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# T CELL SIGNALING: EFFECT OF AGE

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## 1. ABSTRACT

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 kinases, phosphatates, 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.

## 2. 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 ref. 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 reported; however, the data have not been consistent. One dramatic change that has been frequently cited is a decline in the ability of T cells to produce IL-2 (reviewed in ref. 6) and to express the IL-2 receptor (4 and 5). 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. 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. During T cell activation, complex networks of signaling molecules work in concert to transduce the stimulatory signal from TCR to the nuclear target. To unravel the age-related defect, it is important to understand the coordinate role of various signal transduction modulators in T cell signaling.

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 function change upon T cell activation. These investigations resulted in the

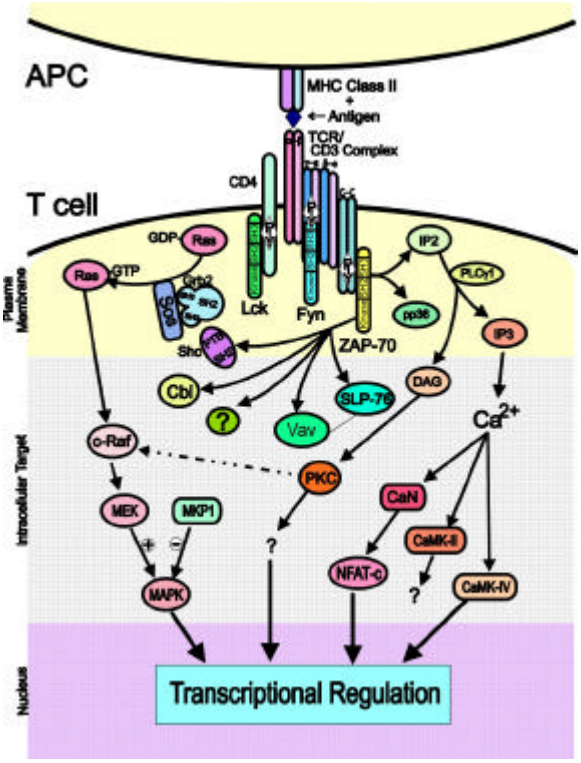


**Figure 1.** A current model for TCR-mediated signal transduction. Interaction of antigen with TCR/CD3 complex initiates a series of biochemical events, of which the earliest is phosphorylation of various proteins on tyrosine residues. Stimulation of PTKs is coupled to the hydrolysis of PLCγ1 which 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.

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. A number of reviews have been written over the last several years on the role of various signaling molecules in different cells including T cells (7-12), The purpose of this paper is to present an overview of the TCR-mediated signaling events and to discuss the age-related alterations in signal transduction in T cell.

## 3. TCR-MEDIATED SIGNAL TRANSDUCTION: GENERAL FEATURE

The events that initiate by antigenic or mitogenic stimuli culminate in the expression of cytokine genes and proliferation is referred to as T cell activation (13,14). 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. One family of genes encodes the antigen recognition component, the alpha/beta (α/β) chains which arise from the rearrangement of constant and variable region genes allowing the diverse repertoire of antigen recognition specificity (15-17). The other two families code for the signal transduction component of the antigen receptor, which contains the CD3 chains: the delta/epsilon (δ/ε) and the gamma/epsilon (γ/ε) chains, and a homo or hetrodimer consisting of one or two members of the zeta (ζ) family (18,19). 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 (figure 1). The cytoplasmic domains of these polypeptides have in common a sequence 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 (20,21). 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 (22-25).

### 3.1 Protein tyrosine phosphorylation

The early signaling event most proximal to the TCR/CD3 complex is the activation of two *Src*-family of protein tyrosine kinases (PTKs): Lck and 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. The importance of ZAP-70 in TCR signaling was demonstrated by the existence of a rare immunodeficiency in human patients with

ZAP-70 mutation (26-29). T cells from these patients fail to produce IL-2 and to proliferate in response to TCR stimulation (26,28,29). Further support for the involvement of Lck, Fyn, and ZAP-70 in TCR-mediated signaling was provided by gene targeting studies. For example, a study in Lck-deficient mice has shown that the basal and the induced level of phosphorylation of ζ-chain and ZAP-70 and other regulatory proteins were greatly reduced (30). This study suggested that although Lck was important for the initiation of TCR signaling, in its absence, Fyn may take over its function. Studies of Fyn-deficient mice have revealed that the TCR-induced calcium mobilization and proliferation were reduced in T cells (31,32). Furthermore, the analysis of $Lck^{-/-}$ $Fyn^{-/-}$ "double-mutants" suggested that Lck and Fyn might have overlapping but not equivalent functions in TCR-mediated signaling (30).

