CD4+ T Cells and Immunosenescence – A Mini-Review

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Key Words

CD4+ cells • Immunosenescence • Vaccination • Infection • Humoral responses

Abstract

Age-related declines in immune function are associated with reduced humoral responses following vaccination or infection. Central to this defect is a decline in naïve CD4+ T cell function. In this review, we discuss factors intrinsic and extrinsic to the CD4+ T cell compartment that affect host immunity and propose means by which deficient CD4+ T cell function can be fully restored in the aged.

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Introduction

Aging is a complex, multifaceted and normal process of life that affects many components of mammalian biology including the immune system. Declines in immune function render the aged more susceptible to infectious diseases resulting in increased morbidity and mortality. For example, West Nile virus (WNV) may infect persons of all ages, but the rate of death and neuroinvasive disease increase with age, especially among those 60–89 years old [1]. Furthermore, the efficacy of vaccination is greatly reduced in the elderly limiting preventative prophylaxis. With a substantial percentage of the global population approaching an advanced age, coupled with the threat of emerging diseases that can severely impact the aged, such

as pandemic influenza (H5N1), WNV and severe acute respiratory syndrome, it is critical that we devise means to improve vaccination of the aged and therapies to increase immunity in this susceptible population.

The hallmark of age-related declines in immunity is reduced humoral responses, which is problematic since the generation of protective antibodies is the goal of most current vaccines. Unfortunately in elderly individuals, current vaccines exhibit reduced efficacy leading to an increased rate of infections in vaccinated individuals. For example, in older individuals, the yearly influenza vaccine is only 40–60% efficacious [2], which may contribute to higher rates of complications and hospitalization of the elderly following influenza infection [3]. Additionally, reduced immune responses have been observed in elderly individuals vaccinated against tetanus, hepatitis and *Streptococcus pneumoniae* [4–6]. As stated previously, the common factor of these vaccines is that they aim to generate protective antibody responses. Reduced antigen-specific antibody production following vaccination in the elderly is the result of reduced antigen-specific B cell expansion and differentiation [7]. The antibodies produced by the aged are of a lower quality compared to antibodies generated in young individuals as they exhibit lower levels of immunoglobulin isotype class switching, reduced somatic hypermutation, and possess a diminished ability to opsonize and neutralize pathogens [7].

Immune responses generated early in life, before the onset of immunosenescence, persist well into later life, as evidenced by the recent isolation of protective antibodies from survivors of the 1918 influenza epidemic [8]. Cen-

Table 1. Strategies to enhance CD4 T cell function in the aged

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1	Develop higher potency adjuvants [19, 23, 33]
2	Sex steroid-based therapies [26, 27]
3	GH and IGF-1 treatments [30, 34, 35]
4	KGF administration [31, 32]
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tral to the generation of productive B cell responses is cognate help provided by CD4+ T cells. However, naïve CD4+ T cell function in the aged decreases due to the accumulation of intrinsic and extrinsic defects, thus limiting effective antibody responses. Therefore, it is the generation of 'new' immune responses against annual or novel vaccines in the aged that is of critical importance. In this review, we will focus on findings from experimental mouse models of aging, discuss age-related factors affecting the generation of primary CD4+ T cell responses and present means by which impaired vaccine responses in the aged may be improved.

Ontogeny of Age-Related Declines in CD4+ T Cell Function

Intrinsic Defects of Immunosenescent CD4+ T Cells

It is well established that naïve CD4+ T cells accumulate intrinsic defects in immune function over time [9, 10]. We refer to these as intrinsic in that they impact only responding naïve CD4+ T cells possessing these defects and manifest themselves without other influence from the aged host microenvironment. Miller and colleagues demonstrated that CD4+ T cells from aged TCR transgenic (TCR Tg) mice did not form immunological synapses with antigen-presenting cells (APC) as efficiently as cells from young mice [reviewed in 10]. In addition, there was a reduction of 50% in recruitment of signaling molecules to these synapses in aged CD4+ T cells compared to young cells [10]. Significant alterations in cytoskeletal rearrangement and cell surface glycosylation contributing to reduced function were also noted in these CD4+ T cells from aged mice [10]. Taken together, these changes negatively influence the intensity of TCR signaling in aged naïve CD4+ T cells, contributing to other observed defects downstream of the initial priming event.

