Implications of dealing with airborne substances and reactive oxygen species: what mammalian lungs, animals, and plants have to say?

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Synopsis A gas-exchange structure interacts with the environment and is constantly challenged by contaminants that may elicit defense responses, thus compromising its primary function. It is also exposed to high concentrations of O_2 that can generate reactive oxygen species (ROS). Revisiting the lung of mammals, an integrative picture emerges, indicating that this bronchi-alveolar structure deals with inflammation in a special way, which minimizes compromising the gas-exchange role. Depending on the challenge, pro-inflammatory or antiinflammatory responses are elicited by conserved molecules, such as surfactant proteins A and D. An even broader picture points to the participation of airway sensors, responsive to inflammatory mediators, in a loop linking the immunological and nervous systems and expanding the role played by respiratory organs in functions other than gas-exchange. A byproduct of exposure to high concentration of O_2 is the formation of superoxide ($O_2^{\bullet -}$), hydrogen peroxide (H_2O_2), hydroxyl radical ($HO^{\bullet}$), and other ROS, which are known to be toxic to different types of cells, including the lung epithelium. A balance between antioxidants and oxidants exists; in pulmonary epithelial cells high intracellular and extracellular levels of antioxidants are found. Antioxidant adaptations related to plant and animal life-styles involve a broad range of overlapping strategies based on well-conserved molecules. Glutathione (GSH) is an abundant and ubiquitous thiol-tripeptide antioxidant, also present in lungs, whose role in providing information on the intracellular redox state of animals and plants is well established. In these organisms, GSH influences gene expression associated with stress, maximizing defense responses. Several enzymatic antioxidants, such as glutathione peroxidase (GPx), glutathione reductase, glutathione S-transferase, and glucose 6-phosphate dehydrogenase participate in the redox system; in animals that are stress-tolerant GPx is a key element against oxidative assaults. Most importantly, alternative roles of ROS as signaling molecules have been found in all plants and animals. For example, alveolar macrophages produce $O_2^{\bullet -}$ that act as second messengers, in addition to having a bactericidal role. The nonradical ROS H_2O_2 signals inflammation in mammalian lungs, apoptosis in different animal tissues, and is also involved in stomatal closure, root development, gene expression, and defense responses of plants. Antioxidant adaptations in some water-breathing animals involve the excretion of H_2O_2 by diffusion through gas-exchange structures. The fine balance among a multitude of factors and cells makes the difference between damage and protection in animals and plants. Knowledge about the mechanisms and consequences of these molecular interactions is now starting to be integrated.

Introduction (Spinelli Oliveira)

Here, we integrate data regarding challenges routinely faced by mammalian lungs and highlight comparable mechanisms found in other animals and in plants.

A brief overview of innate and adaptive host defense systems in mammalian lungs

The innate (IN) immune system is composed of preexisting or rapidly mobilized nonspecific components that detect and combat invaders, often without

This paper summarizes one of the 22 symposia that constituted the ''First International Congress of Respiratory Biology'' held August 14–16, 2006, in Bonn, Germany.
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Integrative and Comparative Biology, volume 47, number 4, pp. 578–591
doi:10.1093/icb/icm078
Advanced Access publication July 31, 2007

invoking an inflammatory response. Nonetheless, the IN immune response and the process of inflammation are interwoven (Hawieger 2001). Cells and mediators also link the IN to the adaptive immune system, which is based on recombinant antigen–receptor specificity. In mammalian lungs, immune responses may be uniquely modulated and/or have features not seen in organs, like the spleen, that are not in direct contact with the external environment (Raz 2007). There are indications that under certain circumstances the lung may transiently become an immune-privileged organ (Hayashi et al. 2004). In these instances, local inflammatory responses are restricted, which prevents the adaptive immune cascade from occurring. Consequently, the large and delicate alveolar gas-exchange area is protected from inflammatory byproducts, which have the potential to cause damage, and the breathing process is efficiently preserved.

Anatomical and physiological barriers

The first line of IN defense in the respiratory tract is constituted by the angulation of the airways and by the mucociliary assembly composed of mucus and cilia. When irritant gases or particles do penetrate into the airways, they activate biosensors that respond to mechanical (Yu, 2005) and chemical (Coleridge and Coleridge 1984) stimuli and initiate reflexes, such as sneezing and coughing, that rapidly move the contaminated air out of the system (Canning et al. 2006). Such a defense system protects the body from foreign invaders. Recently, neuroepithelial bodies have been recognized as sensors that may be connected with different types of sensory afferents (Adriaensen et al. 2006). The lung–brain communication is bi-directional, via the vagus (Widdicombe 2001) and the sympathetic nerves (Soukhova et al. 2003). The lungs can signal the central nervous system through proinflammatory cytokines and mediators released during inflammation (Lin and Yu 2007). These, in turn, send signals to the neuroendocrine and the autonomic nervous systems, and both regulate the inflammatory response (see Biosensors for lung inflammation section).

Biological and biochemical barriers: a battery of diverse cells, mediators, and receptors

The immune system employs a complex network of interrelated mechanisms. An understanding is beginning to emerge of how they function as a unit and at the same time confer distinct immunological properties to specific organs.

Cells

In lungs, as elsewhere, a plethora of cells is involved in IN immune defense: epithelial lining cells, natural killer leukocytes, phagocytes, eosinophils, and mast cells. Epithelial lining cells are more than an anatomical–physiological barrier. Type-II alveolar epithelial cells (AEC-II) secrete surfactants with immunomodulatory functions (see subsequently) and are antigen-presenting cells capable of regulating T-cell activity (Zissel et al. 2000). AEC-II also exerts a tonic inhibition on alveolar macrophages and, consequently, inhibits T-cell-mediated immune responses through the presentation of activated transforming-growth-factor-β (TGF-β). The crosstalk between AEC-II and alveolar macrophages confers a transient immune-privileged state to lungs (Takabayshi et al. 2006). Therefore, potential pulmonary tissue damage, which could be caused by inflammation and adaptive immune responses, is minimized.

Dendritic cells are phagocytic cells first described in the 1970s (Steinman and Cohn 1973). They are now considered the only antigen-presenting cells capable of stimulating naive T-cells (Howard et al. 2004). Thus, although B-cells and T-cells are mediators of adaptive immunity, their function is under the control of dendritic cells, which are able to regulate a broad range of immune responses, including T-cell tolerance to self-antigens, thereby minimizing autoimmune reactions (Banchereau and Steinman 1998). In the respiratory tract, for example, they capture and process antigens, express lymphocyte co-stimulatory molecules, migrate to lymphoid organs, and secrete cytokines that initiate immune responses. Dendritic cells also respond to diverse well-known mediators of the inflammatory response: histamine (Mazzoni et al. 2003), nitric oxide (NO) (Morita et al. 2005), and heat-shock proteins. These proteins are upregulated by several forms of stress and are found in all eukaryotic and prokaryotic cellular organisms (Van Eden et al. 2002).

Receptors

Invaders may interact with a wide variety of receptors (Toll-Like, C-lectin, and scavenger receptors) expressed by the earlier mentioned cells, causing the increase, release and/or activation of numerous universal effectors of host defense. The main mediators are the humoral complement cascade, prostaglandins, histamine, serotonin,

bradykinin, cytokines—such as interleukin (IL), TGF, interferon, tumor necrosis factor (TNF), granulocyte/macrophage colony-stimulating factor (GM-CSF), and granulocyte colony-stimulating factor—, chemokines, reactive oxygen species (ROS) such as O_2^-, H_2O_2, NO—, neuropeptides (e.g., enkephalins), acute-phase proteins (e.g., C-reactive protein), antimicrobial peptides (e.g., defensins and cathelicidins), and peptidoglycan recognition proteins. Negative and positive feedback mechanisms can be triggered, adding complexity to the picture. For example, GM-CSF, a protein secreted by macrophages exerts a stimulatory effect on stem cells generating an increased number of granulocytes and macrophages. As a result, GM-CSF mediates a positive feedback that leads to a rapid increase in the number of macrophages. Some mediators (e.g., the complement cascade of proteins, some cytokines, defensins, lactoferrin, and cathelicidins), as well as some cells (e.g., AEC-II, alveolar macrophages, and dendritic cells), are important links to adaptive immune responses, bringing into action antigen-specific T-cells and antibodies. Depending on the population of T-lymphocytes that is activated and the subsequent set of cytokines that are secreted in the respiratory tract, the balance between tissue damage or microorganism clearance could be altered in favor of the former or latter situation (Boyton and Openshaw 2002).

