The aging process and potential interventions to extend life expectancy

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Abstract: Aging is commonly defined as the accumulation of diverse deleterious changes occurring in cells and tissues with advancing age that are responsible for the increased risk of disease and death. The major theories of aging are all specific of a particular cause of aging, providing useful and important insights for the understanding of age-related physiological changes. However, a global view of them is needed when debating of a process which is still obscure in some of its aspects. In this context, the search for a single cause of aging has recently been replaced by the view of aging as an extremely complex, multifactorial process. Therefore, the different theories of aging should not be considered as mutually exclusive, but complementary of others in the explanation of some or all the features of the normal aging process. To date, no convincing evidence showing the administration of existing "anti-aging" remedies can slow aging or increase longevity in humans is available. Nevertheless, several studies on animal models have shown that aging rates and life expectancy can be modified. The present review provides an overlook of the most commonly accepted theories of aging, providing current evidence of those interventions aimed at modifying the aging process.

Keywords: Aging, anti-aging medicine, caloric restriction, oxidative damage, inflammation, physical exercise

Introduction

Aging is commonly defined as the accumulation of diverse deleterious changes occurring in cells and tissues with advancing age that are responsible for the increased risk of disease and death (Harman 2003). The observation that most of the animals living in a natural environment rarely becomes senescent (because dying earlier for predation, disease, starvation, or drought) (Holliday 2006) suggests that aging is a phenomenon unique to the human species (Hayflick 2000b). In other words, the advancing knowledge of hygiene and biomedicine has led us to discover the aging process, something that was teleologically not intended for us to be experienced (Hayflick 2000b). The immediate consequence of the extended life expectancy is represented by the increasing number of older people in developed countries, an artefact of human civilization (Hayflick 1998, 2000a).

Life expectancy is defined as the average total number of years that a human expects to live. Differently, life span is the maximum number of years that a human can live. While the human life span has substantially remained unchanged for the past 100,000 years at ~125 years, life expectancy has sensibly increased (~27 years during the last century), especially in Western Countries (Hayflick 2000b). The lengthening of life expectancy is mainly due to the elimination of most infectious diseases occurring in youth, better hygiene, and the adoption of antibiotics and vaccines.

Before examining the hypothesized biological factors at the basis of the aging process, it is crucial to underline that aging is not a disease. Based on this assumption, Hayflick estimates that a potential cure of the leading causes of death in old age

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(ie, cardiovascular disease, stroke, cancer) would only lead to a 15 year-increase in human life expectancy (Hayflick 2000b). Therefore, even in this hypothetical condition, we will not become immortal, but we will only be able to experience how death occurs in the absence of disease. Because aging is negatively associated with the ability to respond to stress and positively related to the homeostatic balance and incidence of pathology, death remains the ultimate consequence of aging (Kowald and Kirkwood 1996).

The notion that aging requires treatment is based on the belief that becoming old is undesirable. In the last decades, aging has received a negative connotation and become synonymous of deterioration, approaching pathology, and death. If our society would learn to value old age to the same extent as presently done for youth, then the research aimed at slowing, stopping or reversing the aging process would be as unthinkable as the intervention on the developmental processes of youth. Instead, what is desirable and demonstrably attainable at all times in life, is the prevention or resolution of pathology (Hayflick 2004).

The major theories of aging (eg, the free radical theory (Harman 2003), the immunologic theory (Franceschi et al 2000a), the inflammation theory (Chung et al 2001), mitochondrial theory (Cadenas and Davies 2000)) are all specific of a particular cause of aging, providing useful and important insights for the understanding of physiological changes occurring with aging. However, a global view of them is needed when debating about a process which is still obscure in some of its aspects (Holliday 2006). In this context, the search for a single cause of aging (such as a single gene or the decline of a body system) has recently been replaced by the view of aging as an extremely complex, multifactorial process (Kowald and Kirkwood 1996; Weinert and Timiras 2003). In fact, it is very likely that several processes simultaneously interact and operate at different levels of functional organization (Franceschi et al 2000b). Therefore, different theories of aging should not be considered as mutually exclusive, but may be complementary of others to explain some or all the features of the normal aging process (Weinert and Timiras 2003).

Throughout history, humans have always dreamed of being able to cure aging and diseases, looking for the discovery of a "fountain of youth". This dream has never become reality because of the difficult understanding of the aging process. Any intervention able to delay the development of age-related modifications that are not considered as diseases are indicated with the term "anti-aging medicine" (Butler et al 2002). To date, we know of no intervention that will slow, stop, or reverse the aging process in humans. It is also doubtful that

intervention in the aging process has been achieved in any other life form in view of the absence of a generally accepted definition of aging and prec markers to measure its rate of change (Hayflick 2004). Nevertheless, several studies on animal models have shown that aging rates and life expectancy can be modified.

Evolutionary theory of aging

Evolutionary theory indicates aging as the result from a decline in the force of natural selection. This theory was first extensively formulated in the Forties from the observation of patients with Huntington's disease, a dominant lethal mutation (Haldane 1941). It was noted that, even if subjects affected by this condition should be strongly selected, the Huntington's disease remained in the population. This lack of natural selection can be explained by the late age of onset for the disease (30–40 years) allowing a carrier to reproduce before dying.