Unlike the hormone receptors, the T cell receptor does not possess kinase activity. Thus, induced protein-protein interactions are essential for coupling the stimulatory signals from TCR/CD3 complex to downstream signaling molecules.Tyrosine phosphorylation of regulatory proteins is transduced via transient specific molecular interactions, in which *Src* homology 2 (SH-2) and 3 (SH-3) domains play an important role (33,34). 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 (35,36). Many intracellular substrates of PTKs contain SH-2 domains that recognize phosphotyrosine sites on autophosphorylated kinases, allowing the transduction of the signal to occur during the activation process. In addition to having kinase activity, they also provide docking sites for other signaling molecule (37). The polypeptides that constitute the CD3 and ζ-chain of the TCR/CD3 complex are the important substrates of Lck and Fyn. In the ITAM region of these peptides, there are tyrosine residues that become phosphorylated upon T cell activation. One such protein that has the ability to interact with CD3 and the ζ chain of TCR is ZAP-70. Tyrosine phosphorylated and activated ZAP-70 recruits other SH-2 containing signaling molecules and allows their localization to the activated TCR complex.

### 3.2 TCR-mediated activation of second messengers

Activation of PTKs is coupled to the stimulation of an inositol lipid specific phospholipase C-gamma (PLCγ1) (38-40). 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 protein kinase C (PKC) via the diacylglycerol (DAG) pathway (41-42). Although tyrosine phosphorylation of PLCγ1 has been shown to be essential for its activity, recent studies suggest that phosphorylation of PLCγ1 alone is not sufficient for activation of the second messengers (IP3 and DAG) and that other tyrosine phosphorylated proteins such as pp36 (a 36 kDa phosphorpotein) may play a role in TCR-mediated activation of these second messengers (44). In addition, 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 porbol esters (increases PKC activity), which bypass the TCR requirement and thereby activate signal transduction pathways (45). Although there is convincing evidence indicating the role of calcium in activation of calcium-dependent enzymes, e.g., calcium-calmodulin-dependent phosphatase (calcineurin) and kinases (CaMK-II and IV), the targets of TCR-mediated PKC activation still remains unclear.

### 3.3 TCR-mediated Ras activation

Another PTK mediated signaling event that originates from the TCR involves the guanine nucleotide binding protein Ras ($p21^{ras}$) (46,47). 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 1). 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) (48). 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 sevenless), a 150 kDa guanylnucleotide exchange factor for Ras (49-50). 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 (51,52). 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 1).

Research during the past 4 years has shown that at least three tyrosine phosphorylated proteins become associated with Grb2 following T cell stimulation. One of the Grb2-associated proteins, which becomes tyrosine phosphorylated upon T cell stimulation is SLP-76 (SH2 domain-containing leukocyte protein 76) (53). 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/MAPK that was associated with upregulation of transcription factors and immediate-early genes (54). Studies in Jurkat T cells have shown that SLP-76 overexpression increases the transcriptional activity 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 (55,56). These studies indicated that although SLP-76 plays an important role in the Ras/MAPK signaling pathway, it has no effect on calcium mobilization following T cell stimulation. The second Grb2-associated protein is a 36 kDa phosphorpotein (pp36) associated with SH-2 domain of Grb2 (57-59). In addition, the pp36 has been found to be associated with PLCγ-1 in the plasma membrane via SH2 domain interaction (57-59). The molecular identity of pp36 has not yet been defined. A recent report described the cloning of a cDNA (lnk) from rat lymph node that encodes a protein with molecular weight of 36 kDa (60). Interestingly, the 36 kDa protein becomes phosphorylated on tyrosine upon T cell activation. Whether the lnk gene product has any role on T cell activation has not yet been determined. The third Grb2 binding phosphoprotein is known as Cbl, is a 120 kDa protein that is ubiquitously expressed in resting and stimulated T cells (61-64). 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 (65). 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 (66). 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 (67,68). Moreover, studies in $Vav^{-/-}$ mice have shown that TCR signaling is compromised, in part, due to greatly reduced generation of calcium signals (69,70).

