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Ageing of T-dependent B cell responses

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Abstract

The human immune system is in continuous interaction with environmental factors (pathogens, exercise, stress, pollutants, diet, vaccines, and therapeutics) that condition its efficiency by promoting or moderating multiple immune mechanisms. While the deleterious impact of external factors can be avoided or limited, the immune system itself grows weaker with age. Immune cells persist in the elderly, and the observed decline of cellular immunity is related to cellular senescence. Immunosenescence, which affects both T and B cells, erodes lymphocyte-dependent responses to vaccines and pathogens. Germinal centers (GCs), the organized lymphoid structures where B cells engage in affinity maturation, are regulated by follicular helper (Tfh) and follicular regulatory (Tfr) T cells, the major T cell components of GCs. This review discusses how age-related changes affect Tfh and Tfr cells as key components of B cell immunity, and how they ultimately shape the response of the ageing immune system to vaccines and infectious challenges.

Keywords

B cells, T cells, Ageing

Introduction

The world is growing old fast. By 2050, according to the World Population Prospects 2019 (United Nations, 2019), one person out of six worldwide will be over the age of 65, a proportion increasing to one of every four in Europe and North America. Enjoying an extended lifespan in good health is one of the complex challenges to meet in improving living standards, and supporting social and economic development. The immune system plays a vital role in protecting our body from pathogens while avoiding autoimmunity. It is generally admitted that ageing is associated with an impaired immune system, and deterioration of innate and adaptive immunity as we age weakens the response to vaccination and increases the vulnerability to infection [1, 2]. A powerful illustration of the impact of ageing is to be found in the robust association of age with increased morbidity and mortality from severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) causing coronavirus disease 2019 (COVID-19), the greatest challenge to public health in recent years [3]. Immune responses to vaccination or infection require the collaborative interaction of many types of immune cells, which is regulated in a time- and space-sensitive manner. An example of this multicellular collaboration is the generation of long-lasting immunity by the germinal center (GC) reactions where B cells, CD4⁺ helper T cells, and stromal cells interact to generate antibody-secreting plasma cells and memory B cells that protect against re-infection. The majority of relevant studies in this field have investigated how ageing affects the number and function of naïve and memory T cells. In fact, thymus atrophy with age leads to a smaller production of new naïve T cells [4], which correlates with the expansion and phenotypic changes of the memory T cell compartment [5] and ultimately hinders the development of an effective immune response. Follicular helper (T_{fh}) and follicular regulatory (T_{fr}) T cells are distinct CD4⁺ T cell subsets that critically regulate GC reactions and, consequently, play a pivotal role in developing an efficient and protective B cell response [6-8]. The absence or dysfunction of

the follicular T cell compartment leads to immune disorders such as autoimmune disease, loss of vaccine efficacy, and susceptibility to infection [9-11]. Here we discuss how ageing affects the B cell response and the development and function of follicular T cells, and how these changes consequently impair the response to vaccines and infections.

The T-dependent B cell response

The crucial importance of B cell responses to human health is well-established. The production of high affinity antibodies results from complex interactions between Tfh and B cells in GCs, which constitute specialized microenvironments within secondary lymphoid organs. GCs, whose formation depends on CD4⁺ T cell help, are the site of B cell somatic hypermutation (SHM), class switch recombination (CSR) and hyperproliferation [12]. The GC is formed by the dark zone, where centroblast B cells undergo rapid rounds of proliferation, and SHM of their antibody genes through the expression of activation-induced cytidine deaminase (AID); and by the light zone, where B cells stop proliferating, and higher affinity centrocyte B cells are selected by Tfh cells [13]. Chemotactic cycling of B cells between the GC dark zone and the light zone is orchestrated by the CXCR4/CXCL12 and CXCR5/CXCL13 chemokine axes, in a process that propels the dynamics of GC reactions and, ultimately, of B cell maturation [14]. Although SHM and B cell hyperproliferation take place concomitantly in the GC dark zone, it was shown recently in mice that CSR preferentially begins at the T-B border prior to GC initiation [15]. The key role for Tfh cells in the light zone is to provide cell-to-cell contacts and cytokines enabling B cells to survive and differentiate into either memory B cells or antibody-producing plasma cells [8], accompanied by the active selection of high-affinity GC B cells into the plasma cell

compartment [16]. GCs may arise also ectopically in non-lymphoid tissues at inflammation sites within the target tissues of cancer, autoimmune disease, and infection [17].

