CHAPTER 21

Immune Receptor Signaling, Aging and Autoimmunity

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Abstract

Aging is associated with a myriad of changes including alterations in glucose metabolism, brain function, hormonal regulation, muscle homeostasis and the immune system. Aged individuals, generally still defined as over 65 years old, differ from middle-aged or young donors in many features of the immune system. The major observation is that the elderly population is not able to cope with infections as well as younger adults and recovery generally takes longer. Moreover, some diseases first appear with advancing age and are likely associated with dysfunction of the immune system. Thus, Alzheimer's disease, atherosclerosis, type II diabetes and some autoimmune disorders are linked to changes in immune function. One major immune cell population implicated as being responsible for the initiation and chronicity of immune dysfunction leading to diseases or immunosuppression is the T-cell. Although many changes in B-cell and innate immune function in aging are associated with the appearance of disease, they are not as well studied and clearly demarcated as changes in the T-cell compartment. The adaptive immune system is coordinated by T-cells, the activation of which is required for the initiation, maintenance and termination of responses against pathogens. Changes in the expression and functions of the T-cell receptor (TCR) for antigen and its co-receptors are closely associated with immunosenescence. Certain similar changes have also been found in some other disease states, e.g., rheumatoid arthritis, systemic lupus erythematosus and cancer. In this chapter, we will summarize our knowledge about multichain immune recognition receptor signaling, mainly the TCR, in aging and autoimmune diseases.

Introduction

The percentage of individuals over 65 years old in the world is increasing, not only in developed countries but also to some extent in developing countries.[1] This phenomenon is largely due to improved medication and health care along with decreased malnutrition and death caused by common pathogens such as influenza.[2] However, the elderly population is particularly targeted for vaccination against pneumonia and influenza, because of the lower efficiency of their immune system and their difficulty to cope with infections.[3] A better understanding of aging and age-related diseases affecting the immune system is very important to keep these elderly individuals in the best of health. Public health services also benefit from a reduction in the high costs of maintaining institutionalized ill elderly people. Understanding physiological aging as well as age-related diseases

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Multichain Immune Recognition Receptor Signaling: From Spatiotemporal Organization to Human Disease, edited by Alexander B. Sigalov.

would help to respond better to their specific requirements and to improve the quality of life for longer periods.[4] This is still challenging but several studies have demonstrated significant changes in immune system functions of elderly individuals when compared to their younger counterparts.[5] In the first part of this chapter, we will review which cells exhibit functional changes in aging and then consider receptor signaling in aging and autoimmune diseases such as rheumatoid arthritis (RA) and systemic lupus erythematosus (SLE). Finally, we will discuss possible interventions to modulate multichain immune recognition receptor (MIRR) signaling in order to restore normal immune function.

Immunosenescence

Overall, immune response dysregulation can be termed immunosenescence.[6] This phenomenon is very difficult to explain because it is multifactorial and may have different clinical consequences depending on the individual's health status and immunological history. Several causes for this age-related phenomenon have been put forward without explaining it entirely. There is the old paradigm, certainly still important, that age-related immune deficiency occurs with thymic involution.[7] This is based on the idea that T-cells are lost with time from the periphery but the thymus becomes less able to replace them, resulting in decreased numbers of naïve cells exported. There is a great deal of evidence for decreased, sometimes catastrophically decreased, naïve cells in the elderly.[8] Thymic atrophy is also thought to cause T-cell repertoire shrinkage that renders immunological protection incomplete, explaining the difficulty that elderly individuals encounter in overcoming infection, especially with pathogens that they have not previously encountered. More recently, longitudinal studies have associated immunosenescence with the accumulation of anergic T-cells, mainly CD8+ T-cells specific for antigens from cytomegalovirus (CMV).[9] These can represent >20% of the whole peripheral blood CD8 repertoire. Thus, aging is associated not only with changes in lymphocyte subsets but also with functional changes within these subsets. We will review this briefly now.