Our laboratory has demonstrated that following primary stimulation of naïve TCR Tg CD4+ T cells with peptide-presenting APCs, CD4+ T cells from aged mice produced less IL-2 and expanded poorly compared to

young Tg CD4+ T cells [9]. In addition, naïve TCR Tg CD4+ T cells from aged mice did not provide good cognate help to antigen-specific B cells. Following transfer of naïve TCR Tg CD4+ cells from aged mice into young CD4-deficient mice, there were significant reductions in antigen-specific B cell responses, antibody production and germinal center formation after immunization [9]. While the aged donor CD4+ T cells did migrate appropriately and expressed levels of CXCR5 similar to young T cells, their ability to provide cognate help upon interaction with B cells was significantly less than that of young donor cells. In vitro studies went on to suggest that reductions in humoral responses seen in vivo may be attributed to decreased CD154 expression which is necessary for optimal cognate help [9].

Arguably, the most important factor contributing to the onset of immunosenescence is thymic involution. Following involution, the thymus exhibits greatly reduced output of naïve T cells. Hale et al. [11] demonstrated in the mouse that the number of recent thymic emigrants (RTE) in the periphery peaks at 6 weeks of age and declines thereafter, following similar kinetics to the involution of the thymus. Without a continuous stream of new T cells to replenish the peripheral T cell compartment, it is likely that the resident naïve cells have increased postthymic longevity to compensate for reduced output. This increased longevity of CD4+ T cells in the periphery is sufficient for the accumulation of age-related defects in CD4 function. A study by Jones et al. [12] found that in aged TCR Tg mice, naïve CD4+ V β 5+ TCR-expressing cells were subjected to superantigen-mediated deletion in the periphery and, therefore, this population of TCR-bearing CD4 cells was chronologically younger compared to other age-matched TCR Tg CD4+ cells. When compared against age-matched V β 8.3+ cells not subject to superantigen-mediated deletion, aged V β 5+ TCR Tg cells produced more IL-2 and exhibited greater expansion following stimulation. Further support for the role of thymic involution in the acquisition of age-related declines in CD4 function was evident when young mice were thymectomized. Eight months following surgical ablation of the thymus, CD4+ T cells from thymectomized mice possessed significant defects in IL-2 production and CD25 expression following stimulation with peptide-pulsed APC compared to cells assayed from mock-treated mice [13].

While there is a clear correlation between reduced thymic output and diminished naïve CD4+ T cell function, there are also data supporting that RTEs generated in the aged possess intrinsic defects. RTEs from aged mice have

been shown to produce less IL-2 and expand poorly compared to young counterparts [11, 14]. In addition, RTEs from aged mice were shown to possess defects in Ca^{2+} mobilization following TCR ligation [14]. While these defects in RTEs generated in aged mice are well documented, new CD4 T cells generated from aged bone marrow stem cells (BMSCs) in young hosts have been shown to be highly functional [15]. These newly generated CD4 T cells exhibited robust responses in both primary and memory assays and functioned well to help humoral responses following vaccination. Thus, these data imply that even though aged BMSCs remain functional, the aged thymus microenvironment might play a role in producing defective T cells.

Extrinsic Factors Affecting CD4+ T Cell Function in the Aged

Whereas intrinsic factors have been well defined, to date, relatively little is known about factors extrinsic to the naïve CD4+ T cell compartment that affect the initiation of helper T cell responses. We and others have demonstrated that when young naïve Tg CD4+ T cells, which are devoid of age-related functional defects, are transferred into intact aged mice, they do not expand as well and produce less cytokine than the same cells transferred into young hosts [16; Maue and Haynes, unpubl. obs.]. Therefore, there must be additional extrinsic factors that modulate naïve CD4+ T cell priming in the aged that have yet to be clearly defined.

