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Review article

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TELOMERE SHORTENING AND AGEING OF THE IMMUNE SYSTEM

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Telomeres are protein-DNA complexes localized at the ends of linear chromosomes constituted by short, tandem G-rich hexanucleotide repeats and associated proteins. Their length shortens with each cell division and correlates inversely with age. It can be modified by genetic and epigenetic factors, sex hormones, reactive oxygen species and inflammatory reactions. A critical minimum length of telomeres triggers a cell cycle arrest or senescence of the cell. The immune system is highly sensitive to shortening of telomeres as its competence depends strictly on cell renewal and clonal expansion of T- and B-cell populations. Cells of the immune system are unique among normal somatic cells as they can up-regulate telomerase, the telomere extending enzyme, and limit telomere attrition in the process of cell proliferation undergoing in activated cells. Telomere length is highly variable among humans. Lineage-specific telomere shortening with different kinetics of telomere attrition was observed in CD4+, CD8+ T lymphocytes, B lymphocytes, granulocytes, monocytes and NK cell population. Immunosenescence is characterized by a special remodeling of the immune system induced by antigen exposure and oxidative stress. In ageing immune system adaptive immunity deteriorates because of a progressive decline of naive T and B cells and decrease of absolute numbers of T and B lymphocytes. The innate compartment of the immune system is relatively well preserved although some age-dependent alterations can be also observed. Nonagenarians or centenarians represent phenomenon of successful ageing of the immune system as most of their immune parameters are well preserved.

Key words: *telomeres, telomerase, ageing; immunosenescence, adaptive immunity, innate immunity*

INTRODUCTION

Telomeres are protein-DNA complexes localized at the ends of linear chromosomes constituted by short, tandem G-rich hexanucleotide repeats and

associated proteins. In vertebrates telomeres consist of $(TTAGGG)_n$ sequences and proteins classified into single- and double-strand DNA telomere binding proteins. TRF1 and TRF2 proteins bind specifically to double-stranded DNA and POT1 binds to a single-stranded G-rich telomeric sequence (1, 2). These three DNA-binding proteins form a six-subunit telomere-specific complex termed "shelterin" or "telosome", which includes also three proteins that do not interact directly with telomeric DNA: TIN2, TPP1 and RAP1 (3). The telomeric DNA and accompanied proteins maintain a "capped" structure called also a "functional telomere" and protect the end of the chromosome from illegitimate recombination and fusion with another telomere or DNA end (4). As the ends of chromosomes could be recognized by cellular repair systems as double-strand DNA breaks, telomeric complexes (mainly TRF2 and POT1 proteins of the 'telosome') protect them from DNA repair factors (5). Since DNA-dependent DNA polymerases fail to replicate linear chromosome ends completely, the other function of telomeres is to protect DNA ends from degradation because of end replication problem (6). DNA replication is a semiconservative process and requires a 5' primer for the initiation of DNA synthesis. This requirement prevents complete replication of the 3' end of chromosomes. The incompletely replicated, single-strand DNA is unstable and consequently 50-200 bp of telomeric DNA is lost with each cell division (7). Telomere shortening accompanying cell division was shown to occur both *in vitro* and *in vivo* with age reflecting the cumulative effect of cell divisions. This phenomenon was demonstrated in human fibroblasts (7), hematopoietic stem cells (8), leukocytes (9), keratinocytes (10), epithelial (11) and endothelial cells (12). Telomere length appeared to serve both as a biological indicator of the replication history of the cell and as a determining factor for the replicative capacity of normal somatic cells (13). In contrast, germline and malignant cells do not undergo telomere shortening with every cell division as they express some compensatory mechanisms to overcome telomere loss. One of these mechanisms described to produce telomere elongation is mediated by telomerase, a ribonuclear protein enzyme that can synthesize *de novo* telomere G-rich strands using its own telomere sequence-specific RNA template (14). Telomerase catalytic activity requires the association of two subunits: RNA (TER) and telomerase reverse transcriptase (TERT) (15). hTR (human TER) provides the template for repeat synthesis and is constitutively expressed in human cells. On the contrary, most somatic cells repress hTERT (human TERT) expression at the transcriptional level (16). Most somatic cells in adult organisms do not express telomerase and this enzyme is active only in germ cells, during embryogenesis, in adult stem cells and in activated immune cells, suggesting that its activity is tightly regulated during development and differentiation (17). Although telomere length results from a dynamic balance between elongation and shortening of the chromosome ends (18), mean telomere length within cells is a direct function of the level of telomerase activity (19).

