

Oxidants and asthma

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Many decades of research have produced a significant amount of data showing increased oxidative stress in asthma and indicating a potential role for oxidants in the pathogenesis of the disease, particularly during exacerbations. Putatively relevant pro-oxidative mechanisms have also been identified. Currently available asthma drugs are generally effective for the treatment of the disease, but their effects on oxidative stress have still not been completely elucidated. From the data available in the literature one can conclude that antioxidant compounds may have a potential role in the treatment of asthma, especially of asthma exacerbations. More convincing evidence from controlled clinical trials is required.

hydrogen peroxide (H_2O_2), either spontaneously or under the influence of superoxide dismutases (SODs). Although O_2^- and H_2O_2 themselves are moderate oxidants, both species are critical for the formation of potent cytotoxic radicals in biological systems through their interaction with other molecules. For instance, the lysosomal enzymes myeloperoxidase (MPO) from neutrophils and monocytes/macrophages and the eosinophil peroxidase (EPO) catalyse the oxidation of halides (Cl^- , Br^- , and I^-) by H_2O_2 to form hypohalous acids (HOCl or HOBr). MPO produces predominantly hypochlorous acid (HOCl) whereas EPO produces more hypobromous acid (HOBr). Hypohalous acid production is important in the host defence against infectious agents, but during this reaction the hydroxyl radical (OH^-) is also produced, which is a powerful and indiscriminate oxidant.

Oxidant generation is part of the normal metabolism of many types of cells and is critical for cell homeostasis. To protect itself against exposure to noxious oxidants, the lung has a well developed antioxidant system.^{1–2} When an imbalance occurs between oxidants and antioxidants in favour of oxidants, oxidative stress is said to occur. Many experimental and clinical data suggest that oxidants play a role in the pathogenesis of several respiratory disorders, including bronchial asthma.

In particular, there is increasing evidence that the chronic airway inflammation typical of asthma results in an increased oxidative stress to the airways. Also, many of the triggers for asthma exacerbations, including viral infections and air pollutants, may activate the production of oxidants, triggering increased inflammation which produces asthmatic symptoms.

Eosinophils possess several times greater capacity for generating oxidants than neutrophils, and the EPO content of eosinophils is several times higher than that of MPO in neutrophils.^{3–9} MPO and EPO derived reactive oxygen species can also interact with nitrite (NO_2^-) and H_2O_2 leading to the formation of reactive nitrogen species (RNS; nitrosants). A powerful oxidant, the radical peroxynitrite ($ONOO^-$), is produced from the reaction between O_2^- and nitric oxide (NO) which is increased in the asthmatic airways.^{3–8 10}

As in many other inflammatory conditions, the oxidative “burst” in asthma is a non-specific process initiated by the concurrent action of numerous inflammatory pathways. Indeed, several asthma mediators including lipid mediators, chemokines, adhesion molecules, and eosinophil granule proteins are potential promoters of oxidant production.⁴

OXIDANTS AND INFLAMMATION IN ASTHMA

Sources

The inflammatory cells recruited to the asthmatic airways have an exceptional capacity for producing oxidants. Once recruited in the airspaces, inflammatory cells may become activated and generate reactive oxidants in response to various stimuli. Activated eosinophils, neutrophils, monocytes, and macrophages, and also resident cells such as bronchial epithelial cells, can generate oxidants.^{3–8} The univalent reaction of oxygen to superoxide anion (O_2^-) is an important step in the formation of oxidants. Sources of superoxide anion include primarily the membrane associated NADPH oxidase dependent complex, the cytosolic xanthine oxidase system, and the mitochondrial respiration chain (fig 1).^{3–8} Superoxide anion is then converted to

Effects of oxidants in experimental settings

In experimental models, oxidants can produce many of the features typical of asthma. They induce bronchoconstriction and increase airway responsiveness to several agonists.^{3–8} The release into the airways of tachykinins and neurokinins and the decrease of β_2 adrenergic receptor, cholinesterase, and neutral endopeptidase activities have been documented in these experimental conditions.^{3–8} Oxidants can also lead to increased permeability of the airways in these models.^{3–8} Conversely, reducing agents exert a relaxing effect on airway smooth muscle and can inhibit bronchial smooth muscle contraction and prevent airway hyperresponsiveness in several experimental models.^{3–8}

Experimental exposure to oxidants induces many degrees of injury to bronchial epithelial cells until cell death. EPO can induce lysis of

