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Effects of allergic airway disease on mouse adenovirus type 1 respiratory infection

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ABSTRACT

Virus infection may contribute to asthma pathogenesis. In turn, a Th2-polarized pulmonary environment may increase host susceptibility to infection. We used a cockroach antigen (CRA) model of allergic airway disease to test the hypothesis that Th2 cytokine overproduction increases susceptibility to mouse adenovirus type 1 (MAV-1). CRA sensitization led to upregulated lung expression of IL-4 and IL-13, lung cellular inflammation, and exaggerated airway mucus production. Following intranasal MAV-1 infection, lung cellular inflammation was more pronounced in CRA-sensitized mice than in unsensitized mice at 7 days post-infection but not at a later time point. CRA sensitization did not significantly suppress lung IFN-γ expression, and lung IFN-γ expression was upregulated in both CRA-sensitized mice and unsensitized mice over the course of MAV-1 infection. Despite CRA-induced differences in pulmonary inflammation, MAV-1 viral loads in lung and spleen and MAV-1 gene expression in the lung did not differ between CRA-sensitized and unsensitized mice. Our data therefore suggest that MAV-1 pathogenesis is not affected directly or indirectly by the Th2 polarization associated with allergic airway disease.

Introduction

Asthma is a chronic disorder of the airways characterized by inflammation, increased airway hyperresponsiveness, and obstruction of air flow (reviewed in Eisenbarth et al., 2004). Asthma prevalence continues to rise among 0 to 17 year-olds, increasing from an estimated 3.6% to 6.2% between 1980 to 1996, with the greatest increase observed in 0 to 4 year-olds (Akinbami and Schoendorf, 2002). Acute viral respiratory infections act as triggers for asthma exacerbation, with specific viruses isolated in up to 80–85% of asthma exacerbations in children (Johnston, 2007; Traves and Proud, 2007). Viral infections may also be risk factors for subsequent development of asthma. Children infected with respiratory syncytial virus (RSV) as infants are more likely to develop asthma compared to children without RSV infection (Sigurs et al., 2000), although it is unclear if asthma develops as a direct result of RSV infection or if the virus targets children who are already predisposed to develop obstructive airway disease (Peebles, 2004; Perez-Yarza et al., 2007). Some reports suggest that persistent adenovirus infection serves as a risk factor for childhood asthma (Macek et al., 1994; Marin et al., 2000).

Chronic inflammation characteristic of asthma is dependent on prototypic T-helper type (Th)2 cytokines such as interleukin (IL)-4 and IL-13 (Chatila, 2004). IL-4 and IL-13 activate the intracellular signal transducer and activator of transcription (STAT)6, one key mediator in pathways linked to allergic inflammation and mucus production (Chatila, 2004). Data from mouse models suggest that the Th2-polarized microenvironment in asthma may act as a risk factor for infection. For instance, clearance of *Pseudomonas aeruginosa* from the lungs is impaired in mice with allergic airway inflammation induced by ovalbumin sensitization (Beisswenger et al., 2006). There is also evidence that susceptibility to viral infection is increased in the presence of elevated levels of Th2 cytokines. Mice treated with IL-4 following influenza virus infection show increased viral titers in the lungs compared to controls, and clearance of virus from the lungs is impaired (Moran et al., 1996). Mice constitutively overexpressing IL-4 in the lungs also exhibit a delay in RSV clearance (Fischer et al., 1997).

Studies of human adenovirus pathogenesis are limited by the strict species-specificities of the adenoviruses. Little is known regarding the effects of Th2 cytokines on adenovirus pathogenesis. Mouse adenovirus type 1 (MAV-1) provides an excellent tool to study the pathogenesis of an adenovirus in its natural host. We have previously established MAV-1 as a model of adenovirus respiratory infection (Weinberg et al., 2007; Weinberg et al., 2005). Here we used MAV-1 to test the hypothesis that Th2 polarization increases susceptibility to respiratory infection with an adenovirus. We examine the effects of in vivo Th2 polarization on MAV-1 pathogenesis using allergic airway sensitization with cockroach antigen (CRA). Our results suggest that MAV-1 is relatively resistant to the effects of exaggerated Th2 cytokine production.

