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Contributions of CD8 T cells to the pathogenesis of mouse adenovirus type 1 respiratory infection

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ABSTRACT

CD8 T cells are key components of the immune response to viruses, but their roles in the pathogenesis of adenovirus respiratory infection have not been characterized. We used mouse adenovirus type 1 (MAV-1) to define CD8 T cell contributions to the pathogenesis of adenovirus respiratory infection. CD8 T cell deficiency in β2 $m^{-/-}$ mice had no effect on peak viral replication in lungs, but clearance of virus was delayed in β2 $m^{-/-}$ mice. Virus-induced weight loss and increases in bronchoalveolar lavage fluid total protein, IFN-γ, TNF-α, IL-10, CCL2, and CCL5 concentrations were less in β2 $m^{-/-}$ mice than in controls. CD8 T cell depletion had similar effects on virus clearance, weight loss, and inflammation. Deficiency of IFN-γ or perforin had no effect on viral replication or inflammation, but perforin-deficient mice were partially protected from weight loss. CD8 T cells promote MAV-1-induced pulmonary inflammation via a mechanism that is independent of direct antiviral effects.

1. Introduction

CD8 T cells contribute to the control of many viral infections (Adcock and Lane, 2003; Ehtisham et al., 1993; Kagi et al., 1994; Mullbacher et al., 1999; Walsh et al., 1994). After recognizing infected cells presenting virus-specific peptides by MHC class I, CD8 T cells kill them through the release of perforin (Pfn)- and granzyme (Gzm)-containing granules or by induction of apoptosis via interaction between Fas and Fas ligands (FasL) (Chavez-Galan et al., 2009; Harty et al., 2000; Hoves et al., 2010). CD8 T cells also produce a variety of antiviral and pro-inflammatory cytokines such as interferon (IFN)-γ, interleukin (IL)-2, and tumor necrosis factor (TNF)-α. The collective action of these effectors aids in the control of viral replication during acute infection. However, the actions of CD8 T cells may also contribute to bystander tissue damage (Cannon et al., 1988). CD4 and CD8 T cell responses specific to human adenoviruses (HAdVs) have been described, and patients with acquired or inherited defects in cellular immune function are prone to more severe HAdV disease (Kojaoghlanian et al., 2003; Walls et al., 2003). However, the strict species-specificity of the adenoviruses has precluded extensive studies of the contributions of CD8 T cells to HAdV pathogenesis.

We use mouse adenovirus type 1 (MAV-1) to study the pathogenesis of an adenovirus in its natural host. We have established mouse models of adenovirus respiratory infection (McCarthy et al., 2014; Procario et al., 2012; Weinberg et al., 2005) and myocarditis (McCarthy et al., 2015a). Findings from our laboratory and our collaborators have provided insight into cellular immunity and adenovirus pathogenesis. CD4 and CD8 T cells are recruited to the lungs, heart, and brain of mice infected intranasally (i.n.) with MAV-1 (McCarthy et al., 2015a; Procario et al., 2012; Weinberg et al., 2007), and both CD4 and CD8 T cells recruited to lungs of MAV-1-infected mice include a significant population of $CD62L^{low}$ effector memory cells (Procario et al., 2012). MAV-1-specific epitopes presented by MHC class I to CD8 T cells have not yet been defined, but we have demonstrated increased IFN-γ and GzmB production by CD8 T cells isolated from lungs of infected mice compared to mock-infected mice during acute infection (McCarthy et al., 2015b, 2014), suggesting that CD8 T cells respond specifically to MAV-1. α/β T cells are required for control of viral replication in the spleen and brain and for long-term survival following intraperitoneal (i.p.) inoculation (Moore et al., 2003).

Our recent work suggested the possibility that CD8 T cell dysfunction is associated with delayed virus clearance from the lungs of mice

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that received allogeneic bone marrow transplantation (BMT) (McCarthy et al., 2015c). However, the specific mechanisms by which CD8 T cells contribute to control of MAV-1 replication and to MAV-1-induced pulmonary inflammation are not yet completely defined. In this study, we demonstrate that clearance of MAV-1 DNA from the lungs was delayed in the absence of functioning CD8 T cells, but CD8 T cells were not essential for efficient control of MAV-1 replication in the lungs during acute infection. In contrast, MAV-1-induced airway inflammation and weight loss were markedly reduced in CD8 T cell-deficient mice. Effects of CD8 T cells on airway inflammation did not depend on Pfn or IFN-γ, but Pfn deficiency partially protected against virus-induced weight loss. Depletion of CD4 T cells had no effect on virus clearance or virus-induced inflammation. Even intact CD8 T cell function was insufficient to completely clear MAV-1 from the lungs. Collectively, our data suggest that CD8 T cells exert an immunomodulatory function in the lungs that is independent of their contributions to control of MAV-1 replication in the lungs.

2. Methods and materials

2.1. Mice

C57BL/6J (B6), β2-microglobulin-deficient (β2 $m^{-/-}$, B6.129P2-*B2m*^{tm1Unc}/J), CD8α-deficient (CD8α$^{-/-}$; B6.129S2-*CD8a*^{tm1Mak}/J), Pfn-deficient (Pfn$^{-/-}$; C57BL/6-Prf1^{tm1Sdz}/J), and IFN-γ-deficient (IFN-γ$^{-/-}$, B6.129S7-*Ifng*^{tm1Ts}/J) mice, all on a C57BL/6J background, were obtained from the Jackson Laboratory. In the experiments in Fig. 2, IFN-γ$^{-/-}$ mice bred at the University of Michigan (originally from the Jackson Laboratory, generously provided by Dr. Benjamin Segal) were used. C57BL/6J mice obtained from the Jackson Laboratory and bred at the University of Michigan were used for experiments involving antibody depletion of CD4 T-cells. All mice were maintained under specific pathogen-free conditions. All experiments were approved by the University of Michigan Institutional Animal Care and Use Committee.