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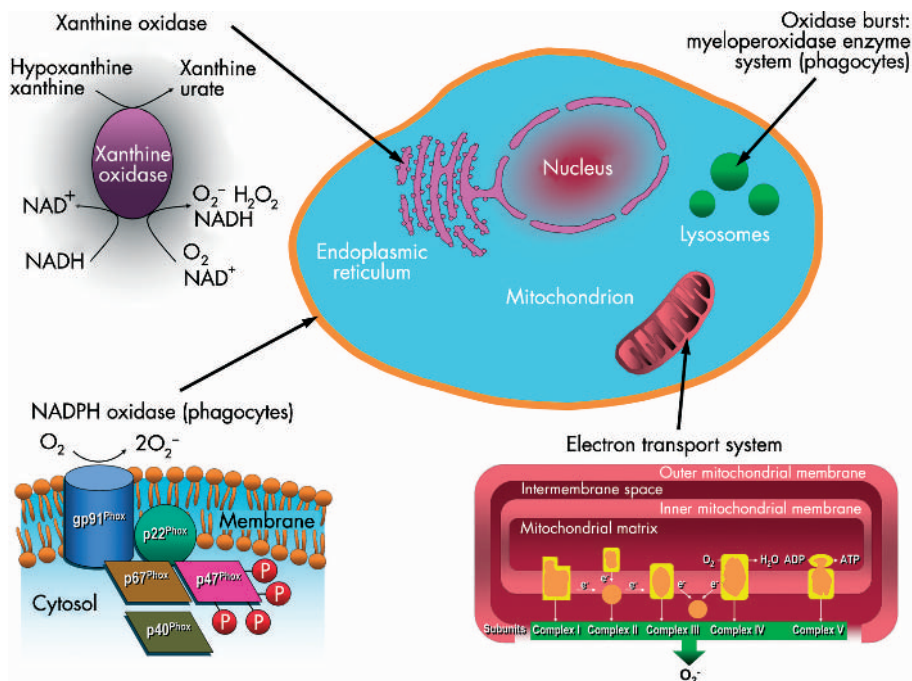


Figure 1 Main cell sources of oxidants.

bronchial epithelial cells at concentrations that have been found in the asthmatic airways.³⁻⁸ Oxidants induce increased apoptosis of bronchial epithelial cells from asthmatic subjects.¹¹⁻¹²

In vitro exposure of structural and inflammatory cells of the lungs to oxidants induces the release of pro-inflammatory mediators including cytokines, chemokines (and their receptors), growth factors, arachidonic acid metabolites, and adhesion molecules (and their ligands) involved in inflammatory cell recruitment in asthma.³⁻⁸

Within the nucleus, oxidants induce changes in gene expression. Interestingly, in vitro oxidative stress can induce activation of nuclear factor κ B (NF- κ B) and activator protein 1 (AP-1), two pivotal regulators of inflammatory processes.¹³ Both these transcription factors are activated in the bronchial epithelial cells of asthmatic subjects.¹⁴ Such activation can potentially explain the increased expression of many pro-inflammatory mediators found in the airways of asthmatic patients.¹⁴

OXIDANTS IN ASTHMA

Many studies suggest that levels of oxidative stress are increased in children and in adults with asthma, not only in their lungs but also in the circulation.

Excessive production of oxidants occurs spontaneously or after stimulation in blood leucocytes of stable asthmatics compared with normal subjects.^{3-8, 15} Levels of EPO and/or MPO are increased in the peripheral blood, induced sputum, and bronchoalveolar lavage (BAL) fluid from patients with stable asthma.^{3-9, 16}

Recently, increased levels of many direct and indirect markers of oxidative stress (including malondialdehyde, thiobarbituric acid reactive products (TBARs), and H_2O_2) have been found in the urine, plasma, sputum, BAL fluid, and lung tissues of patients with asthma.³⁻⁸ Their level is often related to the severity of the disease and is inversely related to the degree of stability.³⁻⁸ Furthermore, analysis of exhaled breath and exhaled condensate has recently allowed direct assessment of H_2O_2 and nitric oxide (NO) and measurement of several indirect byproducts of oxidation in those samples.^{17, 18} The latter are footprints of oxidation on

different substrates such as proteins (nitrotyrosine), lipids (isoprostanes and ethane), and DNA (hydroxydeoxyguanosine).^{17, 18} There is a lot of interest in the effect of these new non-invasive markers on oxidative stress and in their possible clinical application¹⁷⁻¹⁹ as their concentrations are significantly increased in asthma.

