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Corresponding Author: Beatrix Grubeck-Loebenstien, M.D.

Corresponding Author's Institution: Institute for Biomedical Aging Research, Austrian Academy of Sciences, Innsbruck, Austria

First Author: Christoph Arnold, M.D.

Order of Authors: Christoph Arnold, M.D.;juliane Wolf, M.Sc.;Stefan Brunner, DI;Dietmar Herndler-Brandstetter, PhD;Beatrix Grubeck-Loebenstien, M.D.

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**Gain and Loss of T cell subsets in old age –  
Age-related reshaping of the T cell repertoire**

Christoph Arnold<sup>#</sup>, Juliane Wolf<sup>#</sup>, Stefan Brunner<sup>#</sup>, Dietmar Herndler-Brandstetter and  
Beatrix Grubeck-Loebenstein\*

Immunology Division, Institute for Biomedical Aging Research, Austrian Academy of  
Sciences, Rennweg 10, 6020 Innsbruck, Austria

# Equal contributors

\*corresponding author: Beatrix Grubeck-Loebenstein, MD

E-Mail: [beatrix.grubeck-loebenstein@oeaw.ac.at](mailto:beatrix.grubeck-loebenstein@oeaw.ac.at)

Suggested running head: Changes in T cell subsets in old age

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**Abstract**

The immune system is affected by the aging process and undergoes significant age-related changes, termed immunosenescence. Different T cell subsets are affected by this process. Alterations within the bone marrow and thymus lead to a shift in the composition of the T cell repertoire from naïve to antigen-experienced T cells, thereby compromising the diversity of the T cell pool. Additional infection with latent pathogens such as Cytomegalovirus aggravates this process. In this review we focus on the major age-related changes that occur in the naïve and the antigen-experienced T cell population. We discuss the mechanisms responsible for the generation and maintenance of these subsets and how age-related changes can be delayed or prevented by clinical interventions.

**Key words:**

Immunosenescence, T cells, aging, human

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4 **Introduction**  
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6 Worldwide the mean life expectancy is increasing and thereby leading to dramatic  
7 demographic changes. To ensure longevity and healthy aging the maintenance of  
8 appropriate immunity is necessary. However, as individuals age numerous physiological  
9 functions are decreased and the immune system undergoes profound age-related  
10 changes, termed immunosenescence. Changes of the aging immune system are of  
11 particular importance as they contribute to a higher incidence and severity of infectious  
12 diseases, decreased efficacy of vaccinations and possibly autoimmunity and cancer [1-  
13 3]. Although immunosenescence affects many components of both, the innate as well as  
14 the adaptive immunity, the latter is more severely affected by aging. These changes are  
15 numerous and affect a wide range of cell types, ranging from hematopoietic stem cells  
16 and lymphoid progenitors to mature lymphocytes in secondary lymphoid organs and the  
17 periphery [4]. However one of the most prominent features of immunosenescence and  
18 therefore mainly associated with the decline of immunological responsiveness in elderly  
19 persons are changes in the composition of the T cell compartment. The most substantial  
20 age-related changes within the T cell compartment are a decrease in the number of  
21 antigen-inexperienced naïve T lymphocytes combined with an increase in antigen-  
22 experienced memory and effector T cells. The initial trigger responsible for the  
23 dysbalance within the composition of the T cell pool observed in elderly persons is the  
24 involution of the T cell maturation organ, the thymus gland. Along with a decrease in  
25 functional thymic mass with age and the consequent reduction in naïve T cell output  
26 comes the necessity of homeostatic forces to take more responsibility assuming survival  
27 and in keeping T cell numbers constant. For the regulation of the maintenance of the  
28 T cell compartment apoptosis is another key player since it controls the selection of the  
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T cell repertoire in the thymus, the deletion of self-reactive lymphocytes, the regulation of immunological memory and the deletion of effector T cells [5]. Further effects of aging on the immune system are telomere shortening, changes in T cell signaling, impaired DNA repair and antioxidant mechanisms, which may all contribute in regulating T cell survival and shaping of the repertoire. Additionally, pathogens themselves may accelerate age-related changes. One prominent example that has been extensively studied in the context of immunosenescence is the cytomegalovirus (CMV) [6].

