Pathogenesis of an Infectious Mononucleosis-like Disease Induced by a Murine \( \gamma \)-Herpesvirus: Role for a Viral Superantigen?

By Ralph A. Tripp,* Ann Marie Hamilton-Easton,* Honda D. Cardin,* Phuong Nguyen,* Frederick G. Behm,‡ David L. Woodland,*∥ Peter C. Doherty,*∥ and Marcia A. Blackman*∥

Summary

The murine \( \gamma \)-herpesvirus 68 has many similarities to EBV, and induces a syndrome comparable to infectious mononucleosis (IM). The frequency of activated CD8+ T cells (CD62Llo) in the peripheral blood increased greater than fourfold by 21 d after infection of C57BL/6j (H-2b) mice, and remained high for at least a further month. The spectrum of T cell receptor usage was greatly skewed, with as many as 75% of the CD8+ T cells in the blood expressing a V\( \beta \)4+ phenotype. Interestingly, the V\( \beta \)4 dominance was also seen, to varying extents, in H-2d, H-2k, and H-2s strains of mice. In addition, although CD4 depletion from day 11 had no effect on the V\( \beta \)4 bias of the T cells, the V\( \beta \)4+CD8+ expansion was absent in H-2A\( ^{b} \)-deficient congenic mice. However, the numbers of cycling cells in the CD4 antibody–depleted mice and mice that are CD4 deficient as a consequence of the deletion of MHC class II, were generally lower. The findings suggest that the IM-like disease is driven both by cytokines provided by CD4+ T cells and by a viral superantigen presented by MHC class II glycoproteins to V\( \beta \)4+CD8+ T cells.

The murine \( \gamma \)-herpesvirus 68 (MHV-68) is classified as a type 2 \( \gamma \)-herpesvirus (\( \gamma \)HV; references 1, 2), along with Herpesvirus saimiri (3), and a novel \( \gamma \)-HV that has recently been implicated in Kaposi’s sarcoma (4–6). However, the disease process induced in mice infected intranasally with MHV-68 is much more similar to the syndrome associated with prototypic human type 1 \( \gamma \)HV, EBV in people (7), than to that caused by the T lymphotrophic \( \gamma \)HV, \( \gamma \)HV that has recently been implicated in Kaposi’s sarcoma (4–6). How-

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*Abbreviations used in this paper: \( \gamma \)HV, \( \gamma \)-herpesvirus; CTLp, CTL precursor; IM, infectious mononucleosis; MHV-68, murine \( \gamma \)-herpesvirus 68; MMTV, mouse mammary tumor virus.

Peter C. Doherty and Marcia A. Blackman made equal contributions to this manuscript.
Virus Stocks. The original stock of MHV-68 (clone G2.4) was obtained from Dr. A.A. Nash (Edinburgh, U.K.) as a cell-free lysate derived from infected baby hamster kidney cells. This was then propagated in owl monkey kidney fibroblasts (ATCC 1566CR L; American Type Culture Collection, Rockville, MD).

Infection and Sampling. Anesthetized (Avertin, 2,2,2,tribromoethanol) mice were infected intranasally with 400 PFU of MHV-68 at 6–10 wk of age, and sampled at various times after infection. Blood was obtained from the axilla or retroorbital sinus of anesthetized mice.

Cell Cycle Analysis. The cell cycle analysis of CD8+ T lymphocytes was performed as previously described (20). In brief, cells were stained with FITC-conjugated antibodies to CD8α (53-6.72; PharMingen), permeabilized with ethanol, and the DNA was stained with propidium iodide. The cells were analyzed in two-color mode on the FACScan (Becton Dickinson, San Jose, CA) using Modfit/Winlist software (Verity Software Inc., Topsham, ME). The program determines the status of individual CD8+ lymphocytes as being in G0/G1, S, or G2 + M by estimating the DNA content of intact nuclei.

