X-100, respectively. Spontaneous release was generally below 20%. The percentage of specific ^{51}Cr release was calculated by the following formula: (experimental release − spontaneous release)/(maximum release − spontaneous release). The assay was done in duplicate wells. A response was considered positive if specific lysis was higher than 10% and at least twice the value of the negative control (targets treated with medium or infected with VVD4).

Statistical analysis. Statistical analysis was performed with Statview, a statistical package for Macintosh computers (Abacus Concepts, Inc., Berkeley, Calif.). Results are expressed as means ± the SEMs. Differences between means were compared by the Mann-Whitney U test.

RESULTS

Perforin −/− mice efficiently clear primary rotavirus infection, are immune to reinfection, and have delayed viral clearance when depleted of $CD8^+$ T cells. To determine if mice utilize a perforin-dependent mechanism to efficiently mediate clearance of primary rotavirus infection, we infected perforin −/− and control perforin +/+ adult mice with 10^5 SD_{50} of ECw murine rotavirus. After primary infection, perforin −/− and control perforin +/+ mice shed the same quantity of viral antigen and resolved primary rotavirus infection with the same kinetics (Fig. 1). Serial 10-fold dilution titration of ECw virus showed that perforin +/+ and perforin −/− mice were equally susceptible to infection with lower doses of virus: the ECw SD_{50} was similar in C57BL/6 and 129Sv mice (the parental mouse strains of the perforin −/− and +/+ mice), in perforin +/+ and perforin −/− mice, and in BALB/c mice (Table 1). Viral shedding curves were similar to those of Fig. 1 when perforin +/+ or perforin −/− mice were infected with lower viral doses (10^{-1} SD_{50}) (data not shown). In addition, perforin −/− mouse pups infected with 10^6 DD_{50} of EWw rotavirus developed diarrhea that was similar in intensity and duration to that developed by perforin +/+ pups (data not shown). We interpret these observations to indicate that perforin is not necessary for efficient and timely recovery from rotaviral disease or primary infection in suckling or adult mice.

TABLE 1. Perforin −/−, perforin +/+, and immunocompetent mice are equally susceptible to rotavirus infection[a]

Mouse strain	ECw SD_{50}[b]	
	Adult mice	Mouse pups
BALB/c	$10^{7.3}$	$10^{7.5}$
C57BL/6	$10^{6.9}$	ND
129/Sv	$10^{7.4}$	ND
Perforin +/+	10^7	ND
Perforin −/−	$10^{6.8}$	ND

[a] Groups of from four to six mice were infected with 10-fold dilutions of intestinal homogenate from ECw-infected infant pups.

[b] SD_{50} values were calculated by the method of Reed and Muench (26). Results are calculated from a pool of two different experiments except for the 129/Sv mice, for which only one experiment was performed. ND, not determined.

Previous studies demonstrated that infection of immunocompetent suckling mice with EWw virus fully protected them from subsequent challenge with ECw (4). Similarly, perforin −/− mice that had been infected with EWw as pups were completely resistant to reinfection with 10^5 SD_{50} of ECw (data not shown) indicating that perforin is also unnecessary for development of complete immunity to rotavirus reinfection.

The identical kinetics of rotavirus clearance for perforin −/− mice and control mice can be interpreted in two ways: either class I-restricted $CD8^+$ T cells in perforin −/− mice mediate viral clearance independently of perforin, or in these mice $CD8^+$ T cells do not participate in viral clearance. To investigate these two possibilities, we depleted perforin +/+ and perforin −/− mice of $CD8^+$ T cells by administration of an anti-CD8 MAb prior to infection. Both perforin −/− and perforin +/+ mice depleted of $CD8^+$ T cells and sacrificed on the day of viral infection had more than 90% depletion of $CD8^+$ T cells in the spleen and IELs (data not shown). In both groups of mice depletion of $CD8^+$ T cells resulted in resolution of rotavirus infection with a delay of 1 to 2 days compared to the resolution time for untreated control mice (Fig. 1). In our previous studies, β_2 m −/− mice (deficient in class I-restricted $CD8^+$ T cells) cleared primary rotavirus infection with a delay of 1 to 3 days compared to the clearance time for immunocompetent mice (10). Although the difference (1 to 2 days) in shedding between perforin −/− and perforin +/+ CD8-depleted and nondepleted mice was relatively small, the mean number of antigen-shedding days for CD8-depleted mice differed significantly from the mean number of antigen-shedding days for untreated mice ($P = 0.01$ for the difference between depleted and nondepleted perforin −/− mice; $P = 0.008$ for the difference between depleted and nondepleted perforin +/+ mice). Faster clearance in non-CD8-depleted mice clearly indicates perforin-independent participation of $CD8^+$ T cells in efficient clearance of primary rotavirus infection. Nonetheless, $CD8^+$ T cells in the perforin −/− mice are not necessary for clearance of primary infection and their absence can be

