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Serial Review: Redox Signaling in Immune Function and Cellular Responses in Lung Injury and Diseases
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Role of epithelial nitric oxide in airway viral infection ☆

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Abstract

The airway mucosal epithelium is the first site of virus contact with the host, and the main site of infection and inflammation. Nitric oxide (NO) produced by the airway epithelium is vital to antiviral inflammatory and immune defense in the lung. Multiple mechanisms function coordinately to support high-level basal NO synthesis in healthy airway epithelium and further induction of NO synthesis in the infected airway of normal hosts. Hosts deficient in NO synthesis, such as those patients with cystic fibrosis, have impaired antiviral defense and may benefit from therapies to augment NO levels in the airways.

Keywords: Antiviral host defense; Nitric oxide

Contents

Abbreviations: BALF, bronchoalveolar lavage fluid; CF, cystic fibrosis; cGMP, guanosine 3′,5′-cyclic monophosphate; CMV, cytomegalovirus; dsRNA, double-stranded RNA; EPO, eosinophil peroxidase; GAS, γ-activated site; GSH, reduced glutathione; HIV, human immunodeficiency virus; HOCl, hypochlorous acid; HPIVs, human parainfluenza viruses; HRSV, human respiratory syncytial virus; H_2O_2, hydrogen peroxide; IRF, interferon regulatory factor; LPO, lactoperoxidase; MPO, myeloperoxidase; NO_3^-, nitrate; NO, nitric oxide; NOS, nitric oxide synthases; NO_2^-, nitrite; $ONOO^-$, peroxynitrite; PKR, dsRNA-activated protein kinase; poly (IC), polyinosinic-polycytidylic acid; ROS, reactive oxygen species; O_2^-, superoxide; GSNO, *S*-nitrosoglutathione; ssRNA, single-stranded RNA; STAT, signal transducer and activator of transcription.

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Introduction

Respiratory virus infection remains a major cause of morbidity and mortality in human populations worldwide with a broad spectrum of clinical disease ranging from asymptomatic infection, rhinitis, to viral pneumonia. A variety of viruses are associated with respiratory tract infections including rhinovirus, coronavirus, human respiratory syncytial virus (HRSV), influenza A and B viruses, human parainfluenza viruses (HPIVs), and adenovirus [1–3]. Among them, rhinovirus infections are the predominant cause of the common cold and have been associated with exacerbations of asthma, chronic bronchitis, and sinusitis [4–7]. HRSV is the most common cause of bronchiolitis and other respiratory infections in infants and young children [8–10]. Although the factors dictating virulence are complex, the interaction between inherent viral properties and host cellular response ultimately determines severity of virus disease and outcome [11–13].

In this context, the airway mucosal epithelium, a primary target of respiratory viruses, plays an important role in the innate host defense response to virus [14,15]. A critical early component of the innate host defense in the airway is the ability of respiratory epithelial cells to produce high levels of nitric oxide (NO). NO functions as a signaling molecule in initiation of the inflammatory response to viruses, and also has direct antiviral effects [14,16–18]. Here, we review the human airway production of NO and the consequence of NO deficiency on antiviral defense.

NO production in the lung

Nitric oxide synthases

NO is synthesized endogenously by NO synthases (NOS) (EC 1.14.13.39). Three isoforms of NOS, which are products of separate genes [19,20], have been identified, including neuronal (nNOS or NOS I), endothelial (eNOS or NOS III), and iNOS or NOS II [19,21,22]. These enzymes convert L-arginine to NO and L-citrulline in a reaction that requires oxygen, NADPH, and cofactors FAD, FMN, tetrahydrobiopterin, calmodulin, and iron protoporphyrin IX (Fig. 1). NOS I and III, originally identified in neuronal and endothelial cells, respectively, depend on increases in calcium to bind calmodulin which results in enzyme activation and picomolar levels of NO production. NOS II is regulated at the level of transcription, mRNA stability, and translation, and is expressed after exposure of cells to specific cytokines and/or endotoxin [19,23–25]. NOS II is calcium independent, binding calmodulin even at low calcium concentrations intracellularly, and produces nanomolar levels of NO [19,26]. NO is relatively unstable and rapidly oxidized in simple buffers to the metabolic end-products nitrite (NO_2^-) and nitrate (NO_3^-), which can be used as indirect markers to monitor NO formation [19] (Fig. 2).