In Vivo T Cell Dilution and Flow Cytometry. B6 mice were depleted of CD4+ T cells by in vivo treatment with GK1.5 mAb at 2–3-d intervals, as previously described (21, 22), beginning 11 d after infection. Depletion was assessed by flow cytometry using RM 4-4, which is not blocked by GK1.5 (PharMingen). Activated CD8+ lymphocytes were assessed by two-color staining with CD62L (MEL-14-FITC), or CD44 (IM7-FITC) and CD8α (53-6.72-PE) (PharMingen). Lymphocytes were phenotyped using mAb to B220, CD4 (GK1.5), and CD8α (53-6.72) (PharMingen). TCR- Vβ usage was assessed using a panel of mAbs specific for Vβ 2–14, as previously described (23, 24).

Results

Respiratory Infection with MHV-68 Induces an IM-like Syndrome in B6 Mice. Previous data have established that respiratory challenge of C57BL/6J (B6) mice with MHV-68 results in acute viral infection that is eliminated by CD8+ T cells by day 13. Persistent, latent infection is established in B cells, which is accompanied by a significant splenomegaly (9, 12, 13, 25). In the current studies, the profile of PBL was analyzed at various stages of infection. Interestingly, the data showed a greater than fourfold increase in the frequency of activated/memory (26–29) CD8αCD44hiCD62Llo PBL from day 21 after infection (Fig. 1 A), which is reminiscent of the IM that is frequently (∼50% of cases) a consequence of EBV infection in humans (30). This IM-like blood picture was also seen in mice that had been thymectomized at the later time points (days 18 and 25 after infection, Fig. 2 B), corresponding to the expansion of CD8+CD62Llo T cells in the PBL, first observed at day 21 after infection (Fig. 1 A).

There are several possible mechanisms to explain virus-induced massive proliferation in lymphoid tissue that could lead to an IM-like profile in the blood. First, it is well known that viral infection results in clonal expansion of cytokotoxic T lymphocyte precursors (CTLp) specific for viral peptides + class I MHC glycoproteins (31, 32). However, it has been shown that not all of the activated CD8+ T cells in the peripheral blood of EBV-induced IM patients are virus specific (30, 33). Thus, additional mechanisms must contribute to the CD8+ T cell expansion. One possibility is cytokine-induced bystander activation. For example, it has been shown that cytokines contribute to T cell proliferation during viral infection (20, 34, 35). Another possibility is superantigen-driven T cell proliferation. Superantigens are potent stimulatory molecules secreted by microbial pathogens that cause Vβ-specific T cell expansion (36). The existence of an EBV-encoded superantigen has been suggested by some groups (36–38), but remains controversial (39–41).

Vβ Profile of Activated T Cells. As a first step in determining the mechanism of T cell activation, the TCR-Vβ profile was examined. The results showed a striking predominance of Vβ4+ T cells among the CD8+ T cells in the
peripheral blood, and a compensatory decrease in all other V\text{b}s (Fig. 3 A). This V\text{b}4 expansion was also seen in splenic CD8\text{1} T cells (Fig. 3 B), but not in peripheral lymph nodes (data not shown). The V\text{b}4 expansion among the CD8\text{1} peripheral blood T cells was not evident in the first 2 wk of infection, but was consistently seen at day 21 after infection, although the magnitude of the increase varied in individual B6 mice (Fig. 4 A). A variable and much reduced V\text{b}4 expansion was also transiently observed in CD4\text{1} T cells from peripheral blood (Fig. 4 B) and spleen (data not shown). Elevated levels of V\text{b}4\text{1}CD8\text{1} T cells were still observed at 90 d after infection (Fig. 4 A).

