Virology 512 (2017) 144–150

Contents lists available at ScienceDirect

Virology

journal homepage: www.elsevier.com/locate/virology

Protective role of Indoleamine 2,3 dioxygenase in Respiratory Syncytial Virus associated immune response in airway epithelial cells

Devi Rajan[a], Raghavan Chinnadurai[b], Evan O. Keefe[a], Seyhan Boyoglu-Barnum[c], Sean O. Todd[a], Tina V. Hartert[d], Jacques Galipeau[b], Larry J. Anderson[a,*]

[a] Department of Pediatrics, Emory Children's Center, Emory University and Children's Healthcare of Atlanta, Atlanta, Georgia
[b] Department of Medicine, University of Wisconsin Carbone Comprehensive Cancer Center, University of Wisconsin, Madison, WI, USA
[c] VPL, VRC, NIAID, NIH, DHHS, Bethesda, MD, USA
[d] Department of Medicine, Vanderbilt Institute for Medicine & Public Health, Nashville, TN, USA

ARTICLE INFO

Keywords:
Respiratory Syncytial Virus
IDO
Cytokine
Chemokine
Tryptophan

ABSTRACT

RSV is a major cause of severe lower respiratory infection in infants and young children. With no vaccine yet available, it is important to clarify mechanisms of disease pathogenesis. Since indoleamine-2,3-dioxygenase (IDO) is an immunomodulatory enzyme and is upregulated with RSV infection, we studied it *in vivo* during infection of BALB/c mice and *in vitro* in A549 cells. RSV infection upregulated IDO transcripts *in vivo* and *in vitro*. IDO siRNA decreased IDO transcripts ~2 fold compared to control siRNA after RSV infection but this decrease did not affect RSV replication. In the presence of IFN-γ, siRNA-induced a decrease in IDO expression that was associated with an increase in virus replication and increased levels of IL-6, IL-8, CXCL10 and CCL4. Thus, our results show IDO is upregulated with RSV infection and this upregulation likely participates with IFN-γ in inhibition of virus replication and suppression of some host cell responses to infection.

1. Introduction

Respiratory Syncytial Virus (RSV) belongs to the genus Pneumovirus in the family Paramyxoviridae. It is an enveloped negative sense single stranded RNA virus. RSV is a major cause of severe lower respiratory infection including bronchiolitis and pneumonia in infants and young children (Nair et al., 2010; Shay et al., 1999; Hall et al., 2009). Most infants are infected before 1 year of age, and almost everyone gets infected by 2 years of age. In addition, RSV causes respiratory disease in persons with compromised cardiac, pulmonary, or immune systems and in the elderly (Falsey et al., 2005). It is estimated that RSV infection leads to as many as 170,000 hospitalizations and 2.1 million outpatient visits each year in the United States among children younger than 5 years old (Hall et al., 2009; Stockman et al., 2012) and 177,000 hospitalizations and 14,000 deaths among adults older than 65 years (Falsey et al., 2005). RSV infection in infancy has also been associated with later development of reactive airway disease and asthma (Piedimonte, 2002; You et al., 2006; Sigurs et al., 2010). The extent of RSV disease has made it a high priority for vaccine development but neither a vaccine or highly effective treatment is yet available (Hall et al., 2009; Falsey et al., 2005; Stockman et al., 2012; Respiratory syncytial virus—United States, Contract No.35. 2011; Van Drunen Littel-van den Hurk and Watkiss, 2012).

Understanding the pathogenesis of RSV disease provides the foundation for developing new approaches to vaccines and anti-viral drugs. Recent reports show that RSV induces Indoleamine-2,3-dioxygenase (IDO) in dendritic cells (DCs) (Ajamian et al., 2015) and Mesenchymal Stromal Cells (MSCs) (Cheung et al., 2016) and there is a direct correlation between RSV replication and IDO expression (Ajamian et al., 2015). Since IDO is an immunomodulatory enzyme that has been shown to affect a number of immune responses including some linked to RSV (Ajamian et al., 2015; Cheung et al., 2016), it might participate in the pathogenesis of RSV disease. IDO catalyzes the degradation of the essential amino acid L-tryptophan and generates a family of catabolites known as kynurenines. It is expressed in various cell types including activated macrophages and other immunoregulatory cells (Mellor et al., 2001) and reported to play a role in a variety of pathophysiological processes such as antimicrobial and antitumor defense, neuropathology, immunoregulation, and antioxidant activity (Katz et al., 2008; Outinen et al., 2011; Tetsutani et al., 2007; Makala et al., 2011; Carlin et al., 1989a, 1989b; Bodaghi et al., 1999; Terajima and Leporati, 2005; Mao et al., 2011; Boasso et al., 2007). For example, IDO has been reported to cause apoptosis of Th-1 but not Th-2 lymphocytes (Fallarino et al., 2002, 2003) and Th-2 - type responses have been associated with

