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Review Article

Recent Advances in T Cell Signaling in Aging

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ABSTRACT

The immune system of mammalian organisms undergoes alterations that may account for an increased susceptibility to certain infections, autoimmune diseases, or malignancies. Well characterized are age-related defect in T cell functions and cell-mediated immunity. Although it is well established that the functional properties of T cells decrease with age, its biochemical and molecular nature is poorly understood. The available data suggest that changes in the signal transduction machinery are responsible for the impairment of T cell function during aging. T cell activation is initiated when an antigenic peptide is recognized by the antigen receptor of T cells. This recognition event promotes sequential activation of a network of signaling molecules such as phospholipases, kinases, phosphatases, and adaptor proteins that couple the stimulatory signal received from T cell receptor (TCR) to intracellular signaling pathways. The coordinate activation of these signaling molecules is sufficient to stimulate the activation of transcription factors and the expression of the immediate-early genes that are crucial in regulation of T cell function. *Iran. Biomed. J. 3 (1 & 2): 1-13 1999*

Keywords: T cells, Signal Transduction, Aging

INTRODUCTION

Advancing age is accompanied with alterations in most physiological functions and in particular with a substantial decline in the immune responses. These include a decline in both cellular and humoral immunity. Among various cells of the immune system, T cells appear to be more sensitive to the aging process than other immune cells. Significant changes in both functional and phenotypic profiles of T cells have been documented in aging humans and rodents [reviewed in 1-5]. A dramatic decrease in the proliferative response of T cells to activating agents such as bacterial/viral antigens or polyclonal activators (mitogenic lectins) has been consistently observed. In addition, alterations in the profile of cytokine productions have often been documented.

Although the age-related impairments in T cell function are well described, less is known about the molecular mechanisms that are responsible for the diminished T cell function. The early biochemical events that occur after the engagement of antigen with TCR are considered to be essential for cellular response. Cells of the immune system respond to intra- and extracellular signals by turning specific genes on or off and by modulating the extent of active gene transcription. Switching gene expression on and off is the responsibility of transcription factors, operate singly or in association with other proteins. During T cell activation, complex networks of signaling molecules work in concert to transduce the stimulatory signal from TCR to the nuclear target. Because T cell activation decreases with age and because TCR-mediated signaling events are vital to the biological responses, it has been postulated that changes in signal transduction machinery with age might be responsible for the impairment of T cell function. To unravel the age-related defect, it is important to understand the coordinate role of various signal transduction modulators in T cell signaling.

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During the last decade, considerable progress has been made in our understanding of the early biochemical events that occur following engagement of antigen with T cell receptor (TCR)/CD3 complex. Some studies have been focused on the identification and characterization of regulatory proteins/enzymes whose functions change upon T cell activation. These investigations resulted in the discovery of various signaling molecules such as phospholipases, protein kinases and phosphatases, GTP-binding proteins, calcium-binding protein/enzymes, adaptor proteins, and other signal transduction modulators. Other studies have been directed toward understanding the underlying mechanism by which these different signaling molecules are integrated. In this review, I will attempt to describe the recent advances in T cell signaling, with specific emphasis on recent studies focusing on the role of lipid-mediated signal transduction and TCR-associated Ras/MAPK signaling events. I will also discuss the age-related alterations in signal transduction in T cells.

1. SIGNAL TRANSDUCTION AND CELLULAR REGULATION.

Signal transduction is refered to the mechanisms that regulate transmission of a message from extracellular to intracellular compartments leading to proliferation, growth and cellular function. Over the last decade, there has been a great interest in understanding the biochemical and molecular mechanisms of the age-related decline in T cells function. An established paradigm in signal transduction and T cells activation is as follows: antigen, mitogen, or mitogenic antibody (anti-CD3) has cell surface receptors. Upon binding of antigen to TCR/CD3 complex on the surface of T cells, it induces receptor coupling to transducers such as GTP binding proteins or tyrosine kinases, which in turn activate membrane or intracellular enzymes such as phospholipases and various protein tyrosine kinases and phosphatases. These in turn, act on their substrates to produce intracellular molecules that behave as second messengers, activating a cascade of enzymes that lead to phosphorylation and dephosphorylation of multiple substrates, eventually leading to activation of transcription factors, gene transcription, DNA replication, and cellular proliferation.