This marked TCR skewing could reflect a dominant V\text{b}4 usage of H-2K\text{b}– or H-2D\text{b}–restricted MHV-68–specific CD8\text{1} CTLp. Repertoire analysis of CTL specific for a variety of viruses shows that diversity in the recognition of a particular peptide–MHC class I glycoprotein can range from very limited to very diverse (24, 42–46). Preferential usage of a single TCR-\alpha/\beta pair has been described for a long-term EBV–specific CTL response (47), and recently, oligoclonal expansion of T cells in IM patients was reported (39). There is currently no virus-specific CTL assay developed for MHV-68. However, stimulation of T cells with MHV-68, by a standard limiting dilution protocol, showed that the prevalence of microcultures containing effectors capable of CD3-\text{e}–dependent CTL activity (48, 49) ranged from 1:500–1:2000 (Tripp, R.A., and P.C. Doherty, unpublished observations), comparable to the level described for other viruses (20). However, these CTLp frequencies may reflect a gross underestimate if highly activated CD8\text{1} T cells are being driven to apoptosis after in vitro stimulation, as has been described for EBV (50, 51).

Alternatively, the V\text{b}4 bias might be the consequence of a superantigen-driven response, as superantigens stimulate T cells in a V\text{b}–specific manner. Superantigens bind to MHC class II (52–58), but, in contrast to T cell recognition of conventional viral antigen, T cell responses to superantigens are generally not MHC restricted (58–60). Therefore, as a first step in examining a role for a viral superantigen in the T cell activation, we examined the MHC haplotype dependence of the elevated V\text{b}4 pattern seen in B6 mice. TCR profiles were determined for MHV-68–infected H-2k, H-2d, H-2u, and H-2q mice. The data show a clear V\text{b}4 expansion among CD8\text{1} PBL (Table 1), but not CD4\text{1} PBL (data not shown) in mouse strains representing each of the haplotypes. Therefore, although we are currently unable to rule out the possibility of a conventional viral peptide that promiscuously binds to MHC class I (61), the data suggest that the V\text{b}4 expansion seen during the IM phase of MHV-68 infection is driven by a virally-encoded superantigen.
Role of MHC Class II and CD4 in IM. Previous studies established that the splenomegaly characteristic of MHV-68 infection is greatly diminished in mice that lack CD4^+ T cells (9–12) and MHC class II glycoproteins (13). Therefore, we analyzed CD4-deficient MHC class II^−/− mice for evidence of IM. The results showed that both the extent of MHV-68–induced CD8^+ T cell proliferation in the spleen (Fig. 5A) and the relative prevalence of CD8^+ CD62L^− T cells in the blood (Fig. 5, B and C) were much lower than in the MHC class II^+/+ controls. Furthermore, the pattern of TCR-γ 1-Vβ4^+ CD8^+ T cell dominance associated with the increase in frequency for the CD8^+ CD62L^− set in the PBL (Fig. 5A) was not observed for MHC class II^−/− mice (Fig. 5 C). These differences between the MHC class II^+/+ and −/− mice could reflect the absence of the H-21Aβ MHC class II glycoprotein, perhaps because of a requirement for MHC class II presentation of a viral superantigen (62), and/or because of a role for cytokines produced by MHV-68-immune CD4^+ T cells in the antiviral CD8^+ T cell response (63–65). To test this, +/- mice were treated in vivo with the GK1.5 mAb to CD4 from day 11 after virus challenge. The data show that this treatment de-

### Table 1. Vβ4 Expression among CD8^+ T Cells in MHV-68–infected Mice

<table>
<thead>
<tr>
<th>Mouse strain</th>
<th>MHC haplotype</th>
<th>% Vβ4^+ CD8^+</th>
<th>Days after infection</th>
</tr>
</thead>
<tbody>
<tr>
<td>C57BL/6</td>
<td>H-2^b</td>
<td>3.9 ± 0.2</td>
<td>46.8 ± 12.6</td>
</tr>
<tr>
<td>BALB/c</td>
<td>H-2^d</td>
<td>9.0 ± 1.2</td>
<td>29.2 ± 8.9</td>
</tr>
<tr>
<td>B10.BR</td>
<td>H-2^k</td>
<td>3.0 ± 0.8</td>
<td>11.1 ± 3.3</td>
</tr>
<tr>
<td>B10.PL</td>
<td>H-2^d</td>
<td>4.4 ± 1.6</td>
<td>22.0 ± 8.1</td>
</tr>
<tr>
<td>B10.Q</td>
<td>H-2^d</td>
<td>3.9 ± 0.9</td>
<td>8.4 ± 2.4</td>
</tr>
</tbody>
</table>

