Mechanisms of naive CD4+ T cell maintenance in the elderly and its implications for Autoimmunity

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Mechanisms of Naive CD4+ T Cell Maintenance in the Elderly and Its Implications for Autoimmunity

Kornelis S. M. van der Geest, Elisabeth Brouwer, W. H. Abdulahad, and Annemieke M. H. Boots

Abstract

CD4+ T cells are critical players in the immune system. CD4+ T cells coordinate both innate and adaptive immune responses. When naive CD4+ T cells become activated via their antigen-specific T cell receptor in the presence of costimulation, these cells differentiate into effector and memory T cells. Maintenance of a large and diverse naive CD4+ T cell repertoire over time is thus essential for developing immunity to a multitude of novel antigens. Despite a strong decline in thymic production of naive CD4+ T cells with aging, the circulating pool of naive CD4+ T cells is well-maintained in elderly humans. The preservation of naive CD4+ T cells in aged subjects contrasts sharply with that of naive CD8+ T cells, which decline markedly with age. In the current chapter, the mechanisms facilitating the remarkable maintenance of the naive CD4+ T cell pool with age are discussed. Important mechanisms include recognition of self-peptides by T cell receptors and stimulation by homeostatic cytokines, including interleukin-7 and interleukin-2. Furthermore, we address the implications of naive CD4+ T cell maintenance for the development of autoimmune diseases in the elderly. Lastly, two models for the development of aging-associated autoimmunity are proposed, and suggestions for further investigation are provided.

Keywords

Naive CD4+ T cell • Homeostasis • T cell receptor • Interleukin-2 • Interleukin-7 • Thymus • Autoimmunity • Rheumatoid arthritis • Giant cell arteritis

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Contents

Introduction

T cells are critical players in the immune system. CD4+ T cells orchestrate both innate and adaptive immune responses, whereas CD8+ T cells kill infected host cells and tumor cells. A critical step in the activation of T cells is the recognition of specific antigens by their T cell receptor (TCR). A large and diverse TCR repertoire is required to develop T cell immunity against a wide variety of antigens (Nikolich-Zugich et al. 2004). Naive T cells represent a broad and diverse reservoir of thymus-matured T cells that have not yet been recruited into an immune response. The TCR repertoire of naive T cells is therefore less skewed than that of memory T cells (Baum et al. 2012; van der Geest et al. 2015a). Thus, the size of the naive T cell pool determines the ability to mount a T cell response to a myriad of novel antigenic challenges.

As the overall human life expectancy has increased dramatically over the last decade (United Nations 2013), the naive T cell pool has to be maintained over a much longer period of time than before. A factor complicating this maintenance is the loss of thymic function early in life (Fig. 1). In the thymus, T cell progenitors from the bone marrow undergo maturation and become functional, naive T cells. As the thymus is progressively replaced by fat, the production of naive T cells declines sharply after puberty (den Braber et al. 2012). Remarkably, numbers of naive CD4+ T cells remain fairly stable throughout life (Fig. 1) (van der Geest et al. 2015b; Wertheimer et al. 2014). The preservation of naive CD4+ T cells contrasts sharply with that of naive CD8+ T cells, which are nearly absent by the age of 80 (Fig. 1) (Vescovini et al. 2014; Wertheimer et al. 2014).

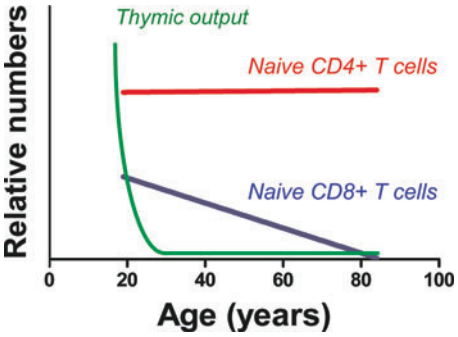

Fig. 1 Effect of age on thymic output and naive T cell numbers. Schematic illustration showing the relative size of thymic output and the circulating pool of naive CD4+ and CD8+ T cells at different ages. As thymic output markedly declines in adolescents, naive CD8+ T cell numbers progressively decline with age. In contrast, naive CD4+ T cell numbers are maintained on to high age

In the current chapter, we will discuss the mechanism responsible for this remarkable preservation of naive CD4+ T cells in the elderly. As the maintenance of T cells differs substantially between mice and men (den Braber et al. 2012), we will focus on findings in human studies, unless stated otherwise. Furthermore, we will address how maintenance of the naive CD4+ T cell pool might contribute to development of autoimmune diseases in the elderly.

Defining Naive CD4+ T Cells

In humans, naive CD4+ T cells are typically identified by their characteristic expression of various surface molecules (Fig. 2). Naive CD4+ T cells express CD45RA, CD27, CD28, and CCR7 on their surface and are able to produce interleukin-2 (Akbar and Henson 2011; Appay et al. 2008). In contrast, naive CD4+ T cells lack expression of CD45RO, CXCR3, CCR4, and CCR6 and are incapable of producing effector cytokines such as IFN-γ, interleukin-4, and interleukin-17, which are characteristic for T helper 1, T helper 2, and T helper 17 cells, respectively. In the vast majority of studies, naive CD4+ T cells have been identified by different combinations of two or more surface markers, i.e., CD45RA +CCR7+ CD4+ T cells, CD45RO-CCR7+ CD4+ T cells, or CD45RA+CD27+ CD4 + T cells. Notably, each of these strategies seems interchangeable to the others (Appay et al. 2008).

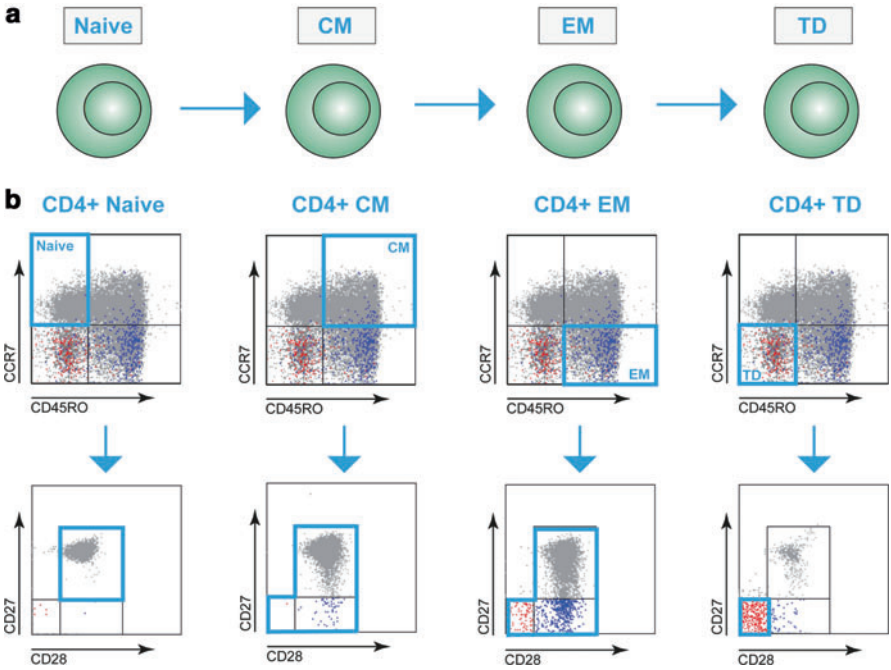

Fig. 2 Phenotype of naive CD4+ T cells. (**a**) Schematic illustration of distinct differentiation subsets of mature CD4+ T cells. (**b**) Flow cytometric gating strategies to identify distinct CD4+ T cell differentiation subsets in peripheral blood of humans. Naive CD4+ T cells are identified as CD45RO-CCR7+CD27+CD28+ CD4+ T cells. *CM* central memory, *EM* effector memory, *TD* terminally differentiated

