An Association between Immunosenescence and $CD4^+CD25^+$ Regulatory T Cells: A Systematic Review[1]

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Objective Age-related increment of the prevalence of $CD4^+CD25^+$ regulatory T (Treg) cells were described controversially, and whether such changes explain immune dysfunction in the elderly is still unclear. The aim of this systematic review is to evaluate the role of the Tregs in immunosenescence. **Methods** Medline and manual searches were performed to identify all published epidemiological and animal studies investigating the efficacy of the association between immunosenescence and Treg cells. **Results** It was founded that the frequency, phenotypic characteristics, and number/function of Tregs were altered significantly with aging. Medical conditions in individuals with advanced ageas well as apoptosis intensity of Treg cells had an impact on the accumulation of Tregs which in turn could deteriorate cytotoxic activity of $CD8^+$ T and NK cells and production of IL-2. The range of immune cells that could be suppressed by Treg cells was quite wide and covered $CD4^+CD25^+$ T cells, NK cells, dendritic cells and even monocytes. These changes were observed both in humans and experimental animals. Besides, it was believed that frequency of Tregs increased with age and was accompanied by intensified suppressive activity for Tregs in patients, for example, with Alzheimer disease (AD) and Parkinson disease (PD). The impaired condition of $CD4^+$ T cells, so-called immunosenescence, rendered transplant recipients less responsive to an allogeneic kidney graft, an effect that was limited to transplant recipients who were aged over 60 years. **Conclusions** Treg cells are associated with immunosenescence. All these changes contribute to the aging-related decline of immune responses and lead to the higher risk of immune-mediated diseases, cancer or infections in aged individuals.

Key words: Aging; Immunosenescence; $CD4^+CD25^+$ T cell; Treg; Case-control studies; Cohort studies; Cross-sectional studies

INTRODUCTION

In humans as well as in many other species, it has been increasingly recognized that the immune system declines with age, a term known as immunosenescence which leads to a higher incidence of infections, neoplasia and autoimmune diseases[1-3]. Immunesenescence, defined as the changes in the immune system associated with age, may represent one of the major predisposing factors contributing to increases in infections, and cancers in aged people[2-3]. These dysfunctions arise from alterations in every component of the immune system[4], but the most consistent and significant alterations are seen in the T lymphocyte compartment, particularly within $CD4^+CD25^+$ regulatory T (Treg) cells[5]. Impaired central tolerance and a reduction in the diversity of the T cell repertoire may be key factors in immune deregulation in elderly and peripheral mechanisms for immune regulation may become increasingly important.

$CD4^+CD25^+$ regulatory T cells are the lymphocytes known to exert suppressive effect on other immune cells. Human Treg cells are rich in $CD4^+CD25^+$ T cells and comprise approximately 1%-5% of peripheral blood CD4 lymphocytes[6]. Treg cells have recently been recognized as a major subset of immune cells maintaining immune self-tolerance in the periphery which in turn could deteriorate cytotoxic activity of $CD8^+$ T and NK cells. However, the mechanisms of $CD4^+CD25^+$ regulatory T cells in the context of immunosenescence remain poorly understood.

To date, a growing number of studies[7-22] have been conducted to investigate this possible causality specifically by linking immunosenescence with Treg cells. A positive association between immunosenescence and Treg cells was observed in some cross-sectional studies and case-control studies[7-21] while no evidence of such association was shown in other case-control studies[22,25]. Thus, this possible association has not been based on sufficient

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clinical or epidemiological evidences, thereby leading to conflicting results. We therefore performed this systematic review from 15 eligible studies involving humans in various age and mice, with the aim of searching the literature for evidence of an association between immunosenescence and Treg cells.

MATERIALS AND METHODS

Data Collection and Selection

MEDLINE (PubMed). OVID, ELSEVIER, and CNKI (China national knowledge internet), WANFANG databases were searched to identify studies published from January of 1980 to February of 2010. As the search strategy, the Medical Subject Headings (MeSH) terms and proximity operators (*; " ") with the following combinations of terms and key words: "aging", "immunosenescence", "$CD4^+CD25^+$ T cell", "Treg" were used in database search.

