INVITED REVIEW ARTICLE

Nagoya J. Med. Sci. **68**. 9 ~ 18, 2006

CELLULAR AND SYSTEMIC DEFENSE SYSTEM AGAINST AGE-PROMOTING STIMULI

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ABSTRACT

Although much progress has been achieved in aging research using lower animals, especially yeast and *C. elegans*, aging in humans remains puzzling. Here I offer my hypothesis of host defense against age-promoting stimuli, which holds that the cell itself has a defense system and multi-organism, an even more sophisticated one against age-promoting stimuli. Here I review recent achievements in aging research and try to explain their findings in the light of my hypothesis. Age-promoting stimuli, including reactive oxygen species, telomere shortening or external stimuli such as UV or radiation induce stress responses in cells. The cellular defense system operates to overcome stimuli or repair damaged cellular components. Stress-induced damaged cells or infectious stimuli activate systemic defense systems, especially the innate immune system. Macrophages in the innate immune system are especially active not only in clearing damaged cells and repairing damaged tissue, but unfortunately, also in inducing age-related diseases.

Key Words: Aging, Defense system, Stress, Macrophage

INTRODUCTION

This review focuses on the stress responses and host defense systems that affect the aging process and age-dependent diseases. Although much progress has been achieved in aging research using lower animals, especially yeast and *C. elegans,* aging in mammalian species remains poorly understood. Here I will discuss mammalian (human) aging and age-associated diseases, starting with several recent important findings.

In general:

All mammalian tissues or organs are composed of somatic cells that originate from a single fertilized egg. Nowadays Embryonic Stem (ES) cells can produce whole mammalian somatic cells. Every somatic cell has one set of genes, mainly identical in composition, although immune T and B cells have rearranged genes. Once a fertilized egg starts to divide, all the developmental processes are triggered by programmed expressions of various genes.

In aging research:

1. Cellular aging: Most mammalian cells in culture undergo a limited number of cell divisions before entering a state of irreversible proliferative arrest termed *replicative senescence*. The

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number of cell divisions in a culture varies with cell type and species, a number that has been termed the *Hayflick limit*. The occurrence of replicative senescence is determined by the number of times that a cell population divides, suggesting that a "mitotic clock records" cell divisions.[1] (Fig. 1).

2. Yeast: Originally discovered in budding yeast, yeast Sir2 is a structural component of silent chromatin and is required for transcriptional silencing of the silent mating-type loci, telomeres, and rDNA repeats. The silencing protein Sir2 is a limiting component of longevity; deletions of SIR2 shorten life spans and an extra copy of this gene increases them.[2]
3. *C. elegans*:

Important aging studies have been conducted using long-lived mutants of *C. elegans*. A pathway that regulates both lifespan and diapause was discovered early on. In response to food limitation and crowding, juvenile worms enter a state of diapause, called *dauer*. Dauers are developmentally arrested, reproductively immature, resistant to oxidative stress, and long-lived. Replete environments stimulate growth to adulthood by activating an insulin/IGF-1 signaling pathway. Loss-of-function mutations in the insulin/IGF-1 receptor homolog daf-2, or in downstream components of a conserved PI3-kinase/PDK/Akt pathway, cause dauer formations even when food is present. This pathway appears to act by downregulating a forkhead/winged-helix transcription factor called *DAF-16*.[3]

Cellular level of aging and defense system (Fig. 2)