*PBL were obtained from control mice or MHV-68–infected mice at the indicated time points after infection. The percentage of Vβ4^+ CD8^+ T cells among total CD8^+ T cells was determined by two-color flow cytometry using biotinylated mAb specific for TCR-Vβ4^+ (KT4) and FITC-conjugated mAb specific for CD8 (53-6.72), using standard protocols.

M HV-68–induced CD8^+ T cell proliferation in the spleen (Fig. 5 A) and the relative prevalence of CD8^+ CD62L^− T cells in the blood (Fig. 5, B and C) were much lower than in the MHC class II^+/+ controls. Furthermore, the pattern of TCR-γ 1-Vβ4^+ CD8^+ dominance associated with the increase in frequency for the CD8^+ CD62L^− set in the PBL (Fig. 5 A) was not observed for MHC class II^−/− mice (Fig. 5 C). These differences between the MHC class II^+/+ and −/− mice could reflect the absence of the H-21Aβ MHC class II glycoprotein, perhaps because of a requirement for MHC class II presentation of a viral superantigen (62), and/or because of a role for cytokines produced by MHV-68-immune CD4^+ T cells in the antiviral CD8^+ T cell response (63–65). To test this, +/- mice were treated in vivo with the GK1.5 mAb to CD4 from day 11 after virus challenge. The data show that this treatment de-
creased the extent of CD8+ T cell cycling in the spleen from ~28% in intact mice to ~16 and 9% at days 17 and 23 after infection, respectively (Table 2). However, >80% of the blood CD8+ T cells still showed the characteristic IM-like CD62Llo profile, and there was little effect on the prevalence of TCR-Vβ4+ T cells in the CD8+ PBL at 23 d after infection (Table 2). These data raise the possibility that the high frequency of Vβ4+CD8+ T cells in the peripheral blood is a consequence of selective protection from apoptosis rather than a direct expansion. However, the data are most consistent with selective expansion of Vβ4+CD8+ T cells. First, although cycling is dramatically reduced, it is not eliminated, and is still elevated compared with naive animals, in which <5% of CD8+ T cells are cycling (Fig. 5A and data not shown). In particular, ~16% of CD8+ T cell in the spleen are cycling at day 17 in CD4-depleted animals, a time point just before the dramatic increase in percentage of Vβ4+CD8+ T cells (Fig. 4). Second, there is no evidence for the massive reduction in numbers of CD8+ T cells that would be necessary to account for the compensatory increase in Vβ4+CD8+ T cells (Table 2). Thus, the data suggest that eliminating >90% of the CD4+ T cells through the time that the IM-like phase of MHV-68 infection is developing did not prevent the emergence of the prominent TCR-Vβ4+CD8+CD62Llo population. Cytokines derived from the CD4+ population are not, therefore, primarily responsible for the selective expansion of the Vβ4+CD8+ T cells.

However, there does appear to be a role for CD4+ T cells in the pathogenesis of IM. The numbers of cycling CD8+ T cells in the spleen were much lower in both the MHC class II −/− (Fig. 5A) and CD4-depleted mice (Table 1). In addition, the frequency of the CD8+ PBL was not significantly increased as a consequence of infection in either group of CD4-deficient, MHV-68-infected mice (Fig. 5C and Table 2). It should be noted that the elevated CD8 frequency in the MHC class II −/− mice is evident before infection, and thus reflects a compensatory increase because of the lack of CD4+ T cells rather than an increase as a consequence of infection. These data suggest that although CD4+ T cells are not involved in the specific Vβ4 expansion, they play a part in the generalized proliferation and activation of the CD8+ subset. Thus, the IM phase of MHV-68 infection appears to be a consequence of at least two separate activation events: a generalized, CD4-dependent, presumably cytokine-driven expansion that precedes, but does not control, the later, perhaps superantigen-driven, expansion of Vβ4+CD8+ T cells. The relationship between cell cycling in the spleen and activated CD8+ T cells in the peripheral blood is likely to be complex, but is an important issue for understanding the pathogenesis of IM, and thus warrants further investigation.