Telomere length and telomerase activity do not always tightly correlate in somatic cells. The example are lymphocytes, that can express telomerase after

activation. The role of telomerase in activated cells is to stabilize telomeres in the process of DNA replication and cell division (20). No tight correlation between telomere length and telomerase activity is observed also in ageing process which in immune system results from the senescence of hematopoietic stem cells (21). It was also observed that some transformed cell lines do not exhibit telomere shortening despite the absence of detectable telomerase activity (22). Subsequent studies revealed the presence of Alternative Lenghtening of Telomeres (ALT) mechanisms that compensate lack of telomerase activity in the cell or complement its activity (23). Although majority of human tumor cells stabilize their telomeres through activation of telomerase, 10-15% of them utilize ALT mechanisms that relies on the homologous DNA recombination machinery. Telomeres composed of repetitive DNA sequences have many sites of homology necessary for homologous recombination within the same chromosome or different chromosomes. It was found that ALT was more prevalent in tumors arising from tissues of mesenchymal origin suggesting the existence of cell type specific mechanisms favouring the activation of ALT (24).

In humans telomeres are relatively short as they extend in a limited range of 10-20 kb, dependent on tissue type (19). *In vitro* studies revealed that with every cell division 40-200 bp are lost until they reach a critical length of 4-5 kb (Hayflick's limit) (25), although this level varies between cell types and individuals (26). Telomeres are normally in a "capped" state which normally cannot be recognized by DNA damage response and repair mechanisms (27). However, telomere shortening is believed to destabilize telomeric loops and increase telomere uncapping (28). The rate of telomere erosion per cell division appears to be independent from telomere length (29). It was suggested that the shortest telomeres could serve as sentinel telomeres that preferentially arrest cell cycle before the other telomeres become critically short. This mechanism could protect chromosomes from illegitimate rearrangements that may arise during intensive proliferation (30). Moreover, it was shown that the presence of very short telomeres and not the average telomere length is critical for cell viability (31). Therefore occurrence of very short, uncapped telomeres triggers either cellular senescence or apoptosis (32).

REGULATION OF THE LENGTH TELOMERE IN AGEING PROCESS

Genetic factors

Although mean telomere length can differ significantly among individuals of the same age, numerous studies suggest that telomere length is a heritable factor despite revealing the high degree of inter-individual variation (33). In these studies telomere length was measured in whole blood white blood cells (WBC) isolated from monozygotic and dizygotic twin pairs. It was found that monozygotic twins had very similar mean telomere length whereas dizygotic twins differed significantly in this respect (9). Some significant correlations

between paternal telomere length and offspring telomere length were also observed (34). In marker linkage studies a few loci with significant influence on human telomere lengths were identified (35). Multi-generational analysis and comparison of parent/child pairs suggested X-linked inheritance of telomere length (36). Analysis of 400 microsatellite markers in over 250 sibling pairs revealed significant linkeage to chromosome 12 and identified a candidate gene for that region, DDX11, a DNA helicase (37). Single nucleotide polymorphisms (SNPs) regarded to be the most common germline genetic variants in the human genome, were also thought to play a role in telomere length regulation (33). It was described that levels of telomerase activity in peripheral lymphocytes after stimulation were under genetic control (38) and differences in telomere lengths found in WBCs were linked to polymorphisms in the hTERT promoter (39), however, not in all populations studied (40). Moreover, it appeared that telomere length was tightly regulated at the level of telomerase activity (41). Inherited mechanisms that control telomerase expression may contribute to differences in mean telomere length between individuals of the same age. Additionally, chromosome-specific telomere length may be regulated by subtelomeric factors, such as proteins (42) and sequence repeats (43). Polymorphism of genes (or their promoters) coding for components of the telomerase complex, such as DKC1 (44) or for proteins of the shelterin complex, such as TRF1, POT1 and TPP1 (45) may also have an impact on telomere homeostasis.