NO in the human airway

NO is produced within the human lung, as evidenced by NO detectable in the exhaled breath, and in the lower airway gases of humans (Fig. 2). Furthermore, reaction products of NO (e.g., NO_2^-, NO_3^-) are detectable at micromolar levels in the bronchoalveolar lavage fluid from human lungs [21,27]. Immunohistochemical studies reveal the presence of the three isoforms of NOS in the airway. NOS III is primarily localized to pulmonary endothelial cells, and NOS I in nonadrenergic, noncholinergic inhibitory neurons [21,28]. NOS II is continuously expressed in normal human airway epithelium under basal airway conditions [24,29–31]. NO is also produced by the upper respiratory tract epithelium within the nasopharynx and paranasal sinuses, most likely by NOS II [32]. There is evidence that epithelial NOS II activity is a major determinant of NO present in exhaled breath [33].

Traditionally termed inducible NOS, NOS II is constitutively expressed only in select tissues like airway epithelium and is more typically synthesized in response to inflammatory or proinflammatory mediators [31]. Although NOS II expression is relatively abundant, at approximately 5–10 mRNA copies per airway epithelial cell, inflammation related to increased endogenous or exogenous reactive oxygen species (ROS), as in asthma or hyperoxia, leads to marked upregulation of the gene expression in human airways. Basal expression of NOS II is very likely important in the innate mucosal host defense of the airway, with further induction related to host response to pathogen(s) or inflammation. The role of NOS II in host defense of the airway is confirmed by mice genetically deficient in NOS II [34,35]. On the other

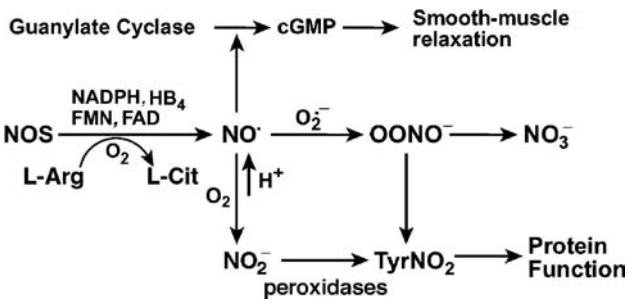

Fig. 1. NO metabolism and function. NOS, nitric oxide synthases; NO˙, nitric oxide; NO_2^-, nitrite; NO_3^-, nitrate; L-Arg, L-arginine; L-Cit, L-citrulline; HB_4, tetrahydrobiopterin; cGMP, guanosine 3′,5′-cyclic monophosphate; $O_2^{\cdot -}$, superoxide; $OONO^-$, peroxynitrite; $TyrNO_2$, nitrotyrosine.

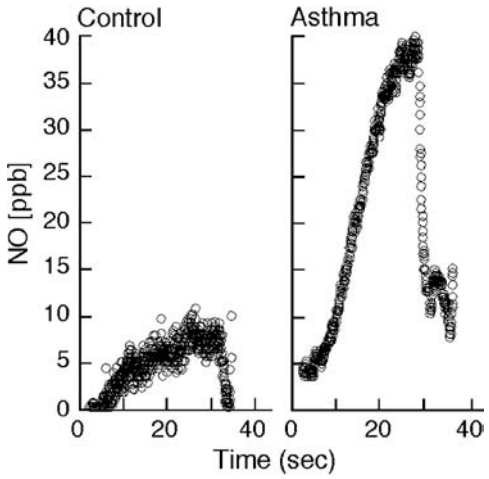

Fig. 2. Accumulation of NO in the lower airway during breath hold of healthy control individual shows that NO is formed in the airway. Levels of accumulation are much greater in an asthmatic individual with airway inflammation [26].

hand, increased NOS II expression in response to virus infection may contribute to airway injury [36].