Inclusion and Exclusion Criteria

The following inclusion criteria were adopted for the studies: (a) type of study -cross-sectional study, cohort study, case-control study; (b) type of sample -aged population and patients in cross-sectional and case-control studies; experimental animals in cohort studies and control studies; (c) type of variable-studies in which qualitative and quantitative changes occur within the immune system during aging, including a decline in $CD4^+$ T cells, loss of naive (antigenic virgin) cells, increase in memory (antigen experienced) cells, decline in T-cell proliferative responses, and narrowing of the T-cell receptor repertoire and so on. The following information was collected from each study: first author's surname, year of publication, countries where studies were conducted, type of study, source of control groups, numbers of cases, and controls with various characteristics respectively.

The exclusion criteria for studies were: (a) individuals who were taking immunosuppressive drugs or who had any disease potentially affecting the immune system including autoimmune diseases, infectious diseases, malignancy, diabetes, and asthma were excluded; (b) literature review articles and editorials.

Statistical Analysis

Because of different methods of assessment and investigated factors, data from each study was heterogeneous and was not suitable for quantitative synthesis of statistical analysis. Therefore, the systematic review was performed to yield available results. We extracted data on baseline characteristics (subjects, sample size, age, immunosenescence-correlated evaluated effects, and main results) of the studies that could potentially confound the link between immunosenescence and Treg cells.

RESULTS

A total of 321 abstracts were identified using the search strategy described above. Of them, 256 were excluded after reading the titles and abstracts. We selected 65 abstracts that full texts were evaluated by two reviewers independently. Studies without agreement for inclusion or exclusion were analyzed by a third reviewer. As a result, 15 papers were finally included in the analyses. There were: (1) 6 individual cross-sectional studies, 2 case-control studies of aged population, (2) 7 experimental studies in mice.

The prevalence of $CD4^+CD25^+$ regulatory T cells have been reported to remain almost stable in young and middle aged healthy adults. Levels of circulating Tregs seem to be age-dependent (Table 1). Cord blood samples, for example, contain a higher proportion of Treg cells compared to adult peripheral blood samples (range 2.3%-9.5%)[23-24]. Alternatively, Treg cells may be less functional in the elderly, leading to the need of higher prevalence to maintain tolerance. Tsaknaridis[7] reported that the suppressive function of Tregs declined by nearly 90% in individuals aged over 50 years suggesting equivalent functions of Tregs in old and young donors[9]. An imbalance of Treg cells homeostasis then predisposes to immune dysfunction in aged individuals explaining their higher risk of immune-mediated diseases: cancer or infections. Treg cells accumulated as a result of aging and/or medical conditions were capable of decreasing cytotoxic activity of $CD8^+$ T and NK cells and production of IL-2[7,10]. Thus, aging may affect the number, phenotypes and function of $CD4^+CD25^+$ regulatory T cells in humans[13]. These changes, as Celine[12] recently reported, were observed both in humans and experimental animals. In addition, it was believed that frequency of Tregs increased with age and was accompanied by intensified suppressive activity for Treg in patients, for example, with Alzheimer disease (AD) and Parkinson disease (PD)[11]. The impaired condition of $CD4^+$T cells, so-called immunosenescence, renders transplant recipients less responsive to an allogeneic kidney graft, an effect that was limited to transplant recipients $\geqslant 60$ years of age[14]. Because of the inherent limitations of conducting experiments in humans, much of what we have learned is owed to the utility of experimental mice models of aging.

Recent studies performed in mice have demonstrated mechanisms responsible for age-related declines in the function of Tregs (Table 2). Key findings in experimental mice models of T cell immunosenescence, were alterations in the number/function of Tregs, defects in activation, differentiation and expansion after stimulation, reduced production of IL-2, cytokines impaired ability to provide cognate help to B cells after immunization[15,19,21]. David[16] examined the frequency and function of Treg from BDC2.5NOD mice of various ages and found that not only was Treg function intact *in vivo* and *in vitro* in old BDC2.5NOD mice but the percentage of peripheral $CD4^+CD25^+$ and $CD4^+Foxp3^+$ regulatory T cells rose in BDC2.5NOD mice in an age-dependent manner in the spleen, thymus, pancreatic lymph nodes (PLNs) and pancreas. These changes were also observed in Balb/c mice, which were older than 20 months[19]. Accumulation of functional Tregs in aged mice could therefore play an important role in the frequent reactivation of chronic infections that occurs with aging[12].

