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Metabolic Syndrome, Hormones, and Maintenance of T Cells during Aging

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Summary

Although the phenotype of T-cell senescence has been extensively investigated, few studies have analyzed the factors that promote the generation and maintenance of naïve and memory T cells that exist throughout the lifespan of the individuals. Unlike senescent T cells, naïve and memory T cells are able to participate in useful immune responses as well as a respond to new activation. Hormones such as leptin, ghrelin, IGF-1, IGFBP3, and cytokines, including IL-7, regulate both thymopoiesis and maintenance of naïve T cells in the periphery. Although chronic viruses such as cytomegalovirus (CMV) are thought to drive T cell senescence, other microbes may be important for maintenance of non-senescent T cells. Microbiota of the gut can induce metabolic syndrome as well as modulate T cell development into specific subpopulations of effector cells. Finally, T-cell generation, maintenance, and apoptosis depend upon pathways of energy utilization within the T cells, which parallel those that regulate overall metabolism. Therefore, better understanding of metabolic syndrome, T cell metabolism, hormones, and microbiota may lead to new insights into the maintenance of proper immune responses in old age.

Prevention of Immune Senescence by Hormones and Apoptosis

Although it is known that there is an increase in senescent T cells with a limited T-cell receptor (TCR) repertoire in old age, much less attention has been focused on the maintenance of useful naïve or early memory T cells. Naïve T cells produced decades earlier can persist into late adulthood and provide an important source of T cells capable of entering into the younger memory pool or responding to new antigens [1]. The two main genetically controlled processes that regulate the size of the naïve T cell and young memory pools are the initial thymic output and subsequent maintenance [2]. Specific factors that regulate both thymopoiesis and maintenance include IL-7, peptide hormones, and sex steroids [3**,4**]. T-cell maintenance is also affected by appropriate T cell activation and activation-induced cell death (AICD) [5**–7]. Functional T-cell apoptosis signaling, which can best be analyzed by *in vivo* analysis, is necessary to remove cells that have become exhausted by replicative senescence or have accumulated oxidative DNA damage [8,9]. Hsu *et al* have shown that successful immune aging is associated with normal AICD in nonagenarians [5]. This review will discuss key factors

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Conflicts of Interest

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related to hormones that regulate thymocyte production, even into late life, as well as factors that maintain apoptosis, prevent accumulation of senescent cells, and provide necessary immunologic "space" for functional naïve and memory T cells.

1. Maintenance of naïve T cells through the metabolic pathway

1.1 Leptin, an adipokine that may regulate thymopoiesis—Leptin is a 16-kDa hormone derived from adipose tissue that acts on specific regions of the brain to regulate food intake, energy expenditure, and neuroendocrine function [10–12**]. Leptin is structurally related to cytokines and acts on receptors that belong to the cytokine receptor superfamily [13**]. Therefore, leptin is also considered to be an adipokine [14]. Interestingly, recent findings suggest that leptin might also play a role in regulating thymopoiesis. Gruver *et al* [3, 15] have shown that the leptin receptor is expressed in the thymic medulla and that leptin protects against stress-induced thymic atrophy. Leptin has a beneficial effect on thymopoiesis as determined by analysis of T cell receptor recombination excision circles (TRECs). Nonagenarians exhibiting higher levels of circulating leptin also exhibited a higher percentage of $TREC^+$ $CD28^+CD95^-$ CD8 T cells in peripheral blood mononuclear cells (PBMCs) [16**]. Thus, leptin may have a beneficial effect on thymopoiesis and maintenance of naïve T cells throughout the lifespan of an individual (Fig. 1).

