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Invited Mini Review

Longevity through diet restriction and immunity

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The share of the population that is aging is growing rapidly. In an aging society, technologies and interventions that delay the aging process are of great interest. Dietary restriction (DR) is the most reproducible and effective nutritional intervention tested to date for delaying the aging process and prolonging the health span in animal models. Preventive effects of DR on age-related diseases have also been reported in human. In addition, highly conserved signaling pathways from small animal models to human mediate the effects of DR. Recent evidence has shown that the immune system is closely related to the effects of DR, and functions as a major mechanism of DR in healthy aging. This review discusses the effects of DR in delaying aging and preventing age-related diseases in animal, including human, and introduces the molecular mechanisms that mediate these effects. In addition, it reports scientific findings on the relationship between the immune system and DR-induced longevity. The review highlights the role of immunity as a potential mediator of the effects of DR on longevity, and provides insights into healthy aging in human. [BMB Reports 2023; 56(10): 537-544]

INTRODUCTION

Aging is a leading factor in the gradual decline of biological functions and an organism's ability to adapt to metabolic stresses; thus, aging is a major cause of many diseases. Therefore, interventions that slow down the aging process are thought to delay or prevent chronic diseases, and improve the quality of life of the elderly.

A major symptom of aging is a decline in immune function, which is associated with the development of various age-related diseases (1). The immune system is classified into two types: innate and adaptive (2). The body's first line of defense is the innate immune system that detects external invasion by viruses and bacteria and the associated cell damage. The second line of defense in the human body is adaptive immune cells, and they can remember external intruders once recognized, and block secondary intrusions to protect the host. Immunosenescence (3), the aging of the immune system, is a major cause of death due to infectious diseases, such as pneumonia and sepsis, in the elderly. Mortality data from the COVID-19 pandemic also indicate that the elderly individual is highly vulnerable to infectious diseases (4). Therefore, it is urgent to develop an effective method to alleviate the decline in immune function to delay aging and maintain a healthy life in an aging society.

Dietary restriction (DR) refers to a reduction in the intake of specific or total nutrients without causing malnutrition. This is often referred to as limiting calorie intake or calorie restriction (CR). Various DR recipes such as low in calories, fasting, fasting-mimicking diet (FMD), and intermittent fasting are being used to study the biological effects of DR and its mechanism of action (5-7). DR reduces the incidence of age-related diseases (8-14), and prolongs the lifespan of animals (15-17). Furthermore, DR such as caloric restriction and fasting can impact stem cell function and regulate tissue homeostasis and regeneration (5). In addition, signaling pathways that mediate DR-induced longevity are conserved in the animal kingdom. Therefore, DR is considered as a reproducible and effective anti-aging nutritional intervention. Recently, scientific findings reported that the anti-aging effects of DR are closely related to the regulation of immunity (8, 18-20). DR can activate or regulate the homeostasis of immune function, and DR prolongs the longevity of animals through regulation of the immune function.

This review provides a brief overview of the effects of DR on longevity and the prevention of the onset of diseases in the animal kingdom. As the most representative and distinct pathways that mediate DR effects, insulin/IGF1 signaling (IIS) and target of rapamycin (TOR) signaling pathways are described. In addition, the relationship between anti-aging regulation of DR and immune function is discussed. This review suggests the potential that studies of homeostatic regulation of immune function via DR may lead to the development of effective anti-aging intervention methods.

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DIETARY RESTRICTION (DR) EFFECTS ON DELAYING AGING AND/OR PREVENTING AGE-ASSOCIATED DISEASES

DR effects on longevity

DR is the most widely studied and reproducible intervention that is known to extend the lifespan of organisms. Here, we describe the effects of DR on the longevity of *C. elegans*, mouse, and monkey.

C. elegans is a useful model organism for studying the genetic and non-genetic interventions that control aging and longevity (21). The physiological aging characteristics of *C. elegans* are very similar to those of human, and it is the first multicellular eukaryote whose genome has been completely sequenced. Thus, *C. elegans* has been widely used to study the mechanisms underlying aging and age-related diseases. The effect of DR on longevity at the organism level has been demonstrated in *C. elegans* (16). Two main approaches have been used to analyze the effects of DR in *C. elegans*. The first was to reduce nematode food intake by reducing the amount of food provided (22). The second was to analyze the effects of DR using mutant worms with low food intake ability (23). In both methods, the lifespan of the nematodes was greatly increased. In addition, genes and signaling pathways that regulate DR-induced longevity have been identified in *C. elegans* (23), and these are well-conserved in other animals (24).