There is a consensus that T-cell functions are altered following TCR ligation. The main critical failure is the decrease in the production of interleukin-2 (IL-2) and consequently a reduced proliferative capacity even of the non-anergic cells.[10] This could help to explain decreased immune responses after antigen recognition. The most common changes demonstrated in T-cell functions and properties in aging are shown in Table 1. Several changes in surface marker expression occur in aging. Most consistently, the nonpolymorphic coreceptor CD28 is decreased leading to an increased number of CD28-negative cells, mostly in the CD8 compartment.[11] Mitogen-induced IL-2 production is severely impaired concomitantly with decreased IL-2 receptor (IL-2R) expression and proliferation.[12] Not only is the intensity of the response changed but also the type of response. Thus, there may be a shift towards Th2 responses with aging.[13] However, changes in T-cells with aging differ within the different T-cell subsets. Some reports have suggested differential susceptibility to apoptosis in CD8+ and CD4+ T-cells in aging.[14] Changes have also been demonstrated in membrane fluidity, DNA damage and telomere length.[15] All these changes lead to the impaired T-cell response with aging.

Elderly individuals may have a normal B-cell count and mount a good humoral response although low B-cell numbers have been described as part of the original immune risk phenotype (a cluster of immune parameters predicting mortality in longitudinal studies of a very elderly population.[9] However, the antibodies produced are commonly of low affinity, providing a less powerful response compared to young individuals.[16] B-cell lymphopoiesis is also reduced, which leads to an increase in the percentage of antigen-experienced cells when compared to newly-produced naïve B-cells.[17] This is analogous to the situation with T-cells described above.

Natural killer T(NKT)-cell cytotoxicity as well as interferon-gamma (IFN-γ) production decreases in aging. However the functionality of NKT-cells in aging is still controversial.[18] The percentage of CD3+Valpha24+ NKT-cells in peripheral blood from elderly donors was found to decrease and the majority of these cells are CD28-positive.[19] However the percentage of Valpha24+ NKT-cells is increased in the CD8+ compartment.

Table 1. Most significant changes in T-cell properties in aging

Decreased	*Increased*
Proliferation with mitogens	CD8+CD28- cells
IL-2 production	CD95 expression
Telomere length	CD45RO+ cells
Telomerase activity	DNA damage
Th1 response: IL-2, IFN-γ	IL-6, TNF-α secretion
Delayed-type hypersensitivity	Th2 response: IL-4, IL-5, IL-10, IL-12
TCR signal transduction	CD4+ T-cell apoptosis
Nuclear factor transcription activity	Anergic CMV-specific CD8+ T-cells
IL-2 receptor expression	
Membrane fluidity	
DNA repair	
CD8+ T-cell apoptosis	
Naïve CD4+ cells	
T-cell repertoire	
CD45RA+ cells	

While adaptive immunity has been clearly shown to be defective in aging, the role of cells from the innate immune system in age-related dysfunction is still a matter for debate. Nevertheless, we can state that the function of macrophages and neutrophils is impaired regarding Toll-like receptor function and expression.[20,21] It remains likely that delayed recovery in elderly individuals can also be caused by defects in innate immunity.

Receptor Signaling in Immunosenescence

Why T-cell functions are decreased in the elderly is still under debate. Defects in T-cell activation or subsequent thereto could be explained by extrinsic or intrinsic factors. It is known that the elderly often manifest a state of low-grade inflammation although this may be the case only for (the majority of) not perfectly healthy individuals, which is reflected by an increase in circulating pro-inflammatory cytokines (e.g., IL-6).[22]

It seems that the number of TCRs on the cell surface is not changed during aging. We will not discuss TCR assembly and signaling in detail here because the previous chapters of the present book cover specifically this area. In Table 2 we depict the TCR signaling alterations in aging in summary. The first step in TCR-mediated signaling is the activation of different tyrosine kinases, leading to the tyrosine phosphorylation of several downstream molecules. The level of tyrosine phosphorylation of p59fyn and ZAP-70 kinases is impaired in T-cells from old mice activated through the TCR/CD3 complex.[23] In human T-cells, an age-related defect is observed in tyrosine-specific protein phosphorylation after activation via TCR–CD3 complexes. In addition, a p59fyn and p56lck activity was recently shown to substantially decrease in T-cells of healthy elderly subjects.[24]

It is now well-documented that with aging other early events related to protein tyrosine phosphorylation following TCR activation are altered, such as the generation of myo-inositol 1,4,5-trisphosphate, intracellular free calcium mobilization and protein kinase C (PKC) translocation to the membrane.[25] It was shown that defects in translocation of PKC following TCR stimulation are present in T-cells of old humans and mice. Data are accumulating showing that more distal events, such as in the ras-mitogen activated protein kinase (MAPK) pathways, are