1.2. Growth hormone (GH) and insulin-like growth factor (IGF)-1—There is extensive evidence that GH deficiency and deficiencies of GH signaling can prolong both lifespan and immune response in aged mice [17,18]. GH signaling is regulated at multiple levels, including the level of growth hormone itself, the level of growth hormone signaling, as well as the levels of IGF-1 and insulin-like growth factor binding proteins (IGFBPs) (Fig. 1) [19]. On the other hand, IGFBP, in addition to binding IGF-1, exhibits autocrine and paracrine actions that affect cell mobility, adhesion, apoptosis, survival, and the cell cycle [20]. Low levels of leptin, IGF-1 and IGFBP3 and high TNF were associated with high mortality among centenarians [21**].

We have recently shown that there is a significant positive correlation between GH and senescent T cells as well as a positive correlation between IGFBP3 and the percentage of naïve CD8 T cells in nonagenarians [16**]. These results suggest that higher levels of IGFBP3 can act to suppress the action of IGF-1 to promote and sustain the levels of naïve T cells during late old age. In addition, lower levels of GH and IGF-1 also play a key role in promoting higher levels of naïve T cells and limiting the percentage of terminally differentiated or senescent T cells.

1.3. Metabolic syndrome and T-cell senescence—The metabolic theory of aging implies that caloric restriction can limit immune senescence in animals, including primates and possibly humans [22**]. This is consistent with new results showing that inhibition of the mammalian target of rapamycin (mTOR) signaling pathway can mimic certain aspects of caloric restriction and can also inhibit immune senescence (Fig. 1) [23**,24]. Rapamycin can extend lifespan through mechanisms similar to caloric restriction [25].

More recently, there has been increased attention focused on the role of metabolic syndrome in immune responses. Metabolic syndrome alters T cell development and T-cells derived from adipose tissue exhibit pro-inflammatory properties [26**,27,28]. Adipose tissue promotes the production and release of high levels of IL-6, TNF-α, and CRP [29**–32] (Fig 2). Although hormones such as leptin and adiponectin [33] are major regulators of metabolic syndrome, other factors include the amount of visceral fat. Visceral or mesenteric fat, but not subcutaneous fat, have a major deleterious effect related to inflammation in metabolic syndrome [31].

One important link between visceral fat and immune response that has only recently been appreciated is that different innate immune response defects can alter the presence of different species of gut microbiota that promote metabolic syndrome [34**,35] and indirectly or directly affect T cell development [36]. Changes in intestinal microbiota are an essential factor in numerous disorders that promote chronic inflammation. In contrast, germ-free mice exhibit lower levels of T cell stimulation and decreased accumulation of $CD44^{+}CD62L^{+}$ T cells, while certain gut microbiota, such as the Cytophaga-Flavobacter-Bacteroidetes phylum, promote development of IL-17 producing Th17 T cells [37]. Other bacteria, such as γ-proteobacteria, exhibit a low correlation with Th17 cells [37]. Toll-like receptors expression and genetic manipulation in mice have been shown to alter the gut microbiota and promote chronic inflammation [38]. Thus, the altered innate responses and gut microflora can cause metabolic syndrome [39], which promotes systemic chronic inflammation and T cell senescence [40]. Regulation of gut microflora with, for example, probiotics, may be an effective way to control both metabolic syndrome and the accumulation of senescent T cells.