Lifelong Preservation of the Naive CD4+ T Cell Pool

Decreased Production of Novel CD4+ T Cells by Primary Lymphoid Organs

The production of naive CD4+ T cells declines substantially early in life. Aging-associated changes in the primary lymphoid organs, i.e., the bone marrow and thymus, are responsible for this decline. Aging of hematopoietic stem cells and the stromal niche in the bone marrow result in decreased output of T cell progenitors (Geiger et al. 2013). Moreover, substantial and progressive replacement of the thymus by fat becomes the most important factor limiting the production of naive CD4+ T cells in adults. This process, termed thymic involution, results in a profound decrease of thymic output from an estimated 16 million cells per day in young adults to less than 1 million above the age of 60 (Westera et al. 2015). Therefore, the contribution of the thymus to the maintenance of the human naive CD4+ T cell pool is limited throughout adult life (Fig. 1). Instead, alternative mechanisms are required to preserve a large and diverse naive CD4+ T cell pool in the elderly.

Homeostatic Proliferation and Survival of Naive CD4+ T Cells in the Periphery

Despite the loss of thymic output, aging by itself is not associated with a decrease of naive CD4+ T cells (Fig. 1) (van der Geest et al. 2015b; Wertheimer et al. 2014). So how can the naive CD4+ T cell pool be maintained for multiple decades, if it is hardly replenished by novel CD4+ T cells from the thymus? It appears that this maintenance is facilitated by slow proliferation and long-term survival of naive CD4+ T cells in the periphery (Fig. 3). An estimated 100 million of naive CD4+ T cells are produced daily by peripheral proliferation in young adults (den Braber et al. 2012; Westera et al. 2015). In the elderly, the naive CD4+ T cell pool becomes less dynamic, and the rate of peripheral proliferation decreases accordingly (Bains et al. 2009). Consequently, a daily production of only 40 million naive CD4+ T cells in the periphery may suffice to maintain the naive CD4+ T cell pool in elderly subjects (Fig. 3) (Westera et al. 2015). Naive CD4+ T cells also persist due to low turnover rates and their relatively long life span of 6 years (den Braber et al. 2012; Vrisekoop et al. 2008; Westera et al. 2015). Although it remains unclear why the naive CD4+ T cell pool becomes less dynamic with age, it is likely that progressive development of immunological memory and the associated broadening of TCR diversity in the memory compartment over time reduce the need to recruit new specificities from the naive T cell repertoire to the effector compartment.

Although age by itself does not alter naive CD4+ T cell numbers in adults, other factors might. Heritable factors appear to strongly influence the maintenance of the naive CD4+ T cell pool in adult humans (Brodin et al. 2015). Gender may partly explain the heritable variability in naive CD4+ T cell numbers. Women maintain higher numbers of naive CD4+ T cells than men (van der Heiden et al. 2016). This gender effect could be the result of differences in sex hormones (Giefing-Kroll et al. 2015). Some evidence indicates that major histocompatibility complex (MHC) genes are another heritable trait influencing maintenance of the naive CD4+ T cells in humans (Schonland et al. 2003). Cytomegalovirus (CMV) infection represents a nonheritable factor with profound effects on adaptive immunity (Sansoni et al. 2014). Although CMV imposes marked effects on the memory T cell pool, its effect on the naive CD4+ T cell pool is subtle (Wertheimer et al. 2014). CMV is associated with a gradual decrease of naive CD4+ T cells late in life only (van der Geest et al. 2015b; Wertheimer et al. 2014). In young subjects, naive CD4+ T cell numbers are similar in CMV seropositive and seronegative individuals. In contrast, aged subjects do not fully maintain their naive CD4+ T cell pool in the presence of a latent CMV infection. The exact cause of this difference remains unknown. So far, no other major factors have been reported to influence the maintenance of naive CD4+ T cells in adults.

It is noteworthy that thymic involution has a more dramatic impact on the maintenance of the naive CD8+ T cell pool in elderly (Fig. 1) (van der Geest et al. 2015b; Vescovini et al. 2014; Wertheimer et al. 2014). By the age of 80, the naive CD8+ T cell pool is nearly absent (van der Geest et al. 2015b). Several findings might explain this discrepancy between naive CD4+ and CD8+ T cells. Peripheral