Table 2. TCR signaling alterations in aging

Intracellular free Ca^{2+}	Lck activation
Myo-inositol 1,4,5-trisphosphate production	ITAM phosphorylation
Protein kinase C translocation	Linker of activated T-cells activation
CD69 expression	Fyn activation
CD25 expression	ZAP-70 activation
Membrane fluidity	Extracellular signal-regulated kinase activation
Cholesterol content	p38 activation
Raft-associated proteins	Proteasome activity
NF-AT distribution	NF-AT translocation
Regulation of cellular cholesterol	NF-κB relocalization
Raft coalescence	

also changed with aging. Whisler et al have shown that elderly subjects had a reduction in MAPK activation.[26] Because MAPK activation is correlated with IL-2 production, it is possible that the impaired signaling may represent the rate-limiting step for IL-2 production.

There is increasing experimental evidence that an appropriate balance between tyrosine kinase and phosphatase activities is essential for the regulation of cellular activation.[27] CD45 is a receptor-like protein tyrosine phosphatase expressed on all haematopoietic cells. It is a positive regulator of Src tyrosine kinases such as Lck by dephosphorylating their negative regulatory C-terminal residue.[28] Other phosphatases are expressed by T-cells but only a few studies tested the hypothesis that phosphatase dysregulation could be responsible for immunosenescence. Changes in the activity/localisation of transcription factors are the direct consequences of any impairment in the signaling cascade. As described above, calcium mobilization is deficient in T-cells from aged donors and the well-known decrease of NF-AT translocation to the nucleus is the direct consequence of this deficiency.[29] The other important transcription factor for IL-2 production is NF-kB, which is constitutively expressed in the cytoplasm and bound to an inhibitory protein, IkB, prior to activation. The decrease in NF-kB activation in mice and in humans is mainly due to a decreased inactivation of IkB by the proteasome.[30] Although there were differences in experimental groups in terms of age, experimental conditions or concentrations of stimuli in the different studies published, there is a general consensus that aging is associated with impairments in the activation of the TCR signaling cascade. One important question still to be solved is the following. Why are so many steps of the TCR signaling cascade shown to be altered, despite apparently unchanged TCR expression in aging? One answer may be that in the earlier studies, the analyses of TCR signaling were not sufficiently sophisticated to reveal subtle changes. This is not to say that the studies previously published are incorrect but that our knowledge in the field of signaling has recently progressed tremendously with the discovery of membrane microdomains which are the initiators of receptor signaling.[31] These will be considered next.

The Role of Membrane Rafts in TCR Signaling: The Aging Rafts

The concept of a spatial organization of signaling molecules in specialized cholesterol- and glycosphingolipid-enriched microdomains called membrane rafts has been introduced recently, suggesting that they provide a platform for lymphocyte signaling.[32] TCR ligation induces a redistribution of phosphorylated proteins into membrane rafts, which are highly compact and relatively small domains (20 to 200 nM) composed of saturated lipids and signaling molecules.[33] The saturation of the lipids as well as the enrichment in cholesterol allows the rafts to move through

the membrane as discrete units. The role of membrane rafts is not limited to signal transduction but also to lipid transport, virus entry, cell movement, as well as cell-cell communication.[34] The accumulation or clustering of signaling molecules via membrane rafts initiates the formation of a signaling platform which increases the efficiency of signaling. The sustained T-cell activation via organised membrane raft signaling ultimately leads to the formation of a mature immune synapse needed to achieve full T-cell activation.[35] The organization and composition of the membrane will directly modulate the formation of such a signaling platform which ultimately influences cellular activation and functions.[36]

It is clear that there is an age-related alteration in the physico-chemical status of the plasma membrane of T-cells leading to decreased fluidity with in vivo aging as well as in in vitro models of senescence.[37] One should be attentive to the detrimental effects of such changes because membrane rafts and immune synapse formation are needed for the sustained activation of the cell. Because the activation of several steps of the signaling cascade is known to be impaired in aging, it is reasonable to test the hypothesis that downstream impairments are caused by upstream alterations. As the first events of the signaling cascade consist of movement of molecules recruited to or excluded from rafts, we will now review raft-associated changes noted in aging.

