New Insights in Oxidant Biology in Asthma

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Abstract

Research over the past 30 years has identified mechanistic biochemical oxidation pathways that contribute to asthma pathophysiology. Redox imbalance is present in asthma and strongly linked to the pathobiology of airflow obstruction, airway hyperreactivity, and remodeling. High levels of reactive oxygen species, reactive nitrogen species, and oxidatively modified proteins in the lung, blood, and urine provide conclusive evidence for pathologic oxidation in asthma. Concurrent loss of antioxidants, such as superoxide dismutases and catalase, is attributed to redox modifications of the enzymes, and further amplifies the oxidative injury in the airway. The presence of high levels of urine bromotyrosine, an oxidation product of eosinophil peroxidase, identifies activated eosinophils, and shows promise for use as a noninvasive biomarker of poor asthma control.

Keywords: asthma; eosinophil; oxidants; nitric oxide; superoxide dismutase

(Received in original form June 24, 2015; accepted in final form July 14, 2015)

Supported, in part, by National Heart, Lung, and Blood Institute grants HL081064, HL103453, and HL109250, and by the Alfred Lerner Chair for Biomedical Research.

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Ann Am Thorac Soc Vol 13, Supplement 1, pp S35–S39, Mar 2016

DOI: 10.1513/AnnalsATS.201506-385MG
Internet address: www.atsjournals.org

A wealth of studies point to an immunologic genesis of asthmatic airway inflammation, but, ultimately, most effector cells produce reactive oxygen species (ROS) and reactive nitrogen species (RNS), which produce pathophysiologic outcomes of airway reactivity, injury, and remodeling (1–4). This article summarizes information on oxidant biology that was presented at the Thomas L. Petty Lecture at the Thomas L. Petty Aspen Lung 2015 Conference. Comprehensive overview of oxidative processes in asthma can be found in work by Comhair and colleagues (5) and Ghosh and colleagues (6, 7).

ROS in Asthma

Enhanced oxidant production is well documented in asthma. Mitochondria are the primary intracellular source of superoxide generation under normal physiologic states (8), but high levels of superoxide can be formed by the reduced nicotinamide adenine dinucleotide phosphate (NADPH) oxidases that are found in granulocytes, including neutrophils and eosinophils (9–12). High levels of hydrogen peroxide can also be formed by NADPH oxidases, Duox1 and -2, expressed in bronchial epithelial cells upon activation by histamine binding to cell-surface receptor (13). Eosinophils and/or neutrophils are present in increased numbers in airways of subjects with asthma and produce much more ROS than in cells found in healthy lungs (14–22). Early studies showed that the amount of ROS generated by eosinophils and/or neutrophils is directly correlated to the severity of hyperreactivity in patients with asthma (16, 21, 23). Experimental exposure of subjects with atopic asthma to allergen, or asthma exacerbations, lead to even greater amounts of superoxide (16, 24), with airspace cells in subjects with asthma producing up to 8×10^6 nmol superoxide/million cells/h (16, 25).

Bromotyrosine, a Biomarker of the Activated Eosinophil

Eosinophils are cellular biomarkers of atopic, or Th2-high, asthma (26–29, 30–33). Activated eosinophils degranulate to release major basic protein, eosinophilic cation protein, and eosinophil peroxidase (EPO) (26–29, 34). EPO is one of the mammalian peroxidase superfamily, which also includes myeloperoxidase (MPO), lactoperoxidase, thyroid peroxidase, and prostaglandin H synthase. All use peroxide for the oxidizing equivalents for catalysis (35). MPO is present in neutrophils and monocytes, and secreted during cell activation (35). EPO and MPO both use hydrogen peroxide to oxidize thiocyanate (36, 37). MPO may also use bromide and chloride as substrates, but at plasma levels of halides, MPO uses

chloride over 500-fold more than bromide (38, 39). On the other hand, EPO can use bromide, but cannot use chloride as a substrate. Multiple studies of various inflammation models using either MPO-knockout or EPO-knockout mice confirm consistently that bromotyrosine generation *in vivo* is absent or reduced to virtually nondetectable levels in the EPO-knockout mouse, and chlorotyrosine formation is absent in the MPO-knockout mouse (40, 41). Thus, chlorotyrosine is specific for MPO, and bromotyrosine is highly selective for EPO *in vivo* (4, 42–45). Bromotyrosine is found at high levels in asthmatic airways and increases with asthma exacerbations (43, 46). Bromotyrosine is stable and excreted in urine, where it can be quantitated and used as a biomarker of the activated eosinophil (42, 47). Urine bromotyrosine levels increase during asthma exacerbation (42), and the presence of high levels of urine bromotyrosine identifies subjects with asthma with poor control, or at risk of exacerbation (48, 49)

