Review

Chemical and biological activity of free radical ‘scavengers’ in allergic diseases

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Abstract

Reactive oxygen species (ROS) are generated constantly in vivo. They can lead to lipid peroxidation and oxidation of some enzymes, as well as protein oxidation and degradation. Cells possess several biological systems, defined as ‘scavengers’, to protect themselves from the radical-mediated damage. Immune cells may discharge their arsenal of toxic agents against host tissues, resulting in oxidative damage and inflammation. Therefore, free radical production and disturbance in redox status can modulate the expression of a variety of immune and inflammatory molecules, leading to inflammatory processes, both exacerbating inflammation and effecting tissue damage. Recently, abnormal immunity has been related to oxidative imbalance, and antioxidant functions are linked to anti-inflammatory and/or immunosuppressive properties. Currently, allergy is one of the most important human diseases. We studied the role of the primary antioxidant defence system, constituted by the antioxidant enzymes superoxide dismutase, catalase and glutathione peroxidase, protecting cells from toxic oxygen. We analyzed how they are involved in blood cells detoxification, and how the imbalance of reactive oxygen species is related to inflammation in allergic diseases by affecting immune cells. Finally, we discuss the published data that relates anti-free radical therapy to the management of human allergic diseases. © 2000 Elsevier Science B.V. All rights reserved.

Abbreviations: CAT, catalase (EC 1.11.1.6); EPO, eosinophil peroxidase (EC 1.11.1.2); FMLP, N-formylmethionyl-leucyl-phenylalanine; GM-CSF, granulocyte-macrophage colony-stimulating factor; GPX, glutathione peroxidase (EC 1.11.1.19); GSH, glutathione; IFN-α, interferon alpha; IFN-γ, interferon gamma; IgE, immunoglobulin E; NADPH, reduced nicotinamide-adenine dinucleotide phosphate; NFκB, nuclear factor kappa-B; NOS: nitric oxide synthase (1.14.13.39); PAF, platelet-activating factor; PMA, phorbol 12-myristate 13-acetate; PMNs, polymophonuclear leukocytes; ROS, reactive oxygen species; SOD, superoxide dismutase (EC 1.15.1.1); TNF-α, tumor necrosis factor alpha

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1. Introduction

The principal physiological function of the immune system is the removal of infectious agents. In this process, cells can be damaged. Therefore, the organism has evolved different ways of eliminating actively its own potentially harmful products, so that the host may survive. Potentially injurious immune reactions may be prevented either by inactivating functionally the responding lymphocytes, or by eliminating the harmful radical reaction products [1]. Cells possess different systems, defined as ‘scavengers’, to protect themselves from the radical-mediated damage and, among these, some enzymes or chemical structures have the role of antioxidants.

Reactive oxygen species (ROS), including hydroxyl radicals (OH), superoxide anion (O$_2^-$), hydrogen peroxide (H$_2$O$_2$) and nitric oxide (NO) are very transient species due to their high chemical reactivity that leads to lipid peroxidation and oxidation of some enzymes, and a massive protein oxidation and degradation [2,3].

Neutrophils constitute 60 per cent of the circulating leukocytes and they are the most abundant cellular components of the immune system. They may discharge their arsenal of toxic agents against host tissues, resulting in oxidative damage and inflammation. The combination of phagocytosis of bacteria and secretion of proteolytic enzymes and immuno-modulatory compounds that assist in the killing and digestion of bacteria, are accompanied by respiratory burst, involving a sudden stimulus-induced increase in non-mitochondrial oxidative metabolism which results in the production of ROS [4].

ROS generation through normal cellular metabolism and by exogenous insults is a constant problem for which cells have developed multiple protective mechanisms to survive [5,6]. ROS are mainly generated by mitochondria at the portion of the electron transport chain as the toxic by-products of oxidative phosphorylation, their energy generating pathway [7]. The interruption of oxidative phosphorylation results in decreased levels of ATP. Whatever the cause(s) and sequence of events are, respiratory chain deficiencies appear to play an important role in the pathogenesis of many diseases [8]. In addition, free radical production and disturbance in redox status can modulate the expression of a variety of immune and inflammatory molecules [9–11], leading to inflammatory processes, both exacerbating inflammation and effecting tissue damage [12]. Recently, it has been suggested that abnormal immunity has been
related to oxidative imbalance [13–17], and antioxidant functions are linked to anti-inflammatory and/or immunosuppressive properties [18–20].