Although this paradigm has not been very extensively studied thus far, data are accumulating to support an important role for lipid rafts in age-related and diseases-related changes in signal transduction (reviewed by ref. 38). Simons et al were able to link membrane rafts with at least forty pathological cases and infections.[38] Miller et al were the first to demonstrate an alteration in several components of this signaling complex in naïve and memory T-cells in aging.[39] Because the latter accumulate with age, analyses of whole peripheral lymphocyte populations mostly reveal these changes. Several raft-associated or recruited proteins, such as the linker of activated T-cells (LAT), PKC and Vav fail to become activated in T-cells of aged mice. Moreover, LAT phosphorylation and redistribution to the T-cell-antigen-presenting cell (APC) immune synapse was impaired following TCR ligation.[39]

The cause of the increased rigidity of the membrane with aging is not known, but there are several possible explanations, of which the cholesterol hypothesis has been most thoroughly tested. Membrane rafts were originally found to float in fractions enriched in cholesterol and glycosphingolipids following centrifugation of Triton X-100-containing lysates. We have analysed the enrichment of cholesterol in membrane rafts of T-cells from young and elderly donors and found a 2-fold increase in the amount of cholesterol in membrane rafts in T-cells from the latter.[40] Such a change could have a detrimental effect on protein movement through the membrane and therefore protein-protein interactions. This idea was also tested and it was found that T-cells exposed to anti-CD3 monoclonal antibodies (mAb) or a combination of anti-CD3 and anti-CD28 mAb induced significantly decreased membrane raft coalescence in T-cells of elderly subjects.[41]

The decrease in CD28 expression in T-cells with aging is a well-known phenomenon which can explain the decrease in raft coalescence, because membrane rafts are reported to be dependent on CD28 ligation for proper functioning.[42] How, then, can we explain the observed impaired coalescence with anti-CD3 stimulation alone? The signaling deficiencies shown in aging are linked to the TCR signaling cascade which is independent of CD28 signal transduction. When molecules associated with pathways of TCR-mediated signal transduction were assessed for activation and localization into membrane rafts, we were able to show severe defects in Lck and LAT activation/localization in T-cells from the elderly following TCR ligation.[41] The age-associated alterations in the properties of membrane rafts include an increase in cholesterol content, impaired coalescence and selective differences in the recruitment of key proteins involved in TCR signaling. The localization of molecules through the membrane is dependent on posttranslational modifications including acylation, farnesylation and palmitoylation.[43] Recently, it was demonstrated that LAT phosphorylation was not optimal in antigen-primed anergic CD4+ T-cells after TCR ligation.[44] More interestingly, LAT association with membrane rafts was defective in these CD4+ T-cells and this was partly explained by the impaired palmitoylation of LAT in these cells. Thus, the anergic state could be a consequence of changes in posttranslational modification of proteins

which interact with membrane rafts. It is likely that anergic CD8+ cells are accumulated in aged humans. A large fraction of these cells are specific for viruses and become anergic for unknown reasons as a consequence of chronic antigenic stimulation, possibly resulting in exhaustion of their replicative potential (replicative senescence). Whether a change in the palmitoylation status of signaling molecules occurs during this process and provides useful information on possible interventions to modulate and restore cellular functions is not known but is under investigation in our group. It should be noted that TCR signaling alterations are different in different T-cell subsets, notably in CD4+ and CD8+ T-cells. Accumulating data show that CD4+ T-cells rely more on membrane rafts whereas CD8+ T-cells do not need such a process to signal properly.[45] This brings us to a discussion of raft heterogeneity in the immune system and its importance in the understanding of immunosenescence.

