Commentary

Nitric oxide: a pro-inflammatory mediator in lung disease?

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Abstract

Inflammatory diseases of the respiratory tract are commonly associated with elevated production of nitric oxide ($NO^{\bullet}$) and increased indices of $NO^{\bullet}$-dependent oxidative stress. Although $NO^{\bullet}$ is known to have anti-microbial, anti-inflammatory and anti-oxidant properties, various lines of evidence support the contribution of $NO^{\bullet}$ to lung injury in several disease models. On the basis of biochemical evidence, it is often presumed that such $NO^{\bullet}$-dependent oxidations are due to the formation of the oxidant peroxynitrite, although alternative mechanisms involving the phagocyte-derived heme proteins myeloperoxidase and eosinophil peroxidase might be operative during conditions of inflammation. Because of the overwhelming literature on $NO^{\bullet}$ generation and activities in the respiratory tract, it would be beyond the scope of this commentary to review this area comprehensively. Instead, it focuses on recent evidence and concepts of the presumed contribution of $NO^{\bullet}$ to inflammatory diseases of the lung.

Keywords: inflammation, neutrophil, nitric oxide, nitrotyrosine, peroxidases

Introduction

Since its discovery as a biological messenger molecule more than 10 years ago, the gaseous molecule nitric oxide ($NO^{\bullet}$) is now well recognized for its involvement in diverse biological processes, including vasodilation, bronchodilation, neurotransmission, tumor surveillance, antimicrobial defense and regulation of inflammatory-immune processes [1–3]. In the respiratory tract, $NO^{\bullet}$ is generated enzymically by three distinct isoforms of $NO^{\bullet}$ synthase (NOS-1, NOS-2 and NOS-3) that are present to different extents in numerous cell types, including airway and alveolar epithelial cells, neuronal cells, macrophages, neutrophils, mast cells, and endothelial and smooth-muscle cells. In contrast with the other two NOS isoforms (NOS-1 and NOS-3), which are expressed constitutively and activated by mediator-induced or stress-induced cell activation, NOS-2 activity is primarily regulated transcriptionally and is commonly induced by bacterial products and pro-inflammatory cytokines. As such, inflammatory diseases of the respiratory tract, such as asthma, acute respiratory distress syndrome (ARDS) and bronchiectasis, are commonly characterized by an increased expression of NOS-2 within respiratory epithelial and inflammatory-immune cells, and a markedly elevated local production of $NO^{\bullet}$, presumably as an additional host defense mechanism against bacterial or viral infections. The drawback of such excessive $NO^{\bullet}$ production is its accelerated metabolism to a family of potentially harmful reactive nitrogen species (RNS), including peroxynitrite ($ONOO^{-}$) and nitrogen dioxide ($NO_2^{\bullet}$), especially in the presence of phagocyte-generated oxidants. The formation of such RNS is thought to be the prime reason why $NO^{\bullet}$

ARDS = acute respiratory distress syndrome; EPO = eosinophil peroxidase; MPO = myeloperoxidase; $NO^{\bullet}$ = nitric oxide; NOS = $NO^{\bullet}$ synthase; $O_2^{\bullet-}$ = superoxide; $ONOO^{-}$ = peroxynitrite; RNS = reactive nitrogen species.

can in many cases contribute to the etiology of inflammatory lung disease [4–6]. Despite extensive research into both pro-inflammatory and anti-inflammatory actions of $NO^{\bullet}$, the overall contribution of $NO^{\bullet}$ to inflammatory conditions of the lung is not easily predicted and seems to depend on many factors, such as the site, time and degree of $NO^{\bullet}$ production in relation to the local redox status, and the acute or chronic nature of the immune response. In addition, our current understanding of the pro-inflammatory or pro-injurious mechanisms of $NO^{\bullet}$ or related RNS is incomplete; this commentary will focus primarily on these latter aspects.