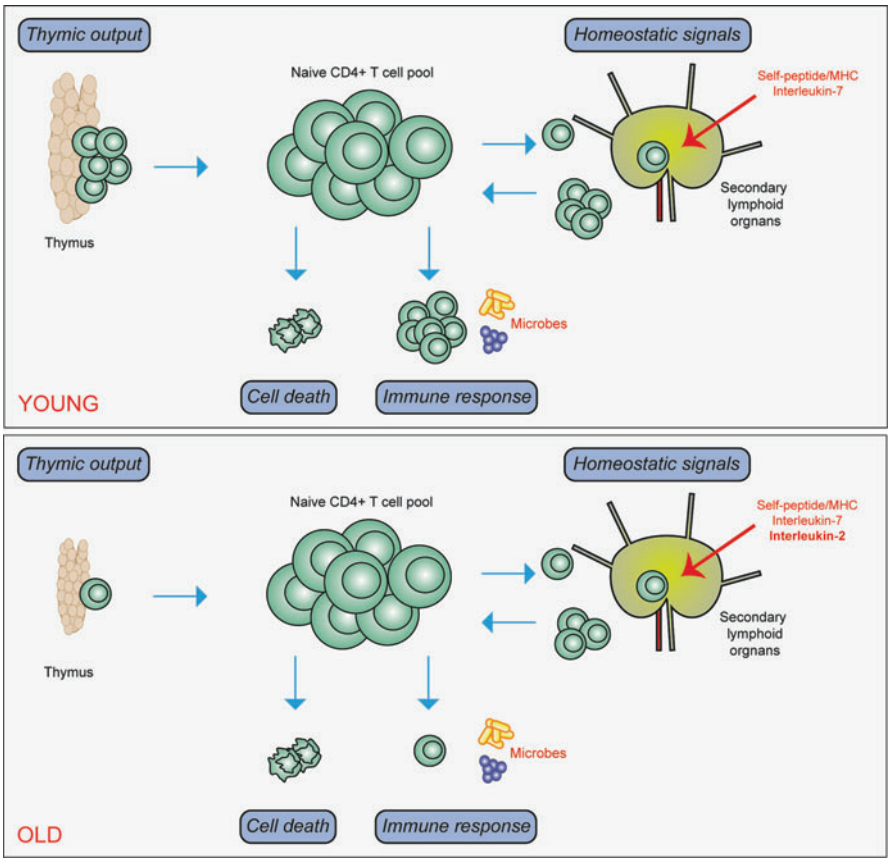

Fig. 3 Homeostasis of naive CD4+ T cells in young and old humans. Schematic illustration of naive CD4+ T cell homeostasis in young subjects (upper panel) and old subjects (lower panel). Briefly, thymic output is high at young age, whereas homeostatic proliferation already contributes markedly to maintenance of the naive CD4+ T cell pool. This homeostatic proliferation is induced by cognate and cytokine triggers in secondary lymphoid organs. Naive CD4+ T cells are lost due to cell death or recruitment toward immune responses against microbes. In contrast, thymic output is limited at old age, and preservation of the naive CD4+ T cell pool in the elderly primarily relies on homeostatic proliferation. Interleukin-2 (IL-2) also becomes important for the homeostasis of naive CD4+ T cells. Although homeostatic proliferation decreases with age, less production of naive CD4+ T cells is needed, since less naive CD4+ T cells are recruited to the memory compartment during antimicrobial immune responses due to existing immunological memory. Consequently, the size of the naive CD4+ T cell pool remains stable over time

turnover rates are higher for naive CD8+ T cells than for naive CD4+ T cells in elderly subjects (Westera et al. 2015). Furthermore, recruitment of naive CD8+ T cells toward the effector and memory compartments during immune responses might be more profound than recruitment of naive CD4+ T cells (van der Geest et al. 2015a). Indeed, CD8+ T cell responses are characterized by more proliferation and expansion than CD4+ T cell responses. Expansion of CD8+ T cells may be more

critical, as these cells directly provide effector functions, i.e., killing of target cells. In contrast, modestly expanded CD4+ T cells might suffice to orchestrate a multitude of other immune cells that will perform the actual effector functions. In addition, animal studies indicate that naive CD8+ T cells, but not naive CD4+ T cells, can readily adopt a memory phenotype upon exposure to cytokines in the absence of TCR stimulation (Cho et al. 2007; Kamimura and Bevan 2007). However, cytokine-induced differentiation of naive CD8+ T cells has not been observed during in vitro studies with human cells (Geginat et al. 2003). Nevertheless, these findings imply that naive CD8+ T cells may expire over time and underline that the preservation of naive CD4+ T cells in aged humans is truly remarkable.

Mechanisms Driving the Peripheral Maintenance of Naive CD4+ T Cells

Substantial effort has been made to understand the mechanisms facilitating the peripheral proliferation and survival of naive T cells in animals. Two types of signals appear to promote the peripheral maintenance of naive T cells in mice: (1) low-affinity TCR engagement by self-peptide/major histocompatibility complexes (MHC) and (2) cytokines of the common γ-chain signaling family, in particular interleukin-7 (IL-7). The current insights obtained from these animal studies are thoroughly reviewed elsewhere (Boyman et al. 2009; Surh and Sprent 2008; Takada and Jameson 2009). Here, we will discuss evidence that similar mechanisms (Fig. 3) may exist in humans.

Evidence for TCR-Dependent Maintenance of Naive CD4+ T Cells

Direct evidence for a role of self-peptide/MHC complexes in naive CD4+ T cell homeostasis is lacking, as transfer of naive CD4+ T cells to MHC-deficient humans is limited by practical and ethical constraints. Similar constrains may also limit transfer of modified naive CD4+ T cells in which expression of the TCR has been silenced. Nevertheless, indirect evidence indicating that self-peptide/MHC complexes also promote the long-term maintenance of naive CD4+ T cells in humans is substantial.

The first evidence came from the observation that naive CD4+ T cells progressively lose expression of PECAM-1 (CD31) with age (Fig. 4a) (Kimmig et al. 2002). CD31 is regarded as a marker of naive CD4+ T cells that have recently egressed from the thymus. These CD31+ naive CD4+ T cells contain high numbers of T cell receptor excision circles (TRECs) (Kimmig et al. 2002). TRECs are circular DNA products created during T cell maturation in the thymus. TRECs are not degraded but are progressively diluted among daughter cells during cell division. Therefore, high TREC content of CD31+ naive CD4+ T cells is suggestive of a limited number of cell divisions, which implies that this cell population consists of recent thymic emigrant cells. CD31 expression by these recent thymic emigrants, however, is