Toll-like receptors (TLR)

A unifying characteristic of most IN immune responses is their reliance on highly conserved proteins—pattern recognition receptor or molecule—to detect invaders (Hoffmann et al. 1999). Pattern recognition reflects the capacity to identify arrays of carbohydrates and/or lipids, which are expressed on microbial surfaces in a way that usually is not found in mammalian cell membranes. Pattern recognition is shared among microorganisms—e.g., the glycolipids of mycobacteria, the lipopolysaccharides (LPS) of Gram-negative bacteria, the lipoteichoic acids of Gram-positive bacteria—and pattern recognition allows the discrimination of microbial "nonself," bypassing the adaptive immune responses based on antigen–antibody receptor systems. TLR exist in all coelomates and are a major class of pattern recognition receptors. They are activated by different signaling molecules, are expressed by different cell types (e.g., in different subtypes of dendritic cells), and at different cellular levels (at cell surfaces or intracellularly).

Elements of TLR signals (transducers, protein kinases, and transcription nuclear factors) have been identified (e.g., the protein MyD88, NF-κB, and various mitogen-associated protein kinases, MAPK). The diversity of TLR signaling is considered important for the generation of organ-specific immunity (Raz 2007). Scavenger receptors are expressed by macrophages, including alveolar macrophages (Palecanda et al. 1999), dendritic cells, and certain endothelial cells; they are involved in IN responses and macrophage control during inflammation (Peiser and Gordon 2001).

Besides humoral factors, lungs share common biological elements with other organs that are equally exposed to the external environment (e.g., the gastrointestinal tract and the skin). Among them are two constitutive and well-conserved proteins, lysozyme (LZ) and lactoferrin.

Mediators

Lysozyme is a widely distributed cationic antimicrobial enzyme and component of IN pulmonary host defenses (Ganz 2004). LZ activity is found in bacteria, bacteriophages, plants, and in mammalian leukocytes and in secretions (nasal, salivary, and lachrymal). In rats, AEC-II secretes LZ into the alveolar space via lamellar bodies (Beers et al. 1994). Greater production of LZ in the respiratory tract of transgenic mice expressing LZ cDNA in epithelium enhanced killing of bacteria *in vivo*. Decreased systemic dissemination of pathogens occurred, resulting in increased survival following infections (Akinbi et al. 2000).

Lactoferrin is a nonheme, iron-binding, multifunctional glycoprotein belonging to the transferrin family and similar to antimicrobial peptides, such as defensins. It is produced by epithelial cells at mucosal surfaces and is secreted in milk, tears, saliva, and by neutrophils. It presents antibacterial, antiviral, and antifungal activities (Levay and Viljoen 1995). In the respiratory tract, it is part of IN responses (Boyton and Openshaw, 2002) and also plays an important antioxidant role protecting the epithelium from ROS damage by scavenging iron, since free iron is a major contributor to the generation of ROS via the Fenton reaction. Both LZ and lactoferrin contribute to the modulation of airway inflammation (Thompson et al. 1990). Lactoferrin expression is upregulated in response to inflammatory stimuli, and it exerts potent local antiinflammatory effect (Conneely 2001). Also, it activates natural killer cells, a type of leukocyte displaying nonspecific cytotoxic activity,

which secretes TNF-α, one of the main inducers of acute phase responses.

Antimicrobial peptides are low molecular amphipathic pattern recognition molecules that destroy microorganisms by permeabilizing their membranes (Zasloff 2002). Defensins designate a group of more than 400 protease-resistant antimicrobial peptides; some are secreted by macrophages and lymphocytes, some are produced by the skin and by epithelial cells lining the gastrointestinal tract, the urogenital tract, and the tracheobronchial tree, where they play an important role in IN defense and in inflammation (Oppenheim et al. 2003). Antimicrobial peptides are found in all multicellular organisms that have been studied so far, including plants, invertebrates, and vertebrates (Broekaert et al. 1995). Some defensins are produced constitutively, whereas proinflammatory cytokines and exogenous microbial products induce others. Defensins also act as signals that initiate, mobilize, and amplify adaptive immune responses. Since they do so by using multiple cellular receptors, they have the capacity to marshal adaptive host defenses against a large range of microbial invaders, including various types of bacteria, fungi, and enveloped viruses (Oppenheim et al. 2003).

Defensins also present an important property; their antibacterial and antifungal activities are inhibited by serum or physiological salt concentrations (Boman 1998). Consequently, they exert their activity mainly in phagocytic vacuoles and on the external surfaces of mucosa and skin (Ganz and Lehrer 1998). This means that in the respiratory tract an alteration in the salinity of the bronchial airway fluid may disable the function of defensins that are found in the epithelium, thereby leading to colonization and infection by microorganisms (Oppenheim et al. 2003).

Peptidoglycan recognition proteins belong to a novel family of IN bactericidal and bacteriostatic proteins that are present at the upper respiratory tract, gastrointestinal tract, eyes, and skin and that function as both recognition molecules and effector molecules. They are highly conserved molecules found in invertebrates (e.g., insects, mollusks, echinoderms) and vertebrates, but not in nematodes or plants (Dziarski and Gupta 2006).

Defense molecules that are mostly specific to lungs: collectins SP-A and SP-D

Surfactants are phospholipid–protein complexes that reduce surface tension at the air–liquid interface of the lung (see Orgeig et al., 2007 this volume). Recent studies have shown that some constituents of surfactants, namely surfactant protein A (SP-A) and surfactant protein D (SP-D), also are components of the pulmonary IN immune system. They are multifunctional proteins that promote opsonization, are directly bactericidal, regulate mediator production (ROS and cytokines) and facilitate clearance of apoptotic cells and DNA (Wright 2006).

SP-A and SP-D are members of the collectin (collagen-like lectin) family of soluble Ca^{2+}-dependent proteins and are produced by AEC- II (see Immunomodulatory processes mediated by SP-A and -D and the role of GM-CSF section). In human lungs, SP-A is mainly secreted into the alveolar space via an alternative pathway that largely bypasses the lamellar bodies (Ochs et al. 2002). In several species, airway cells, such as Clara and submucosal cells also synthesize SP-A and SP-D.

SP-A and SP-D exert a multifaceted immunomodulatory role (proinflammatory and antiinflammatory) that seems to be of paramount importance to the functionality of the respiratory tract. These proteins interact with a broad range of different receptors (e.g., TLR2, TLR4, calreticulin and signal-inhibitory regulatory protein-α, SIRP-α) and they modulate inflammatory responses differently, depending on the challenge and of the type of receptor involved. In the absence of pathogens, the lectin domain in SP-A binds to SIRP-α and the production of proinflammatory mediators is inhibited (Gardai et al. 2003). Opsonization of the pathogen, without eliciting a cascade of inflammatory cells and mediators (an antiinflammatory effect), may be sufficient depending on the challenge. In the presence of a mounting infection and/or cell debris the lectin domain in SP-A binds to the pathogen or cell debris and the collagen domain activates immune cells, promoting the release of inflammatory mediators such as TNF-α, through interaction with the CD91–calreticulin complex. SP-A and SP-D are seen as participants of the innate immune defense repertoire in all vertebrates (Gardai et al. 2003).

Pulmonary surfactants also interact with other members of the IN immune system: e.g., they act synergistically with the acute-phase C-reactive protein in inhibiting the release of proinflammatory cytokines by lung macrophages *in vitro* (Casals et al. 2003).

