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Signaling pathways in aged T cells – a reflection of T cell differentiation, cell senescence and host environment

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Abstract

With increasing age, the ability of the immune system to protect against new antigenic challenges or to control chronic infections erodes. Decline in thymic function and cumulating antigenic experiences of acute and chronic infections threaten T cell homeostasis, but insufficiently explain the failing immune competence and the increased susceptibility for autoimmunity. Alterations in signaling pathways in the aging T cells account for many of the age-related defects. Signaling threshold calibrations seen with aging frequently built on mechanisms that are operational in T cell development and T cell differentiation or are adaptations to the changing environment in the aging host. Age-related changes in transcription of receptors and signaling molecules shift the balance towards inhibitory pathways, most dominantly seen in CD8 T cells and to a lesser degree in CD4 T cells. Prominent examples are the expression of negative regulatory receptors of the CD28 and the TNF receptor superfamilies as well the expression of various cytoplasmic and nuclear dual-specific phosphatases.

Keywords

Aging; signaling; T cell receptor; JAK STAT pathway; dual-specific phosphatase

1 INTRODUCTION

Defects in signaling pathways involved in cell activation have been suspected to contribute to the declining immune function with age. In the current view, the T cell system is disproportionally affected by age [1] which leads to the failing efficacy of vaccination and the increased morbidity and mortality from viral infections such as influenza or the reactivation of chronic viral infections, e.g. in the form of herpes zoster [2–5]. Regulation of signaling pathways is of utmost importance for T cell function. T cell receptor (TCR) signaling has to find the delicate balance between tolerating self-antigens and responding to foreign peptides. Costimulatory signals decide between effective response and anergy. And cytokine receptors regulate T cell proliferation and differentiation through various JAK-STAT pathways. It therefore does not come as a surprise that shortly after signaling components of the TCR pathway were identified, these were probed for abnormalities with aging [6]. TCR induced calcium influx was clearly diminished [7]. The underlying

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mechanisms have remained obscure; however, this defect appeared to account for the decreased IL-2 production, a hallmark for the immune aging of murine naïve T cells. Work by Miller and colleagues provided evidence for at least two mechanisms, both of which impaired or even prevented the formation of a T cell recognition platform [8]. T cells from aged mice had a defect in cytoskeletal organization on encountering antigen-presenting cells, at least in part independent of peptide recognition [9]. And, age influenced the glycosylation of cell surface molecules, in particular CD43, resulting in steric hindrance in the antigen recognition process [10, 11]. In addition, age-dependent alterations in plasma membrane lipids cholesterol-rich microdomains were implicated in defective T cell responses [12]. In contrast to murine T cells, findings in human T cells are more difficult to define and to interpret. Age-related alterations in human T cell responsiveness are more subtle, in particular, when T cells of similar differentiation status are compared [13]. The introduction of flow cytometry to measure signaling events has greatly enhanced our ability to quantify phosphorylated proteins, to examine several signaling events in phenotypically defined single cells and to perform high throughput analysis of larger populations, which is necessary given the inherent multifactorial variability in a human population. In this review, we will review age-related alterations in signaling pathways of human T cells. We will discuss how far age-related response patterns can be interpreted as progressive physiological T cell differentiation processes; what the contributions of adaptations to the aging host environment are; and how cell-internal senescence-associated mechanisms can influence signaling pathways. We will focus our discussion on the TCR receptor and the JAK-STAT pathways, the basic principles of which have been the subject of previous excellent reviews [14, 15].