Another type of genetically determined control of telomere length is maintained at the level of single chromosomal ends as individual telomeres have various lengths within cells (46). Specific telomere lengths are associated with particular chromosomes in humans and some chromosome-specific factors regulate the number of TTAGGG repeats in individual telomeres (47). Further length variation is found between alleles and these intra-allelic differences may reach 6.5 kb (48). These variations are determined in the zygote and strictly maintained during life suggesting that environmental effects are of minor contribution (49). Furthermore, allele-specific relative telomere lengths are transmitted from parents to offspring and preserved in the next generation (50).

Epigenetic factors

Apart from genetic factors telomeres are also under epigenetic control (51, 52). Telomerase activity is influenced by cis-acting subtelomeric genetic elements that determine the epigenetic status of individual telomeres (19). Both elements of the chromatin: DNA and histones are subjected to a variety of post-translational modifications to regulate accessibility of DNA to various proteins. Mammalian telomeres do not contain CpG sequences and are thus not methylated but the subtelomeric regions can be methylated (53). However, both mammalian telomeres and subtelomeres contain nucleosomes and can be subjected to many types of histone modifications associated with heterochromatin such as

acetylation, methylation, phosphorylation, ADP-ribosylation and ubiquitination (54). Changes in either histone modification at the telomeres or DNA methylation at subtelomeres are associated with telomere length deregulation (24). Studies on murine embryonic stem cells revealed that cells lacking one or two out of three methyltransferases: DNMT1 or both DNMT3a and DNMT3b had distinctly longer and more heterogenous telomeres compared to wild-type cells (52). Other studies on primary cells derived from mice lacking the two histone methyltransferases: Suv39h1 and Suv39h2 also presented dramatically elongated and heterogenous telomeres in the analyzed cells compared to the wild-type cells (51). Progressive telomere shortening leads to epigenetic changes both at the telomeres and subtelomeric regions. Studies on telomerase-deficient mice with short telomeres demonstrated decreased levels of histone methylation both at telomeric and subtelomeric region, increased acetylation of histones H3 and H4 and decreased levels of DNA methylation at their subtelomeres. These results indicate that length of telomeres is important for their continued maintenance in a compacted heterochromatic state (55). Moreover, epigenetic modifications of telomeres can reduce accessibility of telomerase to telomeres and activate ALT mechanisms as mice lacking histone methyltransferases revealed elongated, heterogeneous in length telomeres, characteristic for ALT pathway (24).

Since epigenetic modifications of subtelomeric regions contribute to telomere length and to the function of a single cell, a phenomenon of telomere position effect (TPE), primarily discovered in yeast but then described also in humans (56), has to be mentioned. In general TPE is defined as the reversible silencing of genes near telomeres. TPE is induced by epigenetic chromatin modifications converting euchromatin into repressive heterochromatin (57). Telomere length contributes to TPE because longer telomeres improve the positional effect. TPE depends on the amount of silencing factors which can be recruited to telomeres. About 50 proteins contribute to telomere position effect, including Sir-complex proteins (58), Ku heterodimer components (59) and C-terminal domain of Rap1p (60). Telomere length influences the epigenetic status of subtelomeric chromatin as telomere attrition induces decondensation of telomeric and subtelomeric heterochromatin (55). Studies on the role of TPE in senescence of human fibroblasts cultured *in vitro* for an extended period of time revealed that telomere shortening contributed to gene expression of telomeric genes through the local alteration of chromatin structure (61). Although the loss of TPE may not be a basic cause of the cellular senescence, it can be responsible for the age-associated changes of gene expression. Further studies are needed to elucidate the function of genes which expression can be regulated by TPE (57).