1.1 Lipid-Mediated Signal Transduction.

Membrane lipids (glycerolipids and sphingophospholipids) are the essential primarily structural components of the cell membranes. The accumulated evidences indicate that these lipid molecules and the products of their hydrolysis are involved in signal transduction events. Extensive studies have demonstrated the role of glycerophospholipids in cell signaling. The initial discovery showed that the tumor promoting agents such as phorbol esters exert their effect by activating the protein kinase C (PKC). The activation of PKC is linked to the activation of a lipid second messenger, diacylglycerol (DAG). DAG is generated in cells by hydrolysis of glycerophospholipids in response to several key enzymes such as phospholipase C (PLC) and phospholipase D (PLD) (Figure 1). These enzymes are activated by several extracellular stimuli, leading to hydrolysis of the membrane lipids phosphatidylinositol or phosphatidylcholine, respectively [6-8]. The DAG generated from the hydrolysis of these membrane lipids acts, in turn, as a second messengers to activate PKC leading to phosphorylation of a number of intracellular substrates.

When T cells from young human subjects or young experimental animals were stimulated with mitogen/antigen, DAG is generated intracellularly by the hydrolysis of phosphatidylcholine or phosphatidylinositol, leading to PKC activation [reviewed in 9]. This was shown by the ability of PKC to translocate from the cytosol to the membrane (indication of its activation) as demonstrated by kinase activity assays or Western blot analysis. PKC then is involved in phosphorylation cascade leading to activation of the transcription factors, i.e., the Activation Protein-1 (AP-1) and subsequent gene transcription and cellular proliferation [10, 11]. T cells from aged subjects, on the other hand, show decrease responses to mitogen/antigen stimulation, decrease generation of DAG, and PKC activation. The effect of age on DAG generation has been further investigated by using the radioactivity labeled fatty acid (palmitate), which incorporates into membrane lipids, and then the activities of the different lipid metabolizing enzymes were assessed. It was found that DAG generation in response to mitogenic stimuli occurs predominantly in response to PLD hydrolysis of membrane phosphatidylcholine [12]. This activity was reduced in cells from old compared to cells from young subjects. Although the mechanism by which PLD activation diminishes with age is not well understood, recent studies suggest that ceramide (a lipid second messenger) may have a role in inhibition of PLD activation.

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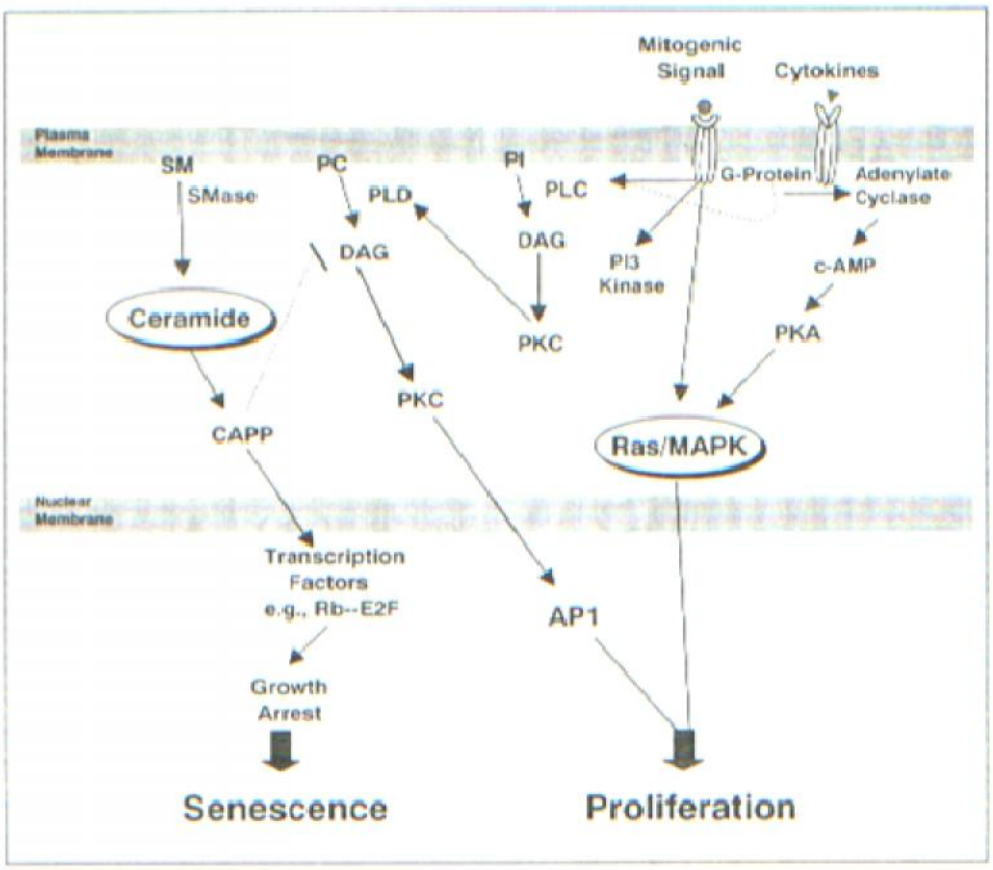

Fig. 1. Schematic diagram of lipid-mediated signal transduction pathways leading to proliferation or growth arrest. Abbreviations: PLC, phospholipase C; PLD, phospholipase D; PI, phosphatidylinositol; SM, sphingomyelin; SMase, sphingomyelinase; CAPP, ceramide-activated protein phosphatase; DAG, diacylglycerol; PKC, protein kinase C; PKA, protein kinase A; AP-1, activation protein-1.