2 TCR threshold calibration – essential for T cell development, detrimental for T cell aging

Calibration of TCR thresholds is an essential element in the life cycle of a T cell. Most notably, thymocytes have an exquisite TCR sensitivity which allows them to be positively selected on self-antigens. Sensitivity drastically changes with T cell maturation [16]. The naïve T cell that leaves the thymus is no longer able to respond to self-antigens with a productive response. Li et al explored the signaling mechanisms that account for this rapid loss in TCR sensitivity and found a correlation with the expression of the miR-181a [17]. Prior studies had shown that miR-181a is one of the most abundant microRNA species expressed in the thymus, in particular, in double-negative thymocytes [18]. Expression levels drop sharply with differentiation into double-positive thymocytes and single-positive T cells. miR-181a controls the expression of several phosphatases, including PTPN22, SHP-2, and the two dual-specific phosphatase (DUSP)5 and DUSP6 in the mouse. With the exception of DUSP5, which is a nuclear phosphatase, all of these phosphatases negatively regulate proximal TCR signaling events. Indeed, overexpression of miR-181a by transfecting the appropriate miRNA precursor lowers the TCR activation threshold and restores the ability of a mature T cell to respond to autoantigens [17]. To address the hypothesis that TCR threshold settings are not final with thymic emigration and that recalibrations continue to occur with aging, we examined whether miR-181a expression levels continue to decline throughout life. Indeed, we found a significant difference in miR-181a expression in naïve CD4 T cells from elderly and young adults [19]. The effect on TCR responsiveness in the middle-age adult is minor; naïve CD4 T cells from 60 year-old healthy adults have nearly normal responses in the expression of activation markers or proliferation after in vitro stimulation with CD3 cross-linking and also more physiologic stimuli such as superantigen stimulation [20]. Only after the age of 70 years, a consistent blunting of TCR-induced ERK phosphorylation is seen. Thus, loss of miR-181a in T cells,

initially necessary to avoid autoimmunity, is eventually counterproductive and interferes with normal responsiveness to exogenous antigen.

miR-181a expression is certainly not the only program involved in TCR threshold setting, and we predict that other mechanisms can be identified that are fundamental for T cell development and differentiation and have unwanted effects if occurring in T cell aging outside of their normal context (Figure 1). TCR signaling calibration occurs during T cell differentiation from naïve to effector and memory T cells. Antigen-experienced T cells in general have faster response time and higher magnitude in responses than naïve T cells, which in part accounts for beneficial effects of immune memory in recall responses. Naïve T cells undergoing cumulative homeostatic proliferation with increasing age acquire memory-like features which may include signaling pathways. Also, memory T cells undergo changes with repeated restimulation. In analogy to other cellular systems, Adachi and Davis have explored the hypothesis that dependent on the differentiation stage, scaffolding molecules channel TCR signals preferentially to one of several signaling pathways [21]. The authors provided evidence that upstream signaling including the phosphorylation of Zap70 is similar irrespective of the differentiation state of the T cells. However, in naïve T cells the TCR-induced signal is preferentially channeled through the scaffolding molecule SLP76 leading to ERK phosphorylation, while in memory T cells Zap70 binds to hDlg to assemble a signaling complex and activate the p38 pathway. Indeed, we also have observed a higher ERK phosphorylation in naïve than in memory CD4 T cells in young individuals which is lost with increasing age. Whether the p38 pathway is preferentially stimulated with age has not been studied but could contribute to the proinflammatory environment seen in the elderly, in particular in those who have increased expansion of CMV-specific effector cell populations. Conversely, von Essen and colleagues found decreased proximal signals, but increased PLC- γ 1 phosphorylation in memory T cells, mainly due to increased expression of PLC- γ 1 induced in primed cells [22]. It is evident from these studies that channeling of TCR signals change with T cell activation and differentiation, and future studies have to explore the influence of age on the preferential pathway usage.

3 Aging and the cell-intrinsic environment

3.1 Reactive oxygen species in aging and signaling

A popular model to explain aging is the free radical theory, where reactive oxygen species (ROS) are responsible for sustained damage to DNA, proteins and lipids. Mitochondria and NADPH oxidase are the major sources of ROS production in most cell types including T cells [23]. With increasing age, mitochondrial ROS leakage increases, mainly due to defective respiratory chain integrity [24, 25]. In addition to their harmful effects, ROS function as important physiological regulators of intracellular signaling pathways [26]. Early studies showed that increased oxidative stress results in displacement of LAT from the cell membrane and thereby inhibits TCR signaling [27, 28]. Taken together with data in murine naïve T cells that LAT phosphorylation is defective and that this defect can be compensated by vitamin E, this observation has led to the hypothesis that an altered redox potential causes signaling abnormalities in aging T cells [29]. It remains to be determined whether T cells develop an abnormal redox potential with age. At least activated T cells from elderly humans express increased levels of metallothioneins which functions as an important redox system [20]. The transcription of metallothioneins is regulated by zinc influx from extracellular sources through the zinc transporter ZIP6 [30] and from intracellular sources through ZIP8 [31]. Moreover, the outcome of increased ROS production on signaling pathways is difficult to predict as ROS are also positive signal mediators in T cell activation. T cells express NADPH oxidases, which can be activated through similar mechanisms as in other cells, e.g. angiotensin II [32]. A proximal oxidant signal is necessary to amplify TCR signals and increase signal strength. Upon T cell activation, mitochondria translocate to the