Evidence for a pro-inflammatory role of $NO^{\bullet}$ in the respiratory tract

To explore a role for $NO^{\bullet}$ (or NOS) in infectious or inflammatory diseases, two general research approaches have been taken: the use of pharmacological inhibitors of NOS isoenzymes, and the targeted deletion of individual NOS enzymes in mice. Both approaches suffer from the shortcoming that animal models of respiratory tract diseases generally do not faithfully reflect human disease. The use of NOS inhibitors to determine the contribution of individual NOS isoenzymes is also hindered by problems related to specificity and pharmacokinetic concerns. However, the unconditional gene disruption of one or more NOS isoforms, leading to lifelong deficiency, can have a markedly different outcome from pharmacological inhibition at a certain stage of disease, as the involvement of individual NOS isoenzymes can be different depending on disease stage and severity. Despite these inherent limitations, studies with the targeted deletion of NOS isoforms have led to some insights, indicating a role for $NO^{\bullet}$ and NOS-2 in the etiology of some inflammatory lung diseases. For instance, mice deficient in NOS-2 are less susceptible to lethality after intranasal inoculation with influenza A virus, suffer less lung injury after administration of endotoxin, and display reduced allergic eosinophilia in airways and lung injury in a model of asthma, than their wild-type counterparts [7–9]. However, although the contribution of NOS-2 is expected in inflammatory conditions, recent studies have determined that NOS-1, rather than NOS-2, seems to be primarily involved in the development of airway hyper-reactivity in a similar asthma model [10]. The linkage of NOS-1 to the etiology of asthma was more recently supported in asthmatic humans by an association of a NOS-1 gene polymorphism with this disease, although the physiological basis for this association remains unclear [11].

Despite the potential contribution of NOS-2-derived $NO^{\bullet}$ to lung injury after endotoxemia, the sequestration of neutrophils in the lung and their adhesion to postcapillary and postsinusoidal venules after administration of endotoxin were found to be markedly increased in NOS-2-deficient mice, and NOS-2 deficiency did not alleviate endotoxin-induced mortality. It therefore seems that the 'harmful' and 'protective' effects of NOS-2 might contend with each other within the same model, which makes the assessment of the potential role of NOS in human disease even more difficult. In this context, it is interesting to note that humans or animals with cystic fibrosis have subnormal levels of NOS-2 in their respiratory epithelium, related to a gene mutation in the cystic fibrosis transmembrane conductance regulator [12]. This relative absence of epithelial NOS-2 might be one of the contributing factors behind the excessively exuberant respiratory tract inflammatory response in patients with cystic fibrosis, even in the absence of detectable respiratory infections. Overall, the apparently contrasting findings associated with NOS deficiency, together with concerns about animal disease models used, make interpretations and conclusions with regard to human lung disease all the more difficult.

Pharmacological inhibitors of NOS have also been found to reduce oxidative injury in several animal models of lung injury, such as ischemia/reperfusion, radiation, paraquat toxicity, and endotoxemia (see, for example, [13–15]). However, results are again not always consistent, and in some cases NOS inhibition has been found to worsen lung injury, indicating anti-inflammatory or protective roles for $NO^{\bullet}$. All in all, despite these inconsistencies, there is ample evidence from such studies to suggest a contributing role of $NO^{\bullet}$ in various respiratory disease conditions, which continues to stimulate research into mechanistic aspects underlying such pro-inflammatory roles and modulation of $NO^{\bullet}$ generation as a potential therapeutic target.

Injurious properties of $NO^{\bullet}$: a role for $ONOO^{-}$?