Recent studies indicate the role of sphingolipid in signal transduction and cellular regulation [reviewed in 13]. Initially, it was discovered that sphingolipid (sphingosine), is involved in inhibition of PKC activation [14]. It is now well known that membrane sphingomyelin is hydrolyzed by sphingomyelinase in response to several extracellular stimuli to generate the lipid molecule ceramide. Ceramide, in turn, behaves as a second messenger involved in signaling event and cellular regulation. Many inducers such as IL-1 [15], TNF-α [16], complement [17], and Fas molecule [18] have been implicated in activation of sphingomyelinase and generation of ceramide, which leads to growth arrest and cellular senescence (Figure 1). In addition, exogenously administered synthetic ceramide mimicked the effects of these inducers, leads to reduce proliferation. For instance, it has been demonstrated that exogenous synthetic ceramide can induce a senescent phenotype by inhibiting DNA synthesis and reducing growth [19]. Ceramide has been shown to inhibit DAG generation and PLD activity in HL60 cells [20], neutrophils [21], and fibroblast [12]. In addition, ceramide is shown to inhibit the induction of DNA binding activity of transcription factor AP-1 and this inhibition has been attributed to inhibition of c-fos transcription [22]. Thus, it appears that ceramide has a specific role in growth arrest such as termination, apoptosis, and cellular aging.

1.2 TCR-Mediated Signal Transduction. The physiological trigger for T cell activation is the engagement of the TCR with antigen presented by the major histocompatibility molecules on the surface of antigen presenting cells (APC). The antigen receptor of T cells is comprised of a multi-chain complex encoded by three families of genes, a homo or hetrodimer consisting of one or two members of the zeta (ζ) family [23, 24]. The α/β heterodimer of the TCR complex is responsible for binding antigenic peptide presented by APCs, and non-covalently associates with the CD3 (γ, δ, ε) and ζ chains of TCR complex [25, 26]. The cyto plasmic domains of these polypeptides have in common a sequence

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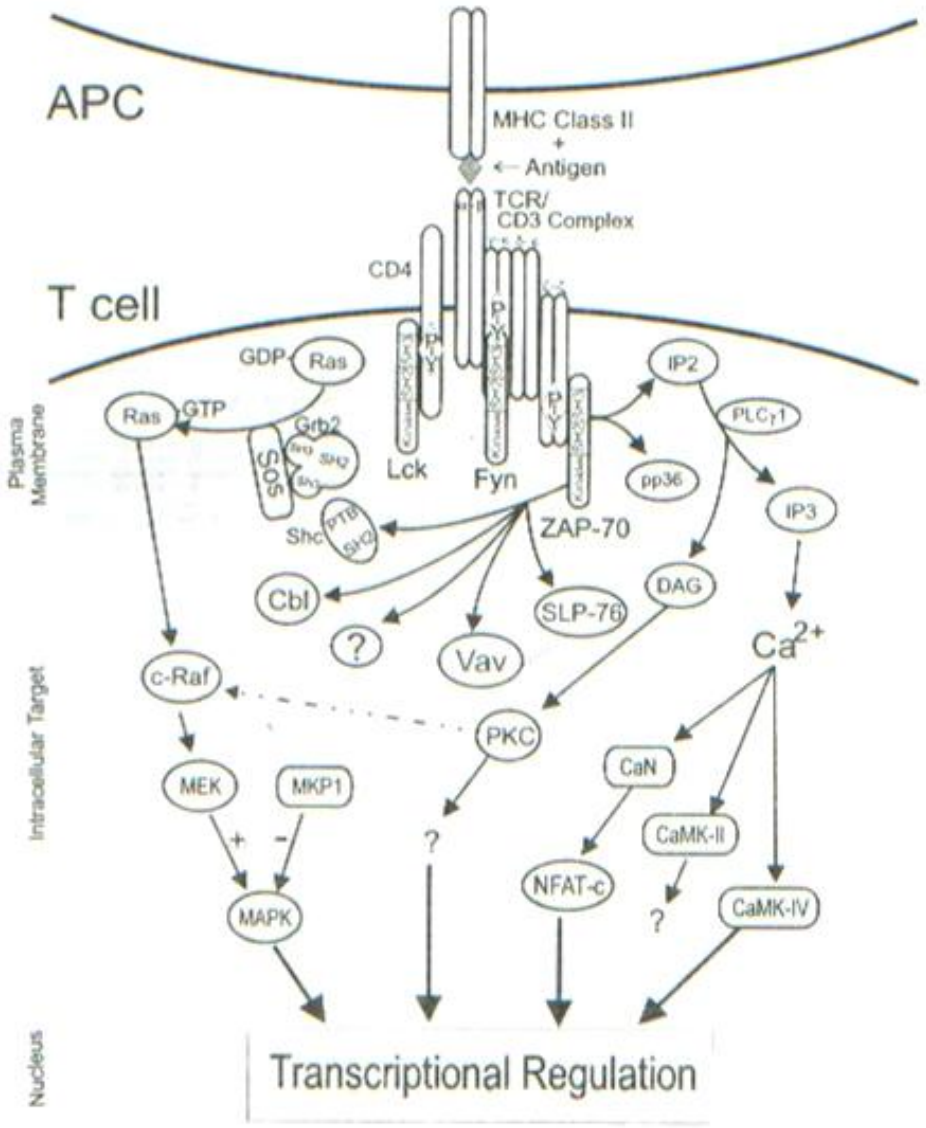

