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Differential requirement for CD28 and CD80/86 pathways of costimulation in the long-term control of murine gammaherpesvirus-68

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Abstract

The costimulatory molecules CD80 and CD86 (B7-1 and B7-2) are upregulated on mature antigen-presenting cells and interact with positive and negative regulators of CD8 T cell function, CD28 and CD152 (CTLA4) respectively. In this study, we examined the role of CD80 and CD86 in the immune response to murine gammaherpesvirus-68 (MHV-68) using CD80/86−/− mice. As we had previously shown that CD28 (the only known activating receptor for CD80 and 86) is not essential for long-term control of MHV-68, we predicted that CD80 and 86 would also be dispensable for an effective response to this virus. However, surprisingly, we observed that CD80/86−/− mice failed to maintain effective long-term control of MHV-68 and showed viral reactivation in the lungs. We did not observe viral reactivation in mice deficient in either CD80 or CD86 alone, indicating that these molecules play overlapping roles in the long-term control of MHV-68. Antiviral antibody responses were dramatically reduced in CD80/86−/− mice, while CD8 T cell expansion and recruitment to the lungs were not significantly affected. The unexpected disparity in the requirement for CD28 and CD80/86 in the response to MHV-68 suggests that CD28 is not the only positive regulatory receptor for CD80/86.

Keywords: CD80; CD86; CD28; Murine gammaherpesvirus; Lung; CD8 T cell; Antiviral antibody

Introduction

Murine gammaherpesvirus-68 (MHV-68) is a naturally occurring rodent pathogen (Blaskovic et al., 1980) which is closely related to Epstein Barr virus (EBV) and the Kaposi's sarcoma-associated human herpesvirus 8 (KSHV, HHV-8) (Efstathiou et al., 1990a, 1990b; Virgin et al., 1997). Intranasal (i.n.) administration of MHV-68 results in acute productive infection of lung alveolar epithelial cells and a latent infection in several cell types including B lymphocytes, dendritic cells, epithelia and macrophages (Flano et al., 2000; Stewart et al., 1998; Sunil-Chandra et al., 1992a, 1992b; Weck et al., 1999). Infectious virus is cleared from the lungs approximately 10 days after infection by a T-cell-mediated process (Ehtisham et al., 1993; Topham et al., 2001). The antibody response develops several weeks after infection (Stevenson and Doherty, 1998). Following the establishment of latency, viral control can be mediated by either T- or B-cell-dependent mechanisms (Kim et al., 2002; Stewart et al., 1998). While CD4 T cells are not essential for primary control of lytic MHV-68, they are required for long-term control and the virus reactivates in the lungs of CD4 T-cell-deficient mice (Cardin et al., 1996).

As predicted by the two-signal hypothesis, both TCR-mediated and costimulatory signals are important in T cell activation during MHV-68 infection. Thus CD40-CD40L interactions appear to be critical for T-cell-mediated control of MHV-68 (Brooks et al., 1999; Lee et al., 2002; Sarawar et al., 2001). CD40 ligation induces upregulation of CD80 and CD86 on antigen-presenting cells (Cella et al., 1996). These molecules interact with CD28 resulting in T cell activation and CTLA4 (cytotoxic T lymphocyte antigen-4) resulting in inhibition of T cell function (reviewed in Chambers et al., 2001; Sharpe and Freeman, 2002). However, surprisingly, neither CD28 nor its downstream signaling molecule PKCθ appears to be essential for the T cell activation events required for either acute or long-term control of MHV-68 (Giannoni et al., 2005; Kim et al.,

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2002; Lee et al., 2002). In this study, we examined the role of CD80/86 in the immune response to MHV-68 and, interestingly, discovered a CD28-independent role for these molecules in the long-term control of the virus.

Results

Differential requirement for CD80/86 and CD28 in the long-term control of MHV-68

Previous studies have shown that CD28−/− mice maintain effective long-term control of MHV-68, while mice lacking CD4 T cells or CD40 initially control the virus but later show viral reactivation in the lungs (Cardin et al., 1996; Kim et al., 2002; Lee et al., 2002). As CD28 is the only known activating receptor for CD80 and 86, we anticipated that long-term control of MHV-68 would also be independent of CD80 and 86. To test this assumption, wild-type and CD80/86−/− (double-deficient) mice were infected intranasally with MHV-68 and virus titers were determined in the lungs at days 16 and 50 after infection. As expected, no replicating virus was detected in the lungs of CD80/86−/− mice at day 16 after infection (Fig. 1), showing that initial control of lytic virus was effective. Latent virus was also assessed in the spleens of wild-type and CD80/86−/− mice using an infectious center assay at day 16 after infection, which is when the peak number of latently-infected cells is observed in this viral model. The frequency of infectious centers in splenocytes from CD80/86−/− mice (856±196, mean±standard error) was not significantly different from that in wild-type mice (526±73, mean±standard error).