Well documented with aging, the CD4+ T cell compartment in mice gradually gains a more effector/memory phenotype ($\text{CD44}^{\text{hi}} \text{CD62L}^{\text{lo}}$) compared to a predominantly naïve phenotype ($\text{CD44}^{\text{lo}} \text{CD62L}^{\text{hi}}$) observed in younger animals, which is likely attributable to lifelong exposure to environmental antigens. In addition, within the effector/memory CD4+ T cell subset there is an increase in the expression of molecules often associated with immune regulation. We and others have observed an increased frequency of cells that express Foxp3, CD25 and GITR. They are competent to secrete IL-10 and possess intracellular stores of CTLA-4 [17–19; Maue and Haynes, unpubl. obs.], all of which may contribute to downregulation of a new immune response. Recently, Lages et al. [18] demonstrated that the increased frequency of Foxp3+ CD4+ regulatory T cells in aged mice has a detrimental effect on the host response to *Leishmania major* infection. They proposed that the increased regulation of IFN- γ was responsible for increased disease severity. Following administration of anti-CD25 therapy, *L. major*-infected mice controlled infection much better

than mock-treated counterparts. Similarly, Sharma et al. [19] showed that the increased frequency of Foxp3+ CD4+ T cells negatively affects the control of tumor growth in aged mice. Antitumor immunity could be restored by addition of proinflammatory stimuli or anti-CD25 treatment. These findings are significant in that they illustrate how age-induced changes in one cell subset can have dramatic effects on immunity as a whole.

Another factor that has been suggested to limit the potency of CD4+ T cells in the aged is defective APC function. However, the acquisition of age-related defects in dendritic cell (DC) function may be specific to different DC subsets. Our laboratory and others have not demonstrated any significant defects in myeloid-derived DC function (ability to prime naïve T cells) [20, 21]. While functional defects may not be evident, decreased numbers, changes in maturation and reduced migration of DCs have been shown to occur in aged mice [reviewed in 16]. Recently, it was shown that aging impairs the function of plasmacytoid DCs (pDCs) to upregulate interferon regulatory factor 7 in response to toll-like receptor stimulation [22]. This defect significantly decreased the production of IFN- α in aged pDCs compared to the responses of young pDCs. It was also shown in vivo that reduced IFN- α production was responsible for reduced clearance of virus in aged mice following HSV-2 challenge. Viral clearance could be increased in aged mice by transfer of young pDCs or bone marrow cells prior to infection. In addition, DCs in aged mice have been shown to express lower levels of costimulatory molecules, which are needed for T cell activation [17], an effect that was attributed to increased regulatory T cell function in the aged and could be reversed following anti-CD25 treatment. Defects such as these, which act to limit the initiation of immune responses, likely contribute to or enhance existing deficiencies in naïve CD4+ T cell activation in the aged.

Promise of Adjuvants and Reconstitution Therapies

There are several strategies that may be used to enhance the response to vaccines in aged individuals (table 1). For many years, the only adjuvants approved for human use were aluminum-containing salts (alum). As indicated by the poor responses of the elderly to alum-containing vaccines, additional adjuvants are needed to enhance vaccine efficacy. Perhaps the easiest solution to enhance the efficacy of vaccination in the aged is to include adjuvants with greater potency in vaccine formula-

tions. Importantly, our laboratory and others have shown that proinflammatory cytokines, or adjuvants that induce the production of these, greatly enhance the initial priming and functional responses of aged T cells [19, 23].

In vitro-generated effector cells from naïve aged CD4+ cells proliferated less well and secreted less IL-2 compared to young CD4+ effectors [21]. When proinflammatory cytokines (TNF α , IL-1 and IL-6) were added during priming, aged CD4+ cells expanded and secreted cytokines at levels comparable to young cells [23]. When these aged proinflammatory effectors were transferred into CD4-deficient hosts, they provided increased levels of B cell help when compared to naïve aged CD4 T cells, as evidenced by increased amounts of antigen-specific antibody and germinal center formation following immunization. Similarly, when naïve aged CD4+ T cells were transferred into CD4-deficient mice and primed with either proinflammatory cytokines or toll-like receptor agonists as adjuvants, the resultant B cell responses were enhanced compared to mice receiving aged CD4+ T cells primed in the presence of antigen and alum alone [Maue and Haynes, accepted]. We have demonstrated that this increased amount of help from aged CD4+ T cells is associated with increased NF- κ B activation and an increase in the production of Th17-associated cytokines, including IL-17, IL-21 and IL-22 [23; Maue and Haynes, accepted]. Importantly, IL-21 has been shown to be critical for the generation of germinal centers and productive follicular helper T cells [24, 25]. Thus, adjuvants that induce Th17-like responses may be used to enhance vaccine responses in the elderly.

