Regulatory T Cells and the Immune Aging Process: A Mini-Review

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Abstract

Constant exposure to new and persisting antigens and the need to replace cellular attrition with newly built cells lead to profound remodeling of the immune system after the age of 50 years. The impact of the immunosenescence process varies amongst the different cellular subsets represented within the immune system. Emerging data suggest that progressive aging significantly affects frequencies, subset distribution and functional competence of regulatory T cells (Tregs). Given the central role of Tregs in immune homeostasis, age-related loss of Treg function would be predicted to cause excessive immunity, encountered in elderly humans as a syndrome of chronic, smoldering inflammation as well as the age-related increase in the risk for autoimmunity. Conversely, age-dependent gain of Treg activity would result in failing immunity, such as the rising risk of malignancies and infections amongst the elderly. Emerging data suggest that some Treg populations, specifically naturally occurring Tregs, seem to accumulate with advancing age, whereas inducible Tregs appear to be less available in the older host. More studies are necessary to elucidate functional compe-

tence of old Tregs, with an emphasis on comparing the efficacy of young and old Tregs for defined functional domains. Mechanisms of declining Treg inducibility are not understood, but may provide an opportunity for targeted immunomodulation in the elderly. On the horizon is the potential to develop novel therapeutic interventions that target Tregs to make the elderly more efficient in fighting cancers and infections and dampen the risk for senescence-associated inflammation.

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Age and T Lymphocytes

Just like other organ systems, the immune system is susceptible to age-related changes, overall resulting in a deteriorating immune competence and a shift in the balance between protective and pathogenic immune responses. Paradoxically, immune aging is associated with muted immunity co-occurring with low-grade and chronic inflammation [1–3]. The process of immune aging has detrimental consequences for the aging host: suc-

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cumming to otherwise innocuous infectious agents, inability to respond to vaccination [4] and a declining ability to fight cancer [5]. In parallel, advancing age is a risk factor for autoimmunity and is associated with a chronic, smoldering inflammatory syndrome [6]. Cellular senescence has been associated with inflammatory tissue damage through the senescence-associated secretory phenotype (SASP) [7, 8]. While both the innate and adaptive branches of the immune system are affected by age, the changes in the T cell compartment are particularly important and bring considerable challenges to the aging organism. The T cell pool contains a number of well-defined, functionally distinct subsets: CD4⁺ T cells, CD8⁺ T cells, $\gamma\delta$ T cells, NKT cells and other nonconventional T cells, each of those being further divided into subpopulations [such as naive versus memory, Th1, Th2, Th17 and regulatory T cells (Tregs)]. While not all T cell compartments are equally affected by age, overall T cell numbers decline with age as thymic involution leads to decreased output of cells. With dwindling thymic T cell production, homeostatic proliferation of peripheral T cells has to compensate and is responsible for maintaining naive T cell numbers. While this is an effective mechanism, it eventually fails, ultimately resulting in a decrease in the total number of naive T cells allowing memory and effector T cells to become dominant [9]. In addition to the numerical decline, T cell receptor (TCR) repertoire diversity progressively contracts over time [9, 10], thus skewing further T cell responses. Repertoire contraction and declining T cell input affects functional subsets differentially [11]. For example, it has been shown for CD4⁺ T cells that Th1 cell numbers decline first, followed later by Th2 cells [12]. It has been proposed that age-related deviations in immune competence may partially be a reflection of changing function in Tregs, profoundly affecting the balance between protective and pathogenic immune responses [13].

In this review, we investigate how Tregs are affected by age both in terms of numbers and function and what the consequences are for the host immune system. While regulatory function has been ascribed to not only CD4⁺ and CD8⁺ T cells, but also to $\gamma\delta$ T cells and natural killer T (NKT) cells [14, 15], only CD4⁺ and CD8⁺ Tregs have been characterized and studied extensively enough to assess the impact of progressive age on their numbers and function. Therefore, we will concentrate on CD4⁺ and CD8⁺ Tregs, while acknowledging that aging-induced changes within smaller nonconventional Treg populations could have a critical impact on the whole immune system.

