Available online at www.sciencedirect.com

SCIENCE DIRECT

VIROLOGY

Virology 338 (2005) 201 – 208

www.elsevier.com/locate/yviro

Rapid Communication

CD4 T cell control of acute and latent murine gammaherpesvirus infection requires IFNγ

Rebecca L. Sparks-Thissen[a,1], Douglas C. Braaten[a], Kai Hildner[a], Theresa L. Murphy[a], Kenneth M. Murphy[a,b], Herbert W. Virgin IV[a,*]

[a]*Department of Pathology and Immunology, Washington University School of Medicine, 660 S. Euclid Ave., Box 8118, St. Louis MO 63110, USA*
[b]*Howard Hughes Medical Institute, Washington University School of Medicine, St. Louis, MO 63110, USA*

Received 16 February 2005; returned to author for revision 4 May 2005; accepted 9 May 2005
Available online 15 June 2005

Abstract

Murine gammaherpesvirus 68 (γHV68, MHV-68)-specific CD4 T cells control γHV68 infection by reducing the frequency of latently infected cells and by inhibiting viral replication. We have previously demonstrated that CD4 T cells do not require CD8 T or B cells to control γHV68 replication, demonstrating a helper-independent activity of CD4 T cells during γHV68 infection. The effector mechanism(s) required for this helper-independent function of CD4 T cells and for the inhibition of the establishment of latency by CD4 T cells are not known. Since IFNγ has been previously shown to be important for control of acute, latent, and persistent γHV68 infection, we tested the hypothesis that CD4 T cells require IFNγ to limit γHV68 latency and replication. We utilized a previously described system in which T cell receptor (TCR) transgenic T cells (DO.11.10) and a recombinant virus (γHV68.OVA) allow for evaluation of high numbers of virus-specific CD4 T cells during both acute and latent infection. We show here that virus-specific CD4 T cells require IFNγ for their anti-viral function in both acute and latent γHV68 infection. We additionally show that an in vitro derived T helper type 1 (TH1) CD4 T cell clone, which produces IFNγ, inhibits γHV68 replication after adoptive transfer into RAG mice. Together, data presented here demonstrate that both CD4 T cell-mediated helper-independent control of γHV68 replication and inhibition of the establishment of γHV68 latency require IFNγ.

Keywords: CD4 T cell; gammaherpesvirus; IFNγ; Herpesvirus latency; TH1 T cell

Introduction

To better understand γ-herpesvirus pathogenesis and to identify immune mechanisms that contribute to the maintenance of the host–virus equilibrium during chronic infection, we and others have studied murine γHV68 (Christensen et al., 1999; Flaño et al., 2001; Kim et al., 2002; McClellan et al., 2004; Sparks-Thissen et al., 2004; Stevenson and Doherty, 1998; Stevenson et al., 1999; Tibbetts et al., 2002, 2003; Tripp et al., 1997; Usherwood et al., 2000). γHV68 infects inbred and outbred strains of mice and establishes latency in B cells, macrophages, and dendritic cells (Flaño et al., 2000, 2002, 2003; Sunil-Chandra et al., 1992; Weck et al., 1999; Willer and Speck, 2003). Chronic γHV68 infection is associated with atherosclerosis, induction of tumors, and severe large vessel arteritis in immunodeficient mice (Alber et al., 2000; Dal Canto et al., 2000, 2001; Sunil- Chandra et al., 1994; Weck et al., 1997). In wildtype mice, CD4 T cells are induced during γHV68 infection and are critically important for the control of γHV68 replication and latency (Christensen et al., 1999; Flaño et al., 2001; McClellan et al., 2004; Sparks-Thissen et al., 2004).

Much of what has been learned about the immune response to γHV68 infection has come from evaluating infection of different genetically modified mice which lack various arms of the adaptive immune system. An alternative approach that we have taken recently is to use a reductive model system in which only one arm of the immune system

* Corresponding author. Fax: +1 (314) 362 4096.
E-mail address: virgin@wustl.edu (H.W. Virgin).
[1] Current address: Department of Biology, Grinnell College, Grinnell, IA 50112, USA.

0042-6822/$ - see front matter © 2005 Elsevier Inc. All rights reserved.
doi:10.1016/j.virol.2005.05.011

is evaluated in the context γHV68 infection. We have previously evaluated infection with a recombinant virus expressing ovalbumin (γHV68.OVA) in mice that express TCR transgenic CD4 or CD8 T cells on either wildtype or RAG−/− genetic backgrounds; this system allows for an evaluation of the function of each of these T cell subsets during acute and latent infection (Braaten et al., 2005; Sparks-Thissen et al., 2004).