In this review we describe the major age-related changes that occur in naïve versus antigen-experienced T cells. Specifically we review the literature on the origin and fate of these subsets. Finally we discuss modes of intervention potentially suitable to counteract deleterious changes.

## Naïve T cells

### *Age-related changes in the generation of naïve T cells*

All circulating blood cells of an individual including the mature lymphocytes originate from common pluripotent hematopoietic stem cells (HSCs), which are maintained in specialized niches within the bone marrow. Common T lymphoid progenitors leave the bone marrow in an immature state and migrate to the thymus, a central lymphoid organ, responsible for the development, selection and output of mature naïve T cells, referred to as recent thymic emigrants (RTE), into the periphery [7, 8]. One of the major changes in the aged immune system is a decline in the naïve T cell number. This has mainly two reasons: With age the function of HSCs decreases due to deficiencies in DNA damage repair [9] and the shortening of telomeres [10], leading to a reduced capacity to generate lymphoid progenitors. It has also been suggested that age-related changes of the stem cell niche, e.g. the decline of the overall amount of hematopoietic tissue, can contribute to the declined HSC function in elderly persons [11]. Secondly, the thymus is severely affected. At birth the thymus is fully developed, but its involution and the replacement of functional tissue by fat starts soon after birth, continues throughout life and is almost complete at the age of 50 [12-14]. Thus in young persons naïve T cells are continuously generated and regenerate the T cell pool to retain the capability of the adaptive immune system to respond to a variety of different pathogens.

As a result of thymic involution the output of peripheral naïve T cells is dramatically reduced (up to -80%) with age, which leads to a reduced ability of the host to respond to new antigens. Low naïve T cell numbers have been described in the periphery as well as lymphoid tissue [15, 16]. Recent striking evidence from young adults thymectomized

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4 as infants in the course of cardiac surgery who have decreased naïve T cell counts  
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6 further emphasizes the role of the thymus for the maintenance of the naïve T cell  
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8 repertoire [17, 18]. Similar results as in humans are reported in studies on mice. After  
9  
10 thymectomy the functionality of the already existing naïve CD4<sup>+</sup> T cells is decreased,  
11  
12 suggesting premature immune aging [19].  
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### 18 *Maintenance of naïve T cells in old age*

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21 Aging affects the naïve CD4<sup>+</sup> and CD8<sup>+</sup> T cell compartments in slightly different ways  
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23 [14, 20-22]. Although the diversity and number of the naïve CD4<sup>+</sup> T cell compartment is  
24  
25 maintained stable for a long time, a dramatic and sudden collapse of diversity occurs  
26  
27 after the age of 70, leading to a more restricted repertoire [23, 24]. Similar changes  
28  
29 occur earlier in life and more gradually in the naïve CD8<sup>+</sup> T cell compartment. In contrast  
30  
31 to naïve CD4<sup>+</sup> T cells, naïve CD8<sup>+</sup> T cells of aged humans seem to be more susceptible  
32  
33 to death receptor-mediated apoptosis, triggered by TNF- $\alpha$  or Fas [25, 26]. It is therefore  
34  
35 suggested that Fas and TNF- $\alpha$  mediated apoptosis might contribute to the gradual  
36  
37 disappearance of naïve, and also of memory, CD8<sup>+</sup> T cells. Generally the CD8<sup>+</sup> T cell  
38  
39 pool is more affected by age-related changes than the CD4<sup>+</sup> T cell pool. This suggests  
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41 that CD4<sup>+</sup> T cells may be more prone to respond to survival assuring mechanisms than  
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43 CD8<sup>+</sup> T cells.  
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51 The reduced thymic output of newly generated naïve T cells is compensated by several  
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53 mechanisms. Homeostatic proliferation has been identified to play a key role for the  
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55 maintenance and restoration of the size of the naïve T cell pool. Thus it has been shown  
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57 that IL-7 plays a essential role in controlling homeostatic proliferation of naïve CD4<sup>+</sup> and  
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59 CD8<sup>+</sup> T cells and supports the survival of naïve CD8<sup>+</sup> T cells [27]. For the survival of  
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4 naïve CD4<sup>+</sup> T cells IL-7 and IL-4 are both essential [28]. It has been proposed that IL-7  
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6 acts in conjunction with TCR signals from contact with self-MHC/peptide ligands, which  
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8 sustains the expression of anti-apoptotic molecules (e.g. Bcl-2). Naïve T cells are thus  
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10 kept alive in a resting state and have a long lifespan [29, 30]. However this extended  
11  
12 lifespan is associated with a prolonged exposure of naïve T cells to unfavorable  
13  
14 environmental factors, which cause DNA damage, which contribute to decreased  
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16 function in old age [31]. If the naïve T cell numbers drop below 4% of total T cells,  
17  
18 homeostatic proliferation increases exponentially. This accelerates telomere shorting  
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20 and may lead to a memory-like phenotype [32, 33]. Naïve T cell survival may differ  
21  
22 between subsets, as CD31<sup>+</sup> (PECAM-1) thymic-naïve T cells decline during aging, but  
23  
24 still display a polyclonal TCR repertoire, while central-naïve CD4<sup>+</sup> CD31<sup>-</sup> T cell numbers  
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26 remain constant during aging, but exhibit increased TCR-mediated signaling and a  
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28 dramatically restricted TCR repertoire [34].  
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### 38 *Age-related functional changes of naïve T cells*