Although the pro-inflammatory and injurious effects of $NO^{\bullet}$ might be mediated by a number of diverse mechanisms, it is commonly assumed that such actions are largely due to the generation of reactive by-products generated during the oxidative metabolism of $NO^{\bullet}$; these are collectively termed RNS. One of the prime suspects commonly implicated in the adverse or injurious properties of $NO^{\bullet}$ is $ONOO^{-}$, a potent oxidative species formed by its almost diffusion-limited reaction with superoxide ($O_2^{\bullet-}$), which is a product of activated phagocytes and of endothelial or epithelial cells [4,5,13]. The formation of $ONOO^{-}$ seems highly feasible under conditions of elevated production of both $NO^{\bullet}$ and $O_2^{\bullet-}$ *in vivo*, and its oxidative and cytotoxic potential is well documented [5,6]. However, because the direct detection of $ONOO^{-}$ under inflammatory conditions is virtually impossible because of its instability and high reactivity, the formation of $ONOO^{-}$ *in vivo* can be demonstrated only by indirect methods. Thus, many investigators have relied on the analysis of characteristic oxidation products in biological molecules, such as proteins and DNA, most notably free or protein-associated 3-nitrotyrosine, a product of tyrosine oxidation that can be formed by $ONOO^{-}$ (and several other RNS) but not by $NO^{\bullet}$ itself (see, for

example, [5]). Indeed, elevated levels of 3-nitrotyrosine have been observed in many different inflammatory conditions of the respiratory tract [16], which illustrates the endogenous formation of $ONOO^-$ or related RNS in these cases. However, without known evidence for functional consequences of (protein) tyrosine nitration, the detection of 3-nitrotyrosine should not be regarded as direct proof of a pro-inflammatory role of $NO^\bullet$. Moreover, although the detection of 3-nitrotyrosine has in most cases been interpreted as conclusive evidence for the formation of $ONOO^-$ *in vivo* (see, for example, [17]), it should be realized that other RNS formed by alternative mechanisms might also contribute to endogenous tyrosine nitration. Indeed, it has recently become clear that the presence of inflammatory-immune cells, and specifically their heme peroxidases myeloperoxidase (MPO) and eosinophil peroxidase (EPO), can catalyze the oxidization of $NO^\bullet$ and/or its metabolite NO_2^- to more reactive RNS and thereby contribute to protein nitration [16,18,19]. This notion is further supported by the fact that 3-nitrotyrosine is commonly detected in tissues affected by active inflammation, mostly in and around these phagocytic cells and macrophages, which can also contain active peroxidases originating from apoptotic neutrophils or eosinophils. Hence, the detection of 3-nitrotyrosine *in vivo* cannot be used as direct proof of the formation of $ONOO^-$, but merely indicates the formation of RNS by multiple oxidative pathways, possibly including $ONOO^-$ but more probably involving the activity of phagocyte peroxidases [16,20]. In this regard, a preliminary study with EPO-deficient mice has recently demonstrated the critical importance of EPO in the formation of 3-nitrotyrosine in a mouse model of asthma [21]. Future studies with animals deficient in MPO and/or EPO will undoubtedly help to clarify this issue.

Protein tyrosine nitration in the lung: does it really matter?

Given the considerable interest in 3-nitrotyrosine as a collective marker of the endogenous formation of $NO^\bullet$-derived RNS, the crucial question remains of whether the detection of 3-nitrotyrosine adequately reflects the toxic or injurious properties of $NO^\bullet$. The formation of $ONOO^-$ (or of other RNS that can induce tyrosine nitration) might in fact represent a mechanism of decreasing excessive levels of $NO^\bullet$ that might exert pro-inflammatory actions by other mechanisms. For instance, $NO^\bullet$ can promote the expression of pro-inflammatory cytokines or cyclo-oxygenase (responsible for the formation of inflammatory prostanoids) by mechanisms independent of $ONOO^-$ [22,23], and the removal of $NO^\bullet$ would minimize these responses. Furthermore, although $ONOO^-$ or related $NO^\bullet$-derived oxidants can be cytotoxic or induce apoptosis, these effects might not necessarily relate to their ability to cause protein nitration (see, for example, [16]). For instance, the bactericidal and cytotoxic properties of $ONOO^-$ are minimized by the presence of CO_2, even though aromatic nitration and other radical-induced modifications are enhanced [5]. Similarly, the presence of NO_2^- in the incubation medium decreases the cytotoxicity of MPO-derived hypochlorous acid (HOCl) toward epithelial cells or bacteria, despite increased tyrosine nitration of cellular proteins (A van der Vliet and M Syvanen, unpublished data). Thus, it would seem that the cytotoxic properties of $NO^\bullet$ and/or its metabolites might instead be mediated through preferred reactions with other biological targets, and these might not necessarily be correlated with the degree of tyrosine nitration. The extent of nitrotyrosine immunoreactivity in bronchial biopsies of asthmatic patients was correlated directly with measured levels of exhaled $NO^\bullet$ and inversely with the provocation concentration for methacholine (PC_{20}) and forced expiratory volume in 1 s [24]. However, an immunohistochemical analysis of nitrotyrosine and apoptosis in pulmonary tissue samples from lung transplant recipients did not identify patients with an imminent risk of developing obliterative bronchiolitis [25]. It is therefore still unclear to what degree tyrosine nitration relates to disease progression.