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Results

Effects of Th2 polarization on MAV-1-induced pulmonary inflammation

Cockroach antigen (CRA) sensitization has been established as a model of allergic airway disease, with increased airway hyperreactivity, mucus production, peribronchial inflammation, and upregulation of lung IL-4 and IL-13 in sensitized mice compared to control animals (Berlin et al., 2006). We used CRA to modify the pulmonary environment in mice prior to infection with MAV-1. After an initial systemic sensitization, mice were repeatedly challenged intranasally (i.n.) with CRA over an 18-day period prior to infection and during the course of infection (summarized in Fig. 1A) in order to localize the response to the airways. We used RT-qPCR to assess the effect of CRA sensitization on expression in the lung of IL-4 and IL-13 (Fig. 1B). Following sensitization but prior to infection, expression of each Th2 cytokine was significantly increased in the lungs of CRA-sensitized mice compared to unsensitized control animals that received phosphate-buffered saline (PBS; 68 ± 19 fold change from PBS-treated control animals for IL-4; 1595 ± 591 fold change for IL-13; mean $\pm$ S.E.M.; $P<0.01$ in each case). These data are similar to studies using CRA sensitization-induced allergic airway disease in which IL-4 and IL-13 protein levels were increased in both lung homogenate and bronchoalveolar lavage fluid of CRA-sensitized mice compared to unsensitized controls (Lindell et al., 2008). Lung expression of both IL-4 and IL-13 continued to be increased in CRA-sensitized mice compared to unsensitized control mice at all time points following MAV-1 infection (data not shown).

We also assessed lung expression of interferon (IFN)-γ following the initial 18-day CRA sensitization and throughout the course of MAV-1 infection (Fig. 1C). Somewhat surprisingly, CRA sensitization did not initially suppress IFN-γ expression in the lungs. Following sensitization but prior to infection, lung IFN-γ expression was instead slightly upregulated in CRA-sensitized mice compared to unsensitized control mice (Fig. 1C, day 0; 3.4 ± 1.6 fold change from PBS-treated control animals), although this difference was not statistically significant. Lung IFN-γ expression was upregulated in both CRA-sensitized and unsensitized control mice following MAV-1 infection. IFN-γ expression was slightly lower in CRA-sensitized mice compared to unsensitized control mice following MAV-1 infection, but there were no statistically significant differences between groups at any time point.

Similar to our earlier findings (Weinberg et al., 2005), MAV-1 infection of unsensitized mice induced a mild pneumonitis (Fig. 2A, left panels). Mononuclear cells began to accumulate around medium and large airways of infected mice as early as 4 d.p.i., and increasing peribronchial infiltrates were noted at 7 and 10 d.p.i. By itself, CRA sensitization also induced a cellular inflammatory response in the lungs, with inflammatory cells surrounding airways of sensitized mice (Fig. 2A, uppermost right panel). Cellular inflammation became more extensive in CRA-sensitized mice over the course of MAV-1 infection (Fig. 2A, right panels). MAV-1-induced cellular inflammation was more pronounced in the lungs of CRA-sensitized mice compared to control mice at 4 and 7 d.p.i. By 10 d.p.i., lungs of MAV-1-infected control mice were histologically similar to lungs of MAV-1-infected, CRA-sensitized mice.

Pathology index scores (Table 1) quantifying lung inflammation in available lung sections reflected the qualitative assessments of lung inflammation described above. As shown in Fig. 2B, lung pathology increased over time in both CRA-sensitized and unsensitized control mice over the course of MAV-1 infection. Pathology was greater in CRA-sensitized mice compared to unsensitized control mice at every time point before and after MAV-1 infection, but only at 7 d.p.i. was this difference statistically significant.