Fig. 2. A current model for TCR-mediated signal transduction. Interaction of antigen with TCR/CD3 complex initiates a series of biochemical events, of which the earliest is phosphorylation of various proteins on tyrosine residues. Stimulation of PTKs is coupled to the hydrolysis of PLCγ1 which results in a rise in intracellular Ca^{2+} and activation of PKC through IP3 and DAG pathways, respectively. Activation of Lck, Fyn, and ZAP-70 induces localization of adaptor proteins Shc, Grb2, and Sos to the cytoplasmic membrane. Associations of these adaptor proteins with Ras, allows the rapid conversion of Ras from the inactive form (GDP-Ras) to the active form (GTP-Ras). Activation of Ras results in sequential phosphorylation and activation of a series of enzymes involved in MAPK cascade that eventually transmit the stimulatory signal received from cytoplasmic membrane into the nucleus. Abbreviations: TCR, T cell receptor; APC, antigen presenting cell; MHC, major histocompatibility complex; PLCγ1, phosphlipase C-gamma 1; DAG, diacylglycerol; IP2, phosphoinositol biphosphate; IP3, inositol 1,4,5-triphosphate; SH2, Src-homology-2; PTB; phosphotyrosine binding; PKC, protein kinase C; NFATc, nuclear factor of activated T cell; CaN, calcineurin; CaMK, calcium calmodulin-dependent protein kinase; MAPK, mitogen-activated protein kinase; MEK, MAPK kinase; MKP1, MAPK phosphatase-1.

motif known as ITAMs (immunoreceptor tyrosine-based activation motifs), that forms the structural basis for interactions with downstream molecules and plays a critical role in transducing extracellular signals from TCR to downstream signaling molecules (Figure 2). Activation of T cells via TCR induces multiple intracellular responses, of which the earliest appear to be the phosphorylation of specific tyrosine residues on numerous cellular proteins [27].

Unlike the hormone receptors, the TCR does not possess kinase activity. Thus, induced protein-protein interactions are essential for coupling the stimulatory signals from TCR/CD3 complex to

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downstream signaling molecules. Tyrosine phosphorylation of regulatory proteins (discussed below) is transduced via transient specific molecular interactions, in which Src homology 2 (SH-2) and 3 (SH-3) domains play an important role [28]. SH-2 domains possess conserved amino acid residues, which form the phosphotyrosine binding sites. In addition, the adjacent residues determine the affinity, the specificity of binding, and provide a mechanism for recognizing different phosphotyrosine containing peptides [29, 30]. The polypeptides that constitute the CD3 and ζ-chain of the TCR/CD3 complex are important substrates of the protein tyrosine kinases (PTKs) such as $p56^{Lck}$, $p59^{Fyn}$, and ZAP-70 [31].

Activation of PTKs is coupled to the stimulation of an inositol lipid specific phospholipase C-gamma (PLCγ1) [32-34]. This enables the TCR activation complex to regulate the hydrolysis of membrane phosphoinositides, which results in the rise of intracellular Ca^{2+} through the activation of the inositol 1,4,5-triphosphate (IP3) pathway, and the activation of PKC via the DAG pathway [35, 36]. The increase in intracellular level of free calcium ion $[Ca^{2+}]_i$ is critical for T cell activation. This was demonstrated by treatment of T cells with agents, such as calcium ionophores (increases calcium flux) and phorbol esters (increases PKC activity), which bypass the TCR requirement and thereby activate the downstream signaling pathways [37].