Heterogeneity in Membrane Rafts and T-Cell Subsets

Membrane rafts are not identical domains where the associated proteins are always the same. It is increasingly evident that a great deal of raft heterogeneity exists.[46] This became even clearer with the recent data from Douglass et al who demonstrated that signaling molecules are not restricted to one particular type of membrane raft.[47] Commonly, membrane rafts have been labelled and identified using the cholera toxin b subunit (CTxB) which targets the ganglioside M1, as a marker for membrane rafts. This study showed that signaling molecules such as LAT and Lck cocluster in domains of the membrane and that this was dependent on protein-protein interactions. This conclusion was based on the fact that Lck and LAT did not colocalize with CTxB fluorescence.[47] Now we know that membrane rafts are not restricted to those positive for this marker but other types such as GM3-rafts and flotillin-rafts also exist.[48-49] When CD4+ and CD8+ T-cells were analyzed for signaling molecule content in membrane rafts, we were able to demonstrate an age-associated decrease in LAT and Lck association and activation in the CD4+ subset while the CD8+ subset suffered less from aging in this respect.[45] There is a paradox here because it is the CD8+ not CD4+ compartment where CD28 expression is most markedly decreased with age and where anergic cells are more prominent. However, our study was limited to exploring molecules associated only with the GM1-rafts. It is possible that other raft domains are altered in the CD8+ compartment but these have simply not yet been investigated. Ongoing studies in this direction will help to better understand the role of membrane rafts in order to increase our knowledge of the spatiotemporal variables in TCR signaling in different T-cell subsets.

Protein-protein interactions direct signaling events but the lipid composition of the membrane facilitates protein movement from one raft to another to control protein-protein interactions. Recently, it was shown that inhibitory receptors such as the inhibitory killer immunoglobulin-like receptor 2DL2 (KIR2DL2) are excluded from membrane rafts and the immune synapse during the first steps of cellular activation but these receptors are recruited when cell functions needed to be down-regulated.[50] Our group has focussed on CMV-specific CD8+ cells which express the killer cell lectin-like receptor G1 (KLRG-1) and exhibit some dysfunctionalities. The expression of KLRG-1 and other important receptors of this type (CD161, KLRF-1 and NKG2A) and their localization in membrane rafts are under investigation. Results of these studies will help us to determine the role of such receptors in the inhibition of TCR signaling in aging.[51] Moreover, we have also shown that phosphatase activity in the membrane rafts of neutrophils could be responsible for age-related changes in susceptibility to apoptosis.[52] Although there is still little data, inhibitory signaling following TCR stimulation will need to be taken into account to explain changes in TCR signaling in aging.

MIRR Signaling and Autoimmunity

Rheumatoid Arthritis

Antibody production is a major step in the initiation and maintenance of autoimmune diseases. It has been shown that uncoupling of the B-cell antigen receptor (BCR) from calcineurin-dependent

signaling pathways prevents self-antigen stimulation, such as by CpG DNA and thus prevents proliferation.[53] Concomitantly, a continuous activation of the extracellular signal-regulated kinase by self-antigen hinders cellular differentiation. In RA, B-cell tolerance is broken and auto-antibodies are secreted. RA patients suffer from a defect in central and peripheral B-cell tolerance.[54] Samuels et al showed that half of RA patients display unexpected immunoglobulin light chain repertoires.[54] Receptor editing and the regulation of recombination may be impaired and defective in RA. Because of this defect, BCR signaling will escape from control and assist in autoimmunity.

It was recently shown that MAPK activation in T-cells results in matrix metalloproteinases production by osteoclasts.[55] T-cells play a central role in the development and maintenance of RA mainly due to cytokine production.[56] Hence, the changes in TCR signaling have critical effects on activation of transcription factors and cytokine production. Synovial T-cells have a particular functional phenotype (hypo-responsiveness to TCR stimulation) which can be explained by the microenvironment of the synovial joint.[57] Chronic exposure of T-cells to tumour necrosis factor alpha (TNF-α) leads to disruption of TCR/CD3 assembly in the membrane which results in severely reduced calcium influx response.[58] In parallel, it was shown that TNF-α also down-regulated CD28 expression.[59] The link between increased TNF-α levels and the maintenance of RA is well-known. Thus, a change in TCR signaling caused by a differential MIRR spatial organization and coreceptor expression could be responsible for the hypo-responsiveness to TCR ligation in RA. This is not restricted to RA because elderly individuals commonly possess increased circulating TNF-α levels which can be seen as part of the "Inflam-Aging" process, a low-grade inflammation primarily caused by cytokines and oxidative stress.[60] Although there are some significant changes in TCR signal transduction, RA is mostly associated with changed B-cell functions as well as extrinsic changes including the cytokine environment. This might explain why changes in TCR signaling are mainly found in synovial T-cells.