### 3.4 Signaling through MAPK/ERK pathway

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 (mitogen-activated protein kinases) or ERKs (extracellular signal regulated kinases) (reviewed in 7, 8, 10). Two intracellular pathways for MAPK appear to co-exist in T cells. One mediated by Ras, the other by PKC (71). Although stimulation of T cells via TCR results in activation of both Ras and PKC, data obtained from transfection experiments show that expression of the inhibitory Ras mutant $N17^{ras}$, which prevents endogenous Ras activation, and suppresses TCR-mediate activation of MAPK (72). This study suggests that Ras and not PKC, couples the TCR to the regulation of MAPKs. T cells express at least two isoforms of MAPK:ERK1 ($p44^{MAPK}$) and ERK2 ($p42^{MAPK}$) that are activated in response to TCR stimulation (73). 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 (74,75). The activity of MAPK requires phosphorylation on both tyrosine and thronine (76). 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 (77). Raf-1 can be regulated by Ras (77) and by PKC (78) and provides the link between Ras or PKC and MAPK (figure 1).

Although studies on T cell signaling and MAPK activation 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 (10). 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 2). Included among these are ultraviolet irradiation, osmolarity changes, heat shock, exposure to inhibitors of protein synthesis, and exposure to inflammatory cytokines (79,80). 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 (79,80). 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 phosphoryaltion of JNK (79). Interestingly, a recent study has shown that IL-2 production is decreased in $SEK\text{-}1^{-/-}$ mice, indicating the importance of SEK-1 in the T cell signaling pathway (81). Whether the decrease in IL-2 production was due to a failure to activate SAPKs was not reported.

### 3.5 Role of protein tyrosine phosphatases in T cell signaling

Protein tyrosine phosphorylation represents a balance between the activities of protein tyrosine kinases (PTKs) and protein tyrosine phosphates (PTPs). Despite the fact that the majority of attention has been focused on PTKs, the role of PTPs in regulation of antigen receptor signaling has been recognized. Particularly important in TCR signaling is the PTP, CD45 (82). CD45 is a transmembrane tyrosine phosphatase that is a critical regulator of the initiation of signal transduction through not only the TCR but also other receptors on immune cells that contain ITAMs (83,84). A single gene encodes CD45; however, multiple isoforms exist due to alternative splicing (85). Mutant T cell lines that have lost CD45 are defective in TCR signaling and in T cell function such as IL-2 production