The expression of nitrotyrosine is increased also in bronchial and bronchiolar epithelial cells, in smooth muscle cells, and in eosinophils of bronchial airways and lung parenchyma of patients with stable asthma.²⁰⁻²² Excessive production of 3-bromotyrosine, another marker of oxidative stress, has been reported in the airways of patients with severe asthma.²³ A significant increase in 3-bromotyrosine has also been described in the proteins of sputum of asthmatic subjects. This finding is of particular interest as the level of 3-bromotyrosine is strongly related to levels of EPO.^{9, 24} However, whether peroxidases simply reflect granulocytic inflammation or whether they actively contribute to tissue damage in asthma remains to be determined.

Taken together, these data indicate that oxidative stress is enhanced in asthma, and that such redox imbalance is not confined within the lungs.

ANTIOXIDANT DEFENCES IN ASTHMA

Many controlled studies suggest that there is a deficiency of antioxidants with few studies indicating an activation of the pathways protecting lung cells from oxidant mediated damage in the lungs or circulation of asthmatic subjects.^{3-8, 15} For instance, a marked reduction in plasma antioxidant capacity occurs during exacerbations.⁸ In stable conditions, CuZn-SOD activity is lower in steroid naive asthmatics than in normal subjects.²⁵ Similarly, peroxynitrite inhibitory activity, an antioxidant system, is reduced in the sputum of patients with stable asthma and its level is positively related to airway responsiveness and negatively related to forced expiratory volume in 1 second (FEV₁) and the degree of sputum eosinophilia.²⁶

Free NO levels are generally low in the respiratory system as the NO produced by endogenous NO synthases (NOSS) is complexed with reducing agents like thiols, including glutathione, to form a major reservoir of NO related

bioactivity in the form of S-nitrosothiols (SNOs) which are relatively resistant to oxidants.¹⁰ Interestingly, airway levels of the endogenous bronchodilator S-nitrosoglutathione (GSNO) are reduced in children with near fatal asthma.²⁷ It has been hypothesised that GSNO could be broken down by oxidants in the asthmatic lung and that its catabolism could have an inhibitory effect on airway smooth muscle relaxation.²⁸

Among antioxidant systems, cyclin dependent kinase inhibitor p21^{CIP1/WAF1}, a protein that protects against oxidative stress, and extracellular glutathione peroxidase are increased in bronchial epithelial cells of asthmatic patients.²⁹⁻³⁰ A link between asthma and selenium deficiency (glutathione peroxidase is one of many selenium dependent antioxidant enzymes) has also been hypothesised.³¹ However, a controlled trial did not show any significant benefit of short term selenium supplementation in patients with intrinsic asthma.³²

Vitamin C has also been studied for its possible inverse relationship with increased risk of asthma³³ but, to date, evidence from randomised controlled trials is insufficient to recommend a specific role for vitamin C in the treatment of asthma.³⁴ The antioxidant vitamin E inhibits IgE responses to allergic stimuli in animals and dietary vitamin E levels are inversely related to the incidence of asthma.³⁴ Recent studies found that supplementation with vitamin C and vitamin E reduced ozone related decrement in lung function in asthmatic subjects, particularly in those with genetically determined increased susceptibility to oxidant stress.³⁵⁻³⁶

Many promising new antioxidants such as nitrones (spin trap antioxidants that inhibit the formation of intracellular oxidants by forming stable compounds) and non-peptidyl SOD analogues³⁷⁻³⁸ are now ready to enter clinical trials.

EFFECT OF ASTHMA DRUGS ON THE OXIDANT/ANTIOXIDANT BALANCE

Although glucocorticoids do not inhibit in vitro oxidant production from eosinophils,³⁹ inhaled glucocorticoids decrease levels of H₂O₂ in exhaled breath condensate of asthmatics.¹⁷⁻¹⁸ Similarly, treatment with low doses of inhaled glucocorticoids decreases total nitrate and nitrite concentrations both in exhaled breath condensate and in sputum of patients with stable asthma.⁴⁰ Treatment with inhaled glucocorticoids also normalises the reduced bronchial epithelial CuZn-SOD specific activity.²⁵ It is unknown whether the efficacy of glucocorticoid treatment in asthma is somehow linked to their influence on oxidant/antioxidant balance.