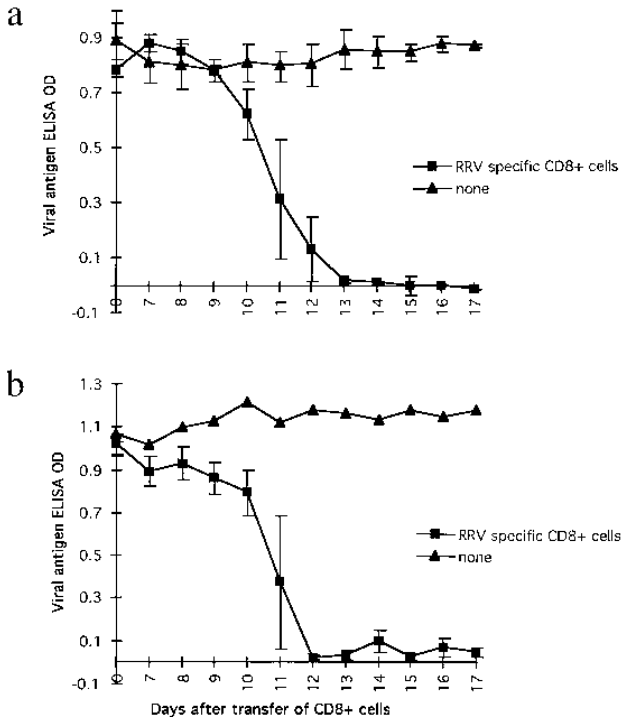

FIG. 2. Fecal viral-antigen-shedding curves of chronically infected Rag 2 −/− mice transferred or not transferred with $CD8^+$ cells from perforin −/− (a) and perforin +/+ mice (b). Rag-2 −/− mice received 10^6 $CD8^+$ spleen cells from mice that had been inoculated IP 18 and 4 days previously with simian rotavirus RRV. Fecal rotavirus antigen was measured by ELISA and results are expressed as OD readings. Each data point represents the mean of three mice ± the SEM except for the single untransferred Rag 2 −/− mouse in panel b. These experiments have been repeated twice with similar results with a total of 10 untransferred mice, 5 mice transferred with $CD8^+$ T cells from perforin +/+ mice, and 4 mice transferred with $CD8^+$ T cells from perforin −/− mice.

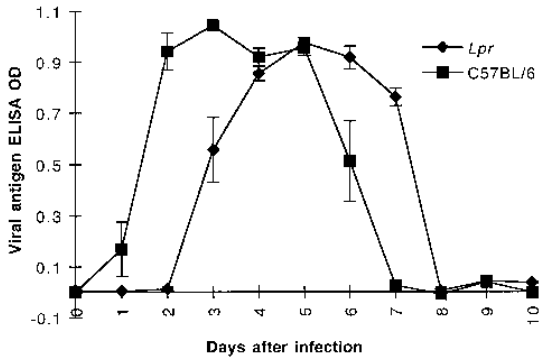

FIG. 3. Fecal viral-antigen-shedding curves for *lpr* and control C57BL/6 mice. Mice were challenged at 6 to 8 weeks of age with 10^5 SD_{50} of the ECw strain of murine rotavirus. Fecal rotavirus was measured by ELISA, and results are expressed as OD readings. Each data point represents the mean of three mice ± the SEM. This experiment has been repeated three times.

compensated for by other mechanisms, such as specific IgA production. This observation confirms our prior studies with β_2m −/− mice (10).

$CD8^+$ T cells from perforin −/− mice clear chronic rotavirus infection in Rag-2 −/− mice. Previously we had shown that passively transferred $CD8^+$ T cells from mice inoculated with heterologous rotaviruses could clear chronic rotavirus infection in SCID mice (7). To confirm that $CD8^+$ T cells can mediate clearance of rotavirus infection in the absence of perforin, we utilized a similar chronic rotavirus infection model. Chronically infected (ECw rotavirus) Rag-2 −/− received purified $CD8^+$ T cells from perforin −/− and perforin +/+ mice previously immunized with RRV. Untransferred Rag 2 −/− mice were used as controls. $CD8^+$ T cells from both perforin −/− and perforin +/+ mice previously inoculated with RRV cleared chronically infected Rag 2 −/− mice with the same kinetics (Fig. 2). Rotavirus-specific antibodies were not detected in stool samples (measured on day 17 after transfer) and serum (measured 3 weeks after transfer) from any transferred Rag 2 −/− mice (data not shown).

Lpr mice efficiently clear primary rotavirus infection. Since fas and fas ligand have been detected in the intestine (24, 37), it is possible that the perforin-independent mechanism used by the $CD8^+$ T cells to clear rotavirus infection was mediated through this alternative cytotoxic pathway. To determine if fas was an indispensable molecule for efficient clearance of rotavirus infection we infected *lpr* and control C57BL/6 mice with 10^5 SD_{50} of ECw. Three experiments with three mice per group were performed. In all three experiments *lpr* and C57BL/6 mice shed viral antigen for similar numbers of days; the mean number of days of virus shedding by *lpr* mice and the mean number of days of virus shedding by C57BL/6 mice were not statistically different ($P = 0.2$). In two of the three experiments the *lpr* mice started shedding viral antigen with a delay of 2 to 4 days in comparison with the start of shedding by the C57BL/6 mice (Fig. 3), but in all cases infection resolution occurred as efficiently as for control mice. Thus, fas does not appear to be necessary for efficient clearance of rotavirus infection. Since C57BL/6 mice depleted of $CD8^+$ T cells have a delayed clearance of ECw primary infection (9a), this result suggests that $CD8^+$ T cells are functional and clear rotavirus infection in the absence of fas.