CD4⁺ Tregs

CD4⁺ Tregs, characterized by the expression of CD25 and the transcription factor FOXP3, are thymically-derived, hence termed naturally occurring, T cells with the ability to regulate both adaptive and innate immune responses [16]. The critical role they play in regulating immune responses and controlling inflammation has been demonstrated in a number of murine models as well as by the human FOXP3 mutation, which leads to immunodysregulation polyendocrinopathy enteropathy X-linked syndrome (IPEX), characterized by multiple autoimmune conditions [17]. There are ample data to suggest that the numbers of CD4⁺ Tregs increase with age [18]. More specifically, in aging mice, the percentage of CD4⁺ Tregs rises in the spleen and the lymph nodes [19–21] (fig. 1; table 1). While this increase in secondary lymphoid organs is well documented, murine studies have not shown any measurable increase in the percentage of Tregs when comparing aged and young mice in peripheral blood [19, 21]. Human studies assessing peripheral blood of aged versus young individuals seem to concur that the percentage of CD4⁺ Tregs is increased in older individuals [21–23]. More supporting data come from human *in situ* studies showing an increased percentage of CD4⁺FOXP3⁺ Tregs in skin biopsies from older individuals compared to younger adults. Interestingly, the increased percentage of Tregs in the skin was observed both before and after antigenic stimulation, suggesting that not only Treg numbers are increased in the steady state in older adults, but those cells are able to expand very potently upon antigenic stimulation. All these data indicate that the CD4⁺ Treg compartment expands with age, relative to the total CD4⁺ T cells. However, since a number of CD4⁺ T cell subsets shrink in the elderly, it is not clear whether this relative increase is due to a loss of naive CD4⁺ T cells or a bona fide expansion in absolute Treg numbers. Data from both mice [19, 20] and humans suggest that the increase in Tregs measured in older subjects is not simply a relative increase due to a decrease in other CD4⁺ T cell subsets, but reflects an increase in absolute Treg numbers (fig. 1; table 1). In addition to an increase in the numbers of CD4⁺CD25⁺FOXP3⁺ Tregs, it has been suggested that CD4⁺CD25[−] T cells, when derived from aged mice, become hyporesponsive and acquire immunosuppressive properties and are able to suppress allogeneic CD4⁺CD25[−] T cells from young mice [24]. This implies that not only Treg numbers increase, but conventional T cells acquire Treg properties, amplifying the general immunosuppressive environment.

Table 1. Treg populations and the aging process

Changes in Tregs	References
<i>Increased with age</i>	
CD4 ⁺ CD25 ⁺ FOXP3 ⁺ nTregs	19–21 (mouse); 21–23 (human)
CD8 ⁺ FOXP3 ⁺ Tregs	20 (mouse); 31 (human)
<i>Decreased with age</i>	
CD4 ⁺ CD25 ⁺ FOXP3 ⁺ iTregs	26 (mouse)
CD8 ⁺ CD44 ⁺ CD62L ⁺ CCR7 ⁺ Tregs	34 (mouse)
CD8 ⁺ CD45RA ⁺ CCR7 ⁺ Tregs	32 (human)
<i>Unknown</i>	
CD8 ⁺ CD39 ⁺ FOXP3 ⁺ Tregs	
CD8 ⁺ CD103 ⁺ Tregs	
γδ Tregs	
NKTregs	

Susceptibility of different Treg subpopulations to aging-induced changes. In the aging host, naturally occurring CD4⁺ and CD8⁺ Tregs expand in numbers. In contrast, inducible CD4⁺ and CD8⁺ Treg numbers decline with advancing age. Information on many of the specialized Treg subsets is still missing.

While CD4⁺FOXP3⁺ Tregs are thymically derived, cells with a similar phenotype can be induced from CD4⁺CD25⁺FOXP3⁺ conventional T cells with TGF-β [25]. These inducible Tregs (iTregs) display the same suppressive function as natural Tregs (nTregs). Studies on iTregs are much more limited, but data suggest that induction of iTregs from conventional CD4⁺ T cells is impaired in aged mice, compared to younger mice [26] (fig. 1; table 1). This is in stark contrast to the findings that nTregs are increased with age and implies that older individuals are less capable of generating iTregs when required.

