Age-Specific T Cell Homeostasis

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Abstract

Multiples defects affect adaptive immunity during aging: T cell production (both at thymic and peripheral sites), T cell activation (including TCR sensitivity, proliferation, and differentiation), T cell functions, and T cell survival. Most of these defects directly impact T cell homeostasis. Indeed T cell homeostasis refers to the maintenance of steady state in the body and the physiological processes

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through which they are regulated. This mechanism ensures a close equilibrium between T cell production and death, two aspects that are drastically affected during aging.

The aim of this chapter is to review the changes in T cell homeostasis developing during aging. We will first review the main homeostatic mechanisms that have been described so far in adult individuals. In a second part, we will document the age-related events that crucially impact T cell homeostasis and the resulting effects on peripheral T cell pools. Finally, the potential benefits of regenerative therapies aiming to restore T cell homeostasis will be discussed.

Keywords

T lymphocytes · Viral infection · Aging · IL-7 · CD8 expansion

Abbreviations

CMV	Cytomegalovirus
HSC	Hematopoietic stem cells
IL	Interleukin
TCR	T cell repertoire
TEC	Thymic epithelial cell

Introduction

Multiples defects affect adaptive immunity during aging: T cell production (both at thymic and peripheral sites), T cell activation (including TCR sensitivity, proliferation, and differentiation), T cell functions and T cell survival. Most of these defects directly impact T cell homeostasis. Indeed T cell homeostasis ensures a close equilibrium between **T cell production** and **T cell death**, two aspects that are drastically affected during aging. Additionally, the maintenance of a relatively constant number of functional and diverse T cells is not an inert process and includes a large range of homeostatic mechanisms that may also be affected by aging (such as T cell survival and/or peripheral expansion and/or regulatory mechanisms). Importantly, those homeostatic mechanisms are highly dependent on both soluble and cellular resources (cytokines availability and MHC expression) that are also impacted during aging as referred by "**resource exhaustion**."

Age-related changes in T cell homeostasis are well documented in murine models: aging is associated with **thymic involution**, reduced naïve T cell production, T cell lymphopenia, loss of T cell diversity, and skewing of naïve/effector-memory ratio toward the more activated fraction. Interestingly, the nature and extent of age-related changes in T cell homeostasis are less consensual in human individuals than in murine models. Although part of these discrepancies may rely on the biological material studied, i.e., peripheral blood versus lymphoid organs, the exact nature of these discrepancies will be discussed. More crucially, it remains to define whether these age-related changes identify an altered T cell homeostasis or an adapted T cell homeostasis. In the latter hypothesis, a novel T cell homeostasis may develop in

reaction to the age-related changes affecting both intrinsic T cell properties and resource availability and thus minimize the impact of age-related defects on T cell homeostasis. It has been suggested that some defects affecting T cell function may preserve T cell homeostasis by limiting inappropriate immune activation. It is currently unclear whether T cell homeostasis in aged individuals contributes to the lower efficiency of immune responses in aged individuals or limits the extent of these defects. Unfortunately, very few data are available on the nature of homeostatic mechanisms developing during aging and whether additional (or replacing) homeostatic strategies are developing.

The aim of this chapter is to review the changes in T cell homeostasis developing during aging. We will first review the main **homeostatic mechanisms** that have been described so far in adult individuals. Factors that participate to T cell production or T cell loss are briefly summarized in Fig. 1. In a second part, we will document the age-related events that crucially impact T cell homeostasis and the resulting effects on **peripheral T cell pools** and subsequently on the quality of immune responses. Three aspects will be particularly documented: (a) the shift on T cell production processes occurring during aging, being predominantly driven by thymic production and peripheral Ag-driven proliferation in young individuals, and gradually shifting toward peripheral Ag-driven proliferation, (b) the exhaustion of survival resources, and (c) the change in antigenic load. Finally, the potential benefits of regenerative therapies aiming to restore T cell homeostasis and the strategies currently envisaged will be discussed.

Homeostasis of T Cell Compartment

The term "**homeostasis**" was initially forged by Walter Cannon (*The Wisdom of the Body*, W. Cannon, 1932) in the continuation of the works of Claude Bernard describing the constancy of biological parameters ("constance du milieu intérieur"). Homeostasis thus refers to the maintenance of steady state in the body and the physiological processes through which they are regulated. Homeostasis applies to a large numbers of biological parameters (body temperature, concentration of sodium, potassium, calcium ions, glucose in the plasma, pH, acidity, carbonic gas concentration, etc.) including the immune system. Envisaging that homeostatic regulation applies to the T cell compartment was initially counterintuitive considering the specific properties of the T cell compartment. (a) Proliferation and expansion are key processes in the development of appropriate immune T cell responses. Following activation, T cells undergo massive expansion allowing differentiation toward effector and/or memory T cell subsets. The limitation by homeostatic processes of such crucial initial expansion of T cell appears theoretically highly deleterious to the development of efficient immune responses. (b) The T cell compartment is highly heterogeneous. It comprises γδT cells and αβT cells, including CD4 and CD8 T cells. Among these latter subsets, additional heterogeneity emerged from differentiation profile (naïve versus memory) and functional properties (Th1/Th2/Th17/Treg, e.g., in the CD4 T cell compartment). Such heterogeneity in

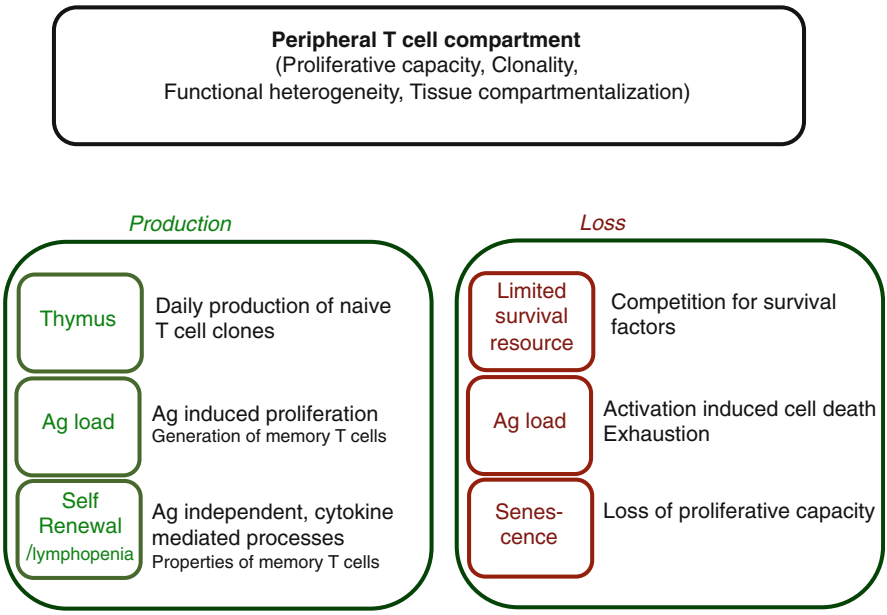

Fig. 1 Balance between T cell production and T cell loss defines T cell homeostasis. The maintenance of constant peripheral T cell numbers is achieved by integration of both production and loss processes. T cell production is ensured by three main processes: thymic production, antigen-induced proliferation, and cytokine-dependent proliferation (including self-renewal of memory T cells and lymphopenia-induced proliferation). On the other hand, T cell death is induced either due to competition for survival resources (mainly cytokines), processes associated to the antigen persistence (activation-induced cell death (AICD), exhaustion), or senescence. Importantly, aging affects most of these processes. Thymic involution is observed during aging and progressively reduces the contribution of thymic export to the mechanisms ensuring T cell production. Exhaustion of resources is also described during aging and may directly impact the rate of T cell loss (by limited availability of survival factors) but also self-renewal. Aging is also associated with an increased antigenic load that exhibits dual effects on T cell homeostasis: it may contribute to T cell production by inducing immune responses and memory T cells in one hand but may also consummate freshly produced naïve T cells and/or lead to exhaustion which consequently favors T cell depletion