Several studies with purified enzymes have suggested that nitration of critical tyrosine residues adversely affects enzyme activity, but there is as yet no conclusive evidence *in vivo* for biological or cellular changes as a direct result of tyrosine nitration [16,20]. For instance, tyrosine nitration was suggested to have an effect on cellular pathways by affecting cytoskeletal proteins or tyrosine phosphorylation, thereby affecting processes involved in, for example, cell proliferation or differentiation [16,26]. Recent studies have provided support for selective tyrosine nitration within certain proteins [27,28] and of selective cellular targets for nitration by RNS (see, for example, [29,30]), and such specificity might indicate a potential physiological role for this protein modification. However, in none of these cases could tyrosine nitration be linked directly to changes in enzyme function. Chemical studies have indicated that tyrosine nitration by RNS accounts for only a minor fraction of oxidant involved, and reactions with other biological targets (thiols, selenoproteins, or transition metal ions) are much more prominent [5,6]. Indeed, the extent of tyrosine nitration *in vivo* is very low (1–1000 per 10^6 tyrosine residues according to best estimates [16]), although different analytical methods used to detect 3-nitrotyrosine in biological systems have often given inconsistent results. It is important to note that recent rigorous studies have unveiled substantial sources of artifact during sample preparation, which might frequently have led to an overestimation of tyrosine nitration *in vivo* in previous studies [31].

On the basis of current knowledge, the formation of 3-nitrotyrosine seems to be merely a marker of $NO^\bullet$-derived oxidants, with as yet questionable pathophysiological significance. In view of the low efficiency of tyrosine

nitration by biological RNS, and the endogenous presence of variable factors that influence protein nitration (antioxidants or other RNS scavengers), it seems unlikely that tyrosine nitration is a reliable mechanism of, for example, enzyme regulation. Nevertheless, the recent discovery of enzymic 'denitration' mechanisms that can reverse tyrosine nitration [32] merits further investigation of the possibility that tyrosine nitration might reflect a signaling pathway, for example analogous to tyrosine phosphorylation or sulfation.

