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CD13/aminopeptidase N and murine cytomegalovirus infection

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Abstract

CD13/aminopeptidase N is a membrane-bound metalloproteinase implicated in human cytomegalovirus (HCMV) infection and pathogenesis. Anti-CD13 antibodies can neutralize HCMV infectivity, and HCMV viremia after bone marrow transplantation induces anti-CD13 autoantibodies which correlate with development of chronic graft vs. host disease. We examined whether murine CD13/APN was similarly implicated in murine cytomegalovirus (MCMV) disease. MCMV infection did induce anti-CD13 antibodies in mice in a strain-specific manner. ICR and 129S mice developed high titers of anti-CD13 antibodies and anti-MCMV antibodies after MCMV infection, whereas CBA and CBAxC57BL/6 f1 hybrid mice produced antibodies against MCMV only. Unlike HCMV, no evidence was found for a correlation between host cell CD13/APN expression and infection, or for the presence of CD13/APN on MCMV particles, although APN inhibitors decreased MCMV plaque formation. Reproduction of CD13/APN autoantibody production in the murine system should make it possible to determine if these antibodies contribute to CMV pathogenesis.

Keywords: Cytomegalovirus; Aminopeptidase N; CD13; Autoantibodies; Autoimmunity; Mouse

Introduction

Cytomegaloviruses are beta-herpesviruses which establish life-long infections in their hosts. Several findings have implicated CD13, also known as aminopeptidase N (APN), in human cytomegalovirus (HCMV) infection and pathogenesis. In a series of bone marrow transplant patients, CD13/APN-specific autoantibodies were found only in patients with confirmed CMV disease after transplant and were highly associated with later development of chronic graft vs. host disease (cGVHD) (Soderberg et al., 1996a, 1996b). Patients seropositive for CMV but negative for the presence of virus did not develop anti-CD13/APN antibodies or cGVHD (Soderberg et al., 1996b). On the molecular level, several groups have shown that human CMV incorporates host cell proteins, one of which is CD13/APN, into its viral envelope (Homman-Loudiyi et al., 2003; Michelson et al., 1989; Naucler et al., 1996; Wright et al., 1995). Some monoclonal antibodies raised against CD13/APN can neutralize human CMV viral particles, and immunoblots of purified human CMV virions detect CD13/APN (Soderberg et al., 1993a). When viral particles are labeled with immunogold-anti-CD13 antibodies, the immunogold particles are later seen on the surface of infected cells (Giugni et al., 1996). Consistent with viral sequestration of CD13/APN, cell surface CD13/APN is down-regulated after human CMV infection in fibroblasts (Phillips et al., 1998). Association of CD13/APN with the viral particle has been suggested as a mechanism for the generation of anti-CD13/APN antibodies after CMV infection by autoimmunization (Naucler et al., 1996). That virus-associated CD13/APN is essential for HCMV infection is suggested by anti-CD13 antibody inhibition of infection (Soderberg et al., 1993a); however, human neuroblastoma cells lacking CD13 expression were susceptible to HCMV infection (Watanabe, 1998), and recently the epidermal growth factor receptor has been shown to be an important mediator of HCMV entry into cells (Wang et al., 2003).

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CD13/APN is a membrane-bound metalloproteinase widely expressed on mammalian cells including monocytes, macrophages, endothelial cells, epithelial cells, and fibroblasts (Curnis et al., 2002; Phillips et al., 1998; Plakidou-Dymock et al., 1993; Riemann et al., 1999). It exists as a heavily glycosylated homodimer with a constitutively active proteinase activity external to the plasma membrane (Riemann et al., 1999). In addition to numerous tissue-specific functions, CD13/APN serves as a major receptor for type I coronaviruses (Breslin et al., 2003; Yeager et al., 1992), including possibly the SARS virus (Yu et al., 2003). More recently, CD13/APN has been shown to be an important mediator of angiogenesis when expressed on endothelial cells of nascent blood vessels (Bhagwat et al., 2001). Antibodies raised against CD13/APN and inhibitors of aminopeptidase activity both block angiogenesis in models of neovascularization (Pasqualini et al., 2000). This may have significance with regard to CMV, since CMV infection is associated with many chronic vascular diseases (Borchers et al., 1999; Hamamdzic et al., 2002; Nieto et al., 1996; Pandey and LeRoy, 1998). CMV-induced anti-CD13/APN antibodies may represent one mechanism by which the virus initiates vascular pathology.