Lung-specific immunity may be achieved through different pathways. At least two are well documented: (1) the presence of unique IN features, such as the high levels of constitutive SP-A and SP-D;

(2) the occurrence of regulatory mechanisms that are respiratory tract-specific: e.g., the secretion of TGF-β by the alveolar epithelium (AEC-II) leading to a local inhibitory effect on alveolar macrophages and on subsequent T- cell-mediated inflammatory responses (Takabayshi et al. 2006).

Implications of the generation of ROS, antioxidants, and adaptations for oxidative stress

The respiratory tract is exposed to high concentrations of O_2 and of oxidants from endogenous (e.g., byproduct of mitochondrial activity and phagocytic respiratory burst) and exogenous (e.g., air pollutants and catalase (CAT) negative microorganisms) sources. This fact suggests another important question: are ROS always deleterious? (See Reactive oxygen species, friend not foe: their role as signaling molecules section).

Reactive oxygen species have been shown to be generally destructive to biological materials and consequently play a role in host defense. In mammals, for example, neutrophils and alveolar macrophages cause cellular damage to pathogens by O_2^- production. Great concentrations of ROS (oxidative stress) are involved in lung dysfunction (Fisher 2002). Nonphagocytic NADPH oxidase has been implicated as the major enzymatic source of ROS in lungs; by catalyzing the single electron reduction of molecular O_2 to superoxide O_2^-. NADPH oxidase knockout mice present a completely blocked phagocyte-mediated ROS production in experimental models (Sato et al. 2002). Data also suggest that NADPH oxidase plays a role in attenuating inflammation after ROS acute lung injury (Segal et al. 2007). The O_2^- generated in alveolar macrophages by activation of NADPH oxidase has a signaling function as second messengers, in addition to their bactericidal role, and signaling may occur by modification of enzymes through reversible oxidation of critical thiols. The high concentration of ROS, nonetheless, is transitory, due to the action of cellular antioxidants, thus fulfilling one important requirement for a signaling molecule (Fisher 2002). Evidence also indicates that NADPH oxidase is an O_2 sensor (Jones et al. 2000), including in-airway chemoreceptors (Fu et al. 2000). The role of moderate concentrations of ROS in the physiological control of cell function in plants and animals is now well established (Dröge 2002).

Effective antioxidant defenses are organized at multiple levels and include prevention, interception, and repair (Sies 1993). Antioxidants are present in appropriate amounts and localization; thus the intracellular levels of ROS are regulated (Reid 2006). They include small molecules—thioredoxin, ascorbic acid, vitamin E (α-tocopherol), uric acid, and GSH—and antioxidant enzymes, such as CAT, glutathione peroxidase (GPx), glutathione reductase (GR), glutathione S-transferase (GST), and superoxide dismutase (SOD) (Besse and Buchanan 1997). SOD converts $O_2^{\bullet -}$ radicals to H_2O_2, which in turn is metabolized by CAT and GPx. Marine animals also deal with H_2O_2 in a special way (see Antioxidants, oxidative stress, mode of life and adaptations to the environment section).

In cells and lining fluids of the respiratory tract lactoferrin and vitamin E associated with lung surfactants (Fisher 2002) have an important role in ROS pulmonary oxidant–antioxidant balance.

GSH is a universal molecule and the most abundant nonprotein source of thiol in mammalian cells; its depletion in the respiratory tract has been associated with increased risk of lung damage and pulmonary diseases (Qamar et al. 1999). Normal intracellular levels of GSH are considered to exert an antiinflammatory effect by inhibiting the secretion of proinflammatory cytokines in mammalian lungs. A link between low pulmonary levels of GSH and abnormalities in the lung surfactant system has also been described (Morris and Bernard 1994).

A model based on studies of plants suggests that interactions between ROS and antioxidants—including GSH, vitamin C and D, and thioredoxin—act as an interface for internal (metabolic) and external (environmental) signals. In turn, this interface plays a key role in modulating acclimation processes or apoptosis (Foyer and Noctor 2005). A view has also emerged that highlights the participation of H_2O_2 in a variety of physiological processes from inflammation signaling in mammals (Soukhova et al. 1999), to key processes in plants (Dröge 2002).

Wide ranges of antioxidant defenses correlate with mode of life and with the environmental challenges faced by animals (see Antioxidants, oxidative stress, mode of life and adaptations to the environment section). The role of strategies of dealing with ROS in animals living in extreme environments or withstanding episodic extremes is also well-documented (see Living in the extremes of Earth: role of free radicals section).

In the following sections, we have assembled brief reports based on symposium talks presented at the ICRB held in August 2006. They exemplify different approaches and the use of diverse models, from vertebrates, including humans and

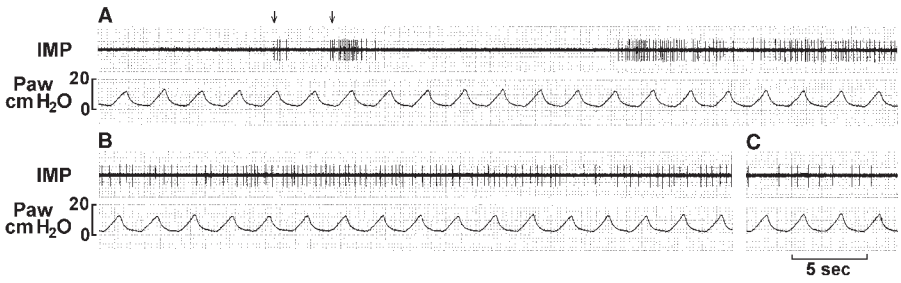

Fig. 1 Activation of a C-fibers receptor (CFR) by IL-1β in an anesthetized, open-chest, and mechanically ventilated rabbit. The receptive field was located close to the left hilum. The traces were IMP = afferent activity (impulses); Paw = airway pressure. Two arrows in (**A**) denote insertion of a needle into the receptive field and the injection of IL-1β (10 μg/ml, 20 μl). (**A**) and (**B**) are continuous recordings. (**B**) and (**C**) are 200 s apart. This CFR was stimulated 18 s after IL-1β injection, reached a peak immediately after activation and still is activated at 5 min after the injection (**C**). Conduction velocity of the afferent was 0.7 m/s.

other mammals, to invertebrates and plants. Here, data from simulated exposure studies aimed at characterizing the effect of fragrance materials (FrM) in humans exemplify an applied approach to inhaled substances (see Quantitative assessment of lungs challenged with fragrance materials in humans section). Although the coverage is far from exhaustive, these reports illustrate the complex interactions that allow organisms to deal with invaders and O_2 byproducts, while highlighting advances and common themes of Respiratory Biology that would benefit from an integrated approach.

Biosensors for lung inflammation (Yu)

There are biosensors in the airways and lung parenchyma, and their afferents run in the vagus nerve in both Aδ and nonmeylinated C fibers. They are named high-threshold Aδ receptors (HTARs) and C fibers receptors (CFRs). In addition, some sensors' afferents are mediated through the sympathetic pathway (Yu 2002). These sensors are also called nociceptors because they are activated by noxious stimuli. In a single-unit preparation, HTARs and CFRs have low background activity and are not very sensitive to changes in lung mechanics, such as lung inflation and deflation, unless the stimulation is extreme. In contrast, both HTARs and CFRs are sensitive to a variety of inflammatory mediators, including histamine, serotonin, prostaglandins, bradykinin, and H_2O_2 (Lee and Pisarri 2001). These nociceptors are not only stimulated by exogenous mediators but also by endogenous inflammatory mediators released in response to LPS (Lai et al. 2005) and oleic acid injections (Lin et al. 2007). These two agents are known to cause sepsis and acute lung injury through release of endogenous inflammatory mediators, including cytokines and chemokines.