Since tissue injury and inflammation lead to increased oxidative stress, it seems logical that good antioxidant status might diminish tissue damage in allergic diseases [6–8]. Herein, we will see the role of the primary antioxidant defence system which is constituted by the antioxidant enzymes, protecting cells from toxic oxygen. We will analyze how they are involved in blood cells detoxification, and how the imbalance of reactive oxygen species is related to inflammation in allergic diseases by affecting immune cells. A further investigation in antioxidant enzymes may open a new pathway in the knowledge of allergic diseases.

2. Reactive oxygen species and free radical scavengers

Generation of hydrogen peroxide takes place through the dismutation of superoxide. Therefore any biological system generating $O_2^-$ will produce $H_2O_2$. However, there are enzymes localized in peroxisomes that produce $H_2O_2$ without intermediation of $O_2^-$. Contrary to $O_2^-$, $H_2O_2$ is able to cross cell membranes and inside the cells it can react with $Fe^{2+}$ or $Cu^+$ to form hydroxyl radicals via the Fenton reaction [21]:

$$Fe^{2+} + H_2O \rightarrow Fe^{3+} + OH + OH^-$$  (Fenton reaction)

$$O_2^- + H_2O_2 \rightarrow O_2 + OH + OH^-$$  (Haber–Weiss reaction)

The metal-catalysed Haber–Weiss reaction may involve the participation of either free iron (or copper) or iron sequestered in the form of nucleotide iron complexes, ferritin, lactoferrin, hemoglobin, and myoglobin.

The wide array of enzymatic and non-enzymatic antioxidant defences includes superoxide dismutase (SOD), glutathione peroxidase (GPX), catalase (CAT), ascorbic acid (vitamin C), $\alpha$-tocopherol (vitamin E), glutathione (GSH), beta-carotene, vitamin A, NADPH, adenosine, coenzyme Q, urate, methionine, cysteine, phenols and flavonoids [22,23].

Superoxide dismutase (EC 1.15.1.1) destroys the highly reactive radical superoxide by conversion into the less reactive peroxide ($H_2O_2$), that can be destroyed by catalase or glutathione peroxidase reactions [21]:

$$O_2 + O_2 + 2H^+ \xrightarrow{\text{SOD}} H_2O_2 + O_2$$

Catalase (EC 1.11.1.6) is a highly reactive enzyme, reacting with $H_2O_2$ to
form water and molecular oxygen; and with hydrogen donors such as methanol, ethanol, formic acid or phenols [22]:

\[
2H_2O_2 \rightarrow H_2O + O_2
\]

\[
ROOH + AH_2 \rightarrow H_2O + ROH + A
\]

Glutathione peroxidase (EC 1.11.1.19) catalyses the reduction of a variety of hydroperoxides (ROOH and H$_2$O$_2$) using GSH, thereby protecting mammalian cells against oxidative damage and, reducing, among others, cellular lipid hydroperoxides [22]:

\[
ROOH + 2GSH \rightarrow ROH + GSSG + H_2O
\]

The flavin containing thioredoxin reductase (EC 1.6.4.5) is an ubiquitous enzyme able to reduce O$_2^-$ and NO by using thioredoxin as a substrate [23]. Transferrin and ferritin sequester iron ions, while ceruloplasmin sequesters copper ions so that these ions are not available to catalyse the Haber–Weiss reaction generating OH or to perform the decomposition of hydroperoxides. Ceruloplasmin has also ferroxidase activity: it oxidizes Fe$^{2+}$ to Fe$^{3+}$ and so inhibits OH formation from H$_2$O$_2$ and iron dependent lipoperoxidation [24].

$\alpha$-Tocopherol is concentrated inside the membranes, in blood lipoproteins and adrenal glands. It quenches and reacts with $^1$O$_2$ and is a scavenger of OH, able to protect membranes from these extremely reactive species. However, its major antioxidant action in biological membranes is to act as a chain breaking antioxidant, donating labile hydrogen to peroxy and alkoxy radicals, thereby breaking the radical chain. It has been proposed that $\alpha$-TO may be reduced by ascorbic acid or reduced glutathione. These are scavengers of ROS and other reactive free radicals. $\beta$-Carotene is a powerful scavenger of $^1$O$_2$. Urate binds iron and copper and scavenges OH, $^1$O$_2$ and peroxy radicals [25].