As aforementioned, the involution of the thymus and subsequent decrease in production of new T cells is a key player in the acquisition of age-related defects in naïve CD4+ T cell function. Several promising therapies aim at regenerating thymic function in the aged [reviewed extensively in 26, 27]. Sex steroids have been shown to play a major role in the function of the thymus. Although thymic involution begins early in life, grossly detectable changes in the thymus occur near puberty when there is an increase in the concentration of circulating sex hormones. The administration of testosterone, estrogens or progesterone results in reversible atrophy of the thymus. Ablation of these sex steroids, either surgical or chemical, has been demonstrated to increase the number of early thymic progenitors (ETPs), RTEs and cellularity of the thymus with a concomitant increase in peripheral T cell function [26, 27]. In addition, other hormone therapies have been used to increase thymic function. It has been observed that there was a decrease in the amount of

growth hormone (GH) produced by the aged compared to young counterparts [28]. Interestingly, GH or insulin-like growth factor-1 (IGF-1) treatment increased thymic cellularity in the aged, albeit at levels still reduced compared to young counterparts [29] and resulted in increased thymocyte responses to mitogen stimulation. Montecino-Rodriguez et al. [30] treated aged mice with IGF-1 and transferred syngeneic bone marrow cells from young mice. They observed that IGF-1 treatment with transfer of young cells resulted in a significantly higher number of cells in the thymus as compared to animals receiving hormone or cells alone. Furthermore, their results indicated that the role of GH or IGF-1 may be to potentiate the colonization of the thymus by bone marrow progenitor T cells. In addition, Koo et al. [34] demonstrated that GH-based treatment of aged mice results in improved T cell responses. Aged mice treated with a GH secretagogue displayed enhanced generation of cytotoxic T lymphocyte responses after transplantation of a lymphoma cell line. The increased production of GH was associated with a significant resistance to the initiation of tumors and reduced metastases.

Keratinocyte-derived growth factor (KGF) has also been used to enhance thymus function. KGF can improve thymic-dependent T cell reconstitution. KGF expanded thymic epithelial cells (TECs) and increased TEC production of IL-7, a cytokine that is essential for proper T cell development [31]. Min et al. [31] showed in aged mice that a single course of KGF treatment resulted in a 4-fold increase in the numbers of TECs, increased numbers of naïve CD4+ cells in the periphery and this effect persisted for at least 2 months. In addition, antibody responses following immunization were significantly enhanced in aged mice receiving KGF treatment compared to mock-treated controls. Similarly, KGF administration resulted in an increase in thymus size in both old and young mice treated with irradiation, cyclophosphamide or dexamethasone and increased the peripheral T cell numbers of aged mice [32]. T cells from these mice did not exhibit any differences in proliferation compared to young T cells either in mixed lymphocyte reactions or following mitogen stimulation.

Conclusions

CD4+ T cell responses are affected by intrinsic and extrinsic factors in the aged. However, it is unlikely that any singular factor or defect is solely responsible for age-related declines in immune function. We propose that it

is a combination of both intrinsic and extrinsic factors working in concert that act to limit CD4+ T cell function in the aged. Further research on these factors is required as we do not fully understand why or how CD4+ T cells acquire functional defects with age. We also know relatively little about how different systems (e.g. immune and

endocrine) interact to contribute to immunosenescence. Through further research, it may be possible to design new therapies that encompass several different age-related defects to improve immune responses in the elderly, which would be invaluable to increasing the efficacy of vaccination.

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