While increased numbers of Tregs could have devastating consequences for the host, it is only physiologically relevant if the suppressive function of the accumulated aged Tregs has remained intact. Phenotypic evaluation of Tregs suggests that the expression of the memory markers CD103 and CD62L is increased in Tregs from aged mice [19, 21] and that the expression of Treg markers CD25, GITR and CD69 is similarly increased in aged CD4⁺ Tregs [27]. The expression of CTLA-4, which mediates, at least in part, the immunosuppressive capability, remains unchanged [21], indicating that functionality of Tregs in aged mice is most likely neither reduced nor enhanced. Similarly, IL-10 production by Tregs is preserved

Fig. 1. nTregs accumulate with age, while iTreg numbers decline. The frequency and absolute number of naturally occurring Tregs (both CD4⁺ and CD8⁺) increases with advancing age in both humans and mice. In contrast, the inducibility of Tregs from non-regulatory CD4⁺ and CD8⁺ precursor cells declines over time.

in aged mice. In terms of FOXP3, which is the prototypic Treg transcription factor that defines commitment to regulatory function, controversy remains with some studies showing no difference in FOXP3 expression between Tregs from aged and young mice [19, 21], whereas others find an increase in FOXP3 expression in Tregs from aged mice compared to young mice [27, 28]. Human studies corroborate the latter set of studies; human CD4⁺FOXP3⁺ Tregs from older individuals show enhanced FOXP3 expression compared to Tregs from young individuals, while the remainder of their phenotypic makeup (GITR, CTLA-4 and CD127^{low}) is unchanged [21, 22]. While increased FOXP3 expression is seen as a surrogate marker of enhanced function, the impact on the suppressive function of Tregs needs to be evaluated. Lastly, the Vβ distribution of murine CD4⁺ Tregs changes with age, albeit in a similar fashion as non-Treg CD4⁺ T cells [21]. Again, the functional significance of this finding is unclear.

More concrete tests of suppressive capacity have shown that purified Tregs from aged and young mice [21, 28] and humans [22] are capable of suppressing CD4 T cell proliferation *in vitro* to the same extent (fig. 2). Similarly, CD8 proliferation and IFN-γ production have been shown to be suppressed to the same extent by Tregs de-

Fig. 2. The impact of aging on the different functional domains of Tregs. Tregs isolated from young hosts are able to efficiently suppress antigen-presenting cell (APC) activation, T cell proliferation and production of cytokines (IFN- γ , IL-2 and IL-17). Aged Tregs maintain suppressive activity towards APCs, T cell proliferation and IFN- γ production, whereas such aged Tregs are much less efficient in downregulating IL-2 and IL-17 production in neighboring T cells.

Table 2. Possible mechanisms of age-related expansion of Tregs

Loss of naive T cells opens 'space'
Changes in Treg homing patterns
Increased conversion of CD4 T effector cells into CD4 T suppressor cells
Antigen-induced expansion of CD4 Tregs
Survival advantage of CD4 Tregs compared to CD4 T effector cells

rived from aged versus young mice [27]. However, Zhao et al. [29] have highlighted an interesting distinction by demonstrating that CD4⁺ Tregs from aged mice are equally capable of suppressing allogeneic CD4⁺ T cell proliferation and IFN- γ production, consistent with the rest of the literature, but were less capable of suppressing syngeneic CD4⁺ T cells. It is possible that the inability of aged Tregs to suppress syngeneic CD4⁺ T cells is due to changes in the CD4⁺CD25⁻ non-Treg compartment. More recent data show that although Tregs from aged mice are able to suppress IFN- γ -producing CD4⁺ T cells, consistent with the previous literature, aged Tregs failed to suppress IL-17-producing CD4⁺ T cells [30] (fig. 2). The implication of this finding is that although Tregs maintain most of their functional properties, which would be responsible for suppressing immune activation against infection and tumors, they may fail to control autoimmune inflammation. Such an abnormality could explain the apparent paradox observed during aging of aberrant inflammation coexisting with immune hyporesponsiveness to infection, vaccination and tumors.

One aspect of Treg function lies in regulating the function of antigen-presenting cells. Some data suggest that there is downregulation of activation markers (CD40,

CD80 and CD86) on dendritic cells as a result of age-induced increases in Treg numbers [19]. Thus, aged Tregs may still be functionally capable of suppressing dendritic cells, and increased numbers of skin-residing Tregs may play a role in the suppression of cutaneous macrophages in the skin of older individuals (fig. 2) [31].