Recently, in anesthetized, open-chest, mechanically ventilated rabbits, we have shown that microinjections (20 μl) of IL-1β into the receptive fields of HTARs and CFRs increased their activity significantly (Fig. 1). This stimulation was attenuated by a natural IL-1 receptor antagonist, thus indicating that nociceptors acting as biosensors can monitor concentrations of inflammatory mediators and cytokines (Yu et al. 2007). Our data support the assumption that nociceptors may be part of a reflex that regulates the intensity of immune responses (Czura and Tracey 2005).

Immunomodulatory processes mediated by SP-A and SP-D, and the role of GM-CSF (Ochs)

Stereological light and electron microscopy studies of mice deficient in SP-A and SP-D shed further light on lung immunomodulatory processes (Ochs 2006). When compared to wild-type, SP-A-deficient mice lacked tubular myelin but the lung structure was normal. SP-D-deficient mice, as well as SP-A/SP-D-double-deficient mice displayed emphysema-like pathology characterized by fewer and larger alveoli, a decreased alveolar epithelial surface area, AEC-II hyperplasia and hypertrophy, and an increased volume of lamellar bodies. Abnormalities in surfactant homeostasis were also observed (Jung et al. 2005). GM-CSF is a cytokine released by various cell types in response to inflammatory stimuli. Growing evidence suggests that GM-CSF is an important regulator of the alveolar epithelium, surfactant homeostasis, and lung host defense (Trapnell and Whitsett 2002). AEC-II and alveolar macrophages express GM-CSF and both subunits of the GM-CSF receptor. GM-CSF is required for the expression of the transcription factor PU.1, which in turn is required for most of the differentiated functions

Table 1 Concentrations ($\mu g/m^3$ of air) of material generating airborne fragrance

	Adult Breathing Zone (152 cm)		Child Breathing Zone (45 cm)	
Fragrance Material	**Maximum**	**Minimum**	**Maximum**	**Minimum**
Benzyl Acetate	380.5	15.2	241.5	7.8
Eugenol	410.4	7.5	266.6	5.2
Hexyl Cinnamaldehyde	125.1	3.9	192.9	3.1
HHCB	122.6	4.3	201.7	3.0
Hydroxy Citronellal	299.3	13.3	250.5	11.4
β-Ionone	273.9	10.1	196.1	6.3
d-Limonene	396.4	27.9	297.7	15.2
Linalool	414.2	20.0	243.5	10.4
Methyl Diydrojasmonate	127.6	3.4	176.9	3.2

HHCB = 1,3,4,6,7,8-hexahydro-4,6,6,7,8,8-hexamethylcyclopenta-γ-2-benzopyran

of alveolar macrophages, including the production of ROS and the catabolism of surfactant (Shibata et al. 2001). Consequently, mice deficient in GM-CSF develop alterations of the lung reminiscent of pulmonary alveolar proteinosis (Dranoff et al. 1994). Additional deletion of GM-CSF in SP-D deficient mice led to pronounced emphysema but less pronounced alterations in AEC-II, indicating that these structural abnormalities are differentially influenced by GM-CSF. These results indicate that GM-CSF, either directly or indirectly, regulates AEC-II metabolism and proliferation, but is not required for the development of emphysema in SP-D-deficient mice (Ochs et al. 2004).

Quantitative assessment of lungs challenged by fragrance materials in humans (Isola and Spinelli Oliveira)

The use of FrM has increased 10-fold since the 1950s but effects on health associated with their use are largely undefined due to scarcity of assessment of exposure and clinical data. Over 2000 different ingredients are used in the manufacture of fragrances, and although the majority has been used for many decades there is a need for continued monitoring to ensure acceptable safety standards (Ford et al. 2000). Here, we exemplify a study of simulated exposure employing nine selected fragrances, which were chosen according to volume of use, volatility and chemical classes. Most tests were conducted at adult (152 cm) and child (45 cm) breathing-zone heights (Isola et al. 2004). Table 1 shows the concentrations in air of the various FrM generated by a surrogate pressurized aerosol air freshener. Data were also collected for a plug-in heated oil air freshener and a fine fragrance, but the pressurized aerosol air freshener yielded the highest concentrations. Note that the differences in fragrance concentrations reflect the different volatilities of each of the materials. These data are important to design exploratory assessments of clinical exposure to fragrance materials that will be useful in establishing guidelines for human exposure. A study of 1189 individuals using questionnaire, patch testing, and skin prick test reactivity (atopy) has revealed that airborne chemicals cause respiratory symptoms in individuals with contact allergy: bronchial hyperactivity, female sex, and psychological vulnerability were independently associated with symptoms, but no association was found between prick test reactivity to proteins and the symptoms elicited by airborne chemicals. The IgE-mediated allergic mechanisms do not play a major role in these circumstances (Elberling et al. 2005). A final report on safety assessment of substituted phenols (for example, p-Chloro-m-cresol (PCMC), thymol, and o-Cymen-5-ol) has shown that clinical patch testing with 2% PCMC may produce irritant reactions and a concentration limitation was established although more studies are needed (Andersen 2006). Human exposure studies including data on personal exposure, biological monitoring measurements and products use, represent a critical component of the risk assessment process and is a rapidly advancing science (Ross et al. 2006). Also it is important to identify conditions in which human volunteers can participate safely in biomedical studies to replace laboratory animals (Combes et al. 2003), taking into consideration that the maximum tolerated dose of chemicals in rodents was poorly correlated with the maximum therapeutic dose values in humans (Matthews et al. 2004). Since 100,000 chemicals are potential for human exposure (Meyer 2003) the challenge to the development

of testing and assessment strategies will persist for many years to come.

Reactive oxygen species, friend not foe: their role as signaling molecules (Hancock)

Recent work demonstrates that ROS are instrumental signaling molecules that are generated in a wide range of cells, and are produced in all aerobic organisms (Maxwell et al. 1999). ROS participate in the control of both cell proliferation and cell development (Waris and Ahsan 2006). The production of reactive species has been shown to lead to the activation of several kinases, including MAPK, and to have profound effects on gene expression in a range of organisms, including humans and plants (Desikan et al. 1999, 2001) and in some species oxidation of transcription factors has been postulated as a possible mechanism (Delaunay et al. 2000). Proteins that are directly modified by the presence of ROS are now being found (Hancock et al. 2006), and signal transduction pathways that are downstream of ROS production are being revealed.

Some of the proteins identified have previously been assigned other functions, for example, ROS has been shown to inhibit glyceraldehyde 3-phosphate dehydrogenase (Hancock et al. 2005), which is well known to be part of glycolysis, so how such proteins fit into signaling pathways has yet to be elucidated.

ROS will alter protein function probably by modification of thiol groups, for example, the –SH group of cysteine residues. Oxidation of thiols may lead to the formation of disulphide bonds, or oxidation to sulphenic acid, but in either case a conformational change in the protein may lead to an alteration of function. Furthermore, thiol groups can react with NO, in a process known as S-nitrosylation, which potentially may also alter the topology of the protein. With ROS and NO both reacting with thiols, it is not hard to envisage that a competition between these signaling compounds might exist, with the end result depending on which reaction takes place. It cannot be assumed that the result will be the same in both cases. Also, ROS and NO can react together, often forming peroxynitrite. This is also a reactive and destructive molecule, but one that is also thought to have a signaling function. Of course, if ROS and NO react with each other, they will modulate the concentration of each other, and hence the amount of NO that is available to partake in thiol reactions might depend on the generation of ROS, but on the other hand, the concentration of ROS that could partake in the oxidation of thiols might depend on the generation of NO. Therefore, anticipating the exact protein modifications that take place, and therefore the signaling that ensues, can be more difficult than often thought.

Such chemistry of ROS, NO, and thiol groups will be common across all organisms, and therefore integrative and comparative studies will lead to a more rapid understanding of how these reactive compounds, and the results of their interactions, lead to the control of cellular function.

