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CALORIC RESTRICTION AND IMMUNOSENESCENCE: A CURRENT PERSPECTIVE

Mohammad A. Pahlavani

Geriatric Research, Education and Clinical Center,South Texas Veterans Health Care System and Department of Physiology, University of Texas Health Science Center, San Antonio, Texas 78284

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1. ABSTRACT

The age-related decrease in immunologic function is believed to be the major predisposing factor contributing to increased morbidity and mortality with age. Hence, the restoration of immunologic function is expected to have a beneficial effect in reducing pathology and maintaining a healthy condition in advanced age. Among various intervention strategies, caloric restriction (CR) has been shown to be the most powerful modulator of aging process. It is the most efficacious means of increasing longevity and reducing pathology. Several mechanisms have been proposed to explain its beneficial and robust action on various physiological systems, including the immune system. Experimental evidence suggests that CR increases longevity and reduces pathology through its action on the immune system. The observation that CR attenuates immunosenescence has provided a rationale for studying whether CR exerts its action through modulation of gene expression. The available data indicate that the effect of CR on signal transduction and gene expression can vary considerably from gene to gene and from one signaling molecule to another. This review summarizes the studies on the influence of CR on aging immune system and discusses the current state of knowledge on the molecular mechanisms responsible for the immunomodulatory action of caloric restriction.

2. INTRODUCTION

The immune system of mammalian organisms undergoes characteristic changes with increasing age, usually resulting in a decreased immune competence, termed "immunosenescence." Research on the effect of aging on immune system has focused on the examination of cellular function using lymphocytes from young and elderly human donors or experimental animals. The effects of aging result mostly from changes in the function of the immune cells, and T cell functions appear to be more severely affected by aging than other immune cells. Distal events such as proliferative response to antigenic or mitogenic challenges, interleukin-2 (IL-2) production and responsiveness, helper and cytotoxic activity show age-related impairments (reviewed in 1-5). There has been a desire to move these investigations forward by employing techniques in molecular biology in order to understand the mechanism underlying the aging immune system. In recent years, signal transduction events leading to the transmission of signals from the cell surface to the nucleus have been studied, and some investigators have indicated that alterations in signal transduction occur with aging in immune cells, i.e., T cells (reviewed in 6).

A variety of intervention strategies and animal models have been used over the last two decades in order to reverse, reduce or delay immunosenescence and the ramifications of its onset (reviewed in 7-9). Until now, the only robust intervention consistently shown to extend the median and the maximum life span in experimental animals and therefore, effective in retarding the process of aging, is caloric restriction (CR). There is an impressive body of evidence showing that in laboratory rodents (mice and rats) a decrease in caloric intake with maintenance of adequate levels of essential nutrients, can increase longevity and postpone the onset and lower the incidence of age-associated diseases (10-12). Caloric restricted rodents have been shown to benefit from a variety of age-retarding alterations. These include decreased rates of tumor formation, reduced levels of oxidative damage, slower progression of numerous disease processes, retardation of a broad spectrum of age-associated pathological parameters, and increased immunological function (10-12). Caloric restriction has been found to influence a wide variety of age-sensitive immune parameters, and overall, the immunological status of animals fed a calorie restricted diet is superior to the immunological status of the non-restricted animals. In this article, the studies on the effect of CR on aging immune system will be reviewed. In addition, recent studies from our laboratory focusing on the effect of CR on the age-related alterations in T cell receptor-associated signal transduction, i.e., Ras/MAPK activity and calcineurin (CaN) and calcium/calmodulin-dependent protein kinase (CaMK-IV) activation will be discussed.

3. DOES CALORIC RESTRICTION ALTER IMMUNOSENESCENCE?

It has been more than half of century since McCay and his colleagues (13) observed that reduction of food intake in rats increased the life span dramatically. Since,

