Progressive Loss of CD8$^+$ T Cell–mediated Control of a γ-Herpesvirus in the Absence of CD4$^+$ T Cells

By Rhonda D. Cardin, James W. Brooks, Sally R. Sarawar, and Peter C. Doherty

From the Department of Immunology, St. Jude Children's Research Hospital, Memphis, Tennessee 38105

Summary

A unique experimental model has been developed for dissecting the integrity of CD8$^+$ T cell–mediated immunity to a persistent gammaherpesvirus under conditions of CD4$^+$ T cell deficiency. Respiratory challenge of major histocompatibility complex class II −/− and +/+ C57BL/6J mice with the murine gammaherpesvirus 68 (MHV-68) leads to productive infection of both lung and adrenal epithelial cells. Virus titers peak within 5–10 d, and are no longer detected after day 15. Persistent, latent infection is established concurrently in splenic and lymph node B cells, with higher numbers of MHV-68$^+$ lymphocytes being found in all lymphoid sites analyzed from the +/+ mice concurrent with the massive, but transient splenomegaly that occurred only in this group. From day 17, however, the numbers of infected B lymphocytes were consistently higher in the −/− group, while the frequency of this population diminished progressively in the +/+ controls. Infectious MHV-68 was again detected in the respiratory tract and the adrenals of the −/− (but not the +/+) mice from day 22 after infection. The titers in these sites rose progressively, with the majority of the −/− mice dying between days 120 and 133. Even so, some CD8$^+$ effectors were still functioning as late as 100 d after infection. Depletion of CD8$^+$ T cells at this stage led to higher virus titers in the −/− lung, and to the development of wasting in some of the −/− mice. Elimination of the CD8$^+$ T cells from the +/+ group (day 80) increased the numbers of MHV-68$^+$ cells in the spleen, but did not reactivate the infection in the respiratory tract. The results are consistent with the interpretation that CD8$^+$ T cell–mediated control of this persistent gammaherpesvirus is progressively lost in the absence of the CD4$^+$ T cell subset. This parallels what may be happening in AIDS patients who develop Kaposi's sarcoma and various Epstein Barr virus–associated disease processes.

Persistent infection of human B lymphocytes with the prototypic type 1 γ-herpesvirus (γHV)[1], Epstein-Barr virus (EBV), is thought to be controlled by virus-specific CD8$^+$ T cells (1–3). Most people are EBV carriers, and subsequent to the infectious mononucleosis phase that often follows the initial invasion of the virus, normally remain asymptomatic into advanced age. This situation can change dramatically in the immunosuppressed host with, for example, EBV-associated lymphoproliferative disease being a too common complication of therapeutic bone marrow and organ transplantation (4–6). The progression of human immunodeficiency virus (HIV) infection to the AIDS can also be accompanied by the development of EBV-associated lymphoma, and by the increased excretion of EBV from the characteristic oral hairy leukoplakia (7, 8). Furthermore, individuals with AIDS show a greatly increased incidence (9) of Kaposi's sarcoma. Recent analysis has implicated a novel type 2 γHV in the etiology of KS (10, 11). The development of AIDS is associated with declining numbers of CD4$^+$ T cells, the primary target of HIV replication (12), rather than the loss of the CD8$^+$ set that is generally considered to be the principal mediator of immune surveillance against viruses (13).

Experimental dissection of the interaction between a γHV and the CD4$^+$ and CD8$^+$ T cell responses has been made possible by the recent development of the murine γHV (MHV-68) model (17). Like EBV, this type 2 γHV (15, 16) is disseminated as a consequence of the productive infection of mucosal epithelial cells (17). The viremia that occurs during the acute phase of the infection also leads to transient virus replication in the thymus and adrenal epithe-

[1] *Abbreviations used in this paper:* BAL, bronchoalveolar lavage; BM, bone marrow; CLN, cervical lymph node; CMC, carboxymethyl cellulose; CMV, cytomegalovirus; EBV, Epstein-Barr virus; γHV, γ-herpesvirus; i.n., intranasal; LCMV, lymphocytic choriomeningitis virus; MHV-68, mouse γHV-68; MLN, mediastinal lymph node; OMK, owl monkey kidney (cells).

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lium, and to the establishment of latent infection in large numbers of B lymphocytes (18). Selective elimination of the responding $CD4^+$ or $CD8^+$ T cells in BALB/c ($H\text{-}2^d$) mice by in vivo treatment with mAbs has established that the initial stage of virus replication in epithelial sites is controlled by $CD8^+$ effectors acting in the absence of the $CD4^+$ subset (19). The characteristic splenomegaly (19, 20) was, however, greatly diminished in the $CD4^+$ T cell–depleted mice, and fewer B cells could be shown to carry the virus.

The present study extends this analysis to long-term MHV-68 infection of $H\text{-}2^b$ mice that are constitutively CD4-deficient as a consequence of homozygous disruption (−/−) of the $H\text{-}2IA^b$ class II MHC glycoprotein (21). Though there are low numbers of $CD4^+$ T cells in these MHC class II −/− animals (2–5% in the periphery), studies with a variety of experimental systems have given no indication that these lymphocytes can function to provide cognate help for antibody production or to promote $CD8^+$ T cell responses (21–24).

