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COMPREHENSIVE INVITED REVIEW

Chronic Inflammatory Disorders and Their Redox Control: From Molecular Mechanisms to Therapeutic Opportunities

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Abstract

A chronic inflammatory disease is a condition characterized by persistent inflammation. A number of human pathologies fall into this category, and a great deal of research has been conducted to learn more about their characteristics and underlying mechanisms. In many cases, a genetic component has been identified, but also external factors like food, smoke, or environmental pollutants can significantly contribute to worsen their symptoms. Accumulated evidence clearly shows that chronic inflammatory diseases are subjected to a redox control. Here, we shall review the identity, source, regulation, and biological activity of redox molecules, to put in a better perspective their key-role in cancer, diabetes, cardiovascular diseases, atherosclerosis, chronic obstructive pulmonary diseases, and inflammatory bowel diseases. In addition, the impact of redox species on autoimmune disorders (rheumatoid arthritis, systemic lupus erythematosus, psoriasis, and celiac disease) and neurodegenerative diseases (Alzheimer's disease, Parkinson's disease, amyotrophic lateral sclerosis, and multiple sclerosis) will be discussed, along with their potential therapeutic implications as novel drugs to combat chronic inflammatory disorders. *Antioxid. Redox Signal.* 15, 2605–2641.

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I. Chronic Inflammatory Disorders: What They Are and What They Do

A CHRONIC INFLAMMATORY DISEASE is a condition characterized by persistent inflammation. A number of human pathologies fall into this category, and a great deal of research has been conducted to learn more about their characteristics and underlying mechanisms. In many cases, a genetic component has been identified that can put people at risk of developing a particular chronic inflammatory disease, and sometimes multiple genes can be involved. Patients develop a chronic inflammatory disease because the immune system has an inappropriate response or an over-response to something it has been exposed to. In some cases, this means that the patient develops an autoimmune disease, in which the immune system starts to attack the body because it has become confused. In other instances, the patient experiences chronic inflammation in response to certain foods or environmental factors such as smoke. The inflammation can wax and wane, but it remains persistent and it often resists treatment. Some examples of chronic inflammatory diseases are atherosclerosis, chronic obstructive pulmonary diseases, irritable bowel diseases, celiac disease, vasculitis, arthritis, lupus, and psoriasis. Some of these conditions have a clear genetic component that can be used to identify patients with congenital cases, and in other instances certain genes may increase the risk of developing the disease. In yet other cases, the onset is apparently random, or is brought on by lifestyle choices made by the patient.

II. Redox Molecules and Their Sources

A. Reactive oxygen species

Oxygen is the life-driving molecule without which all higher eukaryotic organisms could not survive; yet, at the same time, it is partially toxic and a proven harmful cause of cellular deterioration. This is known as the paradox of aerobic life, better termed as the "oxygen paradox" (99). This dark side of oxygen relates directly to the fact that atomic oxygen is a free radical and molecular oxygen is a (free) biradical. Although concerted tetravalent reduction of oxygen by the mitochondrial electron transport chain is a relatively safe process, its univalent reduction (depicted in Fig. 1) leads to the formation of chemically more reactive species, known as reactive oxygen species (ROS). These include molecules that contain oxygen-centered free radicals derived from molecular oxygen (O_2), such as the superoxide radical anion ($^{\bullet}O_2^-$), the hydroxyl radical ($HO^{\bullet}$), and peroxyl radicals ($ROO^{\bullet}$), as well as nonradical derivatives of O_2 like hydrogen peroxide (H_2O_2), hypochlorous acid (HOCl), and peroxynitrite ($ONOO^-$) (98) (Fig. 2). Free radicals can be defined as molecules or molecular fragments containing one or more unpaired electrons in atomic or molecular orbital (98) and such unpaired electrons usually give a considerable degree of reactivity to the compound. The principal mechanism for ROS formation starts with reduction of O_2 by the addition of one electron, which generates $^{\bullet}O_2^-$. The latter species is considered the primary ROS, and its role in redox regulation has been extensively investigated (66). $^{\bullet}O_2^-$ can further interact with other molecules to generate secondary ROS, either directly or through enzyme- or metal-catalyzed reactions (66). Indeed, further reduction of $^{\bullet}O_2^-$ leads to the formation of H_2O_2, which in turn can be converted into the highly reactive $HO^{\bullet}$ (44) (Fig. 3). The fate of most $^{\bullet}O_2^-$ is thus dismutation to H_2O_2 by both enzymatic and nonenzymatic mechanisms. The $^{\bullet}O_2^-$ that escapes degradation by the cellular antioxidant system is not by itself particularly reactive with most biomolecules, and reacts mainly with nitric oxide ($NO^{\bullet}$) and transition metals, such as iron found in iron/sulfur center-containing proteins. Among iron-containing proteins that react with $^{\bullet}O_2^-$ are aconitase (77), guanylyl cyclase (35), and ribonucleotide reductase (49). Thus, the intracellular concentration of $^{\bullet}O_2^-$ and its ability to act as an intracellular signal mainly depends on its reaction with two protons to form H_2O_2, a reaction catalyzed by superoxide dismutase (SOD). Additionally, undegradated $^{\bullet}O_2^-$ may react with $NO^{\bullet}$ to generate $ONOO^-$, may participate in Fenton chemistry through Haber-Weiss reaction (Fig. 3), or may become quickly protonated to the hydroperoxyl radical ($HO_2^{\bullet}$) (262). The latter species may be relevant to *in vivo* lipid peroxidation, which can be initiated by $HO_2^{\bullet}$ through two different pathways: one that is lipid peroxides-independent, and the other one that is lipid peroxides-dependent (3). H_2O_2 is the most studied and best characterized member of ROS, and can be regarded as the most important ROS in redox signaling. The role of these ROS in signal transduction and cell functions will be discussed in the following sections.

Apparently, there is no single source of ROS and this could be due to their site- or function-specific involvement. Both exogenous and endogenous sources contribute to intracellular

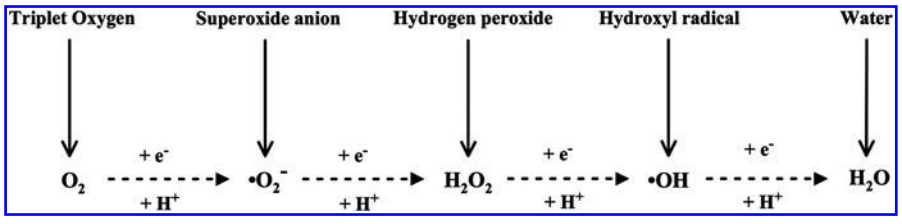

FIG. 1. Univalent reduction of oxygen. This is a chain of reactions that involve oxygen-reducing steps, where oxygen radicals are produced because oxygen accepts only one electron at a time.

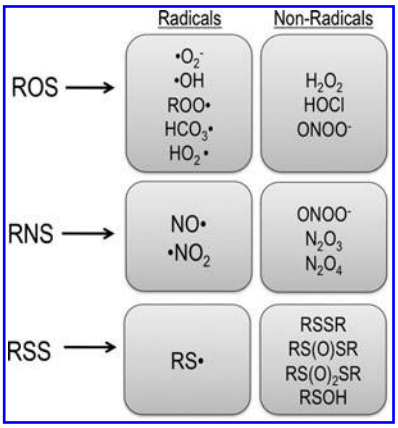

FIG. 2. Reactive oxygen species (ROS), reactive nitrogen species (RNS), and reactive sulfur species (RSS). ROS include the following radical and nonradical species: $^{\bullet}O_2^-$, superoxide radical anion; $^{\bullet}OH$, hydroxyl radical; $ROO^{\bullet}$, peroxyl radical; HCO3$^{\bullet}$, carbonate radical; $HO_2^{\bullet}$, hydroperoxyl radical; H_2O_2, hydrogen peroxide; HOCl, hypochlorous acid; $ONOO^-$, peroxynitrite. RNS include the following radical and nonradical species: $^{\bullet}NO$, nitric oxide; $^{\bullet}NO_2$, nitrogen dioxide; $ONOO^-$, peroxynitrite; N_2O_3, dinitrogen trioxide; N_2O_4, dinitrogen tetraoxide. RSS include the following radical and nonradical species: RS$^{\bullet}$, thiyl radical; RSSR, disulfide; RS(O)SR, disulfide-*S*-monoxide; RS(O)2SR, disulfide-*S*-dioxide; RSOH, sulfenic acid.

ROS formation. Exogenous sources include irradiation (UV, x-ray, and γ-ray), atmospheric pollutants, and chemicals (267). Endogenously, ROS originate mainly from mitochondria, when $^{\bullet}O_2^-$ is formed by electrons leaking between complexes I and III of the electron-transport chain (209). Thus, mitochondrial $^{\bullet}O_2^-$ production accounts for most of its total amount, although several other cellular sources of $^{\bullet}O_2^-$ exist. In this context, phagocytic cells (neutrophils, macrophages, and dendritic cells) deserve a particular attention, because they express NADPH oxidases (Nox) on their plasma membranes. These enzymes use NADPH to reduce O_2, thus generating large amounts of $^{\bullet}O_2^-$ on the membrane surface, that acts as a toxic agent during the engulfment of microbes (109). Recently, a number of Nox isoforms (the so-called Nox family) have been discovered also in nonphagocytic cells, which release $^{\bullet}O_2^-$ into the extracellular space in response to various growth factors and cytokines (145). Other proteins that generate ROS as by-products of their normal function are listed in Table 1. In addition, a number of biomolecules such as glyceraldehydes, FMN_2, $FADH_2$, and certain hormones and neurotransmitters can undergo auto-oxidation in the presence of O_2, thus generating $^{\bullet}O_2^-$ (98).

B. Reactive nitrogen species

The main reactive nitrogen species (RNS) is nitric oxide ($NO^{\bullet}$), which has a central role in cell signaling (117). Additionally, $NO^{\bullet}$ metabolism and reactivity lead to the formation of many other RNS, primarily peroxynitrite ($ONOO^-$), but also nitrogen dioxide ($^{\bullet}NO_2$), dinitrogen trioxide (N_2O_3), and dinitrogen tetroxide (N_2O_4) (248) (Fig. 2). In mammals, $NO^{\bullet}$ is mostly generated by a family of specific $NO^{\bullet}$ synthase (NOS) isozymes, which oxidize the terminal guanido-nitrogen atoms of L-arginine, yielding $NO^{\bullet}$ *via* a five electron oxidative reaction (198) (Fig. 3). There are three main NOS forms, named after the tissues or situations where they were discovered: endothelial (eNOS) (144), neuronal (nNOS) (32), and inducible (iNOS) (288) isozymes. However, $NO^{\bullet}$ can be produced also by other redox enzymes, such as molybdenum-based xanthine oxidoreductase (otherwise known as xanthine oxidase) or nonenzymatically by guanidine-substitute L-arginine analogs in the presence of NADPH (183, 293). In solution, auto-oxidation of $NO^{\bullet}$ results in the formation of nitrite (NO_2^-), which may proceed through highly nitrosating intermediates such as N_2O_3 or N_2O_4; however, a small but important amount reacts with $^{\bullet}O_2^-$ to form $ONOO^-$, which is by itself strongly oxidizing, and when protonated undergoes homolytic scission to produce $^{\bullet}OH$ and $^{\bullet}NO_2$. Moreover, $ONOO^-$ reacts avidly with carbon dioxide (CO_2) to form $^{\bullet}NO_2$ and the carbonate radical, $HCO_3^{\bullet}$ (65, 70). Through its own activity and that of its byproducts, $ONOO^-$ engages in lipid and protein oxidation and nitration, enabling signal transduction and subsequent changes in cellular functioning (91). $NO^{\bullet}$ is the most studied nitrogen radical, since it acts as an important signaling molecule in a large variety of pathophysiologic processes. Due to its unique properties, in 1992 $NO^{\bullet}$ was acclaimed as the "molecule of the year" (56).

C. Biomolecule oxidation products

Polyunsaturated fatty acids (PUFAs) contained in phospholipids of either cell membranes or organelles can be enzymatically or nonenzymatically oxidized *in vivo*, and can trigger a cascade of oxidative reactions that leads to the formation of several secondary breakdown products (Fig. 3). These lipid peroxides not only disrupt the integrity of biological membranes, but may also act as redox signals. The enzymatic oxidation and peroxidation of PUFA occur *via* lipoxygenases and cyclooxygenases, and when followed by dehydrogenase metabolism can yield α,β-unsaturated carbonyl-containing electrophiles (42). Moreover, cholesterol can be oxidized by cytochrome P450 to yield specific hydroxycholesterols (42, 238). ROS- or RNS-mediated oxidation of PUFAs is initiated by molecules such as $HO^{\bullet}$, $HO_2^{\bullet}$, $NO^{\bullet}$, and $^{\bullet}NO_2$ (7, 58). A single initiation event can start a chain reaction that leads to the formation of numerous oxidatively damaged lipids. The *in vivo* decomposition of these lipid peroxides generates a complex mixture of electrophilic products, including epoxides, saturated and unsaturated aldehydes, cyclopentenones, and nitro-fatty acids (NO_2-FAs) (Fig. 3) (7, 246). In brief, fatty acid-derived electrophiles can be divided into two groups: α,β-unsaturated carbonyls and NO_2-FAs. α,β-Unsaturated carbonyls include the reactive aldehydes 4-hydroxy-2-nonenal (HNE), 4-oxononenal (4-ONE), and acrolein (2-propenal), MDA, as well as ω-3 and ω-6 fatty acid derivatives like the cyclopentenone prostaglandins and oxo-eicosatetraenoic acids (oxo-ETEs) (225). NO_2-FAs are mainly produced *in vivo* in response to pathologic stress (226).

These reactive elecrophiles were first recognized for their cytotoxicity since they may alkylate DNA, cause large-scale or irreversible protein disruption, and engage in cell signaling through Micheal addition reaction; the latter occurs with cellular nucleophiles, including cysteine thiols, the imidazole of histidine, and the ε-amino groups of lysine (118). Recently, it has become clearer that elecrophiles at sub-toxic concentrations induce protective or adaptive responses to stress

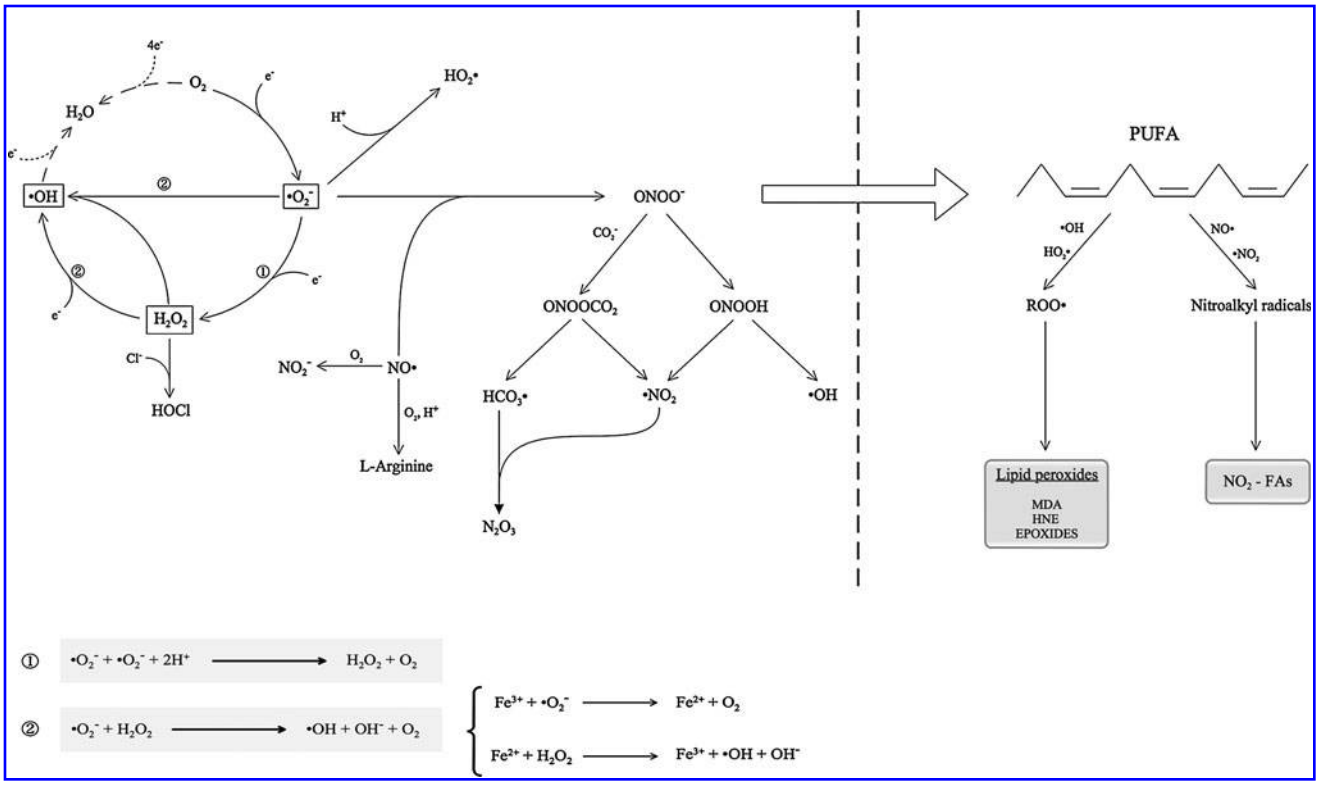

FIG. 3. Interconnections between reactive species and their products. The main pathway of ROS formation starts with the reduction of O_2, which generates $^{\bullet}O_2^-$. This is further reduced to H_2O_2, which is then converted into the highly reactive $^{\bullet}OH$. Further, $^{\bullet}O_2^-$ can be either converted into $ROO^{\bullet}$ or can react with $NO^{\bullet}$ to yield $ONOO^-$. H_2O_2 can be converted to HOCl by the action of myeloperoxidase. The process by which $^{\bullet}O_2^-$ is reduced to $^{\bullet}OH$ is known as Haber-Weiss reaction, which is very slow but can be accelerated by iron *via* the Fenton reaction. O_2, molecular oxygen; H_2O, water; $^{\bullet}O_2^-$, superoxide radical anion; $^{\bullet}OH$, hydroxyl radical; $ROO^{\bullet}$, peroxyl radical; H_2O_2, hydrogen peroxide; $ONOO^-$, peroxynitrite; HOCl, hypochlorous acid; e^-, electron; Fe^{3+}, ferric ion; Fe^{2+}, ferrous ion. $NO^{\bullet}$ is mostly generated by L-arginine. Even if most of $NO^{\bullet}$ ends up as stable products like NO_2^- or N_2O_3, a small fraction reacts with $^{\bullet}O_2^-$ to form $ONOO^-$. Depending on the surrounding melieu, $ONOO^-$ can yield $^{\bullet}NO_2$, HCO3$^{\bullet}$ or $^{\bullet}OH$. $NO^{\bullet}$, nitric oxide; NO_2^-, nitrite; N_2O_3, dinitrogen trioxide; $^{\bullet}O_2^-$, superoxide radical anion; $ONOO^-$, peroxinitrite; $^{\bullet}NO_2$, nitrogen dioxide; $HCO_3^{\bullet}$, carbonate radical; $^{\bullet}OH$, hydroxyl radical. The oxidation of PUFAs and the subsequent hydrogen abstraction lead to the formation of an unstable carbon radical, which undergoes molecular rearrangements. This carbon-centered radical is oxidized again, starting a chain reaction. Each step generates several lipid peroxidation products. PUFA, polyunsaturated fatty acids; HNE, 4-hydroxy-2-nonenal; NO_2-FAS, nitro-fatty acids.