Even earlier than these observations, Darwin explained that a natural selection occurs in organisms dying primarily from predation and environmental hazards and consequently evolving a life span optimized for their own particular environment (Weinert and Timiras 2003). Supporting this hypothesis is the evidence that animals living in a protected environment (eg, a zoo) live longer, potentially reaching their maximum life spans (Holliday 2006). For example, this theory was confirmed by Austad in a natural environment by comparing mainland opossums that are subject to predation to a population of opossums living on an island free of predators (Austad 1993). The evolutionary theory predicts that the protected island opossums would have had the opportunity to evolve a longer life span, if beneficial to their fitness. Indeed, island opossums did live longer and aged more slowly than their mainland counterparts. However, the so-called "disposable soma theory of aging" argues that the somatic organism is effectively maintained only for reproductive success; afterwards it is disposable. Therefore, the somatic maintenance (in other words longevity) has a cost. The equilibrium between resources invested in longevity versus those for reproductive fitness determines life span (Loison et al 1999). This antagonism between reproduction and longevity is supported by experiments in which the limitation of the reproduction by destroying germ line cells can extend life span in both *Drosophila* and *Caenorhabditis elegans*.

Free radical theory of aging

More than 300 theories have been proposed to explain the aging process (Medvedev 1990), but none has yet been

generally accepted by gerontologists. Nevertheless, the free radical theory of aging seems to be the one receiving the widest acceptance as a plausible explanation of the primary chemical reactions at the basis of the aging process (De La Fuente 2002).

The free radical theory of aging was first formulated in the Fifties by Harman who hypothesized a single common process, modifiable by genetic and environmental factors, in which the accumulation of endogenous oxygen radicals generated in cells could be responsible for the aging and death of all living beings (Harman 1957; Finkel and Holbrook 2000). This theory was then revised in 1972 (Harman 1972) when mitochondria were identified as responsible for the initiation of most of the free radical reactions related to the aging process. It was also postulated that the life span is determined by the rate of free radical damage to the mitochondria.

The increasing age-related oxidative stress seems to be a consequence of the imbalance between the free radical production and antioxidant defenses with a higher production of the former (Sastre et al 2000).

All organisms live in an environment that contains reactive oxygen species. Mitochondrial respiration, the basis of energy production in all eukaryotes, generates reactive oxygen species by leaking intermediates from the electron transport chain (Finkel and Holbrook 2000). The universal nature of oxidative free radicals, and possibly of the free radical theory of aging, is suggested by the presence of superoxide dismutase in all aerobic organisms and responsible for scavenging superoxide anions (Finkel and Holbrook 2000). Moreover, cellular oxidative damage is indiscriminate. In fact, oxidative modifications have been shown to occur in of DNA, protein, and lipid molecules (Weinert and Timiras 2003). Elevated levels of both oxidant-damaged DNA and protein have been found in aged organisms (Beckman and Ames 1998; Shringarpure and Davies 2002).

However, even if it is clear that the age-related accumulation of oxidative damage, it is not yet clear whether this process contributes to aging in all organisms. The increased life span of transgenic flies expressing superoxide dismutase indicates that free radical-scavenging enzymes are sufficient to delay aging in *Drosophila* (Tower 2000). Moreover, flies selected for increased longevity have elevated levels of superoxide dismutase and increased resistance to oxidative stress (Arking et al 2000). It has also been demonstrated that long-lived mutant worms are also resistant to oxidative stress and show an age-dependent increase in superoxide dismutase

and catalase activity (Larsen 1993). The life span extension in *Caenorhabditis elegans* models by using synthetic molecules that mimic catalase and/or superoxide dismutase demonstrates that antioxidant compounds may play an important role in delay aging (Melov et al 2000).

The free radical theory of aging is divided into several hypotheses focusing on the exclusive role of particular organelles and types of damaged molecules in the aging process (Weinert and Timiras 2003). For example, it has been hypothesized that mutations in mitochondrial DNA accelerate free radical damage by introducing altered enzyme components into the electron transport chain. Faulty electron transport consequently results in elevated free radical leakage and ultimately more mitochondrial DNA mutation and exacerbated oxidant production. This "vicious cycle" of mutation and oxidant production may then eventually lead to cellular/organ failure, and senescence (Mandavilli et al 2002). Another hypothesis argues that free radicals cause aging because of the accumulation of oxidized proteins in cells. The age-dependent reduction in the capacity of degradation of oxidized proteins may be responsible for the build-up of damaged, dysfunctional molecules in the cell (Shringarpure and Davies 2002).

It has been suggested that oxidative damage may be an important source of somatic mutations at the basis of the so-called "somatic mutation theory of aging". This theory hypothesizes that the accumulation of genetic mutations in somatic cells represents the specific cause of senescence (Beckman and Ames 1998).

The identification of free radical reactions as promoters of the aging process implies that interventions aimed at limiting or inhibiting them should be able to reduce the rate of formation of aging changes with a consequent reduction of the aging rate and disease pathogenesis (Harman 2003). An ideal "golden triangle" of oxidative balance, in which oxidants, antioxidants and biomolecules are placed at each apex, has been described (Carmeli et al 2002). In a normal situation, a balanced-equilibrium exists among these three elements. Excess generation of free radicals may overwhelm natural cellular antioxidant defenses leading to lipid peroxidation and further contributing to muscle damage (Bowles et al 1991; Meydani et al 1993).