The first report on the effect of DR on longevity was published in 1935 by researchers at Cornell University using mouse (15), where the researchers found that the lifespans of the mice could be extended by 33% by feeding them a very low-calorie diet. Furthermore, DR can extend the average and maximal lifespan of mice even when it is started in middle age (12 months) (25). Recently, studies on DR methods that maximize the effects of lifespan extension have been conducted in mouse. To this end, mice were tested using more sophisticated DR methods by considering daily fasting intervals or feeding cycles. It has been reported that DR treatment at night, when mice are most active, is very effective at prolonging their lifespan (26).

If DR can extend animal lifespans, can it extend human lifespans as well? To answer this question, the effect of DR was confirmed in rhesus monkey, which is the closest model organism to human. The rhesus monkey genome shares approximately 93% sequence identity with the human genome, making it an excellent research animal model for human aging (27, 28). Studies at the National Institute on Aging (NIA) (29) and the University of Wisconsin Madison (UW) (17, 30) found the effects of DR on the longevity of monkey. Interestingly, in the UW study, monkeys subjected to DR had significantly extended lifespans, compared to those of the control monkeys (17, 30). However, the NIA reported the opposite effect. The monkeys subjected to DR in the NIA study showed no improvement in their lifespan. Thus, until now, the effect of DR on the longevity of monkeys has remained an issue of debate. This discrepancy is probably due to differences in diet composition, feeding practices, and the heterogeneous genetic background of the monkeys in each study (31). Therefore, it should be emphasized that more sophisticated and consistent experimental methods are needed to obtain more accurate experimental results on DR-induced longevity in rhesus monkey.

In human, there are no results yet that evaluate the longevity effects of DR. While the lifespan extension effect of DR has been confirmed in small animals and monkeys, and will be

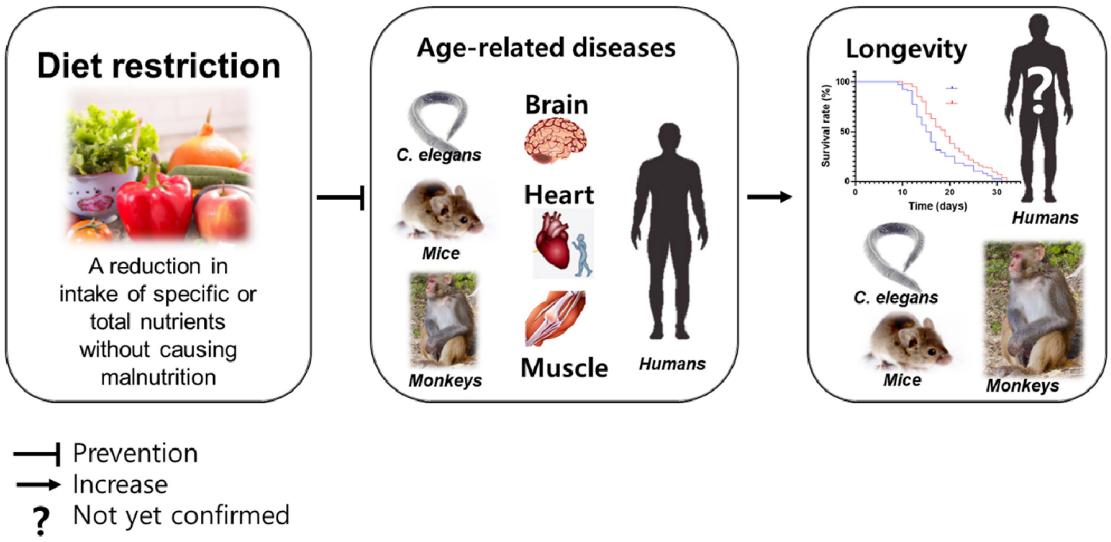

Fig. 1. DR effects in animals. DR prevents age-related diseases in various tissues including brain, heart, and muscle in animals and humans. Furthermore, DR increases the lifespan of animals. An impact of DR on longevity has not yet been confirmed in humans.

discussed further, it has been confirmed that DR can reduce the incidence of age-related diseases in human. Thus, the possibility of lifespan extension by DR remains a possibility in human (Fig. 1).

DR and the prevention of age-related diseases

DR both prolongs the longevity of organisms, and reduces the incidence of age-related diseases. Here, the effects of DR in preventing age-related diseases in *C. elegans*, mouse, monkey, and human are presented.