1.4. T cell metabolism and longevity of T cells—How does organismal metabolism relate to T-cell metabolism? It is worthwhile to note that the maintenance and generation of T cells from older individuals may both be regulated through the PI-3K/AKT/FOXO signaling axis in that T cells use a similar pathway as IGF-1 for regulation of their activation (Fig. 3). Studies by Thompson and colleagues indicate that T cells utilize CD28, which is the homologue of IGF-1R, to trigger its signaling event [41**]. The significance of CD28 in regulating T cells through the PI-3K/AKT/inhibition of FOXO signaling axis has critical biologic implications since this signaling event also increases glucose uptake and glycolytic rate to maintain cellular ATP/ADP levels or macromolecular synthesis in response to T-cell activation [41**]. It is possible that the CD28/PI-3K/AKT and resulting inhibition of the FOXO signaling axis is a unique mechanism developed to separate T cells from the major glucose metabolic system operated by the liver. This would confer an evolutionary advantage for the organism, because the signal that triggers T-cell co-stimulation by CD28 also triggers the energy utilization of the T cell (Fig. 3). In the absence of foreign pathogenic antigens, T cells are maintained in a quiescent state similar to the lower metabolic state of the whole organism to not only conserve energy, but also prevent unnecessary expansion and the necessity for subsequent massive apoptosis of T cells. Both events require very tight regulation to prevent pathogenic consequences to the whole organism. During an immune response, the T cell rapidly regains its ability to respond upon encountering a foreign antigen presented by the antigen-presenting cells. On completion of the T-cell response, down-modulation of the CD28/PI-3K/AKT/inhibition of FOXO signaling axis is a critical event in returning the T cell to its quiescent state. In the periphery, the loss of CD28 expression and altered CD28 signaling are predominant features of T-cell senescence [5,16,42**]. These features are very similar to the life-long enhancement of the IGF-1 signal and its association with shortened lifespan of the organism.

2. Maintenance of naïve T cells through proper regulation of T-cell apoptosis

2.1. Increased activation-induced cell death (AICD) increases thymopoiesis and maintains the naïve T cell repertoire—Zhou *et al* [43**] showed that enforced expression of CD95 (Fas) during thymocyte development and on peripheral T cells can increase thymopoiesis, thymic output, prolong maintenance of naïve or memory T cells in the periphery, and limit development of senescent cells. The mechanism for this was proposed to be heightened sensitivity to apoptosis *in vivo*, which leads to more efficient elimination of pre-senescent cells before development of a senescent apoptosis-resistant phenotype. This enables immunologic space to be available for subsequent immune responses. Murasko and coworkers [44**] showed that Poly(I:C) activation and induction of interferons (IFNs) led to the depletion of T cells in young, but not aged mice. Therefore, appropriate regulation of apoptosis and

removal of cells at the earliest stage of senescence can limit immune senescence and an improved immune response.

Although apoptosis resistance is a property common to all senescent cells, there have been conflicting results between *in vivo* and *in vitro* studies of immune cells from aged humans and mice. Studies *in vitro* show that cells from aged humans and mice are more susceptible to genotoxin-induced cell death [8,9]. However, T-cell death induced by DNA damage and stress is different from AICD during T cell maintenance. Hsu *et al* [6,7] have shown that there is decreased apoptosis after transfer of senescent CD8 T cells from a T cell receptor transgenic (Tg) mouse into a stimulating environment *in vivo*, resulting in lower induction of apoptosis and expansion/infiltration of CD8 T cells in aged mice. Ahmed and colleagues have demonstrated that although generation of T-cell responses is compromised in old mice, homeostatic maintenance, which requires IL-7/IL-7R interaction, was not affected in lymphocytic-choriomeningitis-virus – (LCMV-) specific $CD8^+$ T cells in 22-month-old mice [45**]. Recently, Swain and colleagues [46] found that with increasing organismal age, naïve CD4 T cells become progressively longer-lived. Newly generated naïve T cells derived from aged stem cells have a shorter lifespan like that of young naïve T cells. Conversely, naïve CD4 T cells derived from middle-aged thymectomized mice were longer-lived *in vivo* and their development of functional defects was accelerated. These findings suggested a connection between the accumulation of AICD-resistant T cells from aged mice and the molecular mechanisms associated with the generation of these cells. Because the expression of T-cell AICD inducers, Fas ligand and Bim, are directly down-stream of the FOXO transcription regulator, loss of the FOXO effects may be one mechanism leading to a decline in T-cell AICD in older individuals (Fig. 3).