(200). Indeed, both NO_2-FAs and their protein or GSH adducts are detected clinically in healthy individuals, become elevated postprandially, and are formed by oxidative inflammatory reactions (15, 156). Also, mitochondria are both a source and a target of electrophiles, since their ROS and RNS, together with the peroxidase activity of cytochrome c, lead to the formation of electrophilic lipid species from PUFAs. Proximal targets of mitochondrial electrophiles include electron transport chain components (complexes I, III, and IV), membrane transport proteins and ion channels, as well as

Table 1. Relevant Reactive Oxygen Species–Producing Enzymes

Enzyme	*E.C.*	*Catalyzed reaction*
Lip oxygenase	1.13.11.12	FA + O_2 → FA hydroperoxide
Xantine oxidase	1.17.3.2	Xantine + O_2 → Uric acid + H_2O_2
Nitric oxide synthase	1.14.13.39	L-Arginine + O_2 → L-citrulline + NO + H_2O
Peroxidase	1.11.1.7	Donor + H_2O_2 → oxidized donor + $2H_2O$
Monoamine oxidase	1.4.3.4	Monoamine + H_2O → aldehyde + NH_3
Cytochrome P-450	1.14.14.1	RH + O_2 + $2H^+$ + 2 e^- → ROH + H_2O
Cellobiose oxidoreductase	1.1.99.18	Cellobiose → cellobiono-1,5-lactone
Nitropropane dioxygenase	1.13.11.32	Nitropropane + O_2 → acetone + nitrite
Indoleamine 2,3-dioxygenase	1.13.11.11	L-Tryprophan + O_2 → *N*-formylkynureine
Tryptophan dioxygenase	1.13.99.3	L-Tryptophan + O_2 → glycolaldehyde + CO_2 + NH_3
Galactose oxidase	1.1.3.9	D-Galactose + O_2 → D-galacto-hexodialdose + H_2O_2
Aldehyde oxidase	1.2.3.1	Aldehyde + H_2O + O_2 → carboxylic acid + H_2O_2

FA, fatty acid; O_2, molecular oxygen; H_2O_2, hydrogen peroxide; L-arg, L-arginine; NO, nitric oxide; H_2O, water; NH_3, ammonia.

matrix metabolic enzymes such as α-ketoglutarate dehydrogenase (139).

D. Reactive sulfur species

ROS are known to be inactivated by reaction with cellular thiols to form disulfides (48), which in turn act as mild oxidizing agents. However, naturally occurring molecules that contain sulfur in higher oxidation states, such as disulfide-*S*-oxides, sulfenic acids, and thiyl radicals (termed reactive sulfur species, RSS), are unable to partake in spontaneous redox transformations under physiologic conditions (Fig. 2) (83). Instead, they modulate the redox state of biological thiols and disulfides, acting further as aggressive oxidizing agents. Under oxidative stress, local cellular concentrations of peroxides and $^{\bullet}O_2^-$ that are sufficient to form $ONOO^-$ are also high enough to form RSS (208). In addition, RSS can be formed by oxidation of glutathione (GSH) or glutathione disulfide (GSSG) in the presence of a variety of ROS, including peroxides (73), *S*-nitrosoglutathione (48), and $ONOO^-$ (29). The metabolic fate of RSS is currently unknown and their specificity for thiols makes it unlikely that RSS can be sequestered by electron-donating agents such as ascorbate. On the other hand, the oxidizing power of disulfide-*S*-oxides has important biochemical implications. Although glutathiolation itself is not necessarily noxious to cells, it is expected that proteins (particularly the redox-sensitive ones) will be inhibited by the disulfide-*S*-oxides (83). In addition, oxidation of cellular thiols not only inhibits a number of redox proteins and enzymes, but also consumes GSH and hence tilts the cellular redox balance toward disulfide stress. Further, a hypothetical role for the thiyl radicals and sulfenic acids in protein glutathiolation has been postulated (264), but at present little is known about their *in vitro* and *in vivo* relevance. As a consequence, RSS have to be considered as oxidative stressors with their own particular targets and redox transformation pathways within the cell.

III. Redox Homeostasis: Naturally Occurring and Nutritional Antioxidants

The human body is not unprepared for the formation of reactive species. Cells are equipped with enzymatic and nonenzymatic antioxidant systems to eliminate ROS, lipoperoxides, RNS, and/or RSS and maintain redox homeostasis. Halliwell and Gutteridge have defined antioxidants as substances that are able, at relatively low concentrations, to compete with other oxidizable substrates and, thus, to significantly delay or inhibit their oxidation (99). This definition includes naturally occurring antioxidants of high or low molecular weight, as well as nutritional antioxidants, whose action is strictly linked to their bioavailability. These are discussed in more detail in this section and are schematized in Table 2.

A. Naturally occurring antioxidants

The major endogenous enzymatic antioxidant is superoxide dismutase (SOD, EC 1.15.1.1), which catalyzes the dismutation of $^{\bullet}O_2^-$ to H_2O_2 and O_2. Multiple isoforms of SOD exist in different cellular compartments: SOD-1 (Cu/Zn-SOD) is the major superoxide scavenger found in the cytoplasm, mitochondrial intermembrane space, nucleus, and lysosomes, whereas SOD-2 (Mn-SOD) and SOD-3 (Cu/Zn-SOD) are found in the mitochondria and extracellular matrix, respectively (202). The H_2O_2 is then further rapidly converted to H_2O and O_2 through the action of catalase (EC 1.11.1.6), a heme-containing enzyme that is usually localized within peroxisomes (46). Also, glutathione peroxidase (GPx, EC 1.11.1.9) deals with H_2O_2, but it requires GSH as a reducing agent, which is then oxidized to its GSSG disulfide in the process (151). Some GPx isozymes also reduce lipid

Table 2. Antioxidant Defense Systems Against Free Radical Species

Antioxidant	*Action*	*Localization*
Naturally occurring		
Mn-superoxide dismutase	Removes $^{\bullet}O_2$	Mitochondria
Cu,Zn-superoxide dismutase	Removes $^{\bullet}O_2$	Cytosol
Catalase	Dismutates H_2O_2	Peroxisome
Glutathione peroxidase	Reduces H_2O_2	Cytosol
Glutathione reductase	Reduces glutathione	Cytosol
Peroxiredoxin	Reduces H_2O_2, $ONOO^-$, hydroperoxides	Cytosol, plasma membrane
Transferrin	Sequestration of transition metals	Plasma
Ferritin	Sequestration of transition metals	Cytosol, plasma
Lactoferrin	Sequestration of transition metals	Milk
Caeruloplasmin	Sequestration of transition metals	Plasma
Glutathione	Radical scavenger	
Uric acid	Radical scavenger	Ubiquitous
Pyruvate	Radical scavenger	
Amino acids/Peptides	Radical scavenger	
Nutritional		
Ascorbic acid	Radical scavenger	
Tochopherols	Radical scavenger	
Carotenoids	Radical scavenger	Diet
Quinones	Radical scavenger	
Flavonoids/polyphenols	Radical scavenger, sequestration of metals	

Overall list of naturally occurring and nutritional antioxidants that work together to minimize free radical cytotoxicity. See text for details.
$^{\bullet}O_2^-$, superoxide radical anion.

hydroperoxides and $ONOO^-$ to the corresponding alcohols. Although GPx may be less "convenient," since it consumes the cellular reductant GSH, nonetheless, dependence on GSH allows GPx to fulfill an important role as a redox sensor for GSH levels. GSSG is then rapidly reduced within cells by glutathione reductase (GR, EC 1.8.1.7), a flavin-enzyme that consumes NADPH and reduces disulfides to thiols (119).

Peroxiredoxins (EC 1.11.1.15) catalyze the reduction of H_2O_2, but also of alkyl hydroperoxides and $ONOO^-$ to water and alcohol, at the expenses of cellular thiols, mostly thioredoxin (TRX) but also GSH (284). Oxidated TRX is then reduced by thioredoxin reductase (TR, EC 1.8.1.9), which uses NADPH as an electron donor (8).

High-molecular-weight proteins, such as albumin, ceruloplasmin, transferrin, ferritin, and haptoglobin, which are all present in plasma, bind to redox active metals and limit the production of metal-catalyzed free radicals (97).

The most prominent examples of low-molecular-weight endogenous antioxidants are GSH, uric acid, metallothionein, pyruvate, as well as several peptides and free amino acids (45). Although GSH is a cofactor of GPx, this peptide also exerts antioxidant activity *per se*, and is able to regenerate other antioxidants such as vitamins C and E (186). Due to its high concentration and its central role in maintaining the cell's redox state, GSH is one of the most important cellular antioxidants. Uric acid is formed during purine metabolism, and it also possesses strong antioxidant activity toward ROS in aqueous phase (5). Metallothioneins have the capacity to bind both physiologic and xenobiotic heavy metals through the thiol group of its cysteine residues (25). In particular, metallothioneins are the most abundant intracellular Zn^{2+}-storage proteins, and they likely participate in the uptake, transport, and regulation of this metal in biological systems, thus functioning as intracellular redox sensors. Their activity is based on the reversible dissociation of zinc ions under nonoxidative and oxidative conditions; in the latter event, the sulfur ligands of zinc are oxidized upon capture of oxidant radicals like the superoxide and hydroxyl radicals (173). Further, by binding and releasing zinc ions, metallothioneins may regulate its level within the body, which in turn is a key element for the functional activity of zinc-fingers. The latter structures also act as redox-sensitive molecular switches that control several crucial cellular responses (141). Also, pyruvate has been reported to have antioxidant properties, and is able to scavenge free radicals, especially H_2O_2 and $^{\bullet}OH$, and to prevent radical-mediated tissue injury through anti-inflammatory processes (270). Finally, amino acids are compounds that have a relatively low antioxidant activity on a molar basis, but when present at high concentrations, they can contribute significantly to the overall ROS scavenging activitiy.

B. Nutritional antioxidants

These compounds can be divided into lipid-soluble antioxidants (tocopherols, carotenoids, quinones, and some polyphenols) and water-soluble antioxidants (ascorbic acid and some other polyphenols). They delay or inhibit cellular damage mainly by scavenging free radicals. Among the lipid-soluble, low-molecular-weight antioxidants, the most important group is that of tocopherols, a prominent member of which is α-tocopherol (α-TOH), or vitamin E (205). α-TOH is a highly effective antioxidant within membrane bilayers, where it is capable of breaking chain reactions by scavenging peroxyl radicals (37). Then, the tocoperoxyl radical ($TO^{\bullet}$) that is generated in these reactions can be stabilized, either by donating a second electron to form a quinone derivative, or by being recycled back to α-TOH by vitamin C. A second large family of ROS quenchers is that of carotenoids, which are photosynthetic pigments synthesized by plants that include several hydrocarbon molecules such as β-carotene and licopene. These compounds are able to trap singlet oxygen ($^1O_2$) very efficiently. However, the antioxidative function of carotenoids in mammals is still unclear (206), and so far clinical trials have led to conflicting results. β-Carotene is composed of two retinyl groups, and is broken down in the mucosa of the human small intestine by β-carotene-15,15′-monooxygenase to release retinal, a form of vitamin A. Further, β-carotene can be stored in the liver and body fat, and then can be converted to retinal as needed, thus representing a storage form of vitamin A for humans. Also, α-carotene and γ-carotene have some vitamin A activity due to their retinyl group (though less relevant than that of β-carotene), and so does the xanthophyll carotenoid β-cryptoxanthin (217). All other carotenoids, including lycopene, have no β-ring, and thus they do not display any vitamin A activity; yet, they are physiologically relevant antioxidants, since they efficiently quench singlet oxygen (218). Ubiquinol, another effective lipid-soluble antioxidant, inhibits lipid peroxidation and, much alike vitamin C, can also regenerate the α-$TO^{\bullet}$ radical to form the active α-TOH (127). Vitamin C acts as a strong antioxidant in the plasma, where it exerts synergistic effects with other coantioxidant. During the interaction with free radicals, vitamin C (ascorbic acid) serves as a stable donor and is converted first into "semidehydroascorbate" and then into dehydroascorbate (197).

Both tocopherols and ubiquinol are secondary metabolites of phenol and polyphenol groups. Some polyphenols are lipid soluble, but most of them are water soluble. More than 8000 phenolic compounds are known to date (101), of which almost 2/3 belong to the flavonoid family. Polyphenols are the most abundant antioxidants in the diet, and their *in vivo* antioxidant activity is largely dependent on their bioavailability, that implies: (i) release from the food matrix into the gut; (ii) stability in the gut flora; (iii) modifications in the intestine (glycosylation); (iv) absorption through the intestinal wall to the blood stream; (v) stability in the liver; and (vi) accessibility to the tissue in the target site. Polyphenols, in addition to their ability to donate hydrogen atoms, and thus to act as chain-breaking antioxidants, can also chelate transition metal ions and hence inhibit free radical formation (273).

Interestingly, some RSS found in human diet can function also as antioxidants, and these substances include ergothionine, ovothiols, dialkylpolysulfides, allylisothiocyanate, and related isothiocyanates (38, 100, 122, 295). The chemistry and biomedical applications of many of these compounds have not yet been fully explored, and provide broad opportunities for future research.

IV. Redox Biology: From Physiologic to Pathologic Processes

A. ROS and RNS as mediators of physiologic functions

In the previous sections we have described how reactive species originate within living tissues and how their

concentration is determined by the balance between the rate of radical production and that of their clearance. However, a regulated production of free radicals plays important roles in numerous cell functions, and physiologic manifestations of redox regulation typically involve a temporary increase and/or a temporary shift of the intracellular oxidative/antioxidative ratio toward a more oxidated state. Among the important physiologic functions played by free radicals the following are included: defense against pathogens, apoptosis, regulation of vascular tone, enhancement or regulation of signal transduction, and even the oxidative stress responses that ensure the maintenance of redox homeostasis (74, 123, 162). The most important physiologic functions of ROS and RNS are summarized in Figure 4.

A large NADPH oxidase (Nox)-dependent production of $^{\bullet}O_2^-$ and its derivatives is referred to as "oxidative burst" and plays an important role as first line of defense against both pathogens and cancer cells (220). The activation of Nox is induced by microbial products (e.g., lipopolysaccharide or lipoproteins), or by cytokines like interferon-γ, interleukin-1β, or interleukin-8 (291). Further, Nox can interact with myeloperoxidase, and in phagocytes this leads to the formation of hypochlorous acid (HClO), one of the strongest physiologic oxidants and a powerful antimicrobial agent. The production of ROS occurs also by nonphagocytic Nox isoforms, and exerts a role in the regulation of intracellular signaling in various cell types, including fibroblasts, endothelial cells, vascular smooth muscle cells, cadiac myocytes, and thyroid tissue (188). As part of the immune response human phagocytes generate also NO$^{\bullet}$ *via* iNOS, which is activated by interferon-γ (IFN-γ) as a single signal, or by tumor necrosis factor-α (TNF)-α in combination with a second signal. Conversely, transforming growth factor-β (TGF-β) leads to strong inhibition of iNOS, and interleukin-4 (IL-4) and IL-10 are weak inhibitors of this enzyme (27). Therefore, the immune system may regulate the armamentarium of phagocytes that play a role in inflammation and immune responses. NO$^{\bullet}$ that is secreted as an immune response is toxic to bacteria, because it damages DNA and degrades iron–sulfur centers into free iron ions and iron-nitrosyl compounds.

ROS can also cause programmed cell death by modulating the activity of several cell-death/survival mediators like caspases, FasL, cytochrome c, and Bcl-2. Caspases are highly redox-sensitive enzymes, and can undergo several oxidative modifications such as direct oxidation, glutathionylation, and *S*-nitrosylation, that attenuate their proteolytic activity, and hence inhibit apoptosis (299). Additionally, oxidative stress-induced expression of FasL is responsible for the stimulatation of the whole apoptotic apparatus, promoting mitochondrial permeability transition and ultimately the activation of caspase-9 through the formation of the apoptosome and the release of cytochrome c. The latter event is considered a hallmark of all mitochondrion-dependent apoptotic pathways and it plays a critical role in keeping ROS generation at a low level that is ideal for cell survival (243). Conversely, Bcl-2 is antiapoptotic and a mild oxidative stress is known to promote an NF-kB-induced expression of this protein, thus promoting cell survival, whereas redox-mediated phosphorylation of JNK inhibits Blc-2 and allows apoptosis (116).

The overall regulation of vascular tone is mainly mediated by eNOS-derived NO$^{\bullet}$ produced by the endothelium under basal conditions, as well as upon stimulation by a variety of receptor agonists, and by the shear stress produced by blood flow. Moreover, blood vessels are innervated by nitrergic fibers that generate and release NO$^{\bullet}$, and that are functionally antagonistic to sympathetic nerves in that their stimulation produces vascular relaxation (153). Also, neuronal NO$^{\bullet}$ formed by nNOS in the CNS may contribute to the central regulation of blood pressure, by reducing the vascular sympathetic tone (266). In the periphery, most smooth muscle tissues also happen to be innervated by nitrergic fibers (36). These features make NO$^{\bullet}$ a neurotrasmitter-like molecule that not only is released from nitrergic nerves to mediate smooth muscle relaxation but is also associated with many different behaviors like learning and memory formation, feeding,

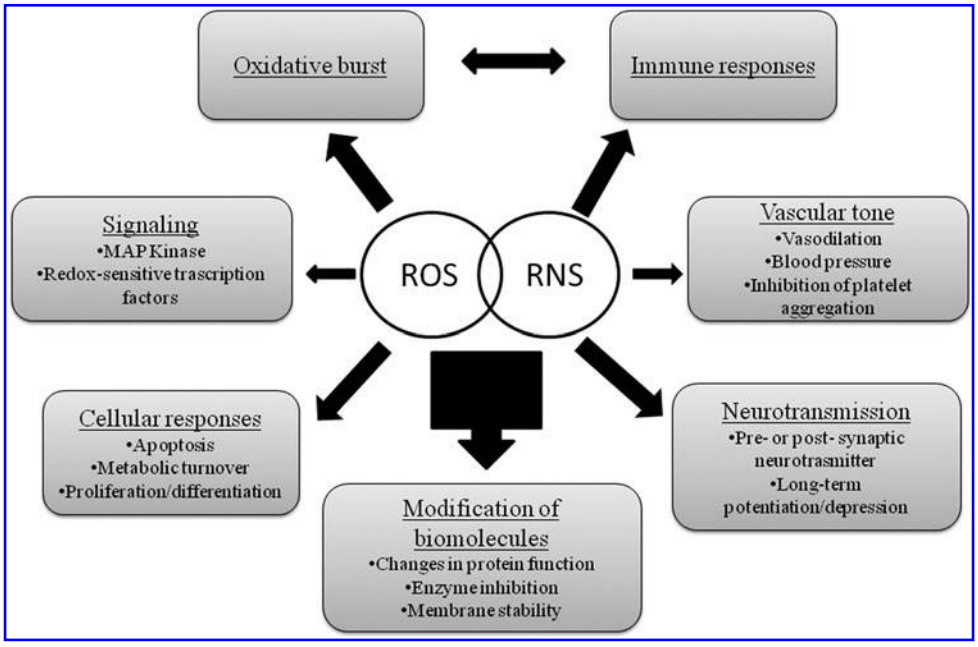

FIG. 4. Physiologic actions of ROS and RNS. Various functions exerted by ROS and RNS are summarized. MAP, mitogen-activated proteins.

sleeping, sensory and motor functions, as well as male and female reproductive events (78, 258).

B. Activators and molecular targets of ROS/RNS

Several growth factors, cytokines, and other ligands can trigger ROS and RNS production in nonphagocytic cells, by binding to their membrane receptors. Such a production is also responsible for a positive feedback on signal transduction triggered by the same receptors, for instance, due to a pro-oxidative shift of the thiol/disulfide redox state of the cell. To date, the role of ROS has been demonstrated for signal transduction triggered by nerve growth factor (NGF) (256), epidermal growth factor (EGF) (10), and platelet-derived growth factor (PDGF) (11). Once produced, these reactive species are able to trigger several signaling pathways (94).

The main mechanism of redox regulation of protein function is achieved through a direct oxidation at the level of sulfur-containing amino acids or at tyrosine (30, 69). The most common protein modifications include *S*-glutathiolation (82), disulfide formation (192), and *S*-nitrosylation (254). Trx, p53, IkB, Ras, Akt, and tyrosine phosphatase are among the proteins that have been found to be regulated through direct oxidation (13).