2.2. Virus and Infections

MAV-1 was grown and passaged in NIH 3T6 fibroblasts, and titers of viral stocks were determined by plaque assay on 3T6 cells as previous described (Cauthen et al., 2007). Adult 6–8 weeks old) mice were anesthetized with ketamine and xylazine and then infected i.n with 10^5 plaque-forming units (pfu) of MAV-1 in 40 µl of sterile phosphate-buffered saline (PBS). Control mice were mock-infected i.n. with conditioned medium at an equivalent dilution in sterile PBS. In some experiments, mice were weighed on the day of infection and then intermittently throughout the course of the experiment. Mice were euthanized by pentobarbital overdose at the indicated time points. Organs were harvested, snap-frozen in dry ice and stored at −80 °C.

2.3. CD4 and CD8 T cell depletion

A rat monoclonal antibody recognizing mouse CD8 T cells (clone YTS 169.4, BioXCell, Inc.) or CD4 T cells (clone GK1.5, BioXCell, Inc.) was administered i.p. at 200 µg/dose. Control mice received equivalent amounts of nonspecific rat IgG2b (clone LTF-2, BioXCell, Inc.). Antibody was administered on days −1, 3, 6, 10, and 13 relative to infection (day 0).

2.4. Isolation of DNA and RNA

RNA was isolated from homogenates of all organs as previously described (Nguyen et al., 2008). Portions of lung, heart, spleen, and brain were homogenized using sterile glass beads in a Mini-Beadbeater (Biospec Products) for 30 s in 1 ml of TRIzol (Invitrogen). RNA (all organs) and DNA (hearts, brains, and spleens) were then isolated from homogenates according to the manufacturer's protocol. DNA was extracted from a separate portion of lung using the DNeasy Tissue kit (Qiagen Inc).

2.5. Analysis of host gene expression

Host gene expression was quantified using reverse transcriptase quantitative real-time PCR (RT-qPCR). RNA was reverse transcribed using MMLV reverse transcriptase (Invitrogen) according to the manufacturer's instructions. For measurements of CD4 (forward primer 5′-AGGTGATGGGACCTACCTCTC-3′; reverse primer 5′-GGGGCCACCACTTGAACTAC-3′) and CD8 (forward primer 5′-CCGTTGACCCGCTTTCTGT-3′; reverse primer 5′-CGGCGTCCATTTTCTTTGGAA-3′), and Pfn (forward primer 5′- AGCACAAGTTCGTGCCAGG-3′; reverse primer 5′- CTCCGTGATGGAAGACCACT-3′), 5 µl of cDNA were added to reactions containing Power SYBR Green PCR Mix (Applied Biosystems) and forward and reverse primers (each at 200 nM final concentration) in a 25 µl reaction volume. Separate reactions were prepared with primers for mouse GAPDH (forward primer 5′-TGCACCACCAACTGCTTAG-3′; reverse primer 5′-GGATGCAGGGATGATGTTC-3′). In all cases, RT-qPCR analysis consisted of 40 cycles of 15 s at 90 °C and 60 s at 60 °C. Quantification of target gene mRNA was normalized to GAPDH and expressed in arbitrary units as $2^{-\Delta Ct}$, where Ct is the threshold cycle and ΔCt = Ct(target) − Ct(GAPDH).

2.6. Analysis of viral gene expression

Viral gene expression was quantified using RT-qPCR. cDNA prepared as described above was amplified using primers targeting the MAV-1 tripartite leader (TPL) sequence (forward primer 5′-CGAGTCGCCTCCTGTGATACT-3′; reverse primer 5′-CAAGTCGATCTGTCGGAGCTT-3′). The 63 bp product amplified by these primers spans the 885 bp intron between exon 2 and exon 3 of the MAV-1 TPL sequence. Quantification of the target gene mRNA was normalized to GAPDH as described above.

2.7. Analysis of viral loads

MAV-1 viral loads were measured in organs using quantitative real-time polymerase chain reaction (qPCR) as previously described (McCarthy et al., 2015a; Procario et al., 2012). Results were standardized to the nanogram (ng) amount of input DNA. Each sample was assayed in triplicate.

2.8. Detection of Infectious Virus in Lung

Lungs were homogenized in sterile PBS (10% weight/volume) using sterile glass beads in a Mini-Beadbeater and then clarified by centrifugation after three freeze/thaw cycles. Plaque assay was then performed as previous described (Cauthen et al., 2007).

2.9. Lymphocyte stimulation

Spleens were harvested from mice and single-cell suspensions of splenocytes were generated by passage through a 70 µm cell strainer followed by lysis of red blood cells with ACK lysis buffer. CD8 T cells were isolated from splenocytes by negative selection using antibody-coated magnetic beads (Mouse CD8α+ T cell kit; Miltenyi Biotec). CD8 T cells were seeded at a concentration of 3×10^5 cells/well in 96-well plates coated with anti-CD3 antibody (clone 145-2C11, BioLegend, 5 µg/ml) and incubated for 24 h. Cytokine protein concentrations in supernatants were determined by ELISA (Duoset Kits, R & D Systems) according to the manufacturer's protocol.