**Discussion**

Initial characterization of MHV-68 has revealed striking biological similarities to EBV. For example, major aspects of the pathogenesis of viral infection are similar in humans and mice, including the initial acute respiratory infection and the establishment of viral latency in B cells (9–12).

In the current studies, we describe a syndrome of T cell activation that occurs late in infection, well after the clearance of infectious virus and the establishment of latent infection in B cells. The activated T cells in the peripheral blood are predominantly CD8+ and express a CD62Llo-, CD44lo-activated phenotype, with as many as 75% of the CD8+ T cells expressing Vβ4+ TCR. These activated cells are a reflection of a more generalized activation in the enlarged spleen, in which CD4+ and B220+ cells are also activated. The activated phenotype is sustained in vivo for >2 mo (Figs. 1 and 4). This pathology has two key features in common with EBV-induced IM, namely, activated CD8+ T cells in the peripheral blood and splenomegaly. Taken together with previously identified similarities between MHV-68 and EBV infection (10), the similarities in the IM

<table>
<thead>
<tr>
<th>Day</th>
<th>CD4 depletion</th>
<th>% CD8+ T cells in PBL</th>
<th>Spleen</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Total</td>
<td>CD62Llo</td>
</tr>
<tr>
<td>17</td>
<td>−</td>
<td>34.1 ± 10.1</td>
<td>26.9 ± 7.2</td>
</tr>
<tr>
<td></td>
<td>+</td>
<td>33.3 ± 6.6</td>
<td>25.6 ± 6.7</td>
</tr>
<tr>
<td>23</td>
<td>−</td>
<td>34.8 ± 5.6</td>
<td>32.9 ± 5.6</td>
</tr>
<tr>
<td></td>
<td>+</td>
<td>38.8 ± 9.2</td>
<td>35.9 ± 9.0</td>
</tr>
</tbody>
</table>

*B6 mice were injected with the GK1.5 mAb to CD4 at 2-d intervals starting from day 11 after infection with MHV-68.

† The number of CD8+ T cells in the PBL was relatively constant (7-11 × 10⁶/ml) in all experiments.

‡ Cytokine in CD8+ T cells from naive animals is <5%.

§ Significantly different from the corresponding values in intact mice (P <0.01) by Wilcoxon rank analysis.

CD4 staining on residual cells was downmodulated.
profile strengthen the relevance of MHV-68 as an experimental mouse model for EBV.

The availability of a mouse model of a γ-herpesvirus-induced IM promises to be a valuable tool in understanding the pathogenesis of the disease. For example, these initial studies show that the T cell–activation profile is unaltered in adult thymectomized mice, suggesting that availability of newly emerging thymocytes is not necessary for IM. In another example, we have been able to establish the relationship between initial infection and the onset of IM. Although this has been difficult to ascertain in EBV-induced IM because, in most cases, IM is the first clinical evidence of infection, the mouse model clearly indicates that the onset of IM is a late event in the viral infection, beginning as early as 2–3 wk after infection, well after the clearance of infectious virus from the lung. Finally, it has been long known that activated CD8+ T cells characteristic of EBV-induced IM are not all virus-specific, and the cause of the lymphoproliferation has been extensively investigated. There are conflicting reports supporting a role for viral antigens (17, 39, 66), superantigens (37, 38), and nonspecific activation (17, 18, 67) in the generation of activated CD8+ T cells. An intriguing possibility suggested by the late kinetics of CD8+ T cell activation is that the IM is a consequence of new viral antigen expression during the establishment of latency. The MHV-68 virus model will allow us to directly address the mechanism of CD8+ T cell activation. Although we don’t yet know whether these T cells are specific for virus, our current studies suggest that both a TCR-mediated event, characterized by Vβ4+CD8+ T cell expansion, and a generalized T cell activation that is probably mediated by CD4-dependent cytokines, are involved in the MHV-68-induced IM.