1.3 Protein Tyrosine Phosphorylation and Ras/MAPK Activation.

The early signaling event most proximal to the TCR/CD3 complex is the activation of two *Src*-family of protein tyrosine kinases (PTKs): Lck ($p56^{Lck}$) and Fyn ($p59^{Fyn}$). Lck is associated with the CD4 and CD8 surface molecules that behave as co-receptors for TCR/CD3 mediated T cell activation. Although Lck is involved in signal transduction in T cells, it does not appear to directly interact with TCR. In contrast, Fyn is physically associated with the TCR/CD3 complex. The third PTK that plays an important role in TCR-mediated signaling is ZAP-70, which belongs to the *Syk*-family of PTKs and is expressed exclusively in T cells and NK cells. In the resting T cells, ZAP-70 is not directly associated with the TCR. However, upon stimulation, ZAP-70 is rapidly recruited to the phosphorylated ζ-chain and other CD3 chains of TCR/CD3 complex.

Another PTK mediated signaling event that originates from the TCR involves the guanine nucleotide binding protein Ras ($p21^{ras}$) [38, 39]. The $p21^{ras}$ protein plays a crucial role as molecular switches, controlling diverse processes including cytokine gene expression and proliferation. Ras bind GTP and have an intrinsic GTPase activity that catalyzes the hydrolysis of GTP to GDP (Figure 2). The signaling event that leads to Ras activation results from tyrosine phosphorylation of a series of adaptor portions known as Shc (src homology 2/α-collagen related) [40]. Shc contains a SH-2 domain, a protein tyrosine binding (PTB) domain, and three tyrosine autophosphorylation sites. The tyrosine phosphorylation of the Shc proteins interact with the SH-2 domain of a small adaptor protein known as Grb2 (growth factor receptor-bound protein 2). Grb2 is a 23 kDa growth factor binding protein that contains a single SH-2 flanked by two SH-3 domains. The SH-3 domains of Grb2 interact with the proline-rich carboxyl terminal domain of a protein termed SOS (son of seveless), a 150 kDa guanylnucleotide exchange factor for Ras [41, 42]. It is believed that Shc localizes the Grb2/SOS to TCR through its interaction with the ζ-chain of TCR/CD3 complex. Thus, the guanylnucleotide exchange activity of SOS is targeted to the plasma membrane location of Ras allows the rapid conversion of Ras from the inactive (GDP-bound) to the active (GTP-bound) form. Ras activation then begins its downstream signaling through association with the c-Raf (a serine/thronine kinase). GTP-bound Ras activates c-Raf and dissociates when converted to the inactive GDP-bound state [43, 44]. Thus, the GTP bound dependent interaction of Ras with c-Raf is required for c-Raf kinase activation. Once activated, c-Raf phosphorylates and activates downstream kinases such as MEK (Figure 2).

Research during the past four years has shown that at least three tyrosine phosphorylated proteins become associated with an adaptor protein, Grb2, following T cell stimulation. One of the Grb2-associated proteins, which become tyrosine phosphorylated upon T cell stimulation is SLP-76 (SH2 domain-containing leukocyte protein 76) [45]. This phosphoprotein contains a central proline rich region that interacts with Grb2, a highly acidic amino terminal domain with tyrosine phosphorylated sites, and a carboxyl terminal SH-2 domain. It has been recently shown that overexpression of SLP-76 resulted in increased activity of Ras and MAPK (mitogen-activated protein kinase) and that was associated with up-regulation of transcription factors and immediate-early genes, i.e., c-fos, and c-jun [46]. Studies in Jurkat T cells have shown that SLP-76 overexpression increases the transcriptional activity

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of a reporter gene driven by three tandem repeat of NFAT (nuclear factor of activated T cell) regulatory sequence or the whole IL-2 promoter [47,48]. The second Grb2-associated protein is a 36 kDa phosphoprotein (pp36) associated with SH-2 domain of Grb2 [49-51]. In addition, the pp36 has been found to be associated with PLCγ-1 in the plasma membrane via SH2 domain interaction [49-51]. The molecular identity of pp36 has not yet been defined. The third Grb2 binding phosphoprotein is known as Cbl, is a 120 kDa protein that is ubiquitously expressed in resting and stimulated T cells [52-55]. Although Cbl appears to be associated with Grb2 via SH-3 domains, its exact role in T cell signaling remains unclear. Another signal transducer downstream of the antigen receptor is a 95 kDa protein known as Vav. This regulatory protein contains several domains (SH-3 and SH-2) that are important for protein-protein or protein-lipid interaction [56]. Vav contains a region which may be involved in guanine nucleotide exchange for small binding proteins such as Rho, Rac, and the Cdc42 family of GTP binding proteins [57]. Gene targeting studies have shown that Vav-deficient T cells proliferate less upon activation and that the decrease in proliferation was the result of a failure to produce IL-2 [58, 59].