Even if antioxidant supplementation is receiving growing attention and is increasingly adopted in Western countries, supporting evidence is still scarce and equivocal. In fact, even if some epidemiological studies shown that dietary supplementation with vitamin E decreases the risk of cancer and cardiovascular disease, such observations are not universal

(Butler et al 2002). The only capability of reducing oxidative damage through antioxidant supplementation is limited. McCall and Frei (1999) stated that "except for supplemental vitamin E, and possibly vitamin C, being able to significantly lower lipid oxidative damage in both smokers and nonsmokers, the current evidence is insufficient to conclude that antioxidant vitamin supplementation materially reduces oxidative damage in humans". Therefore, the longevity-extending potential of antioxidant supplementation (in particular, vitamin E as the most studied one) remains uncertain even in animal studies (Anisimov 2001). The only robust finding that a pharmacological antioxidant can extend longevity has been reported by Melov and colleagues (2000) in an animal model in demonstrating that EUK-134, a compound with both catalase and superoxide dismutase activities, significantly extends longevity in nematodes.

Mitochondrial theory of aging

The age-related physiological decline seems to be due to the accumulation of defects in the several metabolic pathways. Looking for potential candidates to progressive accumulation of damage over a lifetime, it seems reasonable to exclude RNA, proteins and other cellular macromolecules with a rapid turned over. For this main reason, studies exploring mechanisms of aging have always been focused on DNA. In mammalian cells, mitochondria and the nucleus are the only organelles possessing DNA. It appears obvious that the physiological integrity of the cell is strongly linked to the integrity of its genome.

Even if mitochondrial DNA comprises only 1%–3% of genetic material in animal cells, its contribution to cellular physiology seems to be much greater than what expected from its size alone. Mitochondrial DNA, in close proximity to the sites of oxygen radical production and unprotected by the histones that are associated with nuclear DNA, is a sensitive target for oxygen radical attack. In fact, it has been estimated that the level of oxydatively oxidized bases in mitochondrial DNA is 10- to 20-fold higher than that in nuclear DNA (Richter et al 1988; Ames 1989). Moreover, mitochondrial DNA encodes polypeptides of the electron transfer chain as well as components required for their synthesis. Therefore, any coding mutations in mitochondrial DNA will affect the entire electron transfer chain, potentially altering both the assembly and function of the products of numerous nuclear genes in electron transfer chain complexes. Finally, defects in the electron transfer chain can have pleiotropic effects because affecting the entire cellular energetics (Alexeyev et al 2004).

It has been demonstrated by the Framingham Longevity Study of Coronary Heart Disease that longevity is more strongly associated with age of maternal death than that of paternal death, suggesting that mitochondrial DNA inheritance might play an important role in determining longevity (Brand et al 1992). Even if the matter is still controversial (Ross et al 2001), several studies demonstrate that longevity is associated with specific mitochondrial DNA polymorphisms (Ivanova et al 1998; Tanaka et al 1998; De Benedictis et al 1999).

The mitochondrial theory of aging is often considered as an extension and refinement of the free radical theory (Harman 1972; Miquel et al 1980). Mitochondrial DNA mutations accumulate progressively during life and are directly responsible for a measurable deficiency in cellular oxydative phosphorylation activity, leading to an enhanced reactive oxygen species production. This latter results in an increased rate of mitochondrial DNA damage and mutagenesis, triggering the onset of a "vicious cycle" of exponentially increasing oxidative damage and dysfunction, which ultimately culminates in death. Supporting the primary importance of mitochondria in the aging process and in determining longevity, it has been documented that several mutagenic chemicals and lipophilic carcinogens (eg, polycyclic aromatic hydrocarbons) tend to preferentially damage mitochondrial DNA (Wunderlich et al 1970; Allen and Coombs 1980; Niranjan et al 1982; Rossi et al 1988). It can then be hypothesized that a life-long exposure to these environmental toxins may lead to a preferential accumulation of mitochondrial DNA damage and accelerate aging.

The superoxide anion radical (or superoxide) and hydrogen peroxide, respectively the products of the univalent and bivalent reduction of oxygen, are produced during normal aerobic metabolism and constitute physiological intracellular metabolites (Cadenas and Davies 2000). Several reactions in biological systems contribute to the steady state concentrations of superoxide and hydrogen peroxyde, although mitochondria seem to be quantitatively the most important source (Cadenas and Davies 2000).

Although mild amounts of oxidative damage such as that experienced during exercise training (Davies et al 1982) may actually be the stimulus for physiological mitochondrial biogenesis, more severe, more extensive, or more prolonged oxidative damage is clearly toxic (Cadenas and Davies 2000).