In *C. elegans*, DR has been shown to alleviate geriatric diseases, such as sarcopenia. Sarcopenia is the age-related progressive loss of muscle mass and strength (32). During aging, muscle activity declines in *C. elegans*, and DR can prevent age-associated decline in muscle activity (8). DR prevents mitochondrial damage and fragmentation of mitochondrial networks in body wall muscles of aged *C. elegans* (8). In addition, DR confers protection against polyglutamine proteotoxicity (9) in a nematode model, in which a tract of 35 consecutive glutamine residues was fused to YFP (Q_{35}YFP), and expressed in the body wall muscles (10).

In mouse, DR prevents age-related diseases, leading to prolonged good health and survival. Cancer is the main cause of death, accounting for 70-80% of all rodent deaths. Malnutrition-free DR has been shown to prevent or delay the onset of cancer, chronic kidney disease, cardiomyopathy, diabetes, and autoimmune and respiratory diseases in rodent (11-13). The incidence of cancer and multiple sclerosis is reduced by various methods of inducing DR, such as fasting or FMD (6, 33). Furthermore, DR is effective at reducing beta-amyloid deposition in the brains of mice with neurodegenerative diseases, such as Alzheimer's disease, Parkinson's disease, Huntington's disease, and stroke (34, 35).

In primate, the positive effects of DR-induced delay in age-related diseases have been reported by both the NIA (29) and UW (17, 30). Reduced incidences of various age-related diseases, such as cancer, cardiovascular disease, obesity, sarcopenia, and diabetes, have been reported in monkeys subjected to DR (14, 30). In particular, studies from both groups have confirmed that monkeys subjected to DR had a lower incidence of cancer than the controls, thus confirming that tumor suppression is a hallmark of DR. Researchers at the UW have shown that DR can reduce the incidence of cardiovascular disease and the incidence of insulin resistance, while the NIA has reported reduction of the incidence of diabetes.

The beneficial effects of DR have also been observed in human. In human, according to the available information, the effects of DR on various age-related diseases are similar to the positive effects of DR seen in experimental animals. Among the data on the effects of DR on human health, data on the elderly mortality rate in Okinawa (Japan) are very valuable. During the 1940s-1960s, residents of Okinawa Island consumed significantly fewer daily calories (1,785 kcal/day [d]) than those living in the United States (2,980 kcal/d) or mainland Japan (2,068 kcal/d) (36), and older people (aged 65+ years) on Okinawa Island had significantly lower mortality rates from coronary heart disease or cancer than those living in mainland Japan or the United States (37). These data indicate an association between DR and reduction of human adult diseases. As another example, the Calorie Restriction (CR) Society consists of volunteers consuming approximately 1,800 kcal/d, i.e., similar to the prior daily calorie intake of the residents on Okinawa Island, for an average of 6.5 years (38). At 1,800 kcal/d, there was better left ventricular diastolic function than for age- and sex-matched controls (39), and a lower risk of atherosclerosis and hypertension (40). Furthermore, the low-calorie groups maintained lower levels of systolic and diastolic blood pressure than the control group, and the levels of inflammatory markers (e.g., C-reactive protein, tumor necrosis factor-α, and interleukin-6) were also lower than those in the control group (39-41). The results of the Comprehensive Assessment of the Long-Term Effects of Reducing Energy Intake (CALERIE) study, which involved a 25% calorie restriction for just two years, also confirmed that DR provides multiple benefits for non-obese people. The CALERIE study showed positive health-promoting effects, including reduced inflammatory markers and cardiac metabolic risk factors (42, 43). Although DR has many health benefits, it can also cause adverse side effects, such as decreased bone mineral density (44). Therefore, a longer duration and larger cohort study will be needed to develop a DR recipe that can have a positive impact on human health (Fig. 1).