2.2. The PI-3K/AKT/FOXO signaling during DNA damage-induced apoptosis—

The PI-3K/AKT/FOXO signaling axis is involved in control of cell-cycle entry and exit [47]. These events are not only critical during T-cell activation/AICD, but are also an important mechanism for inducing cell cycle arrest and apoptosis during growth factor withdrawal and oxidative stress. A relatively low concentration of reactive oxygen species (ROS) such as H_2O_2 and O_2^- induces cell proliferation by ERK and PI-3K/AKT pathways largely through stimulation of receptor-ligand interactions [48]. In contrast, a large amount of ROS induces deleterious damage and apoptosis in cells by a specific signaling pathway that includes activation of the mitogen-activated protein kinase (MAPK) pathway that ultimately activates the FOXO signaling triggering apoptosis pathway [49]. Thus, the redox status of cells provides a signal for either survival and proliferation or damage and apoptosis. Deficiency of FOXO3 has been shown to be associated with increased induction of the cell-cycle-arrest protein $p21^{CIP1/WAF1/Sdi1}$ and lower expression of several ROS-scavenging enzymes in FOXO3-deficient cells after oxidative stress [50].

The DNA damage-associated cellular senescence also can be connected to the cellular metabolic pathway via a p53-dependent mechanism (Figure 4). ROS, UV light or other genotoxic stresses, that induce DNA damage, principally, double-stranded breaks (DSBs), is detected by the MRE11-RAD50-NBS1 complex, which in turn activates ATM [51]. A key substrate of ATM is p53. ATM phosphorylates p53 leading to its stabilization and activation [52]. P53 has several functions that can regulate T-cell senescence. First, p53 can promote apoptosis of damaged naïve and pre-senescent T cells, creating immunologic space for development of functional T cells [53]. Secondly, p53 can decrease IGF signaling by down-regulation of IGF-1R as well as upregulation of IGFBP-3 [54]. This function would serve to limit further genotoxic stress by lowering the metabolic rate of the cell. Third, it can promote cell cycle arrest and repair of DNA damage [55]. However, prolonged cell cycle arrest is a feature of senescent T cells, and over-activity of this function of p53 may be deleterious to successful immune aging [56]. Lower rate of metabolism is postulated to be associated with

decreased ROS and may be decreased DSBs. Low metabolism also is associated with lower induction of AKT, which physically associates with MDM2 and phosphorylates it at the Ser166 and Ser186 residues to block the effect of ATM phosphorylation of p53 [57]. However, it is likely that for optimum longevity, even at low metabolic rates, the preferential activity of p53 during genotoxic stress is to induce apoptosis and to decrease metabolism of injured cells rather than to induction of cell cycle arrest for the purpose of promoting DNA repair, since this latter function might eventually lead to T-cell senescence [58].

Conclusions

The regulation of the IGF/CD28 signaling pathway, which functions primarily in regulating growth and metabolism of the overall organism, plays a similar role in regulating naive T cells and T cell senescence. Leptin and gut microbiota that can regulate metabolic syndrome can also affect thymopoiesis and maintenance of peripheral T cells. Life-long high stimulation of T cells may become an important mechanism for acceleration of T-cell senescence. The challenge will be to determine whether leptin, IGFBP3, and rapamycin directly regulate the CD28 signaling pathway in the periphery or indirectly regulate this pathway though the enhancement of thymic output or the proper regulation of apoptosis to help preserve naïve T cells during aging.

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Abbreviations

FOXO	Forkhead box O transcription factor
GH	Growth hormone
IGF-1	Insulin-like growth factor 1
IGFBP	Insulin-like growth factor binding protein
mTOR	Mammalian target of rapamycin
TRECs	T cell receptor recombination excision circles
PDK1	Phosphoinositide dependent kinase
PI-3K	Phosphatidylinositol 3-kinase
PIP3	Phosphatidylinositol 3,4,5-triphosphate
ROS	Reactive oxygen species
Th17	IL-17 producing CD4 T-helper cells
TLR	Toll-like receptor