immunologic synapse [33, 34]. TCR-induced calcium mobilization induces mitochondrial ROS release, which functions by inhibiting activities of numerous phosphatases and activating some kinases such as protein kinase D [35, 36]. How age affects these mitochondrial activities, whether activation-induced ROS production is increased, normal or decreased and what the signaling consequences are will be very important for understanding the effect of age on T cell physiology (Figure 1).

3.2 Age-related DNA damage initiates signaling cascades

Age (and also medication commonly used in an elderly population such as statins) may influence plasma membrane composition and therefore the formation of microclusters and TCR signaling [37–39]. Data so far are not conclusive. One additional age-dependent event that alters the intracellular milieu and resets signaling pathways is the accumulation of DNA damage, in part caused by endogenously generated reactive oxygen species [40]. Memory T cells, more so than naïve cells, have increasing number of DNA double-strand breaks (DSB) with age [41]. In addition, several factors including replicative stress, decline in telomerase activity and cumulative DNA damage lead to the shortening of telomeres in lymphocytes, with age and T cell proliferation the major drivers [42, 43]. Telomeres are shorter in end-differentiated T effector cell population, but decrease in all T cell populations with age. Cells have several options to detect and repair broken DNA; similar pathways may be involved in surveillance of telomeric structures [44–46]. Three of the enzymes involved in DNA repair pathways are PI3K-related proteins: the ataxia telangiectasia mutated gene product (ATM), the ataxia telangiectasia-related (ATR), and the catalytic subunit of DNA-dependent protein kinase (DNA-PKcs) [47]. Their kinase activities are not limited to sensing/repairing DSB but also intersect with nuclear and cytoplasmic signaling pathways. We have recently shown that patients with rheumatoid arthritis have an accelerated aging syndrome with increased frequencies of DSBs and activation of DNA-PKcs [48]. In these patients, DNA-PKcs activates the stress kinase pathway and induces T cell death by upregulating the apoptogenic BH3-only proteins Bim and Bmf. DNA-PKcs have also been shown to be an essential mediator in endosomal signaling pathways and to regulate the activation of AKT [49]. Similarly, ATM is not only involved in DNA repair and p53 activation, but it is also activated by oxidative stress in the cytoplasm and intersects with metabolic signaling pathways including AMP-activated protein kinase (AMPK) and mammalian target of rapamycin (mTOR) [40]. While it is clear that both DNA-PKcs and ATM have nuclear and cytoplasmic signaling function, it remains to be determined how much overlaps in their molecular targets exist depending on whether they are activated by DNA damage or cytoplasmic mechanisms and how the increased activation of DNA repair kinases due to DNA damage and telomere attrition with age spills over to other signaling pathways.

4 Age-dependent host environment and T cell signaling pathways

4.1 T cell homeostatic cytokines and autoimmunity in aging

Signaling cascades are initiated in response to exogenous stimuli, frequently cytokines produced in the host. These signals lead to transcriptional activation of multiple genes, including gene products that are involved in positive and more often negative feedback loops of signaling pathways. Alterations in the host environment and in particular the cytokine milieu with age are predicted to modulate signaling pathways and in particular adjust the signaling thresholds at which productive activations occur. Failing T cell homeostasis and increased production of inflammatory cytokines are two factors typical for the aging host [50–52]. T cell homeostasis fundamentally changes with age with the generation of new T cells in the thymus declining and eventually completely ceasing [1]. To avoid lymphopenia, homeostatic proliferation needs to rise. Increased apoptosis

