Absence of a functional defect in CD8+ T cells during primary murine gammaherpesvirus-68 infection of I-Aβ−/− mice

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The murine gammaherpesvirus-68 kills I-Aβ−/− mice despite the presence of virus-specific CD8+ cytotoxic T lymphocytes (CTL). This has raised the possibility that these CTL are functionally abnormal. Here, no difference was observed between I-Aβ−/− mice and I-Aβ+/+ controls in virus-specific CTL function, T cell receptor usage, or surface phenotype. Thus CTL immunity was independent of CD4+ T cells in a chronic herpesvirus infection, but was still inadequate to control virus replication.

CD8+ cytotoxic T lymphocytes (CTL) are thought to be important in the control of persistent viruses. The fact that latent herpesviruses emerge to cause disease when CD4+ T cells are depleted, for example in human immunodeficiency virus (HIV) infection, has consequently focused attention on the possible requirement of CD8+ T cell immunity for CD4+ T cell help. Current thinking, at least for HIV-specific CD8+ T cells, is that a loss of the CD4+ T cell subset leads to impaired CTL function (McMichael & Rowland-Jones, 2001). Specifically, cytokine secretion is maintained while cytotoxicity is lost (Appay et al., 2000). In mice, the spectacular exhaustion of virus-specific CD8+ T cells by lymphocytic choriomeningitis virus, which ranges from physical deletion to a more subtle functional impairment (Zajac et al., 1998), is also more pronounced in the absence of CD4+ T cells.

Murine gammaherpesvirus-68 (MHV-68) persists in immunocompetent mice after intranasal infection without causing overt disease (Virgin & Speck, 1999; Nash et al., 2001), but is lethal to CD4+ T cell-deficient I-Aβ−/− mice after 3–4 months due to chronic lytic virus replication (Cardin et al., 1996). CTL control the acute infection in I-Aβ−/− mice. However, even though the CTL specific for two immunodominant MHV-68 lytic cycle epitopes (Stevenson et al., 1999) are maintained numerically during the chronic illness (Stevenson et al., 1998), they cannot suppress lytic replication completely, even when boosted to high levels by post-exposure, epitope-specific vaccination (Belz et al., 2000). These boosted MHV-68-specific CTL were recently shown to be functionally abnormal (Liu et al., 2002). There was both decreased expression of CD44 and reduced cytotoxicity in comparison with I-Aβ+/+ controls. This has raised the possibility that our earlier analysis of MHV-68 infection in otherwise unmanipulated I-Aβ−/− mice missed some subtle defect in the virus-specific CD8+ T cell response. We have thus looked in more detail at this question of CD8+ T cell functional integrity for MHV-68-specific CTL generated in conventional and CD4+ T cell-deficient mice.

Previously, we documented the persistence of MHV-68-specific CD8+ T cells in I-Aβ−/− mice by MHC class I/peptide tetramer staining and by peptide-induced IFN-γ production (Stevenson et al., 1998). However, recent data (Appay et al., 2000; Liu et al., 2002) have raised the possibility that such criteria are not sufficient to rule out functional deficits. Surface phenotypes may change and the capacity for cytotoxicity may be lost, even when total numbers and IFN-γ production are maintained. In order to assess the MHV-68-specific CD8+ T cell population further, we first analysed CD8+ tetramer+ T cells by flow cytometry for cell surface adhesion molecules and other activation markers (Fig. 1).

Little difference in cell surface phenotype was observed between the CD8+ tetramer+ cells from I-Aβ−/− and I-Aβ+/+ mice, either early or late in infection. Generally, the level of activation (CD62Llo CD44hi) was higher in all mice at acute time-points, corresponding to the higher antigen loads present at this time. Greater activation was also observed at late time-points in the I-Aβ−/− mouse relative...
to the I-A^b+/+ controls, particularly in the CD8^+ H-2Db-p56 population. This was consistent with the selective expansion of these cells observed during chronic MHV-68 infection in I-A^b/-/- mice (Stevenson et al., 1998), and again probably reflects an increased antigen load (Cardin et al., 1996). Overall, there was no evidence from cell surface phenotype for a functional deficit in the MHV-68-specific CD8^+ T cell populations of I-A^b/+ mice, even when these mice showed signs of clinical disease.