Animal studies have shown that circulating cells exhibit membrane alterations when there is a lack of vitamin E in the diet. In fact, the mitochondrial membranes of the reticulocytes and lymphocytes appear bloated and dis-integrated; the number of platelets, which are more adhesive, increases and produces more thromboxanes in comparison with those of normal rats. The response of T and B lymphocytes to mitogenic stimuli, the mixed lymphocyte reaction and the number of cells forming plates are considerably depressed. On the contrary, carotenoids and in particular beta-carotene either directly or indirectly as precursor of vitamin A, enhance T cell proliferation and cytotoxicity, macrophage killing, and TNF-$\alpha$ secretion [24].
3. ROS generation and histamine and nitric oxide release

All allergic diseases are characterized by a specific pattern of inflammation that is largely driven by IgE-dependent mechanisms. Although allergen avoidance is, by far, the most effective way against allergic reaction, immunotherapy is the only clinical treatment to effectively prevent the allergic manifestations. Not only does Immunotherapy act only producing blocking antibodies, but it also favors the transformation of lymphocytes T helper 2 (Th₂) into Th₁. At the same time, the production of interferon gamma (IFN-γ) increases and there is an inhibition in the production of IgE by B lymphocytes (Fig. 1). In these processes stimulated cells generate a considerable amount of reactive oxygen species. Thus, we will describe antioxidant enzymes appearing to work with immunotherapy in order to effect some immune-like functions against inflammation and allergic manifestations (Fig. 2).

Mast cells exist throughout most tissues although they are more prevalent in areas that come into contact with the external environment, such as the skin,

Fig. 1. Immunotherapy favors the transformation of lymphocytes Th₂ into lymphocytes Th₁. By avoiding this transformation, IFN-α production and inhibition of B lymphocytes that secrete IgE, is achieved. The alternative pathways for lymphocytes Th₂ transformation and some of the cytokines involved are shown.
Fig. 2. Antioxidant defence might block histamine (and other chemicals and molecules) released from mast cell. The production of ROS has been detected in cells stimulated with cytokines such as transforming growth factor-β (TGF-β), interleukins (IL-α), granulocyte macrophage colony stimulating factor (GM-CSF) and tumor necrosis factor-α (TNF-α) with peptide growth factors such as platelet derived growth factor (PDGF) and basic fibroblast growth factor (BFGF), with agonists of receptors such as angiotensin II (AGII) and lysophosphatidic acid (LPA), or with drugs as phorbol 12-myristate 13-acetate (PMA).

lungs, and gastrointestinal tract. They have a recognized pathophysiologic role as effector cells in immediate hypersensitivity reactions [24]. Such mast cells, when activated by either immunologic or non-immunologic stimuli, release and generate chemical mediators such as histamine, protaglandins, bradykinins, platelet activating factors (PAF) and leukotrienes which then act on surrounding tissues (Fig. 2).

Hydrogen peroxide stimulates histamine release. It is reduced by addition of catalase and flavonoids, showing a relationship and a balance among antioxidant enzymes, active oxygen and histamine release [26].

IFN-α and IFN-γ enhance monocyte-mediated activity. IFN-α directly activates blood monocyte superoxide anion release in allergic patients [27]. Superoxide generation correlates with the degree of bronchial hyperresponsiveness to inhaled histamine. These results hint that polymorphonuclear leukocytes (PMNs) in asthmatic children, especially in those with attacks, generate more
active oxygen species than those in control subjects, and that airway inflammation caused by O$_2^-$ may be closely related to bronchial hyperresponsiveness [28].

During phagocytosis, neutrophilic and eosinophilic granulocytes undergo metabolic activation and degranulation. This leads to accumulation of H$_2$O$_2$ in the extracellular environment in inflammation with release of histamine. This release reaction was inhibited by azide and catalase [29].

On the other hand, asthmatic patients show an increased expression of inducible nitric oxide synthase (iNOS) in airway epithelial cells and an increased level of NO in exhaled air. The NO derived from airway epithelial cells may be a mechanism for amplifying and perpetuating asthmatic inflammation, through inhibition of Th$_1$ cells and their production of IFN-γ. This would result in an increase in the number of Th$_2$ cells and the cytokines IL-4 and IL-5. Thus, it has been argued that the development of specific iNOS inhibitors may also represent a novel therapeutic approach for asthma and other allergic diseases [30].

Another issue is the molecular mechanisms of ROS in affecting these immuno and inflammatory cell activities. It can be driven by secondary cytokine release. More information on the effects at the transcriptional level, such as on NF-κB are needed [31]. This might be a clinically relevant pathway in the case of exposure to pollutants or respiratory tract infections, and it seems to occur in bronchial epithelial cells [32].