* Correspondence to: Department of Pediatrics, Emory Children's Center, 2015 Uppergate Drive, Room 530, Atlanta, GA 30322, USA.
E-mail address: larry.anderson@emory.edu (L.J. Anderson).

http://dx.doi.org/10.1016/j.virol.2017.09.007
Received 15 June 2017; Received in revised form 31 August 2017; Accepted 7 September 2017

RSV disease. A number of viruses, bacteria, parasites, and various cytokines including IFN-γ induce IDO. Given the earlier studies of RSV and IDO, RSV's immune regulatory effects, and the breadth of IDOs immune regulatory effects, we sought to investigate IDO in RSV infection in the BALB/c mice and human airway epithelial cells (A549) since airway epithelial cells are the primary site for RSV human infection.

2. Material and methods

2.1. Animal studies

Animal studies were performed according to a protocol approved by the Emory University (Atlanta, GA) Institutional Animal Care and Use Committee. Four- to six-week-old, specific-pathogen-free female BALB/c mice (Charles River Laboratory, Wilmington, MA) were housed in microisolator cages, fed sterilized water and food *ad libitum* and challenged intranasally with 1×10^6 PFU of RSV A2 or recombinant 19F (r19F) in serum-free minimal essential medium (MEM) (volume, 50 µl). Lung specimens were collected at different days post infection and stored in RNA lyzol at −80° freezer until use. Before use, lung specimens were thawed and homogenized using minibead beater (Biospec Products, Bartlesville, OK).

2.2. Human airway epithelial cells and viruses

A549 cells were obtained from American Type Culture Collection (ATCC) and grown in minimum essential medium (MEM) supplemented with 10% fetal calf serum (FCS), 1 mM L- Glutamine and 1X penicillin streptomycin. RSV A2 strain was provided by A.G.P.Oomens (Mitra et al., 2012) and r19F by M Moore (Moore et al., 2009). The RSV A2 strain is widely used in RSV studies. We included the r19F strain because it induces different features of disease in mice that might be related to IDO (Moore et al., 2009). For virus preparation, HEp-2 cells were infected with 0.01MOI of RSV A2 and five days later, media was collected and centrifuged briefly to remove the cellular debris, and the clarified supernatant was purified by centrifugation through a 20% sucrose cushion at 14,000 g for 2 h. The infectivity titer was determined in 96 well flat bottom tissue culture plates with ~5000 HEp-2 cells/well and 10-fold serial dilutions of virus in 8 replicates. The infected cells were incubated at 37^0C for 5 days and replication of virus determined by an RSV enzyme-linked immunosorbent assay (ELISA) and tissue culture infectivity dose ($TCID_{50}$) calculated by the Reed Muench method (Reed and Muench, 1938).

2.3. RT-PCR

Supernatant and cell pellet collected from infected AECs were briefly centrifuged to remove the cell debris. Total RNA was extracted from supernatant and cells using Qiagen RNEasy mini kit according to manufacturer's instructions. RSV RNA was assayed by a real-time RT-PCR assay using AgPath-ID™ One-Step RT-PCR Reagents and the Applied Biosystems 7500 Fast Real-Time PCR System (Life Technologies Corporation, Carlsbad, CA). The primers and probes for the RSV matrix (M) gene (forward primer, 5′-GGC AAA TAT GGA AAC ATA CGT GAA-3′; reverse primer, 5′-TCT TTT TCT AGG ACA TTG TAY TGA ACA G-3′; probe, 5′-6-carboxyfluorescein (FAM)-TGT CCG TCT TCT ACG CCC TCG TC- black hole quencher 1 (BHQ-1)-3′) were obtained from Integrated DNA Technologies (IDT) (Coralville, IA). (Kodani et al. 2011). Infected AECs were lysed and total RNA was extracted using an RNeasy plus mini kit (QIAGEN). Normalized RNA was used to convert cDNA using Quantitect reverse transcription kit (QIAGEN). SYBR green (Perfecta Sybr green fast mix, Quanta Biosciences) real-time PCR was performed with primer pairs for IDO and other tryptophan enzymes (Asp et al., 2011). For animal studies, RNA was extracted from lung homogenates and RSV RNA was assayed by a real-time RT-PCR.