Materials and Methods

Mice. The +/+ C57BL/6J (B6) mice were purchased from Jackson ImmunoResearch Laboratories, Inc. (Bar Harbor ME). The C2D mice that are functionally −/− for the $H\text{-}IA^b$ gene (21) were bred under licence from GenPharm International (Mountain View, CA), at St. Jude Children's Research Hospital. Female mice were infected with MHV-68 at 8–10 wk of age, then maintained under otherwise specific pathogen-free conditions in BL3-level containment.

Virus Stocks and Plaque Assay. The original stock of MHV-68 (clone G2.4) was obtained from Dr. A.A. Nash at the University of Cambridge (Cambridge, UK). The virus was grown in owl monkey kidney (OMK; 1566CRL, American Type Culture Collection [ATCC], Rockville, MD) cells maintained in RPMI 1640 (GIBCO BRL, Gaithersburg, MD) supplemented with 10% FCS, 2 mM L-glutamine, and gentamicin. The OMK cells were infected at a multiplicity of infection (MOI) of 0.01, and virus stocks were prepared by sonicating the cells in PBS+0.1% BSA on ice, then stored at −70°C. Uninfected control stocks were prepared from OMK cells in the same manner. Virus titers were determined by plaque assay on NIH 3T3 cells (ATCC CRL1658) grown in DMEM (GIBCO BRL) supplemented with 10% FCS, 2 mM l-glutamine, 4 mM Hepes, and gentamicin. Dilutions of stock virus, sonicated mouse tissues, or sonicates of lymphocytes were adsorbed onto the NIH 3T3 monolayers for 1 h at 37°C, then washed with media and overlaid with 2.0 ml of carboxymethyl cellulose (CMC) diluted 1:1 in 2× modified Eagle's medium supplemented with 10% FCS, glutamine, gentamicin, and nonessential amino acids (13). After 5 d, the CMC overlay was removed, and the monolayers fixed with methanol and stained with Giemsa to determine the number of plaques.

Infection and Sampling. The mice were anesthetized (25) with Avertin (2,2,2 tribromoethanol), then infected intranasally (i.n.) with 4×10^3 PFU of MHV-68 in 40 μl of PBS + 0.1% BSA. At various times after infection, the mice were anesthetized again and bled from the axilla. The inflammatory cells from the lung were obtained (25) by bronchoalveolar lavage (BAL), various tissues were taken for virus isolation, and single-cell preparations were made from the lymph nodes and spleen for cytokine, cytotoxicity, flow cytometry, and infectious center assays. Peripheral blood lymphocytes and bone marrow were isolated (13) and analyzed for presence of virus. The spleens were first weighed before homogenization. The tissues were sonicated in medium on ice, and the presence of virus was determined by plaque assay (13). All samples were stored at −70°C before virus titration.

Infectious Center Assay. The frequency of persistently infected lymphocytes was determined by assaying for infectious centers by overlaying single-cell suspensions on monolayers of NIH 3T3 cells. Various dilutions of the spleen and lymph node cells were plated with 5×10^5 NIH 3T3 cells, incubated overnight at 37°C, and then overlaid with CMC/2× medium. The cells were cocultured for 5–6 d, the overlay was removed, and plaque counts were determined as described above. Aliquots of 10^7 (or higher) spleen or lymph node cells were also sonicated and assayed for the presence of infectious virus.

Redirected Cytotoxicity Assay. Single-cell suspensions of lymph nodes and spleen, or BAL cells that were first adsorbed on plastic to remove most of the macrophages, were assayed for 6 h on ^{51}Cr-labeled FcR^+ P815 cells in the presence of the 2C11 mAb to CD3ε (26, 27). The level of specific ^{51}Cr release is a measure of the total cytotoxicity mediated by all activated T cells. For assays involving in vitro depletions, cell populations were incubated with the appropriate mAb for 30 min at 4°C, then the cells were washed and incubated for 45 min at 37°C in a cocktail of rabbit (Accurate Chemical and Scientific Corp., Westbury, NY) and guinea pig complement (Cedar Lane Laboratories, Hornby, Ontario).

Lymphocyte Depletion and Flow Cytometry. Mice in some experiments were depleted of $CD4^+$ or $CD8^+$ T cells by in vivo treatment with the GK1.5 or 2.43.1 mAbs, respectively, which were administered for at least 8 d at 2–3-d intervals (25, 28). The prevalence of the various lymphocyte subsets was determined (29) in two-color mode on a FACScan® (Becton Dickinson & Co., Mountain View, CA) with Lysis II software. The staining reagents were 53.6.7-FITC anti-CD8α, H129.19-PE anti-$CD4^+$, and RA3-682 FITC anti-B220, all of which were purchased from Pharmingen (San Diego, CA) and are not blocked by the mAbs used for the in vivo depletions.

Cytokine Analysis. The single-cell ELISPOT assay was used to quantify cytokine producing cells in freshly isolated BAL populations (30). Spleen and lymph node cells were restimulated in vitro with MHV-68–infected stimulator cells (31), and culture supernatants were assayed by ELISA for the presence of IFN-γ, IL-2, IL-4, IL-5, IL-6, and IL-10, as described previously (32). The results are expressed as the percentage of spot-forming cells (ELISPOT), or as nanograms (IL-10), or units per ml of culture supernatant (ELISA).

