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RSV-induced prostaglandin E2 production occurs via cPLA$_2$ activation: Role in viral replication

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Abstract

Prostaglandins (PGs) are lipid mediators that participate in the regulation of immunological and inflammatory responses, and PG production can affect viral replication. In this study, we have investigated the mechanism of PGE2 production in airway epithelial cells, following respiratory syncytial virus (RSV) infection, and its role in viral replication. We show that RSV infection strongly induces PGE2 secretion, in a time- and replication-dependent manner, through increased cyclooxygenase-2 (COX-2) expression, which occurs independently from viral or cellular protein synthesis. RSV infection induces arachidonic acid release through induction of cytoplasmic phospholipase A$_2$ (cPLA$_2$) enzymatic activity and its membrane translocation. Specific inhibitors of cPLA$_2$ significantly block RSV-induced PGE2 secretion, indicating a key role of cPLA$_2$ in viral-induced PG production. Blocking PG secretion, through cPLA$_2$ or COX-2 inhibition, results in impairment of RSV replication and subsequent RSV-mediated epithelial cell responses, suggesting that inhibition of PG secretion could be beneficial in RSV infection by reducing proinflammatory mediator production.

Keywords: RSV; Airway epithelial cells; Prostaglandin; Inflammation

Introduction

Prostaglandins (PGs) are ubiquitous fatty acid derivatives produced by a variety of tissues and cell types and participate in the regulation of both physiological and pathophysiological processes. Normal physiological processes include regulation of renal function, vasomotor tone, platelet aggregation and blood clotting, differentiation of immune cells, wound healing, nerve growth, bone metabolism, ovulation, and initiation of labor. Prostaglandins also participate in the regulation of immunological and inflammatory responses. Production of prostaglandin E2 (PGE2) is markedly increased in response to a variety of stimuli including interleukin-1 (IL-1), tumor necrosis factor (TNF)α, antigen/antibody complexes, lipopolysaccharide (LPS), and infectious agents (Arias-Negrete et al., 1995; Corbett et al., 1993; Steer et al., 2003). PGE2 often plays an immunosuppressive role during inflammatory responses, thus preventing overactivation of cellular immunity. For example, PGE2 is able to inhibit cytokine production, as well as T and B cell proliferation (Phipps et al., 1991). A variety of DNA and RNA viruses have been shown to stimulate PGE2 production through cyclooxygenase (COX)-2 expression (Bartz et al., 2002; Nokta et al., 1996; Rossen et al., 2004), although certain viruses, like Epstein–Barr virus, have been shown to suppress PGE2 secretion and COX-2 mRNA accumulation (Savard et al.,