Living in the extremes of Earth: role of free radicals (Hermes-Lima)

It is well known that many species of insects, mollusks, fishes, ''amphibians'' and ''reptiles'' are tolerant to low environmental O_2 tensions for hours or weeks. Several other species are also tolerant to severe freezing or to dehydration of up to 50–60% of their body water (Storey 2006; Bickler and Buck 2007). These conditions are associated with major biochemical adjustments, including depression of metabolic rates and energy production by anaerobic routes. Other species among gastropods, ''amphibians,'' ''reptiles,'' and some mammals, such as bats and ground squirrels, experience hibernation or estivation, for periods of months to a few years, during which aerobic energy production is maintained, but at low metabolic rates (Drew et al. 2002; Ramos-Vasconcelos et al. 2005). The relatively rapid transition from low to restored metabolic rates (e.g., at arousal from hibernation/estivation, at reoxygenation from anoxia/hypoxia, at thawing from freezing) is often associated with overgeneration of ROS, causing physiological oxidative stress (Hermes-Lima and Zenteno-Savín 2002). The oxidative damage to cellular structures, in most animal species so far studied, is minimized by endogenous preactivated antioxidant defenses, which is generated before the occurrence of significant bursts of ROS (i.e., during anoxia, hypoxia, freezing, hibernation, or estivation). This process was named *preparation for oxidative stress*; see Hermes-Lima and Zenteno-Savín (2002). Its first evidence was reported in the early 1990s, when it was shown that garter snakes presented significantly greater levels of CAT (in muscle and lung) and GPx activities (in muscle) after 5 h at freezing temperatures (−2.5°C), as compared to control animals at 5°C (Hermes-Lima and Storey 1993). Now, it is clear that GPx (and/or GSH) is the key antioxidant defenses component involved in preparation for oxidative stress in many species,

Table 2 Antioxidant defenses (AD) and preparation for oxidative stress

Species	Oxidative stress	AD response	References
Oyster: *Crassostrea gigas*	Severe hypoxia (3 to 24 days)	↑ GPX (gene expression)	David et al. (2005)
Gastropod: *Littorina littorea*	Anoxia exposure (6 days)	↑ GSH ↓ several antioxidant enzymes	Hermes-Lima and Zenteno-Savín (2002)
Land snails: *Otala lactea*, *Helix aspersa*	Aestivation (20–30 days)	↑ SOD, GPX and GSH	Ramos-Vasconcelos et al. (2005)
Freshwater snail: *B. tenagophila*	Anoxia (24 h) and aestivation (15 days)	↑ GPX (anoxia +aestivation) ↓ CAT and SOD	Ferreira et al. (2003)
Crab: *Chasmagnathus granulata*	Anoxia (8 h)	↑ CAT and GST ↓ SOD	Oliveira et al. (2005)
Stone crab: *P. granulosa*	Aerial exposure (3 to 12 h)	↑ SOD and CAT	Romero et al. (2007)
Goldfish: *Carassius auratus*	Anoxia (8 h)	↑ CAT and GPX	Hermes-Lima and Zenteno-Savín (2002)
Tilapia:*Niloticus oreochromis*	Severe hypoxia (3 h)	↑ GPX (liver)	M Hermes-Lima (unpublished)
Carp: *Cyprinus carpio*	Hypoxia (5 h)	↑ CAT and GPX	Lushchak et al. (2005)
Leopard frog: *Rana pipiens*	Anoxia (30 h) and dehydration (4 days)	↑ GPX and CAT (anoxia and dehydration)	Hermes-Lima and Zenteno-Savín, (2002)
Wood frog: *Rana sylvatica*	Freezing (24 h)	↑ GPX	Hermes-Lima and Zenteno-Savín (2002)
Five species of hatchling turtles	Anoxia (17–50 days)	↑ CAT (liver)	Dinkelacker et al. (2005)
Hatchling turtle: *Chrysemys picta marginata*	Anoxia (4 h) and freezing (5 h)	↑ GPX, GST, peroxiredoxin (gene expression)	Storey (2006)
Garter snake: *Thamnophis sirtalis parietalis*	Anoxia (10 h) and freezing (5 h)	↑ GPX and CAT (freezing) ↑ SOD and GSH (anoxia)	Hermes-Lima and Storey (1993)
European common lizard: *Lacerta vivipara*	Freezing and supercooling (both for 20 h)	↑ SOD and GPX (supercooling)	Voituron et al. (2006)
Bat: *Myotis lucifugus*	Hibernation (~2 months)	↑ thioredoxin peroxidase (gene expression)	Eddy et al. (2005)
13-Line ground squirrel: *Spermophilus tridecemlineatus*	Hibernation	↑ GPX and GST (gene expression)	Eddy and Storey (2002)

under different conditions (Table 2). Recent studies focus on gene expression involved in antioxidant defenses under different ecophysiological conditions (David et al. 2005), as well as on covalent modifications in enzymes that are part of the antioxidant defenses responses (Willmore and Storey 2005).

Antioxidants, oxidative stress, mode of life, and adaptations to the environment (Wilhelm Filho)

Most animal species are thermoconformers and in these organisms antioxidant defenses fairly reflect environmental temperature and consequently O_2 consumption and/or availability. Active species of fish present relatively greater concentrations of enzymatic and nonenzymatic components of antioxidant defenses than do sluggish species. The cutlass fish *Trichiurus lepturus*, e.g., a long, slender, and very active marine species found throughout the tropical and temperate waters, possesses greater activity levels of SOD, CAT, and GPx (Wilhelm Filho et al. 1993) and greater vitamin E content (Wilhelm Filho et al. 2000), than does Tanduju, the stargazer *Astroscopus sexspinosus*, a sluggish marine species found in shallow waters (Wilhelm Filho 2007). It has also been shown that deep-sea fishes, which are exposed to low O_2 tensions in their environment, possess low antioxidant defenses capability (Guderley 2004).

An interesting antioxidant defenses found in most water-breathing organisms is the excretion of H_2O_2 by simple diffusion through the gills, which was described in three freshwater fish species, the guppy (*Poecilia vellifera*), the armoured catfish *Corydoras paleatus*, and the goldfish *Carassius auratus*, and in one marine species, the clown fish *Amphiprion percula* (Wilhelm Filho et al. 1994, 2000). The ability to eliminate a significant part of the H_2O_2 produced in the tissues through the gills may follow a circadian pattern: in

guppies the rate of H_2O_2 release is twice as great in the afternoon as in the morning (Wilhelm Filho et al. 1994).

Antioxidant levels are adjusted according to seasonal and ontogenetic changes related to foraging and/or spawning in different taxa, such as mussels and fishes, thus possibly minimizing oxidative stress that occurs during these activities (Wilhelm Filho et al. 2000, 2001). For instance, in *Trichiurus lepturus* the concentration of antioxidant enzymes and some non-enzymatic antioxidants possibly follows a temperature gradient, being greater in summer than in winter (Wilhelm Filho et al. 2000). The same species also shows ontogenetic adaptations, in which young specimens generally have greater antioxidant levels than do mature fish (Wilhelm Filho et al. 2000).

Animals that experience large daily changes in O_2 consumption, such as torpid vertebrates and some marine fishes that display daily vertical migrations, appear to prevent oxidative damage by having appropriate high constitutive antioxidant defenses. One example is *T. lepturus*, which lives in coastal waters, rising to eat planktonic crustaceans during the day and returning to the sea bed at night, as compared to *Astroscopus sexspinosus*; or different bat species that routinely become torpid compared to other mammals that do not enter daily torpor (Wilhelm Filho et al. 2000, 2007).

In general, diving mammals present great tissue antioxidant defenses that balance generation of ROS associated with the rapid transition from apnea to reoxygenation. Five different species of diving mammals (the dwarf mink whale *Balaenoptera acutorostrata*, the clymene dolphin *Stenella clymene*, the marine manatee *Trichechus manatus manatus*, and the southern elephant seal *Mirounga leonina*) showed greater antioxidant defenses, than did five terrestrial species, namely the primate *Cebus apella*, the ferret *Grison vittatus*, the raccon *Procyon cancrivorous*, the ant-eater *Tamandua tetradactyla tetradactyla*, and the deer *Ozotocerus besoarticus* (Wilhelm Filho et al. 2002). Recently, it has been suggested that the limited O_2 uptake in insects may be linked to avoidance of oxidative stress (see Hetz in Orgeig et al., in this issue). Also, well-documented are antioxidant defenses to freeze-thawing tolerance and arrested states, both in thermoconformers and in true endotherms that seasonally undergo hibernation/aestivation (Buzádzic et al. 1990).