Perforin −/− mice do not develop rotavirus-specific CTLs. By using perforin −/− mice it has been shown that the capacity of cytotoxic T lymphocytes (CTLs) to lyse target cells that lack fas in short-term CTL assays is perforin dependent (19, 25). To determine if rotavirus-specific CTLs require perforin to kill target cells lacking fas, we tested effector cells from perforin −/− and perforin +/+ mice inoculated orally with ECw and IP with RRV for their capacity to lyse MC57 target cells (that lack fas) infected with RRV or a recombinant VV expressing rotavirus VP7 (10, 11). Spleen cells from naive mice were included as controls. Perforin +/+ mice, but not perforin −/− mice, developed rotavirus-specific CTLs after inoculation with ECw and RRV (Table 2).

IFN-γ-depleted JHD −/− and perforin −/− mice efficiently clear primary rotavirus infection. In addition to cytolysis, $CD8^+$ T cells have also been shown to mediate an antiviral effect through the release of IFN-γ (20, 32). In B-cell-deficient J_HD −/− mice, rotavirus clearance is mediated by $CD8^+$ T cells since CD8 cell depletion leads to chronic infection (10, 26). To determine if IFN-γ was essential for the antirotavirus effect of $CD8^+$ T cells, we depleted J_HD −/− mice of IFN-γ by administration of an anti-IFN-γ MAb. J_HD −/− mice depleted of IFN-γ cleared rotavirus with the same kinetics as those for nondepleted J_HD −/− mice, indicating that IFN-γ is not an essential mediator of the antirotavirus effect of $CD8^+$ T cells (Fig. 4a). To determine if in the absence of perforin $CD8^+$ T cells could mediate the antirotavirus effect independently of IFN-γ, we depleted perforin −/− mice of IFN-γ. Perforin −/− mice depleted of IFN-γ shed rotavirus antigen for the same number of days and at levels comparable to those for untreated control mice (Fig. 4b). In a separate experiment, perforin +/+

TABLE 2. Perforin −/− mice do not develop rotavirus-specific CTLs[a]

Mouse strain of effector cells	Strain used for in vivo priming of effector cells	% Specific lysis of noninfected target cells or target cells infected with the indicated virus strain			
		Noninfected	RRV	VVD4	rVV7
Perforin +/+	ECw	8	**31**	6	**29**
Perforin −/−	ECw	0	−2	−1	0
Perforin +/+	Not primed	5	4	3	6
Perforin +/+	RRV	1	**30**	2	**32**
Perforin −/−	RRV	−1	0	0	2

[a] Effector cells obtained from mice orally infected with ECw, IP infected with RRV, or nonimmunized were restimulated in vitro with RRV and then tested in a 5-h ^{51}Cr release assay against infected or noninfected MC57 target cells. Shown is an effector-to-target ratio of 30. Effector-to-target ratios of 10 and 5 showed a clear dose effect. Values in bold (above 10% and at least double the value of the negative control) are considered positive.

mice depleted of IFN-γ also cleared infection with the same kinetics as those for untreated mice (data not shown). Taken together, these results suggest that $CD8^+$ T cells can clear rotavirus infection in the absence of both perforin and IFN-γ.

IFN-γ −/− mice efficiently clear primary rotavirus infection. To confirm that IFN-γ was not necessary for efficient clearance of rotavirus, we infected IFN-γ −/− and control BALB/c mice with ECw. IFN-γ −/− mice clear rotavirus infection with the same kinetics as those for normal BALB/c mice (Fig. 5a). BALB/c mice and IFN-γ −/− mice depleted of $CD8^+$ T cells have a delay in clearance of primary rotavirus infection of 1 to 2 and 1 to 4 days, respectively compared to the clearance time for undepleted mice (Fig. 5a). The mean numbers of antigen-shedding days for normal BALB/c and IFN-γ −/− mice did not differ significantly ($P = 0.78$). The mean numbers of antigen-shedding days for CD8-depleted BALB/c and IFN-γ −/− mice differed significantly from those for untreated mice ($P = 0.02$ and 0.006, respectively). We conclude that $CD8^+$ T cells are mediating viral clearance in both normal BALB/c and IFN-γ −/− mice.

