Postexposure vaccination massively increases the prevalence of γ-herpesvirus-specific CD8+ T cells but confers minimal survival advantage on CD4-deficient mice

Gabrielle T. Belz*, Philip G. Stevenson*†, Maria R. Castrucci‡, John D. Altman§, and Peter C. Doherty*¶

*Department of Immunology, St. Jude Children's Research Hospital, Memphis, TN 38105; †Division of Virology, University of Cambridge, Cambridge CB2 2QQ, United Kingdom; ‡Istituto Superiore di Sanita, 00161 Rome, Italy; and §Emory Vaccine Center, and Department of Microbiology and Immunology, Emory University School of Medicine, Atlanta, GA 30322

Contributed by Peter C. Doherty, December 27, 1999

Mice that lack CD4+ T cells remain clinically normal for more than 60 days after respiratory challenge with the murine γ-herpesvirus 68 (γHV-68), then develop symptoms of a progressive wasting disease. The γHV-68-specific CD8+ T cells that persist in these I-A$^{b-/-}$ mice are unable to prevent continued, but relatively low level, virus replication. Postexposure challenge with recombinant vaccinia viruses expressing γHV-68 lytic cycle epitopes massively increased the magnitude of the γHV-68-specific CD8+ population detectable by staining with tetrameric complexes of MHC class I glycoprotein + peptide, or by interferon-γ production subsequent to *in vitro* restimulation with peptide. The boosting effect was comparable for γHV-68-infected I-A$^{b-/-}$ and I-A$^{b+/+}$ mice within 7 days of challenge, and took more than 110 days to return to prevaccination levels in the I-A$^{b+/+}$ controls. Although the life-span of the I-A$^{b-/-}$ mice was significantly increased, there was no effect on long-term survival. A further boost with a recombinant influenza A virus failed to improve the situation. Onset of weight loss was associated with a decline in γHV-68-specific CD8+ T cell numbers, though it is not clear whether this was a cause or an effect of the underlying pathology. Even very high levels of virus-specific CD8+ T cells thus provide only transient protection against the uniformly lethal consequences of γHV-68 infection under conditions of CD4+ T cell deficiency.

The murine γ-herpesvirus 68 (γHV-68) is related (1, 2) both to Epstein–Barr virus (EBV) and to human herpesvirus 8 (HHV-8). These three γHVs are characterized by the establishment of life-long latency in B lymphocytes, subsequent to (at least for γHV-68 and EBV) a transient phase of lytic infection in the oropharynx and respiratory epithelium. Increased levels of γHV replication and tumorigenesis (EBV and HHV-8) are common consequences (3) of AIDS induced by HIV. It is far from clear whether the escape of these persistent γHVs from immune control in AIDS patients is a direct reflection of the compromised CD4+ T cell response (4–6), or is because of the subsequent decline in virus-specific CD8+ T cell numbers.

Mice that lack CD4+ T cells as a consequence of homozygous disruption of the H2-IA^b gene (I-A$^{b-/-}$) remain healthy for 2–3 mo after respiratory challenge with γHV-68, then develop symptoms of chronic wasting disease (7–9). Unlike the situation for congenic I-A$^{b+/+}$ mice, the γHV-68 lytic phase in lung epithelium is never completely controlled. Apparently, both CD4+ and CD8+ effectors are important, with the CD4+ set operating by means of γ-interferon (IFN-γ) production (9) and the CD8+ T cells by perforin/granzyme or Fas/Fas ligand-mediated cytotoxicity (10). Virus-specific CD8+ T cells can still be detected at a level of 3–5% in the lymphoid tissue of clinically compromised I-A$^{b-/-}$ mice (8), and, unlike the situation for persistent lymphocytic choriomeningitis virus (LCMV) infection in the absence of CD4+ T cells (11), these lymphocytes are functional in the sense that they can produce IFN-γ after *in vitro* stimulation with a high dose of the cognate peptide.

Immunizing naive I-A$^{b+/+}$ mice by a prime and boost protocol with recombinant vaccinia (vacc) and influenza virus vectors expressing a prominent, lytic phase γHV-68 peptide generated massive numbers of virus-specific CD8+ T cells (12). The greatly enhanced response following γHV-68 challenge of these selectively primed mice controlled virus replication in the respiratory tract but had little effect on the establishment of latency. We ask here whether vaccinating after the establishment of γHV-68 will limit the extent of reactivation to lytic infection in I-A$^{b-/-}$ mice and thus prevent the late-onset wasting disease. Such an approach of this type might be thought useful for minimizing γHV recrudescence and tumorigenesis in AIDS patients. There are some indications that postexposure boosting with HIV vaccines can improve prognosis (13), but this possibility has not been addressed for the intercurrent infections that often cause mortality in AIDS patients.

Materials and Methods

Mice and Sampling. Female C57BL/6J (B6, I-A$^{b+/+}$) mice (The Jackson Laboratory), and MHC class II-deficient (I-A$^{b-/-}$) mice were kept under specific pathogen-free conditions at St. Jude Children's Research Hospital (Memphis, TN). The mice were anesthetized with Avertin and infected intranasally (i.n.) with 600 plaque-forming units (pfu) of γHV-68 at 8–12 wk of age (7). Lymphocyte populations were obtained from anesthetized, exsanguinated mice by peritoneal lavage (PEL), bronchoalveolar lavage (BAL), or by disruption of the spleen. The lungs were removed, homogenized, and freeze-thawed for virus titration.