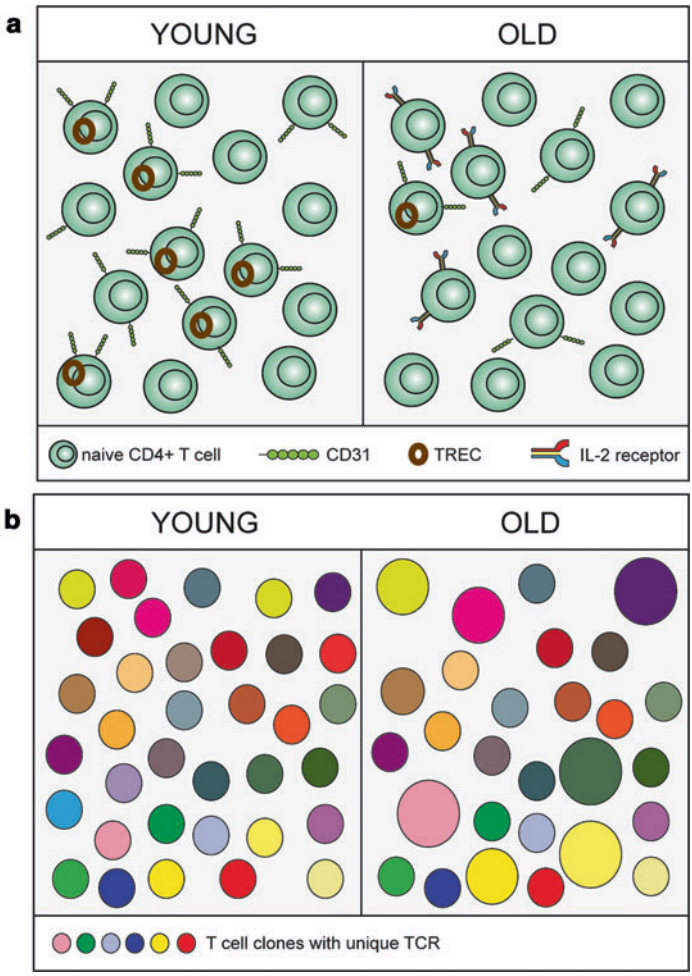

Fig. 4 Signs of TCR engagement in aged naive CD4+ T cells. (**a**) Schematic illustration of naive CD4+ T cells in young subjects (left panel) and old subjects (right panel). In young subjects, CD31+ recent thymic emigrant cells with high T cell receptor excision circles (TRECs) are present, and naive CD4+ T cells lack expression of the IL-2 receptor. In old subjects, CD31+ recent thymic emigrants are decreased and the TREC content of these cells also decreases. Part of the naive CD4+ T cells show dim expression of the IL-2 receptor. (**b**) Schematic illustration showing impact of age on clonal size and diversity within the naive CD4+ T cell compartment. Clonal size reflects the number of cells that are derived from one naive T cell clone and share an identical TCR. In young subjects (left panel), clonal size is comparable among different naive CD4+ T cell clones. In old subjects (right panel), some clones are lost, whereas others expand

lost upon TCR stimulation (Demeure et al. 1996; Kohler et al. 2005; van der Geest et al. 2015b). By the age of 60, the majority of naive CD4+ T cells show evidence of prior TCR engagement, as indicated by loss of CD31 expression (Kohler et al. 2005). Furthermore, it is possible that at that age a substantial part of the CD31+

naive CD4+ T cell population has also undergone TCR stimulation, as CD31 is only gradually lost upon TCR engagement (Demeure et al. 1996; van der Geest et al. 2015b). TCR engagement-dependent accumulation of CD31- naive CD4+ T cells is further suggested by their skewed TCR repertoire in comparison to CD31+ naive CD4+ T cells (Kohler et al. 2005; van der Geest et al. 2015a). TCR-dependent homeostatic proliferation of naive CD4+ T cells is implied by the direct relation between CD31 loss and proliferation rates of these cells (Sauce et al. 2012).

A second indication for TCR-dependent maintenance of naive CD4+ T cells is their progressive acquisition of the interleukin-2 (IL-2) receptor with age (Fig. 4a). Although naive CD4+ T cells in young subjects lack this receptor, a significant proportion of naive CD4+ T cells show dim expression of the IL-2 receptor in aged subjects (Pekalski et al. 2013; van der Geest et al. 2015b). Despite this low per-cell expression level, the IL-2 receptor is fully functional and allows these naive CD4+ T cells to respond to IL-2 (van der Geest et al. 2015b). Naive CD4+ T cell acquires this receptor upon low-affinity TCR engagement in human secondary lymphoid tissues in the absence of costimulation (van der Geest et al. 2015b). As mentioned further on, IL-2 stimulation may further promote the maintenance of these naive CD4+ T cells in elderly subjects.

A third finding that indicates TCR-dependent homeostasis of naive CD4+ T cells is the progressive, aging-associated skewing of the TCR repertoire in this cell population. The number of different naive CD4+ T cell clones, each carrying their own specific TCR, declines modestly with age. This decrease is mirrored by an aging-associated expansion of individual CD4+ T clones (Fig. 4b) (Qi et al. 2014). Consequently, the naive CD4+ T cell repertoire becomes substantially skewed after the age of 65 (Johnson et al. 2012; Naylor et al. 2005). As the TCR is the defining feature of these T cell clones, TCR-dependent signaling thus seems to impact the maintenance of a selected repertoire of naive CD4+ T cell clones in the elderly.

Low-Affinity Self-Peptide/MHC Complexes Promote Naive CD4+ T Cell Maintenance

So which peptides presented by MHC molecules on antigen-presenting cells promote the TCR-mediated preservation of naive CD4+ T cells? Animal studies strongly indicate that self-peptides are critical for the homeostasis of naive T cells and that these self-peptide/MHC complexes are recognized with low affinity by the TCRs. This low-affinity interaction may promote the homeostasis of naive T cells without these cells losing their naive phenotype (Ernst et al. 1999; Goldrath and Bevan 1999; Viret et al. 1999). Although direct evidence is lacking in humans, it is likely that self-peptides are also involved in the TCR-dependent maintenance of human naive CD4+ T cells.

Peripheral proliferation of naive CD4+ T cell is a continuous process occurring during steady state, i.e., in the absence of clinically overt infections (den Braber et al. 2012). An important role for microbial peptides in the homeostasis of naive CD4+ T cells is unlikely, since such microbial peptides (i.e., signal 1) are typically

presented by activated antigen-presenting cells that also provide costimulatory signals (i.e., signal 2). In combination, these two signals induce full differentiation of naive T cells into effector or memory cells (Fig. 5a) (van der Geest et al. 2015b). In contrast, presentation of self-peptide/MHC complexes typically occurs in the absence of costimulation. Therefore, these self-peptide/MHC complexes may promote the homeostasis of their cognate naive CD4+ T cells without inducing an effector or memory phenotype in these cells (Fig. 5b). Although animal studies advocate that naive CD4+ T cell may adopt a state of adaptive tolerance upon TCR stimulation (Fig. 5c), it remains unclear whether adaptive tolerance also exists in humans (Fathman and Lineberry 2007; Schwartz 2003). Furthermore, the factors governing the eventual response to TCR stimulation, i.e., induction of homeostatic proliferation or adaptive tolerance, remain unknown. Adaptive tolerance of naive T cells should be discriminated from clonal T cell anergy that primarily occurs when memory T cells receive TCR stimulation only (Fig. 5d). Whereas adaptive tolerance wanes after removal of self-antigen/MHC complexes, clonal T cell anergy is deeply imprinted in memory T cells and persists long after removal of the TCR stimulus (Fathman and Lineberry 2007; Schwartz 2003).

The recognition of self-peptide/MHC complexes by naive CD4+ T cells in humans likely occurs with low affinity. Indeed, low-affinity recognition of self-peptide/MHC complexes is already a critical step during the maturation and selection of naive CD4+ T cells in the thymus. In other words, naive CD4+ T cells egressing from the thymus are living proof that their TCR can engage in low-affinity binding with self-peptide/MHC complexes. In accordance with this low-affinity recognition of self-peptides, naive CD4+ T cells express low levels of CD5 on their surface, both in humans and in mice (Mandl et al. 2013; van der Geest et al. 2015b). CD5 is a negative regulator of TCR signaling, and its density on T cells is directly proportional to their affinity for peptide/MHC complexes in mice and likely in humans as well (Azzam et al. 1998; Mandl et al. 2013).