We have known since 2011 that the complex Tfh-B cell crosstalk involves a further lymphocyte partner population: the Tfr cell subset consisting of Foxp3⁺ regulatory T cells (Treg), which perform an important function in the control of B cell maturation [18-20]. Tfr cells are present in small numbers in the GC and locate preferentially to the T-B border. Tfh and Tfr cells share many properties. Both express CXCR5, ICOS, PD-1 and Bcl6, but some helper cell markers are specific to Tfh cells, namely IL-21 and IL-4 [18-20]. Tfr cells share also features of Treg cells, express classical suppressive markers such as CTLA-4, GITR, IL-10, and Foxp3, and display immunosuppressive activity *in vitro*. Both Tfh and Tfr cells are able to exit the secondary lymphoid organs and join the peripheral circulation. Due to the limited access to secondary lymphoid organs in humans, circulating Tfh and Tfr cells have been used as indicators of ongoing humoral responses in several physiological and pathological contexts [9, 21-23]. There is compelling evidence that Tfh cells form a heterogeneous cell population that can be stratified according to multiple markers, notably the immunoregulatory molecule PD-1, the costimulatory molecule ICOS, and the CXCR3 and CCR6 chemokine receptors. In humans, chemokine receptor expression defines four populations with distinct helper properties toward naïve B cells: Tfh1 (CXCR3⁺CCR6⁻) and the more efficient helper subtype, Tfh2 (CXCR3⁻CCR6⁻); Tfh17 (CXCR3⁻CCR6⁺); and Tfh1/17 (CXCR3⁺CCR6⁺) cells [23, 24]. In addition, ICOS⁺ PD-1⁺ Tfh cells operate as the chief promoter of memory B cell differentiation into plasma cells [25].

The notion is gaining traction that Tfr cells too form a heterogeneous population. Our work in mice has drawn attention to heterogeneity among Tfr cells depending on their antigen specificity and their ontogeny [26], and we postulated that the self-reactive properties of Tfr cells point to a thymic origin (although we showed also that Tfr cells can be specific for the

immunizing antigen, irrespective of whether it is a self or a foreign antigen). Moreover, we demonstrated that Tfr cells can arise from naïve T cells depending on the inflammatory context [26]. Work by others has highlighted Tfr cell heterogeneity in humans depending on the expression of PD-1, CXCR5, and CD25, in conformity with varying subanatomical localizations and phenotypes for these cells in mesenteric lymph nodes [27]. More recently, Tfr cell heterogeneity has been mapped to diverse functional properties. Several studies have ascribed a negative role to Tfr cells in the control of the magnitude of GC reactions, and in opposition to the emergence of autoreactive B cells [18-20, 28, 29]. Recent evidence has brought to light an unexpected positive regulatory function of Tfr cells on GC reactions, and shown that Tfr cells promote B cell responses in mice following lymphocytic choriomeningitis virus infection and in the context of food allergies [30, 31]. Lastly, a recent study has demonstrated that Tfh cells support Tfr cell development in mice; this effect is mediated notably by sclerostin domain-containing protein 1 (SOSTDC1) secreted by a distinct Tfh cell subpopulation, which promotes Tfr cell differentiation [32]. Overall, Tfh and Tfr cell functions are not so much in opposition as in a mutually supportive relationship for appropriate regulation of the GC reaction [33].

Recent studies have determined the metabolic requirements for Tfh and Tfr cell differentiation and function. The mammalian target of rapamycin (mTOR) is a nutrient-sensing protein kinase and a master regulator of cell growth and metabolism that plays a key role in Tfh and Tfr cell development. The mTOR-1 and mTORC2 protein complexes are essential for Tfh cell differentiation and optimal GC reactions [34]; in addition, humoral homeostasis requires mTORC1 signaling to promote the differentiation and suppressive function of Tfr cells [35]. Later on, during the memory phase, Tfh cells constitutively engage the glycolytic metabolism, and can differentiate into a full spectrum of secondary effectors after recall [36].