susceptibility with age may accelerate this need, and indeed increased T cell turnover is seen in nonhuman primates and humans after a certain age in the memory and also in the naïve T cell compartment [53, 54]. T cell proliferation is driven by various homeostatic cytokines which signal through STAT3 or STAT5 and, to a varying degree, also activate PI3K [55, 56]. We have shown that exposure to homeostatic cytokines sensitizes the TCR signaling machinery, presumably through PI3K-mediated activation of positive feedback mechanisms in the ERK pathway [57]. The TCR signaling threshold is lowered to an extent that T cell responses to autoantigens are enabled. This mechanism may explain in part the frequent production of autoantibodies and the increased predisposition to develop several autoimmune diseases in the elderly. Indeed, lymphopenia is a risk factor for autoimmunity in several animal models due to the activity of homeostatic cytokines [58, 59]. IL-7, as well as IL-21, has been shown to confer this effect [59]. Both of them signal through PI3K in addition to STAT3 (IL-21) or STAT5 (IL-7) and sensitize TCR activation thresholds in vitro [57]. The model of lymphopenia-associated autoimmunity holds for human disease, as best documented by the immune reconstitution inflammatory syndrome [IRIS] where the reconstitution of a functional T cell compartment upon institution of highly active antiretroviral therapy [HAART] in HIV-infected patients is associated with a systemic inflammatory disease [60]. If present in the elderly, such a mechanism must be temporarily restricted or active only in a subpopulation of individuals, because the predominant pattern in T cells from elderly adults is a dampening of the TCR-induced ERK activation (see above).

4.2 Effect of TNF- α on T cell responses

The aging host environment is characterized by the presence of inflammatory mediators independent of acute or chronic disease [52]. Even in the healthy elderly, serum IL-6 and tumor necrosis factor are twofold to fourfold higher than in young adults [61, 62]. Production of inflammatory cytokines is driven by several mechanisms including innate immune activation due to senescence of the adaptive immune system and less effective control of infections and due to degenerative tissue injury [63]. Defective epithelial barrier function, as well as decline in the mucosa-associated lymphoid tissue, may result in a lack of containment of bacterial products as has been most convincingly shown for patients with previous HIV infection [64]. Remodeling of the adaptive immune system with accumulation of effector T cells also favors an inflammatory response [65]. Given its prominent role in autoimmune diseases, the effect of chronic TNF- α exposure has been best studied. TNF impairs TCR signaling through several mechanisms. It lowers expression of the TCR/CD3 complex, in part due to selective targeting of the TCR ζ chain [66, 67]. TNF attenuates tyrosine phosphorylation of the protein tyrosine kinase ZAP-70, the transmembrane adaptor protein LAT and PLC γ , and Ca²⁺ mobilization. N-acetylcysteine, which replenishes intracellular glutathione, completely restores TCR responses of cells treated with TNF- α suggesting that TNF- α acts through the production of reactive oxygen intermediates [68]. Chronic TNF- α exposure is also the most potent downregulator of CD28 expression thereby affecting costimulation, IL-2 production and T cell proliferation [69]. These effects were mostly studied in autoimmune disease where TNF concentrations exceed the levels seen with healthy aging. Most of these effects were proportionally related to TNF concentrations, and it can therefore be expected that, depending on its local concentrations, TNF- α contributes to signal abnormalities in the elderly.

4.3 JAK-STAT pathways and aging

IL-6, one more cytokine consistently elevated in the elderly, signals through STAT3 and several other STAT molecules. One of the common effects of many STAT-dependent cytokines is that they induce the transcription of negative regulators, suppressors of cytokine signaling (SOCS) which interfere with STAT phosphorylation proximal at the level the