Conclusion

By taking a view of biological processes from a range of organisms it can be seen that the structures that have a function in gas exchange do interact with their environment, and this throws up many challenges. They have to pose a barrier to infiltration of pathogens and deal with microorganisms and toxicants that could be deleterious to the tissue, or to the organism as a whole. In mammals, there is a crosstalk between the lung, the nervous system, and endocrine system through proinflammatory cytokines and other mediators, which results in the regulation of the inflammatory and adaptive responses. Consequently, the gas-exchange function of the respiratory organ is preserved. AEC-II seems to play a pivotal role in the modulation of immune responses in lungs, linking components of the innate and adaptive immune system. This complex network of interrelated mechanisms includes multifunctional cells or molecules that are found both in vertebrates and invertebrates or that are widely expressed in respiratory structures of vertebrates (i.e., surfactants). Also, one should consider that the vertebrate innate immunity resembles a mosaic of invertebrate immune responses. Such findings emphasize that throughout evolution there is a variety of solutions to meet similar requirements for protection and functionality. Future studies should aim to reconstruct these interrelationships that are seen in a broad variety of organisms, including plants, within a phylogenetic framework. The presence of relatively high concentrations of O_2 can lead to the generation of ROS, and these may cause damage to the tissues and cells in the immediate locality of their production. Such ROS, however, can also be instrumental in the control of cellular function, such as gene expression, acting as key signaling molecules. The control of such ROS is not only through their generation, but also through their removal by antioxidant enzymes and compounds. As such, the primary functions of gas-exchange structures cannot be compromised, but these structures also have to contribute to the well-being of the whole organism, by interacting with the immune system for example. Studying a variety of organisms allows the similarities and differences in the mechanisms used in such tissues to be uncovered, and will give a greater understanding of how ROS and antioxidants impinge on these systems.

Acknowledgments

We wish to thank the organizers of the ICRB (Anke Schmitz, Stefan K. Hetz, Steve Morris, Thomas Breuer) and especially Steven F. Perry, for the opportunity to discuss respiration within an integrative and comparative framework, and for

the constructive and friendly atmosphere provided during and after the meeting. L. W. Smith, Research Institute for Fragrance Materials, Inc., Woodcliff Lake, NJ, USA; D. Vethanayagam, Division of Pulmonary Medicine, University of Alberta, Edmonton, AB, Canada; R. E. Rogers and C. J. Jeng, Toxcon Health Sciences Research Centre Inc., Edmonton; and N. Prasad, Department of Mathematical and Statistical Sciences, University of Alberta, Edmonton, collaborated in the clinical study with D.A.I.

References

Andersen A. 2006. Final report on the safety assessment of sodium p-chloro-m-cresol, p-chloro-m-cresol, chlorothymol, mixed cresols, m-cresol, o-cresol, p-cresol, isopropyl cresols, thymol, o-cymen-5-ol, and carvacrol. Int J Toxicol 25(Suppl 1):29–127.

Adriaensen D, Brouns I, Pintelon I, De PI, Timmermans J. 2006. Evidence for a role of neuroepithelial bodies as complex airway sensors: comparison with smooth muscle-associated airway receptors. J Appl Physiol 101:960–70.

Akinbi HT, Epaud R, Bhatt H, Weaver TE. 2000. Bacterial killing is enhanced by expression of lysozyme in the lungs of transgenic mice. J Immunol 165:5760–6.

Banchereau J, Steinman RM. 1998. Dendritic cells and the control of immunity. Nature 392:245–52.

Beers MF, Kim CD, Fisher AB. 1994. Synthesis of type II cell lamellar body lysozyme-15 kD protein (lbl-15) by perfused rat lung. Am J Respir Cell Mol Biol 11:240–8.

Besse I, Buchanan BB. 1997. Thioredoxin-linked plant and animal processes: the new generation. Bot Bull Acad Sin 38:1–11.

Bickler PE, Buck LT. 2007. Hypoxia tolerance in reptiles, amphibians, and fishes: life with variable oxygen availability. Annu Rev Physiol 69:145–70.

Boman HG. 1998. Gene-encoded peptide antibiotics and the concept of innate immunity: an update review. Scand J Immunol 48:15–25.

Boyton RJ, Openshaw PJ. 2002. Pulmonary defences to acute respiratory infection. Br Med Bull 61:1–12.

Broekaert WF, Terras FR, Cammue BP, Osborn RW. 1995. Plant defensins: novel antimicrobial peptides as components of the host defense system. Plant Physiol 108:1353–8.

Buzádzic B, Spásic M, Saicic ZS, Radojicic R, Halliwell B, Petrovic VM. 1990. Antioxidant defense in the squirrel *Citellus citellus* 1. A comparison with the rat. Free Radic Biol Med 9:401–4.

Canning BJ, Mori N, Mazzone SB. 2006. Vagal afferent nerves regulating the cough reflex. Respir Physiol Neurobiol 152:223–42.

Casals C, Arias-Díaz J, Valino F, Sáenz A, García C, Balibrea JL, Vara E. 2003. Surfactant strengthens the inhibitory effect of C-reactive protein on human lung macrophage cytokine release. Am J Physiol Lung Cell Mol Physiol 284:L466–72.

Coleridge JCG, Coleridge HM. 1984. Afferent vagal C fibre innervation of the lungs and airways and its functional significance. Rev Physiol Biochem Pharmacol 99:1–110.

Combes RD, Berridge T, Connelly J, Eve MD, Garner RC, Toon S, Wilcox P. 2003. Early microdose drug studies in human volunteers can minimise animal testing: Proceedings of a workshop organised by volunteers in research and testing. Eur J Pharm Sci 19:1–11.

Conneely OM. 2001. Antiinflammatory activities of lactoferrin. J Am Coll Nutr 20:389S–97S.

Czura CJ, Tracey KJ. 2005. Autonomic neural regulation of immunity. J Intern Med 257:156–66.

David E, Tanguy A, Pichavant K, Moraga D. 2005. Response of the Pacific oyster *Crassostrea gigas* to hypoxia exposure under experimental conditions. FEBS J 272:5635–52.

Delaunay A, Isnard A, Toledano MB. 2000. H_2O_2 sensing through oxidation of the YAP1 transcription factor. EMBO J 19:5157–66.

Desikan R, A-H-Mackerness S, Hancock JT, Neill SJ. 2001. Regulation of the *Arabidopsis* transcriptome by oxidative stress. Plant Physiol 127:159–72.

Desikan R, Clarke A, Hancock JT, Neill SJ. 1999. H_2O_2 activates a MAP kinase-like enzyme in *Arabidopsis thaliana* suspension cultures. J Exp Bot 50:1863–6.

Dinkelacker SA, Costanzo JP, Lee RE. 2005. Anoxia tolerance and freeze tolerance in hatchling turtles. J Comp Physiol 175B:209–17.

Dranoff G, et al. 1994. Involvement of granulocyte-macrophage colony-stimulating factor in pulmonary homeostasis. Science 264:713–6.

Drew KL, Toien O, Rivera PM, Smith MA, Perry G, Rice ME. 2002. Role of the antioxidant ascorbate in hibernation and warming from hibernation. Comp Biochem Physiol 133C:483–92.

Dröge W. 2002. Free radicals in the physiological control of cell function. Physiol Rev 82:47–95.

Dziarski R, Gupta D. 2006. The peptidoglycan recognition proteins (PGRPs). Genome Biol 7:232–45.