Gene regulation theory of aging

The gene regulation theory of aging proposes that senescence is the resulting of changes occurring in the gene

expression (Kanungo 1975; Weinert and Timiras 2003). Although it is clear that many genes show changes in expression with age, it is unlikely that selection could act on genes that promote senescence directly (Weinert and Timiras 2003). To date, evidence in this field remains controversial, and aging should be more safely considered as a stochastic process, rather than a programmed mechanism directly governed by genes. At least 15 different genetic manipulations inducing life extension in organisms such as yeast, fruit flies, nematodes, and mice have been demonstrated (Butler et al 2002). However, it is still unknown how the proteins coded by these genes are acting in the regulation of longevity. On the other hand, other studies performed using animal models have suggested that genes supposed to be involved in aging are not able to reverse or arrest the inexorable expression of the molecular disorder that is the hallmark of aging (Hayflick 2004).

It may be that the genome is necessary to govern biological development and maintenance, but unnecessary to cause the animal's aging. Because genes do not drive the aging process, an understanding of the human genome, even beyond what is known today, will not provide insights into a process that is random and thermodynamically driven (Hayflick 2004). Recently, an insulin-like signaling pathway regulating life span in worms, flies, and mice has been identified (Tatar et al 2003). Life span extension results from the activation of a conserved transcription factor in response to a reduction in insulin-like signaling, suggesting that gene expression can regulate life span.

Studies of human centenaries and their relatives have identified a significant genetic aspect of the ability to survive to exceptional ages. By identifying a locus on chromosome 4 that may contain gene(s) promoting longevity (Puca et al 2001), it has recently been supported the theory of a genetic component for exceptional longevity.

If it will be confirmed that changes in gene expression can modulate the aging process, a major step forward the understanding of aging will be completed and a starting point for the development of interventions aimed at delaying aging provided.

Gene manipulations possible in laboratory animals appear to have limited potential for direct application in humans, although they do provide insight into important biological factors in longevity determination in model systems. In contrast, the potential of cell replacement therapy in reversing some of the adverse effects of aging appears to be substantial. Aging is accompanied by some loss of tissue function, which is at least partially due to either the age-related loss of cells from the tissue or an increased proportion of dysfunctional cells.

The recent isolation of nearly totipotent cells, such as human embryonic stem cells, offers a great range of potential opportunities. These cells express telomerase and appear to maintain an immortal phenotype even after extended culture in vitro. Cells and tissues derived from such cultures may provide the unique advantage of possessing a large replicative capacity and broad differentiation potential.

However, it is important to note that formidable hurdles are yet to be overcome. Cells derived from established human embryonic stem cell lines will probably not prove to be immunologically compatible with most patients. This may be resolved by immunosuppressive therapy, genetic modification of the cells to reduce immunogenicity, or possibly the creation of a chimeric immune system in the patient to induce tolerance. The recent discovery of cell reprogramming through nuclear transfer offers a path to the reprogramming of a patient's cell, thereby reverting it to an autologous embryonic stem cell. The ethics of the embryonic stem cell technology and the use of nuclear transfer in medicine is currently a matter of intense debate. Finally, it remains to be seen whether such new tissue (even if it were autologous) would be adequately vascularized and subsequently function appropriately in the patient.

Telomere theory of aging

The cellular senescence theory of aging was formulated in 1965 when cell senescence was described as the process occurring in normal human cells in culture and characterized by a limited number of cell divisions (Hayflick 1965). This limit in "replicative capacity" occurs after a characteristic number of cell divisions and results in terminally arrested cells with altered physiology (Campisi 2003).

Telomeres are specialized DNA sequences located at the ends of eukariotic chromosomes. In humans, telomeres are composed by repeated sequences TTAGGG reiterated in tandem for up to 15 kilobases at birth (Ahmed and Tollefsbol 2001). Telomeres are synthesized by telomerase, a ribonucleoprotein reverse transcriptase enzyme that maintains the lengths of chromosomes (Lingner et al 1997). Telomere sequences stabilize chromosomal ends by binding to proteins that prevent them from being recognized as double-stranded breaks by repair enzymes (de Lange 1992). The attrition of chromosomal termini, caused by loss of telomerase, can lead to breaks and subsequent translocation, fusion, or rearrangement within these DNA regions (de Lange 1992).

The telomerase enzyme, which stabilize chromosomal termini by adding telomere repeats to the ends of chromosomes using a dedicated RNA template (Greider and Blackburn 1989; Artandi 2006), is of considerable interest to gerontologists. Its expression is thought to be necessary for cellular immortalization (Rhyu 1995), and its absence may constitute a fundamental basis for cellular aging (Harley et al 1990; Ahmed and Tollefsbol 2001; Artandi 2006). Immortal cells in general have a stable telomere length and mortal cells have telomeres that shorten with each cell division, thus establishing a link between the presence of telomerase, chromosomal stability, and the mortality of cells. In fact, specialized immortal cell types (such as stem cells, germ cells, and T lymphocytes) express telomerase and will either maintain telomere length or delay telomere attrition. Additionally, telomerase is up-regulated in 85% to 95% of cancer cells, which show no net loss of average telomere length, suggesting that telomere stability may be required for cells to escape replicative senescence and to proliferate indefinitely (Shay and Wright 1996; Artandi 2006).