Normal human cells undergo a limited number of divisions (around 50) in a culture and enter a non-dividing state called *replicative senescence*.[1] Extensive studies have been done to elucidate the causes of replicative senescence. One major cause originates in telomere shortening. The repetitive DNA sequence (TTAGGG in vertebrates) and specialized proteins that cap the ends of linear chromosomes are essential for chromosomal integrity. Although telomerase (an enzyme able to synthesize telomeric DNA de novo) is expressed in the germ line, many human somatic cells do not express it. Telomeric DNA shortening (50–200 bp) occurs during each cell cycle. At an average size of 4–6 kb of telomeric DNA, human cell growth is irreversibly arrested, producing a characteristic (senescent) phenotype.[4] Cells have a defense system telomerase to maintain telomere length, which adds motif-specific nucleotides using its RNA subunit as a template. The telomerase-associated protein (TEP1) binds to telomerase RNA and coordinates the assembly of telomerase holoenzyme[5], while the most important component responsible for the catalytic activity of telomerase is telomerase reverse transcriptase (TERT).[6] Previous studies have shown that TERT is expressed in most malignant tumors but not in normal human tissues, and that its expression is closely associated with telomerase activity, whereas two other components are constitutively expressed in both tumors and normal tissues.[7] It is clear that the level of expression of the TERT subunit is important for telomerase activity. Telomerase works to defend against telomere shortening, which resists cellular aging. What kind of stimuli will induce telomere shortning is an important question. We have analyzed TERT transcriptional regulation,[8] and have cloned a 1.6-kb region of mTERT promoter (1561/+53-Luc) that significantly promotes activity in mouse NIH3T3, C3H10T1/2 and C2C12 cell lines. We showed that the proximal 225-base pair region is the core promoter, which has a GC-rich region and an E-box. We further found that Sp1 or Sp3 has a strong level and c-Myc a weaker level of TERT transactivation in the mouse. Another group clones human TERT promoter. That promoter, a region of 300 bp upstream of the transcriptional start site, contains two E-boxes surrounding several Sp1 binding sites. E-box sites bind several cellular proteins, including the Myc/Mad/Max family of transcription factors.[9]

Other causes of replicative senescence depend on external or internal stresses. Recently, stimuli having little or no impact on telomeres were shown to induce normal cells to arrest growth with