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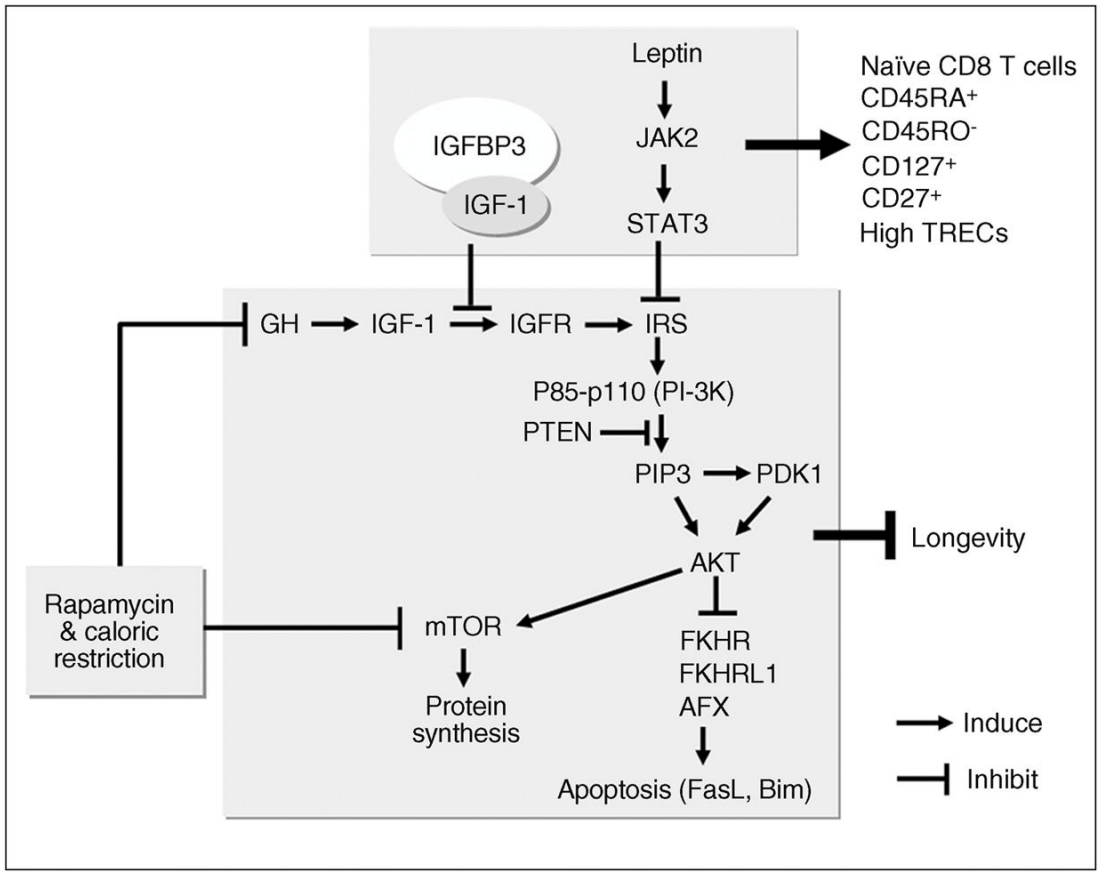

Figure 1.

Leptin, IGFBP3, and rapamycin suppress the IGF-1 signaling pathway to preserve naïve T cells and promote longevity. IGF-1 signaling through the IRS proteins, which bind to the p110 subunit of phosphatidylinositol 3-kinase (PI-3K), leads to the generation of phosphatidylinositol 3,4,5-triphosphate (PIP3) and phosphorylation of AKT by phosphoinositide dependent kinase (PDK1). Phosphorylation of AKT leads to subsequent activation of mTOR and phosphorylates or inactivates a family of apoptosis-inducing forkhead transcription factors, including forkhead box O1 (FOXO1 or FKHR), forkhead box O3 (FOXO3, or FKHRL1), and forkhead box O4 (MLLT-7, FOXO4 or AFX). Life-long enhanced IGF-1 signaling pathway is associated with shortening of lifespan. In contrast, inhibition of mTOR by dietary restriction or rapamycin has been shown to be associated with longevity in mammals. It has recently been shown that increased levels of factors such as leptin and IGFBP3 that can inhibit the IGF-1 signaling pathway, are also associated with preservation of naïve CD8 T cells in human nonagenarians.