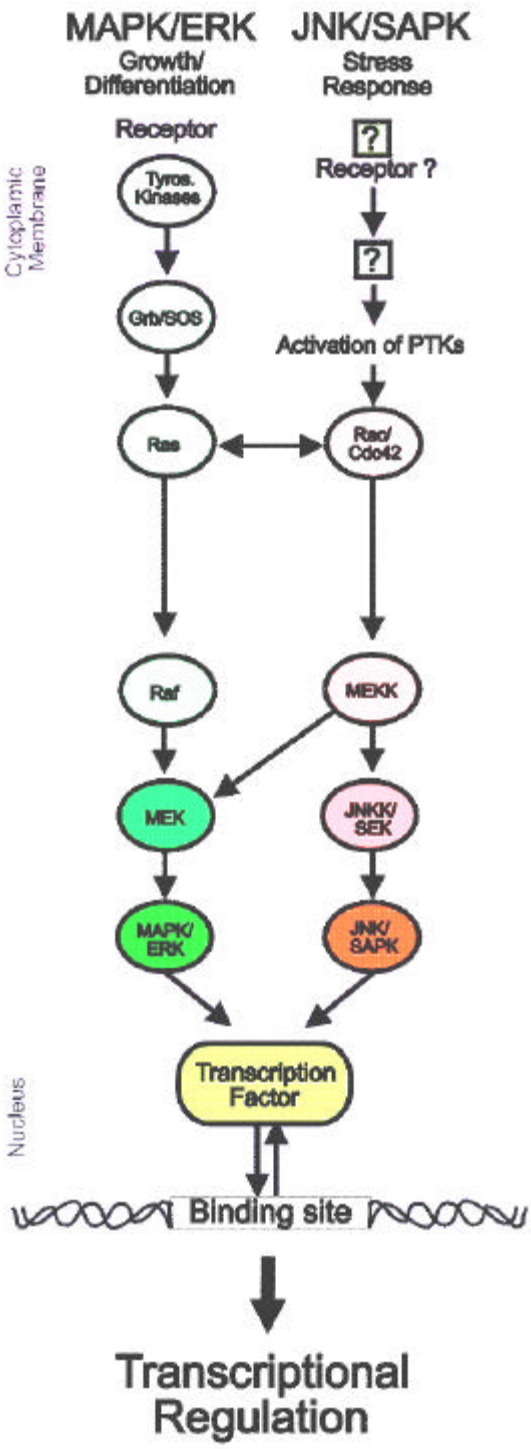


**Figure 2.** Schematic diagram of the MAPK/ERK and JNK/SAPK pathways. Extracellular signals trigger activation events upon interaction with their respective receptors on the surface of the cell. The stimulatory signal is then transmitted into the cells where it activates the appropriate pathway. Abbreviations: 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.

(86). Studies on CD45-deficient mice have shown that in the absence of this phosphatase, TCR signaling is markedly impaired as thymocytes in CD45$^{-/-}$ mice proliferated less in response to stimulation (87-89). One of the proposed functions of CD45 in TCR signaling is to activate the *Src*-family kinases (Lck and Fyn) by dephosphorylating these tyrosine kinases (90). Data from studying T cells in CD45$^{-/-}$ mice have shown that Lck and Fyn were inactive due to a high level of phosphoryation (89). Thus, it appears that CD45 acts to dephosphorylate Lck and Fyn on tyrosine residues (91,92). Dephosphorylation of these residues would enhance Lck and Fyn activities and thereby allow these kinases to perform their positive role in TCR singling.

In contrast to the positive role of CD45 in T cell singling, other PTPs such as SHP-1 (PTP1C) and SHP-2 (PTP1D) have a negative role in the initiation of signal transduction. SHP-1 contains two SH-2 domains and targets the tyrosine phosphorylated proteins in various hematopoietic cells including T cells (93). A form of immunodeficiency phenotype in the murine system has been shown to be due to the mutation of the SHP-1 gene (94). It is believed that the recruitment of SHP-1 to its targets results in suppression of the downstream signaling events (95-98). Overexpression of SHP-1 has shown to result in uncoupling of TCR signaling machinery (99). One study has provided evidence for an association between SHP-1 and ZAP-70 and suggested that ZAP-70 could function as a substrate for SHP-1 (100). Like SHP-1, SHP-2 is a cytoplasmic phosphatase which contains two SH-2 domains in the amino terminal and forms a regulatory function by targeting the tyrosine phosphorylated proteins (101-103). A recent report suggests that SHP-2 is involved in dephosphorylation of the Shc protein and downregulation of TCR-mediated Ras activation (104). The TCR-mediated expression of the surface molecule CTLA4 results in inhibition of T cell activation (105-107). It is believe that CTLA4 exerts its effect by directly linking SHP-2 phosphatase to the Shc adapter protein that couples to the activation of the downstream signaling molecules such as Ras (108,109). Furthermore, other phosphatases may have regulatory roles in the activation of MAPK (110-112). A phosphatase, MKP-1 (MAPK phosphatase-1) has been identified that dephosphorylates MAPK in vivo (111) and in vitro (112). Thus, the TCR-mediated signal regulates MAPK by controlling the activities of a positive regulator (e.g., Ras) and a negative regulator (e.g., MKP1).

