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

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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-
lum, 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-2b) 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-2b mice that are constitutively CD4-deficient as a consequence of homozygous disruption (−/−) of the H-2IAb 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 2D mice that are functionally −/− for the H-IAa 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 sonication of 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, incubated overnight at 37°C, and then overlaid with CMC/2% medium. The cells were cultured for 5−6 d, the overlay was removed, and plaque counts were determined as described above. Aliquots of 10° (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 51Cr-labeled FcR+ P815 cells in the presence of the 2C11 mAb to CD3e (26, 27). The level of specific 51Cr 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 G1.1.5 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° 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-
I 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 medastinal 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-2b) mice confirms and extends the conclusions reached from earlier experiments with CD4-depleted BALB/c (H-2b) mice (19, 63).

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 (d). Virus titers are depicted as the mean log₁₀ 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.

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 10⁷ 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. 1a) 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 CD3ε+ T cells by the redirected CTL assay (26, 27), however, showed the emergence of potent effectors in the BAL by day 10 (Fig. 5a). 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), 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. 5d and Table 1).

Previous experiments with MHV-68 in +/+ mice have shown that the cytokine response to this virus is dominated
Figure 5. Effector CTL activity from the +/+ (m-) and −/− (□) mice was determined as specific ⁵¹Cr release (6-h assay) for freshly isolated BAL, MLN, and spleen populations (m-), respectively) incubated with FcR⁺ P815 targets binding the 2C11 mAb to CD3-ε (redirected assay), at an E/T of 40:1. Time-points 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⁺ (m) 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 (log₁₀ PFU/ml) | Infectious centers | Percent of specific ⁵¹Cr release | Cytokines |
|-------|-----------|-----------------------------------|-------------------|-------------------------------|-----------|
|       |           | Spleen | MLN | CLN | BAL | Spleen | IL-6 | IFN-γ |
| −/−   | 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 |
| Day 84 | 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 |
| Day 80 | CD8⁺      | 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 CD3⁺-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 10⁷ cells from pooled spleen, MLN, or CLN populations (five mice per group). The results at the other timepoints are for pooled (per 10⁷ cells) or individual (per 10⁶ 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.
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. 6a).

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. 6b). 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 6b). 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).

**Figure 6.** The cytokine production profiles are shown for MHV-68-infected +/+ and −/− mice. Freshly isolated spleenocytes from infected +/+ ([ ]) and −/− (○) mice were cultured with irradiated, MHV-68-infected (MOI = 0.01) spleenocytes 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).

**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. 5a) were much higher for the −/− mice at the day 133 timepoint. This divergence from the +/+ controls was not seen for the spleen (Fig. 5c), which contained significant populations of latently infected B cells in both groups (Fig. 2a). 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 (105) 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 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 suggest 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. In the 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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