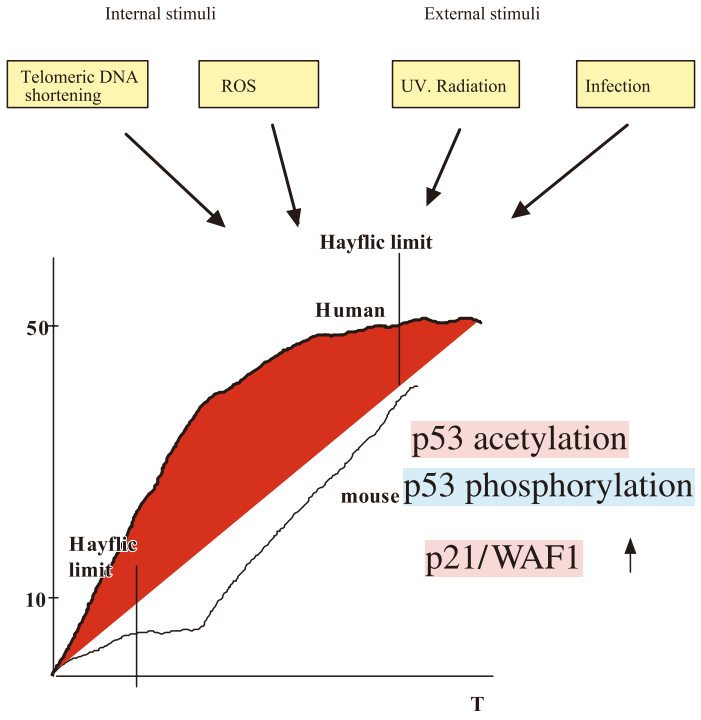

Fig. 1 Cellular senescence

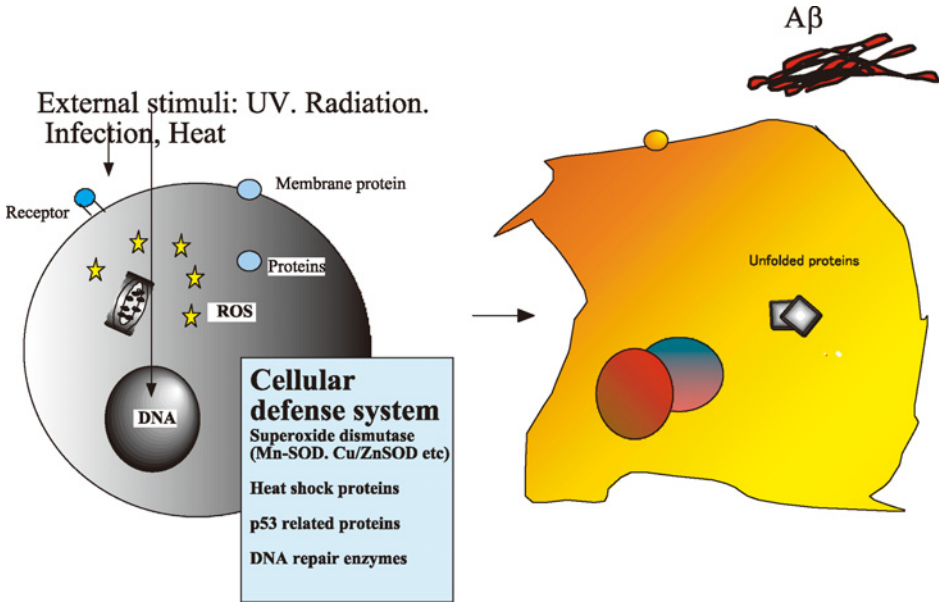

Fig. 2 Cellular defense system

a senescence phenotype.[10] These stimuli include DNA damage (double strand breaks or oxidation), chromatin remodeling, and strong mitogenic signals. Campisi proposed that cellular senescence is one mechanism to prevent the development of cancer, which is caused by these stimuli.[11]

Reactive oxygen species (ROS) are produced internally by glucose metabolism or by external stimuli such as UV radiation, X-ray radiation or microbial infection. ROS induce various kinds of severe damage to cellular components. Harman proposed an aging theory suggesting that ROS generated by metabolism cause cumulative damage over time.[12] Mammals have developed a defense system. The major enzymes crucial to catalyzing oxygen radicals are three types of superoxide dismutase (Mn-SOD, Cu/Zn-SOD, and E-SOD), catalase, and glutathione peroxidase. Along with others, we have analyzed the expression of Mn-SOD induced by pro-inflammatory cytokines such as tumor necrosis factor-α (TNF-α), interleukin-1 (IL-1), lipopolysaccharide (LPS), and interferon-γ (IFN-γ). We have found that the newly identified NF-κ B and C/EBP sites lead to synergistic gene transcription.[13,14]

In cellular senescence, cells undergo cell-cycle arrest. Many investigators are trying to elucidate the signaling pathways that induce cell-cycle arrest by senescence-inducing stimuli. Considerable evidence points to the expression of p21/WAF1, which induces cell cycle/senescence arrest. DNA-damaging stimuli including ROS, UV radiation and X-ray radiation induce p21/WAF1 by several signaling pathways. Telomere shortening and oncogenic RAS induce p53, which in turn induces p21/WAF1. To identify the signaling pathway used to induce p21/WAF1 expression, we tried to discover the transcriptional regulation of p21/WAF1 expression. By isolating 4.6 kb of murine p21 promoter and inserting it upstream of a luciferase reporter gene, we found that this promoter has about 100 bp of a minimal promoter, which has a GC-box where Sp1 and Sp3 bind and work as transcription factors.[15,16] p53-binding sites exist upstream of the minimal promoter of p21/WAF1. Although many DNA-damaging stimuli induce p21/WAF1 expression via the p53 pathway, the histone deacetylase inhibitor sodium butyrate and the trichostatin A-induced expression of p21WAF1 are p53 independent. These inhibitors induce a senescence-like phenotype. We have further shown that p300 collaborates with Sp1 and Sp3 in p21/WAF1 promoter activation induced by histone deacetylase inhibitor.[17]

The best-known defense protein against DNA damaging stresses is p53. Although it has been firmly established that p53 works as a suppressor of cancer development, its role in aging is controversial. We found that the development and aging of the immune system were accelerated in p53-deficient (p53-/-) mice.[18] Tyner *et al.* showed that mice have an enhanced level of p53 (p53+/m) that causes early aging and cancer resistance phenotypes.[19] Scrabble and colleagues described another p53 transgenic mouse model exhibiting suppressed growth and a diminished lifespan.[20] This model is wild-type for p53 and transgenic for the p53 isoform missing 41 codons. Another mouse model with enhanced p53 activity was shown to increase tumor resistance without causing premature aging,[21] a finding that argues against a role for p53 in aging. The transgenic approach sometimes induces other mutations. It has been confirmed that Tyner's p53+/m mice have additional deletions, i.e. 3 genes involved in lipid oxygenation.[22] Recently, Keyes *et al.* have found that p53-related protein p63 works as a defensive protein against aging processes, reporting that p63+/- mice have a shortened life span and display features of accelerated aging.[23] Their results support ours that p53 (p63) works as a defense protein against age-promoting stimuli.