Tickling of the Naive TCR Occurs in Secondary Lymphoid Tissues

By virtue of expression of the chemokine receptor CCR7, naive CD4+ T cells can migrate from the blood toward secondary lymphoid organs, i.e., the lymph nodes and spleen. Of interest, naive CD4+ T cell numbers in the peripheral blood are comparable to those in secondary lymphoid organs (Sathaliyawala et al. 2013; Thome et al. 2014; van der Geest et al. 2015b). Although naive CD4+ T cells lack expression of the activation marker CD69 in the blood, these cells exhibit marked CD69 expression in secondary lymphoid tissues, in the absence of overt infection (van der Geest et al. 2015b). CD69 expression is a well-known activation marker induced upon TCR stimulation rather than cytokine stimulation (Cimbro et al. 2012; Simms and Ellis 1996). Thus, low-affinity stimulation of naive CD4+ T cells by self-peptide/MHC complexes likely occurs in secondary lymphoid tissues (Fig. 3).

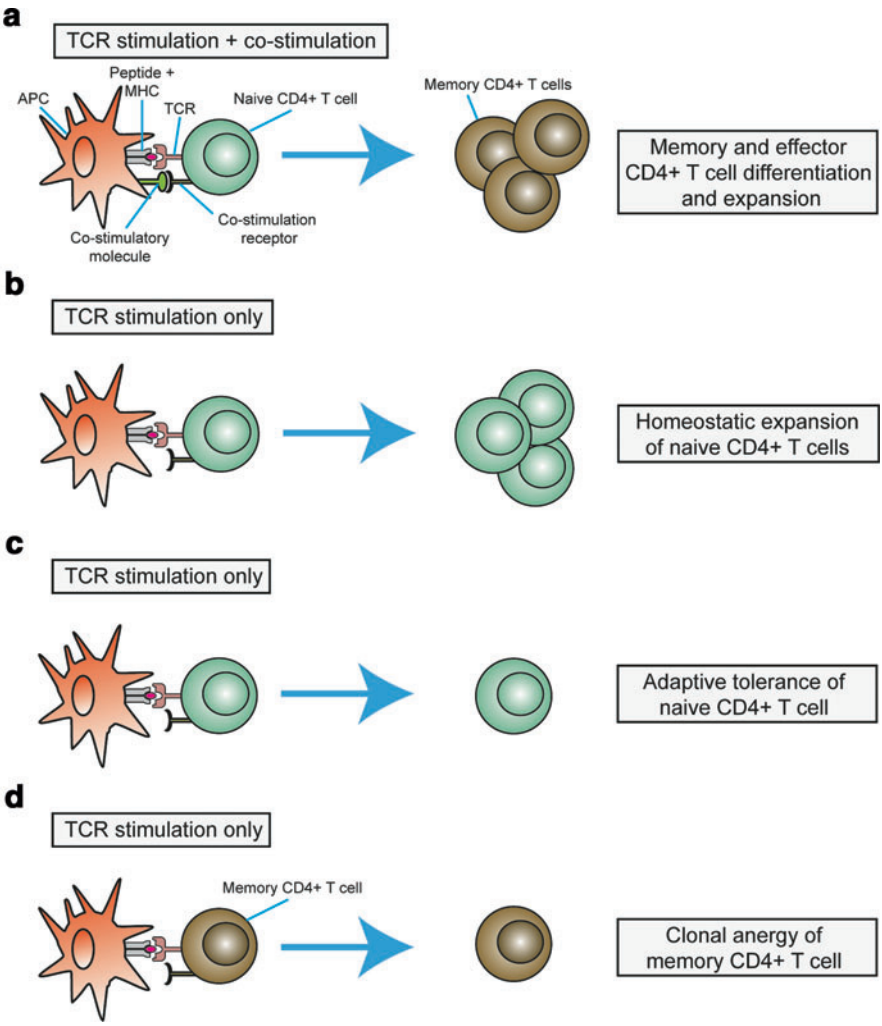

Fig. 5 Fate of naive CD4+ T cells following TCR engagement in the presence and absence of costimulation. (**a**) A naive CD4+ T cell requires two signals for full activation and memory/effector T cell differentiation and proliferation. Signal 1: T cell receptor (TCR) stimulation by a specific peptide/MHC complex. Signal 2: costimulation provided by a costimulatory molecule on the surface of an antigen-presenting cell (APC). (**b**) A naive CD4+ T cell receives TCR stimulation from an APC in the absence of costimulation. This TCR engagement promotes the homeostatic proliferation of the naive CD4+ T cell. (**c**) Animal studies indicate that naive CD4+ T cells receiving TCR stimulation may also adopt a temporary state of adaptive tolerance. This state is associated with a decreased potential to proliferate and produce cytokines. Removal of the TCR trigger results in discontinuation of adaptive tolerance. (**d**) Clonal T cell anergy represents a state of hyporesponsiveness occurring in memory T cells receiving TCR stimulation only. This state of anergy is deeply imprinted and persists after removal of the TCR trigger

Downstream Effects of TCR Signaling in Naive CD4+ T Cells

Both in mice and men, the exact downstream effects linking TCR signaling to proliferation and survival are poorly understood. Nevertheless, some mechanisms have been identified in recent years. Firstly, TCR engagement appears to be a prerequisite for IL-7-mediated proliferation of part of the naive CD4+ T cells (Azevedo et al. 2009). Both TCR signaling and IL-7 receptor signaling activate the PI3K/AKT/mTOR pathway resulting into proliferation and Bcl-2-dependent survival (Fig. 6a). Secondly, TCR stimulation results in expression of the complete IL-2 receptor which promotes IL-2-mediated proliferation and survival of these cells (Fig. 6a). Thirdly, some findings suggest that TCR stimulation by itself may induce proliferation and survival of naive CD4+ T cells. For instance, TCR stimulation induces upregulation of microRNA-21 (miR-21) in naive CD4+ T cells (Teteloshvili et al. 2015b), and expression of miR-21 prevents apoptosis of naive CD4+ T cells (Fig. 6a) (Smigielska-Czepiel et al. 2013). As expected, miR-21 levels are typically higher in CD31- naive CD4+ T cells than in CD31+ naive CD4+ T cells (Teteloshvili et al. 2015a). Overall, more studies linking low-affinity TCR stimulation to proliferation and survival of naive CD4+ T cells are required to identify the pathways/signals involved.