Depleted and undepleted BALB/c and IFN-γ −/− mice started secreting virus-specific intestinal IgA on the same day after infection (Fig. 5b), and serial titers of these antibodies 10 days after infection did not differ between CD8-depleted and nondepleted BALB/c and IFN-γ −/− mice ($P > 0.14$). Nonetheless, for some of the BALB/c and IFN-γ −/− mice depleted of $CD8^+$ T cells, a slower increase in the accumulation of intestinal IgA was evident compared to that for untreated mice (Fig. 5b). We have not seen this effect on the rate of appearance of intestinal IgA in C57BL/6 mice depleted of $CD8^+$ T cells, although these mice also have delayed viral clearance after CD8 depletion (9a). At present it is unclear if this small alteration in the rise in intestinal IgA concentration contributes to the delay in viral clearance in BALB/c and IFN-γ −/− mice and how the depletion of $CD8^+$ T cells would be contributing to this effect.

IFN-γ, in addition to being an antiviral effector molecule, has an immunomodulator role (41). Presumably for this reason, antibody responses have been shown to be altered in

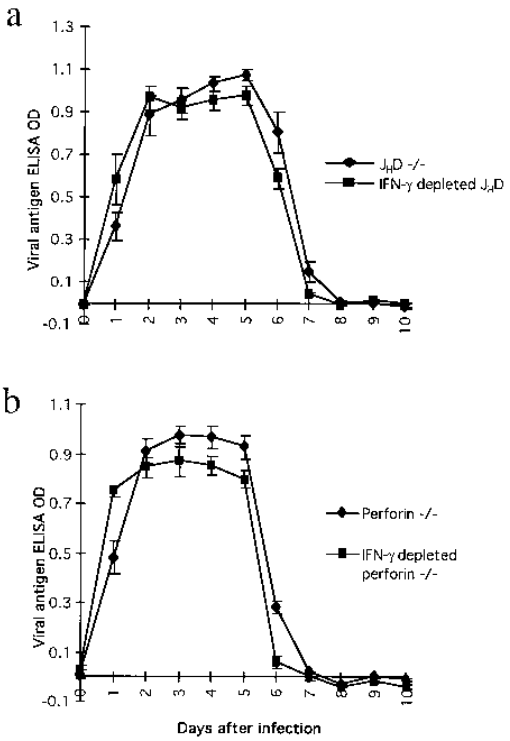

FIG. 4. Fecal viral-antigen-shedding curves of J_HD mice (a) and perforin −/− mice (b) depleted of IFN-γ by administration of XmG1.2 MAb or not depleted. Mice were challenged at 6 to 8 weeks of age with 10^5 SD_{50} of the ECw strain of murine rotavirus. Fecal rotavirus shedding was measured by ELISA and results are expressed as OD readings. Each data point represents the mean of from three to six mice ± the SEM. The experiment whose results are shown in panel a has been repeated twice with similar results. The experiment involving the depletion of IFN-γ in perforin −/− mice has been repeated three times with similar results.

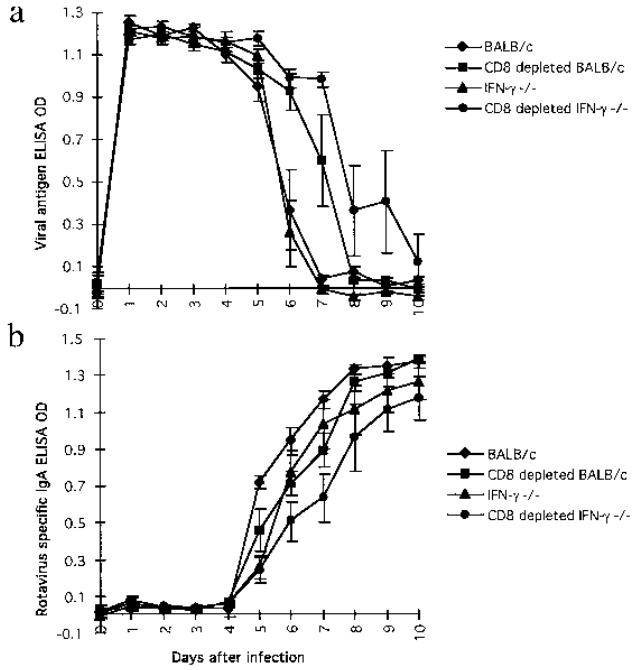

FIG. 5. Fecal viral-antigen (a)- and rotavirus-specific fecal-IgA (b)- shedding curves of CD8-depleted and depleted BALB/c and IFN-γ −/− mice. Mice were challenged at 6 to 8 weeks of age with 10^5 SD_{50} of the ECw strain of murine rotavirus. Fecal rotavirus antigen and rotavirus-specific IgA levels were measured by ELISA, and results are expressed as OD readings. Each data point represents the mean of six IFN-γ −/− mice and five BALB/c mice ± the SEM. Two other experiments have been performed comparing rotavirus-infected IFN-γ −/− mice and BALB/c mice with similar results.