T cell subsets is also reflected in their **proliferative and survival capacities** (Mueller et al. 2013) and consequently their sensitivity to homeostatic processes. (c) The large distribution of T cells that patrol in the whole body, from lymphoid tissues to nonlymphoid tissues by blood and lymph recirculation, is an additional hurdle to homeostatic regulation. The parabiosis studies performed by Klonowski et al. (2004) demonstrate **tissue compartmentalization** of T cells, i.e., tissue-specific T cell compartments are different from peripheral T cell compartment and differ among tissue-specific compartments. Tissue-specific T cell compartments are not equally sensitive to the potential alterations affecting peripheral lymphoid T cell homeostasis. It is currently unknown whether similar homeostatic processes are developing in lymphoid or nonlymphoid tissue and/or whether additional tissue-specific processes are developing. Even in animal models, it is indeed extremely

difficult to properly evaluate the size of T cell compartment in tissue since tissue dissociation and treatment probably impact cell recoveries (Preza et al. 2015). (d) T cell responses are also crucially defined by their specificity. High diversity of T cell clones within T cell clones is a prerequisite to mount specific immune responses directed against the cognate antigen.

Despite these hurdles, T cell homeostasis occurs: the maintenance of constant numbers of peripheral T cells at a given age has been described in peripheral lymphoid compartment in animal models or peripheral blood in humans. The mechanisms involved in T cell homeostasis (that will be referred as homeostatic mechanisms) modulate peripheral T cell pools both quantitatively and qualitatively, ensuring the maintenance of constant numbers of peripheral T cells and the persistence of the most diverse T cell compartment. High diversity applies to the **clonal repertoire** (in order to provide the largest repertoire against antigens) but also integrate functional diversity, allowing persistence of both CD4 and CD8 T cells and different differentiation profile (notably naïve and memory T cells). The nature of the homeostatic processes allowing preservation of **T cell heterogeneity** has been extensively described in young individuals. Three crucial homeostatic rules can be formulated as follow: (i) Homeostatic regulation impacts T cell compartment as a whole: CD4 and CD8 T cells share common critical resource for their survival. (ii) Naïve and memory T cell subsets are independently regulated. (iii) In young animals, T cell production exceeds resource availability: T cell survival relies on competition for survival resources. These three aspects will be further detailed below.

Common Niches for CD4 and CD8 T Cells

Pioneer experiments in the 1990s allowed establishing that B cells and T cells were independently controlled. In CD3εKO mice, B cell numbers are not significantly increased, suggesting that no compensatory mechanism is developing within B cells to replace the missing T lymphocyte compartment (Malissen et al. 1995). In the reverse experimental settings (i.e., in the absence of B cells), no significant increase in T cell numbers is detected, confirming the independent regulation of B and T lymphocytes (Kitamura et al. 1991). Among CD4 and CD8 T cells, clear compensatory mechanisms are observed. In CD4 KO mice, total T cell numbers are equivalent to those recovered in wild-type (wt) animals: CD8 T cells replace in numbers the missing CD4 T cell fraction (Rahemtulla et al. 1991; Cosgrove et al. 1991). Similarly, in CD8 KO mice (Fung-Leung et al. 1991), the absence of CD8 T cells is compensated by an increase in CD4 T cell numbers. Collectively, these observations demonstrate that total T cell numbers are regulated regardless of their CD4 or CD8 profile. Although functionally diverse, the homeostasis of CD4 and CD8 T cells is closely related: both subsets share (at least partially) common resources for their survival. This simple observation led to different interpretations that will be relevant in the context of aging: (a) changes in CD4/CD8 T cell ratio are thus part of homeostatic mechanisms, one subsets potentially compensating for the loss or depletion of the other. (b) Exacerbated expansion of one subset may be detrimental to the counterpart subset.

Coexistence of Naïve and Memory T Cells

T cell also differs by their differentiation status exhibiting specific survival, metabolic, and proliferation properties. Such coexistence guarantees the high efficiency of the immune system ensuring diversity in T cell responses (provided by the large diversity of TCR within the naïve T cell pool) and recall responses for frequently encountered pathogens (provided by the memory pool). Because memory T cells exhibit higher survival capacity and self-renewal properties, one may hypothesize that peripheral T cell compartment survival may be drastically biased toward the memory pool. Such bias may even be exacerbated in context of frequent antigenic stimulation (leading to a rapid consumption of the naïve pool) or when thymic export is reduced, i.e., two features associated with aging.

To address the respective behavior of naïve and memory T cells, both polyclonal and transgenic T cell repertoire were studied (Tanchot and Rocha 1995; Tanchot et al. 1997a). The use of TCR transgenic mice exhibiting restricted reactivity to Ag allows preventing naïve T cell loss due to peripheral antigen-driven activation and their recruitment in the memory pool. Among the various Tg mice used, the HY Tg model was especially relevant since this specific clone directed against the male antigen presented in the MHC H2d context exhibits no cross-reactivity with environmental antigens. Tanchot et al. elegantly studied the substitution of naïve or memory HY Tg T cell clones by thymic export. Naïve HY Tg T cells or memory HY Tg T cells (previously generated by in vivo activation in the presence of the male antigen) were transferred into irradiated hosts that received bone marrow cell from wt mice. The use of an allotypic marker allows distinguishing the thymic-derived population and the peripherally generated naïve or memory fraction. In this setting, preexisting naïve T cells are progressively replaced by newly produced naïve T cells exported from the thymus, whereas the preexisting memory T cell pool is not affected (Tanchot and Rocha 1995; Tanchot et al. 1997a). Collectively, studies performed in both polyclonal and transgenic settings demonstrate that naïve and memory T cell pool were independently regulated. Such independent regulation of naïve and memory T cells may provide a secure environment preventing the extinction of the naïve T cell pool when pressurized by the memory compartment and preserving the coexistence of both subsets (Tanchot et al. 1997a).

The **independence of naïve and memory T cell pools** was thus established in young adult animals in the late 1990s and was based on "standard" phenotypic distinction that probably needs to be reevaluated. Although providing robust arguments toward the independent regulation of naïve and memory T cells, these studies did not fully appreciate the increasingly documented heterogeneity of both naïve and memory T cells. It could be useful to reassess these independent regulations by integrating the memory T cell fraction with reverted CD45RA expression (Appay et al. 2002), the stem cell memory subsets (Tscm) (Gattinoni et al. 2011), and the memory with a naïve phenotype subset (Tnmp) (Pulko et al. 2016) that have enlarged the initial characterization of central (Tcm) and effector (Tem) memory subsets (Sallusto et al. 1999). The distinction of **tissue-resident memory T cells** (Schenkel and Masopust 2014) exhibiting long-term persistence in nonlymphoid tissue also emphasizes the need for tissue-related

studies to fully comprehend peripheral T cell homeostasis. Additionally, the cardinal observation, i.e., the independent regulation of naïve and memory T cell pools, was essentially performed in young adult animals. One may question whether the same homeostatic rules direct T cell homeostasis in aged animals.

Balance Between Production and Consumption

In contrast to erythrocytes, T lymphocytes do not exhibit a predefinite life span (Freitas and Rocha 1993). Their lifetime is conditioned by various parameters: their requirement for survival factors, their appropriate encounter with their nominal antigen (that induces their differentiation profile and modulate their survival and/or turnover capacity (Tough and Sprent 1994)), and the presence of surrounding T cells that may compete for the same survival factors (Jameson 2005; Stockinger et al. 2004). The maintenance of constant number of peripheral T cell lymphocyte is thus a balance between T cell production (relying on thymic export and peripheral expansion) and T cell death (relying on the availability to survival factors).