Although DNA damage is induced by senescence-accelerated stimuli, it can also be spontaneously generated because of errors during DNA replication in dividing cells. Again, the cell itself has a defense system to repair DNA damage or maintain genomic instability. Mutations of such a repair system reveal premature syndromes. Werner syndrome, Cockine syndrome, and ataxia telangiectasia exaggerate the DNA damage and accelerate aging. Mutations in nuclear proteins involved in the maintenance of genomic integrity give rise to another premature syndrome,

Lamin A, which is a major component of the nuclear lamina and nuclear skeleton. Truncation in Lamin A causes Hutchinson-Gilford progerial syndrome (HGPS), a severe form of early-onset premature aging.[24,25]

Yeast as a single organism may also be duplicated to some extent in the cellular aging of multi-organisms. Extensive studies have been done to elucidate the sir2 function in multi-organisms. It has recently been discovered that sir2 is involved in the insulin/IGF-1 signaling cascade. Genetic experiments with *C. elegans* have suggested that SIR2, an NAD-dependent protein deacetylase, acts through the FOXO/DAF-16 transcription factor to prolong life. FOXO mediates the transcriptional output of insulin/IGF-1 signal transduction, which regulates the longevity of species as diverse as worms, flies, and mice.[26] In *C. elegans,* Ligand (37 insulin-like peptides expressed in neurons, intestine, muscle, epidermis, and gonad) bind to DAF-2 insulin-like receptor signals through a kinase cascade to phosphorylate the forkhead transcription factor DAF-16, which is thus sequestered in the cytoplasm. In this state, adults swiftly reach reproductive maturity and age rapidly. Without activation of the pathway, DAF-16 promotes transcription and induces diapause and exceptional longevity. Insulin signaling from IGF-1R (DAF-2) through the PI3-kinase/PDK/Akt pathway to DAF-16/FOXO clearly determines nematode aging. What happens

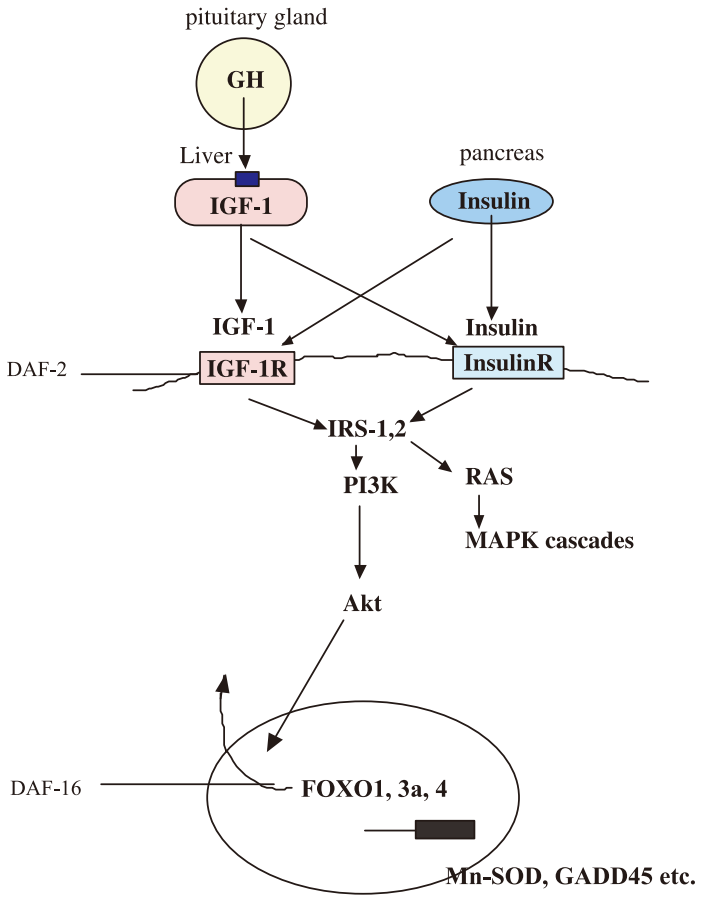

Fig. 3 IGF-1(DAF2)/PI3K/FOXO(DAF16) pathway in mammals

in mammals, which have a similar signaling pathway? One theory of aging has emerged from studies in several laboratories, which suggests that calorie restriction enhances rodent life spans. This theory agrees well with the findings on molecular genetics in lower animals. Food intake promotes release of the growth hormone (GH), luteinizing hormone (LH), follicle-stimulating hormone (FSH), and thyroid-stimulating hormone (TSH) from the pituitary gland via the hypothalamus. GH induces the release of IGF-1 from the liver. Food intake also releases insulin from the pancreas. Both insulin and IGF-1 bind to IGF-1R in somatic cells and promote the PI3-kinase/PDK/Akt pathway, which induces cellular proliferation and accelerates aging (inhibits longevity). The PI3-kinase/PDK/Akt pathway converges with DAF-16. which works as a cellular defense protein, since DAF-16 becomes localized in the nucleus in response to various stresses. As a transcriptional factor, DAF-16 up-regulates Mn-SOD (oxidative protection), GADD45 (DNA-damage repair), and other stress-resistant genes.[27,28] (Fig. 3).