In actively dividing differentiated cells, with each cell division, a small amount of DNA is necessarily lost at each chromosome end, resulting in ever-shorter telomeres and altered telomere structure, eventually leading to the cessation of cellular proliferation (Blackburn 2000; Weinert and Timiras 2003). This progressive shortening of telomeres starts soon after conception, when cells begin widespread differentiation. Although in some of these cells telomerase is inactivated before birth, in others some telomerase activity can be detected after birth (Ulaner and Giudice 1997; Ahmed and Tollefsbol 2001). Thus, telomere shortening and the loss of telomerase in normal somatic cells have been implicated as a potential molecular clock triggering cellular senescence (Harley et al 1990), loss of proliferative capacity, and age-related pathologies (Campisi 1997; Fossel 1998).

The finding that most of the cells expressing telomerase, instead of undergoing cellular aging, maintain a youthful state and proliferate indefinitely has opened new fields of research for a potential "anti-aging" intervention (Bodnar et al 1998; Vaziri and Benchimol 1998). Cells that have been supplied with an exogenous source of telomerase maintain a youthful state and proliferate indefinitely (Bodnar et al 1998). These "rejuvenated" cells are not only immortal, but they have also shown reversal of senescent characteristics (such as increased fragility and subepidermal blistering) (Funk et al 2000). Thus, the biological and potential medical consequences of telomerase expression appear to be highly significant.

Supporting the hypothesized relationship between telomeres and aging, it has been demonstrated that some telomere dysfunctions are involved in the premature aging characteristic of progerias. For example, the DNA helicase protein, that is mutant in the Werner's syndrome, is required for the efficient replication and stability of telomeres. Therefore, by extension, as it happens in the premature aging, telomeres might be, at least partially, responsible for the normal human aging (Artandi 2006).

The human telomerase reverse transcriptase (hTERT), the active component of telomerase, has been identified and cloned and its messenger RNA is undetectable in differentiated cells that do not express telomerase, but is abundant in undifferentiated cells expressing telomerase (Meyerson et al 1997). Although post-transcriptional mechanisms may modify hTERT activity (Liu et al 1999), the expression of hTERT correlates directly with telomerase activity and substantial evidence indicates that hTERT activity is controlled primarily at the level of transcription (Cong et al 1999; Wick et al 1999). The telomerase promoter must be "ON" in cells that can proliferate indefinitely, but "OFF" in cells with limited proliferative lifespan. Unfortunately, little is still known about the switching mechanism that controls telomerase expression, leading to its down-regulation and subsequent cellular mortality in somatic cells.

Moreover, even if studies of telomere shortening and telomerase show great promise in helping to elucidate the underlying basis of cellular aging, it is not yet clear how this knowledge would enhance our understanding of aging of the individual. In fact, it is possible the presence of some tissues in which proliferative failure contributes to the declining physiology associated with aging, but those tissues have not been unequivocally identified. Only up to 70% of immortalized human somatic cell lines (Bryan et al 1997) and about 90% of human cancer cell lines (Shay and Gazdar 1997) have demonstrated in vitro telomerase activity, suggesting that factors other than telomerase are involved in cell replication and senescence. Moreover, many telomerase-negative immortalized cell lines can maintain their telomere lengths (Bryan et al 1995). On the other hand, hybrids of telomerase-negative and telomerase-positive cells have failed to become immortal, so that it is likely that telomerase enzyme alone is insufficient to prevent cell senescence (Bryan et al 1995).

Although studies to this point indicate that telomerase may be intimately involved in cellular senescence and holds great promise, our understanding of these age-related mechanisms is still at the beginning. The amount of

currently available evidence for claiming that preventing telomere shortening would influence any aspect of aging is still insufficient.

Inflammation hypothesis of aging

Even if the involvement of the inflammatory process in several (sub)clinical conditions (eg, atherosclerosis, diabetes, dementia) is well-demonstrated, the importance of inflammation in the aging process was recognized only recently (McGeer and McGeer 1999; Chung et al 2001). Nevertheless, inflammation is growingly considered as a cornerstone of the mechanisms underlying the aging process, so to even generate the neologism "inflamm-aging" (Franceschi et al 2000a). Inflammation is a complex host's normal defense reaction to physiological and nonphysiological stressors. Acute as well as chronic inflammatory responses are constituted by sequential phases, controlled by humoral and cellular stimula: 1) intracellular activation; 2) proinflammatory cells in the tissues; 3) increase of vascular permeability; 4) damaging of tissues and cell death (Huerre and Gounon 1996; Chung et al 2001).

An individual threshold of the capability to cope with stress has been hypothesized. If the age-related inflammation (or inflamm-aging) trespasses this level, the transition between successful and unsuccesful aging occurs. Epidemiologic data support the hypothesis that the period of life during unsuccessful aging (disability) is maximal in the elderly, and minimal in young people and centenarians (Franceschi et al 2000a).

Even when debating about inflammation and its relationship with aging, it is important to underline how this mechanism is associated with others at the basis of different theories of aging. In fact, the close relationship between inflammation and oxidative damage is well-known in literature (Cesari et al 2005). In fact, reactive oxygen species and reactive nitrogen species are heavily implicated in the inflammatory processes. The overproduction or uncontrolled release of reactive species is a major causative factor in tissue inflammation.