Results

Virus Distribution during the Primary Stage of Infection. The kinetics and peak titers of the initial virus growth phase in the respiratory tract after i.n. inoculation with MHV-68 (4×10^3 PFU) were comparable for the MHC class II +/+ and −/− mice (Fig. 1 *a*). Lower titers of MHV-68 were also detected in the adrenals on days 3 and 6 (Fig. 1 *b*), and in the thymus on day 6 (data not shown), indicating that the virus had disseminated rapidly via the blood in both groups of mice. Even so, the spectrum of organs supporting productive infection detectable by plaque assay appears to be very limited. MHV-68 could not be re-

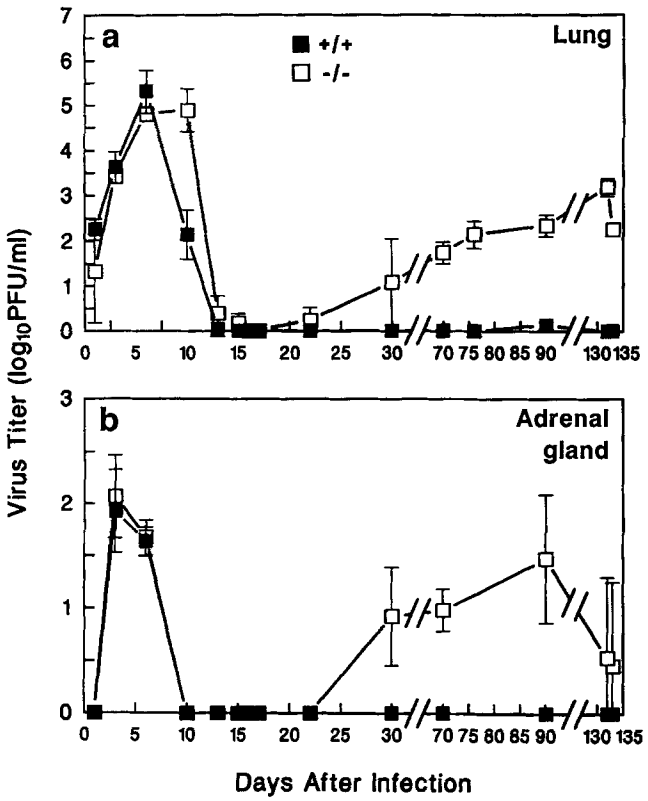

Figure 1. MHV-68 replication in MHC class II +/+ and −/− mice: infectious virus titers are shown for the lungs (*a*) and the adrenal glands (*b*) of the +/+ (■) and −/− mice (□). Virus titers are depicted as the mean $\log_{10}$ PFU/ml of a 10% tissue sonicate, (± SD), for three to five mice per timepoint for three to four separate experiments. Results for single experiments at 16, 17, 76, 132, and 133 d after infection are also included. The limit of detection for the lung was 1 PFU, and 5 PFU for the adrenal gland.

covered during this acute phase from tissue sonicates of the pancreas, salivary glands, kidney, or liver, (data not shown). Replication in the liver and kidneys has been reported for BALB/c mice (17).

This virus does, however, quickly establish a latent infection in large numbers of B lymphocytes (19). Cocultivation with permissive NIH 3T3 fibroblasts established the presence of substantial numbers of cells carrying MHV-68 in the cervical lymph nodes (CLN) from day 3, and in the mediastinal lymph nodes (MLN) and spleen from day 6 (Fig 2, *a–c*). Infectious centers were also detected subsequent to day 15 in the bone marrow (BM, Fig. 2 *d*), peripheral blood cells, and the mesenteric, axial, and brachial lymph nodes (data not shown). The lymph nodes, spleen, bone marrow, and blood cells yielded very few (or no) plaques after sonication, indicating that MHV-68 is predominantly in a nonreplicating (or latent) status within the lymphoid compartment.

Virus titers were greatly diminished by day 10 or 13 after infection for lung sonicates from the +/+ and −/− mice, respectively, with both being negative by day 15 (Fig. 1 *a*). Similarly, the adrenal samples were all negative by day 10 (Fig. 1 *b*). Despite the termination of the replicative phase, however, there was no concurrent decrease in the frequencies of latently infected B cells (17) in the lymphoid tissue (Fig. 2). The numbers of MHV-68$^+$ cells in the spleens of the −/− mice (Fig. 2 *a*) were, however, consistently lower through day 15, reflecting that the extent of splenomegaly is generally much less in the absence of the CD4$^+$ subset (Fig. 3). In general, this analysis of the acute phase of MHV-68 infection in MHC class II −/− B6 (H-2^b) mice confirms and extends the conclusions reached from earlier experiments with CD4-depleted BALB/c (H-2^d) mice (19,