this initial observation, numerous laboratories have investigated the effect of dietary manipulation on various physiological parameters in laboratory animals. The first wave of intense interest in this so-called dietary/food restriction phenomenon occurred in the 1950s and 1960s and demonstrated that food restriction (i.e., undernutrition, not malnutrition) significantly prolonged the survival of rodents (14,15). This prolongation has been observed with a variety of different techniques that reduce the amount of dietary components such as protein, fat, and carbohydrate consumed by rodents (10-12). Over the past decade, it has become apparent that the reduction in total calories is the component of the dietary restriction regimen responsible for the increase in longevity in laboratory rodents (10,11,14). Numerous investigators have demonstrated that caloric restriction (CR) not only increases the survival of rodents, but also retards/reduces the incidence of a variety of age-associated diseases such as neoplastic, renal, and cardiovascular diseases (11,15,16). The mechanism by which CR asserts its action has been postulated to include various components including depression of metabolic rate, retardation of growth, reduction of body temperature, reduction of body fat, delayed neuroendocrine changes, retardation of maturation of the lymphoid cells and enhancement of the immune system, altered gene expression, increase in DNA repair capacities, amelioration of oxidative stress or damage, and enhanced apoptosis (10-12,17-19). Despite the fact that various aspects of the beneficial effects of CR have received considerable amounts of attention over the last decade, a complete understanding of its molecular mechanism has not been gained to date.

Because immune function decreases with age and because CR has been shown to enhance longevity and reduce pathology, there has been a great deal of interest regarding whether CR decreases pathology through its action on the immune system. The beneficial effect of CR on immune function was first reported in 1973 by Walford laboratory, who showed that chronic CR significantly increased lymphocyte function such as mitogen-induced lymphocytes proliferation in mice (20). Since then, a number of different investigators using various strains of mice and rats, and more recently monkey, have demonstrated that the proliferative response of lymphocytes to mitogen is greater in CR animals compared to the control group. Table 1 summarizes the studies that have contributed to the research in the area of CR and immunosenescence. The immunoenhancing effect of CR on modulating the age-related decline in immune function was demonstrated in a variety of lymphoid tissue and cell types from several species, using different stimulatory agents. In the majority of the studies, polyclonal activators such as PHA (phytohemagglutinin), concanavalin A (Con A), pokeweed mitogen (PWM), PPD (purified protein derivative), or mitogenic antibody (anti-CD3) were used to stimulate lymphocytes. In some studies, however, antigens (e.g., alloantigen) or superantigen (staphylococcal enterotoxin B) were used to activate lymphocytes.

As shown in Table 1, various immune parameters decrease with age and CR attenuated the age-related decline in immunologic responses such as mitogen-induced lymphocyte proliferation, cytokine production, antibody response to sheep red blood cells, and natural killer cell activity. In addition, CR increases virus specific antibody production as well as antigen presentation (21). Although some studies indicated that CR has no effect on some of the immunological parameters that were measured, the overwhelming majority of the studies show that CR enhances immune function and this increase ranges from 35% to 450%. For example, in an early study, Fernandes's laboratory reported that mitogen-induced lymphocyte proliferation and IL-2 production were reduced dramatically with age in short-lived autoimmune-prone strain NZB/W mice, and that CR significantly reduced the age-related decline in mitogenesis and IL-2 production by splenocytes from NZB/W mice (22). In another study, they showed that IL-2 activity, as well as IL-2 receptor (IL-2R) expression (number of IL-2R site per cell) was increased significantly in Con A stimulated splenocytes isolated from the 19-month-old F344 rats fed a calorie restricted diet compared to the rats fed *ad libitum* (23). Using a limiting dilution assay, it was shown that the percentage of IL-2 producing cells decreased with age; however, this decline was less in mice fed a calorie restricted diet. For example, 32-month-old mice fed *ad libitum* retained only 15% of their helper T cell function (measured as IL-2 producing cells) compared to 7-month-old control mice. In old mice fed a calorie restricted diet, 53% of helper T cell function was retained (24). The immunoenhancing effect of CR is not only restricted to changes in mitogenesis and cytokine production, but also leads to changes in the percentage and phenotypic expression of lymphocytes. The percentage of T cells ($CD3^+$), cytotoxic/ suppressor T cells ($CD8^+$) and natural killer (NK) cells ($OX8^+$ $OX19^-$) were found to increase in 8-month-old CR Lobund-Wistar rats compared to the control rats fed *ad libitum* (25). In addition, CR has been shown to prevent a rise in memory T cells ($pgp\text{-}1^{high}$) and maintain a higher number of naive/virgin T cells in aged mice (26,27). Thus, these studies indicate that CR enhances the immune function and retards/reduces the age-related decline in immune responses in rodents.