Recombinant Viruses Expressing γHV-68 Epitopes. Oligonucleotides encoding (14) AGPHNDMEI (p56) or NTSINFVKI (p79 plus the NH_2-terminal flanking asparagine) were expressed (15, 16) in vaccinia viruses as described (12). Mice were infected i.p. with 3×10^7 pfu of vacc-p56 or vacc-p79, or with a control vaccinia recombinant (vacc-LCMV^b) that expresses an H-2D^b-restricted LCMV nucleoprotein (NP) peptide (17). The recombinant WSN-p56 influenza virus (12) was generated by reverse genetics

Abbreviations: γHV-68, γ-herpesvirus 68; BAL, bronchoalveolar lavage; EBV, Epstein–Barr virus; HHV-8, human herpesvirus 8; IFN-γ, γ-interferon; i.n., intranasal; LCMV, lymphocytic choriomeningitis virus; NP, nucleoprotein; PEL, peritoneal exudate lymphocytes; vacc, recombinant vaccinia virus; pfu, plaque-forming unit; LDA, limiting dilution assay; PE, phycoerythrin.

¶To whom reprint requests should be addressed. E-mail: peter.doherty@stjude.org.

The publication costs of this article were defrayed in part by page charge payment. This article must therefore be hereby marked "*advertisement*" in accordance with 18 U.S.C. §1734 solely to indicate this fact.

Article published online before print: *Proc. Natl. Acad. Sci. USA*, 10.1073/pnas.040575197. Article and publication date are at www.pnas.org/cgi/doi/10.1073/pnas.040575197

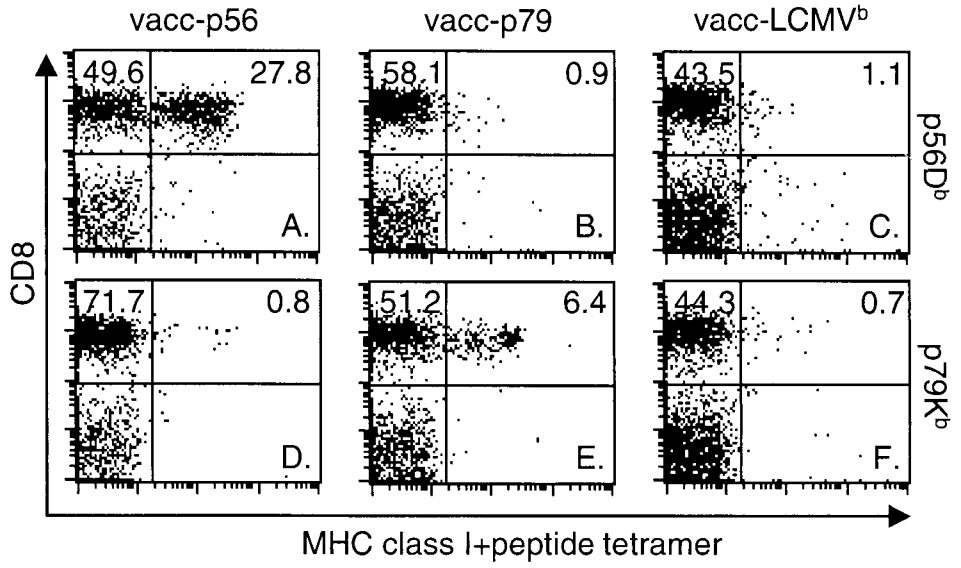

Fig. 1. Virus-specific CD8$^+$ T cells in spleen populations from γHV-68-infected I-A$^{b+/+}$ mice challenged with recombinant vaccinia viruses. The B6 mice were challenged i.p. with 3×10^7 pfu of the vacc-p56, vacc-p79, or vacc-LCMV^b recombinants 1 mo after i.n. infection with 600 pfu of γHV-68. Spleen populations were enriched for the CD8$^+$ set and stained with the p56D^b (*A–C*) and p79K^b (*D* and *E*) tetramers. The results are for a representative animal from each group, with the percent values in the quadrants being for the total lymphocyte population. Cumulated data from three comparable experiments are presented in Table 1.

(18, 19) and plaque-purified on MDCK cells. Influenza A/WSN with the LCMV NP H-2L^d epitope (WSN-LCMV^d) inserted (19) into the neuraminidase stalk was used as an irrelevant control. Mice were infected i.p. with 5×10^5 pfu of WSN-p56 or WSN-LCMV^d.

Virus Titration. An established limiting dilution (LDA) technique (8, 12, 20, 21) utilized congenic (H-2^b) embryonic fibroblasts (2214-CRL; American Type Culture Collection) seeded (10^4/well) overnight into 96-well plates. Dilutions (2- to 3-fold) of lung homogenate in DMEM + 0.1% BSA were added to 16 replicate wells. The monolayer cultures were maintained for 12–13 days in DMEM supplemented with penicillin (100 units/ml), streptomycin (100 μg/ml), gentamicin (10 μg/ml), glutamine (2 mM), and 2.5% FCS (HyClone), then fixed in methanol, stained with Giemsa, and observed for cytopathic effects. Titers were determined by regression plot of the fraction of wells with no cytopathic effect vs. $\log_{10}$ dilution.

Virus titers in lymphoid tissue were determined similarly, except that dilutions of single cell suspensions were added to the monolayers without disruption, and the cultures were maintained for 17–18 days. This variant of the LDA protocol is considered to measure latency by assaying the extent of reactivation (22) to lytic infection (8, 20, 21).