However, surprisingly, CD80/86−/− showed significant viral reactivation in the lungs at day 50 after infection (Fig. 1), whereas no virus was detected in the lungs of wild-type (WT) or CD28−/− mice, confirming our earlier studies (Lee et al., 2002). Comparison with MHC Class II−/− mice suggested that the levels of replicating virus were slightly lower in the lungs of CD80/86−/− than in those of Class II−/− mice (Fig. 1). Thus, these data reveal a differential requirement for CD80/86 and CD28 in the long-term control of MHV-68.

CD80 and CD86 play overlapping roles in the long-term control MHV-68

Our next question was whether CD80 and 86 played distinct or overlapping roles in the long-term control of MHV-68. To address this question, we compared the long-term control of MHV-68 in mice that were deficient in either CD80 or CD86 or in both molecules. We also compared peak virus titers in the lungs of wild type, CD80−/−, CD86−/− and CD80/86−/− mice at day 7 post-infection. Groups of wild type, CD80−/−, CD86−/− or CD80/86−/− mice were infected intranasally with MHV-68 and lung virus titers were determined 7 or 50 days later. Although, on average, lung virus titers at day 7 post-infection appeared to be slightly higher for mice lacking CD80 or 86 than for wild-type mice (Fig. 2), the difference was not statistically significant. The results at day 50 post-infection demonstrated that neither CD80−/− or CD86−/− mice showed reactivation of MHV-68 in the lungs (Fig. 2), whereas replicating virus was detected in the lungs of CD80/86−/− mice. Thus CD80 and CD86 appear to play overlapping roles in the long-term control of MHV-68.

Unaltered lymphocyte numbers and subset distribution in the lungs and spleen of CD80/86−/− mice

Wild-type mice infected with MHV-68 develop splenomegaly and an inflammatory infiltrate in the lungs, comprising mainly T lymphocytes and monocyte/macrophages. To determine whether deficiency of both CD80 and 86 affects the lymphocyte expansion and recruitment that is necessary for these effects, cell numbers and the distribution of lymphocyte subsets were evaluated in the lungs and spleens

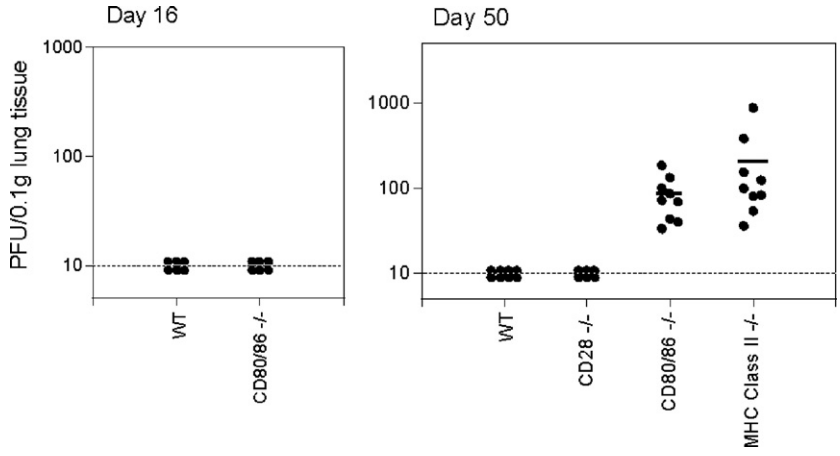

Fig. 1. Differential requirement for CD80/86 and CD28 in the long-term control of MHV-68. Groups of 3–5 mice were infected intranasally with 10^5 PFU MHV-68. At days 16 or 50 after infection, lungs were harvested and virus titers were determined in lung homogenates by plaque assay. Data are expressed as PFU/0.1 g of lung tissue from individual mice and are combined from 2 independent experiments. Horizontal bars represent the mean titer for each group. The detection limit of this assay is 10 PFU/0.1 g of lung tissue.