4. Free radical scavengers and blood detoxification

Neutrophil populations have the innate property of generating highly reactive oxygen free radical species such as O$_2^-$, HO$^-$ and H$_2$O$_2$, and they produce them during inflammatory reactions [33]. CAT, but not SOD, prevented neutrophil activation measured as (1) induction of cytotoxic responses, (2) increase of neutrophil adhesiveness to cell-free surfaces, and (3) inhibition of chemotactic responses to N-formyl-methionyl-leucyl-phenylalanine (FMLP). These findings suggest that H$_2$O$_2$ may play a major role in neutrophil activation [34].

When neutrophils were blocked from direct attachment to endothelial cells, endothelial cell damage was ameliorated. SOD partially inhibited this endothelial cell injury [35]. The respiratory burst is a coordinated series of metabolic events that take place when PMNs and other phagocytes are exposed to an appropriate stimulus such as phorbol 12-myristate 13-acetate (PMA), the chemotactic peptide FMLP or leukotriene B4. In fact, stimulation of PMNs rapidly increases oxygen consumption and produces superoxide [36].

Ig-stimulated eosinophils can also induce damage to respiratory epithelium. Granule basic proteins and reactive oxygen radicals may be responsible for the
injury. Peripheral blood eosinophils and neutrophils obtained from allergic patients, and stimulated with PMA, displayed a significantly higher superoxide generation [37]. CAT inhibited partially the appearance of irregularities on the surface of the epithelium. Moreover, the addition of either CAT or SOD reduced partly the activity of PMA-stimulated eosinophils [38].

GPX also has a protective role against oxidative stress and a regulatory function in arachidonic acid metabolism, which leads to prostaglandins synthesis [39]. Reduced GPX activity contributes to an allergic inflammation and it is associated with intolerance to some food and aspirin (Table 1). It seems likely that the reduced activity of this enzyme in platelets and blood may reflect mechanisms associated with the pathogenesis and severity of allergic disorders [47]. The lowest levels of GPX activity were found during the acute attack, which were significantly less than the control, even in patients with mild symptomatology [48].

SOD is constitutively expressed in leukocytes and other tissues. Significant induction of SOD activity occurs in peripheral blood leukocytes incubated in vitro with paraquat, an agent known to cause intracellular superoxide production [49]. Cu,Zn-SOD activities in platelets from the asthmatics were significantly higher than those from normal healthy subjects. Platelet Cu,Zn-SOD activities were significantly higher in atopic than in non-atopic asthmatics [50]. These results suggest that an oxidant-to-antioxidant imbalance may play a role in the inflammatory situations in the airways of asthmatics.

In fact, an imbalance between oxidants and antioxidants is proposed in

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<sup>a</sup> EPO: Eosinophil peroxidase.

<sup>b</sup> NSAID: non-steroidal anti-inflammatory drugs.

<sup>c</sup> NOS: nitric oxide synthase.
smokers and in patients with airways diseases. This hypothesis has been tested by measuring the Trolox equivalent antioxidant capacity (TEAC) of plasma and the concentrations of lipid peroxidation products as indices of overall oxidative stress. The plasma TEAC was markedly reduced, with increased levels of lipid peroxidation products in healthy chronic smokers as compared with healthy nonsmokers. Plasma TEAC was also low in patients presenting with acute exacerbations of chronic obstructive pulmonary disease (COPD) or asthma [51].

IgE-mediated systemic anaphylaxis can also follow parenteral SOD administration [52]. In addition, olive pollen extracts have a heterogeneous composition, with several important allergens, one of which showed a high degree of homology with a SOD. Otherwise, characterization and identification of latex allergens show that some of the IgE-reactive protein spots have high homology with SOD (Table 1).

Cytokines are implicated in allergic diseases and can modulate effector functions of eosinophils stimulated by another agonist, inducing eosinophil degranulation and superoxide production in vitro. Therefore these cytokines may be important in the release of toxic granule proteins from eosinophils in allergic diseases [53].

