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Forum Editorial

Toward Oxidative Lipidomics of Cell Signaling

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ABSTRACT

Involvement and role of oxidatively modified lipids become increasingly evident in different signaling pathways, although specific mechanisms remain to be explored. The current forum focuses on several aspects of signaling by oxidatively modified lipid molecules during apoptosis and implications of different oxidized phospholipids in elimination of apoptotic cells and regulation of inflammatory response. Studies of signaling mechanisms by oxidatively modified lipids represent a rapidly expanding field of oxidative lipidomics, a new and exciting research focus at the interface of traditional lipid/membrane biochemistry and biophysics with free radical chemistry and cell biology. *Antioxid. Redox Signal.* 6, 199–202.

MEMBRANES AS THE SITE OF REDOX REGULATIONS

It is perhaps not surprising that those membranes defining the cell boundary and acting as gatekeepers for the passage of solutes exploit redox reactions in the performance of their duties. The lipid bilayer matrix of biological membranes is comprised of a very complex assortment of polar lipids. In animal cell membranes, the predominant lipid components are diacyl phospholipids and sphingolipids with lesser amounts of glyceryl ether phospholipids, plasmalogens, and cholesterol. With the exception of sterols, each of the glycerolipid and sphingolipid classes represented in membranes consists of a range of molecular species each distinguished by the length, unsaturation, and presence of branched groups associated with the hydrocarbon chains attached to specific positions on the backbone.

The complexity of membrane lipid composition is bewildering. It is common, for example, to identify more than 100 different types of lipids in morphologically distinct membranes. Taking into account positional isomers as well as stereoisomers, this number can easily reach thousands of individual molecular species of lipids. Not only is the number of molecular species of lipids impressive, but the proportions in which they are present in the membrane vary widely from one membrane type to another. This is presumably a reflection of the different functions that particular membranes perform. To compound the picture further, there is now convincing evidence that the lipids are not distributed symmetrically within the structure. Although the lipid composition and distribution within the membrane lipid bilayer are reasonably well documented, the reasons remain elusive.

Membrane lipid asymmetry is now known to play an important role in transmembrane signaling processes. The creation of lipid domains or rafts by formation of ordered phases in a fluid lipid bilayer is a mechanism for segregating lipid-anchored signaling proteins into a transduction complex. The role of lipid rafts in apoptotic signaling, for example, is a subject of current interest (8). Furthermore, the generation of lipid asymmetry across the two leaflets of the bilayer establishes an entropically unfavorable situation that is linked to signaling processes upon dissipation. Several physiological events have been implicated in the loss of membrane phospholipid asymmetry, including adaptation of plants to growth at low temperatures, the transduction of light signals, endocytosis, and apoptosis (1, 5, 19).

To regulate these signaling systems, there must be biochemical mechanisms in place to sense the composition and disposition of lipids within the structure and to harness the appropriate metabolic pathways involved in lipid turnover. At present, we know little of these homeostatic processes. Nevertheless, there is awareness of the vulnerability of unsaturated molecular species of membrane lipids to free radical oxidation, and the action of antioxidants in protection against

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oxidative stress is well known. What is not yet fully appreciated are the consequences of lipid oxidation on the phase behavior of the membrane bilayer matrix, how this may disturb membrane lipid asymmetry, and the role this may have as a trigger for physiological events.

At the level of the cell, there is mounting evidence that oxidative stress involves, in part, alterations in membrane composition and function. A great deal of information on the role of reactive oxygen species has come, for example, from studies of how oxidants are used in our defenses against infection (2, 17). Free radicals are generated by the action of the NADPH oxidase complex located in the plasma membrane of neutrophils in response to the presence of invading microorganisms (9). The objective is generally believed to be to kill the invader but, because radicals are not targeted, there is invariably collateral damage. One of the consequences is persistent inflammation and damage to surrounding healthy tissues. The situation is ameliorated by removal of excess activated neutrophils from the site of inflammation by targeting them for phagocytosis by macrophages. The targeting process is likely to result from self harm to the neutrophils in the form of oxidized membrane lipids.

INVOLVEMENT OF PHOSPHOLIPIDS IN REDOX REGULATION OF APOPTOSIS

The current forum focuses on several aspects of signaling by oxidatively modified lipid molecules. Essentially most of the articles of the forum address different aspects of phospholipid oxidation in regulating apoptosis as well as elimination of apoptotic cells by phagocytes, hence controlling inflammation—one way or another. By using detailed studies of photodynamically generated molecular species of lipid hydroperoxides (phospholipid hydroperoxides and cholesterol hydroperoxides), Girotti and Kriska (10) describe how these relatively stable molecules can be exploited as second messengers capable of transmembrane transfer of signals, particularly during apoptotic cell death.