Transmission of the stimulatory signals from Ras to the nuclear target appears to involve the regulation of the activity of a family of kinases known as MAPKs or ERKs (extracellular signal regulated kinases) [reviewed in 60-62]. T cells express at least two isoforms of MAPK: ERK1 ($p44^{MAPK}$) and ERK2 ($p42^{MAPK}$) that are activated in response to TCR stimulation [63]. Once they become activated they translocate to the nucleus where they regulate the phosphorylation of transcription factors that are involved in the transcriptional activities of immediate early genes such as c-myc, c-fos, c-jun [64,65]. The activity of MAPK requires phosphorylation on both tyrosine and threonine [66]. The phosphorylation and activation of MAPK is induced by an upstream kinase, MAPK kinase (MEK). There are multiple MEKs including MEK1 (45 kDa) and MEK2 (46 kDa). The link between Ras and MEK is provided by at least one other kinase, MEK kinase (MEKK). One candidate for the MEKK that has been described is the protooncogene c-Raf-1 [67]. Raf-1 can be regulated by Ras [67] and by PKC [68] and provides the link between Ras or PKC and MAPK (Figure 2).

Although studies on T cell signaling have dominated the efforts in understanding the MAPK signaling pathway, increased attention to the role of the stress-associated protein kinases JNKs (c-jun amino terminal kinase), also known as SAPKs (stress-associated protein kinases) and the p38 cascade, demonstrates the diverse nature of the MAPK superfamily of enzymes [62]. While the MAPK signaling pathway is activated in response to many different growth factors that promote proliferation and differentiation, activation of the JNK/SAPK pathway occurs in response to agents that cause stress to the cell (Figure 3). Included among these are ultraviolet irradiation, osmolarity changes, heat shock, exposure to inhibitors of protein synthesis, and exposure to inflammatory cytokines [69, 70]. Due to the differential activation of MAPK and JNK in response to various stimuli, it is believed that these pathways, though related, will be regulated upstream by different molecules. Whereas Ras recruits Raf to the plasma membrane to activate the MAPK pathway, an analogous function may be performed by low molecule weight GTP-binding proteins (Rac and Cdc42) to activate the JNK pathway [69,70]. Like other members of the MAPK family, there are multiple forms of JNKs, including JNK1 (46 kDa) and JNK2 (55 kDa). Both forms appear to be regulated by the upstream kinases. Downstream from MEKK is a protein known as JNKK (JNK kinase) or SEK (SAPK kinase) which is responsible for the phosphorylation of JNK [69]. Interestingly, a recent study has shown that IL-2 production is decreased in transgenic mice that lack the SEK-1 gene, indicating the importance of SEK-1 in the T cell signaling pathway [71]. Whether the decrease in IL-2 production was due to a failure to activate SAPKs was not reported.

2. EFFECT OF AGE ON SIGNAL TRANSDUCTION IN T CELLS

2.1 Age-related Changes in Second Messenger Generation. Activation of T cells results in an increase intracellular level of calcium ion $[Ca^{2+}]_i$ and increase PKC activity through IP3 and DAG signal transduction pathways. The rise in $[Ca^{2+}]_i$ results from the release of intracellular stores and also from the influx of extracellular Ca^{2+}. Several studies have been focused on the effect of age on either total levels of intracellular calcium by using a calcium probe (fluorochrome indo-1) or the influx of extracellular Ca^{2+} by radiolabeled calcium ($^{45}Ca^{2+}$). These studies indicate that the induction of calcium signal generation is altered with age in mice and humans. For example, an early study showed that the basal level of intracellular calcium was slightly higher in T cells from old mice than T cells from

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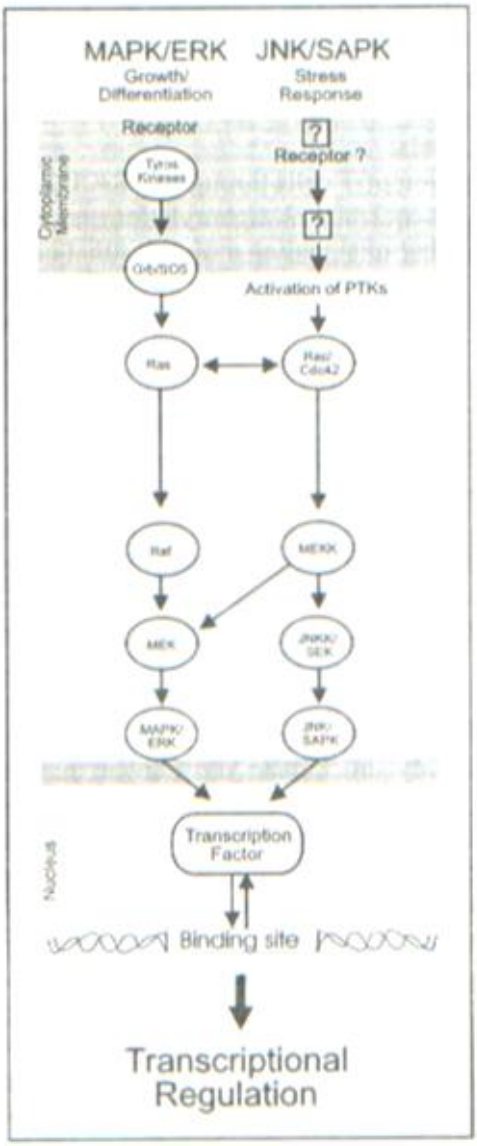