Flow Cytometry. Lymphocytes were washed in PBS/BSA (0.1%)/azide (0.01%) and incubated for 30 min at room temperature with tetrameric complexes (14, 23) of phycoerythrin (PE)-labeled H-2D^b + p56 (p56D^b) or H-2K^b + p56 (p79K^b), then stained on ice with a tricolor-conjugated mAb to CD8α (Caltag, Burlingame, CA). The cells were washed once after a 30-min incubation on ice, and analyzed (24) on a FACScan using CELLQUEST software (Becton Dickinson). The CD8$^+$ set was enriched magnetically (25) from splenic lymphocyte populations by first incubating with mAbs (PharMingen) to I-A^b (M5/114.15.2) and CD4 (GK1.5) mAb, followed by sheep anti-rat Ig and sheep anti-mouse Ig-coated magnetic beads (Dynal, Oslo). Those obtained by PEL or BAL were depleted of macrophages by adherence to plastic.

Pepγ Assay for Peptide-Specific CD8$^+$ T Cells. The CD8$^+$ T cell populations were cultured for 5 h in RPMI supplemented with penicillin (100 units/ml), streptomycin (100 μg/ml), glutamine (2 mM), FCS (10%), and 5 μg/ml Brefeldin A (Epicentre Technologies, Madison, WI), in the presence or absence of 1 μM of the p56 or p79 peptide (8, 14). The cells were then stained with the FITC-conjugated mAb to CD8α (PharMingen), fixed in 1% formaldehyde, permeabilized with 0.5% saponin, and stained with a PE-conjugated mAb to IFN-γ (PharMingen). The percent CD8$^+$IFN-γ$^+$ without peptide (<0.5%) was subtracted from the percent CD8$^+$IFN-γ$^+$ with peptide to give the percent specific CD8$^+$ T cells.

Table 1. γHV-68-specific CD8$^+$ T cells in the peritoneal cavity and spleen 7 days after i.p. challenge of HV-68-infected mice with recombinant vaccinia viruses

	Tetramer		Peptide stimulation	
Immunization	p56D^b	p79K^b	p56	p79
PEL				
vacc-LCMV^b	1.4 ± 0.9	1.7 ± 0.8	0.3 ± 0.2	1.4 ± 0.6
vacc-p56	24.9 ± 6.9*	0.8 ± 0.3*	38.4 ± 8.9*	0.9 ± 0.1
vacc-p79	1.1 ± 0.4*	13.7 ± 2.6	0.2 ± 0.3	38.5 ± 15.4
Spleen				
vacc-LCMV^b	1.1 ± 0.4	1.6 ± 0.1	0.35 ± 0.2	1.2 ± 0.1
vacc-p56	15.2 ± 2.1	1.5 ± 0.2	15.3 ± 1	1 ± 0.1
vacc-p79	0.9 ± 0.3	12.4 ± 6	0.2 ± 0.2	17.1 ± 9.2

The B6 (I-A$^{b+/+}$) mice had been infected i.n. with 600 pfu of γHV-68 4 wk prior to i.p. challenge with 3×10^7 pfu of the recombinant vaccinia viruses. The CD8$^+$ T cells in the PEL and spleen populations were either stained with the tetramers or were restimulated for 5 h *in vitro* before analyzing for intracellular IFN-γ. Each value is the mean ± SD for the percent in the CD8$^+$ set in three separate experiments. The results for those that were given vacc-LCMV^b are equivalent to the virus-specific CD8$^+$ T cell frequencies found normally in γHV-68-infected I-A$^{b+/+}$ mice (12, 14).

*The CD8$^+$ T cells in the PEL (and spleen) samples recovered from mice that had never been exposed to γHV-68 were 2.9% (2.1%) p56D^{b+} and 4.5% (2.2%) IFN-γ$^+$ at 7 days after vacc-p56 infection, while <1% in either site were shown to be p79-specific after the comparable i.p. challenge with vacc-p79.

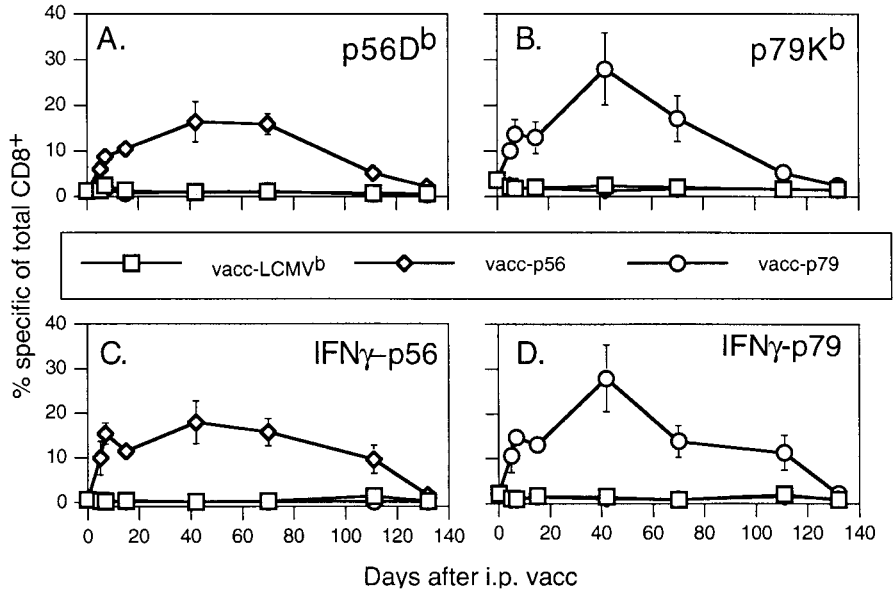

Fig. 2. Persistence of p56- and p79-specific CD8$^+$ T cells in boosted, γHV-68-infected I-A$^{b+/+}$ mice. The mice were infected i.n. with γHV-68 1 mo prior to i.p. challenge with the recombinant vaccinia viruses, as detailed in the Fig. 1 legend. Each data point shows the mean ± SE for three individuals. The titers of latent γHV-68 in the spleen were minimal for all groups.