Sex hormones in the control of telomere length

Hormonal regulation of telomere length is the best described for estrogen which can influence the attrition of telomeres by diverse mechanisms. One of them is

based on the protection of DNA from ROS induced damage (62). It was suggested that estrogens and their derivatives exert an antioxidant effect by scavenging free radicals, inhibiting free radical formation and stimulating enzymes engaged in radical detoxification (63-65). They can also stimulate telomerase activity as an estrogen response element is present in a promoter of the catalytic subunit of the enzyme (66). The activity of telomerase can also be enhanced by posttranscriptional modification of telomerase reverse transcriptase subunit by Akt protein kinase, a downstream mediator of phosphoinositol 3-kinase (67). Estrogens have been shown to stimulate the phosphoinositol 3-kinase/Akt pathway (68). Studies performed on vascular endothelial cells revealed that estrogens increased nitric oxide production which stimulated telomerase activity in these cells (69).

Findings of equivalent telomere length in newborn boys and girls (70) and occurrence of longer telomeres in women than men strongly support links between estrogens and telomere biology (71). Moreover, studies of postmenopausal women who had been on estrogen and progesterone therapy for more than 5 years revealed that long-term hormone therapy may decrease the process of telomere attrition (72).

Environmental stress and inflammation link telomere shortening with oxidative stress

This group of factors includes intrinsic and extrinsic factors acting both at the level of the whole organism and the level of a singular cell. It was show that environmental factors such as high level of stress, smoking, low socio-economical status, obesity, cardiovascular diseases and ageing manifestations like bone demineralization correlate with shorter length of telomeres (73-76). It is widely accepted that at the cellular level these various environmental factors result in an increased oxidative stress which is regarded to be a major contributor to telomere shortening. Oxidative stress can induce different types of DNA damage like oxidation of bases (77) or single- and double-strand breaks (SSBs and DSBs, respectively) (78). Acute stress generates DSBs at higher frequency and may modify the broken DNA ends to make them more resistant to repair, leading to DSBs persistence and inducing of senescence *via* non-telomeric DNA damage response. In consequence telomere-independent stress-induced senescence is triggered. Uncapped telomeres that result from replicative ageing are the other reason for cellular senescence (telomere dependent). It is noteworthy to emphasize that telomere-dependent replicative senescence is not completely stress independent. Dysfunctional mitochondria characteristic for the process of ageing are suggested to form a link between stress-dependent and telomere-dependent physiological ageing of the cell (78). At the end of cell replicative lifespan mitochondria become the major source of oxygen-free radicals in cells because of their metabolic inefficiency and increased ROS generation (79, 80). Telomeric DNA is a preferential target for oxidative damage as GGG repeats are highly sensitive to oxygen species which

generate 8oxoG lesions, the main substrate of the Base Excision Repair (BER) pathway (81). Modulation of the BER pathway may be caused by diet, inflammation or neoplastic transformation (82). Telomeres are suggested to be cellular sensors of mitochondrial function as mitochondrial dysfunction with increased ROS generation leads to accelerated telomere shortening, uncapping of telomeres, activation of DNA repair system and finally cell cycle arrest (78).

Since chronic oxidative stress plays a major role in the pathophysiology of several chronic inflammatory diseases, it was hypothesized that telomere attrition results also from inflammation and exposure to infectious agents. Observations performed on a group of chronic kidney disease patients also revealed that inflammation was associated with telomere attrition. Moreover, age and male gender seemed to be important contributors to the reduced telomere length in these patients (83). It was also shown that level of C-reactive protein (CRP), the plasma concentration of which increases with inflammation (84), was inversely correlated with telomere length in leukocytes of premenopausal women (85).

As today humans live longer, the immune system has to remain active for much a longer time. This extended activity leads often to a chronic inflammation, a characteristic feature of ageing process considered as the major risk factor for age-related chronic diseases. This chronic inflammation often results in the elderly from a persistent infection with *Herpesviridae,* particularly Cytomegalovirus (CMV) (86). Prevalence of CMV infection in the elderly is really high and may reach up to 90% of population in some countries (87). Human immune system evolved to control pathogens and was programmed evolutionarily to react strongly against any infections. The pro-inflammatory genotypes, however, in the process of ageing are responsible for overproduction of inflammatory cytokines that might cause immune-related inflammatory diseases and are supposed to be related to unsuccessful ageing. On the contrary, low responder genotypes engaged in the control of inflammatory status can provide a better chance of successful ageing in an environment with reduced pathogen load and better medical care (88).