Once produced, NO is freely diffusible and enters target cells. Vasodilatory effects are mediated by signal transduction primarily through activation of soluble guanylate cyclase to produce guanosine 3′,5′-cyclic monophosphate (cGMP) [31]. NO and/or its reaction products also mediate effects through protein modifications, particularly nitration of tyrosine and nitrosylation of thiols in proteins [37–39]. NO is recognized to play key roles in virtually all aspects of lung biology, and is clearly involved in antimicrobial host defense [20,21,23]. Hence, reduction of basal NO levels in the lung is associated with several lung diseases characterized by increased susceptibility to respiratory infections, e.g., cystic fibrosis (CF) (Table 1). On the other hand, an excessively high level of NO production is also implicated in the pathophysiology of lung diseases, such as asthma (Fig. 2, Table 1) [29,40,41].

NO and viral infections

Alterations in exhaled breath concentrations of NO have been described for many pulmonary diseases [21,42–52] (Table 1). Exhaled NO is increased in chronic inflammatory airway diseases, such as asthma and bronchiectasis, and in upper respiratory tract infections, including bacterial and viral respiratory illnesses [21,43–45]. Epithelial cells at mucosal surfaces have a variety of inflammatory and immune defense mechanisms to deal with viruses, including the induction of NOS II and increased production of NO [14–18,29]. For example, experimental influenza virus or rhinovirus 16 infection increase exhaled NO in volunteers [53,54]. Furthermore, HRSV infection, Hong Kong influenza A (H3N2) virus, or Adenovirus infection in mice in vivo or in primary human airway epithelial cells in vitro increases levels of NOS II mRNA, protein, and activity in respiratory epithelium and increases production of NO (Fig. 3) [11,14,17,55]. In contrast, exhaled NO is decreased in subjects infected with the Human immunodeficiency virus (HIV)-1, suggesting that viral mechanisms exist to suppress NO-related host defense [56].

Table 1
Alterations of exhaled NO in lung diseases

Increased exhaled NO	Decreased exhaled NO
Asthma	Pulmonary arterial hypertension
Bronchiectasis	Cystic fibrosis
Lymphangioleiomyomatosis	α1-antitrypsin deficiency
Viral respiratory tract infection	Primary ciliary dyskinesia
Active tuberculosis	Acute respiratory distress syndrome
Allergen challenge (late response)	Cigarette smoke
Hyperoxia	Passive smoking
	HIV-1 infection

Antiviral effects of NO

NOS II is sufficient for antiviral defense in human airway epithelial cells [16,34,35,57–61]. Replication of a wide range of DNA and RNA viruses are inhibited in vitro by the addition of chemical donors of NO, or by the overexpression or induction of NOS II. NO inhibits both virus replication and latency of viruses, including coxsackievirus, influenza A and B, rhinovirus, cytomegalovirus, vaccinia virus, ectromelia virus, human herpesvirus-1, and HPIV3 [18,34,57–59,61–65]. Likewise, loss of NO synthesis leads to increase of virus titers with infection, and significantly more severe clinical outcomes [34,35,60,66,67]. For example, mice genetically deficient in NOS II have significantly higher mortality, and decreased virus clearance, with cytomegalovirus (CMV) [35] or coxsackievirus B4 [34] as compared to wild-type. Similarly, in mice treated with a specific inhibitor of NOS II, CMV replication is moderately enhanced as evidenced by 5-fold increase in viral titers, and this results in viral persistence and latency [60].