Fig. 3. Schematic diagram of the MAPK/ERK and JNK/SAPK pathways. Extracellular signals trigger activation events upon interaction with their respective receptors on the surface of the cell. The stimulatory signal is then transmitted into the cells where it activates the appropriate pathway. Abbreviations: MAPK, mitogen activated protein kinase; MEK, MAPK kinase; JNK, c-jun amino terminal kinase; SAPK, stress-associated protein kinase; JNKK, JNK kinase; SEK, SAPK kinase.

young mice and that the induction of intracellular calcium level by mitogen did not change with age [72]. Another report demonstrated that resting T cells from old mice had more uptake of Ca^{2+} than the resting T cells from young mice. However, the induction of Ca^{2+} uptake was less for T cells from old mice than T cells from young mice [73]. In one study, it was shown that the induction of the intracellular calcium level by anti-CD3 but not by PHA, was lower in T cells from old mice compared to T cells from young mice [74]. In contrast to the reported studies in mice, the data in humans on effect of age on calcium signaling in T cells have not been consistent. For example, two studies indicated no age-related changes in the uptake of Ca^{2+} in resting or PHA-stimulated peripheral blood lymphocytes in humans [75, 76]. However, another report showed that the induction of $[Ca^{2+}]_i$ by anti-CD3 and by PHA decreased in T cells from elderly humans subjects compared to T cells from young subjects [77,78].

A common feature of the antigen receptor-mediated signaling is the activation of PLC-γ, resulting in the hydrolysis of phosphoinositide lipids and the production of IP3 and DAG. Production of these second messengers in turn leads to the increase in the intracellular free calcium ion concentrations $[Ca^{2+}]_i$ and the activation of PKC, respectively. Therefore, it has been argued that the age-related changes in calcium signal mobilization might result from alterations in production of IP3. The initial report on the effect of age on IP3 generation indicated that the basal level of IP3 was slightly higher in splenic T cells from old mice compared to the level in young mice. However, concanavalin A (Con A) induction of IP3 generation was lower in splenic T cell from old mice than young mice [72]. In contrast, another study showed no age-related difference in the basal or the induced level of IP3 in T cells from mice [73].

The initial report on PKC activation showed that the basal level of total PKC activity was slightly higher in T cells from old mice, whereas the membrane and the cytoplasmic distribution of PKC were similar in T cells from both old and young mice [72]. In addition, PKC translocation did not change with age when cells were stimulated with phorbol myristate acetate (PMA). However, when cells were stimulated with Con A the induction of PKC translocation was lower (50%) in T cells from old mice than T cells from young mice [72]. This study suggested that the age-related decline in PKC might reflect changes in generation of DAG. One study in human has shown that the basal level of PKC in peripheral blood lymphocytes from elderly subjects was similar to the levels in peripheral blood lymphocytes from young subjects. However, phytohemagglutinin (PHA) induction of PKC was significantly less in peripheral blood lymphocytes from elderly subjects compared to the level in young subjects [76].

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2.2. Age-Related Changes in PTKs and Ras/MAPK Activation

Because of the ubiquitous role of protein phosphorylation in the initiation of physiological signals, it has been postulated that changes in the phosphorylation of the key proteins with age might be the primary cause of defect in T cell function. A number of studies have shown that phosphorylation of various cellular proteins is impaired in T cells from humans [79-81] and rodents [82-84]. In one study, it was shown that T cells from elderly humans were more susceptible to herbimycin A (a PTK inhibitor) which inhibits signal transduction. However, no age-related changes in tyrosine phosphorylation of endogenous proteins were found when cells were stimulated with pervandate (a PTK activator). The increase in sensitivity of T cells from elderly humans to PTK inhibitors was associated with a decrease in the inducibility of tyrosine phosphorylation of the endogenous protein substrate [79]. This study suggested that alterations in upstream signaling events might be the underlying cause of the decline in tyrosine phosphorylation with age.