Immune theory of aging

In 1989, Franceschi proposed the immune theory of aging, or network theory of aging (Franceschi 1989; Franceschi et al 2000a), in which suggested that aging is indirectly controlled by a network of cellular and molecular defense mechanisms. The major parts of the network are constituted by DNA repair enzymes, activation of poly (ADP-ribosyl) polymerase, enzymatic and nonenzymatic antioxidant systems (eg, superoxide dismutase, catalase, glutathione peroxidase), production of heat shock proteins (Franceschi 1989; Franceschi et al 2000b). These mechanisms function to limit the negative effects of a variety of physical, chemical, and biological stressors. The efficiency of the network is genetically controlled and differs among species and individuals, explaining in this way the observed differences in life span.

In the network theory of aging, the immune system represents the most powerful mechanism to face stressors (Franceschi et al 2000a). In particular, Franceschi identified the macrophage as the primary modulator of the vicious cycle existing between innate immunity, inflammation and stress. The macrophage activation due to chronic stress may provide a potential explanation to the subclinical chronic inflammatory status characterizing older persons and, at the same time, a possible feature of the aging process. Lymphocytes are also affected by the continuous age-related antigenic stress, resulting in a chronic stimulation responsible for the expansion of memory cells, the decrease (even exhaustion) of naïve cells, and the shrinkage of the T-cell repertoire.

Supporting this hypothesis and the importance of the immune system in determining the senescence is the evidence of the high incidence of tumors and greater susceptibility to infections from pathogens shown by the older persons. It has been suggested that aged subjects maintaining their immune functions at an exceptionally high level are more likely to have a long life span (Wayne et al 1990; Pawelek et al 1999).

As noted above, theories of aging often overlap each other, suggesting interactions across different systems and mechanisms. In this context it should be considered the association between the immune cell functions (such as those involved in the cytotoxic activity and particularly in phagocytes as regards their microbicidal activity) and the reactive oxygen species generation. The excessive amount of reactive oxygen species not counteracted by the antioxidant defenses can become a potential source of tissue damage (De La Fuente 2002). Moreover, antioxidants maintain the integrity and function of membrane lipids, cellular proteins, and nucleic acids and the control of signal transduction of gene expression in immune cells. Not surprisingly, immune system cells usually contain higher concentrations of antioxidants than do other cells (Knight 2000), given the high percentage of polyunsatured fatty acids in their plasma membranes. Thus, the immune cell functions are strongly influenced by the antioxidant/oxidant balance and, therefore, the antioxidant levels play a pivotal role in maintaining immune cells I) in a reduced environment and II) in protecting them from

oxidative stress, so to preserve their adequate functioning (Knight 2000).

Neuroendocrine theory of aging

It is generally accepted a bidirectional communication between the nervous and the immune systems (Besedovsky and Del Rey 1996). With aging not only a functional decline in the immune and nervous systems occurs, but also an impaired relationship between these two regulatory systems can become evident, with the resulting loss of homeostasis and higher risk of death (Fabris 1991; De La Fuente 2002).

The neuroendocrine theory proposes that aging is due to changes in neural and endocrine functions that are crucial for:1) coordination and responsiveness of different systems to the external environment; 2) programming physiological responses to environmental stimuli; and 3) the maintenance of an optimal functional status for reproduction and survival.

These changes, not only selectively affect neurons and hormones regulating evolutionarily significant functions such as reproduction, growth, and development, but also influence the regulation of survival through adaptation to stress. Thus, the life span, regulated by "biological clocks", would undergo a continuum of sequential stages driven by nervous and endocrine signals. Alterations of the biological clock (eg, reduced responsiveness to the stimuli regulating the clock, excessive or insufficient coordination of responses) would disrupt the clock and the corresponding adjustments (Finkel 1976; Timiras 1978; Weinert and Timiras 2003).

An important component of this theory indicates the hypothalamo-pituitary-adrenal (HPA) axis as the primary regulator, a sort of pacemaker signaling the onset and termination of each stage of life. The HPA axis controls the physiological adjustments aimed at the preservation and maintenance of an internal homeostasis despite the continuing changes in the environment (Weinert and Timiras 2003). During life span, chronic exposure to severe and multiple physical, biological, or emotional stressors may exhaust or weaken this capacity to adapt and to the so-called "disease of adaptation" and death (Selye 1976; Weinert and Timiras 2003). Aging should then be considered as the result of a decrease ability to survive stress, suggesting once more the close relationship between stress and longevity.

The integration of responses to environmental stimuli seems to be carried out by hypothalamus from information derived in various cerebral structures. The hypothalamus itself regulates: 1) nervous functions (eg, sympathetic and parasympathetic visceral functions), 2) behaviors (eg, sexual and eating behavior, rage, fear), and 3) endocrine functions (eg, production and secretion of hypophysiotropic hormones stimulating/inhibiting hormone release from the hypophysis). In response to hypothalamic signals, the hypophysis produces and secrets several hormones acting in the regulation of many important functions of the body. This regulation is controlled by the release of hormones (eg, growth hormone, oxytocin, vasopressin) or by the stimulation of peripheral endocrine glands (eg, adrenal cortex, thyroid, gonads).