T Cell Production: Thymic Export and Peripheral Expansion

Thymic Export

The thymus is the principal site of T cell production, ensuring thymocyte maturation from bone marrow progenitors. During thymic maturation, thymocytes undergo positive and negative selection of their TCR chain and thus produce a diverse repertoire with calibrated reactivity against self-antigen. In young individuals, thymic production capacity exceeds the peripheral storage capacity. The existence of homeostatic mechanisms regulating thymic export depending on the size of the peripheral pool has been discarded using various murine models. Berzins et al. demonstrated that following multiple grafts of thymic lobes, the thymic export provided by the additional thymuses was preserved regardless of the number of thymic transplanted (Berzins et al. 1998). The impact of peripheral T cell depletion on thymic export was also studied: no increase of thymic export is observed (Gabor et al. 1997), suggesting that thymic export rate was independent from peripheral T cell homeostasis and does not provide compensatory mechanisms in the context of peripheral lymphopenia.

Although the thymic export does not appear to undergo homeostatic regulation, T cell that exits the thymus in a transitory state before reaching the mature T cell compartment may be regulated by homeostatic mechanisms. These transitionary cells are called **recent thymic emigrants** (RTE) and exhibit specific properties compared to naïve T cells (Fink 2013). They were initially considered as a specific niche (Berzins et al. 1998), independently regulated from the naïve T cell compartment. These RTE can peripherally expand and maintain a naïve phenotype (Hassan and Reen 2001), thus allowing preservation of the naïve T cell compartment. Different markers are currently available to distinguish human recent thymic emigrants from naïve T cells (expression of CD31 (Kohler and Thiel 2009), PTK7 (Haines et al. 2009) on naïve CD4 T cells). Homeostatic regulation of RTE expansion and survival has been extensively described

in neonatal conditions (Hassan and Reen 2001) and may differ depending on the age (Opiela et al. 2009). Naïve T cell expansion without phenotypic changes may be especially relevant in context of aging where thymic production is drastically reduced and increasing numbers of studies on the mechanisms ensuring naïve T cell proliferation are currently available (Kim et al. 2016; van den Broek et al. 2016). Interestingly, some discrepancy has been discussed between murine and human models, suggesting human naïve T cell homeostasis was essentially driven by homeostatic proliferation of the naïve pool (and presumably the RTE), whereas murine naïve T cell homeostasis was essentially driven by thymic production (den Braber et al. 2012).

Turnover of Memory T Cells

A hallmark of memory T cell survival is their capability to persist in the absence of their specific antigen by undergoing self-renewal (Veiga-Fernandes et al. 2000). Bone marrow is the preferential site for central memory T cell persistence (Becker et al. 2005; Mazo et al. 2005) and self-renewal (Becker et al. 2005). Self-renewal (or homeostatic proliferation) of memory T cells differs from T cell proliferation developing during chronic infection. Cell persistence following acute infection is ensured by slow and steady self-renewal of Ag-specific T cells, whereas T cell maintenance during chronic infection is dependent on **antigen persistence**. Recent works confirm this observation demonstrating that proliferation associated to self-renewal or rechallenge of memory T cells was differently regulated, CXCR4 being essential for self-renewal but dispensable for proliferation associated with secondary challenge (Chaix et al. 2014). The physiological relevance of memory T cell renewal to the maintenance of the peripheral pool remains difficult to appreciate and is currently debated (Di Rosa 2016).

Ag Stimulation

The principal expansion process in periphery is obviously driven by **antigen stimulation** and contributes to the development of the memory T cell pool. During the primary responses, the expansion can be massive (as exemplified by 1000-fold increase in Ag-specific T cell numbers during LCMV) (Blattman et al. 2002). But this massive expansion is followed by a contraction phase leading to the persistence of approximately 10% of the cell numbers detected at the peak of the responses. These studies were essentially performed in repleted young adults, and the outcome of the contraction phase in the context of aging remains to be evaluated. The effector phase is usually considered as independent of any homeostatic processes to prevent any limitation on the development of the immune responses. However, a large range of homeostatic processes may occur during the contraction phase and may modulate T cell homeostasis. Tools are now available to address more specifically these aspects (Garrod et al. 2012).

Lymphopenia-Induced Proliferation

In addition to Ag-induced proliferation, activation and proliferation induced in the context of T cell lymphopenia has also been described. Such phenomenon was initially observed in murine model of extreme lymphopenia, such as irradiated animals or constitutively T cell lymphopenic animals (CD3ε KO, Rag KO). When naïve T cells

are transferred into these lymphopenic hosts, division of most transferred cells is detected (Oehen and Brduscha-Riem 1999). The first interpretation of these results was to consider such activation in the context of lymphopenia as a homeostatic process, permitting peripheral T cell reconstitution in the context of lymphopenia and was accordingly termed "**homeostatic proliferation**" (Fig. 2a). Analyses were performed using different TCR transgenic strains (OT-1, DO11.10, etc.) and thus

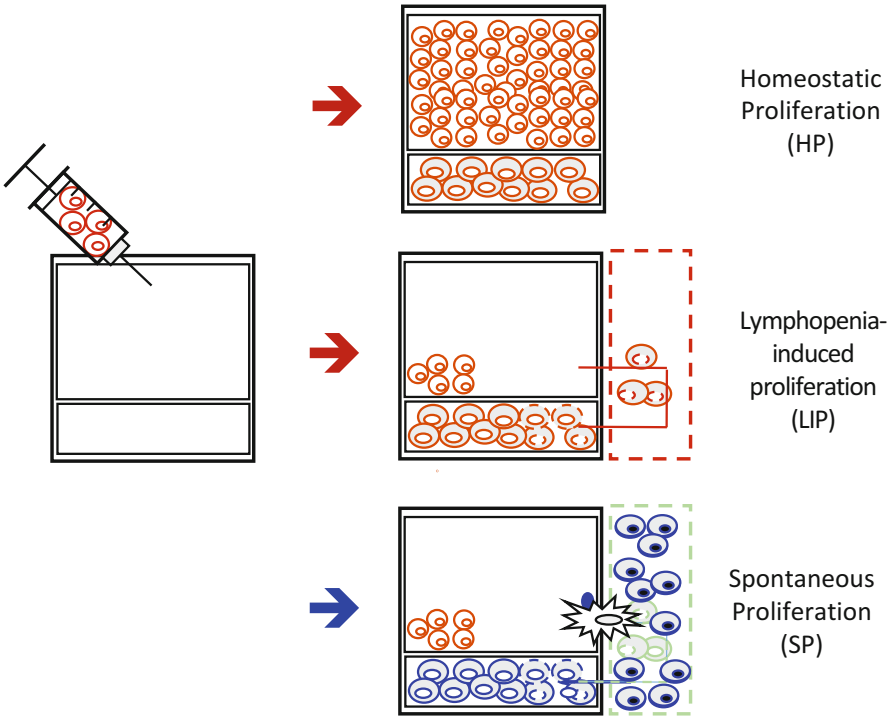

Fig. 2 From lymphopenia-induced proliferation to spontaneous proliferation. Following transfer of naïve T cells into empty hosts (either constitutively (Rag KO or CD3eKO mice) or after induced depletion (irradiation, Ab depletion)), proliferation of the injected cells was observed. This phenomenon was initially termed "homeostatic proliferation" and was interpreted as compensatory mechanisms occurring in context of lymphopenia to ensure replenishment of peripheral T cell pool. However the extent of the proliferation was not efficient in quantity (total T cell number recovered lower than those in normal mice), nor in quality (conversion to a memory-like phenotype). To integrate the limited regenerative potential of this proliferation and the conversion to a "memory-like" phenotype, the phenomenon was subsequently called "lymphopenia-induced proliferation" (LIP). Based on these observations, lymphopenia was thus considered as a potential cofactor of autoimmune disease. In contrary to Ag-induced proliferation, LIP referred to T cell activation and differentiation in the absence of the nominal antigen, the specificity of T cell activation being presumably overcome by the context of lymphopenia. The final step was the observation that the proliferation observed following the transfer of naïve T cells into an empty host was essentially driven by a higher environmental load in lymphopenic hosts. The authors subsequently suggested to term this proliferation as spontaneous proliferation or chronic lymphopenia-induced proliferation