## 4. AGE-RELATED CHANGES IN SIGNAL TRANSDUCTION IN T CELL

### 4.1 Calcium signaling and second messenger generation

Activation of T cells results in a transient increase in intracellular free calcium ion concentrations $[Ca^{2+}]_i$. 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 which are listed in table 1 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 young mice and that the induction of intracellular calcium level by mitogen did not change with

age (113). 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 (115). 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 (118).

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 (132,136). 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 (133-135). One question that has been addressed is whether the calcium signal generation is faulty in all T cell populations from old experimental subjects, or whether only certain subsets of T cell exhibit less response to calcium mobilization and others have a response similar to young subjects. In a study in mice, it was shown that among the T cell populations, the ionomycin-resistant T cell subsets were predominantly of the memory phenotype ($Pgp\text{-}1^{hig}$) and these cells were hyporesposive with respect to the helper and cytotoxic function in both young and old animals (118).

A common feature of the antigen receptor-mediated signaling is the activation of PLC-$\gamma$, 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, Con A induction of IP3 generation was lower in splenic T cell from old mice than young mice (113). In contrast, another study showed no age-related difference in the basal or the induced level of IP3 in T cells from mice (115).

Table 1 lists studies on the effect of age on the generation of the second messenger DAG and the activation of PKC. The initial report 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 (113). 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 (113). This study suggested that the age-related decline in PKC might reflect changes in generation of DAG. One study reported four-fold decrease in membrane-associated PKC activity in T cells from old mice compared to T cells from young mice (122). A study in humans 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, PHA induction of PKC was significantly less in peripheral blood lymphocytes from elderly subjects compared to the level in young subjects (136). Recently, it was reported that the expression of PKC $\alpha$-isomers but not $\beta$–isomers decreased in peripheral blood T cells from elderly human subjects compared to the peripheral blood T cells from young subjects (139). This study suggested that selective alteration in PKC isoenzymes in T cells during aging might contribute to alterations in intracellular signaling events.

### 4.2 Protein tyrosine phosphorylation 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. The studies listed in table 1 show that phosphorylation of various cellular proteins is impaired in T cells from humans (138,142,144) and rodents (123,124,130). 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 (138). 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. table 1 list the studies on the effect of age on the activation of Lck, Fyn, and ZAP-70. The induction of Lck (144) and ZAP-70 (142) 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 (140). Similarly, a recent study in mice showed that the induction of Fyn and ZAP-70 activity decreased with age in T cells (121). 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 (130). 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