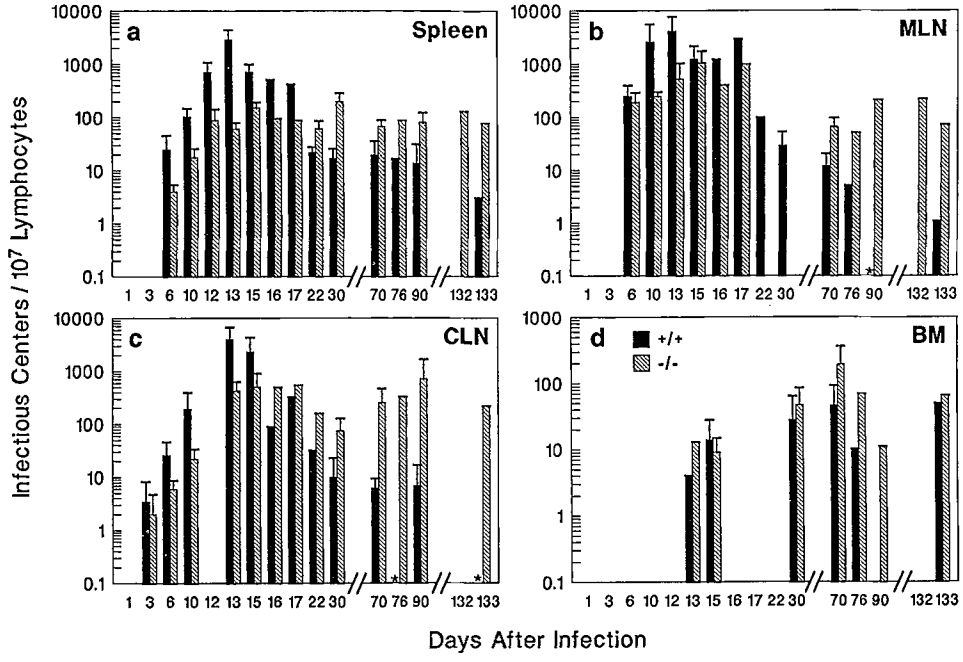

Figure 2. Profiles of MHV-68 latency are shown for lymphoid tissues from the +/+ and −/− mice. Lymphocytes from the (*a*) spleens; (*b*) MLN; (*c*), CLN; and (*d*) BM of +/+ (■) and −/− (▨) mice were isolated at different times after infection for infectious center assay. The results are depicted at the mean number (± SD) of infectious centers per 10^7 lymphocytes pooled from three to five mice per timepoint for repeat or single experiments (see legend to Fig. 1). Timepoints where no infectious centers were detected are indicated by asterisks. Otherwise, timepoints with no data indicate that the samples were not assayed.

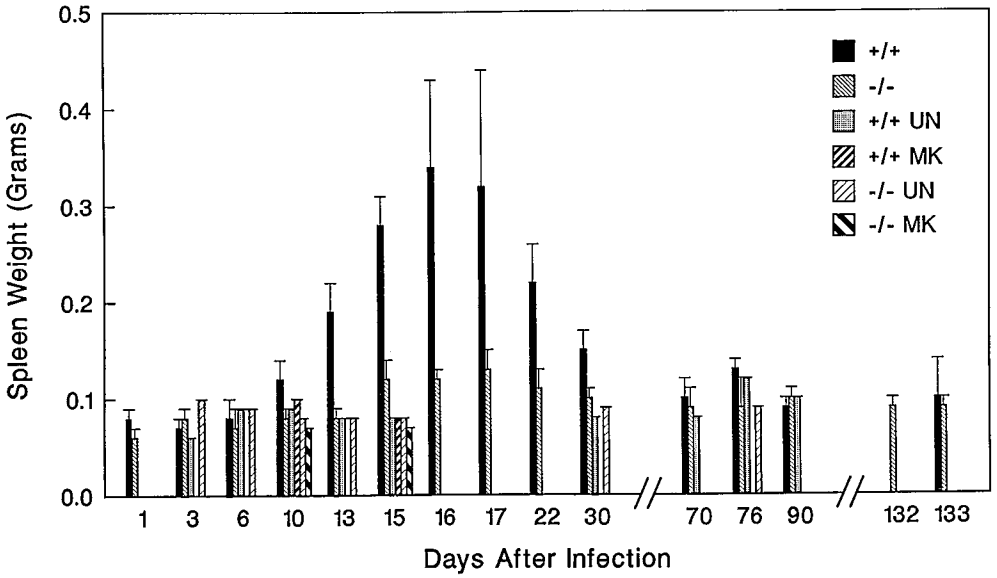

Figure 3. The extent of splenomegaly was determined by weighing spleens from +/+ (■) and −/− (▧) mice at different timepoints after MHV-68 infection. The spleens were into preweighed tubes, and the results are expressed as mean (± SD) spleen weight (grams) for groups of three to five mice per timepoint. Spleens from uninfected (*UN*) and mock-infected (*MK*) mice were measured for control weights, but were not included for each timepoint.

26). An important addition to these studies is that all the lymph nodes, the bone marrow, and peripheral blood cells harbor latent virus, indicating that these persistently infected cells are widely disseminated in the infected mouse.

Characteristics of the Acute Host Response. The fall in lung MHV-68 titers (Fig. 1 *a*) was accompanied by a dramatic increase (Fig. 4, *c* and *d*) in the magnitude of the cell population recovered by BAL. The inflammatory cells were generally more than 30% monocyte/macrophages, with the remaining lymphocyte population being dominated by the $CD8^+$ subset (Fig. 4, *a* and *b*). In the +/+ mice, the lower numbers of $CD4^+$ T cells and few $B220^+$ B cells are consistent with the pattern found for other virus infections of the respiratory tract (25, 28, 33). As yet, there is no reproducible virus-specific CTL assay developed for MHV-68. Measuring the cytotoxic activity of all $CD3\epsilon^+$ T cells by the redirected CTL assay (26, 27), however, showed the emergence of potent effectors in the BAL by day 10 (Fig. 5 *a*). The levels of lysis were higher for the MLN and spleen (Figs. 5, *b* and *c*), which, though they are not supporting virus replication (Fig. 1), they do contain large numbers of latently infected B lymphocytes (Fig. 2). Antibody depletion experiments with the +/+ mice showed that the majority of this CTL activity is mediated by $CD8^+$ T cells, but the $CD4^+$ T cells may also be making some contribution (Fig. 5 *d* and Table 1).