The selective expansion of Vβ4+CD8+ T cells described in this report has two intriguing characteristics. First, the effect is not MHC-restricted, in that Vβ4 expansion is seen among activated CD8+ T cells in the peripheral blood and spleen of MHV-68–infected mouse strains expressing five different MHC haplotypes. Second, the Vβ4+CD8+ expansion is MHC class II–dependent because it is not observed in MHC class II−/− mice, but does occur in mice that were depleted of CD4+ T cells by in vivo antibody administration. There are two possible explanations for the MHC-unrestricted, Vβ4+ T cell expansion. The first possibility is an oligoclonal T cell response to a conventional viral antigen. The MHC promiscuity could be explained by the existence of a dominant epitope capable of binding multiple class I haplotypes, as has been described (61). The second possibility is superantigen-driven T cell activation. We believe the data are most consistent with a superantigen-driven response since Vβ-specific, class II–dependent, MHC-unrestricted stimulation of T cells are hallmarks of superantigen activation. As additional support of this, the response appears to be relatively independent of the α chain of the TCR (Blackman, M.A., unpublished observations) and T cell responses to superantigens are characteristically independent of the non-Vβ components of the TCR (68).

Despite the fact that the Vβ4-specific T cell activation fits several basic criteria for superantigen-driven responses, it is unusual that CD8+ T cells are preferentially activated. Superantigens characteristically stimulate both CD4+ and CD8+ T cells (69, 70), or in the case of weak superantigens, CD4+ T cells are preferentially activated (71). It is possible that the putative MHV-68 superantigen preferentially activates CD8+ T cells. In support of this possibility, a superantigen-like activity in Toxoplasma gondii was shown to selectively activate CD8+ T cells under some experimental conditions (72, 73). It is also possible that MHV-68 activates both CD4+ and CD8+ T cells, but the CD8+ T cells are preferentially driven to apoptosis, resulting in the selective retention of the CD8+ T cells. Selective apoptosis of CD4+ T cells after staphylococcal enterotoxin B stimulation has been described (74). This possibility is consistent with the modest elevation of Vβ4+CD4+ T cells seen in some experiments (Fig. 4B). Thus, although we cannot eliminate the possibility that the Vβ4+CD8+ expansion represents a restricted response to a conventional viral peptide with promiscuous MHC class I binding, the Vβ-specific, MHC class II–dependent, non–MHC-restricted, TCR-α chain–independent Vβ4+CD8+ activation during the IM phase of MHV-68 infection is most readily explained by the expression of a viral superantigen.

One trivial explanation for the Vβ4 expansion is that the effect is mediated by a retroviral superantigen. Perhaps the MHV-68 infection is activating an endogenous superantigen or the virus stock is contaminated with superantigen-expressing murine retroviruses. Although we cannot formally rule out these possibilities, we think that they are unlikely. First, no endogenous retrovirus specific for Vβ4+ T cells has been identified. Also, if the mice were harboring an unknown Vβ4-specific endogenous retrovirus, it would be expected that Vβ4+ T cells would be deleted in these mouse strains, which was not observed (Table 1). Recently, an exogenous mouse mammary tumor virus (MMTV) that activates Vβ4+ T cells was described in the Swiss IBM mouse strain (75). However, this MMTV was shown to require the presence of MHC class II I–E molecules, which are absent in B6 mice, for efficient superantigen presentation. Second, with regard to the possibility that the MHV-68 virus stock is contaminated with murine retroviruses, plaque-purified virus has been cultivated in owl monkey kidney fibroblast cells, which would not be permissive for MMTV replication and would not be a source for introduction of MMTV into the MHV-68 virus stock. In addition, the delayed and sustained expansion of Vβ4+ T cells seen after infection with MHV-68 are not consistent with the kinetics of superantigen expression during a typical MMTV infection (76).

