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Forum Editorial

Oxidative Stress, Redox Imbalance, and the Aging Process

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It has been an arduous task delineating the basic underlying mechanisms involved in aging. This is because of the complex, multifactorial nature of the process, as well as the difficulty in dissociating the effects of age-dependent chronic pathologies versus "normal" aging *per se.* However, several general factors of both the cell biology and biochemistry of aging have emerged that inexorably point to oxidants and attendant oxidative stress as a fundamental controller of longevity.

Genetic studies using *C. elegans, Drosophila,* and now mice show that certain mutations can markedly influence life span (25, 28, 31, 33). It is interesting to note that most of these mutations confer increased longevity by substantially improving resistance to oxidative stress. Alternatively, mutations in antioxidant enzymes, such as ctl-1 (catalase) in C. *elegans,* markedly lower life span (47). The hunt is now on to determine if specific gene polymorphisms influence human aging as well.

These genetic studies are but one of many experimental observations indicating that oxidants influence the rate of aging. In fact, this has been a major rationale of aging research ever since Harman first proposed that reactive oxygen and nitrogen species (ROS/RNS) and the damage that they cause govern species life span (18). This so-called "Free Radical Theory of Aging" (a better modern terminology may be the "Oxidative Stress Theory of Aging"), first described by Harman nearly 50 years ago, is still being rigorously investigated (18, 19, 20). We are fortunate that Professor Harman has written a perspective of the free radical theory for this forum (21). In his perspective, he now issues a clarion call not only to assess the ramifications of oxidative damage to cell function, but also to begin adopting a more applied therapeutic approach to mitigate the ravages of oxidants.

Although it is safe to say that oxidants and oxidative damage remain the central hypothesis being investigated to understand the biology of aging, a broader picture has emerged as to how oxidants may lead to senescence. This view will now be discussed in relation to the articles presented in this forum.

OXIDATIVE STRESS AND MECHANISMS OF CELLULAR SENESCENCE

Increased appearance of oxidants

A broad picture of the components involved in oxidative stress and the consequences to cell dysfunction in aging are beginning to emerge. It appears that both increased oxidant production and a decline in antioxidant defenses contribute to an elevated pro-oxidant state in aged cells. A major factor that heightens cellular oxidant production is mitochondrial decay. Mitochondria are thought to be major sources of ROS, which are produced as by-products of incomplete reduction of molecular oxygen to water in the electron transport chain (3, 5, 8, 24, 42). It has been estimated that nearly 1% of oxygen consumed by mitochondria is converted to superoxide ($O_2^{\cdot -}$), representing a massive and constant source of oxidants (7). More importantly, mitochondria are also considered primary targets for oxidant injury and decay. Damaged mitochondria would be expected to increase the appearance of ROS by decreasing efficiency of electron transport. Decline in mitochondria function would subtly affect a plethora of metabolic pathways via loss of bioenergetics and calcium ion homeostasis. Thus, the role that mitochondria play in aging has come under considerable attention. In this issue, Viña *et al.* review the role of age-related changes in mitochondrial function and offer unique and important insights as to how mitochondria may influence gender differences in aging (49). Overall, they show that estrogens contribute to lower mitochondrial free radical production in females versus males, partly through heightened mitochondrial glutathione (GSH) levels and increased expression of antioxidant genes important for mitochondria.

Along with mitochondrial decay, there is an age-related accumulation of redox-active transition metals, *e.g.,* iron and copper, in a number of tissues examined, which might be responsible for converting less deleterious ROS to more potent

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free radicals (4, 13, 40). Iron- and copper-induced free radical damage may play a particularly important role in normal brain aging (9) and is also considered part of the etiology of neurological disorders, such as Alzheimer's (AD) and Parkinson's diseases (6, 22, 39). The consequences of both mitochondrial dysfunction and transition metal accumulation are reviewed by Zhu *et al.* in this issue (53). They conclude that during AD progression, neurons are exposed to "low, but chronic, levels of oxidative stress whereby neurons elicit adaptive responses to the chronic oxidative challenge." They go on to link the roles of mitochondria, transition metals, and altered signaling pathways to AD progression. This review is augmented by the original research paper by Killilea *et al.,* who demonstrate that iron accumulates exponentially during cell senescence (23). Interestingly, these authors also show that low-dose hydrogen peroxide treatment accelerates iron accumulation; however, *N-tert*-butylhydroxylamine treatment attenuated this increase. These results indicate that hydroxylamines could be a potentially useful therapy to modulate iron-mediated oxidative stress associated with aging or neurological disorders (*e.g.,* AD) where iron accumulation is believed to be part of disease progression.

It therefore appears that the propensity to produce ROS/ RNS increases significantly with age, especially due to mitochondrial decay and accumulation of redox-active transition metals.

Decline in antioxidant defenses

Concomitant with heightened ROS/RNS generation, there is an age-associated decline in some, but not all, antioxidant defenses. In particular, numerous studies show that low-molecular-weight antioxidants, such as vitamin C and GSH, are markedly lower in many aged tissues (17, 27, 41, 45). For vitamin C, this may be due to lower gastrointestinal absorption from the diet and/or uptake into tissues from the blood plasma (32). For GSH, Liu previously showed that an age-related decline in GSH synthetic capacity occurs in some tissues, notably the liver and kidney (29). Decreased GSH levels were mainly attributable to a significant loss of γ-glutamylcysteine ligase, the rate-controlling enzyme for GSH synthesis. Liu and Dickinson currently extend these observations and show herein that age-related changes also occur in GSH synthetase, the second enzyme in GSH biosynthesis, which would also modulate overall GSH synthetic capacity in the aging animal (30).