In particular, high H_2O_2 or strong pro-oxidative changes in the intracellular thiol/disulfide redox state determine increased tyrosine phosphorylation of several proteins, including Lck, Fyn, Syk, and ZAP70 (34, 106, 187, 232). Remarkably, at a dose of $H_2O_2 < 0.2$ m*M*, or after moderate sulfydryl oxidation, the increase in protein phosphorylation is restricted to one or a few prominent targets (187). Instead, concentrations of H_2O_2 around ~ 1 m*M* or relatively high sulfydryl oxidation cause the oxidative inhibition of protein tyrosine phosphatases (102, 230). Also, insulin receptor, whose activation requires the autophosphorylation of three distinct tyrosine residues, is regulated by H_2O_2 at a concentration of ~ 1 m*M*, as well as by pervanadate and thiol-reactive agents (57). Instead, concentrations of $H_2O_2 < 0.1$ m*M* are not enough to trigger the autophosphorylation of the insulin receptor in the absence of the ligand. Such a response to ROS occurs also by means of a pro-oxidative shift in the intracellular GSH redox state (231). Higher concentrations of H_2O_2, however, tend to inhibit rather than enhance insulin receptor singaling and autophosphorylation. Also, MAPK cascades, in particular those mediated by JNK, p38, ERK-1, and ERK-2, are strongly activated by ROS or by a mild oxidative shift of the intracellular thiol/disulfide redox balance (4). In this context, ERK-1 and ERK-2 were found to be activated by $^{\bullet}O_2^-$ but not by H_2O_2 (9). In addition, the serine/threonine protein kinase C (PKC) and other PKC isoforms can be activated by H_2O_2 in a phospholipid-independent process that involves tyrosine phosphorylation of the catalytic domain (87). ROS can also increase the intracellular content of cytosolic Ca^{2+} (63), thus determining the oxidative stress-mediated activation of PKC-α (147) and the transcriptional induction of the AP-1 proteins c-Fos and c-Jun (54). It has also been observed that c-Fos and c-Jun mRNAs are induced by relatively small amounts of H_2O_2, $^{\bullet}O_2^-$, or $NO^{\bullet}$, as well as by other oxidative stress inducers (125). The first eukaryotic transcription factor shown to respond directly to oxidative stress was NF-kB, which is inhibited by antioxidants. In various cell types, NF-kB is activated by μM concentrations of H_2O_2 (171), and also by a moderate pro-oxidative shift in the GSH redox state (76). At least two different mechanisms can mediate this effect: the first involves the ROS-mediated enhancement of IkB degradation (140), and the second involves the oxidative enhancement of upstream signal cascades (201). However, it is well established that oxidative stress can also negatively regulate NF-kB signaling, probably by promoting oxidation at the cysteine 62 in the DNA-binding region of the p50 subunit of this transcription factor, thus strengthening the notion that the redox state of cysteine residues greatly influence proteins' various properties (43). Also, gene expression of the cell cycle inhibitor p21 is enhanced by ROS, *via* a yet unknown mechanism that may involve p53 (59). This observation may be particularly relevant when considering the role of ROS-scavenging mechanisms in the proliferative phase of the cell life. In fact, the ability of ROS to regulate several signaling cascades makes them crucial players in the control of key cellular processes like survival, proliferation, and differentiation. This is rather apparent in the redox control of PI3K/Akt pathway, whereby a moderate level of ROS activates PI3K signaling and promotes cell survival, whereas sustained oxidative stress may inhibit it (132). p38 MAPK, JNK, and PKC can also undergo tyrosine nitration, ultimately preventing protein phosphorylation (233). Further, ROS/RNS-mediated regulation of gene expression occurs also through modulation of chromatin remodeling, since it was recently found that histone deacetylase (HDAC) is a redox-sensitive enzyme (214). Interestingly, both nutritional antioxidants-induced cytoprotection and low levels of oxidative stress or metals determine the induction of antioxidant proteins and phase 2 detoxifying enzymes, by means of NF-E2-related factor 2 (Nrf2) that is a *cis*-acting element held inactive within the cytoplasm by the Kelch-like ECH associating protein 1 (Keap1). Inducers of Nrf2/Keap1 system include ROS/RNS/RSS, hydroperoxides, heavy metals, nutritional antioxidants, quinones, prostaglandins, and growth factors. Extensive studies have revealed that a substantial number of genes are under Nrf2 control, and these include phase 2 detoxifying enzymes, antioxidant proteins, GSH generating enzymes, scavenger receptors, chaperon proteins, and transcription regulators (138). Therefore, Nrf2/Keap1 system may be considered as a central component of cellular defense networks.

C. ROS and RNS as inducers of pathologic processes

Under certain conditions, however, ROS/RNS production is increased more strongly and persistently, and the antioxidative response may not be sufficient to reset the system to the redox baseline. In Table 3, it is summarized the role of oxidative stress in physiologic or pathologic processes according to their levels of production. Indications for such a shift toward more oxidative conditions have been reported in the process of aging. Aging is considered as the ensemble of changes that are associated with progressive degeneration of biological functions, increased susceptibility to diseases, and increased probability of death within a given period of time. The well-known mitochondrial theory of aging, a refined version of the free radical theory of aging, states that the age-related degenerative process is, to a large extent, the consequence of mitochondrial dysfunction-induced free radical damage (104, 160, 268). Although ROS production is difficult

Table 3. Redox Regulation in Health and Disease

Oxidative stress	*Effects*	*Targets*	*Response*
Physiologic	Reversible oxidation Regulation of gene transcription	*Specific* Transcription factors Regulatory proteins	Programmed events 1. Proliferation 2. Differentiation 3. Apoptosis
Pathologic	Irreversible oxidative damage	*General* Macromolecules	Unprogrammed events 1. Survival 2. Necrosis

Depending on their cellular and tissue concentration, reactive species can cause reversible or irreversible oxidation of biomolecules, thus triggering activation of programmed or unprogrammed cell functions, respectively.

to measure in biological tissues, there are various indirect manifestations of oxidative stress in the elderly, including lipid peroxidation, DNA and protein oxidation, and a shift in the redox states of thiol/disulfide redox couples such as GSH, cysteine, and albumin. The observed pro-oxidative changes in elderly subjects strongly suggest that aging of an organism may be associated with changes in redox-sensitive signaling pathways, such as those involved in differentiation-like processes, as well as with a loss of replicative capacity. The discovery that telomeres get progressively shorter in aging human fibroblasts has led to the popular hypothesis that telomere shortening may also be a major cause of cellular senescence (103).

Further, senescent cells show a low ATP consumption and ADP availability, suggesting a particularly high mitochondrial oxidative stress (300). Thus, the rate of mitochondrial ROS production seems to be significantly influenced by the availability of mitochondrial energy substrates. In line with this, it is not surprising that dietary restriction is nowadays the best investigated and most promising experimental strategy to increase life span and to improve the quality of life in the elderly. Additionally, a body of experimental and clinical evidence suggests that the immune system is implicated, with a variable degree of importance, in almost all age-related processes and the term "inflammaging" has been coined to indicate that aging is accompanied by an age-dependent upregulation of the inflammatory response, due to the chronic antigenic stress that bombards the innate immune system thoroughout life and potentially triggers the onset of inflammatory disease (154).

In extreme cases of persistently high ROS/RNS levels, such an oxidative shift may be associated with overt pathologic conditions. Genetically, unstable cells can adapt to cope with stress by adjusting the level of reactive species to an extent that promotes cell survival and proliferation, leading to development of cancer. Conversely, normal or aging cells that failed to maintain a redox balance are prone to oxidative stress-induced cell death, which may also act as a general pathogenic mechanism of degenerative diseases. In particular, the redox system can affect protein function at different levels, that is, through regulating their expression, post-translational modification, or structural stability. According to the type of oxidative modification, proteins may undergo slight conformational changes, or even severe denaturations, and the functional outcome can either be an activation or an inhibition of their biological activity.

It it is worth mentioning that the oxidative stress-induced events that we will discuss below in the different chronic inflammatory disorders might appear as independent phenomena that do not share any common redox regulation. Although the complexity of these diseases makes it difficult to clearly delineate molecular commonalities, it can be proposed that the effect of ROS and RNS in inflammatory processes could be the guideline that would glue them together. For instance, $NO^{\bullet}$, one of the simplest cellular signals, broadly and profoundly impacts inflammation in various chronic disorders, either directly *via* activation of soluble guanylate cyclases or indirectly *via* interaction with ROS and formation of secondary RNS. The cross-talks between these direct and indirect pathways ultimately lead to the formation of distinct classes of signaling molecules that are imbued with both inflammatory and anti-inflammatory activity.

V. Oxidative Stress-Induced Events in Chronic Inflammatory Disorders: Molecular Clues for Therapeutic Intervention

A. Malignant diseases

Malignant neoplasm or cancer comprises a class of diseases in which a group of cells display uncontrolled growth, invasion, and sometimes metastasis. The neoplastic tissue competes with the normal cells and tissues for energy supplies and nutritional substrate and, since it may flourish in a patient who is wasting away, it is to a certain degree autonomous (52). However, all neoplasms ultimately depend on the host for their nutrition and vascular supply; many forms of neoplasia require endocrine support as well as all the releasing machinery of immune cells, especially macrophages (120, 172). The development of cancer in humans is a complex process that implies cellular and molecular changes mediated by diverse endogenous and exogenous stimuli, which ultimately affect DNA.

It is well established that cancer initiation and promotion are associated with permanent chromosomal defects and oncogene activation induced by free radicals. A common form of damage is the formation of oxidized bases of DNA, which are considered an important event in chemical carcinogenesis. Oxidative DNA damage also produces a multiplicity of modifications in the DNA structure, including base and sugar lesions, strand breaks, DNA-protein cross-links, and base-free sites. For example, chronic inflammation resulting from noninfectious diseases like tobacco smoking or asbestosis is a source of oxidative DNA damage that can contribute to the development of lung cancer and other tumors. Equally important to the induction of mutation by ROS is the fact that $NO^{\bullet}$ and $^{\bullet}O_2^-$ are produced by activated macrophages;

therefore, $ONOO^-$ is likely to be formed in the proximity of these cells. Oxidation of guanine at the C8 position results in the formation of 8-hydroxydeoxyguanosine (OH8dG), probably the most studied oxidative DNA adduct. This oxidative damage results in site-specific mutagenesis, is mutagenic in bacterial and mammalian cells, and produces G→T transversions that are widely found in mutated oncogenes and tumor suppressor genes. In addition, ROS can react with dGTP in the nucleotide pool to form OH8dG. Other oxidative DNA lesions, such as 8-oxo-adenine, thymine glycol, 5-hydroxy-deoxycytidine, as well as several uracil analogs, have been shown to be mutagenic (157, 175, 272).

The highly significant correlation between consumption of fats and death rates from leukemia, breast, ovary, and rectum cancers among elderly people may be a reflection of greater lipid peroxidation (174). Malondialdehyde (MDA), a by-product of lipid degradation, is a tautomer that is both highly electrophilic and nucleophilic. This feature allows the formation of MDA oligomers, which have been shown to be mutagenic. MDA also reacts with purines and pyrimidines to form dG, dA, and dC adducts. MDA-DNA adducts appear to be pro-mutagenic, as they induce mutations in oncogenes and tumor suppressor genes, and appear also to correlate with altered cell-cycle control and gene expression in cultured cells. In addition, certain types of cancer cells produce substantial amounts of ROS. The apparent inconsistency between the uncontrolled cell growth in ROS-producing malignant cells and the ROS-induced senescence in normal cells suggests, however, that ROS production may be necessary but not sufficient to induce malignant cell growth. A pro-oxidative shift in the plasma thiol/disulfide redox state has also been observed in patients with various types of advanced malignancies (93).

ROS induce cell proliferation during the tumor promotion stage of carcinogenesis. Both H_2O_2 and $^{\bullet}O_2^-$ induce mitogenesis and cell proliferation in several mammalian cell types. Further, a reduction in cellular oxidants *via* supplementation with antioxidants such as SOD, catalase, β-carotene, and flavonoids inhibits cell proliferation *in vitro*. Oxidative stress also modulates apoptosis, and indeed high concentrations of ROS trigger apoptotic signaling pathways that result in cell loss (244). A number of endogenous substances (prostaglandins and lipid hydroperoxides), redox-cycling compounds (quinines and adriamycin), and growth factors such as TGF-β and TNF-α induce apoptosis *via* the generation of ROS. Instead, antioxidants such as GSH, *N*-acetylcysteine (NAC), and dithiothreitol inhibit the apoptotic process, further supporting the link between ROS production and induction of programmed cell death (163, 229). Although there is no single mechanism that can explain the ROS-induced increase of cell proliferation and/or inhibition of apoptosis, mounting evidence suggests a possible link between ROS production and altered expression of growth regulatory genes. The methylation status of cellular DNA is considered an epigenetic mechanism that influences gene expression. Under normal conditions, DNA is methylated symmetrically on both strands. During the carcinogenesis process, DNA methylation may be such that both hypomethylation and hypermethylation occur. The degree of methylation within a gene inversely correlates with the expression of that gene. Hypermethylation may inhibit transcription of tumor suppressor genes and is associated with decreased gene expression or gene silencing. In line with this, tumor suppressor genes are known to be hypermethylated and subsequently inactivated in several types of cancer (68, 204).

Progressive increases in methylation of CpG islands have been observed in bladder cancer and specific tumor suppressor genes have been reported to be methylated in tumors, such as p16ink4a and p14ARF. Among the agents and situations that can alter the methylation status of DNA, ROS play a prominent role. In particular, oxidative DNA damage can lead to decreased DNA methylation, because it can impair the interaction of methyltransferases with DNA, thus leading to a generalized hypomethylation of cytosine residues at CpG sites. Moreover, also OH8dG formation can lead to hypomethylation of DNA, and can interfere with the normal function of DNA methyltransferases (136). The effects of ROS on malignant diseases are depicted in Figure 5. It is now widely accepted that constitutively elevated levels of oxidative species and dependence on mitogenic and antiapoptotic ROS-signaling in cancer cells represent a specific vulnerability that can be selectively targeted by directly or indirectly acting pro- and antioxidants and redox modulators, known as "redox chemotherapeutics." The latter substances represent a novel class of promising anticancer agents that modulate cellular redox homeostasis through direct or indirect alteration of ROS generation, signaling, and turnover. In this context, it should be recalled that a significant number of attractive molecular targets have now been identified and validated in cancer. Lead optimization of prototype redox chemotherapeutics from natural and synthetic sources has led to the development of advanced clinical candidates with pharmacophores that display attenuated and more targeted redox reactivity, along with less off-target toxicity. In addition, structure-based approaches have identifed redox-silent pharmacophores that target cancer cell redox status and signaling through ligand-based interactions. These include organic endoperoxides (artemisinins), arsenicals, redox cyclers (motexafin gadolinium, menadione, and geldanamycin), metal chelators (disulfiram and triapine), di- and polysulfides, isothiocyanate organosulfur agents, as well as other redox chemotherapics including SOD inhibitors or mimetics, targets of the GSH/GPx/GR and TRX/TR redox systems, and targets of numerous signaling proteins and of metabolic processes like mitochondrial respiration. A detailed list of all these redox chemotherapeutics has been beautifully reviewed by Wondrak and colleagues (283). Particularly promising are some recent studies that have demonstrated feasibility of gene-based redox intervention ranging from intratumoral siRNA injection to adenovirus-based gene therapy. These recent developments may therefore herald a new generation of gene-based therapeutics that modulate expression of crucial redox targets in cancer cells. The impressive number of ongoing clinical trials that examine therapeutic performance of these novel redox drugs in cancer patients demonstrates that redox chemotherapy has already made the crucial transition from bench to bedside. Further translational research will be necessary to enhance the therapeutic benefit provided by early developmental candidates, but it is now evident that redox drugs represent a possible alternative to the classical chemotherapeutic drugs.

B. Diabetes mellitus

Diabetes mellitus (DM) represents a group of metabolic disorders in which there is impaired glucose utilization,

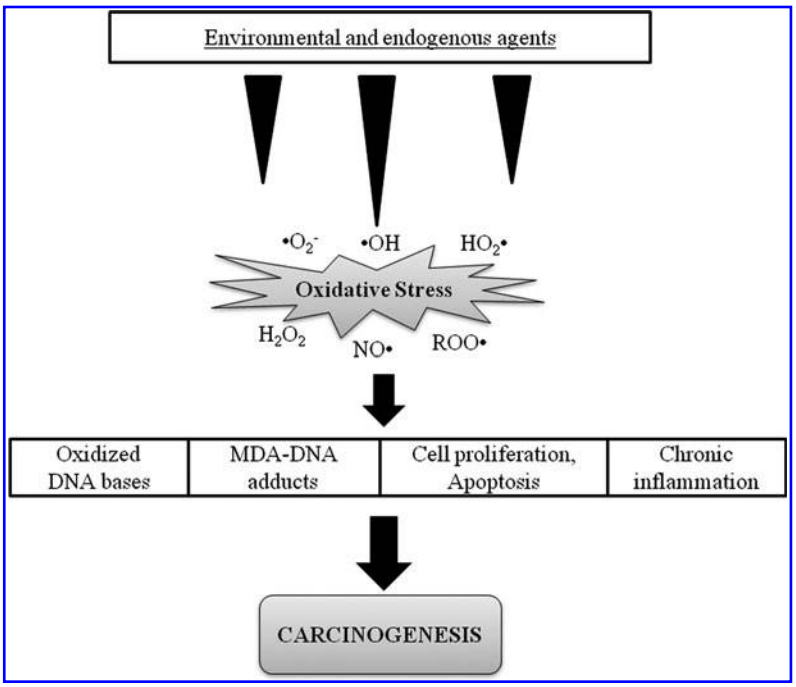

FIG. 5. ROS and their role in the development of malignant diseases. Constant upregulation of oxidative stress contributes to genotypic and phenotypic changes typical of cancer cells. Besides direct DNA damage and modification, ROS and cellular redox status mediate cell signaling pathways that are involved in cell growth and inflammatory responses. $^{\bullet}O_2^-$, superoxide radical anion; $^{\bullet}OH$, hydroxyl radical; $HO_2^{\bullet}$, hydroperoxyl radical; H_2O_2, hydrogen peroxide; $NO^{\bullet}$, nitric oxide; $ROO^{\bullet}$, peroxyl radical; MDA, malondialdehyde; DNA, deoxyribonucleic acid.

leading to hyperglycemia. Primary (or idiopathic) DM is by far the most common and important, and must be distinguished from secondary DM, which includes forms of hyperglycemia associated with destruction of pancreatic islets induced by inflammatory pancreatic disease, surgery, tumors, certain drugs, hemochromatosis, and certain acquired or genetic endocrinopathies. Primary DM basically includes two variants that differ in their pattern of inheritance, insulin responses, and origins: (i) insulin-dependent diabetes mellitus (IDDM), also called type I diabetes and previously known as juvenile-onset and ketosis-prone diabetes, accounting for ~10% to ~20% of all cases of idiopathic diabetes. It results from body's failure to produce insulin mainly due to a T-cell-mediated autoimmune attack of insulin-producing β-cells of the Langerhans islets in pancreas; (ii) noninsulin-dependent diabetes mellitus (NIDDM), also called type II diabetes and previously referred to as adult-onset diabetes, accounting for ~80% to ~90% of all cases. By contrast, NIDDM results from insulin resistance, a condition in which cells fail to respond adequately to circulating insulin due to impaired insulin signaling/glucose homeostasis (21). Chronic extracellular hyperglycemia produces the classical symptoms of polyuria (frequent urination), polydipsia (increased thirst), and polyphagia (increased hunger), and ultimately results in tissue damage and pathophysiologic complications, involving heart disease, atherosclerosis, cataract formation, peripheral nerve damage, retinopathy, and others (16, 33).