Telomeres and telomerase in adaptive immunity: B and T lymphocytes

The adaptive immune response depends on function of T and B lymphocytes. T cells can be divided into CD4+ (“helper”) and CD8+ (cytotoxic) T cells. The function of CD4+ T cells is to stimulate CD8+ T lymphocytes to kill target cells and B cells to produce antibodies. The function of CD8+ cells is to kill cells infected with intracellular pathogens such as viruses or transformed into cancer cells. B cells are responsible for production of pathogen-recognizing antibodies. Both CD4+ and CD8+ T lymphocytes derive from bone marrow progenitors that migrate to the thymus where they differentiate. CD4+ and CD8+ T cells which have yet not had contact with foreign antigens are called naive T lymphocytes and circulate in peripheral blood. Upon contact with antigen naive T cells are activated, expand and become effector cells. After clearance of the antigen, most

of these effective cells undergo apoptosis and only some survive to become long-lived memory T lymphocytes (89).

B cells derive from bone marrow progenitor cells and undergo maturation in the bone marrow. Mature but antigen-naive B cells differentiate into GC B cells in germinal centers (GC) of lymphoid nodules present in peripheral lymphoid organs and then undergo further differentiation into antibody-producing plasma cells or memory B cells (89).

It has been estimated that during a typical immune response, from a naive cell to a million of activated clones of effector cells, 15-20 cell divisions take place (89). Subsequent cell divisions result in telomere shortening and a process of replicative senescence. For the first time shorter telomeres in memory CD4+ T cells compared to naive CD4+ T lymphocytes were described by Weng *et al*. (90) and shorter telomeres in memory CD8+ T cells compared to naive CD8+ T lymphocytes were described by Rufer *et al*. (91). These findings firmly established that normal differentiation of T lymphocytes from naive to memory T cells results in telomere length attrition.

Similarly to T cells, B cells also exhibit telomere shortening with age but at a slower rate than in T cells (92). Studies of B cells showed that in contrast to T cells there was no significant difference between telomere length of naive and memory B cells and no decrease of telomere length during the transition from naive to memory B cells (93, 94).

However, as the clonal expansion is very important for lymphocyte function, lymphocytes employ some mechanisms for telomere maintenance during proliferation. The main mechanism responsible for the stabilization of telomere length in a cell is stimulation of telomerase activity. This strategy is employed by T cells since it was shown that telomerase activity is highly regulated during T cell development and differentiation (95). Similarly to T cells, telomerase is not expressed in resting B cells but is rapidly activated after antigenic stimulation (93). Telomerase activity in different populations of T and B lymphocytes is presented in *Table 1.*

General characteristics of B and T lymphocyte immunosenescence

The most characteristic feature of immunosenescence is deterioration of T-cell compartment because of thymic involution which starts at the puberty and is almost completed by the end of the sixth decade of human life. The progressive, age-dependent decrease in circulating lymphocytes also occurs (97). This reduction in the lymphocyte number is accompanied by a remodeling of circulating lymphocyte subsets and decrease in the absolute number of T lymphocytes and B lymphocytes (97). Humoral response in ageing process also declines because of alteration in immunoglobulin generation and reduced affinity of the produced antibodies (98, 99). Characteristic features of ageing T and B lymphocytes are presented in *Table 2.*

Telomeres and telomerase activity in innate immunity: granulocytes, monocytes, mast cells and NK cells

The innate defense mechanisms including chemotaxis, phagocytosis and natural cytotoxicity engage cells of both myeloid lineage (granulocytes, monocytes and mast cells) and lymphoid lineage (NK cells). Cells of innate immunity similarly to cells of adaptive immunity present telomere shortening in ageing process (*Table 3*). However, mature granulocytes, monocytes and mast

Table 1. Telomerase activity in T and B lymphocytes.