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40 In humans naïve T cells are defined on the basis of their surface expression of CD45RA,  
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42 CD28, CD62L or CCR7 [22, 35]. This population undergoes functional alterations during  
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44 aging. For example CD45RA<sup>+</sup>CD28<sup>+</sup>CD8<sup>+</sup> T cells from elderly persons produce larger  
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46 amounts of the pro-inflammatory cytokine IFN- $\gamma$  after their stimulation with OKT3 and IL-  
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48 2 than those cells of young persons [36]. They also have shorter telomeres and a highly  
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50 restricted TCR repertoire compared with a corresponding population from young  
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52 persons, suggesting increased homeostatic proliferation [37]. The loss of TCR diversity  
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54 in the naïve T cell compartment has also been demonstrated in aged mice [38]. Studies  
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56 in mice have also demonstrated that aged mice accumulate various intrinsic defects of  
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4 naïve T cells related to TCR-mediated signaling, IL-2 production and generation of long-  
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6 term memory cells [39]. Thus low IL-2 production leads for instance to reduced  
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8 expansion and thereby to inefficient generation of effector T cells. This age-related  
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10 defect can be reversed by the addition of exogenous IL-2 [39-41]. Additionally it has  
11  
12 been shown that naïve CD4<sup>+</sup> T cells do not form immunologic synapses upon  
13  
14 stimulation with peptide antigen and antigen presenting cells [42]. This may be due to an  
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16 altered cholesterol/phospholipid ratio in the lymphocyte membrane, leading to an  
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18 impaired TCR-dependent recruitment of signal molecules to the immunological  
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20 synapses [43, 44]. Impaired T cell surface glycosylation [45] and phosphorylation [46-48]  
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22 of key signaling molecules have also been suggested to contribute to age-associated  
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24 defects in TCR signaling. Age-related defects in naïve T cell activation, expansion and  
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26 differentiation may affect their cognate helper function to B cells and lead to reduced  
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28 humoral immune responses [49, 50].  
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### 38 *Naïve T cells in vitro - precautions*

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40 We and others have shown that the level of oxygen to which T cells are exposed is a  
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42 critical parameter. Normally cells in *ex vivo* and *in vitro* experiments are cultured in air  
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44 supplemented with 20% O<sub>2</sub>, but indeed the oxygen level in the *in vivo* environment of  
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46 T cells is lower than in the air and varies from 1 to 10 %, depending on the localization  
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48 of the T cells [51, 52]. Recent studies have shown that different oxygen levels can  
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50 influence the responsiveness of human T cells *in vitro*. *Ex vivo* T cell studies by Larbi et  
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52 al. [53] showed a decreased proliferation and higher susceptibility to apoptosis at low  
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54 oxygen levels following activation. These data could be confirmed by our group. We  
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56 demonstrated that CD3/CD28-stimulated naïve T cells from young and elderly persons  
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cultivated at 3% have a significantly increased rate of apoptosis (Figure 1A) and reduced proliferation (Figure 1B). It is therefore important to consider the oxygen levels in which naïve T cells are cultured *in vitro* when interpreting data from such experiments.