Mucus production in airways of CRA-sensitized and MAV-1-infected mice

Mucus overproduction is a common feature of asthma (Evans and Koo, 2008), and respiratory viral infection is often associated with

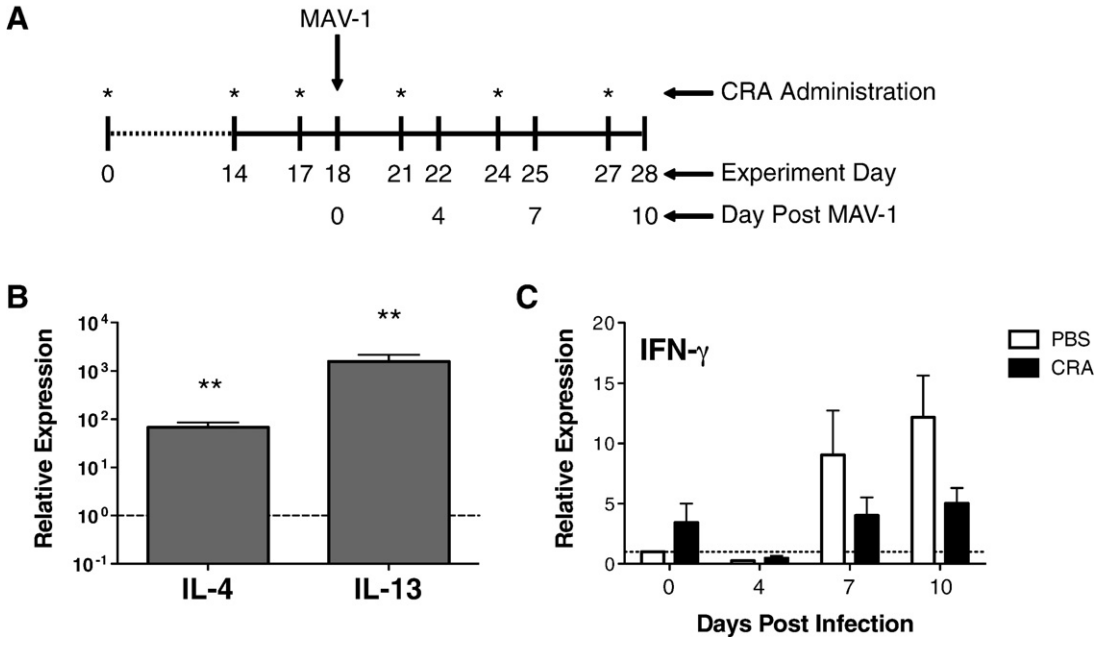

Fig. 1. Cytokine expression in the lungs of CRA-sensitized mice. (A) Experimental protocol for in vivo CRA sensitization and infection. Animals were sensitized intraperitoneally and subcutaneously with CRA at day 0 and subsequently received intranasal CRA at the time points indicated with an asterisk. Unsensitized control mice were instead treated with PBS at each time point. All animals were infected intranasally with MAV-1 on day 18. Animals were euthanized and organs were harvested prior to infection on day 18 and then at 4, 7 and 10 d.p.i. (corresponding to experiment days 22, 25 and 28). (B) RT-qPCR was used to assess IL-4 and IL-13 expression in the lungs of CRA-sensitized and unsensitized control mice on day 18, immediately prior to MAV-1 infection. Data for five mice per group are presented as means $\pm$ S.E.M. $^{**}P<0.01$ compared to unsensitized mice. (C) RT-qPCR was used to assess IFN-γ expression in the lungs of CRA-sensitized and unsensitized control mice prior to infection and then at the indicated time points post-infection. Data for CRA-sensitized mice are presented as fold change from expression levels measured in unsensitized control mice, which are set at 1 for each time point (indicated with horizontal dashed line). Data for five to ten mice per group are combined from three independent experiments and presented as means $\pm$ S.E.M.