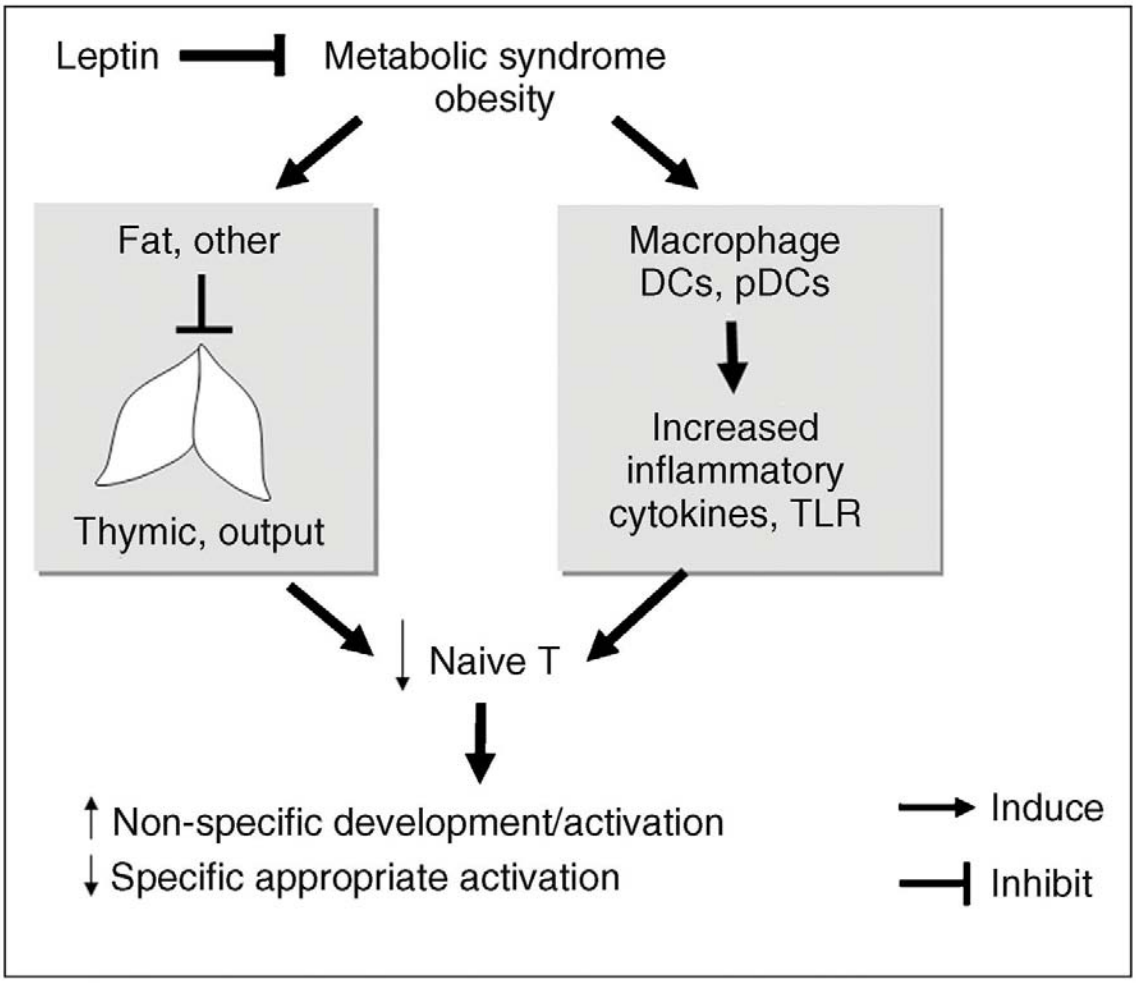

Figure 2.
Potential mechanisms of metabolic syndrome/obesity leading to T-cell senescence. This figure illustrates how obesity, inflammatory factors, and thymic involution might work synergistically to promote T-cell senescence. Excessive amount of visceral fat not