Results

Postexposure Vaccination of I-A$^{b+/+}$ Mice. Mice that had been infected i.n. 1 mo previously with 600 pfu of γHV-68 were given 3×10^7 pfu of vacc-p56, vacc-p79, or vacc-LCMV^b i.p. and assayed 7 days later (12). This challenge with vaccinia recombinants expressing either the p56 or p79 γHV-68 peptides greatly increased the prevalence of CD8$^+$ T cells specific for the cognate tetramer in splenic lymphocyte populations (Fig. 1 *A* and *E*), while the values for the alternate peptide (Fig. 1 *B* and *D*) were comparable to those detected in γHV-68-infected mice injected with the control vacc-LCMV^b (Fig. 1 *C* and *F*). Enhanced frequencies were seen for the p56D^{b+} and p79K^{b+} CD8$^+$ sets in both the PEL and spleen, with broadly comparable values being recorded from both tetramer staining and the Pepγ assay (Table 1). The prevalence was higher in the PEL, presumably reflecting that the peritoneal cavity is the site of inflammatory pathology following i.p. infection (Table 1).

This single, high-dose challenge with the vacc-p56, vacc-p79, or vacc-LCMV^b recombinants had no deleterious effect. All the

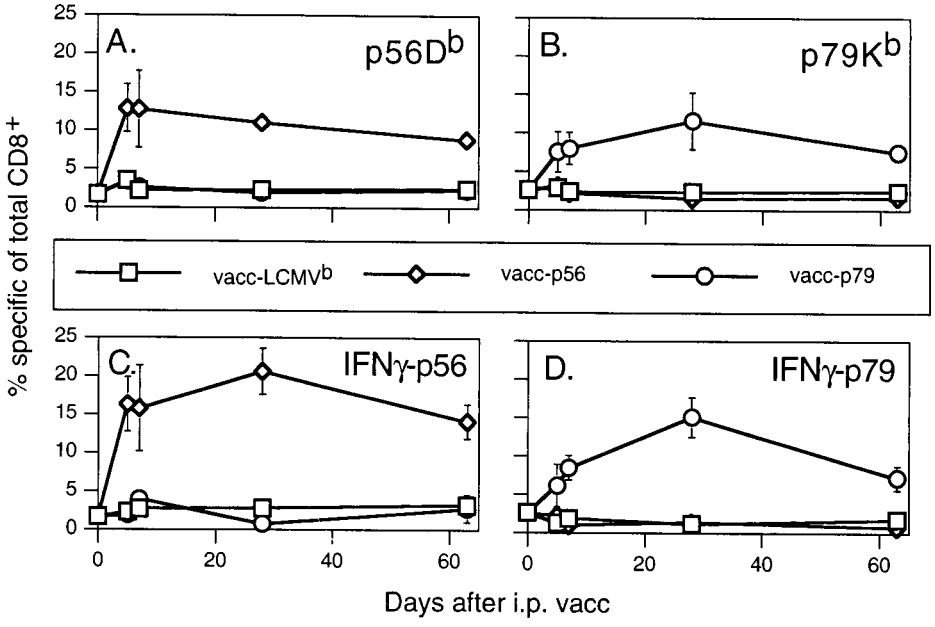

Fig. 3. The γHV-68-specific CD8$^+$ T cell responses following secondary challenge of persistently infected I-A$^{b-/-}$ mice. The mice were infected with γHV-68, then challenged with the vacc-p56, vaccp79, and vacc-LCMV^b recombinants, as described in the Fig. 1 legend. The results are given as mean ± SE for virus-specific CD8$^+$ T cells in the spleens of three mice. Those sampled on day 63 after vaccinia (day 91 after γHV-68) were clinically affected and provide some of the virus titer results presented in Table 2.

Table 2. γHV-68 titers in persistently infected I-A$^{b-/-}$ mice given recombinant vaccinia viruses 63 days previously

Immunization	$\log_{10}$ virus titer	
	Spleen	Lung
vacc-LCMV^b	3.4 ± 0.2	3.0 ± 0.2
vacc-p56	2.8 ± 0.2*	2.6 ± 0.1
vacc-p79	3.1 ± 0.1	2.8 ± 0.1

The mice were infected i.n. with γHV-68, then challenged i.p. with the recombinant vaccinia viruses 28 days later, as described in the Fig. 1 legend. Titers of lytic (lung) and latent (spleen) virus were determined by LDA on embryonic fibroblast monolayers and expressed as mean ± SE $\log_{10}$ for six individuals. The limit of detection was $10^{2.4}$ for the lung and $10^{1.7}$ for the spleen. These virus titration experiments are for groups of six mice, while another study with groups of three mice gave comparable results. The tetramer staining results from three of these mice are presented for the day-63 time point in Fig. 3.

*Significantly less ($P > 0.01$) than the value for the vacc-LCMV^b controls. All the other differences were not significant ($P > 0.05$).