**Table 1.** Effect of age on signal transduction in T Cell

| Species | Age (Mo/Yr) | Inducing Agent | Signaling Molecule (Level / Activity) | Change with Age | Ref |
|---|---|---|---|---|---|
| **Mouse** | 4-24 | Con A | Calcium level | Decrease | 113 |
| | 4-26 | Con A | Calcium level | Decrease | 114 |
| | 2-24 | Con A | Calcium influx | Decrease | 115 |
| | 2-22 | Anti-CD3 | Calcium level | Decrease | 116 |
| | 6-30 | Con A, Anti-CD3 | Calcium level | Decrease | 117 |
| | 2-24 | PHA, Anti-CD3 | Calcium level | Decrease | 118 |
| | 6-30 | Anti-CD3 | Calcium level | Decrease | 119 |
| | 2-25 | Con A, IP | Calcium level | Decrease | 120 |
| | 2-27 | Anti-CD3 | Calcium influx | Decrease | 121 |
| | 2-27 | Anti-CD3 | PIP2 Level | No change | 121 |
| | 2-27 | Anti-CD3 | PLC-γ1 Level | Decrease | 121 |
| | 2-27 | Anti-CD3 | PLC-γ1 phosphorylation | Decrease | 121 |
| | 2-27 | Anti-CD3 | PLC Activity | No change | 121 |
| | 4-24 | Con A | PKC translocation | Decrease | 113 |
| | 4-24 | PMA | PKC translocation | No change | 113 |
| | 4-30 | Con A | PKC translocation | Increase | 122 |
| | 4-24 | Con A | IP3 generation | Decrease | 113 |
| | 2-24 | Con A | IP3 / IP4 generation | No change | 115 |
| | 2-27 | Anti-CD3 | IP3 generation | Decrease | 121 |
| | 2-27 | Anti-CD3 | DAG generation | Decrease | 121 |
| | 3-22 | Anti-CD3 | Tyrosine phosphorylation | Decrease | 123 |
| | 3-22 | Anti-CD3 | Tyrosine phosphorylation | Decrease | 124 |
| | 2-25 | Anti-CD3 | Tyrosine phosphorylation | Decrease | 125 |
| | 3-22 | Anti-CD3 | Tyrosine phosphorylation | Decrease | 126 |
| | 2-22 | Anti-CD3 | Grb2 phosphorylation | Decrease | 127 |
| | 2-22 | Anti-CD3 | Shc phosphorylation | Decrease | 127 |
| | 2-22 | Anti-CD4 | Shc phosphorylation | No change | 127 |
| | 3-22 | Anti-CD3 | ζ-chain phosphorylation | Decrease | 126 |
| | 2-27 | Anti-CD3 | Fyn phosphorylation | Decrease | 121 |
| | 2-27 | Anti-CD3 | ZAP-70 phosphorylation | Decrease | 121 |
| | 3-22 | Anti-CD3 | MEK kinase activity | Decrease | 128 |
| | 3-22 | Anti-CD3 | MAPK kinase activity | Decrease | 128 |
| | 2-27 | Anti-CD3 | MAPK kinase activity | Decrease | 121 |
| | 3-22 | Anti-CD3 | ζ-chain phosphorylation | Decrease | 129 |
| | 3-22 | Anti-CD3 | ZAP-70 protein level | Increase | 129 |
| | 3-22 | Anti-CD3 | Raf-1 kinase activity | Decrease | 129 |
| | 3-22 | Anti-CD3 | MEK kinase activity | Decrease | 129 |
| | 3-22 | Anti-CD3 | MAPK kinase activity | Decrease | 129 |
| **Rat** | 4-26 | Con A | Fyn kinase activity | Decrease | 130 |
| | 4-26 | Con A | Lck kinase activity | Decrease | 130 |
| | 4-26 | Con A | ZAP-70 kinase activity | Decrease | 130 |
| | 4-26 | Con A | Ras activity | Decrease | 130 |
| | 4-26 | Con A | JNK kinase activity | No change | 130 |
| | 4-26 | Con A | MAPK kinase activity | Decrease | 130 |
| **Monkey** | ----- | Anti-CD3 | Calcium Level | Decrease | 131 |
| **Human** | 27-74 | PHA | Calcium level | No change | 132 |
| | 34-78 | PHA, Anti-CD3 | Calcium level | Decrease | 133 |
| | 32-76 | Con A, Anti-CD3 | Calcium level | Decrease | 134 |
| **Species** | **Age** | **Inducing Agent** | **Signaling Molecule** | **Change with Age** | **Ref** |

| (Mo/Yr) | | (Level / Activity) | | |
|---|---|---|---|---|
| 34-75 | Anti-CD3 | Calcium level | Decrease | 135 |
| 26-81 | PHA | Calcium influx | No change | 136 |
| 30-78 | Anti-CD3 | Calcium influx | Decrease | 134 |
| 26-72 | PHA | PLC activity | No change | 137 |
| 34-77 | --- | PKC-α level | Decrease | 149 |
| 34-77 | --- | PKC-β level | No change | 139 |
| 34-82 | PHA | PKC activity | Decrease | 139 |
| 35-80 | Anti-CD3 | Tyrosine phosphorylation | Decrease | 138 |
| 35-80 | Herbimycin | Tyrosine phosphorylation | Decrease | 138 |
| 35-80 | Pervandate | Tyrosine phosphorylation | No change | 138 |
| 34-74 | PHA | Tyrosine phosphorylation | Decrease | 139 |
| 24-72 | Anti-CD3 | Tyrosine phosphorylation | Decrease | 135 |
| 30-70 | Con A, PHA | Tyrosine phosphorylation | Decrease | 142 |
| 21-75 | PHA | Tyrosine phosphorylation | Decrease | 144 |
| 34-74 | Anti-CD3 | Fyn activity | Decrease | 140 |
| 34-74 | Anti-CD3 | Lck activity | No change | 140 |
| 21-75 | PHA | Lck activity | Decrease | 144 |
| 30-70 | Con A, PHA | ZAP-70 | Decrease | 142 |
| 31-79 | Anti-CD3, PHA, IP | MEK activity | Decrease | 141 |
| 31-79 | Anti-CD3, PHA, IP | MAPK activity | Decrease | 141 |
| 34-74 | Anti-CD3 | CD45 phosphatase | No change | 140 |
| 23-76 | Anti-CD3/PMA | MAPK activity | Decrease | 143 |
| 23-76 | Anti-CD3/PMA | JNK activity | Decrease | 143 |
| 23-76 | Anti-CD3/PMA | Raf-1activity | Decrease | 143 |