Like most herpesviruses, cytomegaloviruses are species specific, but the link between CMV and vascular disease seen in humans has been reproduced repeatedly in mice infected with murine cytomegalovirus (Berencsi et al., 1998; Hamamdzic et al., 2001; Hummel et al., 2001; Presti et al., 1998). Therefore, in this study, we have examined whether CD13/APN is also implicated in MCMV infections in mice.

Results

MCMV infection induces anti-CD13/APN antibodies in a strain-specific manner

Four mouse strains were tested for the induction of MCMV and CD13/APN immunity after MCMV infection. These strains, CBA, CBAxC57BL/6 f1 hybrids, ICR, and 129S mice deficient in the interferon gamma receptor (IFNgR−/−), vary in their H-2 haplotype and in their reported resistance to MCMV infection. Groups of 4–6 animals of each of these strains were infected with 10^5 PFU MCMV by IP injection, which is a sublethal dose in adult animals for all strains. Serum was collected after 14 days and analyzed by ELISA for IgG antibodies against MCMV and mouse CD13/APN. All strains had similar levels of IgG antibodies directed against MCMV (Fig. 1). However, anti-CD13/APN antibodies were detected only in the sera from ICR and 129S-IFNgR−/− mice, and no detectable antibodies against CD13 were found in sera from CBA or B6 hybrid mice. Anti-CD13 antibodies in humans were reported in people who developed HCMV viremia while in an immunocompromised state. In order to test whether an immunocompromised state would be associated with induction of higher

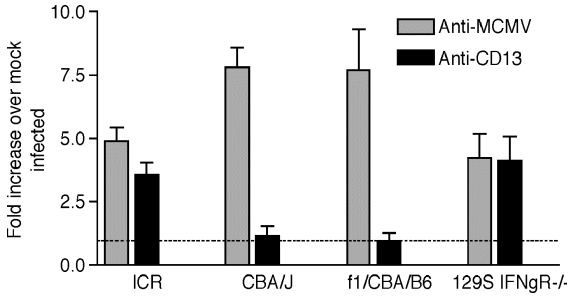

Fig. 1. MCMV infection induces anti-CD13 antibodies in susceptible strains of mice. Groups of four ICR, CBA/J, B6/CBA f1 hybrids, and interferon gamma receptor knock out mice on a 129S background were infected by intraperitoneal injection with 10^5 PFU MCMV. Sera collected 14 days post-infection were assayed in duplicate at a 1:10 dilution for anti-MCMV (gray bars) and anti-CD13 (black bars) antibodies by ELISA. Figure is representative of two similar experiments. Dashed line indicates mean antibody response in mock-infected mice.

levels anti-CD13 antibodies in mice, cyclosporine A was used to induce an immunocompromised state in ICR mice. Three groups of 6 ICR mice were treated with either cyclosporine A alone, cyclosporine A and 10^5 PFU MCMV, or 10^5 PFU MCMV + mock cyclosporine A injections. Mice treated with cyclosporine A and MCMV had increased signs of illness over those given MCMV alone (not shown). However, levels of antibodies to both MCMV and CD13 were not significantly different between the two groups (Fig. 2).

Cell surface APN activity and MCMV susceptibility

To investigate whether expression of murine CD13/APN correlates with MCMV susceptibility as reported for HCMV (Soderberg et al., 1993a, 1993b), five murine cell lines were characterized with respect to MCMV progeny production and cell surface aminopeptidase N/CD13 activity. NIH 3T3, 3T12, and primary mouse embryonic fibroblasts (MEF) are all fibroblast in phenotype. Py-41 and SVEC-4 cells are mouse endothelial cell lines immortalized by the polyoma T antigen and the SV40 T-antigen, respectively (Dubois et al., 1991; O'Connell and Edidin, 1990). In an assay of MCMV progeny production after inoculation at low multiplicity of infection, marked differences were seen between cell lines in susceptibility to productive MCMV infection (Fig. 3A). Peak MCMV titers in supernatants from the three fibroblast lines were close to 10^6 PFU/ml, while SVEC-4 cells produced 10^5 PFU/ml, and Py-41 cells produced less than 1000 PFU/ml. Similar relative results were seen in a plaque assay on the five cell lines (Fig. 4).

In contrast to human cells, attempts to compare CD13/APN expression on murine cell lines by immunological methods were unsuccessful. Two commercially available antibodies specific for murine CD13/APN failed to recognize any proteins on Western blotted cell extracts (not shown). Immunofluorescent flow cytometry staining of cell surface proteins with antibody R3-242 conjugated to phycoerythrin showed that CD13/APN was present on both endothelial and fibroblast cells. However, relative expression