High-level NO synthesis results in a large variety of reactive products which inhibits viral protein and RNA synthesis, and viral replication by modifying target molecules essential for replication [68,69]. Two specific virus targets of

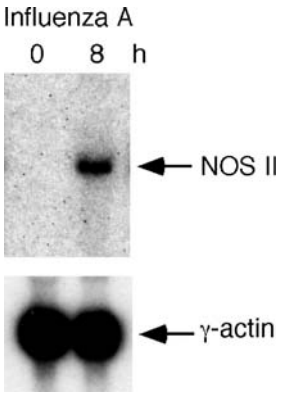

Fig. 3. NOS II induction by influenza A. Northern analysis for NOS II was performed using total RNA from A549 cells (20 μg RNA/lane) uninfected or infected with influenza A virus [$2 \times 10^{5.25}$ 50% of tissue infectious dose ($TCID_{50}$)/ml for 8 h]. γ-Actin is shown as a control [11].

NO, ribonucleotide reductase, the rate-limiting enzyme in DNA synthesis, and viral protease, have been identified on the basis of in vitro exposure of viral protein to NO donors in cell-free systems [62,64,70]. However, targets of NO modification also include host cell proteins, particularly thiol groups and tyrosine [37–39]. NO may also bind to heme iron in proteins [71]. In lung epithelial cells exposed to inflammatory cytokines to induce NOS II, over 40 cellular proteins are modified by tyrosine nitration, with consequences on activity and function [37]. In this context, NO production can be beneficial as an antiviral effector mechanism against viruses [61,62,72,73], but also may be detrimental by contributing to inflammation and injury of the respiratory tract through formation of toxic reactive nitrogen intermediates and cellular protein modifications [72–74]. For example, in murine models of virus-induced pneumonia, NOS II activity and mRNA expression are greatly increased within lung tissue [72–74], but inhibition of NO production improves the histopathologic changes and the survival of virus-infected mice without exerting an effect on the pulmonary viral titer. Therefore, in some cases, pulmonary injury may be attenuated by administration of NO inhibitors without significant effect on viral clearance [72–74].

Abnormalities of NO and virus infection in cystic fibrosis

Cystic fibrosis (CF) is a hereditary disease characterized by dysfunction of the exocrine glands, but respiratory disease is the major cause of morbidity and mortality. Chronic endobronchial infection and inflammation ultimately result in lung destruction and death [75]. Bacterial colonization of CF lung is usually established in the first decade of life and, once established, is difficult to eradicate [76]. Little is known about the natural history of bacterial respiratory tract infection and factors associated with initial colonization in CF lung, but studies show a clear relationship between acute viral respiratory tract infection, including HRSV, HPIV, influenza virus, and adenovirus, and pulmonary exacerbation and disease progression in children with CF [77–81]. In fact, CF children have increased susceptibility to virus infections as compared to non-CF siblings. In one study of CF children with early diagnosis at neonatal screening, hospitalizations in the first year of life were due to respiratory virus infection in over 50% of cases [82]. Surprisingly, despite the inflammatory nature and chronic infections of the lung in CF, NO levels in exhaled breath are lower while nitrite in bronchoalveolar lavage fluid (BALF) is higher from CF patients as compared to healthy controls or individuals with other inflammatory lung diseases [83–85]. Notably, infants with CF before the onset of respiratory symptoms have mean levels of exhaled NO threefold lower than healthy control infants, suggesting that the lower levels of NO in CF are not the result but, rather, a precursor of pulmonary disease [86]. Recent studies support that loss of NO synthesis is a primary pathophysiologic event in the susceptibility of CF patients to virus and other microbial pathogens, through defects in signal transduction to NOS II [16,87–89].