In vitro, short acting and long acting inhaled β_2 agonists have an antioxidant effect on neutrophils and eosinophils. However, to achieve their antioxidant effect, supratherapeutic concentrations are required and therefore the clinical relevance of such an effect is unclear. It has been hypothesised that the antioxidant activity of β_2 agonists is related to the number of hydroxyl groups on the phenol rings within their molecular structure.⁴¹⁻⁴²

Aminophylline reduces the generation of oxidants in an animal model of endotoxin induced bronchial hyperresponsiveness. Theophylline also inhibits platelet activating factor (PAF) induced and interleukin (IL)-5 induced oxidant release from blood eosinophils in a dose dependent manner, indicating that theophylline at high concentrations could have a significant antioxidant effect.⁴³⁻⁴⁴

EXACERBATIONS

Most of the studies on oxidative stress in asthma have focused on the oxidant/antioxidant imbalance that occurs in stable asthma. The superimposed effects of exacerbations have received much less attention.

Episodic worsening of asthma is associated with increased airway inflammation.⁴⁻⁴⁵ There is also evidence of enhanced oxidative stress during exacerbations, both systemically and locally. However, a direct correlation between increased oxidative burden and changes in pulmonary function and/or airway inflammation described during exacerbations remains speculative.⁸ In this context, a hypothesis that links exacerbations of asthma to dietary antioxidant deficiency has been proposed.⁸⁻³² Furthermore, several indirect markers of oxidative stress such as H₂O₂ and isoprostanes are increased in exhaled air, sputum, and BAL fluid during exacerbations and after allergen exposure.⁸⁻¹⁷⁻¹⁸

Respiratory viruses represent the most important causes of asthma exacerbations. Rhinoviruses are the virus type most frequently identified in respiratory tract specimens during exacerbations of asthma, both in children and in adults.¹² Experimental rhinovirus infection of asthmatic patients can induce an inflammatory response in the airways associated with variable airflow obstruction and increased airway hyperresponsiveness.¹² Rhinovirus induced airway inflammatory responses involve eosinophils and neutrophils, possibly recruited via cytokines or chemokines released by bronchial epithelial cells or T cells.⁴⁶ Rhinovirus infection of respiratory epithelial cells causes intracellular oxidant generation which is a crucial step in the activation of NF- κ B and in the following production of proinflammatory adhesion molecules and cytokines.⁴⁷ Reducing agents inhibit both rhinovirus induced oxidant generation and inflammatory mediator production and release.⁴⁷⁻⁴⁸

Taken together, these observations provide evidence of an increased oxidative burden in asthma exacerbations. However, until now, most of this evidence has been indirect and the pathogenetic mechanisms putatively involved have been investigated mostly in vitro.

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REFERENCES

- 1 Comhair SA, Erzurum SC. Antioxidant responses to oxidant-mediated lung diseases. *Am J Physiol Lung Cell Mol Physiol* 2002;**283**:L246-55.
- 2 Cross CE, Van der Vliet A, Eiserich JP, et al. Oxidative stress and antioxidants in respiratory tract lining fluids. In: Clerch LB, Massaro DJ, eds. *Oxygen, gene expression, and cellular function*. Lung Biology in Health and Disease Series. Volume 105. New York: Marcel Dekker, 1997:367-98.
- 3 Barnes PJ. Reactive oxygen species and airway inflammation. *Free Radic Biol Med* 1990;**9**:235-43.
- 4 Barnes PJ, Chung KF, Page CP. Inflammatory mediators of asthma: an update. *Pharmacol Rev* 1998;**50**:515-96.
- 5 Bowler RP, Crapo JD. Oxidative stress in allergic respiratory diseases. *J Allergy Clin Immunol* 2002;**110**:349-56.
- 6 Dworski R. Oxidant stress in asthma. *Thorax* 2000;**55**(suppl 2):S51-3.
- 7 Henricks PA, Nijkamp FP. Reactive oxygen species as mediators in asthma. *Pulm Pharmacol Ther* 2001;**14**:409-20.
- 8 Rahman I, MacNee W. Reactive oxygen species. In: Barnes PJ, Drazen J, Rennard S, et al, eds. *Asthma and COPD*. London: Academic Press, 2002:243-54.
- 9 Aldridge RE, Chan T, van Dalen CJ, et al. Eosinophil peroxidase produces hypobromous acid in the airways of stable asthmatics. *Free Radic Biol Med* 2002;**33**:847-56.
- 10 Ricciardolo FL. Multiple roles of nitric oxide in the airways. *Thorax* 2003;**58**:175-82.
- 11 Bucchieri F, Puddicombe SM, Lordan JL, et al. Asthmatic bronchial epithelium is more susceptible to oxidant-induced apoptosis. *Am J Respir Cell Mol Biol* 2002;**27**:179-85.
- 12 Message SD, Johnston SL. Viruses in asthma. *Br Med Bull* 2002;**61**:29-43.
- 13 Hancock JT, Desikan R, Neill SJ. Role of reactive oxygen species in cell signalling pathways. *Biochem Soc Trans* 2001;**29**:345-50.
- 14 Adcock I, Caramori G. Transcription factors in asthma and COPD. In: Barnes PJ, Drazen J, Rennard S, et al, eds. *Asthma and COPD*. London: Academic Press, 2002:315-21.
- 15 Nadeem A, Chhabra SK, Masood A, et al. Increased oxidative stress and altered levels of antioxidants in asthma. *J Allergy Clin Immunol* 2003;**111**:72-8.