Systemic defense system – immune system: relation to aging and age-associated diseases.

Although we have learned that age-accelerating stresses induce damage to cellular components, cells have their own defense system, which might avoid the accumulation of damage. Organisms have developed another level of defense, i.e. immune system. My hypothesis is that age-associated or stimuli-associated damaged cells (self-altered) induce macrophage activation (Fig. 4). Activated macrophages have two roles. One is the elimination of damaged cells; another is the development of an immune reaction that includes acquired immunity. If inflammation continues, chronic tissue damage will occur, followed by tissue aging or age-related diseases. There are many examples of the ability of the host defense system to repair the tissue damage induced by age-promoting stimuli. Consider sun-burned skin. UV stimuli induce skin damage, which is then removed by tissue macrophages. Since the immune system has developed to combat foreign microorganisms, I will first discuss the relation of infection to aging. In this case the age-accelerating stimulus is infection by microorganisms.

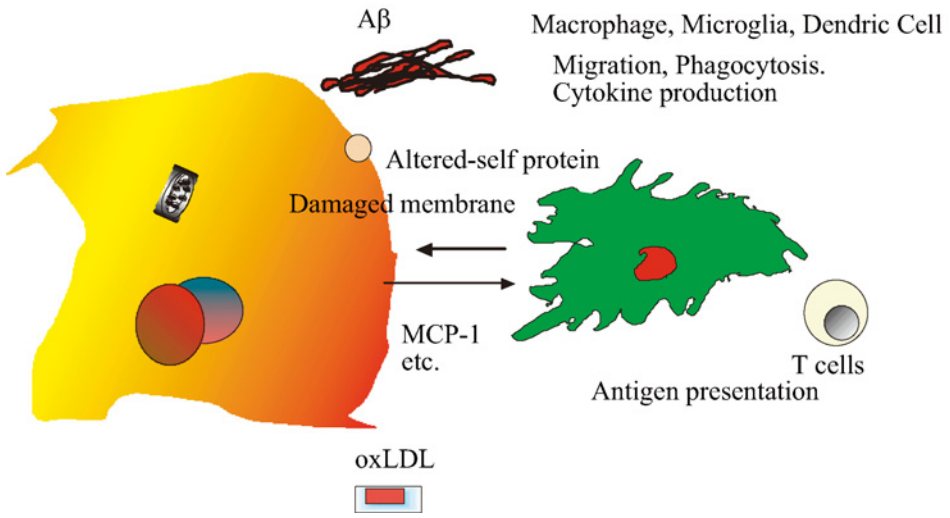

Fig. 4 IGF-1(DAF2)/PI3K/FOXO(DAF16) pathway in mammals

1) Infection

Finch *et al.* proposed the "inflammatory theory of aging".[29] They developed the specific hypothesis that less inflammation during early life leads directly to a decrease in morbidity and mortality resulting from chronic conditions in old age. Their hypothesis is based on the abundance of evidence linking early-life infections with later morbidity, including cardiovascular and respiratory disease, cancer, and diabetes. Some infections directly cause organ damage; the classic example is rheumatic heart disease, which increases adult morbidity from heart valve damage incurred by childhood streptococcal infections. Symptoms of inflammation include elevations of blood CRP as well as interleukin-6, tumor necrosis factor α, and fibrinogen (components of the acute phase of inflammatory response).

Persistent infection will have some effects on the aging of virus-infected tissue, because CD8 T cells contain cell-damaging granules such as granzyme B, e.g., hepatitis C virus (HCV) infects liver cells. This virus is not cleared and usually persists for an entire lifetime. In that case, liver cells may be always vulnerable to attack by CD8+ cytotoxic T cells. A pair of important human viruses, cytomegalovirus (CMV) and Epstein-Barr virus (EBV), induce lifelong persistence, which under normal circumstances remains below the threshold of detection. The most notable aspect of the immunobiology of CMV is the level of T cell response. Very vigorous responses are observed against single epitopes contributing to a substantial fraction of memory CD8+ T cells in healthy seropositive individuals.[30] Moreover, persistent lifelong infection of CMV and EBV has recently been shown to affect T cell repertoires in the elderly, perhaps impairing responses to other antigens.[31]