TABLE 3. Different strains of mice have similar rotavirus-specific serum antibody titers after ECw infection

Ig isotype or IgG subclass	Mean antirotavirus serum titer (titer range)[a] for mice of strain:			
	BALB/c	IFN-γ −/−	Perforin −/−	Perforin −/− depleted of IFN-γ
IgM	698 (150–4,050)	727 (150–1,350)	1,350 (1,350–1,350)	1,621 (1,350–4,050)
IgG	4,520 (4,050–12,150)	2,338 (150–4,050)	17,523 (12,150–36,450)	14,591 (4,050–36,450)
IgA	971 (450–4,050)	679 (150–1,350)	1,350 (450–4,050)	936 (450–1,350)
IgG1	23 (16.7–50)	23 (16.7–150)	24 (16.7–50)	16.7 (16.7–16.7)
IgG2a	1,682 (450–4,050)	634 (150–1,350)	5,841 (1,350–12,150)	10,117 (4,050–12,150)
IgG2b	625 (150–1,350)	552 (50–1,350)	779 (450–1,350)	1,350 (450–4,050)
IgG3	28 (16.7–450)	20 (16.7–50)	16.7 (16.7–16.7)	16.7 (16.7–16.7)

[a] Sera from mice 14 days (BALB/c and IFN-γ −/− mice) or 12 days (perforin −/− and perforin −/− mice depleted of IFN-γ) after oral infection with ECw were assayed for rotavirus-specific antibody titers by ELISA. Results are the means (ranges) for 10 BALB/c mice, 16 IFN-γ −/− mice, 6 perforin −/− mice, and 6 perforin −/− mice depleted of IFN-γ.

IFN-γ receptor −/− mice (34) and IFN-γ-depleted mice (33). In accordance with these findings, serum titers of Ig2a were significantly lower in IFN-γ −/− mice than in BALB/c mice ($P = 0.026$) (Table 3). However, following ECw infection, most IFN-γ −/− mice (14 of 16 mice studied) and all perforin −/− mice depleted of IFN-γ developed rotavirus-specific intestinal IgA with similar kinetics to those of control mice (Fig. 5b; and results not shown). For the IFN-γ −/− mice and BALB/c mice the serum antibody titers of all isotypes and IgG subclasses other than IgG2a did not differ significantly ($P > 0.1$). In addition the serum antibody titers of all isotypes and all IgG subclasses did not differ significantly for IFN-γ-depleted and nondepleted perforin −/− mice ($P > 0.33$).

DISCUSSION

Class I-restricted $CD8^+$ T cells have long been known as effector cells against viruses. These T lymphocytes have been classically characterized by their capacity to kill virus-infected cells in vitro and therefore have been called CTLs. Proof of the importance of in vivo lysis in eliminating a viral infection has been difficult to obtain (21). Recently, by using perforin −/− and *lpr* mice this issue has been greatly clarified. For some viruses (LCMV and adenoviruses) the in vivo antiviral effect of $CD8^+$ T cells appears to be dependent on lysis of the virus-infected cell by a perforin-mediated mechanism (17, 38, 39). For the antiviral effect against VV, $CD8^+$ T cells probably rely on secretion of IFN-γ and not on perforin- or fas-mediated pathways of cell lysis (18).

$CD8^+$ T cells have been shown to play a role in the clearance of murine rotavirus; J_HD mice depleted of $CD8^+$ T cells become chronically infected and β_2m −/− mice have delayed clearance (1 to 3 days) of primary rotavirus infection (10, 26). A modest but highly reproducible and significant effect on clearance of primary rotavirus infection in immunocompetent mice and perforin −/− mice can also be attributed to $CD8^+$ T cells (Fig. 1 and 5a) (9a). This effect is independent of perforin-mediated lysis of virus-infected cells, as perforin −/− mice clear primary rotavirus infection with the same kinetics as those for perforin +/+ mice (Fig. 1). Equal efficiencies of rotavirus clearance with $CD8^+$ T cells transferred from perforin −/− and perforin +/+ mice (Fig. 2) confirm that $CD8^+$ T cells can mediate clearance of rotavirus infection by a perforin-independent pathway.

CTLs have also been shown to mediate lysis of virus-infected cells by a mechanism dependent on the interaction between fas on the target cell and fas ligand on the T cell (1a). This pathway of cell lysis has been proposed as an immunoregulatory mechanism (19). We investigated the possibility that it could also be used as an antiviral mechanism alternative to perforin-mediated cell lysis because fas and fas ligand have both been found in intestinal tissues (24, 37). The facts that C57BL/6 mice depleted of $CD8^+$ T cells have delayed viral clearance and that *lpr* mice clear primary rotavirus infection as efficiently as C57BL/6 mice strongly suggest that the fas system is not the mechanism for $CD8^+$-T-cell-mediated rotavirus clearance. Viral-antigen shedding in *lpr* mice in two of three experiments started with a delay of 2 to 4 days compared to the start of viral shedding in C57BL/6 mice (Fig. 3). The viral-shedding curves for these *lpr* mice resemble the viral-shedding curves for C57BL/6 mice infected with lower doses of rotavirus (data not shown). At present we have no clear explanation for the delay in initiation of viral shedding in these *lpr* mice. However, as noted above, the durations of shedding for *lpr* and control mice were identical.