Eddy SF, Storey KB. 2002. Dynamic use of cDNA arrays: heterologous probing for gene discovery and exploration of animal adaptations in stressful environments. In: Storey KB, Storey JM, editors. Cell and molecular responses to stress. Vol. 3, Amsterdam: Elsevier Press. p 315–25.

Eddy SF, McNally JD, Storey KB. 2005. Up-regulation of a thioredoxin peroxidase-like protein, proliferation-associated gene, in hibernating bats. Arch Biochem Biophys 435:103–11.

Elberling J, Linneberg A, Mosbech H, Dirksen A, Menné T, Nielsen NH, Madsen F, Frølund L, Johansen JD. 2005. Airborne chemicals cause respiratory symptoms in

individuals with contact allergy. Contact Dermatitis 52:65–72.

Ferreira MVR, Alencastro ACR, Hermes-Lima M. 2003. Role of antioxidant defenses during estivation and anoxia exposure in freshwater snails *Biomphalaria tenagophila* (Orbigny, 1835). Can J Zool 81:1239–48.

Fisher AB. 2002. Oxidants and antioxidants: Transatlantic Airway Conference 2002. Am J Respir Cri Care Med 166:S2–3.

Ford RA, Domeyer B, Easterday O, Maier K, Middleton J. 2000. Criteria for development of a database for safety evaluation of fragrance ingredients. Regul Toxicol Pharmacol 31:166–81.

Foyer CH, Noctor G. 2005. Redox homeostasis and antioxidant signaling: a metabolic interface between stress perception and physiological responses. Plant Cell 17:1866–75.

Fu XW, Wang D, Nurse CA, Dinauer MC, Cutz E. 2000. NADPH oxidase is an O2 sensor in airway chemoreceptors: evidence from K+ current modulation in wild-type and oxidase-deficient mice. Proc Natl Acad Sci USA 97:4374–9.

Ganz T. 2004. Antimicrobial polypeptides. J Leukoc Biol 75:34–8.

Ganz T, Lehrer RI. 1998. Antimicrobial peptides of vertebrates. Curr Opin Immunol 10:41–7.

Gardai SJ, Xiao YQ, Dickinson M, Nick JA, Voelker DR, Greene KE, Henson PM. 2003. By binding SIRPalpha or calreticulin/CD91, lung collectins act as dual function surveillance molecules to suppress or enhance inflammation. Cell 115:13–23.

Guderley H. 2004. Locomotor performance and muscle metabolic capacities: impact of temperature and energetic status. Comp Biochem Physiol B 139:371–82.

Hancock JT, Henson D, Nyirenda M, Desikan R, Harrison J, Lewis M, Hughes J, Neill SJ. 2005. Proteomic identification of glyceraldehyde 3-phosphate dehydrogenase as an inhibitory target of hydrogen peroxide in *Arabidopsis*. Plant Physiol Biochem 43:828–35.

Hancock J, Desikan R, Harrison J, Bright J, Hooley R, Neill S. 2006. Doing the unexpected: proteins involved in hydrogen peroxide perception. J Exp Bot 57:1711–8.

Hawieger J. 2001. Innate immunity and inflammation: a transcriptional paradigm. Immunol Res 23:99–109.

Hayashi T, Beck L, Rossetto C, Gong X, Takikawa O, Takabayashi K, Broide DH, Carson DA, Raz E. 2004. Inhibition of experimental asthma by indoleamine 2,3-dioxygenase. J Clin Invest 114:270–9.

Hermes-Lima M, Storey KB. 1993. Role of antioxidants in the tolerance of freezing and anoxia by garter snakes. Am J Physiol 265:R646–52.

Hermes-Lima M, Zenteno-Savín T. 2002. Animal response to drastic changes in oxygen availability and physiological oxidative stress. Comp Biochem Physiol 133C:537–56.

Hoffmann JA, Kafatos FC, Janeway CA, Ezekowitz RA. 1999. Phylogenetic perspectives in innate immunity. Science 284:1313–8.

Howard CJ, Charleston B, Stephens SA, Sopp P, Hope JC. 2004. The role of dendritic cells in shaping the immune response. Anim Health Res Rev 5:191–5.

Isola DA, Rogers RE, Black MS, Smith LW. 2004. Exposure characterizations of three fragranced products. Int J Toxicol 23:397.

Jones RD, Hancock JT, Morice AH. 2000. NADPH oxidase: a universal oxygen sensor? Free Radic Biol Med 29:416–24.

Jung A, Allen L, Nyengaard JR, Gundersen HJG, Richter J, Hawgood S, Ochs M. 2005. Design-based stereological analysis of the lung parenchymal architecture and alveolar type II cells in surfactant protein A and D double deficient mice. Anat Rec A Discov Mol Cell Evol Biol 286:885–90.

Lai CJ, Ruan T, Kou YR. 2005. The involvement of hydroxyl radical and cyclooxygenase metabolites in the activation of lung vagal sensory receptors by circulatory endotoxin in rats. J Appl Physiol 98:620–8.

Lee LY, Pisarri TE. 2001. Afferent properties and reflex functions of bronchopulmonary C-fibers. Respir Physiol 125:47–65.

Levay PF, Viljoen M. 1995. Lactoferrin: a general review. Haematologica 80:252–67.

Lin SX, Yu J. 2007. Effects of arachidonic aid metabolites on airway sensors. Acta Physiol Sinica 59:141–9.

Lin S, Walker J, Xu L, Gozal D, Yu J. 2007. Behaviours of pulmonary sensory receptors during developments of acute lung injury in the rabbit. Exp Physiol 92:749–55.

Lushchak VI, Bagnyukova TV, Lushchak OV, Storey JM, Storey KB. 2005. Hypoxia and recovery perturb free radical processes and antioxidant potential in common carp (*Cyprinus carpio*) tissues. Int J Biochem Cell Biol 37:1319–30.

Matthews EJ, Kruhlak NL, Benz RD, Contrera JF. 2004. Assessment of the health effects of chemicals in humans: I. QSAR estimation of the maximum recommended therapeutic dose (MRTD) and no effect level (NOEL) of organic chemicals based on clinical trial data. Curr Drug Discov Technol 1:61–76.

Maxwell DP, Wang Y, McIntosh L. 1999. The alternative oxidase lowers mitochondrial reactive oxygen production in plant cells. Proc Natl Acad Sci USA 96:8271–6.

Mazzoni A, Leifer CA, Mullen GED, Kennedy MN, Klinman DM, Segal DM. 2003. Cutting edge: histamine inhibits IFN-alpha release from plasmacytoid dendritic cells. J Immunol 170:2269–73.

Meyer O. 2003. Testing and assessment strategies, including alternative and new approaches. Toxicol Lett 11:21–30.

Morita R, Uchiyama T, Hori T. 2005. Nitric oxide inhibits IFN-alpha production of human plasmacytoid dendritic cells partly via a guanosine 3′,5′-cyclic monophosphate-dependent pathway. J Immunol 175:806–12.

Morris PE, Bernard GR. 1994. Significance of glutathione in lung disease and implications for therapy. Am J Med Sci 307:119–27.

Ochs M. 2006. A brief update on lung stereology. J Microsc 222:188–200.

Ochs M, Johnen G, Muller K, Wahlers T, Hawgood S, Richter J, Brasch F. 2002. Intracellular and intraalveolar localization of surfactant protein A (SP-A) in the parenchymal region of the human lung. Am J Respir Cell Mol Biol 26:91–8.

Ochs M, Knudsen L, Allen L, Stumbaugh A, Levitt S, Nyengaard JR, Hawgood S. 2004. GM-CSF mediates alveolar epithelial type II cell changes, but not emphysema-like pathology, in SP-D-deficient mice. Am J Physiol Lung Cell Mol Physiol 287:L1333–41.

Oliveira UO, Araújo ASR, Belló-Klein A, Silva RSM, Kucharski LC. 2005. Effects of environmental anoxia and different periods of reoxygenation on oxidative balance in gills of the estuarine crab *Chasmagnathus granulate*. Comp Biochem Physiol 140B:51–7.