Antioxidant enzymes, particularly GSSG and thioredoxin reductases, and cytosolic thioredoxin levels also decrease in old rats (34, 37, 46). Coupled with changes in cellular repair enzymes, these results suggest that cellular antioxidant defenses and the means to repair oxidative damage generally decline with age. Thus, both increased oxidant appearance and a discernible decline in antioxidant defenses inexorably lead to a heightened pro-oxidant milieu in aging tissue.

CONSEQUENCES OF REDOX IMBALANCE TO CELL FUNCTION

Increased oxidative damage

The aforementioned changes in cellular oxidant appearance and lower antioxidant capacity point toward a more pro-oxidant state in the aging cell (12). A significant result of this shift is readily evident by the increase in steady-state oxidative damage to lipids, proteins, and DNA (5). For example, protein oxidation is heightened markedly with age. It is important to note, however, that protein oxidation does not appear to be entirely random or indiscriminate. Certain proteins and even specific amino acid residues are more prone to oxidation. Critical thiol groups appear to be particular targets for damage. Oxidation of cysteine residues can markedly affect enzyme kinetics and has also been shown to alter the ability of SP-1, a transcription factor necessary for the expression of a number of housekeeping genes, to bind DNA and induce gene expression (51). Oxidation of critical cysteines is normally reversed via thioredoxin/thioredoxin reductase-dependent mechanisms; however, as discussed by Yoshida *et al.* in this special forum, the level of cytosolic thioredoxin declines with age (52). This would likely impair the cellular capacity to respond to oxidation of critical cysteine residues. The review by Yoshida *et al.,* along with that of Stadtman *et al.* (44) on methionine oxidation and its reversal by methionine sulfoxide reductase, provides compelling evidence of the impact that protein thiol oxidation may play in age-related alterations to cell function (48). These changes include protein conformational differences, increased K_m for substrates, and loss of enzyme activity (2). Protein oxidation also affects cell regulatory mechanisms, changes the rate of proteolytic degradation, and alters cell signaling and transcription (15, 43). Finally, the research article by Moreau *et al.* completes this issue's exploration of protein oxidation by showing, for the first time, that oxidation of α-ketoglutarate dehydrogenase becomes increasingly adducted with 4-hydroxynonenal in mitochondria from aging rat heart (35). Interestingly these investigators show that protein adduction does not detrimentally alter enzyme function, again indicating that the type of oxidant and probably the specific nature of the amino acid residue damaged determine whether protein oxidation is deleterious to cell function.

Consequences of a shift in cellular redox balance

A more pro-oxidant cellular environment would be expected to activate stress-signaling pathways that are important for bringing the redox state back into balance (1, 12). Indeed, it appears that potentially redox-sensitive transcription factors, such as nuclear factor-κB, exhibit increased binding to DNA in tissues from aged versus young animals. However, it is also notable that several pathways normally activated by oxidative stress fail to become induced or actually decline with age. Loss of SP-1-dependent gene transcription through thiol modification, as mentioned above, is a prime example of such a loss. Holbrook and co-workers have also shown that extracellular signal-regulated kinase signaling, as well as induction of heat shock protein 70, fails to become induced in aged cells following an oxidative insult (12). Overall, these studies are very instructive as to how important cell survival pathways become truncated with age.

Finally, the age-related redox changes may be directly responsible for loss of resistance to oxidative, toxicological, and pathobiological insults during aging (11, 14, 16, 50). In particular, immunosenescence is a common and hazardous result of the aging process. Evidence is mounting that loss of im-

mune competence is at least partly due to changes in transcriptional regulation, which may be due to altered cellular redox state. In this regard, Daynes *et al.* review how expression of peroxisomal proliferator-activated receptor-α (PPARα) is involved in immunosenescence (10). PPARα, a so-called "adopted orphan nuclear receptor" involved in adaptive immune response in lymphoid cells, appears to decline in CD4 T cells with age; consequently, loss of this transcription factor adversely affects interferon-dependent responses. It is also notable that these investigators find that normal PPARα-dependent signaling can be restored by providing aged cells with vitamin E, again illustrating that if normal redox balance can be maintained, cell function can be regained.

Thus, an important current emphasis of aging research is defining not only the role that cellular redox imbalance plays in acute oxidative damage, but also how mechanisms involved in stress resistance become attenuated with age and the impact that such modulation has on longevity. In this regard, it is important to note that caloric restriction, the only regimen known to increase mean life span, acts, in part, by preventing changes in gene expression and maintaining resistance to oxidative stress (26, 36, 38).

To conclude, this an exciting time for aging research; much has been learned, more needs to be learned. This is especially true regarding the precise role that oxidants play in initiating the vicious downward spiral of oxidative stress and the ability to cope with endogenous and environmental insults. A more thorough understanding of the precise nature of the biology of aging, coupled to further knowledge regarding the molecular mechanisms leading to cellular redox imbalance, should provide important insights on how to age *successfully.* It is hoped that such insights will be instrumental in increasing human health span. As Dr. Harman rightly states in his perspective, ". . . it is now apparent that the Free Radical Theory of Aging can serve as a guide in the increasingly important search for practical methods of further enhancing the human functional life span and decreasing the period of senescence."

ABBREVIATIONS

AD, Alzheimer's disease; GSH, glutathione; PPARα, peroxisomal proliferator-activated receptor-α; RNS, reactive nitrogen species; ROS, reactive oxygen species.

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