Direct and indirect signaling properties of NO•

The biological effects of NO• are mediated by various actions, either by NO• itself or by secondary RNS, and the overall biochemistry of NO• is deceptively complex. Moreover, the metabolism and chemistry of NO• depend importantly on local concentrations and pH; the recently described acidification of the airway surface in asthmatics [33] might significantly affect NO• metabolism in these patients. It is well known that interactions with the ion centers of iron or other transition metals are responsible for many of the signaling properties of NO•; the activation of the heme enzyme guanylyl cyclase and the consequent formation of cGMP is involved not only in smooth-muscle relaxation but also in the activation of certain transcription factors, the expression of several pro-inflammatory and anti-inflammatory genes (including cytokines and cyclo-oxygenase), and the production of respiratory mucus [22–34]. In addition to such direct signaling properties, many actions of NO• might be due largely to secondary RNS that can react with multiple additional targets, in some cases forming nitroso or nitro adducts as potentially unique NO•-mediated signaling mechanisms. As discussed, the formation of protein nitrotyrosine has been postulated as a potential RNS-specific signaling pathway. Even more interest has been given to the reversible *S*-nitros(yl)ation of protein cysteine residues, which has been proposed to affect a number of redox-sensitive signaling pathways, for example by the activation of $p21^{ras}$ or the inhibition of protein tyrosine phosphatases [35,36]. Similar modifications of reactive cysteine residues in transcription factors such as nuclear factor-κB or of caspases contribute to the regulation of gene expression and apoptosis [37–39]. The precise mechanisms leading to protein *S*-nitrosylation *in vivo* are still not clarified, but might involve dinitrogen trioxide (formed during the autoxidation of NO•), iron-nitrosyl complexes, and perhaps $ONOO^-$ [16]; changes in NO• metabolism during inflammatory lung diseases undoubtedly affect such NO•-dependent signaling pathways. In addition, *S*-nitrosylation can be reversed by either enzymic (thioredoxin or glutaredoxin) or chemical (metals or oxidants) mechanisms, and evidence is increasing that this reversible modification is complementary to more widely accepted oxidant-dependent redox signaling pathways [40]. The reported alterations in *S*-nitrosothiol levels in tracheal secretions of patients with asthma or cystic fibrosis further point to altered NO• metabolism in these cases, and might provide new clues to the role of *S*-nitrosylation in controlling such disease processes [41,42]. Unfortunately, technical limitations to detect *S*-nitrosylation in specific protein targets *in vivo* have limited a full understanding of this potential signaling pathway; further research in these areas can be expected to establish more clearly its significance in the pathophysiological properties of NO•.

What is to come?

Despite the by now overwhelming evidence for the increased formation of NO• and NO•-derived oxidants in many different lung diseases, the exact contribution of NO• or its metabolites to inflammatory lung disease is still unclear. Indeed, NO• might have distinctly different roles in different stages of respiratory tract inflammatory diseases, being pro-inflammatory or pro-injurious in acute and severe stages but perhaps being protective and anti-inflammatory in more stable conditions; it is uncertain whether NOS is a suitable therapeutic target in the management of inflammatory lung disease. Caution is clearly needed when interpreting observations of tyrosine nitration in animal models of disease or in human tissues, which does not automatically implicate $ONOO^-$ (as often thought), but rather indicates the formation of RNS by various mechanisms. Furthermore, animal models of chronic lung disease that usually reflect short-term or acute inflammation might not always be applicable to chronic airway diseases in humans. For instance, phagocyte degranulation, a common feature observed in association with human airway inflammatory diseases such as asthma, does not seem to occur in mouse models of asthma [43]. Therefore the importance of granule proteins, such as heme peroxidases, in the pathology of human airway diseases might not be adequately reflected in such animal models. More work with animal models more characteristic of human diseases or with biopsy materials from human subjects will be required to unravel the precise role of NO• in inflammatory lung disease, and might establish more clearly whether the pharmacological inhibition of NOS isoenzymes can be beneficial. This brings up the interesting paradox that, despite presumed adverse roles of NO• in such inflammatory lung diseases as septic shock and ARDS, NO• inhalation has been suggested as a potential therapeutic strategy to improve overall gas exchange [44]. Intriguingly, in a rat model of endotoxemia, inhalation of NO• was found to reduce neutrophilic inflammation and protein nitration [45], again supporting the crucial involvement of inflammatory-immune cells in this protein modification.

For a better assessment of the role of NO• in respiratory tract diseases in humans, the production of RNS and/or characteristic markers would need to be more carefully

monitored during various disease stages. Care should be given to analytical techniques, their quantitative capacity and the possibility of artifacts. The monitoring of exhaled NO•, although convenient and non-invasive, does not reflect the actual production or fate of NO• in the respiratory tract and is not well correlated with NOS activity in the lung [46]. We therefore need to continue research into the local biochemistry of NO• in the lung, taking into account the presence of secreted or phagocyte peroxidases and possible changes in local pH, as in asthmatic airways [33], that might modulate NO• activity and metabolism. This might result in a better understanding of relationships between the various metabolic endproducts of NO• (NO_2^-, NO_3^-, or nitroso and nitro adducts) and its pro-inflammatory or injurious properties.