In the case of CD4 T cell function, we previously evaluated infection with γHV68.OVA in both DO.11.10/BALB and OTII/BL6 mice, both of which express CD4 T cells specific for the MHC Class II epitope of OVA (amino acids 323–339, ISQAVHAAHAEINE) (Barnden et al., 1998; Murphy et al., 1990; Sparks-Thissen et al., 2004). We showed that CD4 T cells demonstrate helper-independent antiviral function, measured as the capacity to inhibit viral replication, during γHV68 infection (Sparks-Thissen et al., 2004). The most convincing data for this conclusion are from experiments with mice that express the OVA CD4 transgenic T cells on a RAG−/− background. In these mice, the OVA-specific CD4 T cells decreased γHV68.OVA replication and modestly delayed death from infection (Sparks-Thissen et al., 2004). In a related series of experiments we also demonstrated that OVA-specific CD4 transgenic T cells present at the initiation of infection in mice containing CD8 T and B cells are able to inhibit the establishment of latent γHV68 infection (Sparks-Thissen et al., 2004). This latter observation corroborated previously published studies demonstrating that CD4 T cells play a key role in effective vaccination against the establishment of γHV68 latency (McClellan et al., 2004; Tibbetts et al., 2003b).

Several previous studies have demonstrated that control of γHV68 infection requires IFNγ. Mice lacking IFNγ (IFNγ−/−) or the IFNγ receptor (IFNγR−/−) have increased persistent replication (defined as preformed infectious virus present at a low level after clearance of acute infection) for months after infection (Dal Canto et al., 2000, 2001; Gangappa et al., 2002; Tibbetts et al., 2002; Weck et al., 1997), and peritoneal cells taken from latently infected animals reactivate infectious virus ex vivo more efficiently than cells from wildtype mice. (Tibbetts et al., 2002). IFNγ−/− and IFNγR−/− mice also develop large vessel arteritis after infection with γHV68 (Dal Canto et al., 2000, 2001; Weck et al., 1997), which is caused by persistent replication of virus in the smooth muscle cells of the aortic media, an immunoprivileged site that requires IFNγ for virus clearance (Dal Canto et al., 2000, 2001; Weck et al., 1997).

γHV68-specific CD4 T cells have been shown to produce IFNγ after antigen stimulation (Brooks et al., 1999; Christensen and Doherty, 1999; Sarawar et al., 1996; Usherwood, 2002). However, it is not known if effective CD4 T cell responses to γHV68 require IFNγ. A study by Christensen et al. demonstrated that B cell deficient mice depleted of both CD8 T cells and IFNγ succumbed to infection and had increased γHV68 titers compared to mice depleted only of CD8 T cells (Christensen et al., 1999). These data indicate that IFNγ derived from either CD4 T cells or other cells (e.g., NK cells, but not CD8 T or B cells) is required for CD4 T cell-dependent control of γHV68 infection. Given these data, we directly tested whether IFNγ is necessary for both CD4 T cell-mediated inhibition of the establishment of latency and the helper-independent function in limiting γHV68.OVA replication. We found that IFNγ is necessary for CD4 T cell-mediated control of latent and productive γHV68 infection. We also show that a stable TH1 differentiated IFNγ expressing DO.11.10 T cell clone is sufficient for control of acute γHV68.OVA replication in RAG−/− mice.

Results and discussion

Inhibition of the establishment of γHV68 latency by CD4 T cells requires IFNγ

We first tested whether virus-specific CD4 T cells require IFNγ for control of γHV68 latency by depleting IFNγ in vivo with the monoclonal antibody H22 during infection (Bancroft et al., 1987; Schreiber et al., 1985). DO.11.10 mice received injections of H22 [or isotype control (Mandik-Nayak et al., 2001)] starting the day before intraperitoneal infection with 100 pfu of either γHV68.OVA or the control virus γHV68.LacZ (Sparks-Thissen et al., 2004; Van Dyk et al., 2000). Sixteen days after infection, peritoneal cells were harvested and analyzed for reactivation from latency and for the frequency of cells carrying latent viral genome. Consistent with our previous results (Sparks-Thissen et al., 2004), γHV68.OVA-infected mice had 15-fold fewer reactivating (Fig. 1A, $P = 0.015$) and 40-fold fewer genome positive cells (Fig. 1B, $P < 0.0001$) than γHV68.LacZ infected mice after treatment with the isotype control antibody, demonstrating that the control antibody does not alter the ability of antigen-specific CD4 T cells to limit latent infection. In contrast, DO.11.10 mice treated with anti-IFNγ had equivalent frequencies of reactivating and genome positive cells regardless of whether the challenge virus expressed OVA (Fig. 1). Thus, the CD4 T cell-dependent decrease in γHV68.OVA latency was completely eliminated by treatment with anti-IFNγ. These data indicate that IFNγ plays an important role in limiting the extent of latent infection by virus-specific CD4 T cells.

CD8 T cell and B cell-independent control of γHV68 replication and virulence by CD4 T cells requires IFNγ

We next asked whether IFNγ was necessary for helper function-independent inhibition of γHV68 replication and virulence by CD4 T cells (Sparks-Thissen et al., 2004). We infected DO.11.10 RAG mice, which contain only OVA-specific CD4 T cells and no other antigen-specific lymphocytes, with either γHV68.OVA or γHV68.LacZ. The mice