only prohibits thymic output but also triggers chronic inflammatory responses that can further promote T-cell senescence. Leptin exhibits a unique effect to suppress obesity and has been shown to be effective in enhancing naïve T cells in the periphery.

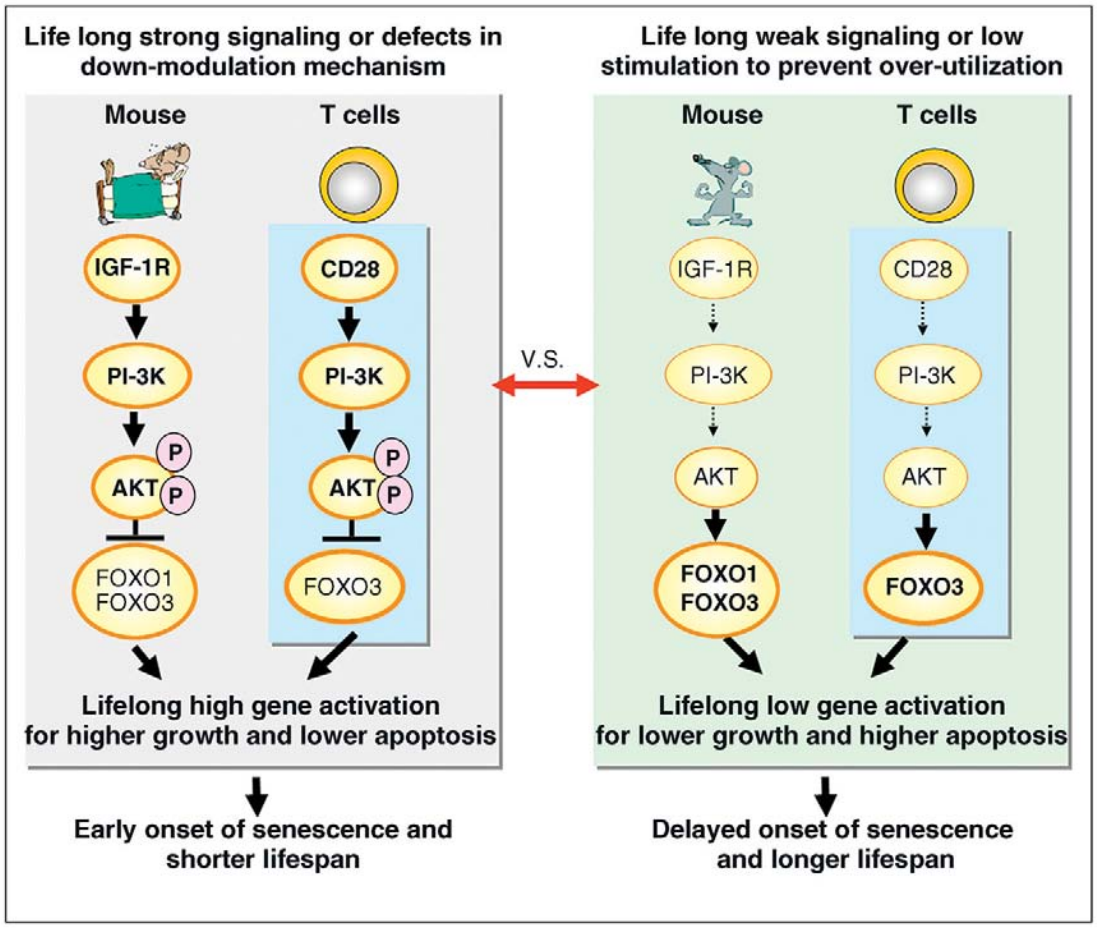

Figure 3.