Interacting determinants in NO–antiviral host defense

Other components and/or pathways are also involved directly or indirectly in the antiviral host defense mechanisms, in part through interactions with NO. ROS, including superoxide (O_2^-) and hydrogen peroxide (H_2O_2), are critical in antimicrobial defense but also contribute to human lung inflammation and injury [90–92]. Increased generation of ROS is intrinsic to viral infections and can be caused by direct effects of virus on cells and/or inflammatory responses of the host [90,91,93,94]. NO and ROS, particularly O_2^-, rapidly form the reactive nitrogen oxide, peroxynitrite ($ONOO^-$), that may cause oxidation and nitration of amino acid residues of proteins or guanine of DNA, lipid peroxidation, and DNA cleavage, all of which can cause cellular dysfunction and injury leading to cell death [91,93,95]. Myeloperoxidase (MPO), eosinophil peroxidase (EPO), and lactoperoxidase (LPO) are members of the mammalian peroxidase superfamily and play specific and complementary roles in host defense through oxidative pathways [90,92,95–99]. MPO is highly expressed in neutrophils, monocytes, and certain subpopulations of tissue macrophages [100], and LPO is present in human airway epithelium [101]. Virus-infected cells produce peroxide that drives the virucidal peroxidase–H_2O_2–halide system to catalyze the H_2O_2-dependent peroxidation of halides and pseudohalides to produce antimicrobial hypohalous acids. Hypochlorous acids (HOCl) play an important role in killing microorganisms including virus but also injure normal tissues [90,96–99]. Recent studies show that members of the mammalian peroxidase superfamily can reduce NO levels [101–105]. NO consumption may be a direct consequence of peroxidase reaction since NO is a peroxidase substrate, or occur through NO reactions with peroxidase-generated free radical species (Fig. 1) [101–106].

Reduced glutathione (GSH), an important water-soluble antioxidant, is present at varying levels in the airway lining fluid [90,107,108]. Systemic deficiency of extracellular GSH is present in CF patients, and related to mutation of the CFTR protein, as CFTR is a channel permeant to chloride anions, and other larger organic anions, including reduced GSH [108–110]. GSH deficiency may decrease overall NO production, through decreasing NOS II expression or by the parallel loss of *S*-nitrosoglutathione (GSNO), an important storage form and source of NO [107,108].

Regulation of NO synthesis

NO biosynthesis is regulated at multiple levels in cells, i.e., NOS gene transcription, mRNA processing, protein expression and dimerization, and enzyme reaction kinetics [19]. All NOSs convert L-arginine to NO and L-citrulline in a reaction that requires oxygen, NADPH, and cofactors FAD, FMN, tetrahydrobiopterin, calmodulin, and iron protoporphyrin IX. Thus, pathways that alter substrate L-arginine availability or cofactor levels are key mechanisms for regulation of NO synthesis [27,49,111]. Further, the concentration of NO in any biologic system is the sum of its rate of enzymatic formation and consumption by other biological molecules, such as in reactions

with ROS. Nevertheless, NOS II is largely subject to predominantly transcriptional regulation [19,25,26,112–114]. Induction of NOS II mRNA expression varies in different human cell types, but typically is increased by cytokines [22,25,113,114]. IFNγ is necessary for induction of NOS II in human airway epithelial cells [114–120] (Fig. 4). NOS II expressed constitutively by airway epithelium is identical to the NOS II that is induced by cytokines or endotoxin [29]. The V_{max} of NOS II assures a high level of NO synthesis and is 10-fold greater than the traditional constitutive NOSs, such as NOS III [121].