The article by Kadl *et al.* (13) deals with the generation of different classes of oxidized phospholipids during execution of an apoptotic program. They suggest that different classes of oxidized phospholipids are formed and/or accumulated in different domains of plasma membrane. In particular, apoptotic blebs may be enriched with oxidized molecular species of phosphatidylcholine. In line with this, Tyurina *et al.* (22) present data demonstrating that cytochrome *c*-catalyzed production of oxidized phosphatidylserine species occurs in the inner leaflet of plasma membrane. Oxidized phospholipids are considerably more polar than their unsaturated progenitors, and therefore they are not so tightly constrained to their location in the cytoplasmic leaflet of the plasma membrane (18). The oxidized lipid is then redistributed to the outer leaflet where it accumulates on the surface of apoptotic cells. According to Fadeel (6), oxidized phosphatidylserine along with nonoxidized phosphatidylserine externalized on the surface of apoptotic cells acts as an important "eat me" signal for phagocytes. This process is likely to contribute to restraint and resolution of an "acute" inflammatory response. In contrast, oxidized molecular species of phosphatidylcholine seem to induce specific proinflammatory genes, thus triggering signaling mechanisms responsible for propagation of chronic inflammation as indicated by Kadl *et al.* (13). In lieu of the significance of oxidized lipid species and their potential to affect interactions of low-density lipoproteins (LDLs) with macrophages, regulation of lipid oxidation in LDLs appears to be of great import. The article by Gomes *et al.* (11) discusses the role of β_2-glycoprotein I and two major lipid-soluble antioxidants (α-tocopherol and ubiquinol-10) in the regulation of LDL lipid oxidation as they relate to inflammatory responses and initiation and progression of atherogenesis.

These very important signaling mechanisms of oxidized phospholipids imply that antioxidant suppression of phospholipid oxidation may be associated with the effects of antioxidants on vital functions during apoptosis/phagocytosis and regulation of inflammatory response. Two articles of the forum address these issues. Forsberg *et al.* (7) demonstrate that, indeed, intrinsic apoptosis executed via mitochondria-based pathways is sensitive to antioxidant interventions (*e.g.,* vitamin E). In contrast, vitamin E does not affect progression of apoptosis through extrinsic apoptotic mechanisms triggered via death receptor-dependent stimuli (*e.g.,* Fas-triggered apoptosis in Jurkat cells), as shown by Serinkan *et al.* (20). These results strongly suggest that cell damage caused by different chemical and physical agents resulting in intrinsic apoptosis relies heavily on accumulation and signaling by oxidized phospholipids. Extrinsic apoptotic mechanisms that are more closely associated with physiologically relevant remodeling functions are less likely to involve phospholipid oxidation reactions and resist antioxidant interventions.

Along with low-molecular-weight lipid- and water-soluble antioxidants, the multilevel defense system against oxidative stress includes enzymatic mechanisms such as superoxide dismutases, catalase, and glutathione peroxidases. Recent work has identified an additional line of enzymatic antioxidant defense functioning at the level of lipid peroxidation products that include glutathione *S*-transferases (GSTs). Sharma *et al.* (21) review the role of different members of the GST family of enzymes not only in reducing different lipid hydroperoxides (fatty acid hydroperoxides and phospholipid hydroperoxides), but also in protecting against aldehydic end products of lipid peroxidation. Using genetic manipulation of expression of different GSTs in cells, they present elegant and persuasive evidence for their importance in the regulation of cell signaling and apoptosis.

Specific features of oxidation-induced apoptosis and phagocytosis of apoptotic cells in brain are discussed by Chong *et al.* (3). Protein kinase B (PKBα or Akt) seems to be central to regulating the balance between neuronal and endothelial cell integrity in the face of oxidative stress and their subsequent apoptotic disposal by phosphatidylserine-dependent microglial phagocytosis (3). An interesting model of increased production of reactive oxygen species caused by cessation of shear stress and resulting in enhanced proliferation of endothelial cells is presented by Milovanova *et al.* (16). In this model, reactive oxygen species-induced signaling and stimulation of proliferation were apparently independent of apoptotic cell death.

An appealing approach to study the role of phospholipids in cell death mechanisms is discussed in the review by Manon

(15). The utility of yeast as a model system is highlighted by the ease with which their lipid composition may be modulated. This makes yeast an ideal system to study very early triggering events of the apoptotic program, such as interactions of Bcl-2 family members and cytochrome *c* with cardiolipin (diphosphatidylglycerol)and its homologues (*e.g.,* phosphatidylglycerol) under conditions of lipid oxidation in mitochondria.

TOWARD OXIDATIVE LIPIDOMICS OF CELL SIGNALING

Recent ground-breaking developments in genomics and proteomics prompted emergence of a new area of lipid research, lipidomics. This is a rapidly expanding field in which multiple techniques are utilized to quantitate a huge variety of chemically distinct lipids in cells and link them to the molecular mechanisms through which they facilitate cellular function (12). Although decades of intensive studies of lipid metabolism discovered major pathways of lipid biosynthesis and degradation, we still do not fully understand at the molecular level the functional and structural role of tens of thousands of different molecular species of lipids. Even less clear are physiological and underlying biochemical effects and functions of lipids chemically modified by enzymatic mechanisms in inflammatory cells (*e.g.,* myeloperoxidase, lipoxygenase). Such reactions result in the formation of chlorinated and nitrated lipids (4). In this emerging broad area, the involvement and role of oxidatively modified lipids become increasingly evident in different signaling and catalytic pathways, although specific mechanisms and pathways remain to be explored (14). This identifies oxidative lipidomics as a new and exciting research focus at the interface of traditional lipid/membrane biochemistry and biophysics with free radical chemistry and cell biology.

ABBREVIATIONS

GST, glutathione *S*-transferase; LDL, low-density lipoprotein.

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