SIGNALING PATHWAYS MEDIATING DR EFFECTS ON LONGEVITY

IIS pathway

Small animals with short lifespans and well-conserved genetic functions have provided genetic information on the mechanisms of aging. Regulation of organismal aging by the IIS pathway was first reported in *C. elegans* (45). Since then, the control of lifespan by the IIS pathway has been confirmed in other animals, such as *Drosophila* and mouse (46). The forkhead transcription factor DAF-16 is the primary target of the IIS pathway. The IIS pathway retains DAF-16 in the cytoplasm, and inactivates it by phosphorylation. However, mutations in genes of the IIS pathway induce dephosphorylation of DAF-16, which then translocates to the nucleus and modulates the expression of various lifespan-regulating genes (47), thereby increasing the lifespan of *C. elegans*. The IIS signaling pathway is involved in DR-induced lifespan extension. DAF-16 is required for longevity under certain conditions, such as solid DR (sDR) and intermittent fasting (IF) (7, 22). However, in some studies, DAF-16 was not found to be required for longevity in other DR recipes (48-50). In addition, IIS signaling pathway is necessary for lifespan extension by intermittent fasting in *C. elegans* (7).

In mouse, similar to in *C. elegans*, mutations in genes in the IIS pathway increase lifespan. The insulin receptor substrate

(IRS) mutant mouse has a longer lifespan than control mice. The lifespan of $IRS1^{-/-}$ (51) and $IRS2^{+/-}$ mice (52) increased by 18%, and $IRS2^{+/-}$ and $IRS2^{-/-}$ in brain correlated with 18 and 14% lifespan extension, respectively, compared to control mice (52). Furthermore, mutant mice with low IGF-1 levels have longer lifespans than control mice. GH receptor-knockout (GHR-KO) mice with low IGF-1 levels live 38-55% longer than control mice (53). IGF-1 receptor heterozygous knockout mice ($IGF\text{-}1R^{+/-}$) also have a very low level of serum IGF-1 and a longer lifespan than that of control mice (54-57). In mouse, intermittent fasting reduces IGF-1 production (58) and fasting reduces growth hormone (GH) and IGF-1 levels (59), suggesting that IIS plays an important role in DR-induced lifespan extension and the delay of age-related diseases.

In human, the effect of corresponding mutations in IIS on longevity remains controversial. In the Itabaianinha cohort (more than 100 humans) in Brazil, a homozygous GH-releasing hormone receptor (GHRHR) mutation that failed to secrete GH correlated with a normal lifespan, and mortality from cancer was similar to that of the general population group (60). However, patients with Laron syndrome in Ecuador, with homozygous mutations in the GHR or GH-induced intracellular signaling molecules (61), had a reduction in cancer-related deaths (62). Protein intake is a key determinant of circulating IGF-1 levels in human. Total and free IGF-1 concentrations are significantly lower in individuals with moderate protein restriction (63). Reducing protein intake from an average of 1.67 to 0.95 $g \cdot kg^{-1}$ of body weight per day for three weeks in six volunteers practicing DR resulted in a reduction in serum IGF-1 levels from 194 to 152 $ng \cdot ml^{-1}$. Therefore, it is possible that DR by reducing protein intake could help maintain healthy aging through the inhibition of IIS.

TOR signaling pathway

TOR is a conserved serine/threonine kinase that integrates nutritional information from the environment to regulate growth in multiple species (64). Thus, a decrease in TOR signaling indicates a decrease in the nutrient status of the environment, like DR, and reduced TOR signaling extends the lifespan of animals.

In *C. elegans*, reduced TOR signaling increases longevity (65). Reducing bacterial food levels in the diet or *eat-2*, a DR-mimicking mutant strain that eats less than the wild-type strain, resulted in an increased lifespan (7, 50, 66). However, inhibition of the TOR signaling pathway in *eat-2* mutant strains did not result in further lifespan extension (50, 67), suggesting that TOR inhibition mediates the effect of DR on longevity in *C. elegans* (68, 69).

Reduced TORC1 signaling extends the lifespan in mouse. S6K1 is a direct TORC1 substrate, and its phosphorylation and activity are modulated by TORC1. A cohort of S6K1-deficient female mice exhibited a 19% increase in lifespan, compared with the wild-type control group (70). However, S6K1-deficient male mice do not exhibit a significant increase in longevity (70). Exposure to the mTOR pathway inhibitor rapamycin resulted in significant median and maximal lifespan extension in both male and female mice (males 9%, females 13%) (71). In addition, if rapamycin treatment was started when the mice had reached middle age (270 d) or old age (600 d), the lifespan of the mice increased under both conditions (71). Another TOR inhibitor, metformin, downregulates TORC1 activity by phosphorylating Tsc2 via AMPK (72), and treatment with metformin prolongs the lifespan of female mice (73).