Abbreviations: Con A, concanavalin A; PHA, phytohemagglutinin; IP, ionomycin plus PMA; PMA, Phorbol myristate acetate.

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 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 (table 1) have provided evidence in support of the view that the induction of MAPK activity decreases with age. For example, a study in humans 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 (141). In addition, it has recently been reported that the induction of Raf-1, MAPK (ERK2), and JNK activities decreased with age in humans (143). Similarly, a study in mice has shown that the induction of MAPK and MEK activity in T cells decreased with age (121,128,129). In this study, MAPK and MEK activity was assessed by monitoring the phosphorylation of the MAPK and MEK substrate in T cell lysates by the mobility shift assay (129). This study showed that a shift in mobility (phosphorylation) of one of the ERK2 substrate (the ribosomal S6 protein kinase $pp90^{rsk}$) was less in the anti-CD3 stimulated T cells from old mice compared to anti-CD3 stimulated T cells from young mice. In addition, this study indicated that the age-related decline in MAPK/MEK activation was not due to changes in the proportion of naive/virgin or memory T cell subsets because MAPK/MEK activity was equally diminished in both naive and memory helper T cells from old mice (128).

Our laboratory has recently investigated the effect of age on signal transduction in T cells from rats. Figure 3 shows data in which the induction of MAPK, JNK, and Ras activation was assessed in T cells from young (6 month) and old (24 months) F344 rats. 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 (130).

Why is MAPK activation reduced in aging T cells? Based on the current model (figure 1), the age-related decrease in MAPK activation could occur by at least two distinct mechanisms. First, 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. Second, the decrease MAPK activity with age could arise from reduced activity of the proximal signaling molecules such as MEK or Ras. In

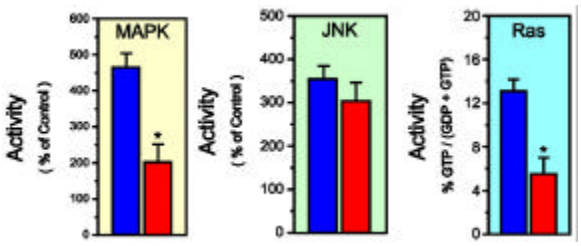


**Figure 3.** Effect of age on the induction of MAPK, JNK, and Ras in T cells from F344 rats. The splenic T cells from young (6 months) and old (24 months) rats were stimulated with Con A for 5 to 10 min. Protein was isolated and the activity associated with the immunoprecipitated p44 and p42 MAPK, p46 JNK or p21ras was measured. Data were taken from Pahlavani et al. (130). The values (*) for the old rats are significantly different from the values for the young rats at $p<0.001$.

another word, less MAPK activity is observed in T cells of old animals because less MEK or Ras activity is present in these cells. 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. The Ras activity was assessed by measuring the accumulation of GTP and GDP-bound $p21^{ras}$ in the immunoprecipitated $p21^{ras}$ protein in the unstimulated and Con A stimulated T cells. As shown in figure 3, 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 (130). This study suggests that aging alters the activation of Ras/MAPK cascade that leads to cytokine gene expression and T cell function.

## 5. 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 underling these changes is 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. An 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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