confirmed that naïve T cells in the absence of their cognate antigen were activated and proliferated in lymphopenic conditions. Interestingly, few Tg strains did not undergo activation and proliferation (such as the HY Tg strain), and among polyclonal T cells, not all were proliferative. This process presumably relied on self-peptide/MHC interactions and IL-7, i.e., the homeostatic factors required for naïve T cell survival. However, extensive analyses led to modulate this assumption: (a) T cell reconstitution did not allow full replenishment of peripheral compartment (Ge et al. 2002; Tanchot et al. 2002); (b) naïve T cells undergoing such lymphopenia-related processes lost their naïve phenotype and exhibited memory-like phenotype (Ge et al. 2002; Murali-Krishna and Ahmed 2000). To integrate these observations, the phenomenon was called "**lymphopenia-induced proliferation**" ascertaining the low homeostatic potential of such process (Fig. 2b). Thirdly, it was subsequently demonstrated that (a) not all naïve T cells undergo activation and proliferation under lymphopenic contexts and that (b) partial lymphopenia did not generate lymphopenia-induced proliferation, thus suggesting that the extent of this "lymphopenia-induced proliferation" was rather limited (Bourgeois et al. 2005). Finally, additional studies aiming to determine the nature of self-antigens that were elicited during such process revealed that the so-called activation and proliferation of T cells was essentially driven by exogenous antigen rather than self-antigen (Min et al. 2004) (Fig. 2c). The absence of lymphopenia-induced proliferation observed for HY Tg CD8 was in fact reflecting the low cross-reactivity of this clone. Based on this third set of data, lymphopenia-induced proliferation appears to integrate two distinct proliferative responses following transfer into an empty host: "**spontaneous proliferation**" reflecting the high influence of the antigenic load on the behavior of peripheral cells and limited "lymphopenia-induced proliferation." Altogether, these observations suggest that the contribution to peripheral expansion of "lymphopenia-induced proliferation" of mature naïve T cells is limited when excluding "spontaneous proliferation" and does not allow naïve T cell maintenance. This observation does not discard the specific properties of recent thymic emigrants that ensure peripheral homeostatic proliferation and contribute to the maintenance of peripheral naïve T cell numbers (Houston et al. 2011).

Competition for Survival Factors

T cell survival is controlled by mainly two sets of survival signals: **cytokines and self-peptide/MHC ligands** (Jameson 2005; Stockinger et al. 2004; Surh and Sprent 2008). The respective role of cytokines and MHC interactions has been extensively reviewed (Tanchot et al. 1997a; Jameson 2005; Tanchot and Rocha 1998) and will be briefly mentioned here. The complexities of these studies emerged from the multiple role of cytokines and MHC interaction for T cell production, survival, proliferation, and differentiation. To note, CD8 T cells recovered from Rag Tg HY animals did not undergo homeostatic proliferation, thus allowing studies of T cell survival independently of any potential lymphopenia-induced or spontaneous proliferation. The crucial role of interaction with self-peptide/MHC complexes has been demonstrated both for CD4 and CD8 T cells (Takeda et al. 1996; Tanchot et al. 1997b). In accordance with the independent regulation of the naïve and memory T cell pools, the requirement for self-peptide MHC complexes differs between naïve and memory

T cells: naïve T cell survival being crucially dependent on MHC molecules, whereas memory T cell survival was less dependent on self-peptide/MHC interactions, although memory T cell effector functions might be affected (De Riva et al. 2007). Cytokines and notably γc cytokines (cytokines that signal through the receptor containing the common gamma chain) are also crucial to T cell homeostasis (Jameson 2002; Rochman et al. 2009; Schluns et al. 2000). Among those γc cytokines, IL-7 is the most important cytokine and is called accordingly the master of homeostasis. IL-7 is involved in survival of both naïve and memory T cells (Jameson 2002; Fry and Mackall 2001) but also constitutes the limiting factor defining the size of the peripheral T cell pool (Fry and Mackall 2001; Guimond et al. 2005). The elegant regulation of IL-7R expression, termed "**altruistic regulation**," also participates to the tight regulation of peripheral T cell pool (Mazzucchelli and Durum 2007). Downregulation of IL-7R following IL-7 ligation on peripheral T cells limits its consumption and presumably potentiates the use of IL-7 resources by a large number of peripheral T cells. IL-15 is another important homeostatic cytokine, being crucial for memory CD8 T cell homeostasis (Rochman et al. 2009; Becker et al. 2002) and confirm the competition for independent factor between naïve and memory T cell compartment. Evaluating the availability of these survival factors during aging is crucially required.

Tissue Homeostasis

Studies on peripheral T cell homeostasis are essentially restricted to blood in humans for obvious technical reasons. It thus provides a limited picture of total T cell homeostasis. PBMC represent less than 2% of the total number of T cells in the body (Ganusov and De Boer 2007), the most recent publication evaluating that blood T cells contribute approximately 0.5% to the total T cell pool (Di Mascio et al. 2009), thus providing access to a limited fraction of peripheral T cell pools. Studies using animal models allow addressing peripheral T cell compartment in secondary lymphoid organs (lymph nodes and spleen) or nonlymphoid organs. Secondary lymphoid organs represent an important proportion of T cells (Ganusov and De Boer 2007; Di Mascio et al. 2009) although the gut is accepted as the largest T cell compartment in the body. Importantly, this notion relies on pioneer works providing first estimations but no clear demonstration and therefore still debated (Ganusov and De Boer 2007; Ganusov and De Boer 2008). Using noninvasive radiolabeling strategies, Di Mascio et al. estimate 15% contribution of the gut to the entire pool of CD4 T lymphocytes (Di Mascio et al. 2009). Collectively, the gut appears to be an important contributor to the T cell compartment, equivalent to the spleen (Di Mascio et al. 2009) but lower than the LN's contribution (Ganusov and De Boer 2007). Enzymatic digestion protocols probably undermine the contribution of these tissues (Preza et al. 2015), and noninvasive imaging strategies should be favored to address this issue and delineate the size of T cell compartment in lymphoid and nonlymphoid tissues. The crucial requirement for evaluation of the size of T cell compartment in nonlymphoid tissue emerged from the recent identification of tissue-resident

memory T cells (Trm) (Steinert et al. 2015). So far, T cells recovered from tissue were essentially considered as effector cells, migrating to the site when required, rapidly disappearing and not submitted to homeostatic regulation. Because of this "effector" status, evaluating the homeostatic processes developing in tissue was not considered as a major issue. However, recent reports indicate that a vast proportion of tissue-resident T cells exhibit memory properties and define tissue-specific properties of these Trm, ensuring regionalization of immunosurveillance (Steinert et al. 2015). The identification of these novel subsets has major consequences in terms of T cell homeostasis; (i) the existence of tissue-specific memory T cells may reveal a much larger number of T cells present in the whole body than previously considered and need to be quantified. (ii) The existence of homeostatic processes that may contribute to resident memory T cell homeostasis remains to be further investigated. (iii) Tissue-specific regionalization of T cell compartment raises the possibility of independent homeostatic system regulating at least partially independently lymphoid and nonlymphoid sites.