TABLE 1

Age-Dependent Levels, Phenotypes, Number/Function of $CD4^+CD25^+$ Regulatory T Cells in Humans

Author (Year)	Subject (Age and Sample Size)	Evaluation Indicators	Main Results
Tsaknaridis L (2003)[7]	Healthy donors (*n*=27; 10 men and 17 women) mean age of 34.0 years (range 22–60 years), with a standard deviation of 10.1 years	Treg cells, $CD4^+CD25^-$, TCR, IL-2 GITR, CTLA-4 , $CD45RO^-$(naive) Or $CD45RO^+$(memory), IL-10, or IL-17	Suppressive activity of human $CD4^+CD25^+$ Treg cells declines with age
Bryl E (2004)[8]	Volunteer donors (average age 34.7±2.8 years for young (*n*=5; two men and three women), 45.3±5.28 years for middle-age (*n*=5;three men and two women) and 76.1±3.6 years for the elderly (*n*=52; men and women)	Regulatory T cell, Cell cycle Apoptosis	Decreased proliferative capability of $CD4^+$ cells of elderly people is associated with faster loss of activation-related antigens and accumulation of regulatory T cells
Gregg R (2005)[9]	Healthy volunteers(total *n*=72) between the ages of 21 and 93 years	$CD4^+$ $CD25^{high}$, $CD4^+$ CD25cells, Proliferation assays, Cytokine assays	The proportion of $CD4^+$ $CD25^{high}$ Treg increases in the peripheral blood with aging
Trzonkowski P (2006)[10]	Four groups were distinguished within the volunteers: young and elderly healthy and young and elderly non-healthy groups (*n*: Yh=35, Eh =36, Ynon=40 and Enon=69, respectively)	T regulatory cells, NK cells CTL, IL-2, Apoptosis	Treg accumulated as a result of ageing and/or medical conditions were capable of decreasing cytotoxic activity of $CD8^+$ T and NK cells and production of IL-2
Rosenkranz D (2007)[11]	AD: (Alzheimer *n*=29) and PD:(Parkinson *n*=40); control young: 38, control old:33	Treg ($CD4^+Foxp3^+$)	Frequency of Treg($CD4^+Foxp3^+$) increases with age and is accomp anied by intensified suppressive activity for Treg in patients
Lages C S (2008)[12]	Healthy elderly individuals (age≥70 *n*=16); healthy donors (age<30 *n*=16); Six-to 8-wk-old C57BL/6 mice(*n*=20). Twenty-month-old C57BL/6 mice(*n*=20)	Regulatory T cells CTLA-4 and GITR IFN-γ	Treg accumulation in aged hosts contributes to the immune suppres sion associated with aging
Hwang K A (2009) [13]	Healthy elderly (age≥65, *n*=32) and young subjects (age≤40, *n*=29) were recruited for this study (mean age ±SD, 77.1 ±7.8 and 30.5 ±5.9)	The effect of aging on the number, phenotypes and function of $CD4^+$ Treg in humans	Aging may affect the capacity of $CD4^+Foxp3^+$T cells in regulating IL-10 production from target $CD4^+$T cells in humans
Trzonkowski P (2010)[14]	Kidney recipients aged≥60(*n*=19) or <60 (*n*=17)	Phenotype, length of telomeres, expression of Foxp3 and proliferative responses were assessed in $CD4^+$ and $CD8^+$ T cell subsets. IL-6, IL-10	The impaired condition of $CD4^+$ T cells, so-called immunosenes cence, renders transplant recipients less responsive to an allogeneic kidney graft, an effect that was limited to transplant recipients of >60 years of age

TABLE 2

Age-Dependent Levels, Phenotypes, Number/Function of $CD4^+CD25^+$ Regulatory T Cells in Mice