2) ROS

We humans suffer from oxidative stress under normal physiological conditions. Superoxide ($O2^{-}$) is formed when an oxygen molecule accepts an additional electron. This occurs regularly, through a non-enzymatic mechanism, as a by-product of the mitochondrial electron transport chain. It is estimated that this chain consumes up to 90% of the oxygen taken up by a cell, ~1% of which is converted to O_2^- under normal physiological conditions.[32] As mentioned in the cellular defense section, enzymes detoxify O_2^-. However, ROS can accumulate in cells with damaged detoxification systems that undergo elevated levels of respiration or that show defects in the electron transport chain. The resultant ROS can then inflict damage on various molecules. Such damaged molecules or cells may be detected by macrophages. Atherosclerosis is one of the major phenotypes among age-associated diseases. Oxidized LDL (oxLDL) is rapidly taken up by macrophages via scavenger receptors.[33,34] The sequential stages are now thought to be as follows. When arterial endothelial cells undergo inflammatory activation, they increase their expression of the vascular cell adhesion molecule-1 (VCAM-1). Blood monocytes adhere to the activated epithelium and migrate into the intima due to chemoattractant gradient. Monocyte chemoattractant protein-1 (MCP-1) and its receptor CCR2 constitute the main chemokine and its receptor. Once resident in the intima, the monocyte acquires characteristics of the tissue macrophage, which expresses scavenger receptors that bind oxidized or glycolated lipoproteins. The macrophage that ingested modified lipids are called *foam cells*. Those cells secrete pro-inflammatory cytokines, growth factors, ROS and MMPs. The macrophage- colony stimulating factor (M-CSF) is important in stimulating the transition of monocyte to the lipid-laden macrophages, inducing scavenger receptor A (SRA) expression, and increasing the production of cytokines. GM-CSF aids the survival of mononuclear phagocytes.

3) Accumulation of aging products detected by macrophage

Budding yeast accumulates circular rDNAs by cell division, which induces cell death. Because Sir2 protein inhibits the production of circular rDNA, Sir 2 is one of the most notable candidate proteins in aging. However, multi-organisms like ourselves are different. We have macrophages

that recognize damaged cells and initiates the stages of immune reaction. One of the age-related accumulated materials is amyloid-β peptide (Aβ). Alzheimer's disease (AD) is characterized by the presence of senile plaques in the brain composed primarily of Aβ. Microglia have been reported to surround the Aβ plaques, which provokes a microglia-mediated inflammatory response that contributes to neuronal cell loss.[35] We have studied that Aβ-induced microglial activation, and have found that Aβ induces M-CSF expression via PI3/Akt to the NFκB signaling pathway.[36] Moreover, we have discovered that several genes related to innate immunity are up-regulated following the activation signal in microglia by Aβ, especially Aβ 1–42, which is increased by aging. Among those genes, chemokines and MMPs, which are related to innate immunity, are similer to atherosclerosis (unpublished results).

CONCLUSION AND PERSPECTIVE

Here, I have proposed a host-defense hypothesis against age-promoting stimuli. I posit that cellular and organism defense systems work to retard the aging process. In particular, our own multi-cellular organism has an innate and adaptive immune system differing completely from that in the aging of yeast, which is a single-cell organism. Another aspect of this review is that age-associated diseases can also be induced by our immune system. Such an innate immune system is especially activated by damaged cells, ROS-induced products, and age-related materials. Although a host defense system sometimes works to delay aging and prevent age-related diseases, that same defense system can induce age-related tissue damage and accelerate aging and age-related diseases. What critical comparisons can be made between good and bad effects? My current position is that once the immune system has eliminated the offending microorganism or stimuli-induced damage, it (the host defense system) stops working and the organism return to its original condition. However, if stimuli continue, the host defense system continues to work, and damage induced by stimuli and the immune system itself accelerate aging and age-related diseases.

At present there are many hypotheses of aging. The major points of my hypothesis are based on recent advances in immunology and molecular biology. Of course, various stress and aging hypotheses as well as inflammation hypotheses have already been proposed. The critical point of my hypothesis is that the cell itself has a defense system and a multi-organism has an even more sophisticated one, both of which are major components of immunology in the broad sense. Human aging and age-related diseases will be more deeply understood by research into both cellular and systemic defense systems against age-promoting stimuli.

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