IFN-γ has been shown to be a mediator of the antiviral effects of $CD8^+$ T cells (20, 32, 36). Having found perforin- and fas-mediated effects unnecessary for rotaviral clearance, we next investigated whether IFN-γ was essential. This does not seem to be the case. J_HD mice become chronically infected with murine rotavirus if they are depleted of $CD8^+$ T cells (10, 26). However, if these mice are depleted of IFN-γ, they clear primary rotavirus infection just like control mice (Fig. 4). Additionally, IFN-γ −/− mice clear primary rotavirus infection efficiently and have a delay in viral clearance if depleted of $CD8^+$ T cells (Fig. 5a). The IFN-γ-independent rotavirus clearance mechanism is not mediated by perforin since perforin −/− mice depleted of IFN-γ clear infection as well as control mice. IFN-γ is produced by human peripheral blood lymphocytes stimulated with rotavirus (40) and inhibits in vitro growth of rotavirus in human cells (1). Our results do not exclude the possibility that IFN-γ has an antirotavirus effect in vivo but they show that this potential effect is not essential for normal clearance of primary rotavirus infection and that $CD8^+$ T cells can mediate an antirotavirus effect in the absence of IFN-γ and perforin.

Similar to what has been reported for other viruses (16, 34), IFN-γ −/− mice infected with rotavirus produced somewhat less virus-specific total serum IgG and significantly less virus-specific IgG2a than control mice (Table 3). The overall intestinal (Fig. 5b) and systemic (Table 3) antibody responses of IFN-γ −/− mice and perforin −/− mice depleted of IFN-γ were not significantly different from those of control mice. Contrary to what has been reported for other viral systems (33), the virus-specific IgG2a level was not lower in IFN-γ-depleted mice than in control mice (Table 3). We have no clear explanation for this finding, but the perforin −/− mice seem to have higher antiviral antibody responses than the BALB/c mice

(Table 3), suggesting that variations in the mouse strain could account for the difference.

Our results strongly suggest that $CD8^+$ T cells can efficiently mediate clearance of rotavirus infection by a mechanism independent of perforin, fas, or IFN-γ. It is interesting to consider what factors might be mediating the antirotavirus effect. Type 1 interferons and TNF are two potential cytokines through which $CD8^+$ T cells could be mediating this effect. Type 1 interferons have been shown to be produced in response to infection with rotaviruses (22) and to have antirotavirus effects in calves and pigs (23, 35). TNF released by $CD8^+$ T cells has been suggested to act against cytomegalovirus (29) and to inhibit hepatitis B virus gene expression in hepatitis B virus transgenic mice (13). Of note, in the transgenic hepatitis B model the capacity of a perforin −/− $CD8^+$ clone to abolish transgenic viral replication was partially inhibited by both anti-IFN-γ and anti-TNF antibodies and was completely inhibited if the antibodies were administered simultaneously (14). A study of mice with gene-targeted mutations in the type 1 IFN receptor (27) and TNF receptors (3) or administration of anti-TNF and anti-type 1 and -type 2 IFN antibodies separately or in combinations to J_HD mice should help clarify the mechanism used by $CD8^+$ T cells to clear rotavirus. Other cytokines could also mediate the antirotavirus effect of $CD8^+$ T cells. For example Rantes, MIP-1α, and MIP-1β have been characterized as major in vitro HIV-suppressive factors produced by $CD8^+$ T cells (6).

Finally, our results could theoretically be explained if the three antiviral mechanisms we have studied (perforin, fas, and IFN-γ) were redundant, that is, if the $CD8^+$ T cells in the perforin −/− mice depleted of IFN-γ cleared the rotavirus infection by a fas-dependent pathway. We think this hypothetical mechanism is unlikely, however, because fas-mediated antiviral effects have not been clearly demonstrated in other in vivo model systems (18). Future studies of perforin −/− mice crossed with *lpr* mice will examine this issue directly.