Author (Year)	Subject (Age and Sample Size)	Evaluation Indicators	Main Results
Gorczynski R M (2006)[15]	8-week-month-old mice(*n*=40); >20-month -old mice(*n*=4)	$CD4^+CD25^+$Treg. $CD4^+$TGF β LPS. IL-2/IL-4 Cytokine	Alterations in the number/function of Treg might themselves be responsible for some of the cytokine changes mediated by FSLE injection into aged mice
Thomas D C (2007)[16]	4-week-old(*n*=7), 8-week-old(*n*=7) or 16–20-week-old(*n*=7) BDC2.5NOD mice	Frequency and function of Treg from BDC2.5NOD mice of various ages.	We found that not only is Treg function intact in vivo and in vitro in old BDC2.5NOD mice but the percentage of peripheral $CD4^+CD25^+$and $CD4^+Foxp3^+$regulatory T cells rises in BDC2.5NOD mice in an age-dependent manner in the spleen, thymus, pancreatic lymph nodes (PLNs) and pancreas
Nishioka T (2006)[17]	various aged C57BL/6 (B6) mice: 2-month-old (*n* = 7), 12-month-old (*n* = 10), or 24-month-old (*n* = 9):	$CD4^+CD25^+$Tcells $CD4^+CD25^-$regulatory/ suppressive function to CD4 T cells	The age-related decline in T cell-mediated immune responses is ascribable to changes in the $CD4^+CD25^-$T cell population and not to a functional augmentation of suppressive $CD4^+CD25^+$ T cells
Sharma S (2006)[18]	Young (2-3-month-old: *n*=5) and old (18-22-month-old *n*=5) BALB/c mice	$CD4^+CD25^+FoxP3^+$Treg, $CD8^+CD25^+Foxp3^+$ Treg, CTL cultures and cytotoxic activity, IL-6	There is a direct correlation between the expansion of Treg cells and immune deficiency in the old, and that depletion of these cells might be critical for restoring immune responses in aged animals
Zhao L (2007)[19]	3 months (young: *n*>8) and 20 months (aged *n*>8) Balb/c mice	Phenotype changes of peripheral $CD4^+CD25^+$Treg cells functional changes of $CD4^+CD25^+$ Treg cells The V ß family	The percentages, phenotypes, the size of TCR repertoire, and function of $CD4^+CD25^+$ Treg cells were altered significantly with aging in mice. The functional defects of $CD4^+CD25^+$ Treg cells may shed light on the role of $CD4^+CD25^+$ Treg cells in the increased sensitivity to autoimmune diseases of aged populations
Chiu B C (2007)[20]	young (5-6 months) and old (21-22 months) mice	$Foxp3^+CD4^+$Treg cells, $Foxp3^-CD4^+$T cells. Monocyte/macrophages; Cytokines	Aging leads to increased numbers of effector/memory Treg cells in secondary lymphoid organs and are associated with impaired dendritic cell co-stimulatory molecule expression
GAO Q (2008)[21]	15 mice were divided into three groups: 2-month (young), the 6-month (middle aged) and the 15-month (aged) groups	$CD4^+CD25^+Foxp3^+$T cell; $CD4^+CD25^-Foxp3^+$T cell	The proportion of $CD4^+CD25^+Foxp3^+$T cell shows an decreasing tendency with an increase of age, however, the proportion of $CD4^+CD25^+Foxp3^+$T cell shows an increasing tendency. These suggest that the two regulatory subsets play different roles in aging

DISCUSSION

In the current systematical review, we have found evidences for an association between immunosenescence and $CD4^+CD25^+$ regulatory T cells, which is still unclear in the elderly. This phenomenon is difficult to explain because it is related with multiple factors and may have different clinical consequences depending on individuals' health status and immunological history. Several causes for this age-related phenomenon have been put forward without complete explanation. Of all age-associated changes in the immune system, regression of the thymus must be the most dramatic, ubiquitous and recognizable. This is based on the idea that T-cells are lost from the periphery over time,but the thymus becomes less capable to replace them, resulting in decreased numbers of naive cells exported. There is a great deal of evidence for decreased, sometimes catastrophically decreased, naive cells in the elderly. Some reports have suggested differential susceptibility to apoptosis in $CD8^+$ and $CD4^+$ T-cells in aging. Changes have also been demonstrated in membrane fluidity, DNA damage and telomere length. All these changes contribute to the aging-related decline of immune responses and lead to a higher risk to immune-mediated diseases, cancer or infections in aged individuals.

Aging is associated not only with changes in lymphocyte subsets but also with functional changes within these subsets. The most common changes demonstrated in T-cell functions and properties in aging are shown in Table 3. Impaired central tolerance and a reduction in the diversity of the T cell repertoire may be key factors in immune dysregulation in the elderly and against this background peripheral mechanisms for immune

regulation may become increasingly important.

$CD4^+CD25^+$ regulatory T cells have recently been recognized as mediators of peripheral immune regulation and play a role in the control of autoimmune and pathogen-specific immune responses. Tregs are currently the main research focus in this field. The significance of $CD4^+CD25^+$ regulatory T cells in the context of immunosenescence remains unknown. Gregg[9] demonstrated that the number of peripheral blood $CD4^+CD25^+$ regulatory T cells increased in association with ageing. The study included donors aged over 60 years, when high numbers were observed with a 2-4-fold increase compared to donors aged between 20 and 30 years. Moreover, both age and health status contributed to the accumulation of Treg cells in the body.