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2000). Respiratory syncytial virus (RSV), an enveloped negative-sense single-stranded RNA virus (Hall and McCarthy, 1995), is a leading cause of epidemic respiratory tract illness in children in the US and worldwide, resulting in more than 100,000 hospital admissions annually in the US alone (Hall and McCarthy, 1995). RSV infection results in the production of a variety of mediators involved in lung immune/inflammatory responses, like cytokines, chemokines, interferons, and upregulating adhesion molecules and MHC antigens on the surface of infected cells (reviewed in Garofalo and Haeberle, 2000). Though RSV infection of the lung results in profound airway inflammation, it is not associated with robust and long-lasting immunological responses so that re-infection is common throughout life (Garofalo and Ogra, 1996). Indeed, RSV infection has been shown to inhibit mononuclear cell responses, as well as lymphocyte proliferation (Franke et al., 1995; Keles et al., 1999), possibly through PGE2 production (Keles et al., 1998, 1999). RSV induction of PGE2 was demonstrated by infecting human cord blood macrophages (Midulla et al., 1993) and dendritic cells (Bartz et al., 2002). When compared with influenza and parainfluenza virus, RSV was the strongest PGE2 inducer. In vivo studies, either in cattle experimentally infected with bovine RSV (Gershwin et al., 1989) or in infants positive for RSV infection (Sznajer et al., 2004), have also shown increased levels of PGE2 in the plasma or endotracheal aspirates. Recently, Richardson et al. have shown PGE2 production and COX-2 expression in alveolar epithelial cells infected with RSV, as well as in the lungs of RSV-infected cotton rats (Richardson et al., 2005). The latter study, however, did not address in depth the mechanism of RSV-induced PGE2 prostaglandin secretion and did not investigate the effect of inhibition of PGE2 production on RSV replication. Indeed, increased production of PGE2 has been shown to result in increased replication of many different types of viruses, including herpes, adenovirus, picorna viruses, and HIV, although PGE2 can inhibit viral replication of a few ones, such as adenovirus, parainfluenza, and measles (reviewed in Steer et al., 2003). In this study, we show that alveolar epithelial cells are strong producers of PGE2 following RSV infection, in a time- and replication-dependent manner, through COX-2 mRNA expression and protein synthesis. RSV-induced COX-2 expression does not require either viral or cellular protein synthesis as it occurs in the presence of cycloheximide, an inhibitor of protein translation. RSV infection of airway epithelial cells induces arachidonic acid (AA) release by activation of cytoplasmic phospholipase A2 ($cPLA_2$), and specific inhibitors of $cPLA_2$ significantly block RSV-induced PGE2 secretion in A549 cells, indicating a key role of $cPLA_2$ in viral-induced prostaglandin production. Inhibition of PGE2 secretion, using either specific COX-2 or $cPLA_2$ inhibitors, results in significant impairment of RSV replication and therefore RSV-mediated airway epithelial cell responses, such as chemokine production, as well as transcription factor activation, suggesting that selective inhibition of PGE2 could be beneficial in RSV infection by dampening the secretion of proinflammatory mediators and possibly promoting stronger immunological responses.

Results

RSV infection induces PGE2 secretion through COX-2 expression in A549 cells

We and others have previously shown that airway epithelial cells, the target cells of RSV infection, play an important role in initiating mucosal immune/inflammatory responses by producing a variety of soluble factors, like cytokines, chemokines, interferons, etc., that regulate communication among cells of the immune system (Garofalo and Haeberle, 2000). Since PGE2 levels have been shown to be elevated in children with severe RSV infection (Sznajer et al., 2004), we investigated whether A549 cells, a lung carcinoma cell line that retains features of type II alveolar epithelial cells (Lieber et al., 1976; Smith, 1977), were able to produce PGE2 following RSV infection. A549 cells is a widely accepted model for studying RSV–epithelial cell interactions (Fiedler et al., 1996; Garofalo et al., 1996; Jamaluddin et al., 1998), and we have previously shown that these cells behave very similar to normal human airway epithelial cells, such as the small alveolar epithelial cells, in terms of chemokine/cytokine gene expression, transcription factor, and signaling pathway activation following RSV infection (Garofalo et al., 1996; Olszewska-Pazdrak et al., 1998; Casola et al., 2001a; Zhang et al., 2001; Pazdrak et al., 2002). A549 cells were infected with purified RSV, and culture supernatants were harvested at different time post-infection (p.i.) to determine PGE2 secretion by ELISA. As shown in Fig. 1A, RSV exposure resulted in a time-dependent increased production of PGE2, starting as early as 6 h p.i., with maximal production at 36 h p.i., time after which infected cells started to loose viability. Addition of UV-inactivated virus, which is unable to replicate, did not induce PG production (data not shown).

The first event in eicosanoid production is the release of arachidonic acid (AA) by PLA_2. COX enzymes catalyze the oxidation of AA to PGH2 which is then isomerized to various prostanoids, including PGE2. There are three known isoforms of COX (reviewed in Smith et al., 2000). COX-1 and COX-2 are similar in size, substrate specificity, and kinetics but differ in distribution and expression. COX-1 is constitutively expressed by many cell types, and PGs produced by this isoform are involved in the regulation of several normal physiological functions (Smith et al., 2000). While COX-2 is constitutively expressed in certain organs or tissues, such as brain, kidney, and testes, in most cell types, its expression is rapidly upregulated by a variety of stimuli, and it is responsible for inducible PG secretion (Smith et al., 2000). Therefore, to determine whether increased PGE2 release was dependent on COX-2 activa-