Homeostasis in Elderly

Feature of Aging: Immunosenescence

Thymic Involution

Thymic involution is a well-described age-related phenomenon in which the thymic space becomes filled with adipose tissue and lipid-laden cells even in healthy individuals (Lynch et al. 2009). This tissue reorganization, which involves a decrease in cellularity and tissue mass, results in decreased naïve T cell output. Indeed, the maintenance of naïve peripheral T cells requires the egress of cells from the thymus in adult animal models (Bourgeois et al. 2008; Cicin-Sain et al. 2007). However, the relationship between thymic activity and naïve T cell homeostasis in humans is a matter of debate, with the recent observations that peripheral proliferation and not thymic output contributes to the maintenance of naïve T cells in young adults (den Braber et al. 2012). Nevertheless, a direct correlation between thymic activity and naïve T cell number was observed in thymectomized individuals (Sauce et al. 2009). Moreover, measurement of thymic function, using **signal-joint T cell receptor (TCR) excision circles** (sjTREC), has shown lower sjTREC levels in elderly individuals who exhibit also a reduction of naïve T cells (Mitchell et al. 2010).

This decline in naïve T cell output is believed to impact the phenotypic and functional properties of the peripheral T cell compartment (Akbar and Henson 2011). This suggests that the altered thymic function is a key factor in the development of immunosenescence (Aw and Palmer 2011; Goronzy and Weyand 2013).

T Cell Lymphopenia

An expected consequence of thymic involution is the development of T cell lymphopenia. Interestingly, whereas such T cell lymphopenia has been documented

in animal models (den Braber et al. 2012; Bourgeois et al. 2005; Bourgeois et al. 2008; Cicin-Sain et al. 2010), the incidence of T cell lymphopenia remains debated in humans. Some reports detect T cell lymphopenia in humans (Mitchell et al. 2010; Fagnoni et al. 2000; Linton and Dorshkind 2004; Rea et al. 1996; Sauce et al. 2012) although some others suggest preserved or limited reduction of naïve CD4 T cell (den Braber et al. 2012; Sempowski et al. 2001; Wertheimer et al. 2014). Different hypotheses have been formulated to explain such discrepancy between murine and human data. (i) Median of ages may differ in studies and contribute to these variations. (ii) Den Braber et al. estimate that the relative contribution of thymic output and peripheral expansion to the peripheral CD4 T cell pool is different between human and mice. Using CD31 expression, the authors estimate that only 11% of naïve T cell originated from the thymus in adults and thus conclude that human CD4 T cell compartment was essentially supported by peripheral expansion (den Braber et al. 2012). The authors thus suggest that peripheral T cell proliferation may importantly contribute to CD4 T cell persistence. Unfortunately, they did not evaluate these proportions in mice using the same experimental strategies, thus limiting their interpretation. The relative impact of peripheral survival of preexisting naïve T cells and peripheral homeostatic proliferation in both species remains to be further dissected (Qi et al. 2014a). (iii) The drastic decay of naïve CD4 T cell in murine model of aging may also reflect different environmental antigenic challenges. This is in line with discussion on the crucial role of limited environmental stimulation in mice kept under specific pathogen-free conditions (Bourgeois et al. 2008; Beura et al. 2016; Nikolich-Zugich 2008). As exemplified in the context of HIV infection, high microbial load induces the gradual erosion of the naïve CD4 T cell pool by inducing constant stimulation of peripheral T cells (Brenchley et al. 2006). Collectively, it appears that **chronic inflammation** and **microbial load** may directly impact the gradual reduction of preexisting T cell pool and notably naïve T cell precursors (Brenchley et al. 2006; Jurk et al. 2014). Differences in the relative contribution of preexisting naïve T cells produced in the thymus and peripheral expansion on the maintenance of peripheral pool may strongly rely on microbial differences. Accordingly, the recent report of Wertheimer et al. elegantly demonstrates that the CMV status critically modulates peripheral T cell homeostasis: the decline of CD4 T cell was essentially observed in CMV+ subjects (Wertheimer et al. 2014).

Change in Naïve/Memory Ratio

The most widely accepted hallmark of aging on periphery T cell compartment is probably the change in naïve/memory proportion skewing toward an increased proportion of memory cells. It reflects both the impact of aging on thymic functionality and the emergence of memory T cell subsets due to chronic stimulation. Decrease in naïve T cell percentages and simultaneous increase in memory T cell percentages has been extensively described (Vallejo 2007). This aspect will not be further discussed in the current chapter because very few data are available on naïve and memory T cell numbers, respectively. Extensive works are currently evaluating the functional properties of the memory T cells in aged individuals but will not be discussed here.

T Cell Homeostasis in Aged Nonlymphoid Tissue

Very little information is currently available on T cell compartment in **mucosa-associated lymphoid tissues (MALT)** such as gut- or lung-associated lymphoid tissue and nonlymphoid tissue such as the liver or skin. The recent identification of **resident memory T cells** emphasizes the crucial role of resident T cells to maintain efficient immune responses against local insults. In murine models, the few reports available suggest that T cell lymphopenia observed in peripheral blood and/or secondary lymphoid organs during aging was not associated with similar decline in tissue. In the **liver**, increased cellularity of total cell and/or T cell has been described in aged animals (Tsukahara et al. 1997). In the gut-associated lymphoid structures (**lamina propria** and/or **epithelium**), increased (Martinet et al. 2014) or preserved numbers of T lymphocytes have been reported. Data in the Peyer's patches are more heterogeneous, presumably due to the variety of ages studied (Martinet et al. 2014; Saffrey 2014; Schmucker et al. 2001). Major changes in the microbiota have been reported and may account at least partially for this sequestration and/or accumulation of gut T cells (Biagi et al. 2012; O'Toole and Jeffery 2015). Collectively, age-related changes appear to differ depending on the organ considered. These observations draw different questions: (i) What is the relevance of blood T cell lymphopenia to the mechanisms of **aging**? (ii) Does T cell lymphopenia in peripheral blood and secondary lymphoid organs reflect T cell redistribution rather than absolute T cell depletion? (iii) **Tissue-specific compartmentalization** may also lead to picture a heterogeneous age-related T cell homeostasis depending on the sites considered. Altogether, it appears crucial to readdressed T cell lymphopenia during aging by performing an exhaustive analysis of the impact of aging on T cell compartment integrating different anatomical locations.

Loss of Diversity

The ability of the adaptive immune system to respond to a wide variety of pathogens depends on a large repertoire of unique T cell receptors (TCRs). TCR diversity is generated by the random rearrangements of the V and J segments of the TCR alpha (TCRA) and V, D, and J segments of the TCR beta (TCRB) genes in the thymus. **Thymic production** of T cells therefore is the sole mechanism to generate **TCR diversity**. With the involution of the thymus with age, the generation of new naïve T cells with new TCRs dwindles and thus is pinpointed as causing defective immune responses in the elderly. By applying next-generation sequencing of replicate TCRB libraries from highly purified T cell subsets, Qi et al. obtained estimates of repertoire richness in the young adult that are higher than previously reported (Messaoudi et al. 2004). Although contracting with age, the repertoire remains surprisingly highly diverse. These data challenge the paradigm that thymic rejuvenation is needed to maintain diversity and prevent immune incompetence in the elderly. However, they also observed an increasing inequality of clonal sizes with age even among naïve T cells (Qi et al. 2014b), suggesting that this clonal selection could result in biased and possibly autoreactive immune responses.