cytokine receptor [70, 71]. SOCS3 transcription is activated by IL-6 among other cytokines [15], interferons predominantly induce SOCS1 and SOCS2 [72]. One would therefore predict that in an inflammatory cytokine environment, the activation threshold of the corresponding STAT-dependent receptor is attenuated. Indeed, Fulop and colleagues first described reduced STAT3 and STAT5 phosphorylation by Western blotting [73]. Recently, Longo and colleagues examined 21 signaling responses evoked by 11 different stimuli in monocytes, B cells and T cell subsets using PhosFlow [74]. Initial results in a training set of 30 healthy individuals of different ages were tested in a second test set. Age-associated signaling nodes mostly involved CD45RA CD8 T cells, possibly reflecting the accumulation of end-differentiated effector CD8 T cells that may have a different receptor profile. A decline in STAT5 phosphorylation after type I interferon and IL-27 and STAT6 after IL-4 stimulation reached significance in CD8 CD45RA T cells. Due to the sample size, the sensitivity of the study was low and several other STAT pathways showed a trend for a decline, in particular cytokine-induced STAT1 and STAT3 phosphorylation. Unmodulated STAT1 phosphorylation showed a trend to increase with age consistent with the interpretation that constitutive activation of the pathway is present which may dampen the subsequent response. Similarly, a study by Shen-Orr and colleagues using PhosFlow analysis of various STAT phosphorylation in response to seven different cytokines concluded that a subset of elderly individuals can be identified where defects in cytokine signaling are common, in particular for CD4 and CD8 T cells. The findings in their study that many of these cells had increased baseline levels of STAT phosphorylation are consistent with the idea that the defects are adaptations to a cytokine environment [75]. However, correlative studies with serum cytokine levels have not been published and may in fact be difficult to interpret, given the STAT promiscuity of some of the cytokine receptors (e.g. for type I interferons or IL-6). Moreover, adaptations may not be the only mechanism accounting for the dampening. Using a system analysis approach incorporating additional readout systems, Shen-Orr postulated a cell-intrinsic common mechanism for defective cytokine responses [75]. In support of such a hypothesis, we found that defects in type I interferon-induced STAT1 and STAT5 signaling in elderly CD4 T cells is acquired during T cell activation and is not mediated by an increased expression of SOCS1 or SOCS3 (own unpublished data).

5 Age-dependent gene expression of molecules involved in signaling

Gene expression studies designed to identify the molecular basis for immunosenescence generally yielded cell surface receptors and cytoplasmic molecules involved in signaling processes topping the analysis (Figure 2). Even before the era of gene arrays, loss of CD28 and CD27 and gain of several negative regulatory receptors including killer cell lectin-like receptor subfamily G (KLRG) and killer immunoglobulin-like receptors (KIR) were noted as hallmarks for cellular aging, in particular for CD8 T cells [76–81]. Fann and colleagues originally compared CD8⁺ CD28⁺ and CD28[−] T cells and found only a limited number of genes differentially expressed, most predominantly several NK cell surface receptors and molecules associated with effector cell function [82]. Cao and colleagues examined the effect of age on global gene expression in CD8 T cells and observed that *Cd8*, *Cd27* and *Cd28* were downregulated, while upregulated genes again included several NK cell-related regulatory receptors, such as *Cd244*, *Cd96*, *Klrf1* and *CD94* [83]. To separately account for aging and T cell differentiation, Lazuardi and colleagues compared gene expression in CD28⁺ and CD28[−] CD8⁺ cells from young and elderly individuals [84]. Analysis showed three clusters based on gene expression profiles: young CD28⁺ cells, old CD28⁺ cells and CD28[−] cells from both young and old donors, which clustered together. This study also documented an increase in NK cell-associated KIR and killer cell lectin-like receptor (KLR) gene expression in CD8⁺ CD28[−] T cells. In our own studies, we found expression of the negative regulatory receptor ILT2 (CD85j) to be even more frequent than KIR and KLR

[85]. Transcriptional control mechanisms of KIR and ILT2 expression were very different suggesting that gene expression is not the result of a shared pathway activated with aging [86, 87]. Obviously, T cell terminal differentiation during aging undertakes tremendous efforts to bias signaling pathways from positive (CD27, CD28) to negative inputs (KIR, KLR, ILT).