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REFERENCES

1. **Bass, D.** Personal communication.
1a. **Berke, G.** 1995. The CTL's kiss of death. Cell **81:**9–12.
2. **Bernstein, D. I., R. I. Glass, G. Rodgers, B. L. Davidson, and D. A. Sack.** 1995. Evaluation of rhesus rotavirus monovalent and tetravalent reassortant vaccines in US children. US Rotavirus Vaccine Efficacy Group. JAMA **273:**1191–1196.
3. **Beutler, B., and H. C. van Huffel.** 1994. Unraveling function in the TNF ligand and receptor families. Science **264:**667–668.
4. **Burns, J. W., A. A. Krishnaney, P. T. Vo, R. V. Rouse, L. J. Anderson, and H. B. Greenberg.** 1995. Analyses of homologous rotavirus infection in the mouse model. Virology **207:**143–153.
5. **Burns, W. B., M. Siadat-Pajouh, A. A. Krishnaney, and H. B. Greenberg.** 1996. Novel protective effect of rotavirus VP6 specific IgA monoclonal antibodies that lack conventional neutralizing activity. Science **272:**104–107.
6. **Cocchi, F., A. L. DeVico, A. Garzino-Demo, S. K. Arya, R. C. Gallo, and L. Paolo.** 1995. Identification of Rantes, MIP-1α, and MIP-1β as the major HIV-suppressive factors produced by CD8+ T cells. Science **270:**1811–1815.
6a. **Dalton, D. K., M. S. Pitts, S. Keshav, I. S. Figari, A. Bradley, and T. A. Stewart.** 1993. Multiple defects of immune cell function in mice with disrupted interferon-gamma genes. Science **259:**1739–1742.
7. **Dharakul, T., L. Rott, and H. B. Greenberg.** 1990. Recovery from chronic rotavirus infection in mice with severe combined immunodeficiency: virus clearance mediated by adoptive transfer of immune $CD8^+$ lymphocytes. J. Virol. **64:**4375–4382.
8. **Dulbecco, R.** 1980. The nature of viruses, p. 881–883. *In* B. D. Davis, R. Dulbecco, H. N. Eisen, and H. S. Ginsberg (ed.), Microbiology, 3rd ed., vol. 1. Harper and Row Publishers, Philadelphia.
9. **Feng, N., J. W. Burns, L. Bracy, and H. B. Greenberg.** 1994. Comparison of mucosal and systemic humoral immune responses and subsequent protection in mice orally inoculated with a homologous or a heterologous rotavirus. J. Virol. **68:**7766–7773.
9a. **Franco, M.** Unpublished data.
10. **Franco, M. A., and H. B. Greenberg.** 1995. Role of B cells and cytotoxic T lymphocytes in clearance of and immunity to rotavirus infection in mice. J. Virol. **69:**7800–7806.
11. **Franco, M. A., I. Prieto, M. Labbe, D. Poncet, C. F. Borras, and J. Cohen.** 1993. An immunodominant cytotoxic T cell epitope on the VP7 rotavirus protein overlaps the H2 signal peptide. J. Gen. Virol. **74:**2579–2586.
12. **Glass, R. I., J. Gentsch, and J. C. Smith.** 1994. Rotavirus vaccines: success by reassortment? Science **265:**1389–1391.
13. **Guidotti, L. G., K. Ando, M. V. Hobbs, T. Ishikawa, L. Runkel, R. D. Schreiber, and F. V. Chisari.** 1994. Cytotoxic T lymphocytes inhibit hepatitis B virus gene expression by a noncytolytic mechanism in transgenic mice. Proc. Natl. Acad. Sci. USA **91:**3764–3768.
14. **Guidotti, L. G., T. Ishikawa, M. V. Hobbs, B. Matzke, R. Schreiber, and F. V. Chisari.** 1996. Intracellular inactivation of the hepatitis B virus by cytotoxic T lymphocytes. Immunity **4:**25–36.
15. **Hoshino, Y., R. G. Wyatt, H. B. Greenberg, J. Flores, and A. Z. Kapikian.** 1984. Serotypic similarity and diversity of rotaviruses of mammalian and avian origin as studied by plaque reduction neutralization. J. Infect. Dis. **149:**694–702.
16. **Huang, S., W. Hendriks, A. Althage, S. Hemmi, H. Bluethmann, R. Kamijo, J. Vilcek, R. M. Zinkernagel, and M. Aguet.** 1993. Immune response in mice that lack the interferon-gamma receptor. Science **259:**1742–1745.
17. **Kagi, D., B. Ledermann, K. Burki, P. Seiler, B. Odermatt, K. J. Olsen, E. R. Podack, R. M. Zinkernagel, and H. Hengartner.** 1994. Cytotoxicity mediated by T cells and natural killer cells is greatly impaired in perforin-deficient mice. Nature **369:**31–37.
18. **Kagi, D., P. Seiler, J. Pavlovic, B. Ledermann, K. Burki, R. M. Zinkernagel, and H. Hengartner.** 1995. The roles of perforin- and Fas-dependent cytotoxicity in protection against cytopathic and noncytopathic viruses. Eur. J. Immunol. **25:**3256–3262.
19. **Kagi, D., F. Vignaux, B. Ledermann, K. Burki, V. Depraetere, S. Nagata, H. Hengartner, and P. Golstein.** 1994. Fas and perforin pathways as major mechanisms of T cell-mediated cytotoxicity. Science **265:**528–530.
20. **Kundig, T. M., H. Hengartner, and R. M. Zinkernagel.** 1993. T cell-dependent IFN-gamma exerts an antiviral effect in the central nervous system but not in peripheral solid organs. J. Immunol. **150:**2316–2321.
21. **Kyburz, D., D. E. Speiser, M. Battegay, H. Hengartner, and R. M. Zinkernagel.** 1993. Lysis of infected cells in vivo by antiviral cytolytic T cells demonstrated by release of cell internal viral proteins. Eur. J. Immunol. **23:**1540–1545.
22. **La Bonnardiere, C., J. Cohen, and M. Contrepois.** 1981. Interferon activity in rotavirus infected newborn calves. Ann. Rech. Vet. **12:**85–91.
23. **Lecce, J. G., J. M. Cummins, and A. B. Richards.** 1990. Treatment of rotavirus infection in neonate and weanling pigs using natural human interferon alpha. Mol. Biother. **2:**211–216.
24. **Leithauser, F., J. Dhein, G. Mechtersheimer, K. Koretz, S. Bruderlein, C. Henne, A. Schmidt, K. M. Debatin, P. H. Krammer, and P. Moller.** 1993. Constitutive and induced expression of APO-1, a new member of the nerve growth factor/tumor necrosis factor receptor superfamily, in normal and neoplastic cells. Lab. Invest. **69:**415–429.
25. **Lowin, B., M. Hahne, C. Mattmann, and J. Tschopp.** 1994. Cytolytic T-cell cytotoxicity is mediated through perforin and Fas lytic pathways. Nature **370:**650–652.
26. **McNeal, M. M., K. Siobhan Barone, M. N. Rae, and R. L. Ward.** 1995. Effector functions of antibody and CD8+ cells in resolution of rotavirus infection and protection against reinfection in mice. Virology **214:**387–397.
27. **Muller, U., U. Steinhoff, L. F. Reis, S. Hemmi, J. Pavlovic, R. M. Zinkernagel, and M. Aguet.** 1994. Functional role of type I and type II interferons in antiviral defense. Science **264:**1918–1921.
28. **Offit, P. A., and K. I. Dudzik.** 1990. Rotavirus-specific cytotoxic T lymphocytes passively protect against gastroenteritis in suckling mice. J. Virol. **64:**6325–6328.
29. **Pavic, I., B. Polic, I. Crnkovic, P. Lucin, S. Jonjic, and U. H. Koszinowski.** 1993. Participation of endogenous tumour necrosis factor alpha in host resistance to cytomegalovirus infection. J. Gen. Virol. **74:**2215–2223.
30. **Ramsay, A. J., J. Ruby, and I. A. Ramshaw.** 1993. A case for cytokines as effector molecules in the resolution of virus infection. Immunol. Today **14:**155–157.
31. **Riepenhoff-Talty, M., T. Dharakul, E. Kowalski, S. Michalak, and P. L. Ogra.** 1987. Persistent rotavirus infection in mice with severe combined immunodeficiency. J. Virol. **61:**3345–3348.
32. **Ruby, J., and I. Ramshaw.** 1991. The antiviral activity of immune CD8+ T cells is dependent on interferon-gamma. Lymphokine Cytokine Res. **10:**353–358.