Inflammaging

Elderly frequently presents a systemic **chronic low-grade inflammation** that has been termed "inflammaging" (Franceschi and Campisi 2014), which is characterized by increased levels of pro-inflammatory cytokines (IL-1, IL-6, IL-8, tumor necrosis factor (TNF)-a, and C-reactive protein (CRP)). However, the cellular sources of these cytokines are not known. The increased inflammatory cytokines are a proposed driver of less successful aging (increased morbidity, sarcopenia, or frailty) and shortened healthspan (Franceschi et al. 2007). The inflammatory scenario constitutes a highly complex response to various internal and environmental stimuli mediated mainly, but not exclusively, by the high levels of pro-inflammatory cytokines. This results in the generation of **reactive oxygen species** (ROS) causing both oxidative damage and eliciting an amplification of the **cytokines' release**, thus perpetuating a vicious circle of chronic systemic pro-inflammatory state where tissue injury and healing mechanisms proceed in parallel and damages accumulate slowly and asymptomatically over decades. Moreover, the shift of cytokine production toward a pro-inflammatory profile is accompanied by endocrine and metabolic alterations that could explain some age-related pathologies.

Mechanisms Affecting T Cell Homeostasis

Resources Exhaustion and Progenitors

Cellular senescence not only hallmarks specific differentiated immune cells, as discussed below, but also occurs in **hematopoietic progenitors**. Hematopoietic stem cells (HSCs) are adult stem cells from which, upon exposure to specific differentiation stimuli, all blood cells originate. In humans, the number of HSCs isolated from bone marrow dramatically increases with age, whereas their capacity to proliferate in vitro in cloning formation assays (CFU-F) and to repopulate the **bone marrow** progressively decreases (Sauce et al. 2009; Geiger et al. 2013).

Several groups have reported that murine HSCs accumulate DNA damage and senescence markers with age (Flach et al. 2014; Yahata et al. 2011). This is caused not only by an increase in oxidative stress linked to dysfunctions in energy metabolism but also by the progressive repression of the replication factor MCM4 at each cell division (Flach et al. 2014). Defects in HSC replicative capacities could increase the pool of HSCs with impaired differentiation potential over time. Indeed, murine and human studies have reported that HSCs from old subjects are skewed toward **myeloid differentiation** with a restricted **lymphoid maturation** capacity (Beerman et al. 2010; Van Zant and Liang 2012).

Clonal expansion of individual HSCs within the HSC pool in the bone marrow at a given time has also been reported (McKerrell et al. 2015). It is currently unclear whether the aging-associated myeloid bias of hematopoiesis is a consequence of such a clonal shift or whether aging directly impacts on the differentiation potential of HSCs themselves. Further downstream, impaired differentiation in the lymphoid lineage upon aging is linked to impaired IL-7 stimulation of CLPs owing to lower

levels of IL-7, reduced expression on IL-7 receptor (IL-7R) on T cell progenitors, and reduced expression of differentiation regulators such as NOTCH 1 and GATA3 (GATA-binding protein 3) in HSCs (Gruver et al. 2007).

Therefore, replicative and age-induced HSC senescence affects immune system homeostasis (Flach et al. 2014; Yahata et al. 2011) by reducing their ability to produce naïve CD4+ and CD8+ T cells. Accordingly, in mouse models, deletion of two **senescence-associated cell cycle regulators** such as p16INK4A and p14/p19ARF increases the in vitro HSC replicative potential compared to wild-type cells (Sperka et al. 2012).

Thymic Involution

Although the precise mechanisms involved in age-associated thymic involution are not totally depicted, an emergent consensus suggests that both thymic **stroma** and newly made thymocytes are defective (Linton and Dorshkind 2004). During thymopoiesis, sequential developmental steps occur. Briefly, lymphoid progenitors, identified by a lack of both CD4 and CD8 expression, enter into the thymus (double-negative (DN) thymocytes). Thereafter, these cells differentiate, acquiring both CD4 and CD8 molecules (double-positive (DP) thymocytes). Subsequently, they mature through positive and negative selection, which give rise to single-positive (SP) T cells (CD4 or CD8), who can then exit into the periphery (Anderson and Jenkinson 2001). Since the thymus requires a continuous flow of lymphoid progenitors, any HSC properties alterations would contribute toward thymic dysregulation.

The thymic stroma plays a crucial role in thymopoiesis by providing the signals necessary to promote proliferation and differentiation due primarily to the influence of cortical and medullary epithelial cells (Anderson and Jenkinson 2001); thus age-related **intrinsic defects** in the **thymic niches** could potentially promote thymic involution (Aw and Palmer 2011; Chinn et al. 2012). This includes downregulation of keratin and MHC class II on **thymic epithelial cell** (TEC) together with changes of cortical and medullary junction (in particular with an accumulation of adipose tissue and senescent cells) (Aw et al. 2008; Bertho et al. 1997; Gray et al. 2006; Dixit 2010; Gui et al. 2007). Moreover, **extrinsic defects** within the thymic stromal niche can impact T cell development in aged subjects (Zhu et al. 2007). For instance, aged TEC produces less IL-7 (Gray et al. 2006; Aspinall and Andrew 2000), which is a cytokine essential for thymopoiesis (Hong et al. 2012).

Thus, contributing factors toward thymic involution imply both the alteration of **thymic microenvironment** and a defective cross talk between thymocytes and TEC. Altogether, this leads to the incapacity to preserve a functional thymic structure and to maintain an active thymopoiesis (Gui et al. 2007) in older individuals.

In addition to the intrinsic defects of aged TEC, there is an accumulation of fat, which may inhibit thymic function (Dixit 2010). Although the exact mechanism of **lipid deposition** in the human thymus is not known, the presence of excess lipid-derived metabolic intermediates such as ceramides and free cholesterol triggers caspase-1 activation leading to increased IL-1β concentrations and tissue damage. However, since fat aggregates are observed before thymic involution, this suggests that thymic adiposity is not initiating the process but could worsen the detrimental impact of age on thymic activity (Dixit 2010).

Another statement is that thymic involution is triggered during puberty. This is based on the fact that **sex steroids** are deleterious on thymocytes and that thymic size can be restored by chemical or surgical castration in older rodents (Sutherland et al. 2005). However, several reports assessing thymic properties (number of RTE, cellularity, epithelial space) have shown that thymic involution occurs early in life, prior to puberty. After an early decline of 3% of thymic tissue lost per year until adulthood, involution persists at a steady rate of 1% per year (Steinmann 1986). These new observations question the exact role of sex hormones on thymic involution (Montecino-Rodriquez et al. 2005).

Cytokine Availability

Among survival factors, **IL-7** is considered as the crucial limiting factor. Evaluating the concentration of IL-7 in mice is extremely difficult for technical reason: physiological concentrations are essentially below detection limit (Guimond et al. 2009). IL-7 is produced by various stromal cells but also DCs (Guimond et al. 2009). Aging has been associated with decrease IL-7 production by BM stromal cells and in peripheral blood (Passtoors et al. 2015). However, the net availability is difficult to address because it depends on the **production** rate but also the **consumption** rate of IL-7, the latter being directly related to the number of consuming cells and the expression of IL-7R. An additional level of complexity relies on the physiological activity of IL-7 signaling that may induce either T cell proliferation or survival depending on **IL-7 signaling** thresholds (Palmer et al. 2011). Altogether, these parameters are modulated during aging (Sauce et al. 2012). Indeed, plasmatic level of IL-7 is increased in elderly who are fully able to respond to this cytokine-induced stimulation through intact STAT signaling pathway. This results in a higher degree of cell cycling: the lower the number of naïve T cells, the higher their turnover (Sauce et al. 2012). Developing a mathematical model focusing on the respective survival and proliferation capacity of IL-7 signaling, Reynolds et al. demonstrate that naïve CD4 T cell loss during aging may predominantly rely on peripheral expansion due to higher net IL-7 availability (Reynolds et al. 2013). Thus, more than IL-7 concentration per se, it is probably the global "**IL-7 network**" that needs to be considered (Passtoors et al. 2015). Interestingly, using this strategy, Passtoors et al. identify IL-7R expression as a marker for better metabolic health profile and/or healthy aging.