Previous experiments with MHV-68 in +/+ mice have shown that the cytokine response to this virus is dominated

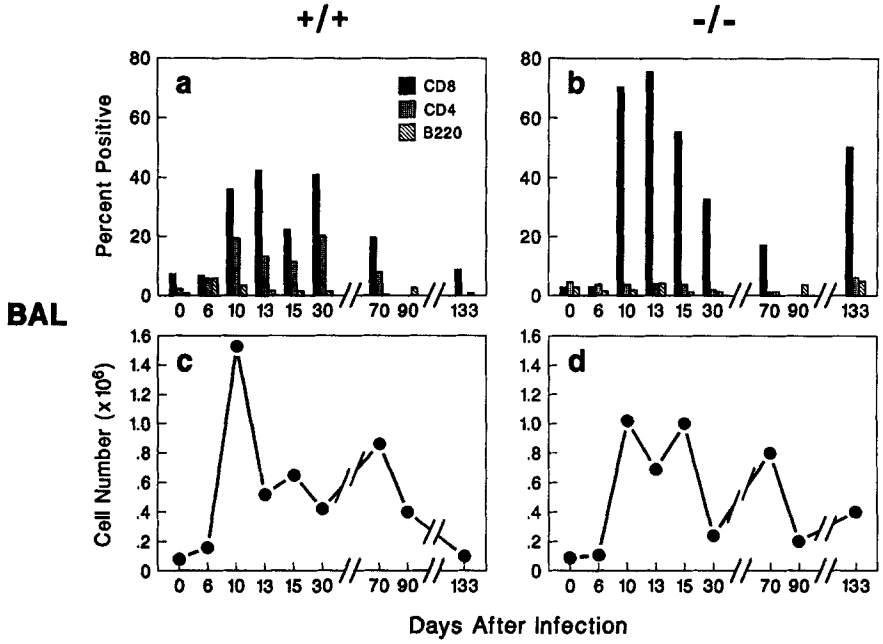

Figure 4. The characteristics of the inflammatory response in the lung are shown for the MHV-68–infected +/+ and −/− mice. The BAL population was collected and pooled from three to five mice per timepoint in single or repeat experiments (see to Fig. 1). After absorbance on plastic dishes, cell numbers were determined (*c* and *d*), and the samples were analyzed by flow cytometry (*a* and *b*).

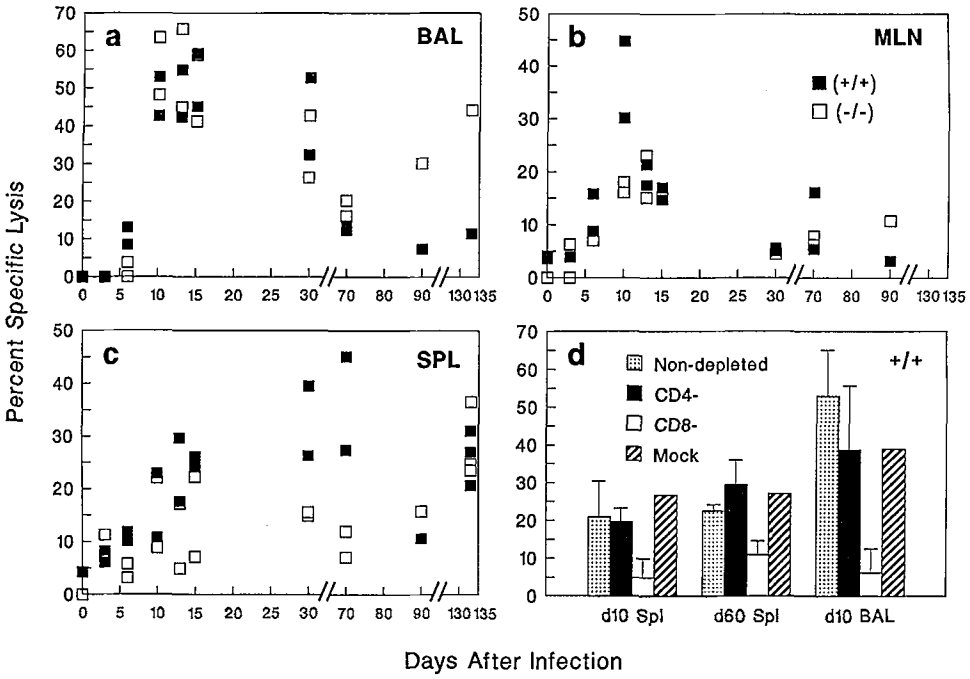

Figure 5. Effector CTL activity from the +/+ (■−) and −/− (□) mice was determined as specific ^{51}Cr release (6-h assay) for freshly isolated BAL, MLN, and spleen populations (*a–c*, respectively) incubated with FcR^+ P815 targets binding the 2C11 mAb to CD3-ϵ (redirected assay), at an E/T of 40:1. Timepoints through 70 d after infection are from two separate experiments with three to five mice per group, the day 90 results are for a single experiment with three to five mice per group, while the day 133 results represent separate assays of three mice per group. (*d*) Freshly isolated BAL and spleen populations were depleted of $CD4^+$ (■) or $CD8^+$ (□) lymphocytes by in vitro treatment with the GK1.5 or 2.43.1 mAbs, respectively, followed by incubation with a cocktail of rabbit and guinea pig complement. Fractions of the cells were either untreated (*nondepleted*) or treated with complement alone (*mock*). Results represent the means (± SD) of three separate experiments for nondepleted, CD4-depleted, and CD8-depleted samples. The mock treatment results are from two separate experiments.