TABLE 3

Most Significant Changes in T cell Properties in Aging

Decreased	Increased
Proliferation with mitogens	$CD8^+CD28^-$ cells
IL-2 production	CD95 expression
Telomere length	$CD45RO^+$ cells
Telomerase activity	DNA damage
Th1 response: IL-2. IFN- γ	IL-6, TNF-α secretion
Delayed-type hypersensitivity	Th2 response: IL-4, IL-5, IL-10, IL-12
TCR signal transduction	$CD4^+$ T cell apoptosis
Nuclear factor transcription activity	Anergic CMV-specific $CD8^+$ T cells
IL-2 receptor expression	
Membrane fluidity	
DNA repair	
$CD8^+$ T cell apoptosis	
Naive $CD4^+$ cells	
T cell repertoire	
$CD45RA^+$ cells	

Evidences from animals also indicated the increased percentage of Treg cells in peripheral lymphoid organs in aged SJL/J mice, which was in consistent with the findings reported in aged BALB/c mice[18-19] and C57BL/6 mice[12,17] suggesting that an age-related increase of Treg cells was a common phenomenon among different animal species. For scientific, ethical and economic reasons, it is important to design animal experiments to analyze the data correctly, and to use the minimum number of animals necessary to achieve the scientific objectives. We have learned much from the experimental mice models of aging. Recent studies performed in the mice have demonstrated mechanisms responsible for age-related declines in the function of Treg cells. Of particular interest, the result has demonstrated for the first time that aging leads to increased numbers of effector/memory Treg cells in secondary lymphoid organs and are associated with impaired dendritic cell co-stimulatory molecule expression(expressing CD44). Furthermore, the aged mice lose the balance between naive T cells and effector/memory T cells or $Foxp3^+$ Treg cells. These imbalances of homeostasis result in a shrunk or reduced pool of naive $CD4^+$ T cells by the involvement of compromised thymic replenishment and reduction of a naive $CD4^+$ T cells repertoire due to chronic activation. Age-related immune imbalance may contribute to increased susceptibility to emerging infections and neoplasm in animals and humans.

Age-related increment of the prevalence of $CD4^+CD25^+$ regulatory T cells is now described controversially, but a majority of studies report that Treg cells, which is linked to the thymic origin of Treg cells, can be also generated in the periphery. Advanced age and medical conditions, as well as apoptosis intensity of Treg cells has an impact on the accumulation of Tregs, which in turn can deteriorate cytotoxic activity of $CD8^+$ T and NK cells and production of IL-2. The range of immune cells that can be suppressed by Treg cells is quite wide and covers $CD4^+CD25^+$ T cells, NK cells, dendritic cells and even monocytes. Of note, some reports state that Treg are apoptosis-resistant. It suggests that they exist in the body for a long time as a polyclonal bystander subset and may exert their suppressive action repetitively upon stimulation[13]. Since Treg cells can be generated in the periphery each and every time when infection occurs, it is likely that their level differs between individuals with different histories of morbidity. It may be of special interest in the elderly where long survival of Treg cells would lead to their accumulation. Moreover, the pro-inflammatory environment present in the elderly, in particular in the frail ones, will provide strong stimulatory signal to Treg cells[14]. Thus, in our study, we test hypothesis that Treg cells may accumulate with aging. They are associated with immunosenescence, and then, an increased frequency and/or dysfunction of Treg cells may contribute to the development of infection, malignancy and inflammatory diseases in the elderly.

To date, the percentage of individuals over 65 years in the world is increasing, not only in developed countries but also to some extent in developing countries. However, the elderly population is particularly targeted for vaccination against pneumonia and influenza, because of the lower efficiency of their immune system and their difficulty to cope with infections. A better

understanding of aging and age-related diseases affecting the immune system is extremely important to keep these elderly individuals in the best of health. Public health services also benefit from a reduction in the high costs of treating ill elderly people. Understanding physiological aging as well as age-related diseases would help to respond better to their specific requirements and to improve the quality of life for a longer period.

In summary, our present study has indicated that the levels, phenotypes, number/function of $CD4^+CD25^+$ regulatory T cells are altered significantly with aging both in mice and humans. The declined ability of the thymus to produce Treg cells and the increased levels of the peripheral $CD4^+CD25^+$ regulatory T cells of aged subjects have suggested that the homeostasis of Treg cells in the periphery of aged individuals is altered. The functional defects of $CD4^+CD25^+$ regulatory T cells may shed light on the role of Treg cells in the increased sensitivity to immune-mediated diseases of the aged population.

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