Hyperactivation

Two processes may theoretically participate to the exacerbation of immune responses: inflammation that may exacerbate immune responses and increased antigenic loads.

Microbial Load

It is now clear that microbial load impacts the shaping and the quality of immune responses. Aging appears to impact mucosal-associated lymphoid site (MALT), affecting epithelial and lymphoid structures, immune responses (Saffrey 2014; Ogra 2010), and consequently **microbial translocation** (O'Toole and Jeffery 2015). This field of investigation may rapidly provide important insight on the mechanisms of peripheral T cell activation.

Ag Persistence

Thymic involution dictates that there is extremely limited generation of naïve T cells in elderly individuals. This means that immunity is largely maintained by turnover of existing populations of cells (Linton and Dorshkind 2004) but is potentially unable to ensure proper elimination of newly encountered pathogens fueling bystander inflammation by innate responses. Importantly, **Ag persistence** also affects pre-existing memory T cells. Although the quantity of T lymphocytes remains stable over the lifetime of an individual, the functional quality and proportion within the T cell pool can be dramatically altered (Pawelec et al. 2004). This is particularly true for T cells that are specific for agents that cause persistent infection, in which repeated episodes of cellular expansion in response to virus reactivation can drive terminal differentiation. Infection with **CMV** in particular seems to drive specific T cells to extreme differentiation particularly during aging (Appay et al. 2002; Sauce et al. 2003). In parallel, other recent studies show that CMV seropositivity is predictive of early mortality of elderly individuals (Wikby et al. 2002).

CMV is a beta-herpesvirus that is usually acquired during an asymptomatic primary infection in early childhood, after which the virus establishes lifelong persistence. It is estimated that CMV infects 50–90% of a given population, depending on socioeconomic status, geographical location, and hygiene levels. A particular facet of CMV-specific responses is that T cells reactive against CMV are significantly more differentiated than influenza- (Maczek et al. 2005), EBV- (Appay et al. 2002; Sauce et al. 2003), hepatitis C- (Lucas et al. 2004), or HIV-specific populations (Appay et al. 2002) in the same subjects. Another feature of the T cell compartment in elderly humans is that many of the **clonal expansions** of CD8+ T cells in elderly subjects are CMV-specific (Hadrup et al. 2006; Weekes et al. 1999).

Clonal Expansion

One of the hallmarks of immune aging is loss of **TCR repertoire diversity** due in large part to the dominance of memory over naïve T cells. However, in addition to that reduction, the CD8 compartment often shows additional loss of diversity, in the form of large, often clonal expansions of T cells bearing the same TCR, named **T cell clonal expansions** (TCE) (Messaoudi et al. 2004; Posnett et al. 1994). The presence of expanded clones is more pronounced in CD8+ than in CD4+ T cell compartments, suggesting that the clonal expansion in these two subsets is controlled by different mechanisms.

Two types of TCE can be classified according to the mechanism of their generation and/or maintenance. Large Ag-independent TCE are thought to arise and/or to be maintained independently of antigenic stimulation, due to age-related changes in perceiving homeostatic signals. This is based upon (i) the activation status of these cells (Ku et al. 2001; Messaoudi et al. 2006); (ii) their level of cytokine receptor expression, principally IL-7R (CD127) and IL-15R; and (iii) their ability to proliferate upon adoptive transfer (Ku et al. 2001) independently of lymphopenic environment. In contrast, Ag-reactive TCE are linked to the persistence of viral antigens exemplified by herpes viral infections (Pawelec et al. 2004; Fletcher et al. 2005).

TCE can occupy up to 50% of the human memory CD8 T cell pool with a reduced diversity (Posnett et al. 1994; LeMaoult et al. 2000). Therefore, drastic disturbance of peripheral immunity can be expected to impair the ability to mount protective responses against **pathogenic challenge**. Indeed, expanded clones of CMV-specific T cells might smother other memory T cell populations through competition for space or growth factors (Messaoudi et al. 2004). As the size of the CMV-specific T cell compartment increases with age while the total size of the T cell pool remains unchanged, some memory T cell pools that are present at low frequency might be lost. This may lead, in turn, to the reactivation of silent viruses such as VZV, which causes shingles, in elderly subjects (Berger and Florent 1981). The observation that CMV seropositivity correlates with reduced EBV-specific T cell responses in old age (Khan et al. 2004) is supporting this possibility. Therefore CMV-induced CD8+ **memory inflation**, which results in **repertoire shrinkage**, places CMV-seropositive elderly into an at-risk group (Hadrup et al. 2006).

Therapeutic Approaches

Two medical approaches can be combined to handle aging: defining biomarkers of healthy versus pathological aging and, subsequently, designing strategies to restore/improve T cell homeostasis.

Biomarker of Aging: Immune Risk Phenotype

Longitudinal studies have defined an immune risk phenotype (IRP) in healthy elderly individuals, which is predictive of significantly decreased survival of patients above the age of 80 (Wikby et al. 2002). The IRP phenotype is composed of a cluster of immune parameters including **CMV seropositivity**, a **CD4/CD8 T cell ratio of <1** due to increased CD8+ T cells, an increased proportion of highly differentiated **CD8+ CD28- T cells**, the presence of CD8+ T cell **clonal expansions**, and elevated serum levels of **pro-inflammatory cytokines** (Wikby et al. 2002; Olsson et al. 2000). There is evidence that all these changes may be primarily due to the effects of persistent infection with CMV in elderly subjects (Pawelec et al. 2004). Thus, CMV might have a more insidious effect on the immune system than previously appreciated; however, it is unclear how the various immune changes that comprise the IRP are linked and why CMV infection in particular appears to reduce the survival of elderly individuals in the IRP group. It should be noted that not all elderly subjects who are CMV seropositive have the defined IRP (Olsson et al. 2000). This suggests that other factors in addition to CMV infection may also be involved.

As dysregulated immunity predominates in the elderly, it is often difficult to know which changes are really beneficial or harmful. Of note, since no clear biomarkers of immunosenescence have been defined, regenerative therapies aiming at restoring T cell homeostasis can be difficult to monitor.

Restoration Strategies

Four main restoration strategies can be considered: (a) **immune restoration** of the thymus, (b) act on inflammatory environment, (c) decrease the **antigenic load,** and (d) maintain or enhance **T cell function** (activation) of remaining intact cells. It must be clearly stated however that none of the above therapy has been proven in human to have a beneficial impact on age-related T cell homeostasis to limit **immunosenescence**.

Restoration of the Thymus

The rejuvenation of a functional thymus is likely to be essential for maintaining or restoring effective immunity with aging. This would require that thymic activity is increased and that the newly produced naïve thymic cells are released to the periphery (and not stopped by homeostatic feedback from a "full" periphery). Several possibilities can be envisaged to help to maintain the function of the thymus (Sutherland et al. 2005).

The first is physical **grafting** of functionally intact thymus to old individuals. Indeed, transplantation of cultured thymic fragments to neonatal patients with DiGeorge syndrome has been quite successful (Markert et al. 1999).

The second could be the administration of **IL-7** in order to compensate the age-related decrease of IL-7 thymic content. IL-7 administration which is a cytokine playing a crucial role in the development and maintenance of the peripheral T cell pool results in partial thymic rejuvenation but also in lymphoma development (Aspinall 2006). Indeed, IL-7 is a survival factor but is also a pro-inflammatory cytokine exacerbating immune responses (Seruga et al. 2008). Its use should be considered with caution, especially in context of aging, where the incidence of autoimmunity is increased (Vadasz et al. 2013).