- 16 **Monteseirin J**, Bonilla I, Camacho J, *et al*. Elevated secretion of myeloperoxidase by neutrophils from asthmatic patients: the effect of immunotherapy. *J Allergy Clin Immunol* 2001;**107**:623–6.
- 17 **Kharitonov SA**, Barnes PJ. Exhaled markers of pulmonary disease. *Am J Respir Crit Care Med* 2001;**163**:1693–722.
- 18 **Kharitonov SA**, Barnes PJ. Biomarkers of some pulmonary diseases in exhaled breath. *Biomarkers* 2002;**7**:1–32.
- 19 **Corradi M**, Pesci A, Casana R, *et al*. Nitrate in exhaled breath condensate of patients with different airway diseases. *Nitric Oxide* 2003;**8**:26–30.
- 20 **Dweik RA**, Comhair SA, Gaston B, *et al*. NO chemical events in the human airway during the immediate and late antigen-induced asthmatic response. *Proc Natl Acad Sci USA* 2001;**98**:2622–7.
- 21 **Kaminsky DA**, Mitchell J, Carroll N, *et al*. Nitrotyrosine formation in the airways and lung parenchyma of patients with asthma. *J Allergy Clin Immunol* 1999;**104**:747–54.
- 22 **Saleh D**, Ernst P, Lim S, *et al*. 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**:929–37.
- 23 **MacPherson JC**, Comhair SA, Erzurum SC, *et al*. Eosinophils are a major source of nitric oxide-derived oxidants in severe asthma: characterization of pathways available to eosinophils for generating reactive nitrogen species. *J Immunol* 2001;**166**:5763–72.
- 24 **Wu W**, Samoszuk MK, Comhair SA, *et al*. Eosinophils generate brominating oxidants in allergen-induced asthma. *J Clin Invest* 2000;**105**:1455–63.
- 25 **De Raeve HR**, Thunnissen FB, Kaneko FT, *et al*. Decreased Cu,Zn-SOD activity in asthmatic airway epithelium: correction by inhaled corticosteroid in vivo. *Am J Physiol Lung Cell Mol Physiol* 1997;**272**:L148–54.
- 26 **Kanazawa H**, Shiraishi S, Hirata K, *et al*. Decreased peroxynitrite inhibitory activity in induced sputum in patients with bronchial asthma. *Thorax* 2002;**57**:509–12.
- 27 **Gaston B**, Sears S, Woods J, *et al*. Bronchodilator S-nitrosothiol deficiency in asthmatic respiratory failure. *Lancet* 1998;**351**:1317–9.
- 28 **Richardson G**, Benjamin N. Potential therapeutic uses for S-nitrosothiols. *Clin Sci (Lond)* 2002;**102**:99–105.
- 29 **Puddicombe SM**, Torres-Lozano C, Richter A, *et al*. Increased expression of p21(waf) cyclin-dependent kinase inhibitor in asthmatic bronchial epithelium. *Am J Respir Cell Mol Biol* 2003;**28**:61–8.
- 30 **Comhair SA**, Bhatena PR, Farver C, *et al*. Extracellular glutathione peroxidase induction in asthmatic lungs: evidence for redox regulation of expression in human airway epithelial cells. *FASEB J* 2001;**15**:70–8.
- 31 **Omland O**, Deguchi Y, Sigsgaard T, *et al*. Selenium serum and urine is associated to mild asthma and atopy. The SUS study. *J Trace Elem Med Biol* 2002;**16**:123–7.
- 32 **Baker JC**, Ayres JG. Diet and asthma. *Respir Med* 2000;**94**:925–34.
- 33 **Fogarty A**, Lewis S, Weiss S, *et al*. Dietary vitamin E, IgE concentrations, and atopy. *Lancet* 2000;**356**:1573–4.