Cytokines Influencing the Homeostasis of Naive CD4+ T Cells

In addition to TCR stimulation, cytokines have been implied in the homeostasis of naive T cells. In particular cytokines of the common γ-chain family appear to promote the proliferation and survival of T cells. These include IL-7, as well as IL-2 and interleukin-15 (IL-15). These cytokines typically result into activation of the JAK-STAT pathway, thereby promoting proliferation and Bcl-2-dependent survival of these T cells (Fig. 6a) (Rochman et al. 2009). The common γ-chain is universally expressed by all T cells, and the sensitivity to these three cytokines relies on the presence of additional receptor chains: the IL-7 receptor α-chain (CD127) for IL-7, the IL-2 receptor α-chain (CD25) and IL-2 receptor β-chain (CD122) for IL-2, and the IL-15 receptor α-chain (CD215) and IL-2 receptor β-chain for IL-15 (Fig. 6b) (Boyman et al. 2009). Of these three cytokines, IL-7 was long regarded as the only cytokine relevant for the maintenance of naive CD4+ T cells (Mackall et al. 2011). Recent studies, however, indicate that IL-2 becomes important in the maintenance of naive CD4+ T cells of aged humans (Pekalski et al. 2013; van der Geest et al. 2015b).

Interleukin-7 Induces Proliferation and Survival of Naive T Cells

IL-7 promotes both the proliferation and survival of naive CD4+ T cells. IL-7-mediated signaling activates STAT5 which leads to upregulation of the antiapoptotic factor Bcl-2 (Fig. 6a), thereby preventing apoptosis of both CD31+ and CD31- naive

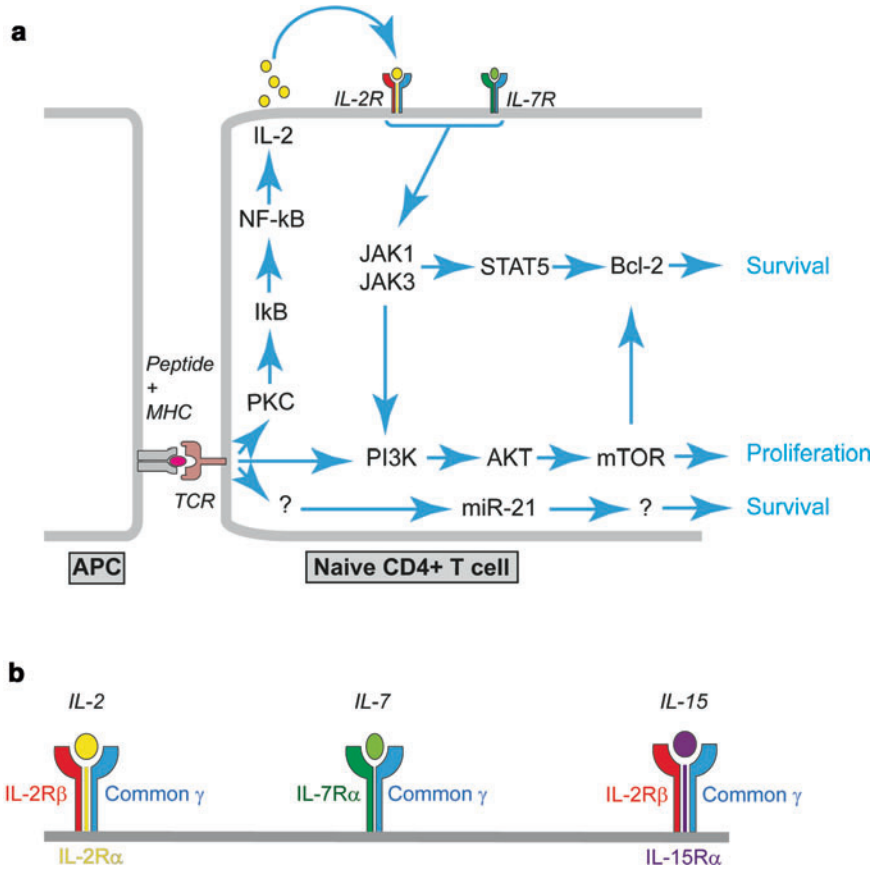

Fig. 6 Signaling pathways and receptors involved in the homeostasis of naive CD4+ T cells. (**a**) Schematic illustration showing intracellular signals provided by TCR stimulation and IL-2 receptor or IL-7 receptor stimulation and their interactions. These signals result into enhanced survival and proliferation of naive CD4+ T cells. (**b**) Receptor units required for IL-2, IL-7, and IL-15 signaling

CD4+ T cells (Azevedo et al. 2009). Sustained exposure of naive CD4+ T cells to IL-7 is required to make these relatively quiescent cells proliferate (Swainson et al. 2006). IL-7 by itself can induce marked proliferation of CD31+ naive CD4+ T cells in a PI3K-dependent manner (Fig. 6a), but not of CD31- naive CD4+ T cells (Azevedo et al. 2009). Although the exact cause of this difference is unknown, it explains why naive CD4+ T cells in umbilical cord blood, which are nearly all CD31+ naive CD4+ T cells, proliferate more substantially upon IL-7 stimulation than naive CD4+ T cells in adults (Soares et al. 1998).

Important insight into the role of IL-7 for naive CD4+ T cell homeostasis has been obtained from clinical trials with recombinant IL-7. Administration of recombinant IL-7 induces profound proliferation and Bcl-2 expression in circulating naive CD4+ T cells (Levy et al. 2012; Sportes et al. 2008). IL-7 treatment also increases the

remaining thymic production of naive CD4+ T cells, as indicated by broadening of the T cell receptor repertoire and an increase of T cell receptor excision circles (Levy et al. 2012; Sportes et al. 2008). Thus, IL-7 can exert profound effects on the homeostasis of naive CD4+ T cells.

Conflicting data have been reported on the availability of IL-7 in aged humans. IL-7 is constitutively produced in lymphoid tissues, and substantial levels can be measured in plasma. Various groups have found that plasma levels of IL-7 decrease with age (Banerjee et al. 2011; Kang et al. 2004), whereas others found no change (Ferrando-Martinez et al. 2011) or actually an increase of IL-7 (Sauce et al. 2012). Currently, it is unclear if plasma levels of IL-7 correlate with naive CD4+ T cell numbers in the elderly. Furthermore, it is unknown whether aging affects local concentrations of IL-7 in secondary lymphoid structures.

Interleukin-2 Promotes Maintenance of Naive CD4+ T Cells in the Elderly

The importance of IL-2 for the preservation of memory T cells and regulatory T cells is well documented (Boyman and Sprent 2012). In contrast, interleukin-2 was long thought irrelevant for the homeostasis of naive T cells (Boyman et al. 2009), until two studies challenged this notion by demonstrating the importance of IL-2 for naive CD4+ T cell maintenance in aged humans (Pekalski et al. 2013; van der Geest et al. 2015b). It appears that a significant proportion of naive CD4+ T cells having gained dim expression of a complete IL-2 receptor, develop and accumulate with aging in humans. Functionality of this receptor was demonstrated by increased STAT5 activation upon IL-2 stimulation. As a combination of the IL-2 receptor β-chain and common γ-chain also allows for some IL-15-mediated signaling, it is not surprising that this IL-2 receptor expressing naive CD4+ cells could also respond slightly to IL-15 stimulation (Pekalski et al. 2013; van der Geest et al. 2015b). Thus, IL-2 stimulation likely induces the proliferation and survival of these naive CD4+ T cells (Fig. 6a) (van der Geest et al. 2015b).