Nitric Oxide and Airway Inflammation

In addition to ROS, RNS are also increased in asthma. Nitric oxide (NO) is higher in asthmatic airways as compared with nonasthmatic airways (1, 3, 50, 51). Measurement of the fraction of exhaled NO (FeNO) is a sensitive biomarker of airway inflammation (52–57), and was approved by the U.S. Food and Drug Administration for evaluation of antiinflammatory treatment responses in asthma (57–62). NO synthases (NOSs) produce NO by converting L-arginine to L-citrulline (63). The inducible NOS (iNOS; *NOS2*) is induced by cytokines, including IFN-γ, IL-1β, TNF-α, IL-4 and/or IL-13 (63–65). The Th2 cytokines are not essential for iNOS expression; hence, FeNO does not perfectly correlate with Th2 biomarkers, such as eosinophils or bromotyrosine (66). The higher-than-normal FeNO in asthma is associated with greater transcriptional activation of the *NOS2* gene and iNOS protein expression (3, 64, 67, 68). The signal transduction effects of NO are classically related to binding to guanyl cyclases, but its byproducts also have biologic effects. NO reacts with oxygen or ROS to form nitrite, nitrate, and RNS, such as peroxynitrite. The addition of NO to thiol residues (nitrosothiol) is called nitrosation, and can alter function and/or structure of proteins (69–71). NO addition to tyrosyl residues (nitrotyrosine) is termed nitration, and occurs in the setting of increased RNS. Nitration usually leads to inactivation of proteins (72–75). EPO may use nitrite for nitration of protein-bound tyrosyl residues (76); in fact, up to 70% of nitrotyrosine formed in the murine asthma model is produced by EPO (40). Studies show that subjects with well controlled asthma have higher levels than individuals without asthma of airway NO, nitrate, and nitrotyrosine, and very low levels of S-nitrosothiols (1, 77). Nitrotyrosine is present at very high levels in airways of patients during asthma attacks (4, 78, 79). Unbiased metabolomic profiling has recently revealed a unique NO-associated endotype of asthma typified by changes in taurine transport and bile acid metabolism, which are known systemic effects of NO (80).

Redox Abnormalities in Asthma

The lung has a wide variety of antioxidants (e.g., glutathione, catalase, and superoxide dismutases (SODs) (81), but increased ROS and RNS in asthma overcome antioxidant defenses (5, 82). Airways of individuals with asthma have higher-than-normal levels of glutathione. Maintenance of the optimal intracellular thiol/dithiol redox ratio is important to cell functions and survival. Protein cysteinyl thiols are susceptible to oxidation, and cells can resist oxidation of thiols by protein thiolation (83), particularly with glutathione to generate S-glutathionyated proteins (i.e., mixed disulfides) (84). Recent work suggests alterations in glutathionylation in asthmatic airways (85, 86). The intracellular redox in asthmatic airway cells appears to be shifted to greater intracellular reducing potential, with higher ratio of the reduced-to-oxidized glutathione (87), perhaps as a response to the repetitive oxidative stress (2). Recently, quantitative nuclear imaging