Table 3. Capacity of reisolated virus to generate a γHV-68-specific CD8$^+$ T cell response

Viral isolates from I-A$^{b-/-}$ mice given	% Tetramer$^+$ mean ± SD†		Ratio H:L Mean ± SD‡	
	p56	p79	p56	p79
vacc-LCMV^b	16.0 ± 4	17.7 ± 6	1.5 ± 0.3	2.3 ± 0.9
vacc-56	13.8 ± 3	18.2 ± 3.7	1.50 ± 2	1.3 ± 0.3
vacc-p79	15.8 ± 3.5	16.8 ± 7	1.4 ± 0.3	2.5 ± 1.2

*Clones of γHV-68 were isolated by LDA from the lungs of I-A$^{b-/-}$ mice that had been infected i.n. with γHV-68, boosted with the recombinant vaccinia viruses, and sampled 63 days later, as described in the Fig. 1 legend and Table 2. Individual, naive B6 mice were then infected i.n. with 40:1 of each γHV-68 isolate, with no attempt being made to determine the actual amount of virus that was given.

†The BAL cells were collected 13 days after infection and stained for CD8α and p56D^b or p79K^b. The results show the mean ± SD for 15–18 I-A$^{b-/-}$ mice, reflecting the analysis of 2–3 viral isolates from each of six I-A$^{b-/-}$ mice. The variability was such that none of these differences was statistically significant at the 5% level.

‡Ratios of the highest to lowest values for the 2–3 isolates were calculated for the individual mice, then expressed as mean ± SD for the particular group.

mice remained clinically normal for at least 140 days (Fig. 2). The remarkable feature, when compared with the situation for pathogens that are readily eliminated, like the influenza A viruses (26), is that the p56- and p79-specific CD8$^+$ T cell numbers in spleen remained at high levels for at least 70 days, and did not return to the relatively low frequencies detected normally in I-A$^{b+/+}$ mice persistently infected with γHV-68 (8, 14) for more than 100 days (Fig. 2). We have not yet developed an approach that allows the *in vivo* measurement of γHV-68 reactivation from latency in normal mice, but it does seem likely that there is continuing restimulation with these lytic-phase epitopes.

Boosting γHV-68-Specific CD8$^+$ T Cells in the I-A$^{b-/-}$ Mice. Challenging the γHV-68-infected, CD4$^+$ T cell-deficient I-A$^{b-/-}$ mice with vacc-p56 and vacc-p79 also led to substantial, prolonged increases in prevalence for the p56D^b- and p79K^b-specific sets (Fig. 3). As with the response to γHV-68 alone (8, 14), this expansion of virus-specific CD8$^+$ T cells induced by a single, large dose of a recombinant vaccinia virus (Fig. 3) seemed to be independent of CD4$^+$ T help for both the initial clonal burst and for the maintenance of high-frequency p56D^b- or p79K^b-reactive populations through at least the subsequent 60 days. Exposure to these vaccinia viruses did not compromise the γHV-68-infected I-A$^{b-/-}$ mice in any obvious way, and they remained healthy for at least an additional month.

The increase in p56D^b-specific CD8$^+$ T cell frequency in the γHV-68-infected I-A$^{b-/-}$ mice was associated with a decrease (relative to the vacc-LCMV^b control) in the extent of virus latency in the spleen, but the effect was small and the titers of lytic virus in the lung were not significantly modified (Table 2). The greater numbers of p79K^b-specific cells did not lead to any improvement in the control of persistent, active γHV-68 infection in these I-A$^{b-/-}$ mice. Though the challenge with vacc-p56 (but not vacc-p79) was associated with a significant increase in survival time (Fig. 4), the virus titration studies (Table 2) were not pursued further because all mice eventually succumbed to the γHV-68-induced wasting disease.

Virus isolates were cloned from the persistently infected, boosted I-A$^{b-/-}$ mice to determine whether the continued, high prevalence of γHV-68-specific CD8$^+$ T cells had selected for escape mutants (Table 3). Normal B6 mice were infected i.n. with at least 15 isolates from each of the vacc-LCMV^b, vacc-p56, and vacc-p79 groups, and individual BAL samples were assayed 13 days later for the presence of CD8$^+$p56D^{b+} and CD8$^+$p79K^{b+} lymphocytes. No significant differences were found for the γHV-68 isolates recovered from persistently infected I-A$^{b-/-}$ mice that had been challenged i.p. with vacc-LCMV^b, vacc-p56, or vacc-p79 (Table 3). Thus, though the six I-A$^{b-/-}$ mice sampled from each group all showed some degree of clinical impairment, there was no indication that the development of symptoms reflected the emergence of viral variants that were no longer recognized by the CD8$^+$ effectors.