Cell type / population	Telomerase activity	Reference
T lymphocytes		
thymocytes	+++	96
resting CD4+ T lymphocytes (peripheral blood)	+ / -	92, 96
resting CD8+ T lymphocytes (peripheral blood)	+ / -	92, 96
activated CD4+ T lymphocytes	+++	92, 96
activated CD8+ T lymphocytes	+++	92
B lymphocytes		
naive B lymphocytes	+ / -	93
memory B lymphocytes	+ / -	93
GC B lymphocytes	+++	93
activated naive B lymphocytes	+++	93
activated memory B lymphocytes	+++	93

+ / - low or undetectable telomerase activity
+++ high telomerase activity
GC germinal center B lymphocytes

Table 2. General characteristics of T and B immunosenescence.

Cell type / characteristic	Reference
T lymphocytes	
↓ decrease in the number of T lymphocytes (CD3+)	97
↓ decrease in the number of helper / inducer T lymphocytes (CD4+)	97
↓ decrease in the number of suppressor / cytotoxic T lymphocytes (CD8+)	97
↓ decrease in the number of naive T lymphocytes (CD95-)	100
↑ increase in the number of activated T lymphocytes	97
↑ increase in the number of T lymphocytes with markers of NK activity	97
↑ increase in the number of CD28-CD4+ T lymphocytes	101
↑ increase in the number of CD28-CD8+ T lymphocytes	101
↑ increase of type 1 response CD8+ T lymphocytes	102
↑ increase of type 2 response CD8+ T lymphocytes	102
↑ upregulation of inflammatory response	103
B lymphocytes	
↓ decrease in the number of B lymphocytes	97
↓ downregulation of immunoglobulin generation (through class switch)	99
↓ reduced antibody affinity	98
↑ increased level of autoantibodies	98

cells do not undergo cell division, therefore telomere length in these cells reflects telomere attrition of myeloid progenitor cells (104). Similarly, telomerase is expressed in myeloid progenitor cells, however, not in mature granulocytes, monocytes and mast cells (104). NK cells similarly to the other cells of the ageing immune system reveal age-associated telomere loss (105). Moreover, mature NK cells ($CD56^{dim}CD16^{+}$ cells) reveal significantly shorter telomeres than immature ones ($CD56^{bright}CD16^{-}$ cells) (106). However, on the contrary to mature granulocytes, monocytes and mast cells, NK cells can undergo cell proliferation after antigen stimulation ($CD56^{bright}$ NK cell subset) (107) and some decreasing with age levels of telomerase activity were detected also in mature cells (105).

Immunosenescence in innate immunity

Currently immunosenescence is often defined as the imbalance between inflammatory and anti-inflammatory networks that results in the chronic pro-inflammatory status called inflammaging (109). Within this hypothesis, healthy ageing and longevity result not only from lower propensity to reveal inflammatory responses but also from efficient anti-inflammatory mechanisms, which in majority of seniors fail to fully neutralize chronic inflammatory processes (110). The major risk factor for age-related chronic diseases like Alzeimer's disease, atherosclerosis, diabetes, sarcopenia or cancer is a chronic inflammation state caused by antigenic burden and exposure to damaging agents (88). The progression of these diseases seems to be dependent on the genetic background of individuals suggesting that pro-inflammatory genotypes are related to unsuccessful ageing described also as frailty. Paradoxically, low innate immunity responder genotypes might better control inflammatory responses resulting in successful or healthy ageing (88). General characteristics of immunosenescence in cells of innate immunity is presented in *Table 4.*

Interestingly, studies on centenarians revealed increased number of granulocytes, increased phagocytic activity and increased cytokine production by granulocytes (111). These results differ from results obtained in less elderly seniors indicating that centenarians represent a specific group of seniors. Their

Table 3. Telomere shortening and telomerase activity in granulocytes, monocytes, mast cells and NK cells.

Cell type\ \characteristic	Telomere shortening	Cell proliferation after activation	Telomerase activity in mature cells	Telomerase activity after activation	Reference
granulocytes	+	-	-	-	104
monocytes	+	-	-	-	104
mast cells	+	-	-	-	104
NK cells	+	+	+/-	+	105, 108

+/- low to undetectable level

immune system appears to be rebuilt in a different way contributing to successful ageing.