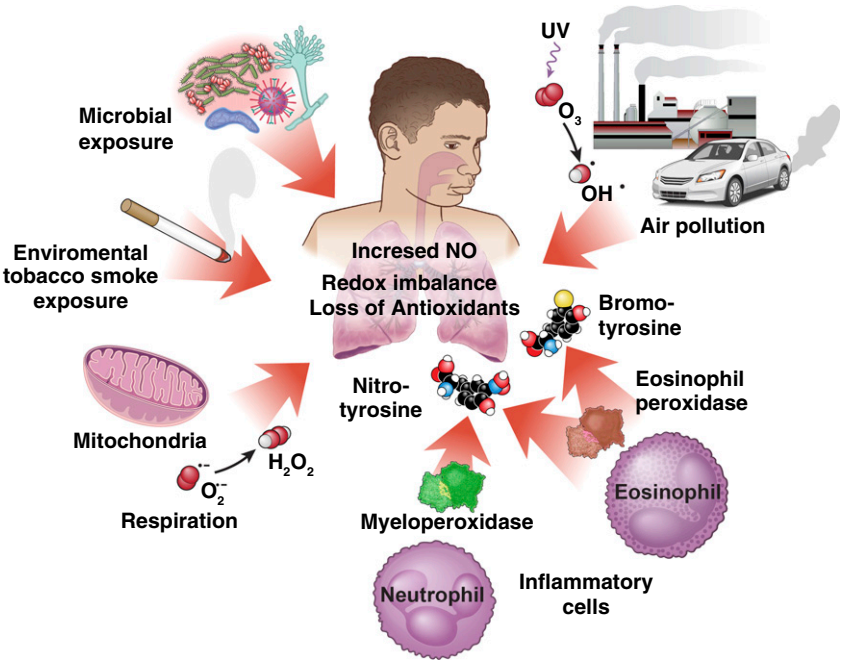

Figure 1. Oxidant biology in asthma. Reactive oxygen and nitrogen species and proteins oxidatively modified by nitration and/or bromination are increased in asthma. The higher levels of oxidants are generated endogenously by inflammatory cells and epithelial cells, and amplified by inhalational exposure to microbes, allergens, pollutants, and environmental tobacco smoke. Loss of antioxidants and alterations in thiol/dithiol balance further augment pathologic oxidative processes. NO = nitric oxide.

using the radiopharmaceutical, [^{99} ^{m}Tc-exametazime (HMPAO)], which is retained in tissues dependent on intracellular reduced glutathione levels, confirmed a greater intracellular reducing potential in asthma *in vivo*, and predominantly in lower central regions of the lungs (88).

Redox-Mediated Loss of Antioxidant Activities

In addition to greater ROS and RNS, asthmatic lungs have lower-than-normal SOD and catalase activities (2, 77, 87, 89–91). The loss of SOD is associated with more severe airflow obstruction and greater airway hyperreactivity and remodeling (2, 5, 89–91, 92). The redox thiol/dithiol imbalance in asthma results in systemic change in cytosolic copper-zinc SOD (CuZnSOD), such that CuZnSOD is susceptible to autoinactivation by hydrogen peroxide (2, 5, 89–91, 92, 93). On the other hand, the decrease in mitochondrial manganese SOD (MnSOD) activity is associated with nitration of tyrosyl residues in the protein (2, 91). Similarly, catalase activity is decreased in the asthmatic airway in association with increased oxidation of specific tyrosyl residues in the protein (77). Murine asthma models verify a mechanistic role of SOD loss in pathophysiology of asthma. The CuZnSOD transgenic mice have less airway inflammation and hyperreactivity in comparison to wild-type mice in a murine asthma model (94). In human studies, exposure to second hand smoke is associated with even lower levels of serum SOD in asthma, and, overall, more severe asthma (93). Increased ROS in asthma is usually attributed to leukocyte activation, but murine models of asthma also point to metabolic–mitochondrial origins of greater ROS in the asthmatic airway smooth muscle (95). Subjects with asthma who were provided coenzyme Q, an essential component of mitochondrial electron transport chain and a mitochondrial antioxidant (96), recovered SOD activity and redox thiol balance to healthy, nonasthmatic levels (97).

Conclusions

Altogether, the inflammation in asthma is defined by oxidant biology and alterations of airway redox (Figure 1). Redox abnormalities are amplified by infections, exposure to pollutants, and/or allergen in subjects with atopic asthma. EPO-mediated reactions produce high levels of brominating and oxidizing species that damage proteins. High levels of NO production and reactive nitrogen oxides contribute to the oxidant pathophysiology. Loss of enzymic antioxidant activities further fuels redox disturbances and injury to the airway. Future studies to develop noninvasive signature biochemical biomarkers of oxidative pathways, and/or to design therapies targeting redox mechanisms to limit formation of damaging oxidant species, may be useful to provide optimal care of the patient with asthma. ■

Author disclosures are available with the text of this article at www.atsjournals.org.

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