Major hormones of the adrenal medulla are the catecolamines epinephrine and norepinephrine, functioning as neurotransmitters for the sympathetic division of the autonomic nervous system and rapidly responding to any external or internal stress through circulatory and metabolic adjustments (Weinert and Timiras 2003). With aging, a reduction in sympathetic responsiveness is characterized by: 1) a lower number of catecholamine receptors in peripheral target tissues; 2) a decline of heat shock proteins that increase stress resistance; and 3) a decreased capability of catecholamines to induce heat shock proteins.

The hormones of the adrenal cortex are glucocorticoids (responsible for the regulation of lipid, protein, and carbohydrate metabolism), mineralcorticoids (regulating water and electrolytes), and sex hormones. Among the latter is dehydroepiandrosterone, which has shown to decrease with aging.

Dehydroepiandrosterone replacement therapy has been advocated in humans, despite unconvincing results (Daynes and Araneo 1992). Glucocorticoids, as well as other steroid hormones, are regulated by positive and negative feedbacks between the target hormones and their central control by the hypophysis and hypothalamus. With aging and in response to chronic stress, not only feedback mechanisms may be altered, but also glucocorticoids themselves become toxic to neural cells, thus disrupting feedback control and hormonal cyclicity (Sapolsky et al 1986; Sapolsky 1992; Weinert and Timiras 2003).

The circulating levels of growth hormone, testosterone, estrogen, dehydroepiandrosterone, and other hormones decrease with age. Although some hormone replacement strategies have been shown in clinical trials to modify some of physiological attributes associated with aging, negative side effects occur frequently with those interventions shown to have some benefit, such as growth hormone.

Although the epidemiological data are overwhelmingly positive regarding some health benefits of estrogen replacement therapy, a recent study has raised a concern about ovarian cancer after long-term use. It has been shown that melatonin supplementation increases the mean life

expectancy of mice by approximately 5%, but in association with an increase in spontaneous tumor incidence.

In order to adequately address hormone decline occurring with aging, it is crucial the understanding of the complex hormonal cascade, an intricate interplay between signals, pathways, and production and delivery systems.

Estrogen replacement therapy represents a special case of hormone replacement therapy and deserves particular attention because of its long clinical history and apparent record of success in increasing quality of life in postmenopausal women. Estrogen is particularly recommended for the prevention of osteoporosis, but it has been suggested it may reduce the risk of dementia and cardiovascular disease. It has been estimated that favorable changes in plasma lipids may account for approximately 25% of the cardioprotective effect of estrogen.

The conclusion that estrogen protects postmenopausal women against cardiovascular disease is now being questioned, based mainly on experiments examining secondary prevention in women with preexisting heart disease.

Estrogen replacement therapy has been called the first true anti-aging therapy. However, no results have yet been reported of randomized studies that compare effects of this therapy with placebos, beginning at the menopausal transition, in women with no known preexisting coronary heart disease or dementia.

It has been demonstrated that circulating levels of growth hormone drop with increasing age. It has also been shown that GH replacement in adults with pituitary disease and GH deficiency has beneficial effects on body composition, reducing fat and increasing lean body mass, muscle strength, and bone mass. Rudman and colleagues investigated whether GH injections in older men would restore muscle mass typical of younger men. They found that insulin growth factor (IGF)-1 levels did rise and that lean body mass increased while fat mass decreased, suggesting that GH injections did reverse the changes in body composition that were due to age and deconditioning. Recent data obtained with mice suggest that lifelong overproduction of GH reduces longevity in mice, whereas underproduction or an inability to respond to GH increases it. Transgenic mice overexpressing GH exhibit severe kidney lesions and increased incidence of neoplasms, and overproduction of GH in adult humans leads to a condition known as acromegaly, which is characterized by excessive growth of certain organs and tissues, but also premature heart and lung failure.

The evidence from both nematodes and fruit flies suggests that decreased activity of the insulin-like signaling pathway is associated with increased life expectancy, rather than the reverse. Thus, further research is needed before the GH supplementation in humans encouraged by many "antiaging" clinicians can be considered either safe or useful for long-term intervention.

Neuroendocrine-immuno theory of aging

In the hierarchy of multisystem regulation throughout the sequential stages of life, there is a significant role for the interaction and integration of the neuroendocrine and immune systems. Such interaction occurs through 1) neuropeptides and cytokines present in the immune system mediating both intraimmune communication and between the neuroendocrine and immune systems, 2) several hormones from the posterior (vasopressin) and anterior (thyroid-stimulating hormone, prolactin, adrenocorticotropic hormone, growth hormone) hypophysis, and 3) reciprocal action of cytokines on neuroendocrine functions.

Besides of neuroendocrine interactions, the immune system must control and eliminate foreign organisms and substances in the host body while at the same time recognizing, and therefore sparing from destruction, the molecules from oneself. In most older persons, immunosenescence is characterized by a decreased resistance to infectious diseases, a decreased protection against cancer, and an increased failure to recognize self (leading to consequent autoimmune pathology) (Franceschi et al 2000b). Both the neuroendocrine and immune systems are characterized by a high degree of plasticity and are able to modify their functioning according to demand. Plasticity is most efficient at early ages, but persists at advanced age.