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References

1. Moncada S, Palmer RMJ, Higgs EA: **Nitric oxide: physiology, pathophysiology, and pharmacology.** *Pharmacol Rev* 1991, **43**:109–142.
2. Weinberger B, Heck BE, Laskin DL, Laskin JD: **Nitric oxide in the lung: therapeutic and cellular mechanisms of action.** *Pharmacol Therapeut* 1999, **84**:401–411.
3. Fang FC: **Mechanisms of nitric oxide-related antimicrobial activity.** *J Clin Invest* 1997, **100**:S43–S50.
4. Gaston B, Drazen JM, Loscalzo J, Stamler JS: **The biology of nitrogen oxides in the airways.** *Am J Respir Crit Care Med* 1994, **149**:538–551.
5. Beckman JS, Koppenol WH: **Nitric oxide, superoxide, and peroxynitrite: the good, the bad, and the ugly.** *Am J Physiol* 1996, **271**:C1424–C1437.
6. Grisham MB, Jourd'Heuil D, Wink DA: **Nitric oxide. I. Physiological chemistry of nitric oxide and its metabolites: implications in inflammation.** *Am J Physiol* 1999, **276**:G315–G321.
7. Nathan C: **Inducible nitric oxide synthase: what difference does it make?** *J Clin Invest* 1997, **100**:2417–2423.
8. Xiong Y, Karupiah G, Hogan SP, Foster PS, Ramsay AJ: **Inhibition of allergic airway inflammation in mice lacking nitric oxide synthase 2.** *J Immunol* 1999, **162**:445–452.
9. Kristof AS, Goldberg P, Laubach V, Hussain SNA: **Role of inducible nitric oxide synthase in endotoxin-induced acute lung injury.** *Am J Respir Crit Care Med* 1998, **158**:1883–1889.
10. De Sanctis GT, MacLean JA, Hamada K, Mehta S, Scott JA, Jiao A, Yandava CN, Kobzik L, Wolyniec WW, Fabian AJ, Venugopal CS, Grasemann H, Huang PL, Drazen JM: **Contribution of nitric oxide synthases 1, 2, and 3 to airway hyperresponsiveness and inflammation in a murine model of asthma.** *J Exp Med* 1999, **189**:1621–1629.
11. Grasemann H, Yandava CN, Storm van's Gravesande K, Deykin A, Pillari A, Ma J, Sonna LA, Lilly C, Stampfer MJ, Israel E, Silverman EK, Drazen JM: **A neuronal NO synthase (NOS1) gene polymorphism is associated with asthma.** *Biochem Biophys Res Commun* 2000, **272**:391–394.
12. Kelley TJ, Drumm ML: **Inducible nitric oxide synthase expression is reduced in cystic fibrosis murine and human airway epithelial cells.** *J Clin Invest* 1998, **102**:1200–1207.
13. Ischiropoulos H, Al-Mehdi AB, Fisher AB: **Reactive species in ischemic rat lung injury: contribution of peroxynitrite.** *Am J Physiol* 1995, **269**:L158–L164.
14. Akaike T, Noguchi Y, Ijiri S, Setoguchi K, Suga M, Zheng YM, Dietzschold B, Maeda H: **Pathogenesis of influenza virus-induced pneumonia: involvement of both nitric oxide and oxygen radicals.** *Proc Natl Acad Sci USA* 1996, **93**:2448–2453.
15. Tsuji C, Shioya Y, Hirota Y, Fukuyama N, Kurita D, Tanigaki T, Ohta Y, Nakazawa H: **Increased production of nitrotyrosine in lung tissue of rats with radiation-induced acute lung injury.** *Am J Physiol* 2000, **278**:L710–L725.
16. van der Vliet A, Eiserich JP, Shigenaga MK, Cross CE: **Reactive nitrogen species and tyrosine nitration in the respiratory tract. Epiphenomena or a pathobiologic mechanism of disease?** *Am J Respir Crit Care Med* 1999, **160**:1–9.
17. Sadeghi-Hashjin G, Folkerts G, Henricks PAJ, Muijers RBR, Nijkamp FP: **Peroxynitrite in airway diseases.** *Clin Exp Allergy* 1998, **28**: 1464–1473.
18. Eiserich JP, Hristova M, Cross CE, Jones AD, Freeman BA, Halliwell B, van der Vliet A: **Formation of nitric oxide-derived inflammatory oxidants by myeloperoxidase in neutrophils.** *Nature* 1998, **391**:393–397.
19. Wu W, Chen Y, Hazen SL: **Eosinophil peroxidase nitrates protein tyrosyl residues. Implications for oxidative damage by nitrating intermediates in eosinophilic inflammatory disorders.** *J Biol Chem* 1999, **274**:25933–25944.