The effects of mTOR antagonists on health have been investigated in human. Selective TORC1 inhibition improves immune function and reduces infections in elderly individuals (74). Improved immune function and reduced infection rates were observed in 264 elderly subjects who received a low-dose combination of a catalytic (BEZ235) and allosteric (RAD001) TORC1 inhibitors (74). In addition, upregulation of antiviral gene expression and an enhanced response to the influenza vaccine were observed in the elderly, even one year after initiation of the study drug (74). Rapamycin treatment is a potential antiaging therapy for human skin (75). Rapamycin treatment of the skin reduced the expression of p16INK4A, a marker of aging, which reflects a decrease in cellular senescence. Additionally, rapamycin treatment increased collagen VII levels, which are important for basement membrane integrity (75). However, studies using data from patients prescribed metformin and rapamycin have important limitations, because these drugs are prescribed for the treatment of life-shortening diseases. Rapamycin and its derivatives also have side effects, such as type 2 diabetes (76).

DR-INDUCED LONGEVITY IS REGULATED BY IMMUNE SIGNALS

Although little is known about the function of the immune system in animal longevity, Soo *et al*. recently reported that the innate immune system plays an important role in determining longevity, and that the same genes drive both immunity and longevity (77). Consistent with this finding, Fabian *et al*. (78) reported that aging and immune responses are modulated by a small number of conserved genetic pathways in animals, including in human. The authors compiled genetic information regarding regulation of the aging process and immune responses through public databases and in-house manual curation in *C. elegans*, D. melanogaster, mouse, and human. They combined genes within the Gene Ontology (GO) and KEGG terms related to aging or immunity, and included annotations from aging (GenAge (79), AgeFactDB (80)) and immunity (insect innate immunity database IIIDB (81), InnateDB (82), and immunome knowledge base IKB (83)) databases. They identified several conserved genes known to regulate both aging and immune responses. Ten highly conserved immune-aging genes were identified in the four species, six of which are signaling components of the IIS and TOR pathways. These are akt-1/Akt1/AKT2, age-1/Pi3K92E/PIK3CD, daf-2/InR/IGF1R, DAF-16/

foxo/FOXO3, let-363/Tor/MTOR, and rsks-1/S6k/RPS6KB2 (gene name sequence: *Caenorhabditis elegans*/*Drosophila melanogaster*/mammal). The other four immune-aging genes are mpk-1/rl/MAPK1, pmk-1/p38a, b/MAPK14, mek-2/Dsor1/MAP2K1, and let60/Ras85D/HRAS, which act on the ERK and p38 MAPK signaling pathways (78). Therefore, these results suggest that the regulation of aging and immune function are closely related to each other, and suggests the need for detailed studies of the role of immune function in aging regulation.

In fact, recent studies have reported that DR-induced longevity is regulated by innate immune signaling components. In *C. elegans*, the bZIP transcription factor ZIP-2 is an innate immune signaling component molecule that is upregulated in response to infection by *Pseudomonas aeruginosa* (PA14), and is necessary for survival against PA14 infection (84). Hahm *et al*. reported that ZIP-2 is a key mediator of the effects of DR on healthy aging in *C. elegans*. They found that ZIP-2 activity increased in response to DR, and *zip-2* was necessary for DR-induced longevity and physical activity improvement in worms subjected to DR (8). They found that ZIP-2 activity was increased by inhibition of the TOR signaling pathway and rapamycin treatment (8). They concluded that *zip-2* extends longevity through TOR/S6K inhibition by DR. In addition, Hahm *et al*. found that the F-box gene *fbxc-58* is a zip-2 downstream effector molecule that protects worm against PA14 infection. They found that *fbxc-58* was upregulated by DR or S6K mutation, and extended the longevity of worm through DR (18). Consistent with the results in *C. elegans*, acute DR boosts innate immunity in *Drosophila* (19). Lee *et al*. reported that DR via yeast restriction enhanced *Drosophila* survival against PA14 infection, and they confirmed that reduced TOR signaling protected flies from pathogenic bacterial infection. In addition, they confirmed the beneficial effects of yeast restriction on *Drosophila* immunity following rapamycin treatment. The p38-MAPK signaling pathway is an important innate immune pathway that is highly conserved from *C. elegans* to human. Wu *et al*. reported that the p38-MAPK signaling pathway is related to longevity extension by DR in *C. elegans*. They found that DR maintained the level of the p38-ATF-7 (ATF-7 is a transcription factor downstream of p38) innate immune response at the basal activation level (20), and that maintaining p38-ATF-7 activity at the basal level is an important factor for longevity in *C. elegans*. Thus, these results imply that the regulation of immune signals by DR is an important mechanism for extending longevity.