While most of the data from both mice and humans are in agreement that CD4⁺ Treg numbers increase with age, it is still unclear why CD4⁺ Tregs accumulate with progressing age. Several mechanisms may contribute to the age-induced expansion of Treg frequencies (table 2): (a) increased thymic output of Tregs at the expense of naive T cells with advancing age; (b) changes in the homing of Tregs; (c) increased conversion of conventional CD4⁺ T cells to Tregs in the periphery and (d) better survival of CD4⁺ Tregs in the periphery compared to conventional CD4⁺ T cells. While limited data exist on the topic, a group that has looked into the percentage of CD4⁺FOXP3⁺ Tregs in the thymus found that there is no difference in the percentage of Tregs in the thymus of older mice; therefore, increased peripheral numbers of Tregs are probably not due to increased thymic output [26]. Since increased Treg numbers have been measured in circulating blood (at least for humans), it is also unlikely that the

increased peripheral tissue numbers of Tregs are due to a redistribution of Tregs from blood to tissue. Induction of Tregs, at least in vitro, appears to be impaired in mice [26], rendering it unlikely that increasing Treg numbers in the elderly are due to increased conversion of non-Treg CD4⁺ T cells to Tregs. Measurement of in vivo proliferation of Tregs using BrdU showed no enhanced proliferation of aged Tregs [26], suggesting that increased numbers also do not reflect excessive proliferative expansion. Evidence for reduced apoptosis rates in Tregs from aged hosts came from Choungnet et al. [26] who demonstrated a reduction of the pro-apoptotic molecule Bim in aged Tregs. This suggests that Tregs lose Bim expression with age, which increases their longevity.

CD8⁺ Tregs

Just like CD4⁺ T cells, CD8⁺ T cells lose naive precursor cells with progressive age. Contraction of the naive CD8 T cell compartment occurs at an even greater extent than in the CD4 T cell population [11]. Also, aging CD8 T cells sustain a number of functional defects spanning their ability to proliferate, to become activated, the magnitude of response to antigens and their cytotoxic ability [32]. While a major role of CD8⁺ T cells lies in lysing virally infected cells, there are a number of smaller CD8⁺ subpopulations with regulatory capacity. Tregs belonging to the CD8⁺ T cell compartment, although less well-studied than their CD4⁺ counterpart, are equally important in regulating immune responses [15]. Because the CD8⁺ Treg pool comprises of a variety of subsets, each with a unique phenotype, it is difficult to construct a simple picture of how their numbers and function are altered with advanced age. However, some clear parallels to the CD4⁺ Tregs can be drawn.

Like CD4⁺ Tregs, the percentage of CD8⁺FOXP3⁺ Tregs has been shown to be significantly increased in the blood of older individuals [33], as well as in the spleen and lymph nodes of aged mice [20] (fig. 1; table 1). A corresponding increase in the absolute number of CD8⁺ Tregs has been shown in murine spleen and lymph nodes [20], suggesting that this is a genuine increase in numbers and not just a relative increase due to declining numbers of other CD8⁺ T cell subsets.

In contrast, inducible CD8⁺CCR7⁺ Tregs, which maintain a naive phenotype even after a 6-day in vitro induction, clearly decrease with age [34] (fig. 1; table 1). More specifically, peripheral blood lymphocytes from elderly donors have a significantly lower capacity of inducing

CD8⁺CD45RA⁺CCR7⁺FoxP3⁺ Tregs than younger ones following stimulation with anti-CD3 mAb and IL-15 in vitro. While the mechanism responsible for the reduction of inducible CD8⁺ Tregs is not known, their reduction is independent of the reduced number of precursor CD8⁺CD45RA⁺ naive T cells [34]. Moreover, the expression levels of both FOXP3 and CD45RA in CD8⁺CCR7⁺ Tregs from older individuals were also lower than from younger donors. This decreased expression of FOXP3 is suggestive of a reduced suppressive capacity and could possibly contribute to the rising risk for autoimmune disease in the elderly host. In vivo treatment with IL-15 also induced a smaller population of CD8⁺CD122⁺ T cells in the liver and spleen of aged mice compared to younger mice [35], suggesting a potential role for altered IL-15 signaling. The cytokine IL-15 is important in establishing central memory CD8⁺ T cells, and especially CD8⁺CD122⁺ T cells, which contain a population of central memory T cells expressing CD44⁺CD62L⁺CCR7⁺ that display Treg function [36]. The Treg (CD44⁺CD62L⁺CCR7⁺) portion of the IL-15-induced CD8⁺CD122⁺ T cells is considerably decreased in older mice [36]. In contrast, CD8⁺CD122⁺ T cells in old mice had a high percentage of the effector memory population of CD44⁺CD62L⁻CCR7⁻, which were shown to have a weaker regulatory function [36]. These findings are similar to the observation that CD4⁺ iTregs cannot be efficiently induced in aged mice, suggesting a consistent pattern whereby naturally existing Tregs (whether CD4⁺ or CD8⁺) are increased with age, whereas iTregs decline with age.