33. **Sarawar, S. R., M. Sangster, R. L. Coffman, and P. C. Doherty.** 1994. Administration of anti-IFN-gamma antibody to beta 2-microglobulin-deficient mice delays influenza virus clearance but does not switch the response to a T helper cell 2 phenotype. J. Immunol. **153:**1246–1253.
34. **Schijns, V. E., B. L. Haagmans, E. O. Rijke, S. Huang, M. Aguet, and M. C. Horzinek.** 1994. IFN-gamma receptor-deficient mice generate antiviral Th1-characteristic cytokine profiles but altered antibody responses. J. Immunol. **153:**2029–2037.
35. **Schwers, A., B. C. Vanden, M. Maenhoudt, J. M. Beduin, J. Werenne, and P. P. Pastoret.** 1985. Experimental rotavirus diarrhoea in colostrum-deprived newborn calves: assay of treatment by administration of bacterially produced human interferon (Hu-IFN alpha 2). Ann. Rech. Vet. **16:**213–218.
36. **Smith, P. M., R. M. Wolcott, R. Chervenak, and S. R. Jennings.** 1994. Control of acute cutaneous herpes simplex virus infection: T cell-mediated viral clearance is dependent upon interferon-gamma (IFN-gamma). Virology **202:**76–88.
37. **Suda, T., T. Okazaki, Y. Naito, T. Yokota, N. Arai, S. Ozaki, K. Nakao, and S. Nagata.** 1995. Expression of the Fas ligand in cells of T cell lineage. J. Immunol. **154:**3806–3813.
37a. **VanCott, J.** Unpublished data.
38. **Walsh, C. M., M. Matloubian, C. C. Liu, R. Ueda, C. G. Kurahara, J. L. Christensen, M. T. Huang, J. D. Young, R. Ahmed, and W. R. Clark.** 1994. Immune function in mice lacking the perforin gene. Proc. Natl. Acad. Sci. USA **91:**10854–10858.
39. **Yang, Y., Z. Xiang, H. C. Ertl, and J. M. Wilson.** 1995. Upregulation of class I major histocompatibility complex antigens by interferon gamma is necessary for T-cell-mediated elimination of recombinant adenovirus-infected hepatocytes in vivo. Proc. Natl. Acad. Sci. USA **92:**7257–7261.
40. **Yasukawa, M., O. Nakagomi, and Y. Kobayashi.** 1990. Rotavirus induces proliferative response and augments non-specific cytotoxic activity of lymphocytes in humans. Clin. Exp. Immunol. **80:**49–55.
41. **Young, H. A., and K. J. Hardy.** 1995. Role of interferon-gamma in immune cell regulation. J. Leukocyte Biol. **58:**373–381.