- 34 **Kaur B**, Rowe BH, Ram FS. Vitamin C supplementation for asthma (Cochrane Review). *Cochrane Database Syst Rev* 2001;**4**:CD000993.
- 35 **Romieu I**, Sienna-Monge JJ, Ramirez M, *et al*. Genetic polymorphism of GSTM1 and antioxidant supplementation influence lung function in relation to ozone exposure in asthmatic children in Mexico City. *Thorax* 2004;**59**:8–10.
- 36 **Romieu I**, Sienna-Monge JJ, Ramirez-Aguilar M, *et al*. Antioxidant supplementation and lung functions among children with asthma exposed to high levels of air pollutants. *Am J Respir Crit Care Med* 2002;**166**:703–9.
- 37 **Salvemini D**, Cuzzocrea S. Therapeutic potential of superoxide dismutase mimetics as therapeutic agents in critical care medicine. *Crit Care Med* 2003;**31**(suppl 1):S29–38.
- 38 **Floyd RA**, Hensley K, Forster MJ, *et al*. Nitrones, their value as therapeutics and probes to understand aging. *Mech Ageing Dev* 2002;**30**:123:1021–31.
- 39 **Lantero S**, Oddera S, Silvestri M, *et al*. Budesonide down-regulates eosinophil locomotion but has no effects on ECP release or on H2O2 production. *Lung* 1999;**177**:219–28.
- 40 **Kanazawa H**, Hirata K, Yoshikawa J. Nitrogen oxides reduce albuterol-induced bronchodilation in patients with bronchial asthma. *Respiration* 2002;**69**:490–5.
- 41 **Gillissen A**, Jaworska M, Scharling B, *et al*. Beta-2-agonists have antioxidant function in vitro. 1. Inhibition of superoxide anion, hydrogen peroxide, hypochlorous acid and hydroxyl radical. *Respiration* 1997;**64**:16–22.
- 42 **Gillissen A**, Wickenburg D, van Zwoll D, *et al*. Beta-2-agonists have antioxidant function in vitro. 2. The effect of beta-2-agonists on oxidant-mediated cytotoxicity and on superoxide anion generated by human polymorphonuclear leukocytes. *Respiration* 1997;**64**:23–8.
- 43 **Lapenna D**, De Gioia S, Mezzetti A, *et al*. Aminophylline: could it act as an antioxidant in vivo? *Eur J Clin Invest* 1995;**25**:464–70.
- 44 **Yasui K**, Agematsu K, Shinozaki K, *et al*. Effects of theophylline on human eosinophil functions: comparative study with neutrophil functions. *J Leukoc Biol* 2000;**68**:194–200.
- 45 **Global Initiative for Asthma**. *Global strategy for asthma management and prevention*. NHLBI/WHO Workshop Report. NIH Publication 02-3659. Bethesda, MD: NHLBI, 2002.
- 46 **Papi A**, Caramori G, Adcock IM, *et al*. Molecular mechanisms of respiratory virus-induced inflammation. In: Johnston SL, Papadopoulos NG, eds. *Respiratory infections in allergy and asthma*. Lung Biology in Health and Disease Series. Volume 178. New York: Marcel Dekker, 2003:210–28.
- 47 **Papi A**, Papadopoulos NG, Stanciu LA, *et al*. Reducing agents inhibit rhinovirus-induced up-regulation of the rhinovirus receptor intercellular adhesion molecule-1 (ICAM-1) in respiratory epithelial cells. *FASEB J* 2002;**16**:1934–6.
- 48 **Biagioli MC**, Kaul P, Singh I, *et al*. The role of oxidative stress in rhinovirus induced elaboration of IL-8 by respiratory epithelial cells. *Free Radic Biol Med* 1999;**26**:454–62.