In an early study, we reported that CR significantly increased the immune response in aged rats (28). In that study, at 6 weeks of age Fischer 344 rats were subjected to a calorie restricted diet (40% reduction in calories). After 5, 12, 21, and 28 months of age, Con A induction of proliferation and IL-2 production (activity) by spleen lymphocytes were measured. We found that the proliferative response of lymphocytes to Con A in both calorie restricted rats and *ad libitum* fed rats declined significantly with increasing age. No differences were observed in mitogenesis and IL-2 production between calorie restricted rats and the *ad libitum* fed rats at 5 and 12 month of age. However, the induction of proliferation and IL-2 expression was significantly higher in 21 and 28-month-old calorie restricted rats compared to the rats fed *ad libitum*. In addition, we found that the increase in IL-2 activity was paralleled by an increase in the levels of the IL-2 mRNA transcript. This was the first study to demonstrate that CR alters IL-2 expression at the level of transcription.

Table 1. Influence of Caloric Restriction and Aging on Immunologic Function

Species	Strain	Age (Mo)	Lymphoid Cells	Immune Parameters	Change with Age	Change with CR	Ref.
MOUSE							
	B6D2F1	6-30	Splenocyte	**Proliferation (PHA)**	*Decrease*	*Increase*	26
				Proliferation (ConA)	*Decrease*	*Increase*	
				Proliferation (LPS)	*Decrease*	*Increase*	
				Proliferation (SEB)	*Decrease*	*Increase*	
				IL-2 activity	*Decrease*	*Increase*	
				IFN-g	*Increase*	*No change*	
				CD4+pgp-1 (naive)	*Decrease*	*Increase*	
				CD4+ (Memory)	*Increase*	*Decrease*	
	C3B10RF1	3-33	Splenocyte	**NK activity**	*Decrease*	*Increase*	41
				CTL generation	*Decrease*	*Increase*	
	NZB/W	7-10	Splenocyte	**Proliferation (ConA)**	*Decrease*	*Increase*	22
				Proliferation (LPS)	*Decrease*	*Increase*	
				IL-2 activity	*Decrease*	*Increase*	
				Antibody (SRBC)	*Decrease*	*Increase*	
				Cytotoxicity	*Decrease*	*Increase*	
	C6CBAF1	7-30	Splenocyte	**PHTC (precursor)**	*Decrease*	*Increase*	24
				PCTL(precursor)	*Decrease*	*Increase*	
	BDF1	3-30	Splenocyte	**Proliferation (CD3)**	*Decrease*	*Increase*	25
				$CD4^+$ T cell	*Decrease*	*Increase*	
				$CD8^+$ T cell	*No change*	*Increase*	
				Calcium flux	*Decrease*	*Increase*	
	NZB/W	3-8	Saliva gland	**TGF-**b **1 mRNA**	----------	*Increase*	42
				IL-6	*Increase*	*Decrease*	
				TNF-a	----------	*Increase*	
	NZB/W	3-11	Splenocyte	**Proliferation (ConA)**	*Decrease*	*Increase*	43
				IL-2 activity	*Decrease*	*Increase*	
	BXKF1	8-21	Periton. Mac.	**IL-6**	*Decrease*	*No change*	44
				TNF-a	*Decrease*	*No change*	
	C3B10RF1	8-36	Blood	**IL-6**	*Increase*	*No change*	45
				TNF-a	*Increase*	*No change*	
	B6CBAT6F1	3-30	Splenocyte	**Helper T cells**	*Decrease*	*Increase*	46
			Blood	**Naive Helper T cells**	*Decrease*	*Increase*	
				Naive CTL	*Decrease*	*Increase*	
			Thymus	**Size**	*Decrease*	*No change*	
				Thymocytes No.	*Decrease*	*Increase*	
			Spleen	**Splenomegaly**	*Increase*	*Decrease*	
	C57BL/6J	5-29	Splenocyte	**Proliferation (PHA)**	*Decrease*	*Increase*	47
				Proliferation (ConA)	*Decrease*	*Increase*	
				Proliferation (PWM)	*Decrease*	*Increase*	
				Proliferation (LPS)	*Decrease*	*Increase*	
				Proliferation (PPD)	*Decrease*	*Increase*	
				Antibody Res. (SRBC)	*Decrease*	*Increase*	
	NZB/W	4-9	Blood MNC	**IL-2 activity**	*Decrease*	*Increase*	48
				IFN-g	*Decrease*	*Decrease*	
				IL-5	*Increase*	*Decrease*	
				IL-10	*Increase*	*Decrease*	
	CB6B10RF1	3-26	Splenocyte	**T cell Proliferation (Influenza Virus)**	*Decrease*	*Increase*	49
			Blood	**Antibody Res. (Influenza Virus)**	*Decrease*	*Increase*	
				Antigen Present. (Influenza Virus)	*Decrease*	*Increase*	
	NZB/W	3-6	Splenocyte	**% LY-1+ B cells**	*Increase*	*Decrease*	50
	MRL/lpr		Lymph Node	**% LY-1+ B cells**	*Increase*	*Decrease*	
			Thymus	**% LY-1+ B cells**	*Increase*	*Decrease*	
			Blood	**% LY-1+ B cells**	*Increase*	*Decrease*	
	CB6B10RF1	10-31	Splenocyte	**Proliferation (PHA)**	*Decrease*	*Increase*	51
				Proliferation (ConA)	*Decrease*	*Increase*	
				Proliferation (PPD)	*Decrease*	*Increase*	
	SAM-P/1	2-11	Splenocyte	**Thy-1.1+ T cells**	*Decrease*	*No change*	52