IL-2 can be detected in plasma of healthy individuals, and its plasma levels remain stable with age (Kim et al. 2011). In contrast to IL-7, which is constitutively produced in lymphoid tissues, IL-2 is produced by T cells upon their activation. Importantly, naive CD4+ T cells themselves are capable of producing IL-2 (Kimmig et al. 2002; van der Geest et al. 2015b). Therefore, TCR stimulation may facilitate an autocrine loop of IL-2 production promoting the maintenance of naive CD4+ T cells in aged humans (Fig. 6a).

Implications for Aging-Associated Autoimmune Diseases

Maintenance of a large and diverse TCR repertoire may be critical for effective immunity against microbes and tumor cells in the elderly (Gorochov et al. 1998; Manuel et al. 2012; Messaoudi et al. 2002; Saurwein-Teissl et al. 2002; Wang et al.

2012). This topic has been extensively reviewed by others (Goronzy and Weyand 2013; Nikolich-Zugich et al. 2004). Similarly, a diverse naive CD4+ T cell repertoire may also be important for development of autoimmune diseases in the elderly, a notion which is less well explored. In the remainder of this chapter, we will focus on the relation between naive CD4+ T cell maintenance and aging-associated, autoimmune diseases.

Aging-Associated Autoimmune Diseases Occur Long After Thymic Involution

Aging is associated with development of autoimmunity and autoimmune diseases. Increased autoimmunity is indicated by appearance of antinuclear antibodies and rheumatoid factors in sera of otherwise healthy, elderly subjects (Nisihara et al. 2013; van der Geest et al. 2015c; van Schaardenburg et al. 1993). In general, the risk for developing an autoimmune disease increases with age (Crowson et al. 2011). This is especially true for CD4+T cell-driven autoimmune diseases, such as rheumatoid arthritis and giant cell arteritis (McInnes and Schett 2011; Weyand and Goronzy 2013). The importance of CD4+ T cells in these diseases is substantiated by their presence at the sites of inflammation, as well as the association of these diseases with certain HLA-DR4 alleles. Although the exact pathogenic autoantigens presented by these MHC molecules remain unknown in both diseases, an array of citrullinated peptides appears to be recognized by CD4+ T cells in rheumatoid arthritis (Firestein 2003). Interestingly, both diseases typically develop many decades after thymic involution has occurred (Crowson et al. 2011).

Although not extensively explored, maintenance of a large and diverse naive CD4+ T cell repertoire may be a prerequisite for development of autoimmune diseases. The presence of a diverse naive CD4+ T cell repertoire was critical for development of autoimmunity in an animal study. In this study, limited CD4+ T cell repertoires were unable to induce IgG autoantibody production by autoreactive B cells, whereas combining these limited CD4+ T cell repertoires resulted in a more diverse repertoire that allowed the development of IgG autoantibodies (Busser et al. 2003). The presence of a diverse self-peptide responsive naive CD4+ T cell repertoire may also be important for autoimmune diseases in humans, as the development of full-blown rheumatoid arthritis is associated with progressive autoimmunity against a growing number of self-peptides, i.e., epitope spreading (Arend and Firestein 2012). In accordance with this notion, absolute numbers of naive CD4+ T cells tend to be increased in seropositive arthralgia patients, when compared to healthy controls, and are actually increased in patients that have progressed to full-blown rheumatoid arthritis (Chalan et al. 2013). Notably, some reports indicated that rheumatoid arthritis patients demonstrate decreased percentages of naive CD4+ T cells (Ponchel et al. 2002). This proportional decrease, however, likely reflects the expansion of other CD4+ T cell populations rather than a true decrease of naive CD4+ T cells (Ponchel et al. 2002). Thus, maintenance of a large and diverse naive CD4+ T cell repertoire may enable the development of autoimmune diseases in the

elderly. We will further discuss two models that might explain the origin of autoreactive CD4+ T cell clones that cause aging-associated autoimmune disease long after thymic involution.

Lifelong Maintenance of Autoreactive CD4+ T Cell Clones

The first model focuses on the lifelong maintenance of naive CD4+ T cells in the periphery (Fig. 7a). In this model, naive CD4+ T cell clones that have been produced early in life persist over time and cause autoimmune diseases many years after these cells were produced by the thymus. Mechanisms promoting the maintenance of these cells therefore contribute to the development of autoimmune diseases later in life. So why do these CD4+ T cell clones only cause problems after many years of survival? One proposed explanation is that over time only the naive CD4+ T cells with the highest affinity for self-peptide/MHC complexes become expanded (Goronzy and Weyand 2005). Thus, aging may be associated with positive selection of the most "autoreactive" naive CD4+ T cells in the periphery.

By itself, however, the lifelong selection of naive CD4+ T cells with the highest affinity for self-peptide/MHC complexes may be insufficient for the development of autoimmune disease in the elderly. Although differences in affinity for self-peptide/MHC complexes may exist between naive CD4+ T cells, these cells typically have an affinity that is on the lower end of the spectrum. Strongly autoreactive T cells undergo apoptosis or adopt a regulatory phenotype in the thymus (i.e., negative selection) (Fig. 7a). In addition, the affinity for self-peptide/MHC complexes does not increase over time, as T cells do not undergo affinity maturation. So, how can these naive CD4+ T cells become involved in autoimmune diseases later in life?

Likely, both naive CD4+ T cell intrinsic and extrinsic factors could play a role. One intrinsic factor might be that naive CD4+ T cells become more biased toward Th1 and Th17 differentiation over time (Hendricks and Fink 2011). Another intrinsic factor may be the enhanced sensitivity of naive CD4+ T cells to IL-2 (Pekalski et al. 2013; van der Geest et al. 2015b). Additional stimulation of autoimmune-prone naive CD4+ T cells by IL-2 could enhance the inappropriate activation of these cells in the elderly. A third intrinsic factor might be aging-associated changes of TCR signaling. Although the TCR does not undergo affinity maturation through somatic hypermutation of TCR encoding genes, posttranslational modification of the TCR may impact its affinity for self-peptide/MHC complexes and response to these complexes. Animal studies have shown that glycosylation represents an important form of posttranslational modification that influences the affinity for self-peptide/MHC complexes in the thymus (Zhou et al. 2014). To what extent aging alters posttranslational modifications of TCR in peripheral T cells remains unknown. Animal studies also indicate that aging affects the posttranslational modification of surface molecules that regulate TCR signaling (Garcia et al. 2005). Furthermore, intracellular signaling pathways downstream of the TCR may also change with age (Fulop et al. 2003). Extrinsic factors may include loss of peripheral tolerance by regulatory B and T cells or enhanced activation of naive CD4+ T cells by the aging-