20. Ischiropoulos H: **Biological tyrosine nitration: a pathophysiological function of nitric oxide and reactive oxygen species.** *Arch Biochem Biophys* 1998, **356**:1–11.
21. Duguet A, Iijima H, Eidelman DH: **Nitrotyrosine formation is reduced in eosinophil deficient mice after antigen challenge.** *Am J Respir Crit Care Med* 2000, **161**:A920.
22. Watkins DN, Garlepp MJ, Thompson PJ: **Regulation of the inducible cyclo-oxygenase pathway in human cultured airway epithelial (A549) cells by nitric oxide.** *Br J Pharmacol* 1997, **121**:1482–1488.
23. Idriss SD, Gudi T, Casteel DE, Kharitonov VG, Pilz RB, Boss GR: **Nitric oxide regulation of gene transcription via soluble guanylate cyclase and type I cGMP-dependent protein kinase.** *J Biol Chem* 1999, **274**:9489–9493.
24. Saleh D, Ernst P, Lim S, Barnes PJ, Giaid A: **Increased formation of the potent oxidant peroxynitrite in the airways of asthmatic patients is associated with induction of nitric oxide synthase: effect of inhaled glucocorticoid.** *FASEB J* 1998, **12**:929–937.
25. Hansen PR, Holm AM, Svendsen UG, Olsen PS, Andersen CB: **Apoptosis and formation of peroxynitrite in the lungs of patients with obliterative bronchiolitis.** *J Heart Lung Transpl* 2000, **19**:160–166.
26. Eiserich JP, Estévez AG, Bamberg TV, Ye YZ, Chumley PH, Beckman JS, Freeman BA: **Microtubule dysfunction by posttranslational nitrotyrosination of α-tubulin: a nitric oxide-dependent mechanism of cellular injury.** *Proc Natl Acad Sci USA* 1999, **96**:6365–6370.
27. Souza JM, Daikhin E, Yudkoff M, Raman CS, Ischiropoulos H: **Factors determining the selectivity of protein tyrosine nitration.** *Arch Biochem Biophys* 1999, **371**:169–178.
28. Roberts ES, Lin HI, Crowley JR, Vuletich JL, Osawa Y, Hollenberg PF: **Peroxynitrite-mediated nitration of tyrosine and inactivation of the catalytic activity of cytochrome P450 2B1.** *Chem Res Toxicol* 1998, **11**:1067–1074.
29. Zou MH, Leist M, Ullrich V: **Selective nitration of prostacyclin synthase and defective vasorelaxation in atherosclerotic bovine coronary arteries.** *Am J Pathol* 1999, **154**:1359–1365.
30. Gole MD, Souza JM, Choi I, Hertkorn C, Malcolm S, Foust RF 3rd, Finkel B, Lanken PN, Ischiropoulos H: **Plasma proteins modified by tyrosine nitration in acute respiratory distress syndrome.** *Am J Physiol* 2000, **278**:L961–L967.
31. Yi D, Ingelse BA, Duncan MW, Smythe GA: **Quantification of 3-nitrotyrosine in biological tissues and fluids: generating valid results by eliminating artifactual formation.** *J Am Soc Mass Spectrom* 2000, **11**:578–586.
32. Kamisaki Y, Wada K, Bian K, Balabanli B, Davis K, Martin E, Behbod F, Lee YC, Murad F: **An activity in rat tissues that modifies nitrotyrosine-containing proteins.** *Proc Natl Acad Sci USA* 1998, **95**:11584–11589.
33. Hunt JF, Fang K, Malik R, Snyder A, Malhotra N, Platts-Mills TA, Gaston B **Endogenous airway acidification. Implications for asthma pathophysiology.** *Am J Respir Crit Care Med* 2000, **161**:694–699.
34. Wright DT, Fischer BM, Li C, Rochelle LG, Akley NJ, Adler KB: **Oxidant stress stimulates mucin secretion and PLC in airway epithelium via a nitric oxide-dependent mechanism.** *Am J Physiol* 1996, **271**:L854–L861.
35. Caselli A, Camici G, Manao G, Moneti G, Pazzagli L, Cappugi G, Ramponi G: **Nitric oxide causes inactivation of the low molecular weight phosphotyrosine protein phosphatase.** *J Biol Chem* 1994, **269**:24878–24882.
36. Lander HM, Milbank AJ, Tauras JM, Hajjar DP, Hempstead BL, Schwartz GD, Kraemer RT, Mirza UA, Chait BT, Burk SC, Quilliam LA: **Redox regulation of cell signaling.** *Nature* 1996, **381**:380–381.
37. Marshall HE, Stamler JS: **Exhaled nitric oxide (NO), NO synthase activity, and regulation of nuclear factor (NF)-kappaB.** *Am J Respir Cell Mol Biol* 1999, **21**:296–297.