Table 1. *continued*

				Ig+ B cells	*Decrease*	*No change*	
				Antibody Response	*Decrease*	*Increase*	
				Proliferation (ConA)	*Decrease*	*Increase*	
				Proliferation (LPS)	*Decrease*	*Increase*	
	CB6B10RF1	5-29	Splenocyte	**Proliferation (PHA)**	*Decrease*	*Increase*	53
				Proliferation (ConA)	*Decrease*	*Increase*	
				Proliferation (PPD)	*Decrease*	*No change*	
				Proliferation (LPS)	*Decrease*	*No change*	
				Antibody Response	*Decrease*	*No change*	
			Lymph Node	**Proliferation (PHA)**	*Decrease*	*Increase*	
				Proliferation (ConA)	*Decrease*	*Increase*	
				Proliferation (PPD)	*Decrease*	*No change*	
	BALB/C?	5-29	Splenocyte	**Proliferation (ConA)**	*Decrease*	*Increase*	54
				Proliferation (LPS)	*Decrease*	*Increase*	
				Antibody (SRBC)	*Decrease*	*Increase*	
	NZB/W	2-11	Splenocyte	**Proliferation (ConA)**	*Decrease*	*Increase*	55
RAT							
	F344	6-24	Splenocyte	**Proliferation (ConA)**	*Decrease*	*Increase*	56
				IL-2 activity	*Decrease*	*Increase*	
	F344	6-28	Splenocyte	**Proliferation (ConA)**	*Decrease*	*Increase*	28
				Proliferation (LPS)	*Decrease*	*Increase*	
				IL-2 activity/mRNA	*Decrease*	*Increase*	
				IL-3 activity	*Decrease*	*Increase*	
	B.Norway	6-30	Splenocyte	**Proliferation (ConA)**	*Decrease*	*Increase*	57
				IL-2 activity	*Decrease*	*No change*	
				IFN-g	*Increase*	*Increase*	
	F344	4-19	Splenocyte	**Proliferation (PHA)**	*Decrease*	*Increase*	23
				Proliferation (ConA)	*Decrease*	*Increase*	
				IL-2 activity	*Decrease*	*Increase*	
				IL-2R/cell	*Decrease*	*Increase*	
	F344	6-27	Splenocyte	**IL-2 activity**	*Decrease*	*Increase*	58
				Cytotoxicity	*Decrease*	*No change*	
	F344	4-26	Alveol. Mac.	**Number of cells**	*No change*	*Decrease*	59
				Hsp70 mRNA	*Decrease*	*Increase*	
	F344xBN	6-30	Splenocyte	**Proliferation (PHA)**	*Decrease*	*Increase*	27
				Proliferation (ConA)	*Decrease*	*Increase*	
				Proliferation (ConA)	*Decrease*	*Increase*	
				CD4+ (naive)	*Decrease*	*Increase*	
				CD4+ (Memory)	*Increase*	*Decrease*	
	F344	6-24	Splenic T cell	**MAPK activity**	*Decrease*	*Increase*	35
				JNK activity	*No chg.*	*No change*	
				Ras (p21) activity	*Decrease*	*Increase*	
				Calcineurin activity	*Decrease*	*Increase*	
				CaMK-IV activity	*Decrease*	*No change*	
	F344xBN	5-31	Splenocyte	**Proliferation (PHA)**	*Decrease*	*Increase*	60
				Proliferation (ConA)	*Decrease*	*Increase*	
	F344	6-24	Splenic T cell	**IL-2 mRNA**	*Decrease*	*Increase*	32
				NFAT activity	*Decrease*	*Increase*	
				AP-1 activity	*Decrease*	*No change*	
	Wistar	4-27	Splenocyte	**Antibody (SRBC)**	*Decrease*	*Increase*	61
MONKEY							
	Rhesus	1-25	Blood MNC	**Intracell. Calcium (anti-CD3 stimulated CD4+ T cells)**	*Decrease*	*No change*	62
	Rhesus	0.5-1	Blood MNC	**Proliferation (ConA)**	----------	*Decrease*	63
		3-25		**Proliferation (PHA)**	----------	*No change*	
				Proliferation (PWM)	----------	*No change*	