In conclusion naïve T cells display remarkable changes during aging, in number as well as in functionality.

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4 **Antigen-experienced T cells**  
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9 *Generation of memory T cells*  
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11 Immunological T cell memory is a key feature of the adaptive immune system in all  
12 vertebrates to ensure protection against previously encountered pathogens. Two models  
13 have been proposed for the generation of memory T cells following a primary infection.  
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15 A linear model which postulates a consecutive activation of a naïve T cell, its  
16 subsequent expansion into an effector population that can potently eliminate the  
17 pathogen and the development of T cell memory following a contraction phase [54]. The  
18 integration of various factors and environmental parameters, such as signal strength,  
19 costimulatory signals and the surrounding cytokine milieu determines the outcome of the  
20 differentiation of an effector to a memory T cell. In stark contrast, the model of the  
21 asymmetric T cell division favors a more practical approach and a division of labor [55].  
22 While a naïve T cell is primed by an antigen presenting cell, cytosolic and membrane  
23 components of the T cell shift and aggregate towards/away from the contact zone and  
24 remain throughout the first cell division. Unequal inheritance of those components to the  
25 progeny ensures the simultaneous generation of an effector cell, fully equipped with  
26 cytotoxic mediators at the proximal site of the immunological synapse, and a distal  
27 daughter cell that becomes the first memory T cell to that particular antigen.  
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53 *Age-related changes within the memory T cell compartment*  
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55 During the course of healthy aging the peripheral T cell compartment is populated by  
56 increasing numbers of memory T cells. This is due to the age-dependent decline in the  
57 output of naïve T cells (see above) and results in the filling of the resulting  
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4 immunological space with naïve and memory T cells by homeostatic means. In contrast  
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6 to naïve T cells memory T cells rely on IL-7 in concert with IL-15, cycle and self-renew *in*  
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8 *vivo* three- to fourfold faster than naïve T cells and are capable of vigorous proliferation  
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10 under lymphopenic conditions [56]. Additionally, homeostatic turnover of naïve CD8<sup>+</sup>  
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12 T cells may induce a memory-like phenotype [57, 58], thereby complicating the  
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14 quantitative analysis of naïve, antigen-inexperienced T cells in elderly persons [22, 59].  
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16 In addition to the decrease in naïve T cell numbers, antigenic stimulation by persistent  
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18 viral infections can challenge the tightly regulated orchestra of clonal expansion,  
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20 contraction and homeostasis of memory T cell and may thus lead to the massive  
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22 accumulation of clones of certain specificities [60, 61]. This culminates in a dramatically  
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24 reduced diversity of the memory T cell pool in elderly individuals [20, 24]. Similar to the  
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26 situation in naïve T cells, this age-related effect is more pronounced within the CD8<sup>+</sup>  
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28 T cell compartment [62]. It is also of interest that new T cell subsets appear in the aged  
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30 CD8<sup>+</sup> memory compartment, such as a population of CD25<sup>+</sup> T cells [59, 63]. These  
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32 memory T cells, which are neither regulatory, nor recently activated, produce IL-2 and  
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34 IL-4 and represent an early stage in the differentiation of CD8<sup>+</sup> T cells, with longer  
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36 telomeres (indicating a shorter replicative history) and a polyclonal TCR repertoire.  
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38 Elderly persons with a high frequency of CD8<sup>+</sup>CD25<sup>+</sup> memory T cells seem to have a  
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40 better functioning immune system as indicated by an intact humoral immune response  
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42 after influenza vaccination.  
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### 55 *Function and maintenance of memory T cells*