Increased oxidative stress has been proposed to be one of the major triggers of hyperglycemia-induced diabetic complications. In DM, oxidative stress is associated with a pro-oxidative shift of the GSH redox state in blood. Further, hyperglycemia stimulates ROS/RNS formation from a variety of sources, including oxidative phosphorylation, Nox, lipoxygenase, cytochrome P450, NOS activity, hexosamine metabolism, as well as glucose auto-oxidation (14). Additionally, Nox is also capable of generating ROS, not only within the islet vasculature but also in the islet β-cells *via* a protein kinase C-dependent mechanism (193). Moreover, the Schiff's reaction during the glycation process results in the formation of glycated proteins in the plasma of diabetic patients, termed advanced glycation endproducts (AGE), and in the activation of the AGE receptor (RAGE), which further stimulates ROS production and decreases intracellular GSH levels. At the cellular level, the AGE/RAGE couple was found to induce a cascade of cytotoxic pathways, mainly *via* activation and nuclear translocation of NF-kB, whose downstream signaling contributes to endothelial dysfunction, inflammation, and ultimately to the aforementioned development of diabetic complications (16, 282). Oxidant stress can indeed initiate an inflammatory process, which leads to activation of endothelial cells that synthesize inflammatory cytokines and chemokines and recruit additional inflammatory cells to the site of injury (189). The presence of inflammatory infiltrates and anti-islet antibodies, and the identification of diabetogenic T cells suggest that inflammation is a central player in the pathogenesis of diabetes. Further, destruction of the pancreatic β-cells in type-1 diabetes occurs through pathogenic T cells that are reactive with as yet unknown islet antigens, and their expansion is a result of a failure of the homeostatic mechanisms that prevent cleaning of self-antigens. Interestingly, cross-linking of the T-cell receptor (TCR) activates a T-cell oxidase through recruitment of Fas and Fas ligand (121). Also, the impairment of regulatory T cells is likely to contribute to the autoimmune destruction of pancreatic β-cells (111).

Hyperglycemia enhances cell-mediated low-density lipoprotein (LDL) peroxidation in endothelial cells (178), and the signal transduction triggered by insulin is mediated mainly by insulin receptor substrate (IRS) proteins, whose phosphorylation in turn activates different pathways. While the phosphorylation of IRS1 on tyrosine residues is critical for insulin-stimulated responses, its phosphorylation on serine residues has a dual role: either to enhance or to terminate the insulin effects. The unbalance between the positive IRS1 tyrosine phosphorylation and the negative IRS1 serine

phosphorylation is strongly stimulated by diabetogenic factors, which include free fatty acids, TNF-α, and oxidative stress. The latter can lead to both activation of serine/threonine kinases and inhibition of IRS1 *via* its phosphorylation (148). Understanding the mechanisms of IRS1 inhibition and identification of kinases involved in these processes may reveal novel targets for development of strategies to prevent insulin resistance. On the other hand, vitamin E is depleted in DM and this impacts on its protective effect, exerted mainly through suppression of lipid peroxidation. Also, vitamin C levels have been found to be reduced in plasma of diabetes patients; however, the relationship between the levels of this vitamin and diabetic complications remains unclear (274). Various consequences of oxidative stress in diabetic subjects involve accumulation of MDA, although its levels are not indicative of an active role in the onset of DM. Some of the most significant roles of oxidative stress linked to DM are depicted in Figure 6. Insulin resistance is a major contributor to progression of the disease and to complications of type II diabetes. Currently, numerous events are recognized as crucial triggers; for instance, oxidative stress-related defects of the oxidative phosphorylation machinery and of the mitochondrial β-oxidation lead to excessive accumulation of intracellular triglycerides in muscle and liver, with subsequent insulin resistance (222).

Many studies have suggested that β-cell dysfunction results from prolonged exposure to high glucose or free fatty acid levels, or to a combination of both. β-Cells are particularly sensitive to ROS, because they are low in antioxidant enzymes such as catalase, GPx, and SOD. Therefore, the ability of oxidative stress to damage mitochondria and markedly blunt insulin secretion is not surprising. Exploitation of antioxidants in the clinical treatment of type II diabetes as an adjunct therapy is warranted, because of the many reports of elevated markers of oxidative stress in patients with this disease. In this context, beneficial effects of antioxidant agents, including trace elements, especially against the damage of the cardiovascular system due to DM have been documented. In fact, the use of PPARα agonists to reduce fatty acid oxidation, and of trace elements like zinc and selenium as antioxidants, along with physical exercise to induce mitochondrial adaptation, have been shown to contribute to the prevention of diabetes-induced cardiac dysfunction (275). In general, antioxidant treatment seems to exert beneficial effects in DM, with preservation of β-cell function *in vivo*. Antioxidant treatment suppresses β-cells apoptosis without changing their rate of proliferation, supporting the hypothesis that in chronic hyperglycemia apoptosis induced by oxidative stress causes reduction of β-cell mass (128). The antioxidant treatment also preserves the amounts of insulin content and insulin mRNA. Well-tuned, balanced, and responsive antioxidant defense systems are thus vital for proper prevention against DM.

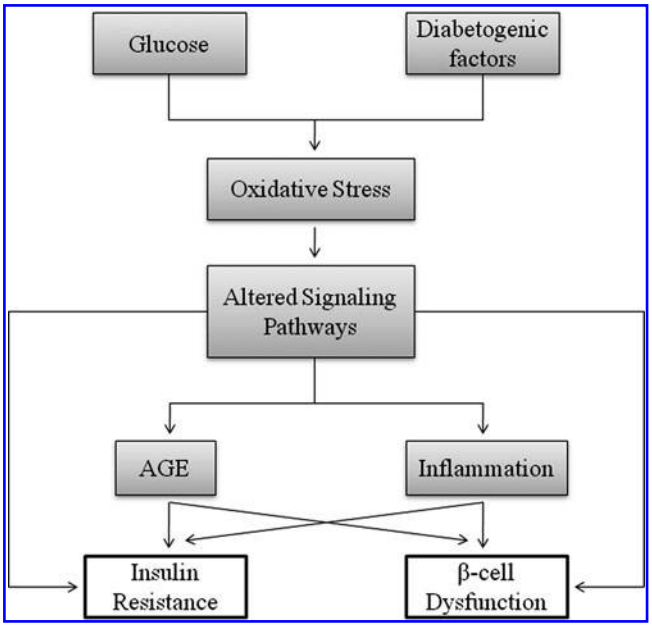

FIG. 6. Oxidative stress and diabetes mellitus. Hyperglycemia and other diabetogenic factors cause oxidative stress, which negatively regulates insulin signaling, thus favoring insulin resistance and inflammation-induced β-cells dysfunction. AGE, advanced glycation end products.

C. Cardiovascular diseases and atherosclerosis

Cardiovascular diseases (CVD) consist of a group of multifactorial disorders affecting the cardiovascular system, which can all be associated with a variety of risk factors, including hypercholesterolaemia, hypertension, smoking, diabetes, stress, and physical inactivity. Although evidence for a direct link is still missing, oxidative stress plays a role in various CVDs, such as atherosclerosis, ischemic heart disease, cardiomyopathies, cardiac hypertrophy, and congestive heart failure (185). The term CVD could indeed refer to any disease that affects the cardiovascular system; however, it is commonly restricted to those conditions that are related to atherosclerosis. The latter pathology seems to be the underlying cause that, by the time that heart problems are detected, is already at a quite advanced state.

Atherosclerosis is a disorder of large- and medium-sized arteries, and is characterized by endothelial dysfunction and accumulation of lipids, cellular debris, and fibrous elements within the intima of the vessel wall. This buildup leads to plaque formation, vascular remodeling, luminal obstruction, abnormalities of blood flow, and thus in a diminished oxygen supply to target organs.

The presence of lipid-laden macrophages is the first step of atherogenesis, and in fact their transformation into foam cells is mediated by a dysregulated uptake of cholesterol-enriched modified LDL through scavenger receptors like CD36 and SR-A. At variance with the LDL receptor, scavenger receptors are not downregulated by increased cholesterol content, and therefore they allow a continued engulfment of modified LDL and the subsequent transformation of macrophages into foam cells (90). It is now also generally recognized that atherosclerosis is an inflammatory disease, whose progression and consequences depend on dynamic interactions between inflammatory cells of both innate and adaptive immunity; these cells are recruited in response to sub-endothelial lipid accumulation, and to the local wound-healing response of surrounding vascular smooth muscle cells (224). Therefore, a chronic and insidious process develops over decades, with clinical consequences that stem from gradual occlusion of the arterial blood supply, and ultimately lead to chronic ischemia.

However, atherosclerosis can also lead to serious if not fatal complications within minutes, like in the case of acute coronary syndromes and emboli, the latter being caused by plaque disruption.

Lipoprotein oxidation is the key event in the development of atherosclerosis. Many cell types are able to oxidize LDL, including monocytes, macrophages, neutrophils, endothelial cells, smooth muscle cells, and fibroblasts. It should be recalled that oxLDL do not bind any longer to LDL receptor, but they acquire an increased affinity for the scavenger receptors. It is still unclear which oxidative mechanism or radical species are involved, but potential candidates include Nox, myeloperoxidase, cytochrome P450, the mitochondrial electron transport chain, $ONOO^-$, xanthine oxidase, caeruloplasmin, and lipoxygenase. The last enzyme has received much attention with the discovery that not only it modifies LDL *in vitro* to a form taken up by the scavenger receptor, but disruption of its gene reduces atherosclerosis in transgenic mice. It is conceivable that different mechanisms of LDL oxidation may predominate at different stages of plaque development. In particular, transition metal-mediated oxidation seems to occur only in advanced lesions, whereas oxidation mediated by myeloperoxidase or by RNS occurs throughout plaque development (251). The susceptibility of LDL to oxidation *in vivo* is influenced by both LDL composition (intrinsic factors), and surrounding microenvironment (extrinsic factors). Among the intrinsic factors, fatty acid composition is of primary importance: a high proportion of PUFAs confers greater susceptibility to oxidation, whereas a high proportion of monounsaturated fatty acids protects against it. Since the propagation of LDL oxidation begins after the endogenous antioxidants have been consumed, oxLDL formation is also highly dependent on the antioxidant content in lipoproteins, which is mainly *a*-tocopherol with a contribution of ubiquinol-10 and carotenoids. The molar ratio of PUFA to total antioxidants in LDL is $\sim$150:1, although there is considerable between-subject variation depending on fatty acid and lipophilic antioxidant content in the diet (135).

Besides initiating oxLDL formation, oxidative stress is also involved in the induction of the expression of protein kinases, such as focal adhesion kinase, and intercellular adhesion molecules like ICAM-1. Binding of oxLDL leads to the activation of monocytes and macrophages, and stimulates the expression of Mn-SOD, which, in turn, increases the concentration of H_2O_2 by perturbing the steady-state levels of ROS. This process is associated with massive macrophage apoptosis, and thus contributes to the formation of the atherosclerotic lesion. Cytokines and other molecules such as TNF-α, IL-1β, angiotensin II, and IFN-γ may further enhance the process, thus inducing $^\bullet O_2^-$ production by the membrane-bound Nox in endothelial cells (168).

Another hallmark of atherosclerosis is endothelial dysfunction, which is strictly associated with endothelial lipid accumulation that ultimately triggers foam cells formation (28). Decreased bioactivity of NO, due to either impaired synthesis or exaggerated degradation, is a major feature of endothelial dysfunction. Indeed, classic studies demonstrated that $^\bullet O_2^-$ can inactivate NO, thereby causing endothelial dysfunction by reduction of NO bioavailability. It appears that levels of $^\bullet O_2^-$ are increased in atherosclerotic vessels, and inactivation of NO by $^\bullet O_2^-$ plays a key role in endothelial dysfunction of atherosclerotic arteries (180). Further, it seems unexpected that eNOS is also a source of $^\bullet O_2^-$, yet *in vitro* studies have clearly demonstrated that both eNOS and nNOS produce $^\bullet O_2^-$, in addition to (or instead of) NO under conditions that uncouple NO synthases (184). Effects of $^\bullet O_2^-$ on NO-mediated vasomotor responses have been studied extensively during the past two decades, and recently a rather new concept has emerged, whereby $^\bullet O_2^-$ and other ROS may modulate also NO-independent relaxation in vascular muscle. Although direct biochemical evidence for reduced NO bioavailability has been obtained in various experimental models, this approach is rather difficult to reproduce in patients; however, plasma nitrite levels have been recently measured in humans, indicating endothelial dysfunction and a clear correlation with cardiovascular risk factors (107, 137).

Conventional therapies against atherosclerosis include statins, which are the most popular and widely prescribed drugs; their properties include cholesterol reduction, immunosuppression, and antioxidant effects. However, statins have several adverse side effects, such as myopathy, hepatotoxicity, peripheral neuropathy, and even autoimmune responses; hence, there has been an increasing interest in developing novel therapies aimed at reducing cholesterol content, and at exerting anti-inflammatory effects, in a more selective manner (19).

Lipid peroxidation and atherogenesis may be reduced by vitamin E. A study on atherosclerosis-susceptible Apolipoprotein-E1 knockout mice revealed that induction of vitamin E deficiency by disruption of the α-tocopherol transfer protein gene increases the severity of atherosclerotic lesions in the proximal aorta. In addition, dietary supplementation with vitamin E led to increased LDL resistance to copper-induced oxidation *in vitro*, an effect that was dose dependent (165, 259, 298).

Widely used cardiovascular agents like statins or angiotensin receptor blockers have well-documented beneficial effects on the vascular redox state, which is reflected also in the improvement of the clinical symptoms. Novel strategies and recently patented therapeutics include thiazolidinediones, folates, tetrahydrobiopterin, cyclopentone prostaglandins, and aldose reductase inhibitors, which all have well-defined effects on the vascular redox balance, but still have unclear therapeutic efficacy (276). A better understanding of redox-sensitive intracellular signaling pathways could disclose the critical steps to be tackled by novel agents, to reverse vascular dysfunctions, inhibit atherosclerosis progression, and ultimately improve clinical outcome.

Probucol, which is a drug with modest cholesterol-lowering ability but remarkable antioxidant properties, has been evaluated for its effects on the development of atherosclerosis in both hypercholesterolemic rabbits and nonhuman primates. A focus of these trials was the relationship between the efficacy in reducing atherosclerotic-lesion formation and that in enhancing the resistance to LDL oxidation *in vitro*. Probucol, at the very high doses used in these studies, was partially effective in reducing atherosclerotic lesions, yet without a close correlation with the inhibition of LDL oxidation (134). Further, an analog of probucol that was moderately less effective than the parent compound in inhibiting LDL oxidation had no effect on atherosclerosis, leading to the speculation that a threshold level of LDL oxidation is needed to inhibit atherosclerosis. In view of the *in vitro* data summarized above, which suggest that intracellular redox state is important in

controlling the proinflammatory status of endothelial cells, it seems likely that the disparity between probucol and its analog in inhibiting atherosclerosis might be related to variability in intracellular permeability rather than in inhibition of LDL oxidation.

The beneficial effect of vitamin E on the cardiovascular system has been explored also in supplementation studies, where it was administered in combination with other antioxidants, in both primary and secondary prevention trials (219).

Thus, diet and nutrition play a fundamental role in cardiovascular prevention and in maintaining physiologic homeostasis. Recent literature emphasizes the potential therapeutic effect of flavonoids in controlling the pathogenesis of chronic cardiovascular disease, driven by cardiovascular risk factors and oxidative stress. The pharmacological mechanism of action of flavanols has yet to be identified, but they likely include enhanced NO bioactivity, modulation of the immune system, and enhanced endothelial homeostatic vascular repair (89). It has been speculated that the intrinsic antioxidant capacity of flavanols, and of flavonoids in general, may underline their positive vascular effects; yet, the possibility that they may influence the overall level of oxidative stress through secondary mechanisms cannot be ruled out. At any rate, sustained improvements in endothelial dysfunction have been found upon regular intake of flavonols with the diet. Clinical studies suggest that endothelial function can be improved in CVD patients by consumption of flavonol-rich cocoa, which can also contribute to normalize microvascular response, inflammatory markers, and metabolites of NO (108). A pilot study of Oligonol supplementation (a patented lychee fruit extract particularly rich in low-molecular-weight flavanol) has also been recently found by Hackman and colleagues to improve endothelial function, to reduce platelet reactivity and to increase circulating levels of flavonoids after a single intake (http://clinicaltrials.gov/ct2/show/NCT01162174). While flavonols represent a promising class of food components that determine lower cardiovascular risk and can positively affect cardiovascular parameters in the short term, no long-term randomized controlled dietary intervention trials with hard clinical endpoints are available to date.

Hence, despite accumulated evidence and notwithstanding the fact that oxidative stress is pathophysiologically important for atherosclerosis, antioxidant strategies yield a poor performance in limiting either atherosclerosis or cardiovascular problems derived thereafter. As yet, there is no consensus that antioxidant supplementation has any effect on the disease process in patients at risk of atherosclerosis. Thus, these observations have questioned the cause–effect relationship between oxidative stress and atherosclerosis, providing a real challenge to the design of new redox-based therapeutics. A relevant open issue is also the ability of a given drug to modulate the expression of redox-sensitive genes in cells of the vessel wall, rather than that to affect LDL oxidation. In this context, given the anti-inflammatory role of Nrf-2 and the fact that it appears to be an important factor in the protection of vasculature against oxidative stress and inflammation, it was unexpected that independent studies recently found that Nrf-2 expression promotes atherosclerotic lesion formation, probably *via* a positive regulation of CD36 (12). On this basis, the initial expectation of a promising efficacy of Nrf-2-targeted therapies to treat cardiovascular diseases needs to be reconsidered. The overall effects of ROS on atherosclerosis are depicted in Figure 7.

D. Chronic obstructive pulmonary diseases

Chronic obstructive pulmonary diseases (COPD) are a group of conditions that share a major symptom, dyspnea, and are accompanied by chronic or recurrent obstruction to air flow within the lung. Because of the increase of environmental pollutants, cigarettes smoking, and other noxious factors, the incidence of COPD has increased dramatically in the past few decades. Usually, it presents with prototypical symptoms such as chronic bronchitis, bronchiectasis, asthma, and emphysema. There is mounting evidence that COPD may have an autoimmune component, because many subjects with COPD still have active inflammation in the lungs after smoking cessation, which may continue to worsen for many years. This sustained inflammation is thought to be mediated by auto-antibodies and auto-reactive T cells. However, it is not fully understood how tobacco smoke and other inhaled particles damage the lungs, ultimately causing COPD. The most important processes that may be noxious to lungs are: (i) oxidative stress produced by the high concentrations of free radicals in tobacco smoke; (ii) cytokine release due to inflammation elicited by irritant particles in the airway; and (iii) impairment of antiprotease enzymes such as α1-antitrypsin by tobacco smoke and free radicals, thus allowing protease activity to damage the lung (112).

The oxidant burden in the lungs is enhanced in smokers by the release of ROS from alveolar macrophages, which also release a host of mediators that attract neutrophils and other inflammatory cells into the lungs. Here, neutrophils and macrophages generate ROS mainly *via* Nox activity. Circulating neutrophils from cigarette smokers and patients

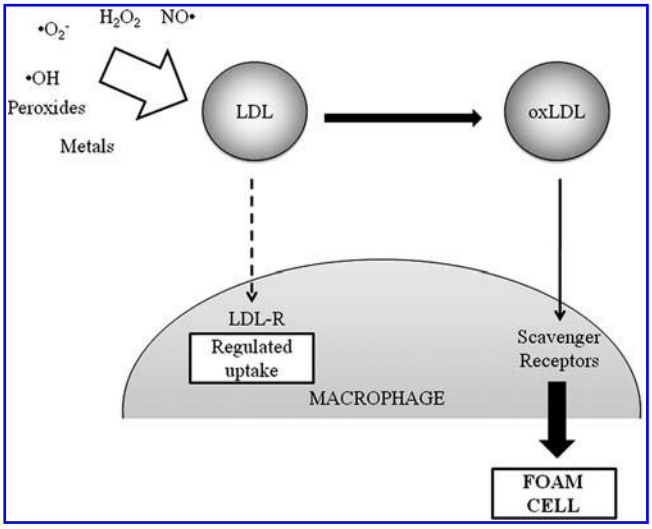

FIG. 7. Role of oxidative stress in the development of atherosclerosis. Increased oxidative stress is responsible for enhanced oxidation of circulating LDL, which are constantly taken up by scavenger receptors of macrophages, which develop into foam cells. $^{\bullet}O_2^-$, superoxide radical anion; $^{\bullet}OH$, hydroxyl radical; $HO_2^{\bullet}$, hydroperoxyl radical; H_2O_2, hydrogen peroxide; $NO^{\bullet}$, nitric oxide; LDL, lowdensity lipoprotein; oxLDL, oxidized low density lipoprotein; LDL-R, low density lipoprotein receptor.

with exacerbations of COPD release more $^{\bullet}O_2^-$ than matched controls. Cigarette smoking is also associated with increased content of myeloperoxidase in neutrophils, which correlates with the degree of pulmonary dysfunction (112, 212). The generation of ROS in epithelial lining fluid may be further enhanced by the presence of increased amounts of free iron in the airspaces of smokers. In addition, macrophages obtained from smokers release more free iron *in vitro* than do those obtained from nonsmokers. Also, ROS-mediated lipid peroxidation products, mainly MDA, HNE, acrolein, and F_2-isoprostanes, play an important role in COPD. These substances can initiate additional lipid peroxidation and DNA damage, as well as covalent modification and functional impairment of proteins (215, 269).