Systemic Lupus Erythematosus

Recent studies have revealed an immunological disorder mainly in lymphocytes of SLE patients. SLE T-cells show an altered CD4:CD8 ratio, which is due to a decreased proportion of CD4+ T-cells and a concomitant increase in the proportion of CD8+ T-cells.[61] This parallels the inverted CD4:CD8 ratio which is a hallmark of the "immune risk phenotype" (IRP) predicting mortality in the very elderly.[62] T lymphocytes from SLE patients present abnormalities in TCR signaling which may also be to some extent similar to those found in the elderly. Abnormal expression of key signaling molecules and defective functions of T lymphocytes play a significant role in the pathogenesis of SLE. It is well-accepted that the expression of TCR zeta chain is defective in the majority of SLE patients.[63] However, TCR-mediated stimulation of SLE T-cells shows over-phosphorylation and different calcium response patterns when compared to healthy individuals but the outcome is the decreased IL-2 production. This phenomenon has been recently explained by Juang et al who demonstrated that cAMP response element modulator binds to the IL-2 promoter and suppresses IL-2 production.[64] While it is not known why the zeta chain is down-regulated in SLE, there are several possibilities to explain the increased activation state of SLE T-cells that leads to their dysfunction upon stimulation. Of these, the altered structure of the receptors, the modulation of membrane clustering, the altered association of signaling molecules to membrane rafts or impaired inhibitory signaling may be important. For example, SLE T-cells have been shown to form greater amounts of GM1-rafts which are remarkably similar to those observed in T-cells of elderly donors. The alterations in the membrane raft signaling machinery represent an important mechanism that is responsible for the basal hyper-activated state of T-cells in SLE.[65] Jury et al were the first to describe a role for membrane rafts to explain the changes in SLE T-cell responses.[66] This study clearly showed that although there is a decrease in overall Lck expression at the cellular level, there is an increased activation of Lck in membrane rafts which explains the basal activation of SLE T-cells. This in turn leads to the inability to produce IL-2 upon stimulation to the same level as T-cells from control groups.[66] There is a correlation between aging and SLE since we previously described an increased basal phosphorylation of Lck in membrane rafts in T-cells from elderly

donors which interferes with the proper signaling and IL-2 production.[41] Above, we discussed the putative role of phosphatases and inhibitory molecules in the changes of TCR signaling in aging. It was demonstrated that in SLE, CD45 is over-associated to membrane rafts which can explain changes of Lck activity by dephosphorylating Tyr505.[66] The localization of CD45 is important for its activity. Immunosenescence and SLE are very different phenomena. The first is a normal process with slow changes that results from immune remodelling over the lifespan, while the latter displays an earlier and more intense dysfunction of the immune system. However, despite this difference, it may be possible to increase our understanding of each by studying the other. The changes in TCR signaling in aging, RA and SLE, are described in Figure 1. Some investigators hypothesize that autoimmune diseases are premature amplifications of certain changes which occur more slowly during "normal" aging,[67] such as defects of T-cell selection, receptor functioning and apoptosis resistance. This notion can easily be extended to include changes in membrane properties and MIRR signaling. The modulation of such properties is of great interest for putative interventions to achieve improved immune functions or to control autoimmune diseases.

Therapeutic Strategies Targeting the MIRR Signaling

In order to influence cellular activation, the modulation of membrane rafts would be of importance not only for the TCR but all MIRRs. Using methyl β-cyclodextrin, a cholesterol-extracting molecule, it is possible to inhibit TCR signaling by modulating membrane raft properties. The activation of Lck and LAT and their association with membrane rafts are inhibited by methyl β-cyclodextrin.[40] It is of note that cyclodextrins are commonly used as vehicles for many drugs because they are functional excipients that increases drug solubility.[68] Some anti-fungal creams (Itraconazole),[69] hepatitis C drugs (PG301029),[70] anti-inflammatory drugs (Meloxicam®),[71] and many others[72] contain cyclodextrins. The use of cyclodextrins as vehicles can be valuable when T-cell function is specifically targeted. However, cyclodextrins are nonspecific polymers and one should take this into account for drug delivery as well as for side effects.