To describe the theoretical trajectories of the aging process, Rowe and Kahn (1987) described three curves: the first characterized by disease and disability; the second, known as "usual aging", characterized by absence of overt pathology but presence of some decline in function; and the last, the so-called "successful aging", with little or no physiological loss and no pathology. Mechanisms of successful aging are based on: 1) persistence of normal function and plasticity, 2) compensatory responses to restore normal function, 3) interventions to replace deficient functions, 4) changing of health outcome by modifying risk profiles, 5) prevention of disease, and 6) strengthening of social interactions and support (Rowe and Kahn 1998). It has been postulated that a successful example of this "functional remodeling" may be

mediated by neuroendocrine and immune systems (Mobbs et al 2001).

Caloric restriction

A single chapter in this review is deserved by caloric restriction, the only nongenetic intervention that has consistently shown to slow the intrinsic rate of aging in mammals (Dirks and Leeuwenburgh 2006). It is defined by the reduction in caloric intake while maintaining essential nutrient requirements. Traditionally, experimental mammalian models of caloric restriction reduce caloric intake by ~40% of the *adlibitum* diet throughout the lifespan of the animal. This reduction has resulted in a 30%–40% increase in maximum lifespan (Weindruch et al 1986).

How is caloric restriction able to increase lifespan? It is likely that this intervention can obtain beneficial effects by acting at various levels of function and involving a number of molecular cellular, and systemic changes. Not only is longevity increased, but also metabolic (eg, increased tissue sensitivity to insulin), neuroendocrine and immune (eg, increased defenses against stress, infections, cancer), and collagen responses (eg, reduction of cross-linking) are significantly enhanced (Mobbs et al 2001). It is noteworthy that such functional changes might also be modulated by changes in gene expression profile. Caloric restriction may promote longevity by a metabolic reprogramming with a transcriptional shift (perhaps triggered by insulin) toward 1) reduced energy metabolism, and 2) increased biosynthesis and turnover of proteins. It has also been demonstrated that caloric restriction markedly influences the expression of pathological phenotypes in rodent species selectively bred as models of human pathology (Weinert and Timiras 2003).

Even if the short-term effects in humans are promising (Walford et al 1999; Weyer et al 2000; Fontana et al 2004), long-term studies are not surprisingly difficult to conduct in humans. The lack of data from human models is mainly due to the difficulties of adhering to this rigorous intervention and the length of the human life span.

The most famous reports about the effects of caloric restriction on humans' health were obtained from the Biosphere 2 experiments. Biosphere 2 is a closed ecological space located in the deserts of Arizona. In 1991, eight individuals entered the biosphere for a two-year period to study the effects from living in a closed system. Because of unexpected technical problems, the access to food was limited for the entire duration of the study, so that the actual caloric intake of the participants was approximately 30% lower than what expected. Physiological and biochemical measurements were

assessed over the time spent inside the biosphere (ie, while crew members experienced caloric restriction) as well as 18 months after exiting the biosphere and returning to their normal diets. The physiological modifications experienced by the Biosphere 2 participants were similar to those found in caloric restricted rodents and nonhuman primates: decline in metabolic rate, body temperature, and systolic and diastolic blood pressure, and reductions in blood glucose, insulin, and thyroid hormone levels.

It has been shown that the Okinawan population is characterized by reduced morbidity and mortality, and the greatest percentage of centenarians in the world lives in this island. It has been hypothesized that the long disability-free life expectancy of this population might be due to the diet, based on vegetables, grains, soy, fruits, fish and seaweed, and characterized by a low caloric intake (~20% less than the rest of Japan and ~40% less than United States). It is noteworthy that this diet is very similar to the caloric restriction interventions designed for experiments in animal models.

Despite of the abundant data showing health benefits and the reduction of the aging rate by use of a caloric restriction intervention in mammalian animal models, it is likely that these beneficial effects will be lost in the translation to human models (Dirks and Leeuwenburgh 2006). Consequently, the previous great expectations about long-term caloric restriction in humans as the new "fountain of youth" have recently been resized.

Conclusion

It has been argued that more than half of the budget of the US National Institute on Aging is spent on Alzheimer's disease (Hayflick 2000a). Nevertheless, the elimination of this clinical condition will have only a minimal impact on life expectancy and will not help the advancing of our knowledge of fundamental biology of aging. Greater attention has to be given to a rarely posed question: why are old cells more vulnerable to disease than young cells? The answer to this issue will not only advance our fundamental knowledge of aging, but also promote the understanding of age-related diseases (Hayflick 2000a).

Several and important step forward the understanding of the aging process have been done, so that it is no more an obscure issue of biology (Holliday 2006). Nevertheless, further studies are still needed and numerous cues solved. In particular, it is important to clarify to which extent and at which price the aging process can be limited or reversed. In pursuing a solution to these issues, we should keep clear in mind what Hayflick wrote in a *Nature* commentary: "If

the main goal of our biomedical research enterprises is to resolve causes of death, then every old person becomes a testimony to those successes. Biogerontologists have an obligation to emphasize that the goal of research on ageing is not to increase human longevity regardless of the consequences, but to increase active longevity free from disability and functional dependence" (Hayflick 2000b).

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