Table 1. *T Cell Depletion of Persistently Infected Mice*

Group	Treatment	Virus titer in lung (log_{10} PF U/ml)	Infectious centers‡			Percent of specific ^{51}Cr release		Cytokines	
			Spleen	MLN	CLN	BAL	Spleen	IL-6	IFN-γ
								U/ml	
−/−	Saline	0.98 ± 0.30	70	194	39	35.0	7.4	9.6	0
Day 84	$CD4^-$	1.43 ± 0.58	60	88	40	ND	18.0	16.2	2.3
	$CD8^-$	3.66 ± 0.58*	170	191	442	ND	7.3	15.3	3.2
−/−	Saline	2.2 ± 0.04	130	(12.4 ± 9.7‡)		43.4	7.7	12.4 ± 16.1	0
Day 114	$CD8^-$	3.7 ± 1.2*	150	(51.2 ± 47.8)		ND	0	30.9 ± 21.5	0
+/+	Saline	0	4	(0)‡		44.5	32.9	9.6 ± 21.5	31.5 ± 37.1
Day 80	$CD4^-$	0	6	(0)		38.4	26.9	22.6 ± 4.7*	8.1 ± 6.8
	$CD8^-$	0	52	(1.6 ± 0.8)*		17.6	10.1	29.7 ± 12.7*	83.5 ± 55.0*

The mice were treated with the GK1.5 mAb to CD4, the 2.43.1 mAb to CD8, or with saline at 2–3-d intervals commencing 10 d before sampling. The percent of $CD8^+$ T cells in the spleens of the CD8-depleted mice were 0.3 (day 84), 0.5 (day 114), and 1.3 (day 80). The CD3ϵ-dependent CTL assay (26, 27) is described in the legend to Fig. 5. Cytokines (mean ± SD) were measured by ELISA of culture supernatants from restimulated spleen cells as described in the legend to Fig. 6.

*Significantly different ($P < 0.05$ or <) from the saline control by Wilcoxon rank analysis (virus data) or, for the cytokine results, Mann U. Whitney rank sum, test or Student's *t* test, depending on whether the data was normally distributed.

‡The day 84 results show infectious centers per 10^7 cells from pooled spleen, MLN, or CLN populations (five mice per group). The results at the other timepoints are for pooled (per 10^7 cells) or individual (per 10^6 cells, mean ± SD, in parentheses) spleen cells. At day 114, −/− results are for five mice (+ saline) and four mice ($CD8^-$), one of which was severely debilitated and was harvested on the previous day. At day 80, the +/+ results are for six mice (+ saline) and five mice ($CD8^-$).

ND, not done because of insufficient cell numbers.

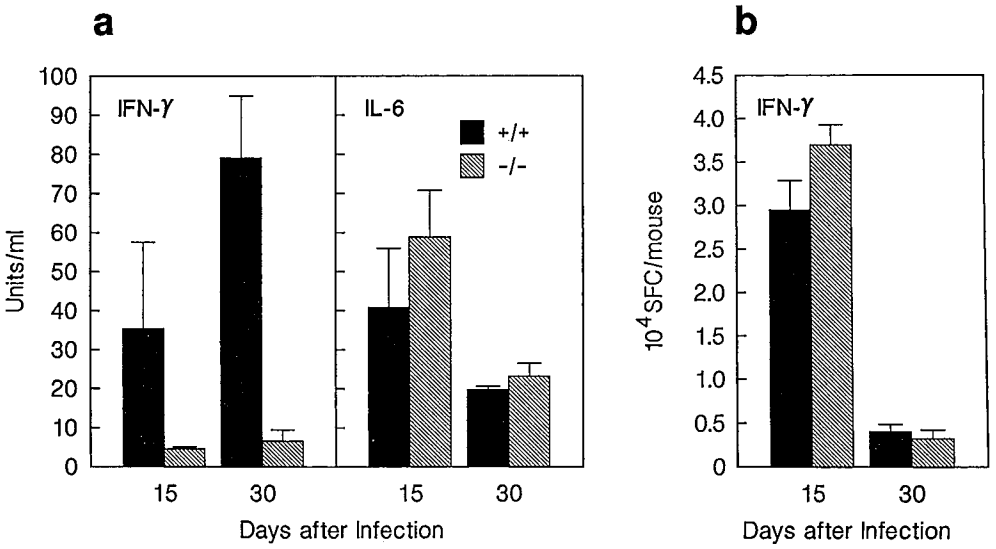

Figure 6. The cytokine production profiles are shown for MHV-68–infected +/+ and −/− mice. Freshly isolated splenocytes from infected +/+ (■) and −/− (▨) mice were cultured with irradiated, MHV-68–infected (MOI = 0.01) splenocytes isolated from uninfected mice as APCs. The lymphocyte cultures were incubated at 37°C and supernatants were collected at 24, 72, and 96 h after infection for detection of cytokine production by ELISA (31). (*a*) IFN-γ and IL-6 were measured in culture supernatants by ELISA. Results are expressed as the mean units of IFN-γ or IL-6 per ml supernatant after 72 h of stimulation. Little or no IL-2, IL-4, or IL-5 was detected. (*b*) Frequencies of IFN-γ–producing cells in BAL populations were determined using the ELISPOT technique (30).