Inhibitory receptors of the KIR, KLR and ILT families have been best study in the context of NK cell activation [88, 89]. They contain cytoplasmic immunoreceptor tyrosine-based inhibition motifs (ITIMs), which initiate inhibitory signals following their phosphorylation by providing a docking site for tyrosine phosphatases. When NK cells contact susceptible target cells, both activating and inhibitory receptors are recruited to the cSMAC [90–92]. Whether NK cells execute cytolytic function on target cells depends on the ratio of activating and inhibitory signaling in the immune synapse [93–96]. If inhibitory signals dominate, they abrogate actin cytoskeleton rearrangement, reduce granule exocytosis, and prevent the cytolytic activity of NK cells [97, 98]. Rather than spreading over the cell membrane, inhibitory signaling is confined to the immune synapse in contact with a susceptible target cell [99]. In initial published studies these receptors had a similar coinhibitory function in T cells as in NK cells [100]. Such a result is to be expected if T cell activation is studied after in vitro cross-linking of inhibitory and stimulatory receptors. However, it is currently less clear whether and how these negative regulatory receptors cluster with TCR and stimulatory receptors and how their ITIMs are phosphorylated in the physiological setting of T cell activation. In several studies of superantigen or antigenic peptide recognition, functionality of the inhibitory receptors has been reported consistent with the paradigm derived from their function in NK cells [101–103]. However, it is difficult to envision a model where effector T cells accumulate with age, but are equipped with receptors negating their activity. There are also observations that these receptors do not simply paralyze TCR-mediated T cell activation. KLRG1 preferentially inhibits AKT activation and therefore T cell proliferation [104]. KIR2DL2, upon superantigen stimulation of KIR2DL2⁺ CD4⁺ T cell clones, inhibited IFN- γ production and proliferation, but not the cytotoxicity suggesting that negative regulatory receptors act to dissociate effector function [105]. The mechanism underlying this dissociation may be a difference in the recruitment kinetics of cell surface receptors to the TCR activation platform. Also, for many viral antigens such as CMV, the effect of CD85j or KIRs on CMV-specific CD8⁺ T cells seems to be limited, since cytolytic function and cytokine production of KIR⁺ or CD85j⁺ CMV peptide-specific T cells are normal upon antigen-specific stimulation (own unpublished observation). One possible explanation is that the inhibitory function is overcome by high signaling strength and therefore is involved in repertoire selection [106]. Alternatively, negative regulatory receptors may not co-aggregate with TCRs but modify other signaling events, e.g., cytokine-mediated stimulation, and prevent memory inflation without effector cell function [107, 108]. In summary, effector cell differentiation and immune aging upregulate the expression of receptors that have the potential to recruit phosphatases and attenuate tyrosine and serine phosphorylation (Figure 2). How these receptors act in time and space needs further studies, and it is therefore currently unclear whether the expression is detrimental for immune competence or beneficial in maintaining homeostasis.

The gain of negative regulatory receptors is reminiscent of T cell exhaustion as seen in chronic active viral infections. However, exhausted, senescent and aged T cells have different expression patterns of inhibitory receptors [109]. Whether the inhibitory receptors that are important in T cell exhaustion also play role in T cell senescence is not well characterized, and data so far are mostly limited to the murine species. In these studies, the expression of PD-1, CTLA-4, LAG-3 and TIM-3 all increase with age, which at least partly reflects the increased frequency of memory T cells with age [110–112]. In contrast, end-differentiated effector T cells in the human elderly are not truly exhausted in their ability to

respond to stimuli as discussed above. Moreover, the expression of PD-1 is inversely correlated with the expression of age-related inhibitory receptors [113], and exhausted T cells do not express KLRG1 or age-associated KIRs [114].

In addition to surface-expressed regulatory molecules, gene array studies of CD8 T cells in the elderly identified also several changes in the expression of downstream signaling molecules [83]. The MAP kinase pathways were disproportionately affected, in particular through the upregulated expression of the dual-specificity phosphatase genes *Dusp2, 4, 5, 6* and *10*. DUSP5 and DUSP6 are regulated through miR-181a and constitutively expressed as discussed above. In contrast, DUSP4 is an inducible nuclear phosphatase which is upregulated after TCR activation and dephosphorylates ERK and JNK. Expression peaks several days after stimulation suggesting that it is primarily involved in signal processing controlling T cell proliferation and differentiation. Indeed, forced overexpression has been shown to mimic cellular senescence. In our studies of age-related defects in CD4 T memory cell responses, we found that activation-induced expression of DUSP4 was increased and more sustained in the elderly [115]. Sustained ERK and JNK activities are known to control and to bias the acquisition of effector functions. We were able to show that increased DUSP4 expression impaired the differentiation into helper cells that regulate the induction of antibody production [115]. When B cells from young adults were cocultured with activated CD4 memory cells from unrelated young or elderly healthy individuals, B cell differentiation into plasmablasts was severely impaired with elderly CD4 memory T cells. This defect could be completely compensated when the expression of DUSP4 in the T cell population was silenced. One possible mechanism is that DUSP4, through its ability to curtail nuclear ERK and JNK phosphorylation, reduces the ability to transcribe CD40L, ICOS and IL-4 when activated CD4 T cells are restimulated by antigen-presenting B cells, thereby leading to compromised T helper function. Interestingly, the increased inducibility of DUSP4 expression may again be related to the altered internal cellular milieu (Figure 2). DUSP4 transcription is controlled through the AMPK-Egr1 pathway [116]. AMPK is an important sensor of the energy state through AMP levels regulating its activation [117–119]. Increased activation-induced transcription of DUSP4 may therefore reflect an unfavorable cellular energy state and increased AMPK activity.