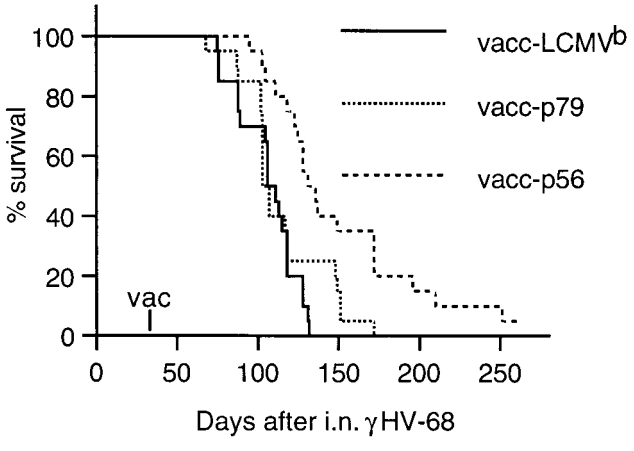

Fig. 4. Survival profiles for γHV-68-infected I-A$^{b-/-}$ mice boosted with recombinant vaccinia viruses. The I-A$^{b-/-}$ mice were infected i.n. with γHV-68 and boosted with the recombinant vaccinia viruses, as described in the Fig. 1 legend. The survival profiles were then determined for groups of 20 mice, with any animals that were severely affected being terminated. Vaccinia virus infection alone did not cause any mortality in I-A$^{b-/-}$ mice, and the pattern of illness in the vacc-LCMV^b group was indistinguishable from that for other controls given γHV-68 alone (data not shown). The mice given vacc-p56 lived significantly longer ($P = 0.0003$ by logrank test) than those injected with vacc-p79 or vacc-LCMV^b.

Disease Status and γHV-68-Specific CD8$^+$ T Cell Numbers. We then asked whether the limited protection conferred by postexposure vaccination of γHV-68-infected I-A$^{b-/-}$ mice (Fig. 4) could be enhanced by i.p. boosting with both vacc-p56 and vacc-p79, followed by i.p. challenge with WSN-p56 (Table 4). However, unlike the situation described previously for vacc-p56-primed mice that had not yet encountered γHV-68 (12), the further stimulation with WSN-p56 had no additional effect on virus-

Table 4. Comparison of apparently normal and clinically compromised γHV-68-infected I-A$^{b-/-}$ mice that had been boosted with vacc-p79, vacc-p56, and WSN-p56

Group	Body weight (g)	Log_{10} virus titer		$CD8^+$	
		Spleen	Lung	p56D^{b+}	p79K^{b+}
Healthy	21.0 ± 0.7*	2.2 ± 0.3*	3.1 ± 0.1*	11.9 ± 1.4*	3.9 ± 1.6
Sick	13.4 ± 1.6	2.7 ± 0.1	3.6 ± 0.2	4.5 ± 1.8†	2.8 ± 1.6†

The data were derived from I-A$^{b-/-}$ mice that were infected i.p. with 5×10^5 pfu of γHV-68, boosted i.p. 32 days later with 3×10^7 pfu of both vacc-p79 and vacc-p56, then infected i.p. after an additional 39 days with WSN-p56. The tetramer results are for the splenic $CD8^+$ set. The ''healthy'' mice looked clinically normal, whereas the ''sick'' animals were hunched and had ruffled fur. The six healthy and four sick mice were sampled at 138 (4 and 2), 151 (1 and 1), and 171 (1 and 1) days after the original γHV-68 challenge. The Pepγ assay was also used at day 151 and day 171. The percent $CD8^+$ T cells staining with the p56D^b tetramer and for IFN-γ after stimulation with the p56 peptide were, respectively: Healthy, 9.9 and 8.9, 9.5 and 10.2; Sick, 7.6 and 6.4, 3.5 and 2.9. The comparable results for p79-specific $CD8^+$ T cells were: Healthy, 3.5 and 1.8, 2.8 and 1.9; Sick, 5.5 and 3.2, 2.4 and 1.0.

*Significantly different ($P < 0.03$) from the result for the ''Sick'' group.

†These values were no different from those for γHV-68-infected, I-A$^{b-/-}$ controls, some of which were also given vacc-LCMV^b and WSN-LCMV^d. The cumulated results (% $CD8^+$ in spleen) for control mice sampled (groups of two to four) at intervals from 71–139 days after the initial exposure to γHV-68 were: p56D^{b+}, 3.9 ± 1.7; p79K^{b+}, 2.2 ± 0.9. The significantly ($P < 0.01$) greater frequencies in healthy, boosted (vacc-p56, vacc-p79, WSN-p56) mice sampled at the same time points were: p56D^{b+}, 12.9 ± 3.2; p79K^{b+}, 5.2 ± 2.2. The results for the controls were identical to previously published data (12).

specific $CD8^+$ T cell numbers (compare Fig. 3 and Table 4) and the mice still developed severe symptoms. Evidence of clinical impairment was associated with a slight, but consistent increase in virus-titers, and a fall in the prevalence of the $CD8^+$p56D^{b+} set to the levels found in γHV-68-infected I-A$^{b-/-}$ mice that had not been boosted (see footnote to Table 4).

Discussion

The I-A$^{b-/-}$ mice lack both $CD4^+$ T cells and a high-quality, class-switched antibody response (27). The fact that persistently infected I-A$^{b+/+}$ mice remain clinically normal following *in vivo* mAb depletion of the $CD4^+$ and $CD8^+$ subsets is considered to reflect protection mediated by circulating antibody (28). The same experiment with convalescent Ig$^{-/-}$ μMT mice led to increased γHV-68 latency in the spleen, greater evidence of replicative infection in the respiratory tract, and 30% mortality within 21 days of commencing the mAb treatment (9). A prominent site of γHV-68 latency in the μMT mice is the macrophage (21).