Protein-protein interactions as well as signaling molecule localization depend on posttranslational modifications of the proteins. The posttranslational modification of amino acids extends the range of functions of the protein by attaching other functional groups such as acetate, phosphate, lipids and carbohydrates. Palmitoylation, myristoylation, farnesylation and prenylation are very important for protein localization and interaction with adjacent molecules.[73] Hundt et al recently showed that LAT palmitoylation was defective in anergized CD4+ T-cells.[44] This can explain its altered association with membrane rafts and the central supra-molecular activation cluster (c-SMAC) of the immune synapse. The control of such a process could benefit cells which are hyper- or hypo-responsive. There is already some encouraging progress in this direction. For example, Garcia et al were able to reverse the age-related decrease in murine CD4+ T-cells using O-sialoglycoprotein endopeptidase.[74] The expression of activation markers such as CD25 and CD69, was restored using this approach.

MIRR assembly and signaling depend on the physico-chemical properties of the membrane. It has been shown that modulating the lipid composition of the extracellular milieu may lead to significant changes in membrane composition that ultimately result in perturbation of cellular functions.[75] We have seen previously that LAT can be displaced from membrane domains by changing its palmitoylation status. Stulnig et al have shown that the addition of polyunsaturated fatty acids to T-cell cultures in vitro leads to modifications of membrane rafts,[76,77] in particular, displacement of LAT.[76] This has direct effects on TCR signaling.[77] Therefore, we have here a real possibility to modulate T-cell and B-cell immune functions via their receptors by nutritional supplementation or by changes in food intake. Using this model, in our own study, healthy young donors were supplemented intravenously for 2 hours with a mixture of lipids (Intralipid 20%) which contains mainly palmitic, oleic and linoleic acids.[78] Blood samples were collected before and after injection and T-cells were isolated for further analysis. This study demonstrated that increases in lipid plasma levels have a direct effect on T-cell functions including signaling following TCR stimulation, IL-2 production and cell proliferation. This is of particular interest when we consider that this lipid