The basal ability to produce high NO levels indicates that airway epithelial cells are primary effector cells of the host defense system. Healthy human airway epithelium has abundant expression of NOS II due to continuous transcriptional activation of the gene in vivo. Basal NOS II expression in airway epithelium is linked to STAT1 activation, and is related to physiologic levels of IFNγ in vivo. Removal of airway epithelial cells from the airway milieu results in rapid loss of NOS II transcription within a few hours of placing cells into culture. IFNγ induces NOS II gene expression in cells in vitro, with gene response in a dose-dependent manner from a range of 10 to 10,000 U IFNγ/ml, which encompasses healthy physiologic to pathologic levels of IFNγ found during virus infection [25,26,74]. Overall, constitutive NOS II expression in the airway is related to airway factors, including physiologic levels of IFNγ with STAT1 activation, and is important for mucosal host defense under basal conditions. Virus infection may further induce NOS II gene expression, resulting in more NOS II protein and NO synthesis that may function to inhibit virus proliferation, but also may contribute to the oxidative injury of the airway.

Analysis of the 5′-flanking region of the human NOSII shows that cooperative DNA binding of multiple signal transduction proteins is involved in activation of the NOS II promoter. A region of human NOS II 4.9 kb upstream of the transcription start site (GenBank Accession No. AF017634) [122] contains several sites that serve as composite binding elements, i.e., region encompassing the γ-activated site (GAS) and binding sites for activator protein (AP)-1 and nuclear factor kappa B (NF-κB). Prior work has emphasized the importance of AP-1 binding sites in activation of NOS II promoter in response to cytokine combinations [113,119]. AP-1 is a complex composed of proteins of the Fos (c-Fos, FosB, Fra-1 and Fra-2) and Jun (c-Jun, JunB, and JunD) proto-oncogene families [123–125]. In general, Fos and Jun family proteins function as dimeric transcription factors that bind to AP-1 regulatory elements in the promoter and enhancer regions of genes [123,125]. Other studies show that NF-κB activation and binding to κB DNA elements in the 5′-flanking region of the NOS II gene play a role in the cytokine induction of NOS II in A549 lung epithelial cell line in vitro [114]. We have shown that NOS II gene expression requires a physical interaction between c-Fos and signal transducer and activator of transcription (STAT)-1 to participate in cooperative transcriptional activation of the gene [122].

Viral mechanisms of airway inflammation and NO induction

IFN-γ, produced by lymphocytes in the airway mucosa within 24 to 36 h following viral infection, is almost certainly involved in NOS II induction in the course of viral infection through the activation of STAT-1. However, NOS II induction by virus early in infection is mediated by proteins responsive