To the best of our knowledge, whether the immune system is involved in DR-induced longevity in mammal has not been established. However, several studies have reported that DR increases immune function in mammal and human. Fasting and FMD can improve immune response through regeneration of immune system. Chronic use of the FMD promotes a reversal of the age-dependent decline in the lymphoid-to-myeloid ratio (85), and prolonged fasting promoted hematopoietic stem cell based regeneration that affects to lymphocyte number and

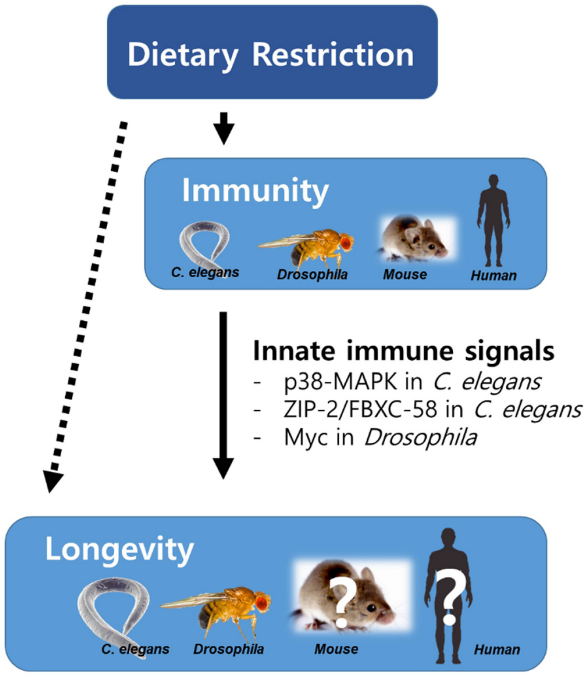

Fig. 2. DR extends longevity through modulation of immune signals. DR activates immune responses or prevents age-related immunosenescence in animals, including humans. DR increases the lifespan of animals through innate immune signals such as p38-MAPK-, ZIP-2-, or Myc-related signals in *C. elegans* and *Drosophila*. An impact of DR on longevity through immune signals has not yet been confirmed in mice or humans.

a reverse of immunosenescence (86). Mature functional T cells are generated in the thymus. Shrinkage of the thymus with aging reduces immune surveillance. Age-related changes in the adaptive immune system, such as thymic degeneration, reduced production of naive T cells, reduced T-cell proliferation, and reduced activity of cytotoxic T lymphocytes, are accompanied by a weakening of immune function. DR can inhibit immune aging by preserving T-cell function and repertoire, and promoting the production and/or maintenance of naive T cells in mouse, primate, and human (87-89). Furthermore, the concentrations of pro-inflammatory cytokines were lower in the DR group (89). These results raise the expectation that DR can enhance the lifespan of human by supporting a healthy immune system (Fig. 2).

CONCLUSIONS

This review explained the relationship between DR, a representative anti-aging intervention, and the immune system. Among the anti-aging intervention methods reported so far, DR is known to be the most effective and reproducible in animal, and the decline in immune function is recognized as a

cause of aging, or a result of aging. Actual decline in immune function is closely related to aging and the occurrence of aging-related diseases. Many reports have demonstrated that the anti-aging effect of DR is induced by regulation of the function of the immune system. Through DR, it is possible to alleviate the decline in function of various immune cells due to aging, and increase the activity of the immune response. In addition, a comparative analysis between species on the relationship between aging control signals and immune function control signals studied in human and various animal models suggests that the same signaling pathways regulate aging and immune function. These results suggest that DR enhances immune function, and this immune function enhancement can lead to lifespan extension. In fact, recent studies have provided support for the notion that DR-induced increases in lifespan in small animals (*C. elegans* and *Drosophila*) are induced by immune signaling regulation (8, 18-20). Therefore, maintenance of homeostasis of the immune system is thought to play an important role as one of the longevity regulation mechanisms by DR. To date, the effect of DR on the human lifespan has not been demonstrated. However, the effects of DR on the suppression of aging-related diseases are regulated by signal transduction pathways that are highly conserved and functional in other animal models, and immune function-enhancing effects of DR have also been reported in human. Therefore, this review suggests that anti-aging research should focus on the mechanism of the immune system as an intervention for healthy aging, and should also focus on the relationship between the DR mechanism and immune function.

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CONFLICTS OF INTEREST

The authors have no conflicting interests.

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