2.4. SiRNA Knock down

A549 cells were seeded in 96-well plate at a concentration of 5000 cells/well 1 day prior to transfection with ON-TARGETplus Human IDO1 (3620) siRNA-SMARTpool and ON-TARGETplus Non-targeting pool from Dharmacon GE. During transfection, the cells were conditioned with serum-free 10 mM HEPES containing α-MEM for 30 min. 2 µl of 100 µM siRNA solution and 4 µl DharmaFECT 1 reagent was added to 250 µl α-MEM containing 10 mM HEPES. The siRNA solution and the DharmaFECT reagent were mixed and incubated at room temperature for 30 min. 50 µl of the siRNA transfection mixture was added to each well. The cells were then incubated for 5 h followed by replacement of the transfection medium with A549 culture medium.

2.5. Cytokine assays

Multiplex luminex assays were performed from the collected supernatant according to the manufacturer's instructions for IFN-γ, IL–12 (p40 / p70) IL–13, RANTES, MIP–1α, MIG, MIP–1β, IL–1β, IL–2, IL–4, IL–5, IL–6, IL–2R, MCP–1, Eotaxin, IL–8, IL–10, IL–15, IL–17, IL–1RA, GM–CSF, TNF-α, IL–7, IP-10, IFN-α (Human cytokine 30-plex panel, Life technologies) using Luminex xMAP technology.

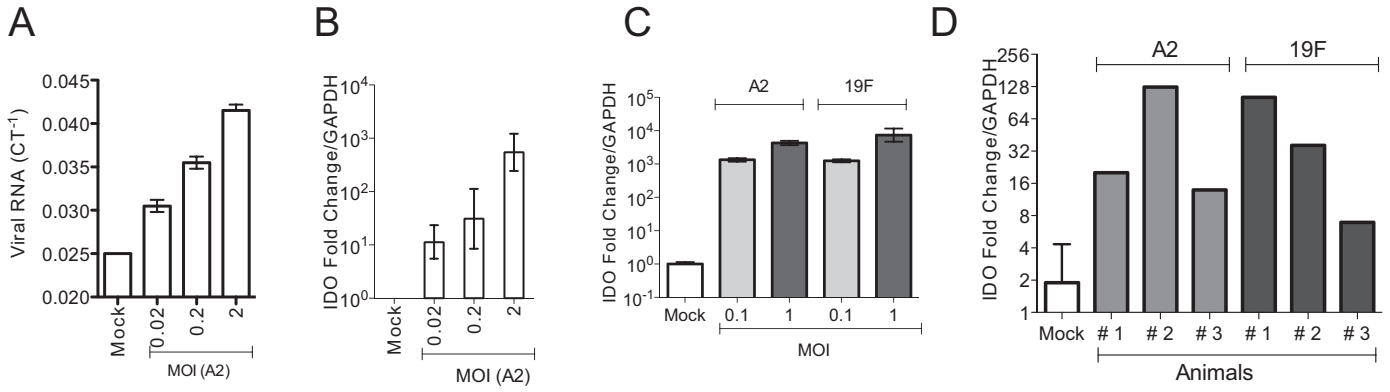

Fig. 1. RSV induces IDO *in vitro* and *in vivo* (A) The relative amount of viral RNA detected in supernatants of A549 cells infected with RSV A2 in real time (RT) PCR and represented as inverse cycle threshold (Ct) values. Ct levels reflect the number of cycles required to exceed the background level; inverse Ct levels (1/Ct) are proportional to the amount of target nucleic acid in the sample. RT-PCR underwent 40 cycles of amplification. The data are represented as average ± SD from a representative experiment. (B) A549 cells were infected with indicated MOI of RSV A2 for 72 h. RNA was extracted from the cells and the expression levels of IDO mRNA were quantitated by quantitative SYBR Green real-time PCR. GAPDH mRNA levels were used as internal controls. The $\Delta\Delta C_t$ method was applied to calculate the fold change. (C) IDO expression using two different RSV strains is shown. (D) IDO expression in lung tissue homogenate of BALB/c mice challenged with RSV A2 or r19F 8 days post challenge is shown.