In terms of function, the ability of aged naturally occurring CD8⁺ Tregs to suppress the proliferation and cytokine production of effector CD4⁺ T cells remains comparable between younger and elder individuals [33]. Interestingly, a defined subset of CD8⁺ Tregs lacks CD28 expression [37]. End-differentiated CD8⁺CD28⁻ T cells are considered as a hallmark of immune aging, since they have been known for a long time to expand with progressing age, while the frequency of naive CD8⁺CD28⁺ T cells decreases with age [38]. This indicates that the increase in CD8⁺FOXP3⁺CD28⁻ Tregs is consistent with the increase in overall numbers of CD8⁺CD28⁻ T cells. Whether the functional spectrum of CD8⁺CCR7⁺CD45RA⁺ Tregs, which display a naive phenotype, and CD8⁺CD28⁻ Tregs, which resemble end-differentiated effector T cells, are comparable or distinct is currently not known. The expression of CCR7 enables CD8⁺CCR7⁺ Tregs to traffic like a naive cell and access storage places that are occupied by naive cells, such as lymph nodes. In contrast, end-differentiated CD8⁺CD28⁻ Tregs should display a distribu-

Table 3. Predicted and observed consequences of increased and decreased Treg numbers

	Age-related increase in Treg function	Age-related loss of Treg function
Tumor cell development and growth	Impaired anti-tumor responses	More effective anti-tumor responses
Protection from infections	Declining antimicrobial immune responses	Improved anti-pathogen immunity
Self-tolerance	Autoimmunity mostly in the young	Autoimmunity mostly in the elderly
Tissue regeneration, wound healing	Tissue degeneration	Effective tissue regeneration in the elderly

Gain and loss of Treg function with progressive age has predictable consequences for the immune competence of the host. Trends for age-related morbidities in the general population are printed in bold; aging individuals become more susceptible to malignancy and infection (increased Treg function) but, at the same time, are more likely to develop autoimmunity (reduced Treg function).

tion pattern like end-differentiated T cells, assigning them to CCL5-producing tissue sites, such as inflammatory lesions. In essence, the tissue distribution patterns and, thus, the functional impact of these CD8⁺ Treg populations are predicted to be quite different.

In summary, naturally occurring CD8⁺ Treg numbers appear to increase with age, while maintaining immunosuppressive function, whereas induction of CD8⁺ Tregs in the periphery declines with age (fig. 1; table 1). Whether aged Tregs are fully functional or are biased to preferentially suppress specific CD4⁺ Th subsets and whether Bim also plays a role in maintaining higher numbers of CD8⁺ Tregs or another mechanism is responsible for the increase in numbers are questions that remain to be answered.

Immunosenescence of Tregs – Functional Consequences

Since naturally occurring Tregs increase with age, but iTregs decline with age, it is difficult to delineate what the functional consequences are for the aging host. An increase in Tregs would be predicted to cause disease phenotypes that are consistent with aging (table 3). First, cancer is a disease mainly affecting the aging population [5]. Moreover, cancer incidence has been shown to correlate with an increase in Treg numbers. In gastric cancer tissue, increased prevalence of CD8⁺FOXP3⁺ Tregs has been described, whose phenotype is that of effector memory cells lacking both CD45RA and CD27 [39]. CD4⁺ Tregs infiltrating cancer tissue and expressing an effector memory phenotype of CD44⁺CD62L[−]CCR7[−] have been shown not only to be prevalent, but also to be functionally able to suppress CD4⁺ T cell responses [40]. Percentages of CD4⁺ Tregs and FOXP3 expression correlate with lung cancer