Members of the *Src* (Lck and Fyn) and *Syk* (ZAP-70) family of PTKs play a critical role in TCR-mediated signal transduction. The induction of Lck [81] and ZAP-70 [80] activity has been shown to decrease with age in humans. One study reported that Fyn activity but not Lck activity by anti-CD3 was less in T cells from elderly subjects than T cells from young subjects [85]. Similarly, a recent study in mice showed that the induction of Fyn and ZAP-70 activity decreased with age in T cells [86]. More recently, our laboratory reported that the kinase activities (autophsphorylation) associated with Lck and ZAP-70 but not Fyn were significantly less (by 56% and 76%, respectively) in T cells from old rats compared to T cells from young rats [84]. Furthermore, our study showed that the decrease in Lck and ZAP-70 activities with age was not due to changes in their corresponding protein levels.

Stimulation of T cells through TCR/CD3 complex results in sequential phosphorylation and activation of a number of signaling molecules that eventually lead to the activation of the Ras/MAPKs signaling cascade. MAPKs in turn phosphorylate and activate a variety of regulatory proteins and transcription factors involved in regulation of cytokine genes (e.g., IL-2). Because the age-related decline in T cell function (i.e., IL-2 expression and proliferation) has been well documented [reviewed in 87] and because activation of MAPK is essential for the induction of cytokine gene expression, it has been hypothesized that the age-related decline in T cell function might occur as a result of a decrease in MAPK activation. Several studies have provided evidence in support of the view that the induction of MAPK activity decreases with age. For example, a study in human has shown that MAPK (ERK1 and ERK2) and MEK activities decreased with age when cells were stimulated with anti-CD3, PHA, PHA plus PMA, or PMA plus ionomycin [88]. In addition, it has recently been reported that the induction of Raf-1, MAPK (ERK2), and JNK activities decreased with age in humans [89]. Similarly, a study in mice has shown that the induction of MAPK and MEK activity in T cells decreased with age [85, 90, 91]. Our laboratory has recently investigated the effect of age on signal transduction in T cells from rats. We found that aging had no effect on the basal level of MAPK or JNK activity or the protein levels of these regulatory proteins. However, Con A induction of MAPK activity but not JNK activity was significantly less (by 65%) in T cells isolated from old rats compared with T cells isolated from young rats. Furthermore, we found that the age-related decline in MAPK activity was correlated with a decrease in phosphorylation of $p44^{MAPK}$ protein [84].

Why MAPK activation is reduced in aging T cells? Based on the current model (Figure 2), the age-related decrease in MAPK activation could occur by at least two distinct mechanisms. First, the decrease MAPK activity with age could arise from reduced activity of the proximal signaling molecules such as MEK or Ras. In another word, less MAPK activity is observed in T cells of old animals because less MEK or Ras activity is present in these cells. Second, the decrease in the activity of MAPK may be due to the upregulation of the MAPK phosphatase (MPK-1). That is, similar levels of MAPK activity are present in the activated T cells from young and old animals; but in response to stimulation, the activity of MPK-1 that is involved in dephosphorylation of MAPK increases in the T cells from old animals. Our laboratory has addressed the question of whether Ras activation alters with age and if the changes are correlated with an alteration in the expression of $p21^{ras}$ protein. We found that the percentage of GTP-$p21^{ras}$ observed in the Con A-stimulated T cells from old rats was significantly less (53%) than the level observed in T cells isolated from young rats. The age-related decrease in Ras activation was not associated with changes in the $p21^{ras}$ protein level [84]. This study suggests that aging alters the activation of Ras/MAPK cascade that leads to cytokine gene expression and T cell function.

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CONCLUDING REMARKS

The age-associated decline in the immune system is reflected by a sum of dysfunction and dysregulation of the immunologic responses. Many of these defects implicate a deficit in the functional properties of T cells. Diminished proliferative response of T cells to antigenic/ mitogenic challenges, altered cytokine expression, accumulation of the hyporesponsive memory T cells, and decreased calcium mobilization have all been considered as contributors to immune senescence. Although the molecular mechanisms underlying these changes are not fully understood, T cells provide an excellent model for studying cellular aging. One of contemporary hypothesis is that aging is an active genetically programmed event. T cells undergo a series of genetically programmed processes, first maturation into functionally competent cells and later development into hyporesponsive senescent cells. How then can aging affect the cellular mechanism directing normal T cell function? Signal transduction is ubiquitously involved in the initiation of physiological signals that lead to growth and proliferation and even programmed cell death. The current research demonstrates that signal transduction events are an important cellular mechanism for both T cell development and T cell function. A number of recent studies, including ours, have proposed that changes in signal transduction machinery are one of the underlying causes of the age-related decline in T cell function. Alterations in some of the early signaling events such as calcium mobilization, tyrosine phosphorylation, Ras and MAPK activation have been linked to the age-associated decrease in the induction of cytokine (IL-2) expression and T cell proliferation. Impairment in the proximal signaling molecules at the cell membrane or the cytoplasmic level may contribute to the secondary defect of the other downstream nuclear events such as transcription. 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 aging alters the activation of various signaling molecules resulting in diminished T cell responsiveness is far from complete.

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