It is noteworthy that GSH is concentrated in epithelial lining fluid compared with plasma, and has an important protective role toward epithelial cells, both in the airspace and intracellularly. Decreasing the levels of GSH in alveolar epithelial cells leads to loss of barrier function, paralleled by an increase in permeability (216). Thus, a simple increase of GSH levels in lung cells could represent a promising approach to the treatment of COPD. In fact, extracellular augmentation of GSH has been tried through intravenous administration, oral ingestion, or aerosol inhalation of nebulized GSH. However, all these routes of administration led to undesirable side effects, demonstrating that a direct GSH therapy is not yet appropriate to combat COPD. Alternative formulations of GSH, such as liposomal delivery, may improve its bioavailability, but at present it seems that direct administration of GSH is not promising enough to treat COPD (211).

The availability of cysteine is critical for the regulation of GSH biosynthesis. NAC, a cysteine-donating compound with reducing activity, is a precursor of GSH that may also reduce cystine to cysteine; the latter reaction is important *in vivo* for the elevation of intracellular GSH in lungs. NAC is also used as a mucolytic agent, to reduce mucus viscosity and to improve mucociliary clearance. Although small-scale trials failed to demonstrate any overt clinical benefit of NAC, a few meta-analyses have shown a small but significant efficacy in the treatment of the clinical symptoms of COPD (88).

A number of oxidative stress-sensitive proteins involved in the regulation of a variety of inflammatory genes, such as NF-kB, AP-1, p38, and JNK, are altered in patients with COPD. This is particularly evident with the p65 protein component of NF-kB, whose expression is increased in bronchial epithelium of smokers and of patients with COPD. Inhibition of NF-kB pathway by means of small molecules is currently under investigation, as a potential tool to downregulate the inflammatory component of COPD. To this aim, several compounds are in preclinical development for targeting NF-kB signaling, but as yet a substance that can be suitable for clinical application is still missing (210).

An interesting alternative to modulate the transcriptional activity of NF-kB may be through inhibition of TR. A TR inhibitor, fotemustine, which is currently in phase III trials for the treatment of various cancers, may in fact potentially improve also the symptoms of COPD (227).

Particular interest has been directed to polyphenols, in particular curcumin. Recent studies have reported that curcumin has multiple properties to protect against cigarette smoke-mediated oxidative stress: it acts as an oxygen radical and $HO^{\bullet}$ scavenger, increases GSH levels by induction of glutamate cysteine ligase, and as an anti-inflammatory agent through inhibition of NF-kB and IL-8 release from lung cells (24). Further, dietary polyphenols not only act as antioxidant/anti-inflammatory agents, but also increase the efficacy of glucocorticosteroids in COPD (213). The effects of ROS on COPD are depicted in Figure 8.

E. Inflammatory bowel disease

Inflammatory bowel disease (IBD) is a group of inflammatory conditions of the colon and the small intestine. The major types of IBD are ulcerative colitis (UC) and Crohn's disease (CD), that show some overlapping clinical features; in fact, in 10%–15% of cases it is not possible to differentiate between the two disease conditions. However, differences exist in the nature and location of the lesions between UC and CD. The former disorder is restricted to the large intestine and is associated with continuous mucosal inflammation, including crypt abscesses and ulcers, which typically spread from the most caudal part of the rectum. Instead, the latter disease can affect any part of the gastrointestinal tract, and is characterized by segmental and transmural inflammation, fistulas, oedema and granulomas in the whole intestinal wall (47). There is compelling evidence that dysregulation of the mucosal immune system is a major factor contributing to the pathogenesis of IBD. As a matter of fact, the mucosal immune system senses and interprets the local microenvironment, recognizing (and avoiding reactions against) the commensal flora (tolerance), while retaining its capacity to respond to episodic challenges from pathogens. The basis of IBD is the presence of genetically determined alterations, which cause an over-reaction of the mucosal immunity to normal intestinal microflora (287). These responses may be induced by defects in the epithelial barrier (and hence increased intestinal permeability), adherence of bacteria, or decreased expression of a group of proteins collectively termed "defensins." As a consequence, the mucosal immune system produces excessive ROS/RNS and lipid peroxides, as well as pro-inflammatory mediators that include cytokines, growth factors, and adhesion molecules. A large body of evidence supports the notion of increased free radical production during IBD, and oxidant-mediated injury is known to play an important role in the pathophysiology of this disease. In the lamina propria of IBD patients there are an increased number of activated inflammatory cells that release ROS (highly produced by infiltrating neutrophils, macrophages, and dendritic cells) to an extent that exceeds the intestinal antioxidant defense system, thus contributing to intestinal oxidative injury. Recently, it has been suggested that oxidant-mediated insults are crucial in both primary and subsequent secondary pathophysiologic mechanisms that underlie intestinal inflammation (287). In addition, nutritional deficiencies have been reported in IBD, such as lower levels of antioxidant vitamins A, C, and E, and decreased amounts of trace elements like zinc and selenium, which are cofactors of antioxidant enzymes like SOD and GPx, respectively. Further, GSH levels, as well as those of SOD and metallothionein, are also significantly depleted in UC and CD, compared to healthy colon (155). This collected evidence suggests that depletion of small antioxidants like ascorbate and glutathione occurs both in UC and CD, whereas a decrease in enzymatic antioxidants like superoxide dismutase is more likely in CD.

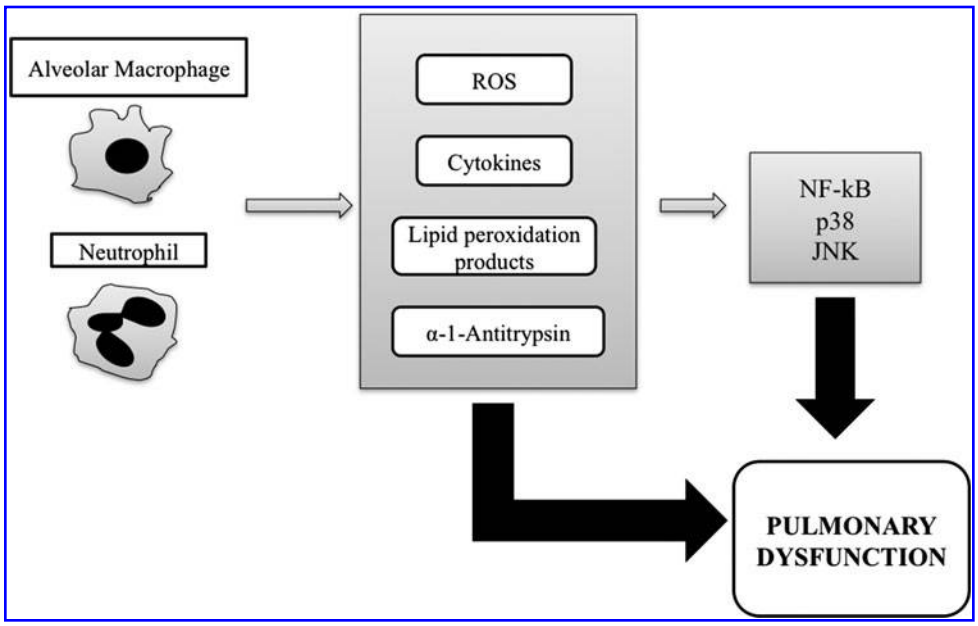

FIG. 8. Mechanisms of oxidative stress-mediated COPD. Inflammatory response is mediated by oxidants either inhaled or released by activated alveolar macrophages and neutrophils, leading to production of ROS, lipid peroxides, as well as pro-inflammatory mediators. NF-kB, nuclear factor kappa-light chain-enhancer of activated B cells; JNK, c-Jun N-terminal kinases.

The overall oxidative scenario of IBD is summarized in Figure 9. There are several potential mechanisms by which free radical production might alter tissue inflammation. It has been suggested that the sequence of events during ischemia-reperfusion starts with the generation of $^{\bullet}O_2^-$, followed by the activation of specific endothelial and neutrophil adhesion molecules; these events lead to microvascular injury, mediated by ROS produced by neutrophils, and by the activity of elastase and other proteases within the surrounding environment. In addition, the potential relevance of GSH in IBD has been suggested by animal studies, demonstrating that GSH depletion alters T-cell and macrophage function. As a further potential link between oxidative stress and gut inflammation, there is evidence supporting the concept that NO production modulates lymphocyte migration into both intestinal Peyer's patches and nonlymphoid intestinal regions (113, 161).

The most interesting and feasible strategy for managing IBD clinical symptoms is that of attenuating oxidative stress in

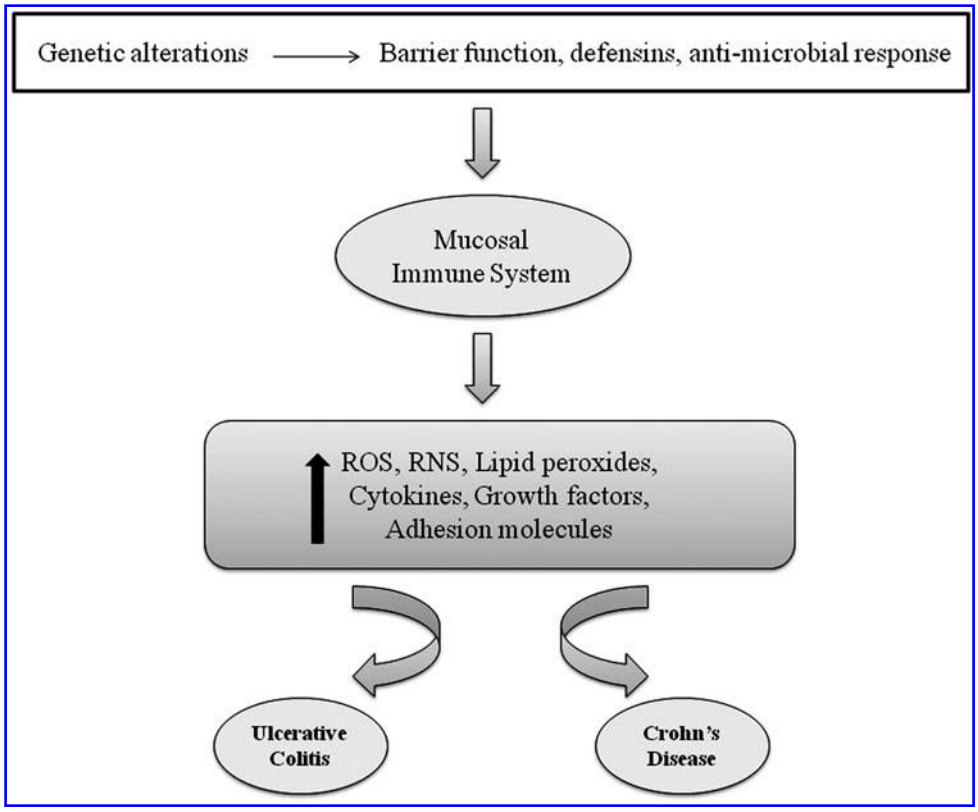

FIG. 9. Pathophysiology of IBD. Intestinal inflammation in IBD results from alteration in interaction between resident microbes and the mucosa. This can result from the influence of environmental factors and/or host factors, which vary depending on genetic predisposition affecting barrier function, defensins expressions, and innate and adaptive immunity toward microbes. As a consequence, mucosal immune system produces excessive reactive species as well as inflammatory mediators that determine UC and CD. CD, Crohn's disease; UC, ulcerative colitis.

patients, a therapeutic approach that has been carried out for 50 years. Commonly used drugs, in particular sulfasalazine and its active moiety 5-aminosalicylic acid, are potent free radical scavengers (179). In addition, there have been attempts to specifically prevent or attenuate intestinal oxidative stress through either the inhibition of ROS/RNS-producing enzymes, or scavenging of their products. Most investigations have been carried out in animal models of colitis and have been extensively reviewed elsewhere (142). Specific antioxidant trials in IBD patients are rare, and they all miss appropriate controls. Some of them have been even withdrawn. In fact, only two studies remain widely accepted, which in the mid-980s reported high positive response rates (>80% remission) when patients with severe CD or UC were treated with free or liposomal-encapsulated bovine Cu/Zn-SOD (190). Since then, no further SOD-based clinical trials in IBD patients have been reported. Obviously, the therapeutic exploitation of natural SOD has its limitations, in terms of limited cell permeability, short circulating half-life, immunogenicity, and cost of production. Several innovative antioxidants that have been recently developed have overcome (at least in part) these constraints, yet they await further clinical validation (228). Before we start introducing any drug with antioxidant activity into the gut, however, we must learn more about the status of the endogenous antioxidant defenses in the normal and inflamed intestinal mucosa. To date, data on the mucosal concentration, activity, and localization of the most important antioxidant enzymes in CD and UC are scarce or, at most, fragmentary. We also need to understand their association with parameters of oxidative damage. In combination with animal models designed to evaluate the functional relationship between the (transgenic) expression of antioxidant enzymes and the development and course of intestinal inflammation, these studies might give a fresh impulse to the application and development of antioxidant therapy for IBD.

F. Autoimmune diseases

Autoimmune diseases arise from an overactive self-response of the immune system against substances and tissues normally present in the body. This may be restricted to certain organs or involve a particular tissue in different places. There is an on-going discussion about when a disease should be considered autoimmune, leading to different criteria such as the Witebsky's postulates (223). These are a series of criteria developed by Ernst Witebsky, used to determine if a condition should be considered autoimmune: (i) direct evidence from transfer of pathogenic antibody or pathogenic T cells; (ii) indirect evidence based on reproduction of the autoimmune disease in experimental animals; and (iii) circumstantial evidence from clinical clues. Autoimmune diseases are complex diseases, where both genetic and environmental factors are involved. Excessive oxidative stress is thought to have an important role in their pathogenesis by promoting inflammation, inducing apoptotic cell death, and breaking down the immunological tolerance, as summarized in Figure 10. The role of apoptosis seems of major interest, because inefficient clearance of dying cells can result in the accumulation of apoptotic remnants, which can ultimately determine the formation of autoantibodies, thus triggering an autoimmune chain reaction. The most relevant disorders that belong to this group are discussed below.

1. Rheumatoid arthritis. Rheumatoid arthritis (RA) is a rather common autoimmune disease with an incidence of ~1% in Western world; it affects many tissues and organs, but most extensively synovial joints. RA produces an inflammatory response of the synovium (synovitis) that is secondary to hyperplasia of synovial cells, excessive synovial fluid, and development of a "pannus" in the synovium itself. The progress of the disease often leads to the destruction of articular cartilage and ankylosis of the joints, and in addition

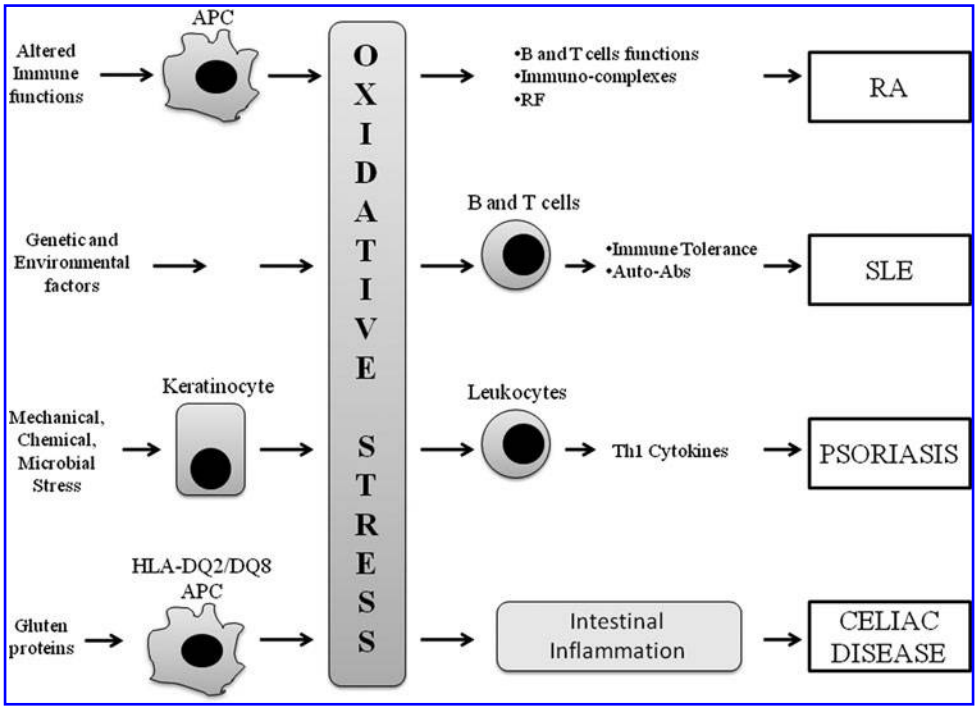

FIG. 10. Oxidative stress and autoimmune diseases. Schematic representation of the basic mechanisms underlying oxidative stress involvement in autoimmune diseases. Details are described in the text. Abs, antibodies; APC, antigen presenting cell; RA, Rheumatoid arthritis; RF, rheumatoid factor; SLE, Systemic lupus erythematosus; Th1, T-helper 1.

it can produce diffuse inflammation in the lungs, pericardium, pleura, and sclera, as well as nodular lesions that are most common in the subcutaneous tissue. The key features of RA pathogenesis are: (i) a genetic link with HLA-DR4 and related allotypes of major histocompatibility complex class II (MHC-II) proteins, and with the T cell-associated protein PTPN22; (ii) a causative link with cigarette smoking; (iii) a dramatic response to blockade of TNF-α and to depletion of B lymphocytes, without a comparable response to depletion of T lymphocytes; (iv) the presence of autoantibodies to IgGFc, known as rheumatoid factors (RF), and anticitrullinated peptides antibodies (ACPA) (71).

These data suggest that RA involves abnormal B cell-T cell interaction, with presentation of antigens by B cells to T cells *via* HLA-DR that triggers production of RF and ACPA. Inflammation is then driven either by B cell or T cell products, which stimulate release of TNF-α and other cytokines. If the release of TNF-α is stimulated by B cell products in the form of immuno-complexes containing RF or ACPA, RA can be seen as a form of type III hypersensitivity. If, instead, the release of TNF-α is stimulated by T cell products such as IL-17, RA might be considered closer to type IV hypersensitivity. However, this terminology may be getting somewhat outdated and useless. The debate on the relative roles of immune complexes and T cell products in RA has been going on for 30 years, but nowadays there is little doubt that both B and T cells are essential to the disease, though neither cell type appears necessary at the site of inflammation. This observation favors immuno-complexes (based on antibodies synthesized elsewhere) as the authentic initiators of RA, although they are not the sole propagators of the associated inflammation. TNF-α seems to be the dominant cytokine, but also other chemical mediators are likely to be involved in RA inflammation. Blockade of TNF-α is not beneficial to all patients or all tissues: lung disease and nodules may even get worse. Blockade of IL-1, IL-15, and IL-6 also has beneficial effects, and IL-17 may be of particular relevance (6). Further, as with most autoimmune diseases, it is necessary to distinguish between the factors that trigger the disease, and those that may permit its persistance and progression.