Studies on NK cells in immunosenescence revealed contradictory results, especially in regard to NK cell number and NK cytotoxic activity. Most studies revealed increase of the absolute and relative number of NK circulating cells in aged people (112, 113) although some authors did not find age-related changes in the number of NK cells (114). NK cytotoxic activity has been reported to be unaffected (115), decreased (116) or increased (117) in elderly. These differences resulted probably from methodology and subject characteristics. Some investigators followed the strict criteria of the SENIEUR Protocol in the selection procedure of elderly subjects (118), others studied cells from apparently healthy individuals (119). Centenarians, however, presented well-preserved cytotoxic activity and increased number of circulating NK cells (120).

CONCLUSIONS

During immunosenescence adaptive immune response progressively deteriorates with age. The alterations concern both T and B cells and, respectively,

Table 4. General characteristics of immunosenescence in cells of innate immunity.

Cell type / characteristic	Reference
Neutrophils	
↓ decreased number of circulating cells	121
↓ decrease in phagocytic capacity	122
↓ decrease of superoxide production after activation	123
↓ decreased sensitivity to G-CSF	124
↓ decreased sensitivity to IFN-γ and growth hormone	125
↓ decreased expression level of CAM on the cell surface (ICAM-3)	121
↓ decreased number of cells expressing L-selectin on the cell surface	121
Monocytes	
↓ decreased number of circulating cells	121
↓ decreased release of reactive oxygen and nitrogen intermediates	126
↓ decreased cytotoxicity against tumour cells	126
↓ decreased IL-1 secretion	126
↓ decreased expression level of CAM on the cell surface (ICAM-3)	121
↓ decreased number of cells expressing L-selectin on the cell surface	121
NK cells	
↑ increased number of circulating cells	112, 113
↑ increase in the number of mature $CD56^{dim}$ subset	127
↓ decreased NK activity per cell	128
↓ decreased responsiveness to IFN-α	129
↓ decreased secretion of IFN-γ after stimulation with IL-2	130
↓ decreased proliferation after stimulation with IL-2	131

cellular and humoral response. Innate immunity is thought to be largely preserved although some age-dependent changes are also observed. Important immune parameters can be, however, well preserved in centenarians in the process of successful ageing. The main problem of immunosenescence concerns a chronic-proinflammatory status which results from an imbalance between inflammatory and anti-inflammatory networks. Inflammaging is defined to be age-dependent up-regulation of the inflammatory response and is a consequence of lifelong antigenic burden, very often chronic viral infections (CMV) and exposure to damaging agents. Inflammatory responses associate with different age-related diseases and finally contribute to unsuccessful ageing. Successful ageing is represented by nonagenarians and centenarians who often reveal well preserved immune parameters and exhibit better mechanisms controlling inflammatory responses.

As the immune system depends on the ability of lymphocytes to undergo cell divisions in response to antigenic challenge, telomere length may limit the number of divisions. Age-dependent decline of the total number of T and B lymphocytes and decrease of naive T and B cells result from stem cell ageing. Serial experiments on transplantation of haematopoietic stem cells (HSC) revealed that these cells undergo ageing process and their self-renewal capacity is not unlimited. Telomere shortening is one of the mechanisms that can limit the self-renewal of HSC. The others include oxidative and proliferative stress (132, 133). On the other hand, we can observe successful ageing with functional immune cells presenting significantly shorter telomeres compared to the young counterparts.

There are no direct studies on the contribution of telomere length to the function of particular cells of the immune system. Some indirect assumptions concerning this contribution in ageing process were made on the basis of functional analysis of different cells of the immune system and their telomere attrition. Some further studies on molecular level are necessary to explain telomere position effect (TPE) and its contribution to the function of a single cell. Although TPE is not considered to be a fundamental mechanism of the senescence, it could be responsible for some changes in gene expression associated with ageing. The biological function of genes differentially expressed in senescent cells should be then also elucidated to discover molecular mechanisms regulating the process of ageing.

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