metastasis rates in elderly individuals. While human studies of Tregs in cancer can only be correlative, Sharma et al. [20] were able to illustrate the link between cancer and Tregs in the murine system by demonstrating that increased numbers of Tregs in aged mice are indeed responsible for lack of anti-tumor responses. Recent data show that a tumor challenge results in a higher tumor load in aged mice compared to young mice and that tumor load correlates to an increase in CD4⁺ Tregs. Second, aging-related susceptibility to infectious diseases also goes hand in hand with Treg numbers [41]. It is thought that an increase in Treg numbers leads to impaired anti-pathogen responses and may contribute to the high risk of disease reactivation typically encountered in individuals older than 60 years of age [41]. Indeed, Lages et al. [21] have demonstrated that the presence of increased Treg numbers in aged mice is responsible for the lack of an appropriate response against *Leishmania major* by suppressing IFN- γ production. More recent studies from Williams-Bey et al. [27] have provided evidence on another functionally important link between aging, Treg function and infectious susceptibility. Following influenza infection, aged mice, in addition to starting with higher Treg numbers, expand their Treg pool to a much greater extent than young mice, which results in decreased IFN- γ production by antiviral CD8⁺ effector T cells [27]. Human data show that an increase of Tregs in the skin correlates with a reduced response to VZV challenge [31]. This mechanism could be of particular relevance in reactivation of herpes zoster infection, a typical complication in the aging population. Here, age-related defects in Treg biology could provide an explanation for the combination of poor vaccine responsiveness and increased susceptibility to viral reactivation [41]. Age-related reprogramming of CD4 T cells has been associated with a shift in intracellular kinases and phosphatases, majorly im-

pacting the outcome of immune stimulation [42, 43]. Whether similar mechanisms hold for Tregs is currently unknown. Lastly, causal links between increased Treg numbers and incidence of neurodegenerative disease have been suggested [44], since in a mouse model of optic nerve injury, neuron survival was higher in the absence of Tregs. This opens up the possibility that increased Treg numbers in older individuals may negatively impact neuron survival and the ability of the central nervous system to withstand and recover from injury.

Another important aspect of the immune aging process is a syndrome of smoldering, low-grade inflammation [6] and the increasing risk to develop autoimmune disease in the elderly [45]. In light of insufficient data from aging humans, it is not clear whether this inflammatory syndrome results from enhanced immune responsiveness, from lack of physiologic immune suppression or both. Given the critical role of Tregs in immune homeostasis, any decline in Treg competence would inevitably lead to a dysbalance of protective and pathogenic immunity and would favor chronic relentless and possibly tissue-damaging inflammation (table 3). In view of increasing frequencies of Tregs with advancing age, it is predictable that they are either less efficient in downregulating inflammation or fail to be recruited to the sites where such inflammation originates. Another possible mechanism involves functional skewing such that Tregs in the elderly preferentially suppress selected T cell populations, while leaving others relatively unaffected [30], and therefore allow certain proinflammatory cells to persist. Finally,

Treg diversity must be considered. Evidence suggests that iTregs may be more susceptible to age-related insufficiency, whereas nTregs appear to be rising with advancing age. Failure to oppose the inflammatory syndrome of the senescent immune system may reflect a decline in the inducibility and durability of iTregs. Appropriate studies are required to address the relevant issues. Is there a division of labor between nTregs and iTregs? Is the inflammatory syndrome of the elderly susceptible to Treg-mediated suppression? Do Tregs, either nTregs or iTregs, dampen life-saving anti-tumor responses? Is anti-pathogen immunity in the elderly subject to Treg-dependent regulation?

Given the important role that Treg dysbalance appears to play in the development of diseases afflicting primarily the elderly population, the possibility of harnessing Treg function has a significant therapeutic potential [46]. Selective depletion or inhibition of Tregs is a possibility that has been explored in cancer models [47] and is a strategy that could be applied, not only to prevent and treat cancer, but also infections and could be used as an adjuvant to enhance responses to vaccination. By the same token, expanding Tregs could prove beneficial in treating the inflammation associated with aging. While there are still major technical obstacles to be overcome for both the targeted depletion and the ex vivo expansion of functional Tregs, the development of a successful biologic therapy based on Tregs will also depend on our ability to delineate the precise contribution of Tregs to the initiation and perpetuation of the diseases of the aging host.

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