It is becoming clear that ROS play a role in autoimmunity. However, their exact role in the different phases of the immune response (*i.e.*, priming, expansion, and effector phase) is still unknown. When ROS are released extracellularly, matrix molecules like collagens and proteoglycans can be damaged and structurally modified. This might result in increased inflammation and immune activation against neo-epitopes in the joint during arthritis, in both experimental and human RA. Data obtained from rat and mouse models with polymorphic members of Nox complex p47phox showed that T cells play an essential role in the induction of arthritis, and indicate a role for ROS in T cell activation. However, T cells hardly or not at all produce Nox-dependent ROS; therefore, it is more likely that they are influenced by ROS produced by other cells. Among the latter the best candidates are the antigen-presenting cells (APC), which include dendritic cells, macrophages, and B cells (247). It is also likely that ROS produced by APC during antigen presentation affect molecules on T cell surface, or proteins linked to the membrane on the inner side, thereby affecting signal transduction and subsequent cell response. Another possibility is that ROS affect membrane composition of T cells, causing changes in membrane protein organization, and thereby affecting intracellular responses. A remarkable amount of ROS is produced in arthritic joints; therefore, many research efforts have tried to link T cell function with oxidative stress in RA patients (79). Remans and colleagues have recently reported a possible mechanism that explains how oxidative stress could influence T cells upon RA (221). They have shown that T cells from the synovial fluid have higher intracellular levels of ROS than peripheral T cells isolated from blood of the same patients. In addition, the intracellular GSH levels in T cells from synovial fluid were found to be decreased, whereas TRX was increased. In line with this, it was shown that depletion of intracellular GSH led to a shift toward a Th2 response, characterized by increased IL-4 production and inhibition of IFN-γ and IL-12 release (203). Also, a role for GSH in the prevention of apoptosis has been described, because GSH depletion makes the cellular environment more oxidizing, and hence more favorable for apoptosis induction. In fact, an oxidizing environment leads to apoptosis that shifts toward necrosis under intensively oxidizing conditions. It could be possible that the fate of a T cell is dependent on the level of ROS produced during cellular contacts in the thymus or at the periphery. This could cause both deletion of autoreactive T cells and/or induction of regulatory T cells. Very high levels of ROS induce T cell death, whereas intermediate or modest levels reduce T cell reactivity during autoantigen recognition; conversely, absence of ROS might promote T cell activation. In keeping with this, in RA patients the redox balance is shifted toward oxidation, and antioxidant levels are often significantly lower than in healthy controls or subjects suffering from osteoarthritis (79). Taken together, manipulation of the redox balance might be a successful therapeutic strategy to combat RA, underscoring the concept of an antioxidant treatment for this disease (Fig. 11).

TRX has been shown to inhibit antibody-induced arthritis in mice, when administered as recombinant protein or when overexpressed as a transgene, although the study was not genetically controlled. Retinoids (*e.g.*, derivatives of vitamin A) have immuno-modulatory effects and prevent ROS production in stimulated human polymorphonucleated cells (PMNs). Other well-known antioxidants like vitamin C and vitamin E have only low or no efficacy. Scavenging of ROS in the joint apparently prevents its destruction, but does not influence the immune response itself (79). Despite the general dogma that ROS have a damaging effect, experimental data suggest that induction of ROS production could actually be beneficial. Indeed, a natural compound (phytol) has been recently reported to induce, very efficiently and in a Nox-dependent manner, oxidative burst of neutrophils *in vitro* (194). Interestingly, administration of phytol to rats with an acute or chronic arthritis significantly decreased disease severity, due to a direct downregulation of arthritogenic T cells. These findings reveal novel possibilities for the identification of new therapeutics for the treatment of RA. Remarkably, these new therapeutic strategies could help RA patients with a defective oxidative burst, but also those with a functional Nox complex. It should be stressed that the possibility that Nox-activating pharmaceuticals can treat autoimmune conditions like RA opens new avenues for combined therapies, where compounds like phytol could be administered together with other disease-modifying antirheumatic drugs (DMARDS), which form a group of substances that are frequently used in the treatment of RA.

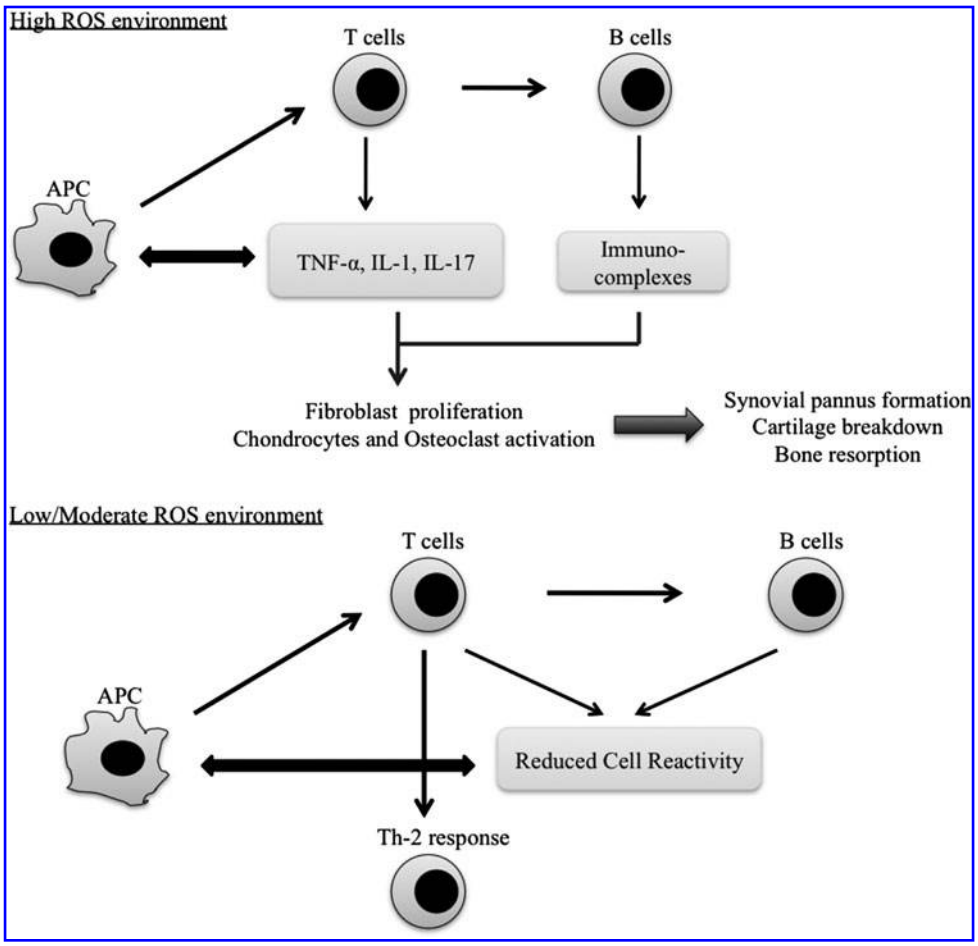

FIG. 11. Oxidative stress and rheumatoid arthritis. In an environment in which ROS levels are high, T and B cells become autoreactive and cause the activation of fibroblasts, chondrocytes, and osteoclasts. On the contrary, when ROS levels are low or moderate, such autoreactive responses are reduced, and T cells can be even polarized to Th-2. TNF-α, tumor necrosis factor-α; IL-1, interleukin-1; IL-17, interleukin-17; Th-2, T helper-2.

2. Systemic lupus erythematosus. Systemic lupus erythematosus (SLE) is a prototypic autoimmune disease characterized by the production of antibodies against components of the cell nucleus, in association with a diverse array of clinical manifestations. The primary pathologic findings in patients with SLE are inflammation, vasculitis, immune complex deposition, and vasculopathy. However, the exact etiology of SLE remains elusive. An extremely complicated and multifactorial interaction among various genetic and environmental factors is probably involved, and multiple genes may contribute to disease susceptibility. The interaction of sex, hormonal milieu, and the hypothalamo-pituitary-adrenal axis modifies this susceptibility, as well as the clinical symptoms of SLE (181). Defective immuno-regulatory mechanisms, such as the clearance of apoptotic cells and of immuno-complexes, are important contributors to the development of SLE. Loss of immune tolerance, increased antigenic load, excessive T cell activation, defective B cell suppression, and the shift of Th1 toward Th2 cytokines, all lead to B cell hyperactivity with production of pathogenic autoantibodies. Finally, certain environmental factors are probably required to trigger SLE and sustain its progression, though the actual role of redox stress remains to be clarified. Very recently, in fact, unbalance between oxidative stress and Th1-derived cytokines was proven to be one possible cause for the pathogenesis of SLE, because a significant increase in the level of lipid peroxidation, measured as MDA, and a significant reduction in antioxidant enzymes SOD, catalase, and GPx, was found in SLE patients (239). Further, literature data provide evidence that GSH is depleted in T cells from patients with SLE. This may be a key factor underlying abnormal activation and predisposition of T lymphocytes to pro-inflammatory necrotic cell death. An increase in the levels of MDA-modified proteins and a decrease in the concentration of thiol groups among SLE patients were also observed. Circulating autoantibodies in the blood of SLE patients exhibited a significantly enhanced reactivity against catalase and SOD-modified proteins. The same observations were reported in different protein fractions extracted from cultured cells. These findings further support the role of oxidative stress, and especially of lipid peroxidation products, in the progression of SLE (20). Thus, the view that markers of oxidative and nitrosative stress strongly correlate with SLE pathogenesis and disease activity has gained increasing recognition in the last few years (182, 279). In line with this, SLE patients exhibit elevated levels of various markers of protein oxidiation and reduced activity of antioxidant enzymes (294). Administration of NAC, which serves as a GSH precursor, and of cysteamine has been found to improve the clinical outcome of murine lupus, since both compounds suppressed mortality of female SLE mice, and additionally NAC suppressed autoantibody formation (255, 263). On this basis, a phase I-II NAC-based clinical trial of SLE was launched, and after 1 year of treatment patients showed reduced disease severity and reduced muscle fatigue, the latter being the most disabling symptom for ~50% of SLE patients (255). Altogether, these

findings suggest that oxidative stress plays a role in the pathogenesis of SLE and that antioxidants may be a beneficial adjunctive therapy in the treatment of this human disease. In this context, the possibility that SLE (and also RA) could develop due to intake of supplementary antioxidants (*e.g.*, vitamins A, C, and E, carotenes, and lycopene) was ruled out by epidemiological studies (51).

3. Psoriasis. Psoriasis is a chronic immune-mediated hyperproliferative inflammatory skin disease, and a dysregulation of cytokine networks is widely recognized as the main cause. Skin is a major target of oxidative stress, mainly because of ROS that originate from the environment and from skin metabolism. Complex cellular interactions among epidermal keratinocytes, mononuclear leukocytes, neutrophils, dendritic cells, and activated T cells, together with growth factors, chemokines, and cytokines, are involved in the development of psoriasis. Recent concepts of the pathogenesis of this disease have focused on the importance of the innate immune system and the role of dendritic cells, neutrophils, and antimicrobial peptides. Mechanical, chemical, or microbial stress to skin seems to initiate psoriasis on the basis of a genetic predisposition. Two fundamentally different cell types interact during the formation of a psoriatic lesion: epidermal keratinocytes and leukocytes. Keratinocytes probably act as active participants in the recruitment and activation of leukocytes into psoriatic lesions. Cytokine interactions in psoriasis have previously been illustrated as a "type 1 pathway," which assumes a linear relationship between proximal inducers like IL-23 or IL-12, production of IFN-γ and TNF-α by Th1 cells, and downstream activation of signal transducer and activator of transcription 1 (STAT1), as well as of NF-κB (159). The skin is constantly exposed to oxidative stress induced by ROS, which are generated from both endogenous sources and external pro-oxidant stimuli (164). Increased infiltration of PMNs in psoriatic lesions leads to the release of ROS *via* Nox/myeloperoxidase and protease activity, overall leading to lipid peroxidation and oxidative damage of cell membranes and proteins. Psoriatic lesional skin was shown to be positive for oxLDL staining, whereas there was no positive staining in nonlesional skin samples of the same individuals. Accumulation of oxLDL in psoriatic skin was thought to play an important role in the inflammatory events that cause progressive skin damage. Interestingly, psoriatic keratinocytes express inducible iNOS mRNA and protein, that in principle might lead to high-output NO synthesis from L-arginine, and hence to arrest of epidermal hyperproliferation. However, this pathway does not appear to be effective in psoriasis, where it has been clearly demonstrated that an abnormal overexpression of arginase 1 limits the availability of L-arginine by metabolizing it into L-ornithine and urea (1). Altogether, it can be suggested that L-arginine is a possible therapeutic agent to reduce psoriatic symptoms.

In plasma and erythrocytes of psoriatic patients, increased levels of MDA associated with decreased plasma β-carotene, GSH, and vitamin E, as well as catalase, SOD, and GPx activities in erythrocytes, were observed (285). Moreover, transduction pathways mediated by redox-sensitive proteins like MAPK activator protein-1, NFκB, and JNK–STAT have been shown to take part in the progress of psoriasis. More recently, the activation of peroxisome proliferator-activated receptors (PPARs), whose natural ligands are PUFAs and their oxidation products, has been recognized as a major event in psoriasis. PPARs contribute to the hyperproliferative phenotype by inducing the heparin-binding EGF-like growth factor, which is over-expressed in psoriasis and is known to cause epidermal hyperplasia (296). In line with this, antioxidants such as dimethylfumarate (DMF) have proven to be effective therapeutics against psoriasis, by increasing GSH levels in various tissues and exerting anti-inflammatory effects like downregulation of cytokines and adhesion molecules (292). In fact, a drug containing DMF as the main ingredient has been registered in Germany for oral therapy of moderate to severe psoriasis since 1994. Interestingly, a new DMF monosubstance preparation has been recently found beneficial also in a phase II clinical trial in relapsing–remitting MS, and activation of the Nrf2 transcriptional pathway that controls phase 2 detoxifying enzyme gene expression (that is crucial for antioxidative responses) was proposed as the main mechanism of action (129).

Also, the vitamin A derivative tazarotene may be used for plaque psoriasis. Although it can produce longer remissions than topical steroids, the induction of local irritation, the high cost, and teratogenicity limit its use (260). More recently, supplementation with antioxidants like coenzyme Q_{10}, vitamin E, and selenium has been found to be beneficial for the management of patients with severe forms of psoriasis, in terms of significant improvement of clinical conditions; in fact, a faster normalization of oxidative stress markers like superoxide production, Cu/Zn-SOD, and catalase activity was observed in circulating granulocytes and epidermis *versus* placebo, and also plasma levels of nitrites/nitrates were improved in treated *versus* untreated subjects (133). Rottlerin is a natural polyphenolic compound that was initially marketed as a PKCδ inhibitor, and more recently was patented as an antihypertensive drug. Further *in vitro* results suggest a potential use of rottlerin also in the treatment/control of psoriasis, demonstrating that this compound is an antioxidant and a potent inhibitor of NF-kB that regulates cell cycle and apoptosis in keratinocytes (169). Another clinically promising agent for the treatment of psoriasis is curcumin, a natural antioxidant that exerts pleiotropic activities (105). Finally, psirelax is an emerging topical medication to treat patients with psoriasis, and its formulation includes a 55%–75% mixture of natural antioxidants (*e.g.*, vitamin E, wheat germ oil, and safflower oil), natural skin softening agents (*e.g.*, sweet almond oil and sesame oil), and natural preservatives (*e.g.*, paraben, tea trea essential oil, thyme essential oil, grapefruit seed extract, and vitamin E) (http://clinicaltrials.gov/ct2/show/NCT01000714).

4. Celiac disease. Celiac disease is a common autoimmune disorder of the small intestine that is mainly triggered and maintained by the storage proteins (gluten) of wheat, barley, and rye in genetically predisposed individuals. Patients display various degrees of intestinal inflammation, ranging from mere intraepithelial lymphocytosis to severe subepithelial (lamina propria) mononuclear cell infiltration, with total villous atrophy coupled with crypt hyperplasia. Celiac disease has become one of the best-understood HLA-linked disorders. Although it shares many immunologic features with IBD, celiac disease is uniquely characterized by: (i) a defined trigger (gluten proteins from wheat and related cereals); (ii) the necessary presence of HLA-DQ2 or HLA-

DQ8; and (iii) the generation of circulating autoantibodies against the enzyme tissue transglutaminase-2 (TG2), which deamidates certain gluten peptides, thus increasing their affinity for HLA-DQ2 or HLA-DQ8. This generates a more vigorous $CD4^+$ T-helper 1 activation, which can result in intestinal mucosal inflammation, malabsorption, and several secondary symptoms and autoimmune diseases. Moreover, gluten elicits innate immune responses that act in concert with the adaptive immunity. Among the nutrients that are less absorbed during celiac disease are vitamins, in particular A, D, E, and K. Further, individuals with celiac disease show a major reduction in their antioxidant levels, due to a reduction in GSH, GPx, and GR (234), as well as high levels of prooxidant molecules, especially lipid peroxides (252). Further, the inflammatory damage of the small intestine causes selenium deficiency, thus impairing the activity of selenoproteins involved in oxidative stress, such as GPx and TR (249).

Exclusion of gluten from the diet reverses many disease manifestations, and a life-long gluten-free diet remains at present the only treatment of celiac disease. However, food restriction is difficult to maintain in the long term, and can lead to social isolation because modern diets are largely based on gluten-containing food. Such a restriction could be poorly efficient or even totally ineffective in patients with refractory celiac disease or associated autoimmune diseases. A recent study has shown that an isomer of conjugated linoleic acid activates antioxidants and cytoprotective phase 2 enzymes, and downregulates maturation of dendritic cells from a murine model of celiac disease, suggesting new opportunities for dietary intervention (22).

To date, the use of natural antioxidants and appropriate dietary supplements may offer some benefits and could be an important complement to the classic therapy of celiac disease, thus deserving further investigations.

G. Neurodegenerative diseases

The central nervous system (CNS) is particularly sensitive to oxidative stress. One reason for this peculiarity is the high O_2 consumption by CNS, which accounts for $\sim$20% of the total amount under basal conditions, and is used to metabolize $\sim 4 \times 10^{21}$ molecules of glucose per minute. A second reason is the high production of ROS and RNS, which originate from specific neurochemical reactions (*e.g.*, dopamine oxidation), in addition to the sources already discussed in section II. A third reason is the increasing deposition of metal ions in the brain with aging. Neurons are postmitotic cells, and their general inability to divide explains loss of function of the brain, as neurons die without being replaced. Among mechanisms for neuronal death, apoptosis, and excitotoxicity play a pivotal role, and ROS and RNS are involved in both processes (17). However, oxidative stress is not the primary cause of neurodegenerative diseases, which instead are mainly caused by mitochondrial impairment. Apart from this component, neurodegenerative diseases can be classified as: (i) single-gene diseases, like Huntington's disease and Friedreich's ataxia; (ii) diseases following patterns of Mendel's laws (*i.e.*, heterozygote gene diseases), like familial amyotrophic lateral sclerosis (ALS), familial Parkinson's disease (PD), and familial Alzheimer's disease (AD); (iii) diseases resulting from a complex interaction among multiple predisposing genes (*i.e.*, multigenic diseases), among which sporadic AD or PD are more common. Their onset occurs later in life than familial forms, and environmental contributions and chance occurrences could play a role; the mechanisms from gene to disease seem to have common features, as observed in SOD1-G93A transgenic mice (a model of ALS that is discussed below); (iv) diseases due to protein misfolding and aggregation, for example, of Aβ42 protein in AD; and (v) diseases due to proteasome dysfunction on ubiquitinated material (ubiquitin forms covalent bonds with other proteins, to mark them for degradation by an ATP-dependent, nonlysosomial proteolytic system called "proteasome") (177, 265). The basic mechanisms of oxidative stress-induced neurodegenerative diseases are summarized in Figure 12.