by $CD4^+$ T cells producing IFN-γ and several cell types (including acutely infected B lymphocytes) making IL-6 (reference 31 and unpublished data). This pattern was confirmed in the present study, with the amounts of IFN-γ that could be detected after in vitro restimulation of spleen cells from the −/− mice being much lower, while the levels of IL-6 were comparable for the two groups (Fig. 6 *a*). In contrast to the results for the spleen, the numbers of IFN-γ–producing cells detected by single-cell ELISPOT analysis of the BAL were similar for the +/+ and −/− mice (Fig. 6 *b*). Activated $CD8^+$ T cells are generally considered to produce IFN-γ, but at a lower level than the $CD4^+$ subset. If this ELISPOT analysis is assaying virus-specific effectors, the prevalence in the BAL at the acute phase of MHV-68 infection is in the range of 1:20 for the T cell component in both groups of mice (compare Figs. 4 and 6 *b*). Analyzing sorted $CD8^+$ T cells from the BAL has not been successful (data not shown), perhaps because these highly activated lymphocytes require support from monocyte/macrophages for survival during the 24-h incubation time of the ELISPOT assay (30).

Virus Reactivation and Persistence. Virus was again detected consistently in lungs and adrenals from the −/− mice subsequent to day 22 after the initial challenge, while the only evidence of virus replication in the +/+ group was a single plaque in a lung sample from day 90 (Fig. 1, *a* and *b*). Virus was also detected in the kidneys of one −/− mouse at day 70, even though there was no evidence of virus replication in this site during the acute infection (data not shown). Furthermore, the numbers of latently infected cells in the lymph nodes and spleen (and peripheral blood cells, but not the BM) decreased progressively for the +/+ mice, while the counts stayed high for the −/− group (Fig. 2). Though the lung titers in the −/− group were always at least 10-fold lower than in the acute phase of the disease, these mice developed evidence of wasting and 21 of 25 died by day 133: the results at the late timepoints in Figs. 1–4 are for the few survivors. The +/+ mice all remained clinically normal.

Both the numbers of $CD8^+$ T cells in the BAL (Fig. 4, *b* and *d*) and the levels of CD3ε- dependent CTL activity (Fig. 5 *a*) were much higher for the −/− mice at the day 133 timepoint. This divergence from the +/+ controls was not seen for the spleen (Fig. 5 *c*), which contained significant populations of latently infected B cells in both groups (Fig. 2 *a*). In other virus models (such as influenza) where the virus is rapidly eliminated, this CD3ε-dependent effector function does not persist beyond 2–3 wk after infection (Tripp, R.A., unpublished data). Though the total numbers of $CD8^+$ T cells did not decline (data not shown), restimulation of the −/− spleen cells in vitro after day 30 gave no indication of a significant IFN-γ recall response (Table 1). Spleen cells from +/+ mice, however, continued to produce significant levels of IFN-γ (data not shown), as previously reported (31).

Maintenance then Loss of $CD8^+$ T Cell–mediated Control. Given that the acute phase of MHV-68 infection is terminated by the $CD8^+$ subset, the obvious question was whether the reactivation or reappearance of infectious virus in the lungs and adrenals by day 22 in the −/− mice (Fig. 1) reflected that the $CD8^+$ effectors rapidly become nonfunctional in the absence of $CD4^+$ T cell "help." There is a precedent for thinking that such "immune exhaustion" (34, 35) can occur from experiments with lymphocytic choriomeningitis virus (LCMV).

Depleting the −/− mice with the 2.43.1 mAb to $CD8^+$ commencing at 74 or 104 d after infection established that there is still some $CD8^+$ T cell–mediated control of MHV-

68 at this late stage of the disease process. In the first experiment (day 84, Table 1), removing the $CD8^+$ T cells caused a >40-fold increase in lung virus titers within 10 d of commencing the mAb treatment, compared with a >3-fold effect in −/− mice given the GK1.5 mAb to $CD4^+$ as a control. The repeat experiment showed a >30-fold increase after in vivo elimination of the $CD8^+$ subset in the −/− mice (day 114, Table 1), while no virus was detected in lung sonicates from +/+ controls that were treated concurrently (Table 1). Only the CD8-depleted −/− animals showed any signs of obvious debility and, in the second experiment, the mice were weighed every 2 d to confirm the impression of wasting. All −/− mice exhibited weight loss after treatment with the 2.43.1 mAb, with one decreasing 40% within 6 d. This mouse was sampled 1 d early, and had the highest lung virus titer yet recorded (10^5) during the phase of MHV-68 reactivation (data not shown).

The numbers of latently infected B cells in −/− pooled spleen populations also tended to increase after the removal of the $CD8^+$ subset (day 84 and 114, Table 1), with this treatment being statistically significant for the +/+ mice that were analyzed on day 80 (Table 1). The +/+ mice also showed evidence of reduced CD3ε-dependent CTL activity in the BAL and spleen after both $CD4^+$ and $CD8^+$ T cell depletion, but only the latter treatment had any effect on the frequency of MHV-68^+ B cells, which were still much less prevalent compared to the −/− mice (Table 1). It thus seems that the numbers of persistently infected B cells are being continuously controlled by $CD8^+$, but not $CD4^+$, effectors. However, this $CD8^+$ T cell surveillance function becomes progressively compromised in the long-term absence of the $CD4^+$ subset.