Abbreviations: CR, caloric restriction; ConA, concanavalin A; PHA, phytohemagglutinin; LPS, lipopolysaccharide; PWM, pokeweed mitogen; PPD, purified protein derivative; SEB, staphylococcal Enterotoxin B; IL-2, interleukin-2; IL-3, interleukin-3; IFN-γ, Interferon-gamma; SRBC, sheep red blood cell; NK, natural killer; pHTL, precursor of helper T lymphocyte; pCTL, precursor of cytotoxic T lymphocyte; HSP70, heat shock protein-70; NFAT, nuclear factor of activated T cells; AP-1, activation protein-1; MAPK, mitogen-activated protein kinase; JNK, c-jun amino terminal kinase; CaMK, calcium/calmodulin-dependent protein kinase.

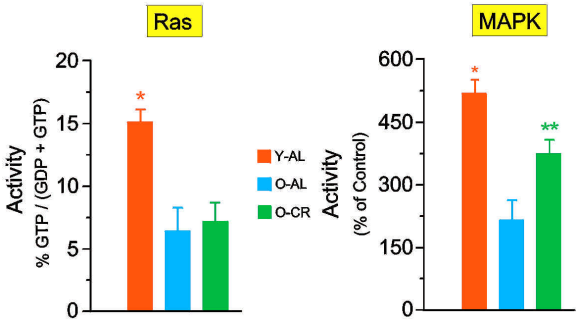

Figure 1. Effect of caloric restriction and aging on the induction of Ras and MAPK activation in T cells from rats. Splenic T cells from control (AL) young and old rats or caloric restricted (CR) old rats were incubated with or without Con A for 10 min to measure Ras activity or for 15 min to measure MAPK activity. The Ras activity is expressed as the percentage ratio of GTP-$p21^{ras}$ over total (GDP-$p21^{ras}$ plus GTP-$p21^{ras}$). The data for MAPK activity is expressed as the percentage of activity in Con A (CA)-stimulated cells relative to the unstimulated cells. Each point represents the mean ± SD for data obtained from three experiments (for the Ras activity) or from four experiments (for the MAPK activity) and each experiment was pooled from two rats. Data were taken from Pahlavani, M.A and D.A. Vargas (35). * The value for young rats was significantly different from the values for the control old rats at the $p<0.001$. ** The value for caloric restricted old rats was significantly different from the value for the age-matched control at the $p<0.05$.

Transcription of the IL-2 gene is regulated by the binding of several transcription factors (NFAT, NF-κB, AP-1, AP-3, and OCT), of which NFAT (nuclear factor of activated T cell) plays a predominant role (29,30). Because NFAT play a critical role in regulation of IL-2 transcription, we were interested in determining whether the DNA binding activity of T cell/IL-2-specific transcription factor NFAT and/or ubiquitous transcription factor AP-1 is affected by aging and whether the changes are alter with CR. Using nuclear extracts from Con A-stimulated T cells isolated from young and old rats fed *ad libitum* and old rats fed a calorie restricted diet, the induction of NFAT and AP-1 binding activity was measured with a gel shift assay. We found that the induction of both NFAT and AP-1 binding activity was significantly less in nuclear extracts from T cells isolated from old rats compared to the level from young rats (31). In a subsequent study, we found that the DNA binding activity of NFAT but not AP-1, was significantly higher in nuclear extracts isolated from old rats fed a calorie restricted diet than old rats fed *ad libitum* (32). The increase in NFAT binding activity in calorie restricted rats correlated with an increase in IL-2 gene expression. Furthermore, we found that the increase in NFAT binding activity with CR was associated with an increase in the expression of c-fos, which is a component of the NFAT protein complex (32). Thus, our study indicated that CR alters the transcription of IL-2 through changes in the NFAT transcription factor.