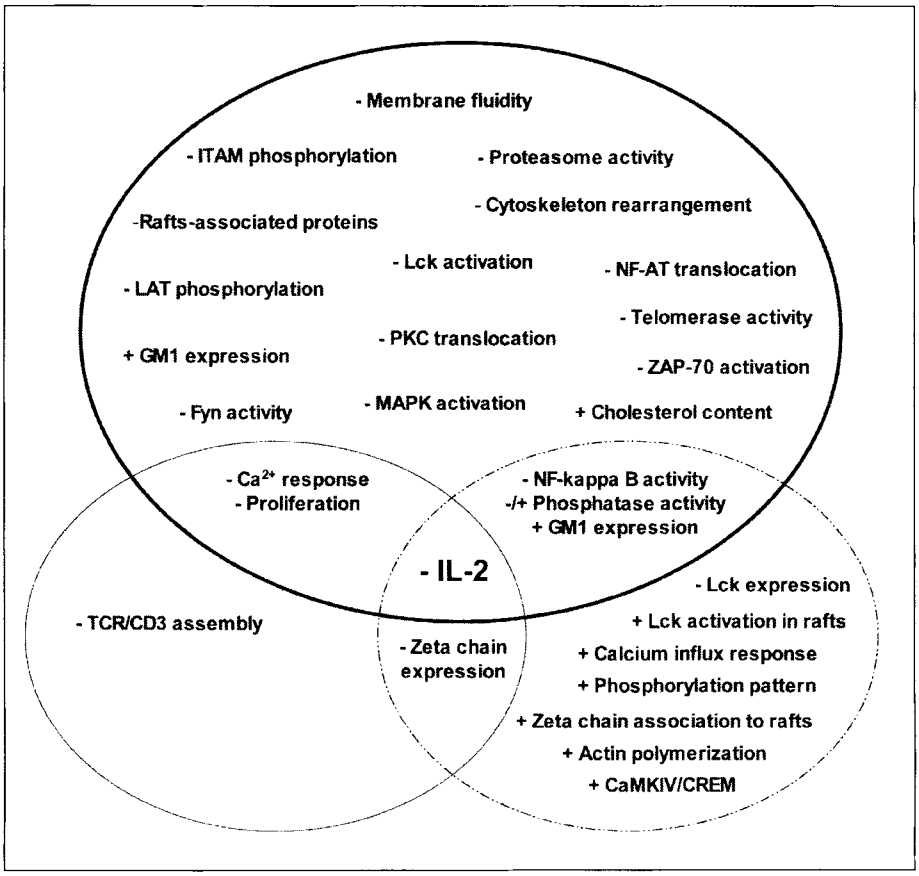

Figure 1. Comparative changes in TCR signaling in aging vs RA and SLE. The main changes in TCR signaling are depicted for T-cells from elderly donors (bold line), RA patients (continuous line) and SLE patients (discontinuous line). RA T-cells from the synovial joint mostly display resistance to activation. Peripheral T-cells from RA patients do not exhibit such changes. Decrease in the activation of molecules involved in TCR signal transduction is the hallmark of T-cells from elderly donors. SLE T-cells exhibit a very different pattern which consists of increased early signal transduction such as calcium influx and protein tyrosine phosphorylation but the end result is still a reduced capacity to produce IL-2. This is explained by CREM binding to the IL-2 promoter. Quantitatively, RA T-cells exhibit minor changes when compared to aging T-cells and SLE T-cells. The role of cytokines in autoimmune diseases is predominant, which explains why less attention has been paid to TCR signaling. Nevertheless, this pro-inflammatory environment will certainly influence TCR signaling. Increase in signaling events is represented by (+) while a decrease is represented by (-). Common changes are also mentioned. PKC, protein kinase C; ITAM, immunoreceptor tyrosine-based activation motif; LAT, linker of activated T-cells; MAPK, mitogen-activated protein kinase; GM1, gangliosides M1; CREM, cyclic adenosine monophosphate response element modulator; CaMKIV, Ca^{2+}/Calmodulin-dependent Protein Kinases IV.

supplementation is often given to hospitalized patients. One should reconsider the balance between beneficial and side effects of this supplementation in the case of immuno-depressed patients.[79] It is known that elderly individuals have a very different nutritional intake than young people.[80] Increased lifespan is due to better health services, vaccination and a better quality of life which

includes food intake. Nevertheless, this can be improved even more because elderly individuals often have disturbed eating patterns which may not provide optimal nutrition.

Aging is associated with an increase in the number of anergic virus-specific CD8+ T-cells. Patients with certain autoimmune diseases or cancer may also display an increase in anergic and hypo-responsive T-cells. The possible causes of such phenomena have been described in this chapter focussing on the role of defects in TCR signaling. The accumulation of these anergic cells may influence the functioning of the other cells in several ways (e.g., competing for antigen, competing for cytokines, secreting suppressive cytokines, etc). Using tetramer technology, it is possible to detect antigen-specific cells and in combination with functional tests such as IFN-γ production[81] to assess which cells are responding and which belong to the anergic population. It may be also possible to selectively deplete the anergic population and thereby reconstitute immune function, however, it is not an easy task to perform on humans as yet.

Concerning other viral infections, it is worth mentioning that HIV, which enters T-cells via the TCR and TCR coreceptors such as CD4 causes a shift in phospholipid synthesis to neutral lipids and also causes polyunsaturated fatty acid peroxidation and deregulation of cytokine production.[82] We have the example here of a virus which is able to modulate the TCR environment in order to subvert cell proliferation according to its needs (viral replication), or for cell death initiation. How viruses can modulate TCR signaling is of great interest to open new windows for future strategies to modulate T-cell signaling and improve function in immuno-deficient individuals (elderly) as well as in pathological situations (auto-immune diseases, cancer).

Conclusion

TCR signaling changes with age as well as in autoimmunity and there are some resemblances between these two phenomena. The most important point is that protein-protein interactions and T-cell activation in an elderly population are very different from those in young healthy individuals and it helps to explain the changes in cellular function. Membrane rafting is critical for the assembly of the signaling platform for the TCR and BCR (and also for other receptors that we did not discuss here). The final outcome of protein rafting is the formation of the immunological synapse which is needed for sustained activation resulting in a complete immune response. We can document changes in molecular events with age and autoimmunity, but we are not yet able to explain these changes. Understanding the events that lead to changes in the TCR signaling cascade would be of great benefit considering the large number of diseases in which membrane raft dysfunction is thought to play a role.

Acknowledgements

The authors' own work was supported by the Deutsche Forschungsgemeinschaft (SFB 685) and the European Commission (QLK6-CT-2002-02283, "T-CIA"). The authors were all supported by the European Commission (EU contract 6FP-CT-2003-506850, "ZINCAGE"). Tamas Fulop was supported by the Canadian Institute of Health Research (No 63149), the Research Center on Aging and the University of Sherbrooke.

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