Discussion

No comparable generalized recrudescence of a DNA virus infection has been described for any rodent model. Depleting $CD4^+$ T cells with an appropriate mAb led to defective control of murine cytomegalovirus (CMV) in the salivary gland, and to reactivation after such treatment of latently infected mice (36, 37, 13). Unlike MHV-68 (19), systemic CMV infection can be limited by $CD4^+$ T cells acting in the absence of the $CD8^+$ subset (38). The effector mechanism is IFN-γ dependent, with CMV dissemination during recurrent infection being facilitated by subversion of the specific antibody response. Failure to neutralize MHV-68 could be contributing to the progressive involvement of new B lymphocyte and epithelial cell targets in the MHV-68–infected MHC class II −/− mice. However, the consequences of eliminating the $CD8^+$ subset late in the course of MHV-68 infection are such that, even if the lack of antibody is allowing indolent virus to spread, the extent of the infectious process is still being limited by the $CD8^+$ effector population for some months.

It is known that concurrent $CD4^+$ T help can sometimes, though not always, have a significant effect on the acute clonal expansion and differentiation of the virus-specific $CD8^+$ population (33). The $CD8^+$ T cell response after infection with Herpes simplex virus was substantially diminished by mAb depletion of the $CD4^+$ subset commencing before virus priming (39). The −/− mice used here developed fewer virus-specific $CD8^+$ CTL precursors after infection with an influenza A virus (24), though clearance from the lung was not compromised (24, 40), and the acute CTL response to LCMV and to Sendai virus was substantially normal (41, 42). Results from the influenza experiments suggests that the smaller numbers of CTL precursors were transiently consumed to give a sufficient population of CTL effectors (24). Comparable cytokine profiles were found for −/− mice infected acutely with Sendai virus or influenza virus, though the level of IFN-γ production was much higher for the Sendai model (24, 42).

The requirement for the $CD4^+$ subset to sustain effective $CD8^+$ T cell surveillance in the MHV-68 model is likely to reflect that the "helpers" function continuously to provide a cytokine-rich milieu in responding lymphoid tissue. Restimulation of lymphocyte populations from MHV-68 infected +/+ mice has shown the long-term presence of IFN-γ–producing T cells (31). Little IL-2 is detected, and there is no tendency for IL-4 producers to emerge in the long-term in either the +/+ or −/− mice (data not shown). The cytokines may be promoting continued clonal expansion of the $CD8^+$ responder population, or diminishing the extent of effector T cell loss by (for example) blocking apoptosis. The in vivo dissection of rates of virus-specific T cell production and loss presents major difficulties with even the well-established viral models (43, 44), and is clearly not feasible at this early stage of the analysis with MHV-68.

Like MHV-68 (19), LCMV cannot be eliminated by $CD4^+$ T cells acting in the absence of the $CD8^+$ subset (45, 46). Mice infected with macrophage or lymphotropic variants of LCMV develop a persistent infection, which is eventually cleared in normal mice, but not in CD4-depleted mice (35). The progressive exhaustion of the $CD8^+$ subset in this and in the "high virus dose immune paralysis" model of LCMV infection (34) has obvious parallels to the events that we describe here in the MHC class II −/− mice infected with MHV-68. The differences are that LCMV is known to infect a wide variety of cell types in vivo, (including a percentage of T lymphocytes), but unlike the herpesviruses, this RNA virus has no mechanism for establishing latent infection.

It is not clear whether the MHV-68 replicative phase in the lung and the latently infected B cells in lymphoid tissue are controlled by the same or different sets of virus-specific effectors (47), and will not be understood until at least some of the epitopes recognized by the responding T cells are defined. The mAb depletion experiments with BALB/c mice (19) had already established that the termination of the acute, replicative phase in epithelial sites is mediated by the $CD8^+$ subset. Even so, the larger numbers of MHV-68^+ B cells in the persistently infected −/− mice may reflect that MHC class II–restricted $CD4^+$ effectors operate to limit the extent of infection in the normal +/+ animal.

However, treating the persistently-infected +/+ mice with an mAb to the $CD8^+$ but, not the $CD4^+$, subset increased the numbers of MHV-68^+ B cells. Though $CD4^+$ CTL can be shown to develop after the infection of mice that lack MHC class I glycoproteins with (for example) LCMV and Sendai virus (48, 49), there is currently no evidence that such effectors are functioning directly to eliminate latently infected B cells in the MHV-68 model.

Infection of MHC class II −/− mice with MHV-68 thus offers a unique experimental system for assessing the mechanisms that underlie compromised immune surveillance in a model of viral latency, and for screening possible chemotherapy-, cytokine-, or cell-based protocols to limit γHV-associated disease. This is the first time that we have had the opportunity to analyze experimentally a situation where there is continuing control of a DNA virus by $CD8^+$ effectors. The fact that the progressive escape from effective surveillance by $CD8^+$ T cells is a direct consequence of the absence of the $CD4^+$ subset offers a direct parallel to the situation in AIDS.

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Address correspondence to Dr. Peter Doherty, St. Jude Children's Research Hospital, 332 North Lauderdale, Memphis, TN 38105.

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