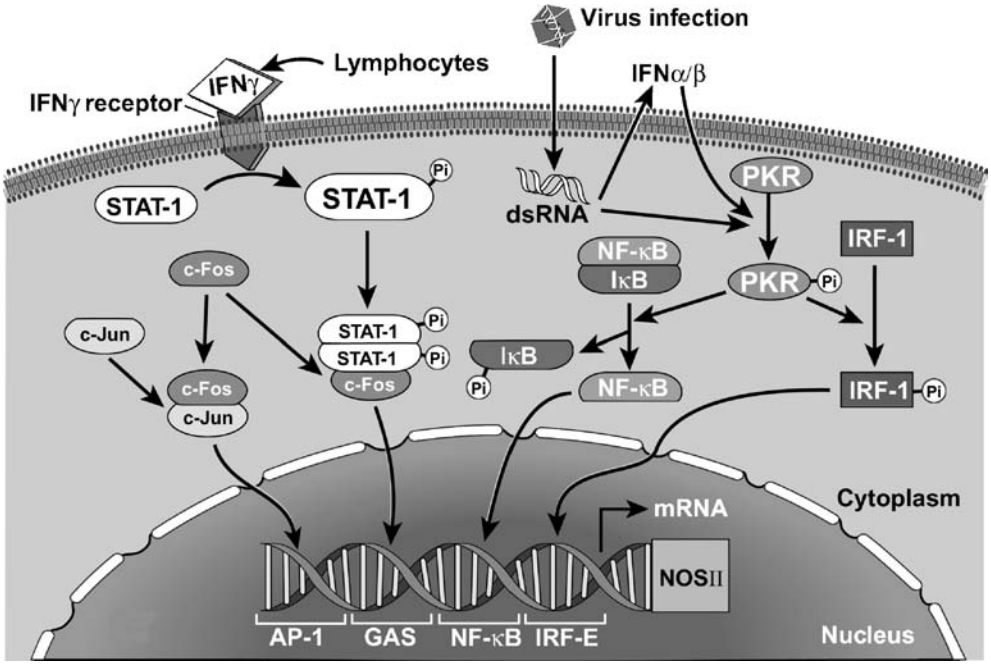

Fig. 4. Mechanisms of viral-induced NOS II expression in human airway epithelial cells. STAT, signal transducer and activator of transcription; dsRNA, double-stranded RNA; PKR, dsRNA-activated protein kinase; NF-κB, nuclear factor kappa B; IRF-1, interferon regulatory factor 1; AP-1, activator protein-1; GAS, γ-activated site; IRF-E, interferon regulatory factor element.

to the viral replicative intermediate, double-stranded RNA (dsRNA) [11]. Most viruses induce the synthesis of dsRNA at some time during their replication cycle [126]. For single-stranded RNA (ssRNA) viruses, dsRNA is the replicative intermediate in infected cells. For DNA viruses, dsRNA accumulates as the result of overlapping convergent transcription. Intracellular dsRNA formed during viral replication [126] binds to and activates a serine/threonine kinase, dsRNA-activated protein kinase (PKR), which activates signal transduction that leads to the activation of NOS II [11,16,17,126–130].

PKR is critical for host antiviral mechanisms and for the activation of NOS II, as assessed by experiments in murine embryo fibroblast cells derived from mice genetically deficient in PKR [11,129]. PKR exhibits two distinct kinase activities upon activation by dsRNA, autophosphorylation/activation and phosphorylation of substrates [131], which regulate transcriptional events by phosphorylation of proteins related to signal transduction pathways. For example, NF-κB activation by dsRNA in human airway epithelial cells is due to PKR activation and phosphorylation of the inhibitor of NF-κB (IκB). PKR also leads to transcriptional and posttranslational effects on interferon regulatory factor 1 (IRF-1), which may subsequently activate NOS II gene expression [132–135]. IRF-1 protein is induced and activated by dsRNA in human airway epithelial cells [11]. Thus, PKR is essential in the signaling pathway for dsRNA induction of NOS II. Intriguingly, PKR is also essential for endotoxin-induction of NOS II in murine cells, confirming a central role for PKR in a generalized microbial-response signaling pathway to NOS II [11].

Defects in signal transduction to NOS II in CF

Unlike normal airway epithelium, NOS II expression under basal conditions of the CF airway epithelium is absent in vivo. Further, NOS II expression is not inducible in CF airway cells in vitro, despite multiple stimuli including HPIV3, influenza virus, dsRNA mimic (poly(IC)) or combinations of cytokines (IFNγ, IL-1β, and TNFα) and endotoxin [16,89]. Loss of expression is linked to impaired activation of STAT-1 [16,89]. PKR, IRF-1, and NF-κB activation induced by poly(IC) are similar in CF and healthy control cells [16]. Loss of constitutive and inducible gene expression and the subsequent absence of high-level NO synthesis have been linked to host susceptibility to microbial invasion of the airway.

STAT-1 is required for IFN signal transduction in the cell and essential for the host response to virus infection [136,137]. Despite numerous downstream targets of STAT-1 activation, loss of NOS II has been identified as one primary factor in the susceptibility of STAT-1 null animals to virus [138–140]. Previous studies have shown that nonfunctional JAK1 or Tyk2, receptor-associated kinases in the IFN signaling pathway, also result in decreased STAT-1 protein and activation, and a defective antiviral state, although the response to IFNα or γ is intact [139,140]. The Tyk2-deficient cells display a phenotype remarkably analogous to CF airway epithelial cells: impairment of STAT-1 activation, increased virus replication in cells, and lack of NOS II induction, but most all other IFN-dependent pathways are intact. Altogether, these studies support that NOS II is one of the central mediators of the antiviral effects of STAT-1 activation in the human airway.