T cell activation is initiated when an antigenic peptide is recognized by the antigen receptor of the T cell (29,30). This recognition event promotes sequential activation of a network of signaling molecules such as kinases, phosphatases, and adaptor proteins that couple the stimulatory signal received from TCR to intracellular signaling pathways. The coordinated activation of these signaling molecules is sufficient to stimulate the activation of transcription factors, and the expression of immediate-early genes that are crucial in the regulation of T cell function. Because T cell responses such as proliferation, differentiation, and gene expression are critically dependent on signal transduction cascades, several studies have been focused on the effect of age on the activation or the levels of signal transduction molecules (reviewed in 6). For example, our laboratory has recently shown that the induction of Ras and MAPK activity but not JNK activity by Con A decreased with age (33). In addition, we found that this decrease was paralleled by a decrease in the induction of TCR-associated protein tyrosine kinases, Lck ($p56^{lck}$) and ZAP-70 activities, but not Fyn activity (33). A recent study in human has confirmed our finding and showed that the induction of $p56^{lck}$ and ZAP-70 activities decreased with age (34). Thus, the available data indicate that the induction of Ras/MAPK activities and Lack/ZAP-70 activities in T cells decreases with age. Recently, we were interested in determining whether CR alters the age-related decrease in Ras or MAPK activity in T cells. Our results summarized in figure 1 show that the induction of Ras and MAPK activity was significantly less in T cells from control old and CR old rats than T cells from control young rats (35). More importantly, we found that 40% caloric restriction partially reverses the age-related decline in MAPK but not Ras activation. In contrast to MAPK activity, the JNK activity did not change significantly with age or with CR. Furthermore, our results showed that the changes in Ras/MAPK activation with age or with CR were not associated with changes in their corresponding protein levels (35). We have postulated that the increase in MAPK activation with CR could occur at least by two distinct mechanisms. The increase in the MAPK activity with CR could arise from increased activity of the proximal signaling molecules such as MEK. In other words, more MAPK activity is observed in T cells of CR old rats because more MEK activity is present in these cells. The other possible mechanisms might involve down-regulation of MAPK phosphatase (MPK-1), which plays a role in the regulation of MAPK activity. That is, similar levels of MAPK protein are present in T cells from control old and CR old rats; but in response to stimulation, the activity of MPK-1 that is involved in dephosphorylation and down-regulation of MAPK, decreases in the T cells from CR old rats.

Activation of T cells results in a transient increase in intracellular free calcium ion concentrations, which leads to the activation of calcium/calmodulin-dependent enzymes such as calcineurin (CaN) and the multifunctional CaMK-II and CaMK-IV/Gr. During the past several years, it has been demonstrated that the

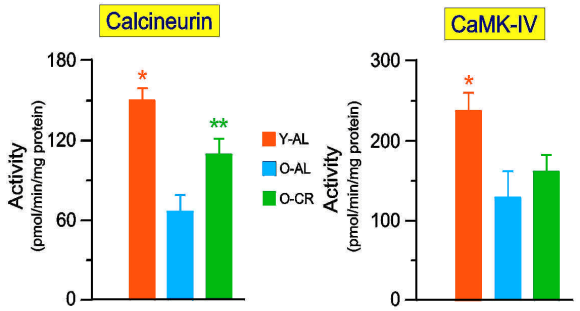

Figure 2. Effect of caloric restriction and aging on the induction of calcineurin (CaN) and CaMK-IV activities in T cells from rats. Splenic T cells from control (AL) young and old rats or caloric restricted (CR) old rats were cultured in the presence or absence of Con A. After 5 to 10 minutes of incubation, cells were lysed and the protein extracts were assayed for CaN phosphatase activity and CaMK-IV activity. Each point represents the mean ± SD for data obtained from four experiments for the CaN assay, and three experiments for the CaMK-IV assay, each experiment was pooled from two rats. Data were taken from Pahlavani, M.A and D.A. Vargas (35). * The value for young rats was significantly different from the values for the control old rats and caloric restricted old rats at $p<0.05$. ** The value for caloric restricted old rats was significantly different from the value for the age-matched control at $p<0.05$.