Indeed, asthma is characterized by an accumulation of activated eosinophils in the airway. Bronchial epithelial cells have been shown to release cytokines including granulocyte-macrophage colony-stimulating factor (GM-CSF). Eosinophil peroxidase (EPO) stimulates epithelial cells to release GM-CSF and forms a self-stimulatory cycle [54]. On the other hand, in bronchial asthma, inflammatory cells, infiltrating the airway mucosa, release oxygen radicals that cause tissue damage and amplify the airway inflammation. Therefore, antioxidant enzymes may have a protective effect on the airways, and a beneficial effect on bronchial asthma [43]. In addition, oxidative stress can be measured rather easily and non invasively by hydrogen peroxide, pentane, CO and NO in exhaled air [55–59].

5. Antioxidant enzymes also against cytotoxicity?

Cell-mediated cytotoxicity is a kind of immune response mainly due to T and NK cells. Examples of such reactions are the T cell infiltration of tumor beds and the T cell infiltration of blood vessels and alveoli [60]. Oxygen radicals have been detected in a variety of stimulated blood cells (Fig. 2) [61]. For instance, during various biological processes as inflammation or septic shock, free radical damages are not only caused by a direct generation of oxygen radicals by phagocytes, but also by a TNF-α-mediated generation in target cells. The oxidative effect of TNF-α is beneficial in physiological conditions as it can destroy cancerous or virus infested cells. But this effect can be deleterious in a
situation of deficiency in some antioxidants. TNF-α-induced free radicals can increase the replication of virus as HIV-1 and destroy immunocompetent cells such as T cells. This last action explains the defect in cellular immunity observed in oxidative stress and the immunostimulatory effect of many antioxidants. In this event, catalase has the better protecting effect whereas Cu,Zn-SOD has little effect [62]. So, antioxidants have been demonstrated as protective against TNF-α cytotoxicity.

On the other hand, addition of IFN-α or IFN-γ enhanced the monocyte-mediated cytotoxicity of a colon carcinoma cell line. However, inhibitors of H₂O₂-myeloperoxidase system suppressed both IFN-α- and IFN-γ-induced cytotoxicity of these cells [63].

6. Summary and prospects

There is considerable interest in the therapeutic use of antioxidants. This may involve the use of naturally occurring antioxidants or completely synthetic molecules. In addition, there is evidence that some drugs already used clinically may exert part or all their effect by antioxidant mechanisms. Infusions of SOD in liposomes (usually with catalase) have been reported to protect animals against O₂ toxicity. Cu,Zn-SOD has an anti-inflammatory effect in animal models of acute inflammation, in part because it can decrease the number of neutrophils entering sites of inflammation. A wide variety of Cu,Zn-SOD conjugates are available, including polyethylene glycol (PEG)-SOD, Ficoll-SOD, lecithinized SOD, polyamine conjugated SOD, cationized SOD, genetically engineered SOD polymers, pyran-SOD and albumin-SOD complexes. All have longer circulating half-lives than the unconjugated SOD molecules [25]. In addition, several low-molecular-mass compounds that react with superoxide anion have been described. Most contain transition-metal ions. Examples include iron porphyrins, a complex of manganese ions with the chelatins agents desferrioxamine and copper ions chelated to amino acids or to anti-inflammatory drugs [25,64].

It is well established that immunotherapy can prevent or reduce allergy both increasing IFN-γ production and decreasing IgE production. Nevertheless, sometimes immunotherapy is not the best indicated treatment. Drugs, traditionally used against clinical manifestations of allergy, include coumarins, glucocorticoids, anti-leukotrienes and anti-histaminics (Fig. 3).

We know today that immune cell response to allergens induces an accumulation of ROS that brings about mast cell in order to release histamine, the main chemical mediator of inflammation in allergy. Additionally, active oxygen becomes toxic when an imbalance arises between antioxidant enzymes and free radical production during an allergic reaction. Consequently, could the in-
vestigation in antioxidant enzymes open a new and complementary pathway in the treatment of allergic diseases?

We believe that the established connections between antioxidant enzymes and several allergic diseases (Table 1) may be a sign of their functional importance in the mechanism of the immunopathologic reaction named allergy. In fact, oxidative stress and antioxidant enzymes have been widely studied in many other pathologies and they have also been determined separately as an indicator of oxidative damage in many patients suffering from an allergic reaction. For instance, unsaturated lipid components of membrane are essential for a correct immune function, and represent one of the main targets of free radical damage. However, a complete study in this field has not been described to date for allergic patients.

This work provides further clues to the interaction between oxidative stress and allergic diseases. Therefore, in spite of considering potential negative consequences of antioxidant therapy, efforts are necessary to fully understand the relevance of ROS and free radical scavengers in the immune system and
allergy: without any doubt one of the most important human diseases at the beginning of this new century.

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