T cell responses in ageing individuals

The abnormal immune response in elderly individuals is associated with dysfunction of the thymus, the bone marrow, and secondary lymphoid organs in both humans and mice. These changes are collectively termed immunosenescence. Thymic involution leads to the progressive loss of functional thymopoiesis, and the resulting decrease of naïve T cell output to the periphery erodes T cell receptor (TCR) diversity [4]. Besides the contraction of the naïve T cell repertoire and population size, the T cells of the elderly are handicapped by intrinsic modifications that compromise their functions. The naïve CD4⁺ T cells of aged humans carry a defect in TCR signaling due to age-associated changes in the expression of dual-specific phosphatase 6 (DUSP6), microRNAs miR-181a, and the transcription factor complex YY1 that reduce extracellular signal-regulated kinase (ERK) activation upon TCR engagement [37, 38] (**Figure 1**). Another important change in the T cell compartment is the accumulation of effector and memory T cells with a pronounced senescent-like phenotype [39]. Defective low-nutrient sensing, and DNA damage in T cells are major features of immunosenescence that limit both cell proliferation and activation. T cells exhibit also abnormal glucose homeostasis in humans and mice as the basal levels of activation of mTOR signalling pathways increase with age, leading up to heightened basal glycolytic metabolism [40]. In parallel, erosion of telomeres and loss of telomerase activity contribute to the proliferative disability of ageing human T cells [41], a process that is less observable in mice due to their much shorter lifespan. The CD4⁺ memory T cells from elderly persons exhibit also high expression of the protein phosphatase encoded by the *DUSP4* gene, which is known to prevent Tfh cell differentiation following influenza vaccination; overexpression of the orthologue gene in murine CD4⁺ T cells has been found to suppress humoral responses to protein immunization [42] (**Figure 1**). During chronic HIV-1 infection of human subjects,

which is associated with T cell exhaustion, effector T cells display lower levels of CD28 and CD27, two co-stimulatory molecules required for cognate interaction of T cells with antigen presenting cells, even as surface expression of the inhibitory receptors, killer cell lectin-like receptor G1 (KLRG1) and CD57, is increased [39, 43]. Therefore, functional defects leading to T cell senescence may be actively maintained, in part, by augmented inhibitory receptor signaling (KLRG1 and CD57), and by depressed signaling through activating receptors (CD28 and CD27).

Among CD4⁺ T cells, the Treg subset constitutes the main regulator of immune responses, maintaining immune homeostasis and preventing autoimmunity [44, 45]. Treg cells can be divided into two main populations, namely thymus-derived Treg (tTreg) cells, and Treg differentiated in the periphery (pTreg). Interestingly, in addition to thymic involution, Treg cells increase with age in both humans and mice, a mechanism that may help preserve central tolerance during ageing but could also negatively impact the ability to control infections [46, 47]. It has been proposed that Treg build-up likely results from an increase in tTreg over pTreg cell generation [48]. Ageing is also associated with alterations of the composition and homeostasis of the Treg compartment. Memory Treg (CD45RO⁺) increase with age while naïve Treg (CD45RA⁺) collapse [49]. In mice, the decline of IL-2 with age results in reduced IL-2R α (CD25) expression by Treg cells, while increased IL-2R β (CD122) expression leads to an expansion of the aged Treg subset through an IL-15 sensing mechanism and the downregulation of a pro-apoptotic molecule, mitochondrial membrane protein Bim [50]. This effect is likely amplified by the increase in IL-6 with age, which promotes ICOS expression on murine Treg cells and further antagonizes the pro-apoptotic activity of Bim [51].

B cell responses in the elderly

Murine B cell progenitors require signaling by IL-7, a cytokine provided by bone marrow stromal cells that is essential to B cell proliferation and survival. The development of B cell progenitors from the bone marrow is thus impaired by the diminished IL-7 receptor signaling observed in older mice. In contrast, the generation of human B cell precursors in the bone marrow declines only moderately with age [52, 53]. Unlike the murine cells, human B lymphocytes are not essentially dependent on IL-7 signals for their development, as shown by the persistence of B cells in patients with loss-of-function mutations of the *IL7R* gene [54]. Mature naïve and memory B cells in the periphery see their numbers substantially decline with age in both humans and mice [55]. Moreover, SHM and CSR are negatively affected by the age-dependent, intrinsic decrease of AID expression in memory B cells, with a consequent deterioration of repertoire diversity and impairment of the high-affinity protective antibody response. E47, a key transcription factor regulating AID, shows a substantial decrease with age in human and mouse B cells [55] (**Figure 1**). Two microRNAs in inverse correlation with AID, miR-155 and miR-16, are increased in human B cells from elderly subjects, and are candidate negative regulators of AID expression [56] (**Figure 1**). Therefore, age-related suboptimal responses to vaccination are likely due to the quantitative and qualitative defects arising in both T and B cells.