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57 Similar to naïve T cells, where it has been shown that naïve T cells from young mice  
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59 exhibit a better functional profile than naïve T cells from aged animals [40, 41], the  
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4 functionality of memory T cells strongly depends on the age of the host at the time the  
5 antigen is encountered. Studies in mice have shown that CD4<sup>+</sup> memory T cells  
6 generated during youth function well into old age, *in vivo* as well as *in vitro*, in terms of  
7 proliferation, cytokine production and cognate helper function, compared to memory  
8 T cells generated later in life [64]. Once generated memory T cells have different  
9 possible destinies: they can either survive as memory T cells, go into apoptosis or  
10 differentiate into effector T cells. The homeostatic maintenance of memory T cells  
11 throughout lifetime is tightly regulated and preserves T cell repertoire diversity to combat  
12 new pathogens as well as the host's ability to mount vigorous recall responses to  
13 recurrent infections [65]. Only recently have we begun to understand how and where  
14 memory T cells are maintained and sheltered in times of serenity. In this respect, the  
15 bone marrow and its mesenchymal stromal cells (MSCs) have been paid particular  
16 attention. Among other proteins, MSCs express proteoglycan ligands to CD44 which is  
17 present on memory T cells and mediates their local retention in the bone marrow. They  
18 furthermore produce IL-7 and IL-15 for the homeostatic maintenance of memory T cells  
19 [66]. It has been proposed that memory T cells, when in contact with stromal cells in the  
20 bone marrow, are suppressed and display reduced allogenic and mitogenic proliferation,  
21 a state of T cell anergy and reduced apoptosis as well as modulated cytokine production  
22 [66]. As for today, we only know little about the aged bone marrow and its role as  
23 survival niche for memory T cells. Recent data from our laboratory suggests that the  
24 bone marrow of elderly persons seems still intact as a frontline defense against  
25 recurrent infections (Herndler-Brandstetter *et al.*, in preparation). Further sites of  
26 residence include the gut and other mucosal surfaces, which are not well characterized  
27 in terms of age-related changes in the harboring potential of memory T cells. Further

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4 studies will have to shed light on how memory T cells are maintained throughout a  
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6 lifetime, with special respect to these sites.  
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### 10 11 *Generation of terminally differentiated T cells* 12

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14 Over the last decade, scientific evidence has accumulated that persistent viral infections  
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16 play a major role in driving the T cell compartment into exhaustion [6] with highest rates  
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18 of exhausted T cells observed in elderly persons. Persistent infection with HCV [67], HIV  
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20 [68-73] and CMV [20, 74] but not EBV or VZV have been shown to cause inflation of  
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22 exhausted T cells already early in life. Depending on the type of persistent viral infection,  
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24 T cells are repeatedly stimulated by viral antigens thereby contributing to the massive  
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26 accumulation of virus-specific CD4<sup>+</sup> and CD8<sup>+</sup> T cell clones in both, mice [75] and  
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28 humans [76, 77]. Although persistent CMV-infection is systemically controlled by the  
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30 immune system and viral particles are detectable only in times of reactivation, life-long  
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32 exposure to CMV has been demonstrated to severely impair the T cell system. It  
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34 increases the number of highly differentiated, exhausted CD4<sup>+</sup> and CD8<sup>+</sup> T cells [74, 78]  
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36 with an average of 10% – in the elderly up to 50% – of the overall T cell pool being  
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38 specific for CMV [77]. One of the most robust markers in describing these exhausted  
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40 T cells is the lack of the costimulatory molecule CD28, a member of the tumor necrosis  
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42 factor receptor (TNFR) family that interacts with CD80 and/or CD86 expressed on  
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44 activated antigen presenting cells. Along with an appropriate TCR/MHC engagement,  
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46 CD28 signaling provides the obligatory second stimulus to achieve full T cell activation  
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48 and differentiation. Recently, it has been shown that signaling via the CD28 receptor  
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50 overcomes the T cells auto-inhibitory pathway to sustain full T cell activation and IL-2  
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52 production [79]. The loss of CD28-mediated Akt (Ser473) signaling has also been  
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4 associated with decreased telomerase activity [80] further contributing to the exhaustion  
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6 of CD28<sup>-</sup> T cells. In general, the CD8<sup>+</sup> T cell compartment is more affected by the  
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8 accumulation of terminally differentiated T cells than the CD4<sup>+</sup> T cell compartment [81,  
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10 82]. Exceptions represent rheumatoid arthritis and inflammatory bowel diseases, where  
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12 the expansion of CD28<sup>-</sup> T cells is predominant in the CD4<sup>+</sup> T cell compartment [83, 84].  
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### 19 *Maintenance of terminally differentiated T cells*