Therapies with NO as antiviral agent

Chemical donors of NO or induction of NOS II inhibit virus replication in vitro [16,34,35,57–64]. Since loss of NO synthesis is a primary pathophysiologic event in the susceptibility of CF patients to virus and other microbial pathogens [16,87], overexpression of NOS II or NO donors is rational strategy to protect from viral infection. These approaches are successful in reducing virus infection of CF airway epithelial cells in culture [16] (Fig. 5). Important for application to patient care, providing NO donor after virus infection also significantly reduces virus replication [58,59,62,63,141,142]. Thus, exogenous application of NO to the lung is a prospect for future antiviral therapy especially in the CF neonate in whom virus infection is a life-threatening event.

Conclusion

NO is a critical component of the antiviral host defense mechanisms in airway epithelium. Healthy human airway epithelium has abundant expression of NOS II due to continuous transcriptional activation of the gene in vivo. NOS II gene expression increases rapidly with viral infection in response to the viral replicative intermediate dsRNA activation of PKR signaling events. Multiple mechanisms function to support NO synthesis later in the course of virus infection including the IFN/STAT-1 pathways (Fig. 4). Thus, the airway

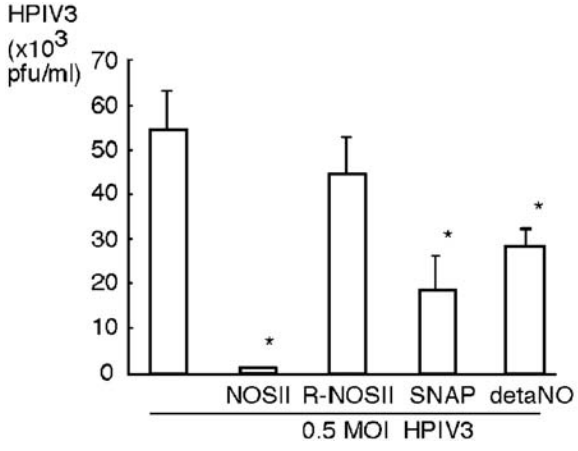

Fig. 5. NOS II overexpression or NO donors protect CF cells from HPIV3 infection. Plaque assay of media overlying CF cells 24 h after HPIV3 infection (0.5 multiplicity of infections). Twenty-four hours prior to infection, CF cells were transfected with NOS II expression construct (NOSII), reverse sequence NOS II expression construct (R-NOSII), or left untreated. At the time of infection, some untreated CF cells were exposed to NO donors [SNAP, or deta NONOate (detaNO)]. Untreated cells have higher titer of infectious virus production than cells with NOS II-transgene or with NO donors [16]. Reprinted from S. Zheng et al., *Immunity*, Vol. 18, pp. 619–630, copyright (2003) with permission from Elsevier.

epithelium has highly efficient NO synthetic machinery which is amplified in viral infection. Loss of NO synthesis in lung diseases such as CF predisposes one to increased virus/microbe infection due to defects in signal transduction through STAT-1. Clinical epidemiology suggests that virus infections impact on early morbidity and tempo of disease progression in CF. In fact, although extension of CF children's life expectancy occurred with the introduction of antibiotics, vaccination against virus such as measles was one of the earliest interventions that led to improved survival in the 1960s [143]. Prior to viral vaccinations, CF neonates who contracted measles, a virus like HPIV3 in the paramyxovirus family, had progressive decline with worsening of pulmonary infections and early death [144,145]. Thus, therapies aimed at improving antiviral host defense may further improve and extend the life of CF individuals. On the other hand, chronic high levels of NO lead to oxidative stress and airway injury, so the optimal timing, duration, and amount of NO supplementation which may benefit patients will be critical in planning therapy.

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