Persistently infected μMT mice seem to remain relatively normal (20, 29), whereas the I-A$^{b-/-}$ mice eventually die (7). The presence of B lymphocytes means that the pool of latently infected cells will be larger in the I-A$^{b-/-}$ mice, which also lack IFN-γ-producing $CD4^+$ effectors (9, 30, 31). The $CD8^+$ T cells have the capacity to secrete IFN-γ (14), but either the amount made is insufficient or their targeting to MHC class I glycoprotein + peptide does not allow them to fulfill the same role as the MHC class II-restricted $CD4^+$ subset. Recent experiments with a different viral model have shown that cytokine production by the $CD8^+$ set is transient, and ceases rapidly after dissociation between the T cell receptor and the targeted MHC class I glycoprotein (32). At the time that the I-A$^{b-/-}$ mice are wasting, there are still significant numbers (at least 3–5% of the $CD8^+$ set) of virus-specific, IFN-γ competent T cells in the lymphoid tissue (8). The idea that the total amount of IFN-γ produced may be important could explain why the massive expansion of the virus-specific $CD8^+$ population mice induced by the vacc-p56 recombinant in the γHV-68-infected led to some increase in survival time.

The continued (but relatively low level) reactivation to lytic infection in the I-A$^{b-/-}$ mice presumably results in the cumulative development of virus-induced cytopathology, leading to the progressive dysfunction of one or more key organs. The partial control by $CD8^+$ CTL is clearly enhanced by increasing the availability of these effectors, but the numbers eventually fall and a threshold is reached where the extent of irreversible damage ensures the development of symptoms. The mice do not obviously die from pneumonitis or from the thickening of the large arteries that has been described for IFN-γ receptor$^{-/-}$ mice given a large dose of γHV-68 by the i.p. route (31), though they can develop such vascular lesions. Despite the fact that γHV-68 has been shown to replicate in a variety of anatomical sites, including the adrenals (7), the nature of the underlying pathology is not clearly understood.

Much has been written about the possible role of persisting antigen in the long-term maintenance of virus-specific $CD8^+$ T cells (33–35). Previous comparison of γHV-68-infected I-A$^{b+/+}$ and I-A$^{b-/-}$ mice established that the higher virus load in the I-A$^{b-/-}$ group was indeed associated with the continued presence of more $CD8^+$ p56D^b-specific T cells in the lymphoid tissue, with this population still constituting 3–4% of the total $CD8^+$ pool (8). It is indeed remarkable that postexposure challenge of either the I-A$^{b+/+}$ or I-A$^{b-/-}$ mice with a vacc-p56 or vacc-p79 recombinant can increase the prevalence of the respective virus-specific $CD8^+$ set to >10% in the very long term. These vaccinia recombinants tend to cause a fairly limited infectious process, which is generally controlled within 5–7 days (36).

The perturbation in virus-specific $CD8^+$ T cell numbers caused by giving a single dose of a recombinant vaccinia virus to persistently infected mice reinforces earlier impressions that epitope expression in cells infected with γHV-68 is in some sense suboptimal (37). Perhaps IFN-γ acts to enhance antigenicity (38), though the protective effect of IFN-γ is still apparent in mice depleted of $CD8^+$ T cells (9). The HVs are known to use a variety of strategies to interfere with peptide presentation by MHC class I glycoproteins (39, 40), but nothing is yet known about γHV-68 in this regard.

The recruitment of $CD8^+$ T cells may also be modified by the viral M3 protein, which has been shown recently to have chemokine binding properties (41). It is likely that these large complex viruses have evolved a spectrum of mechanisms that allow persistence in the face of a continuing host response (42, 43), many of which have yet to be defined. The present analysis shows that the lethal consequences of removing one key element of immunity is not readily reversed by enhancing another.

We thank Joe Miller for technical help and Vicki Henderson for assistance with the manuscript. These experiments were supported by National Institutes of Health Grants AI29579 and CA21765, and The American Lebanese Syrian Associated Charities. G.T.B. is a C.J. Martin Fellow of the Australian National Health and Medical Research Council (Fellowship regkey 977 309).