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21 The persistence and accumulation of exhausted T cells is still a matter of debate. Some  
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23 reports using heavy glucose favor an extended lifespan rather than accelerated  
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25 proliferation [85]. Along this line other reports suggest a certain resistance to apoptosis  
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27 [25, 26, 86]. Contrariwise, different authors stress their susceptibility to apoptosis [87-89]  
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29 and are therefore in favor of a more continuous production model, either antigenic  
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31 derived or homeostatically.  
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### 38 *Functional changes in terminally differentiated T cells*

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40 The gene expression profile of CD8<sup>+</sup>CD28<sup>-</sup> T cells fundamentally differs from  
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42 CD8<sup>+</sup>CD28<sup>+</sup> T cells, both at the mRNA level as well as in microRNA usage, which in part  
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44 explains the differences observed in apoptosis and the modulation of the activation  
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46 threshold [90-95]. The loss of CD28 is associated with a change of cellular function in  
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48 T cells including decreased TCR-mediated activation and proliferation as well as a  
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50 diminished ability to secrete IL-2 but high levels of cytotoxic mediators (perforin and  
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52 granzymes) that enable them to exhibit immediate effector functions. The finding that  
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54 CD28<sup>-</sup> T cells have shorter telomeres than their CD28<sup>+</sup> counterparts [96, 97] completed  
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56 the dogma of the 'senescent' CD28<sup>-</sup> T cell arising from chronic TCR stimulation with no  
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4 further proliferative capacity. Only recently have we begun to understand the complex  
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6 processes taking place during the aging of the human immune system. It has been  
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8 shown that CD28<sup>-</sup> T cells are not truly senescent as they can still proliferate if provided  
9  
10 appropriate costimulation, especially by 4-1BBL and OX40L [98] and/or cytokines, such  
11  
12 as IL-2 and IL-15 [87, 99].  
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### 15 16 17 18 19 *Consequences of the accumulation of terminally differentiated T cells*

20  
21 The age-dependent accumulation of exhausted CD28<sup>-</sup> T cells, which preferentially  
22  
23 produce the pro-inflammatory cytokines IFN- $\gamma$  and TNF- $\alpha$ , is thought to contribute –  
24  
25 together with components of the innate immune system – to the low-grade pro-  
26  
27 inflammatory background observed in elderly persons (inflamm-aging) [100]. The  
28  
29 enhanced prevalence of CD28<sup>-</sup> T cells in elderly persons, together with other  
30  
31 parameters, such as a disturbed CD4:CD8 ratio and CMV-seropositivity, has led to the  
32  
33 definition of the so-called ‘immune risk phenotype’ (IRP) predicting a higher 2-year  
34  
35 mortality in a longitudinal study of octa- and nonagenarians [101]. The efficacy of  
36  
37 booster vaccinations is severely decreased in the elderly [1, 102] and an insufficient  
38  
39 antibody response following influenza vaccination in elderly persons has been correlated  
40  
41 with a high frequency of CD8<sup>+</sup>CD28<sup>-</sup> T cells [103]. Persistent infection with CMV and the  
42  
43 consequent accumulation of pro-inflammatory CD8<sup>+</sup>CD28<sup>-</sup> T cells have also been  
44  
45 associated with an enhanced risk of coronary heart disease and impaired vascular  
46  
47 function [104-106]. In particular, vascular inflammation caused by vessel wall injury and  
48  
49 endothelial cell (EC) dysfunction is triggered by persistent infection with CMV [107, 108]  
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51 and leads to increased arterial blood pressure, consequently contributing to the  
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53 development of atherosclerosis [109]. An accumulation of CD28<sup>-</sup> T cells was also  
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identified in persons suffering from rheumatoid arthritis and ankylosing spondylitis [83, 110]. In conclusion, persistent infection with CMV and/or the accumulation of CD28<sup>-</sup> T cells may thus be involved in the pathogenesis of a broad variety of age-associated diseases.