Earlier studies have suggested the presence of a viral superantigen expressed by EBV. Smith et al. described a Vβ-specific expansion in the peripheral blood of patients with IM, which was absent in two cases examined after resolution of the acute phase of the disease (37). Other studies, however, have not shown a Vβ-specific expansion in vivo during IM nor after in vitro stimulation with EBV-infected cells (40, 41). More recently, Sutkowski et al.
reported an MHC class II-dependent, but MHC-unrestricted proliferation of naïve T cells in response to EBV-transformed B cells in which the virus was reactivated (38). Analysis of early activation markers indicated a selective Vβ13 activation. In contrast, in another recent study, Callan et al. showed clonal or oligoclonal populations of activated T cells in the peripheral blood of IM patients, in which the Vβ-specific expansion varied among individuals, suggesting viral antigen-driven proliferation (39). It should be noted that variation in the Vβ profile from individual patients does not in itself rule out a role for a viral superantigen. It is possible that there is sequence variation in different isolates, analogous to MMTV, resulting in expansion of different Vβ populations of T cells. However, in support of a conventional virus-specific response, Callan et al. showed that, in some cases, the specificity of the β chain was identical to that identified in EBV-reactive T cell clones by an independent group (39).

There are also reports of viral superantigens in other herpesviruses. For example, evidence for a CMV-encoded superantigen has been reported (77). In addition, an open reading frame of the H. saimiri genome, ORF 14, which has significant homology to a mouse mammary tumor virus-encoded superantigen (78), encodes a protein that binds to MHC class II molecules and stimulates T cell proliferation, although the Vβ profile of the proliferating T cells has not been reported (79).

It is interesting to speculate on the role of a putative superantigen in MHV-68 infection. In the case of MMTV superantigens, expression of the viral superantigen is essential for productive infection (62, 80–82). MHC class II molecules on virally infected B cells present a superantigen that activates a subset of T cells expressing the appropriate Vβ element. The activated T cells subsequently promote the proliferation and differentiation of infected B cells, resulting in clonal expansion and the establishment of memory cells that serve as a stable reservoir for the virus (81). It is clear from our studies that expression of the putative superantigen is not essential for infection or the establishment of latency of MHV-68 because MHC class II −/− mice still became infected and established latency (13), although they did not exhibit IM (Fig. 5). It is possible that superantigen-driven T cell activation is required for expansion of the pool of latently infected B cells. A role for a putative EBV superantigen in establishing an equilibrium between latency and activation, resulting in the maintenance of a constant EBV burden, has been postulated (38). In this light, more detailed studies of latency and malignancy in MHV-68-infected B6 and MHC class II −/− mice should be revealing (13). An intriguing feature of the putative MHV-68 viral superantigen is that the effect on the TCR-Vβ4+ CD8+ T cells is not apparent by day 14, when all lytic virus has been cleared from epithelial sites, and only becomes obvious from ~2 wk after evidence of latent MHV-68 infection is first detected in B lymphocytes. It is likely that the putative superantigen is a viral gene product that binds to MHC class II glycoproteins on the surface of persistently infected B cells but, if this is so, the kinetics of IM development suggest either that the protein is not expressed during the acute stage of the disease, or that the emergence of the TCR-Vβ4+ CD8+ T cells is in some way inhibited during the time that the effectors that deal with the lytic phase of the infection are operating. One possible scenario is that the superantigen is not expressed on infected epithelial cells during acute infection, but can be expressed on B cells during reactivation of latent virus.

In conclusion, we have described an IM phase of MHV-68 infection of mice characterized by at least two activation events: a CD4-dependent expansion that is presumably cytokine dependent, and a preferential expansion of Vβ4+ CD8+ T cells, perhaps driven by a viral superantigen. This M HV thus provides a valuable experimental model for understanding the pathogenesis of IM and the equilibrium between the lytic and latent stages of herpesvirus infection.

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**References**


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