Oppenheim JJ, Biragyn A, Kwak LW, Yang D. 2003. Roles of antimicrobial peptides such as defensins in innate and adaptive immunity. Ann Rheum Dis 62(Suppl 2):ii17–21.

Orgeig S, Bernhard W, Biswas SC, Daniels CB, Hall SB, Hetz SK, Lang CJ, Maina JN, Panda AK, Perez-Gil J, Possmayer F, Veldhuizen RA, Yan W. 2007. The anatomy, physics and physiology of gas exchange surfaces: Is there a universal function for pulmonary surfactant in animal respiratory structures? Integr Comp Biol (In press).

Palecanda A, Paulauskis J, Al-Mutairi E, Imrich A, Qin G, Suzuki H, Kodama T, Tryggvason K, Koziel H, Kobzik L. 1999. Role of the scavenger receptor MARCO in alveolar macrophage binding of unopsonized environmental particles. J Exp Med 189:1497–506.

Peiser L, Gordon S. 2001. The function of scavenger receptors expressed by macrophages and their role in the regulation of inflammation. Microbes Infect 3:149–59.

Qamar R, Parveen A, Farrukh A, Schiffmann D, Mossman BT, Kamp DW, Mohammad A. 1999. Glutathione redox system in oxidative lung injury. Crit Rev Toxicol 29:543–68.

Ramos-Vasconcelos GR, Cardoso LA, Hermes-Lima M. 2005. Seasonal modulation of free radical metabolism in estivating land snails *Helix aspersa*. Comp Biochem Physiol 140C:165–74.

Raz E. 2007. Organ-specific regulation of innate immunity. Nat Immunol 8:3–4.

Reid MB. 2006. Of balance and unbalance. J Applied Physiol 101:1011–2.

Romero MC, Ansaldo M, Lovrich GA. 2007. Effect of aerial exposure on the antioxidant status in the subantarctic stone crab *Paralomis granulosa* (Decapoda: Anomura). Comp Biochem Physiol C (In press).

Ross JH, Driver JH, Lunchick C, Wible C, Selman F. 2006. Pesticide exposure monitoring databases in applied risk analysis. Rev Environ Contam Toxicol 186:107–32.

Sato K, Kadiiska MB, Ghio AJ, Corbett J, Fann YC, Holland SM, Thurma RG, Mason RP. 2002. In vivo lipid-derived free radical formation by NADPH oxidase in acute lung injury induced by lipopolysaccharide: a model for ARDS. FASEB J 16:1713–20.

Segal BH, Davidson BA, Hutson AD, Russo TA, Holm BA, Mullan BA, Habitzruther M, Holland SM, Knight PR. 2007. Acid aspiration-induced lung inflammation and injury are exacerbated in NADPH oxidase-deficient mice. Am J Physiol Lung Cell Mol Physiol 292:L760–8.

Shibata Y, Berclaz PY, Chroneos ZC, Yoshida M, Whitsett JA, Trapnell BC. 2001. GM-CSF regulates alveolar macrophage differentiation and innate immunity in the lung through PU.1. Immunity 15:557–67.

Sies H. 1993. Strategies of antioxidant defense. Eur J Biochem 215:213–9.

Soukhova GK, Ahmed M, Fletcher EC, Yu J. 1999. Hydrogen peroxide in the lung parenchyma stimulates vagally mediated phrenic activity. Chest 116:1365–8.

Soukhova G, Wang Y, Ahmed M, Walker JF, Yu J. 2003. Bradykinin stimulates respiratory drive by activating pulmonary sympathetic afferents in the rabbit. J Appl Physiol 95:241–9.

Steinman RM, Cohn ZA. 1973. Identification of a novel cell type in peripheral lymphoid organs of mice. I. Morphology, quantitation, tissue distribution. J Exp Med 137:1142–62.

Storey KB. 2006. Reptile freeze tolerance: metabolism and gene expression. Cryobiology 52:1–16.

Takabayshi K, Corr M, Hayashi T, Redecke V, Beck L, Guiney D, Sheppard D, Raz E. 2006. Induction of a homeostatic circuit in lung tissue by microbial compounds. Immunity 24:475–87.

Thompson AB, Bohling T, Payvandi F, Rennard SI. 1990. Lower respiratory tract lactoferrin and lysozyme arise primarily in the airways and are elevated in association with chronic bronchitis. J Lab Clin Med 115:148–58.

Trapnell BC, Whitsett JA. 2002. GM-CSF regulates pulmonary surfactant homeostasis and alveolar macrophage-mediated innate host defense. Ann Rev Physiol 64:775–802.

Van Eden W, Van Der Zee R, Van Kooten P, Berlo SE, Cobelens PM, Kavelaars A, Heijnen CJ, Prakken B, Roord S, Albani S. 2002. Balancing the immune system: Th1 and Th2. Ann Rheum Dis 61(Suppl 2):ii25–8.

Voituron Y, Servais S, Romestaing C, Douki T, Barré H. 2006. Oxidative DNA damage and antioxidant defenses in the European common lizard (*Lacerta vivipara*) in supercooled and frozen states. Cryobiology 52:74–82.

Waris G, Ahsan H. 2006. Reactive oxygen species: role in the development of cancer and various conditions. J Carcinog 5:14.

Widdicombe JG. 2001. Airway receptors. Respir Physiol 125:3–15.

Wilhelm Filho D. 2007. Reactive oxygen species, antioxidants and fish mitochondria. Front Biosci 12:1229–37.

Wilhelm Filho D, Althoff SL, Dafré AL, Boveris A. 2007. Antioxidant defenses, longevity and ecophysiology of South American bats. Comp Biochem Physiol C (In press).

Wilhelm Filho D, Giulivi C, Boveris A. 1993. Antioxidant defences in marine fish. I-Teleosts. Comp Biochem Physiol 106C:409–13.

Wilhelm Filho D, González-Flecha B, Bredeston L, Boveris A. 1994. Gill diffusion as a physiological mechanism for hydrogen peroxide elimination by fish. Braz J Med Biol Res 27:2879–82.

Wilhelm Filho D, Sell F, Ribeiro L, Ghisland M, Carrasquedo F, Fraga CG, Wallauer JP, Simões-Lopes PC, Uhart MM. 2002. Comparison between the antioxidant status of terrestrial and diving mammals. Comp Biochem Physiol 133A:885–92.

Wilhelm Filho D, Torres MA, Marcon JL, Fraga CG, Boveris A. 2000. Comparative antioxidant defenses in vertebrates - emphasis on fish and mammals. Trends Comp Biochem Physiol 7:33–45.

Wilhelm Filho D, Torres MA, Tribess TB, Pedrosa RC, Soares CH. 2001. Influence of season and pollution on the antioxidant defenses of the cichlid fish acará (Geophagus brasiliensis). Braz J Med Biol Res 34:719–26.

Willmore WG, Storey KB. 2005. Purification and properties of the glutathione S-transferases from the anoxia-tolerant turtle, *Trachemys scripta elegans*. FEBS J 272:3602–14.

Wright JR. 2006. The ''wisdom'' of lung surfactant: balancing host defense and surface tension-reducing functions. Am J Physiol Lung Cell Mol Physiol 291:L847–50.

Yu J. 2002. An overview of vagal airway receptors. Acta Physiol Sinica 54:451–9.

Yu J. 2005. Airway mechanosensors. Respir Physiol Neurobiol 148:217–43.

Yu J, Lin S, Zhang J, Otmishi P, Guardiola J. 2007. Airway nociceptors activated by pro-inflammatory cytokines. Respir Physiol Neurobiol 156:116–9.

Zasloff M. 2002. Antimicrobial peptides of multicellular organisms. Nature 415:389–95.

Zissel G, Ernst M, Rabe K, Papadopoulos T, Magnussen H, Schlaak M. Muller-Quernheim J. 2000. Human alveolar epithelial cells type II are capable of regulating T-cell activity. J Investig Med 48:66–75.