38. Li J, Billiar TR: **The role of nitric oxide in apoptosis.** *Semin Perinatol* 2000, **24**:46–50.
39. Mannick JB, Hausladen A, Liu L, Hess DT, Zeng M, Miao QX, Kane LS, Gow AJ, Stamler JS: **Fas-induced caspase denitrosylation.** *Science* 1999, **284**:651–654.
40. Finkel T: **Oxygen radicals and signaling.** *Curr Opin Cell Biol* 1998, **10**:248–253.
41. Gaston B, Sears S, Woods J, Hunt J, Ponaman M, McMahon T, Stamler JS: **Bronchodilator *S*-nitrosothiol deficiency in asthmatic respiratory failure.** *Lancet* 1998, **351**:1317–1319.
42. Grasemann H, Gaston B, Fang K, Paul K, Ratjen F: **Decreased levels of nitrosothiols in the lower airways of patients with cystic fibrosis and normal pulmonary function.** *J Pediatr* 1999, **135**:770–772.
43. Malm MGK, Erjefält JS, Persson CGA: **Eosinophil degranulation: mouse–man comparison [abstract].** *Am J Respir Crit Care Med* 2000, **161**:A542.
44. Honda K, Kobayashi H, Hataishi R, Hirano S, Fukuyama N, Nakazawa H, Tomita T: **Inhaled nitric oxide reduces tyrosine nitration after lipopolysaccharide instillation into lungs of rats.** *Am J Respir Crit Care Med* 1999, **160**:678–688.
45. Michael JR, Barton RG, Saffle JR, Mone M, Markewitz BA, Hillier K, Elstad MR, Campbell EJ, Troyer BE, Whatley RE, Liou TG, Samuelson WM, Carveth HJ, Hinson DM, Morris SE, Davis BL, Day RW: **Inhaled nitric oxide versus conventional therapy: effect on oxygenation in ARDS.** *Am J Respir Crit Care Med* 1998, **157**:1372–1380.
46. Mehta S, Lilly CM, Rollenhagen JE, Haley KJ, Asano K, Drazen JM: **Acute and chronic effects of allergic airway inflammation on pulmonary nitric oxide production.** *Am J Physiol* 1997, **272**:L124–L131.

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