The third possibility is to play on thymic epithelial cells (TEC), either by generating thymic epithelial progenitor cells or by administrating keratin growth factor. This factor has been described to maintain thymic architecture and cellularity, as well as to restore immunity in aged mice (Min et al. 2007). Similarly, beneficial effects have been observed in mice using sex steroid ablation (Sutherland et al. 2005), growth hormone (French et al. 2002), ghrelin (Dixit et al. 2007), and IL-22 (Dudakov et al. 2012).

The last would be to act on **nutrition**. Since nutrition plays a role in the development of various diseases such as infections, cancer, and cardiovascular diseases, nutritional interventions could be beneficial for the prevention, retardation, or even reversal of established immunosenescence. In human, large numbers of supplementation studies have been performed, and some beneficial effects in aged individuals have been obtained by using vitamins and minerals and/or antioxidants (Lesourd 1997). The best example of non-pharmaceutical intervention is **caloric restriction** (CR) without malnutrition, which extends healthspan and life span. Since CR protects against lipid accumulation in the aging thymus, it results in preservation of thymic integrity and the peripheral T cell pool in old mice (Yang et al. 2009). In humans, the effects of CR as an anti-inflammatory intervention have been recently

established in a randomized controlled multicenter study called the Comprehensive Assessment of Long-term Effects of Reducing Intake of Energy (CALERIE) (Ravussin et al. 2015). The study reveals that 2 years of CR decreased T3, TSH, TNF-α, and CRP which have been previously described to impact on longevity. It remains to be determined if such improvement includes better T cell homeostasis and preservation of repertoire diversity.

Anti-Inflammatory Agents

There are other less potent well-known **inflammation-modulating drugs** that have few side effects and can be used with safety even in very old subjects. Such drugs include statins and nonsteroid anti-inflammatory drugs (NSAID). These are pleiotropic drugs having multiple targets in the inflammatory response. They could act in preventing several age-related immune-associated diseases such as atherosclerosis, dementia, and sarcopenia. Large longitudinal studies were already conducted and prove the beneficial effect of such drugs (Foody et al. 2006). The statins developed and used primarily for atherosclerosis prevention and treatment have also shown significant beneficial effects on susceptibility to a wide variety of cancers. There are several other drugs that could have anti-inflammatory effects, such as those targeting either the selectins (pan-selectin inhibitors), the TLR, the NF-κ B pathway by inhibiting inhibitor κ B kinase (IKK) activation, or the matrix metalloproteinases (MMPs). These strategies would not aim to completely restore the altered immune response with aging but could attenuate some of the consequences due to the low-grade inflammation and its clinical sequelae.

Strategies to Reduce the Infectious Antigen Load

It is important to recognize the existence of chronic latent infections, mainly **herpesviruses**, providing a continuous antigenic load and chronic stimulation resulting in exhaustion of the T cells involved (Pawelec et al. 2004). Not only persistent viruses but also the presence of subclinical bacterial infections might also contribute to chronic antigenic stress. Thus, strategies combating these sources of chronic stimulation could have a general immunorestorative role on age-associated immune dysfunction. Recent studies on aging have indicated that CMV is an important contributor of immunosenescence-related signature. Nevertheless, at the moment, there is no major viable anti-CMV vaccine. However, Oxman and colleagues (Oxman et al. 2005) published a large clinical trial on the utilization of a vaccine to prevent herpes zoster and postherpetic neuralgia in older adults. This is not a vaccine aimed to eradicate the latent herpes zoster (VZV) infection but to significantly decrease VZV clinical infections (Levin et al. 2016). This could, however, diminish the immune system-exhausting effect of this persistent virus. This could open new avenues for other types of vaccination against latent immunosuppressing viruses. Finally, the reduction of the viral load, as in HIV infections as well as in herpes zoster, can be used. The newer generations of **antiviral drugs** could be used with an acceptable safety profile in the elderly, such as acyclovir and famciclovir (Scheinfeld 2005). These agents could reduce viral load and extend the time to exhaustion for viral antigen-specific T cells.

Maintaining or Enhancing T Cell Function

Cytokines and Hormones

One of the most striking functional alterations observed in T cells is the decrease of the production of IL-2. This cytokine is essential for the clonal proliferation of antigen-reactive T cells and for antibody production.

It is now accepted that the existence of a **neuroendocrine-immune network** implies that hormonal replacement can have beneficial effects on immunity in the elderly as well. It seems that estrogen can have powerful immunomodulating effects, albeit mainly during stress, by modulating the innate immune response (Kovacs 2005).

Another hormone, which could play an important role in modulating and maintaining the immune system, is insulin, which can directly influence innate immunity via shared signaling pathways involving PI3K. Thus, insulin resistance developing with aging even at the level of innate immunity could have anti-inflammatory effects. Restoring insulin sensitivity by drugs such as glitazone could therefore have a beneficial immunorestorative effect in the elderly via the modulation of peroxisome proliferator-activated receptor (PPAR)γ (Berstein et al. 2005).

Insulin-like growth factor-1 (**IGF-1**) has also been shown to promote the survival and function of peripheral T cells as well as increasing the function of B cells, NK cells, and macrophages. It is well known that the IGF-1 level decreases with age, so future trials could be warranted to establish whether its supplementation or increase through other **hormones** (i.e., growth hormones) would be an effective restorative strategy despite potential toxic effects (Bonkowski et al. 2006).

Dehydroepiandrosterone (**DHEA**) has been considered for immunorestoration, because it dramatically decreases with age in both sexes. The direct action of DHEA is still questionable (Butcher et al. 2005) as no specific receptor has been identified. Moreover, DHEA is having an opposite effect on immune function compared to another adrenal hormone, cortisol, limiting its usage (Buford and Willoughby 2008).

Vitamin D (1,25(OH)2 D3) could intervene in the interaction between APCs and T cells and as such modulate T cell activation (Fabre-Mersseman et al. 2014). Vitamin D insufficiency, a common problem in apparently healthy elderly people, could lead to the disruption of immune tolerance and play a role in the development of chronic inflammatory disease and the autoimmune disorders often seen with aging. This could be related to the failed upregulation of T regulatory cells for suppressing autoreactive T cells (Adorini et al. 2003). As most elderly people nowadays are under vitamin D treatment for osteoporosis, it would be worth evaluating the long-term effects of this therapy on immunity.

Restoration of Telomerase Activity

CD8+ T cells show the greatest **telomere length erosion**, a sign of proliferation due to chronic antigenic stimulation (Effros 2004). It is tempting to suggest interventions aimed at restoring the telomere repeats. An obvious approach could be telomerase gene therapy. However, there are multiple problems with this approach including

eventual carcinogenesis. Recently, a study using natural product-derived telomerase activator, TA-65, in human, has shown removal of senescent CD28- CD8 T cell (Harley et al. 2010). Another emerging therapeutic concept is to use non-hydrolyzed forms of naturally occurring imidazole peptide-based compounds, carnosine and carcinine. This would slow down the rate of telomere shortening in specific tissues (Babizhayev et al. 2014). Potential adverse effect of such intervention includes the additional risk of maintaining old "senescent" cells artificially rejuvenated, which may unbalance the already fragile homeostasis in elderly. It remains to be determined if such approaches will ever occur in human.

Conclusion

Age-specific T cell homeostasis is highly dependent on the T cell survival of pre-existing pool. Because aging affects thymic production, intrinsic T cell properties, and survival factors, age-related T cell homeostasis is obviously highly altered and may limit the efficiency of immune responses in elderly. Interestingly, **homeostatic regulation** appears to be essentially preserved during aging although adapted to the novel equilibrium provided in aged individuals: reduction of thymic export and constant stimulation of naïve (and memory) T cells leading to a skewing toward memory T cell pools. The **preservation of the naïve T cell pool** may constitute a main criterion of healthy aging, preserving diversity of T cell responses and reflecting low bystander activation.

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