1. Alzheimer's disease. Alzheimer's disease (AD) is a neurodegenerative disorder characterized by a progressive decline in cognitive function and extensive neuronal loss. The brain of affected patients shows numerous amyloid plaques and neurofibrillary tangles that are the two histological hallmarks of AD and two of the probable causes of its pathogenesis. They are deposits of proteins distributed throughout the brain of AD patients, particularly in the entorhinal cortex, hippocampus, temporal, frontal, and inferior parietal lobes. Amyloid plaques are primarily composed of aggregates of β-amyloid (Aβ), as well as other protein aggregates (*e.g.*, hyperphosphorylated Tau, ubiquitin, and presenilins 1 and 2), whereas neurofibrillary tangles are aggregates of hyperphosphorylated Tau protein (143). The production of ROS and its involvement in AD pathogenesis are supported by the significant amount of lipid peroxidation detected in the brain of AD patients, as well as by the increased levels of HNE found postmortem in their cerebrospinal fluid (CSF). Further, β-amyloid-induced damage promotes the generation of ROS, thus contributing to cell death and neurodegeneration, and induces also glial recruitment and activation, thus triggering local inflammation. Further, oxidative stress promotes abortive cell cycle re-entry, and hence apoptosis, of nerve cells of the adult brain, and gene duplication without cell division, leading to aneuploidy and DNA damage (207). In addition, oxidative stress can damage DNA, leading to strand breaks and large deletions, and can affect various enzymatic and mitogenic pathways, like those triggered by EGF and VEGF, stress-activated protein kinases JNK and p38, JAK/STAT, protein kinase C, and histone deacetylase. Interestingly, oxidative stress has been shown to decrease neurogenesis in the adult brain, thus limiting its neuroregenerative capacity (297). A summary of AD pathogenesis is shown in Figure 13. For the treatment of AD, the only strategy aimed not only at reducing the deleterious activities of ROS, but also at promoting the regenerative capacity of the adult brain, is the use of antioxidants. These drugs have been experimented in rodent models of AD, and include acetylcholinesterase inhibitors (galantamine and tacrine) and *N*-methyl-*D*-aspartate-glutamate receptor (NMDA-R) inhibitors, like memantine (261). The reduction of oxidative stress has been tested as a therapy for AD. Ionic zinc and copper are able to accelerate the aggregation of Aβ, the main component of β-amyloid deposits, and to promote its neurotoxic redox activity, inducing oxidative cross-linking of the peptide into stable oligomers (the so-called metal hypothesis of Alzheimer's disease) (39). Therefore, increasingly sophisticated pharmaceutical approaches are now being implemented, to attenuate abnormal Aβ-metal

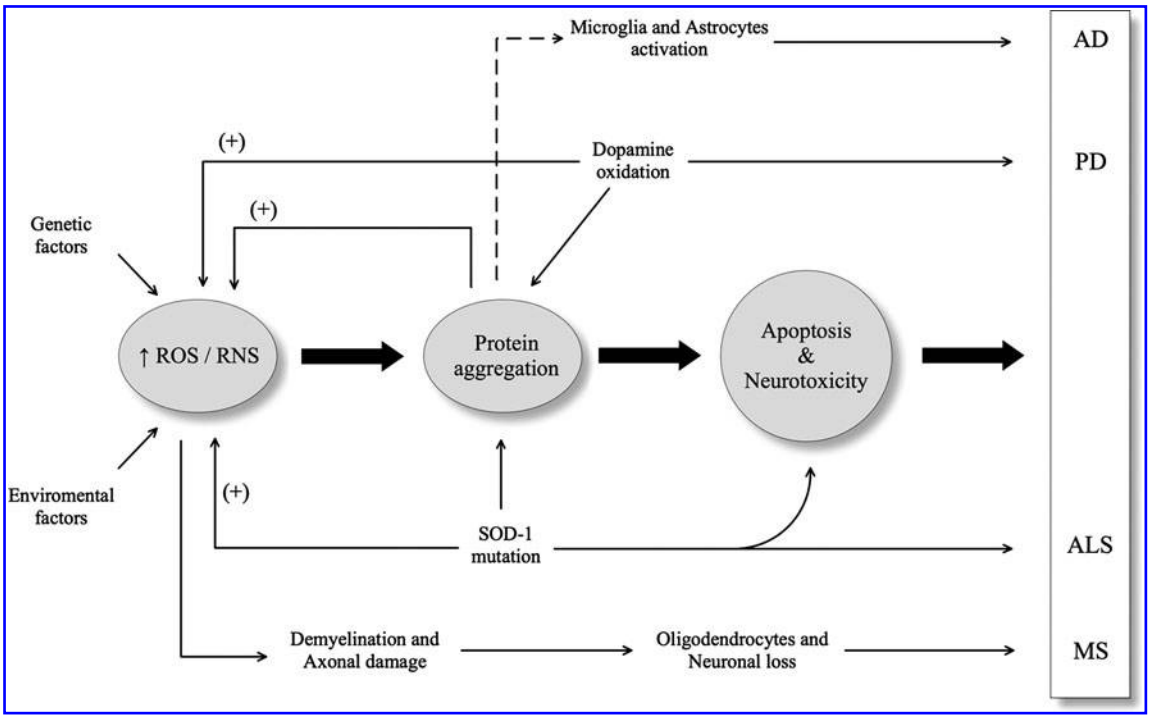

FIG. 12. Oxidative stress and neurodegenerative diseases. Schematic representation of the basic mechanisms underlying oxidative stress involvement in neurodegenerative diseases. Details are described in the text. AD, Alzheimer's disease; ALS, amyotrophic lateral sclerosis; MS, multiple sclerosis; PD, Parkinson's disease.

interactions without causing systemic disturbance of essential metals. Small molecules targeting these interactions, such as PBT2, are currently under clinical trials, and hold promise as disease-modifying agents for AD based on the metal hypothesis (39). Indeed PBT2, a zinc/copper ionophore, has been shown to facilitate the clearance of Aβ aggregates in the cortex of animal models of AD. The ionophoric properties of PBT2 liberate copper and zinc ions trapped by amyloid, facilitating the reuptake of these essential metal ions into cells and hence promoting memory functions such as long-term potentiation. Oral PBT2 treatment is strikingly effective in transgenic mouse models of AD, markedly improving learning and memory within days, accompanied by a reduction in interstitial Aβ content (2). Also, the redox-sensitive AGE/RAGE pathway has been recently exploited as a potential therapeutic target for AD (167).

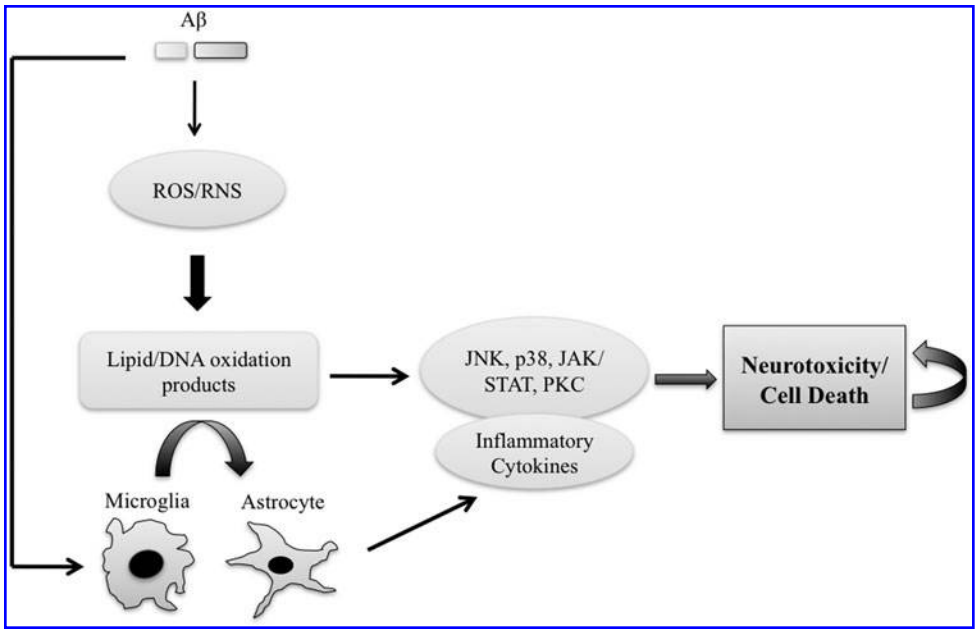

FIG. 13. Oxidative stress and Alzheimer's disease. Aβ-induced oxidative stress induces microglia and astrocytes activation, triggering local inflammation and ultimately neuronal cell death. Aβ, β-amyloid; JAK, Janus kinase; JNK, c-Jun N-terminal kinases; PKC, protein kinase C; STAT, signal transducer and activator of transcription.

Overall, among the promising agents to prevent AD are: (i) aged garlic extract, (ii) curcumin, (iii) melatonin, (iv) resveratrol, (v) *Ginkgo biloba* extracts, (vi) green tea, (vii) vitamin C, and (viii) vitamin E. While the clinical value of antioxidants for the prevention of AD is often elusive, some of these compounds can be recommended based upon: (i) epidemiological evidence, (ii) known benefits for prevention of other maladies, and (iii) benign nature of the substance. Yet, further long-term studies can be recommended, to better understand their mode of action (75).

Vitamin E supplementation in moderately severe AD is to date the most promising approach, but it reveals the limitations of general antioxidant therapies: they simply lower oxidative stress and, therefore, the complexity of the redox system. The multiple contributing factors that foster the clinical manifestations of AD should be unravelled, to design really effective antioxidative therapies (61, 149). Thus, further studies will be needed to identify and validate antioxidants as beneficial drugs for treating AD, particularly in relation to their effects on adult neurogenesis.

2. Parkinson's disease. Parkinson's disease (PD) is a common neurodegenerative disorder that produces muscular rigidity, bradykinesia, tremor of resting limbs, and loss of postural balance. The basic neuropathology of PD involves degeneration of pigmented neurons in substantia nigra, resulting in depletion of striatal dopamine (DA) and its metabolites. The pathologic hallmarks of PD are large cytoplasmic inclusions called Lewy bodies, which occur predominantly in the melanin-containing neurons of substantia nigra pars compacta (SNpc), and contain aggregates of α-synuclein. Another gene encoding for a protein termed parkin is involved in autosomal recessive Parkinsonism. Parkin is one member of the family of ubiquitin ligases, and may be involved in normal turnover of α-synuclein. Although the exact cause of PD is still obscure, both environmental and genetic factors have been implicated in its pathogenesis. For instance, epidemiological studies have suggested a possible link between pesticide exposure and PD symptoms (124). Recent evidence points toward a putative role of mitochondrial dysfunction and oxidative stress in the pathogenesis of PD, and analysis of postmortem brains from PD patients suggests an important role for them in degeneration of the pigmented dopaminergic neurons in SNpc (41). Apparently, there is a specific chemical fingerprint indicative of the damaging oxidative events, that is, higher levels of cholesterol hydroperoxide, MDA, and protein adducts of HNE and of OH8dG, which point to the presence of ROS-induced DNA nicks. One of the suggested causes of oxidative stress in the SNpc is the production of ROS during normal DA metabolism. In human SNpc, the oxidation products of DA may polymerize to form neuromelanin, which may also be toxic by inducing programmed cell death (23). Further, according to postmortem studies the SNpc of PD patients shows a significant (~60%) reduction in GSH and a moderate (~30%) increase in GSSG levels. This could be a critical primary event that weakens or abrogates the natural antioxidative defense of the cell, thereby triggering degeneration of the nigral neurons and causing PD (271). Since dysregulation of metal ion homeostasis is a potential catalyst to further produce reactive species, the highly oxidative environment for DA interaction with α-synuclein, and the resulting oxidant-mediated toxicity and protein aggregation, is one of the most likely underlying mechanisms for PD. Thus, the distruction of neuronal cells occurs as a result of self-propagating reactions that involve DA, α-synuclein, and redox-active metals (Fig. 14).

Over the last decade, neuroprotective approaches for PD have been tried with the aim of slowing the rate of disease progression by decreasing oxidative stress. There has been much interest in the use of supplemental vitamin E as an antioxidant able to prevent or slow down the progression of PD, through inhibition of nigral cell death. In one study, oral intake of high doses of vitamin E (400–4000 IU/die for 5 months) failed to increase the levels of the vitamin in CSF of patients (199). However, these subjects had already presented with clinical symptoms of PD when vitamin E was administered; therefore, >80% of the critically important neurons in the SNpc were already lost at the beginning of treatment. Instead, regular consumption of vitamin E-rich food during life may have the potential to decrease the risk or delay the onset of PD (86). In one population-based case–control study of the possible association of food or supplement dietary intake of vitamins A, C, and E (all endowed with antioxidant activity) and PD, no significant differences were observed between patients and control subjects. Another study investigated whether a high dietary intake of vitamin E (10 mg/die), β-carotene (1 mg/die), vitamin C (100 mg/die), and flavonoids (10 mg/die) could decrease the risk of PD, and found that individuals with higher vitamin E intake developed PD significantly less often than those with lower vitamin E intake. Also, intake of β-carotene inversely correlated with PD, but not in a statistically significant manner, whereas intake of vitamin C and flavonoids was not beneficial at all. Taken together, the authors concluded that a high intake of dietary antioxidant supplements may protect against the occurrence of PD (84). The use of melatonin and α-lipoic acid has also been investigated for PD treatment, but their effects, though promising, were not fully characterized. Further, in an open-label clinical study, GSH was administered to early and untreated patients, showing positive results; however, the study sample was small, and did not include proper controls; therefore, its conclusions require further confirmation (237).

Another antioxidant with a therapeutic potential is coenzyme Q_{10}, and indeed a phase II clinical trial of PD showed a ~45% reduction in motor deficits at a maximal dose of 1200 mg per day (242). However, a subsequent study that included a withdrawal phase on patients with middle stage PD failed to observe significant motor improvements at a dose of 300 mg per day (253). It was concluded that the effective dosage of coenzyme Q_{10} can vary with the disease stage. Another investigation tested the combination of coenzyme Q_{10} and creatine in a mouse model of PD, documenting an additive neuroprotective effects against DA depletion in the striatum and against loss of tyrosine hydroxylase neurons in the SNpc, as well as reduction in lipid peroxidation and pathologic accumulation of α-synuclein in the same SNpc neurons (290). In addition, modulation of biometals holds promise for the treatment of PD. In fact, controlling the bioavailability of metals could prevent not only the increase of oxidative stress through metallo-redox reactions, but also their interaction with other proteins like α-synuclein. In this context, novel therapies have been directed toward metal-associated targets, or aim at chelating metals themselves (80). These new drugs are referred to as "metal protein attenuating

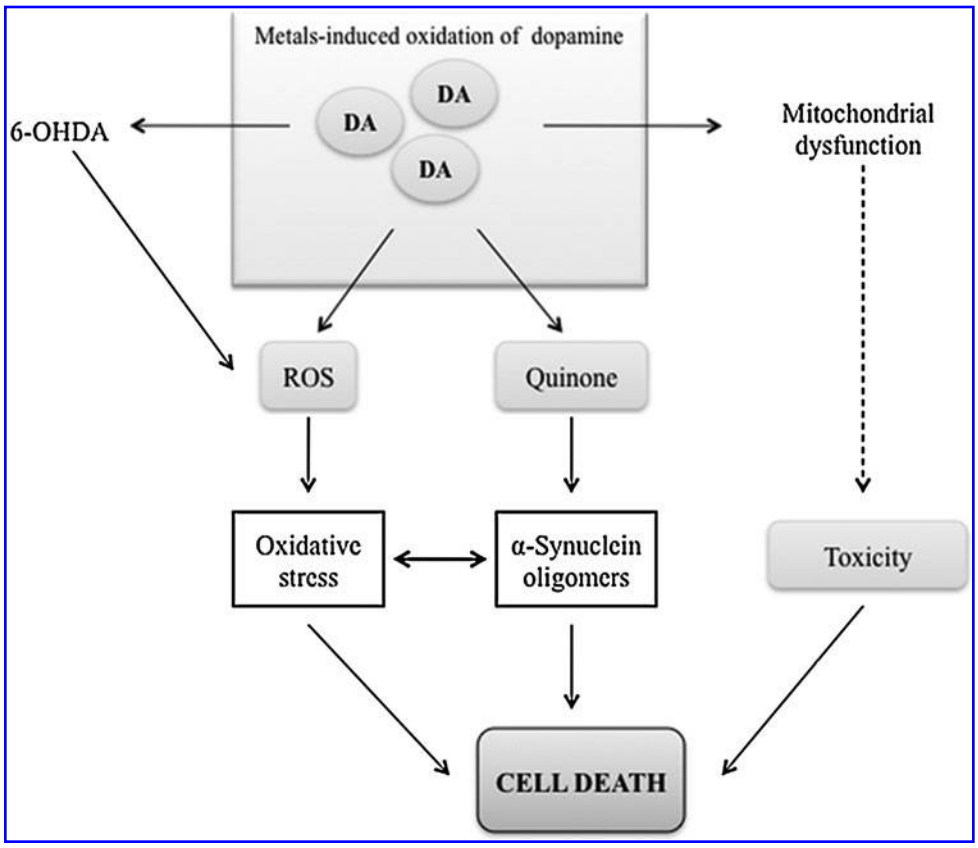

FIG. 14. Oxidative stress and Parkinson's disease. Dopamine-induced oxidative stress contributes to accumulation of α-synuclein oligomers or directly causes cell damage of neuronal cells. Dysregulated oxidation of dopamine seems also to be involved in mitochondrial dysfunction. DA, dopamine.

compounds (MPAC)," which may interfere with DA, α-synuclein and redox-active metals. Clioquinol (5-chloro-7-iodo-8-hydroxyquinoline, CQ) is the prototype MPAC and acts by competing with proteins for metal ions (64). Treatment of animals with CQ for 8 weeks before induction of PD lesions resulted in ~50% decrease in nigral cell loss (131). Further, cultured neuronal cells that express the human A30P mutant α-synuclein were rescued by either catalase or CQ, suggesting that MPAC represent indeed a new pharmacologic opportunity to slow down the progression of PD (280).

3. Amyotrophic lateral sclerosis. Amyotrophic lateral sclerosis (ALS) is a neurodegenerative disease that affects primarily motor neurons in the spinal cord and brain stem, ultimately leading to progressive weakness and atrophy of skeletal muscles, weakness of chest muscles and diaphragm, and dysfunction of the larynx and pharynx, thus leading to respiratory problems, bronchopneumonia, and death. Approximately 10% of the cases are inherited in an autosomal dominant manner, and 1/5 of these familial ALS patients carry mutations in the Cu/Zn-SOD (SOD-1) gene, suggesting an involvement of ROS. The toxicity of mutant SOD-1 seems to be due to a gain of function of the altered enzyme, in such a way that its catalytic activity is enhanced with abnormal substrates like $ONOO^-$; this gain sustains nitration of tyrosine, and subsequent oxidative stress (81). The latter may also be related to impaired ability of mutant SOD-1 to bind zinc, because *in vivo* the mutant enzyme is likely to denature more quickly than does the normal form, and releases zinc ions along with the potentially pro-oxidant copper ions. Oxidative stress may also be involved in misfolding of mutant SOD-1, to yield abnormal protein aggregates that can be found as early as in 1-month-old SOD1-G93A mice (240). Also, the disorganization of intermediate filaments could be due to mutant SOD-1-induced toxicity, as these cytoskeletal proteins are vulnerable to oxidative damage. A particular intermediate filament, peripherin, has been found in neuronal inclusions of patients with sporadic ALS, as well as in transgenic mice with SOD-1 mutations. In addition protein carbonyl and nitrotyrosine modifications, that are indexes of protein oxidation, was found to be elevated by ~85% in patients with sporadic ALS compared to controls, suggesting that oxidative stress may indeed be involved in all types of ALS (53). Other mechanisms that have been implied in ALS, such as excitotoxicity and defective axonal transport, may be consequences of oxidative stress. What remains as yet unclear is whether this increased redox-stress is a primary defect or a secondary consequence of the disease. A recent study has demonstrated that SOD1-G93A ALS transgenic mice produce elevated levels of Nox2 and $^{\bullet}O_2^-$ in spinal cord microglia (286). However, the mechanisms whereby mutations in SOD-1 lead to dysregulation of $^{\bullet}O_2^-$ production by Nox2 remain poorly understood. It can be proposed that inflammation produces ROS *via* Nox2 activation in microglia, and this leads to further injury in a vicious cycle that is repeated until death occurs. In the context of the same pathogenetic model, it is important to understand how Nox enzymes control pro-inflammatory signaling that is relevant for the progression of ALS, such as that mediated by IL-1β and TNF-α. Also, the redox-dependent processes that control NF-kB activation by these cytokines have been shown to engage Nox (specifically Nox1 and Nox2) through the intervention of redox-active signaling endosomes named

"redoxosomes" (40). A controlled production of ROS and the subsequent NF-kB activation by redoxosomes is dependent on the uncoupling of SOD1 from these structures; however, the redox-dependent uncoupling appears to be defective in certain ALS-associated SOD-1 mutants, and may lead to increased redox stress when ligands trigger redoxosomal pathways (40). Finally, levels of MDA, an index of lipid oxidation, increased over time in mutant SOD-1 mice, as compared to controls (96). In patients with sporadic ALS there was a marked elevation over healthy controls in plasma 2-thiobarbituric reactive substances, which are also well-known products of lipid peroxidation. However, plasma concentrations of antioxidants like α-tocopherol, β-carotene, ubiquinol-10, and GSH, and SOD activity in red blood cells were not significantly different between ALS patients and healthy subjects (196).