In addition to these cell-intrinsic alterations, stromal cell decline in ageing humans and mice compromises the structure of secondary lymphoid organs and the outcome of the immune response [57, 58]. Histological studies of human and mouse secondary lymphoid organs show evidence of disruption in the organization of the T and B areas, an increase in fibrosis, and impairment of the reticular cell network involving fibroblastic reticular (FRCs) and follicular dendritic cells (FDCs). FRCs and FDCs are important in organizing the T cell zone and B cell follicles through the production of CCL19/CCL21 and CXCL13, respectively, and they

facilitate the mobility of, and interactions between, T cells, B cells, and dendritic cells. Impairment of the FRC function with age damages T cell proliferation, the organization and size of secondary lymphoid organs, and also the interactions between dendritic cells and T lymphocytes in the response to infection by influenza or Chikungunya virus or after protein immunization [59-62]. In addition, FDCs display lower CXCL13 production and their capacity to retain immune complexes is impaired [63, 64], which ultimately reduces GC size and function with age in mice [65] and humans [66]. The outcome is, again, deficient B cell maturation and generation of high-affinity antibodies. In short, the humoral response is compromised during ageing by intrinsic defects in T and B lymphocytes, and by deterioration of the microenvironment and the macro-anatomical structure of lymphoid organs (**Figure 1**).

Properties of follicular T cells during ageing

GCs are indispensable for antibody affinity maturation, and appropriate GC reactions require Tfh and Tfr cells [7, 67, 68]. Tfh and/or Tfr cell dysfunction can lead to immune disorders [8, 10, 11]. The magnitude and quality of the GC response decline with age, but the identity of the cellular players responsible for this age-associated impairment is still unclear [69]. In mice, age-dependent changes in CD4⁺ T cells contribute to the defective GC and antibody responses to protein immunization or influenza infection [70, 71]. Ageing is expected to cause Tfh and Tfr cell dysregulation (reviewed in reference [72]), because their precursor cells, respectively CD4⁺ naïve T cells and Treg cells, are subject to intrinsic modifications with age (**Figure 1**). Increased protein phosphatase DUSP6 activity due to the age-dependent decline of miR-181a in naïve human T cells alters TCR engagement and signal strength during priming [37, 38], which is ultimately detrimental to Tfh and Tfr cell differentiation. Memory T cells, which are responsible for protection from acute infection, and constitute the

renewable source of effector T cells such as Tfh cells, display an increase in DUSP4 expression with age that undermines their differentiation into Tfh cells in human donors vaccinated against influenza and in immunized mice [42]. Moreover, as described above, mTOR signalling is required *in vivo* to integrate immune signals and metabolic cues. Dysregulation of this process with age was demonstrated in T cells [40] and likely affects the development and function of Tfh and Tfr cells specifically, with a consequent deterioration of the output of the B cell response.

The helper function of Tfh cells is also affected by age, and exhibits defective B cell stimulation in humans vaccinated against influenza and in immunized mice [73, 74]. A range of molecules taking part to Tfh and/or Tfr cell function are negatively impacted by age. The costimulatory molecule ICOS required for Tfh and Tfr cell development and function [75], the cytokine IL-21, which is the main helper B cell cytokine produced by Tfh cells [76, 77], and CD40 ligand (CD40L), which is highly expressed by Tfh cells and is essential to GC initiation and function [78], are all diminished with age in T cells from human recipients of influenza vaccine and from immunized mice [42, 73]. Moreover, the aged microenvironment also affects the GC responses to infection or vaccination [59, 71]. The age-dependent alteration of stromal cells results in smaller B cell follicles, and in a disruption of the T-B boundary that may affect Tfh and Tfr cell development. Indeed, Tfh and Tfr cell differentiation is a multiple-step process requiring cell migration and interaction with distinct antigen-presenting cells in the T cell zone, at the T-B border, and inside the B cell follicle. The changes in lymph node architecture and function affect the coordination of the immune response [57, 58], ultimately compromising Tfh and/or Tfr cell differentiation.