We then determined the cytotoxic activity of MHV-68-specific CTL from I-A^b+/+ and I-A^b/-/- mice (Fig. 2). Spleen cells were recovered and used directly to kill peptide-pulsed syngeneic targets. The splenocytes of MHV-68-infected I-A^b/-/- mice showed at least as good cytotoxicity against epitope-pulsed targets as equivalent I-A^b+/+ populations (Fig. 2A). The cytotoxic activity of spleen cell populations late in infection thus reflected the frequencies of antigen-specific CTL measured by tetramer and intracellular IFN-γ staining (Stevenson et al., 1998): recognition of the H-2K^b-p79 epitope was maintained at a similar level in I-A^b+/+ and I-A^b/-/- mice, while the H-2Db-p56 population was preferentially expanded in the I-A^b/-/- group. There was no evidence for a loss of cytotoxic activity against either immunodominant epitope in the absence of CD4^+ T cells. The ex vivo killing of virus-infected targets by all spleen cell populations was low, variable and usually undetectable above the background lysis of uninfected targets (data not shown). This probably reflected the action of viral evasion genes such as K3 (Boname & Stevenson, 2001). Because the peptide concentrations used in cytotoxicity assays were saturating (Stevenson et al., 1999), it was possible that these assays detected relatively low affinity CTL that would contribute little to in vivo immunity. Thus we titrated the peptide requirement for cytotoxicity, comparing early and late populations and I-A^b+/+ and I-A^b/-/- mice (Fig. 2B). A similar level of sensitivity to antigen was observed in all populations, with cytotoxicity titrating out at peptide concentrations of 10^{-10} - 10^{-13} M. Thus there was no evidence for a loss of CTL responsiveness to low levels of antigen late in infection of the I-A^b/-/- mice.

As a final comparison of the different virus-specific CTL populations, we quantified T cell receptor Vβ usage (Fig. 3). That individual littermates of a genetically homogeneous strain can differ markedly in the composition of their antiviral CTL responses is well recognized (Pala et al., 1986), and the CD8^+ T cell response is known to alter in Vβ repertoire between the acute and persistent phases of Epstein–Barr virus infection even in immunocompetent individuals (Annels et al., 2000). Thus considerable variation between individual mice was expected. However, a CD4^+ T cell dependence of CD8^+ T cell memory might still be expected to manifest itself as significant shifts in repertoire usage between acute and chronic infection in the I-A^b/-/- mice. There was no evidence for this.
The H-2D\textsuperscript{b}-p56 response used predominantly V\textsubscript{b}2 in the I-A\textsuperscript{b}-/- mice, with lesser contributions from V\textsubscript{b}8 and V\textsubscript{b}11. This pattern was maintained at late times. The H-2D\textsuperscript{b}-p56 response in I-A\textsuperscript{b}+/+ mice was more evenly split between V\textsubscript{b}2 and V\textsubscript{b}8, especially at early times, but essentially showed little difference from the I-A\textsuperscript{b}-/- pattern. The H-2K\textsuperscript{b}-p79 response was fairly evenly split between V\textsubscript{b}5, V\textsubscript{b}8, V\textsubscript{b}9 and V\textsubscript{b}13 at early time-points in both I-A\textsuperscript{b}+/+ and I-A\textsuperscript{b}-/- mice. V\textsubscript{b}5 and V\textsubscript{b}9 predominated at late time-points in the I-A\textsuperscript{b}+/+ group, whereas all groups were maintained in the I-A\textsuperscript{b}-/- mice. Overall, there was no evidence for significant changes in the H-2D\textsuperscript{b}-p56 or H-2K\textsuperscript{b}-p79 repertoires between early and late times after infection in the absence of CD4\textsuperscript{+} T cells. Similar results were obtained for tetramer\textsuperscript{+} CD8\textsuperscript{+} T cells recovered from lungs by bronchoalveolar lavage (not shown).

Our conclusion is that the virus-specific CD8\textsuperscript{+} T cell populations in MHV-68-infected but otherwise unmanipulated I-A\textsuperscript{b}-/- mice show no signs of functional...
that the level of CD8+ conjugated V(K1.5) and anti-I-A b (M5/114) followed by negative selection impairment results. The peak cytotoxicity against MHV-68 resources, for example cytokines, such that functional antigen loads there may be intense competition for limited journal of general virology difference between the normal CTL function observed here exhaustion, even though these cells probably encounter viral antigens fairly frequently (Belz & Doherty, 2001). The difference between the normal CTL function observed here and the compromised function in populations further expanded by antigenic challenge (Liu et al., 2002) suggests that the level of CD8+ T cell stimulation determines the necessity of CD4+ T cell help. In situations with high antigen loads there may be intense competition for limited resources, for example cytokines, such that functional impairment results. The peak cytotoxicity against MHV-68 was not impaired in the I-Ab-/- mice, but the numbers involved are small compared with lymphocytic choriomeningitis virus infection (Butz & Bevan, 1998; Zajac et al., 1998). Immune evasion by MHV-68 (Boname & Stevenson, 2001) may contribute to the relative lack of antigen presented, and is a more likely explanation than a CTL deficiency for the incapacity of I-Ab-/- mice to control chronic infection. In contrast to MHV-68, vaccinia virus provides a large stimulus to murine CTL and presumably stretches the response sufficiently to reveal an effect of CD4+ T cell exhaustion. A lack of CD8+ T cell exhaustion can perhaps be seen as one more aspect of the adaptation of a herpesvirus to its natural host.

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