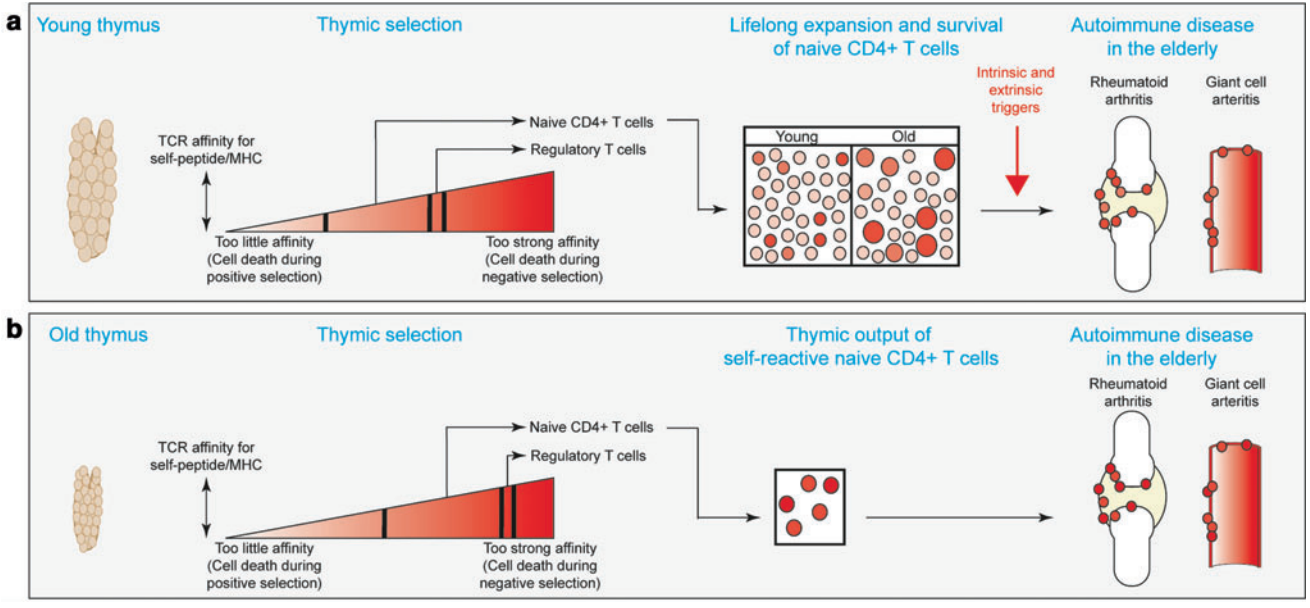

Fig. 7 Two models explaining how autoimmune disease develops long after thymic involution. (**a**) In the first model, naive CD4+ T cells are produced early in life and maintained on to high age before these cells become involved in autoimmune responses. During their maturation in the young thymus, immature T cells are selected based on TCR affinity for self-peptide/MHC complexes. Both no/too little affinity and too much affinity result into cell death. Cells with low/intermediate affinity for self-peptide/MHC complexes are positively selected. Part of the cells with intermediate affinity become dedicated to the regulatory T cell lineage. The remaining cells with low but sufficient affinity develop into naive CD4+ T cells. These naive CD4+ T cells are than preserved for decades in the periphery. Over time, naive CD4+ T cell clones with the highest affinity for self-peptide/MHC complexes will selectively expand. Eventually, a combination of intrinsic and extrinsic triggers stimulates these naive CD4+ T cell clones, thereby promoting the development of autoimmune disease. (**b**) In the second model, limited output of naive CD4+ T cells by the aged thymus is responsible for development of aging-associated autoimmune diseases. In this model, the thymic selection of naive CD4+ T cells is disturbed. Cells with relatively higher affinity for self-peptide/MHC complexes are allowed to mature and enter the circulation. These autoimmune-prone CD4+ T cells then promote the development of autoimmune diseases in aged subjects

associated inflammatory environment, i.e., *inflammaging* (Baylis et al. 2013; Duggal et al. 2013; Franceschi et al. 2007; van der Geest et al. 2015c). Both aging-associated damage in tissues and adaptation of a senescence-associated secretory phenotype by aged stromal cells may drive the *inflammaging* process in the elderly. Thus, aging-associated triggers may be required to gear up peripherally maintained naive CD4+ T cells toward autoimmune responses in the elderly.

Autoreactive T Cells from the Thymus of Aged Subjects

In the second model, strongly autoreactive T cells are produced by remnants of the aged thymus (Fig. 7b). Indeed, a limited daily output of naive CD4+ T cells persists long after thymic involution (den Braber et al. 2012). Currently, data from human studies supporting or contradicting this model are lacking. However, some evidence from animal studies suggests that central tolerance is indeed disturbed in the aged thymus. Thymic epithelial cells of old mice show decreased expression of the autoimmune regulator (AIRE) gene, which is required for the negative selection of autoreactive T cells in the thymus (Coder et al. 2015). Furthermore, naive T cells produced by the thymus of aged mice show increased affinity for self-peptide/MHC complexes, as indicated by higher levels of CD5 expression (Deshpande et al. 2015). Thus, autoimmune diseases in aged humans may potentially be caused by autoreactive clones produced by the aged thymus.

Future Directions

To date, evidence for both models is limited and additional studies are needed. To study the first model, it would be interesting to investigate the naive CD4+ T cell repertoire before onset of autoimmune disease, for instance, in patients with seropositive arthralgia who are at risk for developing rheumatoid arthritis. So do patients progressing to full-blown autoimmune disease have a broader naive CD4+ T cell repertoire? What is the influence of MHC class II molecules on the size of the naive CD4+ T cell repertoire? With respect to the second model, much may be learned from longitudinal study cohorts with children who underwent thymectomy during cardiac surgery. Although at young age these children do not develop more autoimmune diseases than healthy subjects, it would be interesting to see if complete thymectomy may protect against development of CD4+ T cell-driven autoimmune diseases later in life.

Concluding Remarks

The production of naive CD4+ T cells by the thymus declines substantially early in life. Consequently, lifelong maintenance of the naive CD4+ T cell repertoire relies on the proliferation and long-term survival of these cells in the periphery. A complex

and not yet fully understood interplay between low-affinity recognition of self-peptide/MHC complexes and homeostatic cytokines contributes to the preservation of the naive CD4+ T cell pool in aged humans. It is possible that the preserved naive CD4+ T cell pool in the elderly includes naive CD4+ T cell clones that could potentially cause autoimmune diseases later in life. The exact triggers for these clones to cause autoimmune disease remain to be elucidated. Alternatively, autoimmune diseases in elderly subjects may be driven by autoreactive CD4+ T cells produced by the aged thymus in which central tolerance is failing.

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