6 Conclusions

There is increasing evidence to implicate altered signaling pathways in the immune defects seen with increasing age. In a recent study of in vitro replicative senescence of T cells, alterations in cell surface marker expression and TCR-induced signaling were highly predictive for CD8 T cell age. Rivet and colleagues used a microfluid chip analysis to quantify 25 static biomarkers and 48 dynamic signaling measurements [120]. A combination of Lck and ERK phosphorylation and cell surface expression of CD28 and CD27 was highly accurate in predicting the number of cell doublings, which the authors used as a surrogate marker for T cell age. Moreover, in a multivariate analysis, signaling measurements alone was only mildly less predictive for T cell age, due to their correlation with the expression of cell surface markers. These findings underscore the close relationship of immune aging and signaling pathways and attests to their functional importance. The last years have seen a development from the encyclopedic listing of signaling defects in the elderly to a conceptual progress in interpreting them in the context of cellular differentiation and/or senescence as well as the environment in the aging individual. Obviously, alterations in signaling thresholds and in signal propagation and amplification can be beneficial adaptations as well as functional decline. They may provide an explanation for the observation that the elderly is prone to autoimmunity as well as to infections. A contextual understanding is necessary to target the defects in translational medicine, e.g. to improve vaccine responses.

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Abbreviations

AKT	Protein kinase B
AMPK	AMP-activated protein kinase
ATM	Ataxia telangiectasia mutated
ATR	Ataxia telangiectasia-related
DNA-PKcs	Catalytic subunit of DNA-dependent protein kinase
DSB	Double strand break
DUSP	Dual-specific phosphatase
HAART	Highly active antiretroviral therapy
IRIS	Immune reconstitution inflammatory syndrome
ITIM	Immunoreceptor tyrosine-based inhibition motif
KIR	Killer cell immunoglobulin-like receptor
KLR	Killer cell lectin-like receptor
KLRG	Killer cell lectin-like receptor subfamily G
mTOR	Mammalian target of rapamycin
ROS	Reactive oxygen species
SOCS	Suppressors of cytokine signaling
TCR	T cell receptor

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Highlights

- Mechanisms of TCR threshold calibration in T cell differentiation and aging are shared.
- The cytokine milieu in the aging host attenuates signaling cascades.
- The aging T cell is biased towards negative regulatory pathways.
- Negative regulatory receptors (ILT2, KIRs and KLRG1) interfere with pro-proliferative signals.
- Increased expression of dual-specific phosphatases attenuates MAP kinase pathways.

Figure 1.

TCR signaling pathways in T cell differentiation and T cell aging. The schematic diagram illustrates alterations in the MAP kinase pathways that occur with T cell differentiation and aging and that are at least in part shared. ERK phosphorylation is counterbalanced by DUSP6 which is regulated by miR-181a; miR-181a expression declines with T cell differentiation as well as aging. Also with T cell differentiation, TCR signaling shifts from preferential ERK to p38 phosphorylation through to different choices in scaffolding molecules. Whether this shift also occurs by T cell aging has still to be determined. These signaling events are modified by mitochondrial ROS release, which is known to be influenced by aging in various cellular systems, but has been less studied in aging T cells. Upon activation, aged CD4 memory T cells have an increased expression of DUSP4 which controls nuclear ERK and JNK phosphorylation and is regulated through the activity of the energy sensor AMPK

Young CD8 T cells

Elderly CD8 T cells

Figure 2.

Age-related alterations in receptors and signaling molecules of CD8 T cells. Immune aging is associated with the accumulation of end-differentiated effector T cells that exhibit striking differences in the expression of signaling receptors. CD28 and CD27 expression is lost, expression of several ITIM- and ITSM-containing negative regulatory receptors is gained. Several JAK-STAT pathways are subdued, in part through the loss of the relevant cytokine receptor and in part due to increased constitutive activation of the pathway, possibly as a result of increased cytokine concentration and the corresponding increase in SOCS expression.