calcium/calmodulin-dependent phosphatase calcineurin is crucial for the regulation of the transcription factor NFAT that is involved in IL-2 transcription (reviewed in 36). In response to an increase in the intracellular levels of calcium, calcineurin is activated, which dephosphorylates the cytoplasmic component (NFAT-c) of the NFAT protein complex. The dephosphorylated form of NFAT-c translocates into the nucleus and forms a complex with the nuclear components (Fos/Jun-Elf-1) of NFAT resulting in the stimulation of IL-2 transcription (36). In addition, recent studies have demonstrated that the calcium/ calmodulin-dependent kinase type IV/Gr (CaMK-IV) plays an important role in the up-regulation of the transcriptional activity of the c-fos promoter through phosphorylation of the transcription factor CREB and serum response factor (SRF) (37-39). In view of our present finding on the effect of CR and aging on signal transduction and IL-2 gene expression and given the potential important role of CaN and CaMK-IV, we have been interested in studying whether the activation or the level of these calcium regulating enzymes is altered with age and whether CR alters the changes. The results of our recent study, which is summarized in figure 2, show that the induction of CaN phosphatase activity and CaMK-IV kinase activity by Con A decreased with age (40) and that CR partially reversed the age-related decline in CaN activation but not CaMK-IV activity (35). Furthermore, our data showed that the decrease in CaN and CaMK-IV activity with age or with CR was not due to changes in their protein levels (35). Our data demonstrate that the influence of CR on signal transduction events can vary considerably from one signaling molecule to another. For example, CR partially reverses the age-related decline in MAPK and CaN activities, but it appears to have no effect in Ras or CaMK-IV activation. At the present time, it is not known why CR alters the age-related decline in MAPK and CaN activities, but not Ras or CaMK-IV activity. Thus, it would be of interest in the future to determine the mechanism by which CR alters the activity of one group of signaling molecules and not others.

4. CONCLUDING REMARKS

Intervention in the aging immune system by various experimental manipulations has provided immunogerontologists with the opportunity to examine the basic mechanism underlying immunosenescence. Among various intervention strategies, CR has received particular attention during the last two decades, perhaps because it has provided a powerful model to study the underlying mechanisms of aging process. Caloric restriction is the most efficacious intervention method known thus far that increases median and maximum lifespan in laboratory animals. The increase in longevity with CR directly correlates with the decrease in the age-associated diseases such as infectious, autoimmunity, and cancer. Thus, the observation that reduced caloric intake is associated with increase longevity and reduced pathology in experimental animals has provided a rationale for immunoenhancing hypothesis of CR. As indicated by the literature summarized in Table 1, the overwhelming majority of the reported studies indicate that CR modulate the immune function and restore or delay the immunosenescence in laboratory animals. Although the mechanism by which CR alters immunosenescence remains unclear, we have speculated that CR mediates its effect by altering gene expression, e.g., expression of IL-2 gene, at the level of transcription. At the present time, it is unclear how CR alters gene expression at the level of transcription. Studies from our laboratory support the view that the mechanism of CR involves changes in the activities of a transcription factor, i.e., NFAT that plays a predominant role in the regulation of IL-2 transcription.

Signal transduction is ubiquitously involved in the initiation of physiological signals that lead to growth and proliferation and even cell death. The current research demonstrates that signal transduction events are an important cellular mechanism for both T cell development and T cell function. Alterations in some of the early signaling events such as tyrosine phosphorylation, Ras and MAPKs, and calcium signaling, have been linked to the age-associated decrease in the induction of cytokine (IL-2) expression and T cell proliferation. The observation that CR attenuates the age-related decline in IL-2 expression has provided a rationale for our study to determine whether CR exert its effect on activation or the levels of the upstream signaling molecules. We have recently demonstrated that CR partially reversed the age-related decline in MAPK and calcineurin activities; however, it appears to have no effect in Ras or CaMK-IV activation. Research is currently in progress in our laboratory to determine how CR alters the activity of one group signaling molecules and not the others. Although much has been

learned about the early biochemical processes and how various signaling pathways are integrated leading to T cell growth and function, our understanding of how CR alters the activation of various signaling molecules resulting in modulation of immunosenescence is far from complete.

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Send correspondence to: Mohammad A. Pahlavani, Ph.D., GRECC (182), Audie Murphy VA Hospital, 7400 Merton Minter Blvd., San Antonio, TX 78284, Tel:210-617-5197, Fax: 210-617-5312, E: mail: Pahlavani@uthscsa.edu

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