In the context of therapeutic approaches against ALS, several antioxidants have been checked as potential means to manage this disease; these substances include NAC, vitamins C and E, *N*-acetylmethionine (NAM), dithiothreitol, or its isomer dithioerythritol. Patients with a history of heavy exposure to metals were also given meso-2,3-dimercaptosuccinic acid, and none of these antioxidants was harmful, but did not prolong patient survival either (278). So far, two antioxidants are still used in clinical trials. The manganese porphyrin AEOL-10150 markedly prolonged survival of symptomatic ALS mice (31, 55). Two phase I studies that tested the safety of AEOL-10150 have demonstrated that administration of this drug at doses up to 2 mg/kg per day is safe and well-tolerated, with an excellent pharmacokinetic profile in ALS subjects (195). The second antioxidant currently under study is KNS-760704, which confers protection against oxidative stress-related neurotoxic cascades in ALS (92).

Novel approaches to shut down the production of mutant SOD-1 are under development for potential benefit of those patients bearing this mutation (245). However, effective treatments for ALS patients have remained as yet elusive, despite some efficacy in animal models (62). Interestingly, a recent study suggests that most of the data on mutant SOD-1 transgenic mice may actually reflect noise in survival distribution (236), thus questioning even the suitability of this animal model to mimic human sporadic ALS. It can be proposed that inhibiting a specific biological target like Nox2 with potent and selective inhibitors could represent a more effective strategy to combat ALS in the future.

4. Multiple sclerosis. Multiple sclerosis (MS) is an inflammatory, demyelinating disease of the CNS that begins most often in late adolescence and early adult life. MS is characterized by perivenous infiltration of lymphocytes and APC into the parenchyma of the brain, and is characterized by different forms: primary progressive (PPMS), relapsing progressive (RPMS), secondary progressive (SPMS), and relapsing remitting (RRMS); the latter is the most prevalent form that represents $\sim$80% of all cases (191). Genetic factors such as expression levels of IL-1β, IL-1 receptor, Apolipoprotein-E1, and HLA-DR2, which were found to potentially increase the risk of MS, and DQ polymorphisms are not associated with the course and severity of the disease, despite their substantial contribution to its susceptibility. On the other hand, environmental factors such as pathogens or chemical pollutants have also been suggested as causes of MS, and it is likely that both genetic and environmental components play a role in disease onset and development (85). The etiology of MS has not been fully elucidated yet, but it is believed that immunological mechanisms are the most important cause of disease initiation and progression (250). Binding of putative MS antigens, especially components of myelin like myelin basic protein (MBP), myelin-associated basic glycoprotein (MOBP), myelin oligodendrocyte glycoprotein (MOG), to targets like the trimolecular complex, the T-cell receptor (TCR) and MHC-II proteins on APC, may trigger either an enhanced immune response against the bound antigens or anergy (191). Indeed, autoantibodies against MBP and MOG have been found in MS patients (67). In addition to the autoimmune response, oligodendrocyte death, axon damage, and even neuronal loss have been associated with the inflammatory attack of CNS that occurs in the course of MS (60, 72).

Accumulated evidence indicates that oxidative stress plays a major role in the pathogenesis of MS. ROS and RNS are mainly generated in excess by activated microglia, and have been implicated as mediators of demyelination and axonal damage typical of MS (26). In addition, free radicals can activate certain transcription factors, like NF-κB, which upregulate the expression of many genes involved in human MS and in its EAE (experimental autoimmune encephalomyelitis) animal model: TNF-α, iNOS, ICAM-1, and VCAM-1 are some of them (281). Additionally, redox reactions are involved in the activity of matrix metalloproteinases (MMPs), which are important to T cell trafficking into the CNS (152). Earlier studies have found evidence of lipid peroxidation in the CSF and plasma of MS patients. Further, a weakened cellular antioxidant defense, especially due to impairment of SOD and GPx, was found in red blood cells of these subjects. Another study documented that oxidative damage of CNS was provoked by the release of iron from injured cells, and by low levels of enzymatic and nonenzymatic antioxidants (particularly ubiquinone and vitamin E) in plasma and lymphocytes of MS patients (257). In addition, Karg and colleagues (130) found a $\sim$40% increase in lipid peroxidation, elevated levels of GSSG, and reduced vitamin E:lipid ratio in plasma during the active phase of MS. Analysis of CSF showed also a significantly higher concentration of isoprostanes and MDA, along with increased GR activity and decreased GPx activity. Moreover, direct examination of MS plaques revealed an increase in free radical activity, and decreased levels of relevant antioxidants like GSH, α-tocopherol, and uric acid (146). Further, Vladimirova and colleagues demonstrated that activated mononuclear cells of MS patients produce high amounts of ROS and $NO^{\bullet}$, and that oxidative damage to DNA (mitochondrial DNA included) develops in association with inflammation in chronic active plaques (277). The involvement of the redox- and immuno-mediated neuronal cytotoxicity in MS is schematically represented in Figure 15.

Different mechanisms have been proposed to explain how low levels of antioxidants or high levels of ROS might specifically contribute to CNS damage in MS. For instance, lower levels of antioxidants may promote increased activity of lipoxygenase (that catalyzes one branch of the arachidonate cascade) (115, 166), thereby increasing the immuno-inflammatory processes within the brain. In line with this, others have suggested that excessive ROS can stimulate T-cell activity *via* the arachidonate cascade, or they can produce

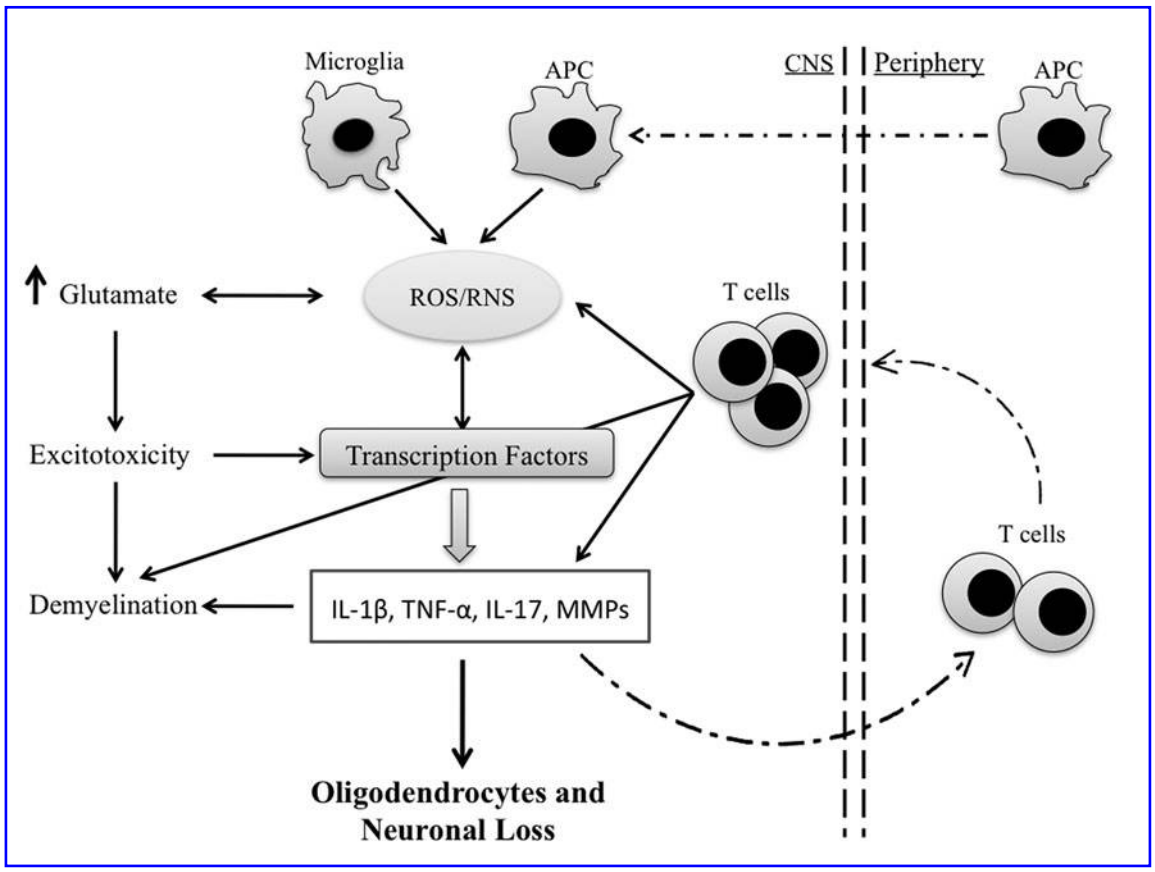

FIG. 15. Oxidative stress and multiple sclerosis. In the CNS the production of ROS/RNS by activated microglia or infiltrating macrophages or dendritic cells causes direct or glutamate-induced demyelination or production of pro-inflammatory mediators, which contribute to the recruitment of autoreactive T cells from the periphery. APC, antigen presenting cells; CNS, central nervous system; IL-1β, interleukin-1β; MMPs, metalloproteinases.

direct/indirect damage to the blood–brain barrier (BBB), or to myelin (50). Because of the pathogenic role of ROS and RNS in MS pathology, antioxidants might prevent free radical-mediated tissue destruction, and inhibit some of the early pro-inflammatory events such as T-cell activation and trafficking into the CNS, ultimately leading to inflammation and tissue destruction. Overall, treatment with antioxidants might in principle prevent propagation of tissue damage and improve both survival and neurological outcome of the disease. It should be recalled that the main problem in the treatment of neurodegenerative diseases (MS included) is to develop drugs able to reach the brain by crossing the BBB, which is the major obstacle between the brain microenvironment and peripheral blood. It is BBB that reduces the efficacy of antioxidants, much alike that of several other potential therapeutics. Incidentally, design of novel antioxidant drugs that enables their transport through BBB will depend on new knowledge of BBB structure and transport mechanism. In this context, Hall and colleagues found that the antioxidant tirilazade mesylate, a member of the lazaroids family that is known to scavenge lipid peroxyl radicals and to inhibit iron-dependent lipid peroxidation, was effective in reducing the incidence and severity of acute EAE (95). Another study showed that oral administration of NAC, which can effectively raise intracellular GSH levels, inhibited the induction of acute EAE. This protection was associated with enhancement of the specific lymphocyte proliferative response to the immunizing antigens (like spinal cord homogenate) at early stages (150). A synthetic salen-manganese complex (Euk-8) may be regarded as a lead compound for a new class of synthetic catalytic scavengers with combined SOD and catalase activity. Repeated injection of Euk-8 at the time of EAE induction delayed the onset and markedly reduced the severity of the disease in mice (170), supporting hopes for the treatment of human MS. Recently, it was shown that α-lipoic acid, a well-known antioxidant that is able to cross the BBB, suppressed inflammation, demyelination, and axonal damage in EAE mice when supplied in the diet; its effect occurred by inhibiting T cell trafficking into the spinal cord, perhaps through the inhibition of MMP-9 activity (176). Furthermore, inhibition of the inducible/inflammatory form of iNOS, as well as the use of antisense iNOS knockdown animals, suppressed EAE in rodents. Moreover, Hooper and colleagues demonstrated that inhibition of iNOS, or scavenging of $NO^{\bullet}$ or $ONOO^{-}$ by uric acid, inhibited neurological deficits in mice with EAE, whereas withdrawal of iNOS inhibitors resulted in the appearance of neurological signs within 24 h (114). In this context, the administration of uric acid precursors has been demonstrated to be effective in EAE mice (235). In contrast, recent data have shown that inhibition of $NO^{\bullet}$ production is lethal; thus, it is not yet clear whether it can be beneficial to treat EAE. Based on encouraging findings in EAE animals, it

Table 4. Biomarkers of Oxidative Damage Associated with Chronic Inflammatory Disorders

Disease/Biomarker		
Malignant disease	Rheumatoid arthritis	Alzheimer's disease
MDA	F_2-isoprostanes	MDA
GSH/GSSG ratio	GSH/GSSG ratio	HNE
8-OH-dG	Systemic lupus erythematosus	GSH/GSSG ratio
NO_2-Tyr	MDA	F_2-isoprostanes
Diabetes mellitus	GSH/GSSG ratio	NO_2-Tyr
MDA	Psoriasis	AGE
GSH/GSSG ratio	MDA	Parkinson's disease
NO_2-Tyr	GSH/GSSG ratio	HNE
AGE	Celiac disease	GSH/GSSG ratio
F_2-isoprostanes	GSH/GSSG ratio	Carbonylated proteins
Atherosclerosis		Iron levels
MDA		Amyotrophic lateral sclerosis
HNE		MDA
F_2-isoprotanes		Carbonylated proteins
NO_2-Tyr		NO_2-Tyr
Chronic obstructive pulmonary disease		GSH/GSSG ratio
MDA		Multiple sclerosis
HNE		F_2-isoprostanes
F_2-isoprostanes		MDA
GSH/GSSG ratio		GSH/GSSG ratio
Inflammatory bowel disease		
GSH/GSSG ratio		

MDA, malondialdehyde; HNE, 4-hydroxy-2-nonenal; AGE, advanced glycation end products; 8-OH-dG, 8-hydroxy-20-deoxyguanosine; GSH, reduced glutathione; GSSG, oxidized glutathione; NO_2-Tyr, 3-nitro-tyrosine.

has been suggested that dietary antioxidant intake, for example, of vitamin E or selenium, may help to inhibit MS progression (110). However, Jensen and colleagues showed that antioxidant supplementation did increase and normalize GPx activity, as well as linoleic acid content in erythrocytes and hematogenous cells within 3 weeks, yet with no effect on MS severity (126). Therefore, despite media reports of individuals who appear to benefit from restrictive dietary regimens, there is minimal scientific evidence that antioxidant intake is effective in slowing down the rate or severity of MS progression.

Three treatments based on natural antioxidants are under study in phase I–II clinical trials of MS: *Ginko biloba* extracts, α-lipoic acid/essential fatty acids, and vitamin E/selenium. The result of these studies will serve as a basis for a phase III trial to assess the long-term effectiveness of an antioxidant therapy for MS (158). Further, a phase I–II clinical trial is currently recruiting patients to test the effect of idebenone, a synthetic drug that mimics the natural coenzyme Q_{10}. Available data suggest that idebenone may be able to limit demyelination and death of brain cells, thereby slowing down or halting the progression of neurological dysfunction (18). Antioxidant cocktails or their combinations with conventional therapies that involve immunosuppressors or immunomodulators might have more likely beneficial effects, by acting synergistically. Well-designed clinical studies based on antioxidant intake, as well as observational investigations based on larger cohorts of subjects, observed over a longer period of time, are needed to establish whether MS patients may really benefit from antioxidant therapeutics (111, 289). In fact, ω-3 fatty acid supplementation was shown to decrease matrix metalloproteinase-9 production by immune cells in relapsing-remitting MS (241).

VI. Conclusions

The redox control of chronic inflammatory disorders is a rather challenging issue, due to the large number of reactive species (*e.g.*, ROS, RNS, RSS, and lipid peroxides) and multiple routes for their synthesis and degradation that operate simultaneously in the healthy body. Indeed, these reactive species can be crucial mediators of several biological processes and the relatively large number of Nox or the presence of the three different NOS isoforms is just an example to indicate that nature has made sure to take advantage of free radicals. This concept is supported by the fact that their production and signaling is driven by important mediators, which serve a broad range of regulatory functions, including hormones, growth factors, and cytokines. Little is known about the intersections of one reactive species with another under physiologic conditions, and even less is understood of the dysregulations that underlie disease conditions. Further, chronic inflammatory diseases may share common and overlapping signaling pathways, inasmuch as not only each species can be distinctive of more than one disease, but also an individual who is affected by one chronic inflammatory disorder can be affected by another one and this is summarized in Table 4, where several oxidative biomarkers are distinctive of numerous diseases. Nevertheless, major human disorders are more likely to be dependent, in a way or another, on redox regulation, and chances are that from this group of substances next-generation therapeutics will be developed in the coming future. Whenever this is going to happen, it is sure that the new drugs will hit the disease by acting at the heart of key biological processes that span from maintenance of DNA integrity to regulation of membrane composition. These are the key cellular events under redox control, further supporting

the concept that with too little or too much radicals we cannot live a healthy life. Up to date, it seems that most clinical trials against chronic inflammatory diseases are focused on the use of antioxidants rather than on inhibiting the oxidants-producing enzymes, yet we acknowledge that a diet rich in antioxidants will have to be paralleled by a strong avoidance of as many oxidant sources as possible.

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Abbreviations Used

$A\beta$ = β amyloid
ACPA = anticitrullinated peptides antibody
AD = Alzheimer's disease
AGE = advanced glycation end-product
ALS = amyotrophic lateral sclerosis
APC = antigen-presenting cell
BBB = blood–brain barrier
CD = Crohn's disease
CNS = central nervous system
COPD = chronic obstructive pulmonary disease
CSF = cerebrospinal fluid
CVD = cardiovascular disease
DA = dopamine
DM = diabetes mellitus
DMARDS = disease-modifying antirheumatic drugs
EAE = experimental autoimmune encephalomyelitis
EGF = epidermal growth factor
GPx = glutathione peroxidase
GR = glutathione disulfide reductase
GSH = glutathione
GSSG = glutathione disulfide
$HCO_3^{\bullet}$ = carbonate radical
HDAC = histone deacetylase
HNE = 4-hydroxy-2-nonenal
H_2O_2 = hydrogen peroxide
$HO_2^{\bullet}$ = hydroperoxyl radical
HOCl = hypochlorous acid
IBD = inflammatory bowel disease
ICAM = intercellular adhesion molecule
IDDM = insulin-dependent diabetes mellitus
IL = interleukin
IRS = insulin receptor substrate protein
LDL = low density lipoprotein
MBP = myelin basic protein
MDA = malondialdehyde
MHC = major histocompatibility complex
MOBP = myelin-associated basic glycoprotein
MOG = myelin oligodendrocyte glycoprotein
MMP = matrix metalloproteinase
MPAC = metal protein attenuating compound
MS = multiple sclerosis
NAC = *N*-acetylcysteine
NAM = *N*-acetylmethionine
NGF = nerve growth factor
NIDDM = noninsulin-dependent diabetes mellitus
N_2O_3 = dinitrogen trioxide
N_2O_4 = dinitrogen tetroxide
$NO^{\bullet}$ = nitric oxide
$^{\bullet}NO_2$ = nitrogen dioxide
NO_2^- = nitrite
NO_3^- = nitrate
NOS = nitric oxide synthase
Nox = NADPH oxidase
O_2 = molecular oxygen
$^{\bullet}O_2^-$ = superoxide radical anion
$^{\bullet}OH$ = hydroxyl radical

Abbreviations Used (Cont.)

$ONOO^-$ = peroxynitrite
PD = Parkinson's disease
PMN = polymorphonucleated cell
PPAR = peroxisome proliferator-activated receptor
PUFA = polyunsaturated fatty acid
RA = rheumatoid arthritis
RAGE = receptor of advanced glycation end-product
RF = rheumatoid factor
RNS = reactive nitrogen species
$ROO^\bullet$ = peroxyl radical
ROS = reactive oxygen species
RSS = reactive sulfur species
SLE = systemic Lupus erythematosus
SNpc = substantia nigra pars compacta
SOD = superoxide dismutase
STAT = signal transducer and activator of transcription
TCR = T-cell receptor
TG = transglutaminase
TGF-β = transforming growth factor-β
TNF-α = tumor necrosis factor-α
TOH = tocopherol
TR = thioredoxin reductase
TRX = thioredoxin
UC = ulcerative colitis
VCAM = vascular cell adhesion molecule

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