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4 **Interventions to decelerate age-related changes of the T cell repertoire**  
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9 *Strategies to counteract age-related defects in naïve T cells*

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11 Experiments in mice [40] and studies in humans [111] indicate some promising  
12 approaches for the rejuvenation of naïve T cells leading to the production of new naïve  
13  
14 T cells that function as well as young cells and better than those from aged individuals.  
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16 Thus it has been shown that the defects of old naïve T cells can be restored when IL-2  
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18 treated cells or naïve T cells from young mice are reimplanted into aged mice [49]. The  
19  
20 production of rejuvenated naïve T cells can also be achieved by increasing  
21  
22 immunological space by whole body irradiation [112]. Another strategy aims at restoring  
23  
24 thymopoiesis in the elderly. Factors such as IL-7, growth hormone and sex hormone  
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26 ablation have thereby been tried (reviewed by [113]).  
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36 *Caloric restriction*

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38 Increasing lifespan of cells and organisms has long been of great interest for the  
39  
40 scientific community. Caloric restriction (CR) is today the only known method to prolong  
41  
42 median as well as maximal lifespan in all tested animals, from invertebrates to rodents  
43  
44 and even vertebrates including non-human primates. While it is not fully understood how  
45  
46 CR fulfills this life prolonging effect, several probably concerted hypotheses have been  
47  
48 postulated, one including the mTOR signaling pathway which we will discuss shortly  
49  
50 hereafter. CR not only increases the median and maximal lifespan of a variety of  
51  
52 organisms, but also improves specific functions that seem to acquire failures with age,  
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54 for instance the immune system. Nikolich-Zugich reviewed the impact of CR on the  
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56 immune system and showed, that in rodents and non-human primates CR was able to  
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4 attenuate the natural shift from naïve to memory-phenotype T cells and maintain a  
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6 higher number of naïve T cells in aged animals while decreasing the total number of  
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8 peripheral lymphocytes [114]. Still it remains unclear whether this is due to an increased  
9  
10 thymic production of naïve T cells, improved maintenance of naïve T cells in the  
11  
12 periphery or reduced T cell activation. Furthermore the age-related increase of pro-  
13  
14 inflammatory cytokines, such as IL-6, IFN- $\gamma$  and TNF- $\alpha$ , and the resulting pro-  
15  
16 inflammatory state of an aged immune system (inflamm-aging) can be reversed by CR.  
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18 Finally, the decreased proliferative capacity of T cells in an aged immune system due to  
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20 the shift from naïve to memory-phenotype T cells can be avoided and even retracted by  
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22 CR.  
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### 31 *mTOR, autophagy and aging*