The biology of Tfr cells during ageing is still largely unexplored. Two main studies have revealed that Tfr numbers increase with age in humans [74, 79] and in mice [74, 79], which reduces the Tfh/Tfr cell ratio and leads up to weaker B cell responses. The enlargement of the

Tfr subset is, in part, secondary to the age-dependent expansion of the total Treg compartment in humans and mice [74, 79]. We know also that IL-2, a major repressor of Tfh and Tfr cells, declines with age [46], and that the interleukine promoters of Tfh cell development, IL-6 in mice [51] and IL-12 in humans [80], are conversely increased. Although Tfh and Tfr cell numbers increase as a result of age-dependent changes to the cytokine environment, the outcome of B cell responses is also determined by the evolving functional properties of these two cell subsets. Tfr cells see their suppressive capacity increases with age but, very recently, Almanan and colleagues demonstrated that aged mice exhibit increased systemic IL-10 produced by a Foxp3⁻CD4⁺ T cell compartment with a bona fide Tfh cell phenotype. These IL-10-producing Tfh cells are present in murine and human secondary lymphoid organs. Interestingly, neutralization of IL-10 restores B cell responses in aged mice [81]. Thus, immune suppression with age seems reversible. Therefore, investigating the impact of the magnitude and functional properties of the Tfh and Tfr cell compartments will be mandatory to improve the overall success of humoral responses in aged populations.

Consequences of ageing on responses to vaccines and infections

Elderly persons are more susceptible to infections, which results from suboptimal immune responses to infectious challenges. The elderly carry the greatest burden of mortality from influenza, and represent 71% to 85% of seasonal influenza-related deaths and hospital admissions. Vaccination is the best way to prevent infection. The WHO and the ECDC agree in recommending annual influenza vaccination for the elderly, defined as persons of 65 or more years of age. Still, T cell-dependent vaccines are less effective in older persons than in children and younger adults [82]. In the context of the COVID-19 pandemic, elderly individuals are also those most at risk of severe disease and death. Interestingly, many lines of

evidence point to impaired B cell responses during natural infection with SARS-CoV-2. It has been shown that GCs are lost in acute COVID-19, while GC B and Tfh cells are markedly diminished [83]. Moreover, hospitalized COVID-19 patients exhibit a strong cytotoxic Tfh cell response that is negatively correlated with the abundance of antibodies to the SARS-CoV-2 spike protein [84]. Critically ill patients with COVID-19 display also the hallmark of extra-follicular B cell responses that correlate strongly with poor clinical outcomes [85]. It was demonstrated, however, that recovered patients exhibit robust circulating Tfh cell responses to the viral spike protein [86]. Overall, these data suggest a basis in the biology of the GC for suboptimal immune responses to SARS-CoV-2, and for the decline in immunity in the elderly that multiplies their vulnerability to infection. The elderly are the age group with the highest risk of severe COVID-19, and we must make progress in relevant vaccine design and protocols as a pressing issue. Of note, it was shown very recently that two injections of the adenoviral vectored ChAdOx1 nCoV-19 COVID-19 vaccine candidate were needed to enhance protective immunogenicity in aged mice while a single dose was sufficient in adult mice [87].

Concluding remarks

Declining immune function in the elderly, especially with regard to Tfh and B cells, augments the risk and severity of infection and undermines the response to vaccination. A better understanding of the mechanisms of the functional decline of these cells during ageing holds promise for the translation of new findings into treatments and vaccines that will benefit older adults.

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Figure 1: Impact of aging on T-dependent B cell responses

Comparative diagrams of T-dependent B cell responses in the contexts of adult and elderly immunity, illustrating the molecular and cellular impacts of aging on the environment and the cell partners of T-dependent B cell responses.

Treg, Regulatory T cell; Tfh, T Follicular Helper cell; Tfr, T Follicular regulatory cell; FDC, follicular dendritic cell; FRC, Follicular Reticular cell.

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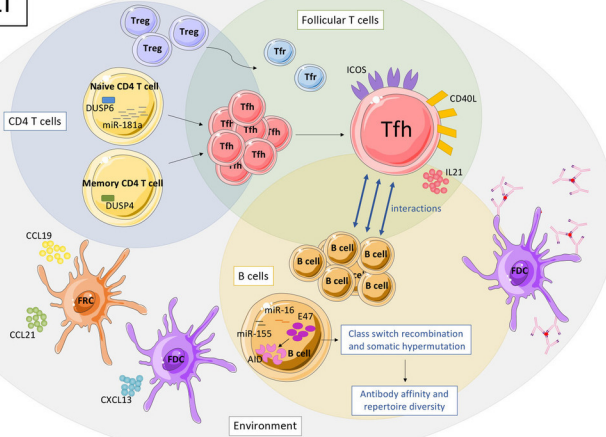
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ADULT



Legend :

- CCL19
- CCL21
- CXCL13
- DUSP4
- DUSP6
- miR-181a
- miR-155
- miR-16
- ✿ ICOS
- CD40L
- IL21
- ✿ AID
- E47
- ✿ Immune complex

ELDERLY