1. Efstathiou, S., Ho, Y. M., Hall, S., Styles, C. J., Scott, S. D. & Gompels, U. A. (1990) *J. Gen. Virol.* **71,** 1365–1372.
2. Virgin, H. W., Latreille, P., Wamsley, P., Hallsworth, K., Weck, K. E., Dal, C. A. & Speck, S. H. (1997) *J. Virol.* **71,** 5894–5904.
3. Mueller, N. (1999) *J. Acquired Immune Defic. Syndr.* **21,** Suppl. 1, S5–S10.
4. Haynes, B. F., Pantaleo, G. & Fauci, A. S. (1996) *Science* **271,** 324–328.
5. Jassoy, C. & Walker, B. D. (1997) *Springer Semin. Immunopathol.* **18,** 341–354.
6. Rosenberg, E. S., Billingsley, J. M., Caliendo, A. M., Boswell, S. L., Sax, P. E., Kalams, S. A. & Walker, B. D. (1997) *Science* **278,** 1447–1450.
7. Cardin, R. D., Brooks, J. W., Sarawar, S. R. & Doherty, P. C. (1996) *J. Exp. Med.* **184,** 863–871.
8. Stevenson, P. G., Belz, G. T., Altman, J. D. & Doherty, P. C. (1998) *Proc. Natl. Acad. Sci. USA* **95,** 15565–15570.
9. Christensen, J. P., Cardin, R. D., Branum, K. C. & Doherty, P. C. (1999) *Proc. Natl. Acad. Sci. USA* **96,** 5135–5140.
10. Doherty, P. C., Topham, D. J., Tripp, R. A., Cardin, R. D., Brooks, J. W. & Stevenson, P. G. (1997) *Immunol. Rev.* **159,** 105–117.
11. Zajac, A. J., Blattman, J. N., Murali-Krishna, K., Sourdive, D. J., Suresh, M., Altman, J. D. & Ahmed, R. (1998) *J. Exp. Med.* **188,** 2205–2213.
12. Stevenson, P. G., Belz, G. T., Castrucci, M. R., Altman, J. D. & Doherty, P. C. (1999) *Proc. Natl. Acad. Sci. USA* **96,** 9281–9286.
13. Balter, M. (1999) *Science* **286,** 1470–1471.
14. Stevenson, P. G., Belz, G. T., Altman, J. D. & Doherty, P. C. (1999) *Eur. J. Immunol.* **29,** 1059–1067.
15. Restifo, N. P., Bacik, I., Irvine, K. R., Yewdell, J. W., McCabe, B. J., Anderson, R. W., Eisenlohr, L. C., Rosenberg, S. A. & Bennink, J. R. (1995) *J. Immunol.* **154,** 4414–4422.
16. Mackett, M., Smith, G. L. & Moss, B. (1984) *J. Virol.* **49,** 857–864.
17. Yanagi, Y., Tishon, A., Lewicki, H., Cubitt, B. A. & Oldstone, M. B. (1992) *J. Virol.* **66,** 2527–2531.
18. Enami, M., Luytjes, W., Krystal, M. & Palese, P. (1990) *Proc. Natl. Acad. Sci. USA* **87,** 3802–3805.
19. Castrucci, M. R., Hou, S., Doherty, P. C. & Kawaoka, Y. (1994) *J. Virol.* **68,** 3486–3490.
20. Weck, K. E., Barkon, M. L., Yoo, L. I., Speck, S. H. & Virgin, H. W. (1996) *J. Virol.* **70,** 6775–6780.
21. Weck, K. E., Kim, S. S., Virgin, H. I. & Speck, S. H. (1999) *J. Virol.* **73,** 3273–3283.
22. Sunil-Chandra, N. P., Efstathiou, S. & Nash, A. A. (1992) *J. Gen. Virol.* **73,** 3275–3279.
23. Altman, J. D., Moss, P. A. H., Goulder, P. J. R., Barouch, D. H., McHeyzer-Williams, M. G., Bell, J. I., McMichael, A. J. & Davis, M. M. (1996) *Science* **274,** 94–96.
24. Tripp, R. A., Hou, S. & Doherty, P. C. (1995) *J. Immunol.* **154,** 5870–5875.
25. Hou, S., Hyland, L., Ryan, K. W., Portner, A. & Doherty, P. C. (1994) *Nature (London)* **369,** 652–654.
26. Flynn, K. J., Riberdy, J. M., Christensen, J. P., Altman, J. D. & Doherty, P. C. (1999) *Proc. Natl. Acad. Sci. USA* **96,** 8597–8602.
27. Stevenson, P. G. & Doherty, P. C. (1999) *J. Virol.* **73,** 1075–1079.
28. Stevenson, P. G., Cardin, R. D., Christensen, J. P. & Doherty, P. C. (1999) *J. Gen. Virol.* **80,** 477–483.
29. Usherwood, E. J., Stewart, J. P., Robertson, K., Allen, D. J. & Nash, A. A. (1996) *J. Gen. Virol.* **77,** 2819–2825.
30. Dutia, B. M., Clarke, C. J., Allen, D. J. & Nash, A. A. (1997) *J. Virol.* **71,** 4278–4283.
31. Weck, K. E., Dal Canto, A. J., Gould, J. D., O'Guin, A. K., Roth, K. A., Saffitz, J. E., Speck, S. H. & Virgin, H. W. (1997) *Nat. Med.* **3,** 1346–1353.
32. Slifka, M. K., Rodriguez, F. & Whitton, J. L. (1999) *Nature (London)* **401,** 76–79.
33. Zinkernagel, R. M., Bachmann, M. F., Kundig, T. M., Oehen, S., Pirchet, H. & Hengartner, H. (1996) *Annu. Rev. Immunol.* **14,** 333–367.
34. Doherty, P. C., Topham, D. J. & Tripp, R. A. (1996) *Immunol. Rev.* **150,** 23–44.
35. Ahmed, R. & Gray, D. (1996) *Science* **272,** 54–60.
36. Rolph, M. S. & Ramshaw, I. A. (1997) *Curr. Opin. Immunol.* **9,** 517–524.
37. Stevenson, P. G. & Doherty, P. C. (1998) *J. Virol.* **72,** 943–949.
38. Hengel, H., Esslinger, C., Pool, J., Goulmy, E. & Koszinowski, U. H. (1995) *J. Gen. Virol.* **76,** 2987–2997.
39. Johnson, D. C. & Hill, A. B. (1998) *Curr. Top. Microbiol. Immunol.* **232,** 149–177.
40. Ploegh, H. L. (1998) *Science* **280,** 248–253.
41. Parry, C. M., Simas, J. P., Smith, V. P., Stewart, C. A., Minson, A. C., Efstathiou, S. & Alcami, A. (2000) *J. Exp. Med.,* **191,** 573–578.
42. Spriggs, M. K. (1996) *Annu. Rev. Immunol.* **14,** 101–130.
43. Strockbine, L. D., Cohen, J. I., Farrah, T., Lyman, S. D., Wagener, F., DuBose, R. F., Armitage, R. J. & Spriggs, M. K. (1998) *J. Virol.* **75,** 4015–4021.