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33 The mammalian target of rapamycin (mTOR), a central integrator of diverse intra- and  
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35 extracellular signals such as growth factors, nutrients, energy status or stress signals  
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37 [115] could be an explanation for the life-prolonging effect of CR. In case of sufficient  
38  
39 nutrients and other positive signals and/or in the absence of stress signals, in other  
40  
41 words, if the cell is doing fine, mTOR is active and thereby inhibits the catabolic process  
42  
43 of autophagy while promoting anabolic processes important for growth and proliferation  
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45 via different downstream molecules. Two of the best characterized ones are S6 kinase 1  
46  
47 (S6K1) and the 4E binding protein 1 (4EBP1) [116]. In the course of CR, nutrients are  
48  
49 rare and the enzymatic activity of mTOR is inhibited, leading to an upregulation of  
50  
51 autophagy while cell growth and proliferation cease. This is reasonable for the cell since  
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53 autophagy describes amongst other things the recycling of cellular components to gain  
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55 new building blocks for critical proteins by degrading momentarily not needed proteins  
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4 and even organelles [117]. It has been shown that autophagy can be induced in all  
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6 somatic cells of an organism by fasting or CR which represents mTOR inhibition in the  
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8 course of nutrient withdrawal [118]. It is known that chronological aging of cells leads to  
9  
10 malfunctions in various cellular mechanisms and autophagy is not an exception. It has  
11  
12 been shown that with the aging of an organism autophagic capacity declines, leading to  
13  
14 the accumulation of already quantitatively increased potentially harmful protein  
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16 aggregates that are normally degraded via autophagy [118]. When in other somatic cells  
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18 the autophagic efficiency declines with chronological aging, the same is certainly true for  
19  
20 T cells. Interestingly, it has been found that in T cells that display replicative senescence  
21  
22 characteristics, autophagic capacity is also decreased [119]. One might conclude, that  
23  
24 the higher the differentiation stage of a T cell is, the lower its autophagic capacity is and  
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26 therefore its probability to survive stress situations.  
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### 36 *Pharmacological interventions*

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38 Besides CR, there are other methods to prolong lifespan via autophagy. Recently we  
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40 have shown that the natural polyamine spermidine promotes longevity in yeast, flies,  
41  
42 worms and human PBMCs in an autophagy dependent fashion [120]. Furthermore mice  
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44 fed with a spermidine rich diet have an increased lifespan [121]. Along the line of mTOR  
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46 inhibiting autophagy, rapamycin, a well known inhibitor of mTOR and  
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48 immunosuppressive drug, prolongs lifespan in various organisms in a mTOR dependent  
49  
50 fashion [122-124]. Interestingly, autophagy not only prolongs the lifespan but also  
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52 increases the resistance to disadvantageous environmental circumstances [125]. In the  
53  
54 immune system, mTOR is additionally responsible for the differentiation of CD8<sup>+</sup> T cells.  
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56 Araki et al have recently shown, that in mice inhibition of mTOR by rapamycin shortly  
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4 after an acute lymphocytic choriomeningitis virus infection improved not only the quantity  
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6 but also the quality of virus-specific CD8<sup>+</sup> T cells [126]. Therefore they propose that  
7  
8 treatment with rapamycin after vaccination could enhance memory T cell formation.  
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### 11 12 13 *Conclusion*

14  
15 Immunosenescence describes the wide range of changes within the immune system  
16  
17 that occur with increasing age. In this review we summarize the most prominent  
18  
19 alterations in naïve and antigen-experienced T cells. Special emphasis is placed on  
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21 terminally differentiated T cells that accumulate in the elderly, display a decayed  
22  
23 functionality and contribute to a low-grade pro-inflammatory background, a phenomenon  
24  
25 called inflamm-aging. These age-associated dysfunctions within T cells have a strong  
26  
27 clinical impact, the most important being reduced efficacy of vaccination and decreased  
28  
29 resistance to infections. Our continuously improving comprehension of the aged immune  
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31 system reveals strategies to overcome the detrimental effects of immunosenescence,  
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33 some of which are discussed in this review.  
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## **Figure 1**

**Effect of oxygen level on apoptosis and proliferation of CD8<sup>+</sup>CD45RA<sup>+</sup>CD28<sup>+</sup> T cells from young (n=5) and elderly persons (n=3), stimulated for 5 days with CD3/CD28 DynaBeads**

(A) Flow cytometric analysis of Annexin V / 7-AAD of CD8<sup>+</sup>CD45RA<sup>+</sup>CD28<sup>+</sup> T cells at 3% and 20% oxygen after 5 days. A representative contour plot (left) and a bar chart (right; mean  $\pm$  SEM, \*p<0.05, student's t-test)

(B) Proliferation of CFSE-labeled CD8<sup>+</sup>CD45RA<sup>+</sup>CD28<sup>+</sup> T cells at 3% and 20% oxygen after 5 days. One representative histogram is shown.

Figure  
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