The CD28 signaling pathway in T cells is equivalent to the IGF-1 pathway in the whole organism. Stimulation of the CD28/PI-3K/AKT pathway and thereby inhibition of the FOXO signaling axis is a unique mechanism developed to separate T cells from the major glucose metabolic system. This would confer a great evolutionary advantage on the organism, because the signal that triggers T-cell co-stimulation by CD28 also triggers the energy utilization of T cells. However, life-long over-utilization of this pathway is associated with acceleration of T-cell senescence, including loss of CD28 expression and development of AICD-resistant T cells.

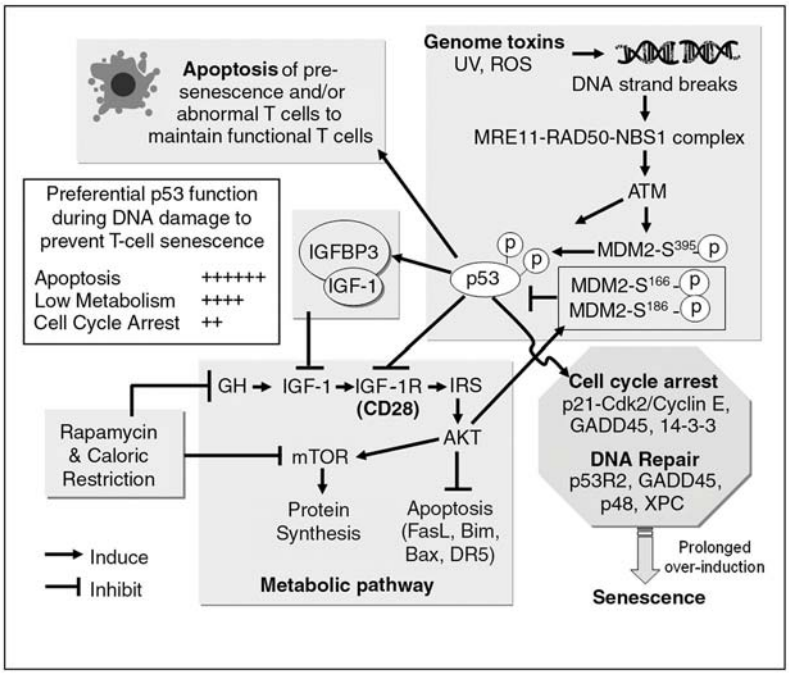

Figure 4.

Reactive oxygen species, UV light or other genotoxic stresses, that induce DNA damage, principally, double-stranded breaks, is detected by the MRE11-RAD50-NBS1 complex, which in turn activates ATM. A key substrate of ATM is p53. ATM phosphorylates p53 leading to its stabilization and activation. ATM also phosphorylates the E3 ubiquitin ligase MDM2 at the Ser^{395} residue, which inhibits ubiquitinization and degradation of p53. One key function of p53 is to inhibit cell cycle and promote apoptosis. A second key function is p53 can inhibit expression of an IGF-1 receptor (IGF-1R), while at the same time increasing expression of IGFBP-3, which decreases IGF-1 signaling. A third key function is to induce cell cycle arrest to enable DNA repair. AKT can also phosphorylate MDM2 at the Ser^{166} and Ser^{186} residue which blocks the effect of ATM phosphorylation of p53, resulting in inhibition of apoptosis. Thus, appropriate regulation of DNA damage can lead to inhibition of the growth hormone signaling pathway and this leads to lower oxidative stress. Thus, limitation of DNA damage by low ROS and control of DNA repair by several mechanisms can promote healthy immune aging.