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# Aging Affects Human B Cell Responses

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## Abstract

Aging represents a complex remodeling in which both innate and adaptive immunities deteriorate. Age-related changes in humoral immunity are responsible for the reduced vaccine responses observed in elderly individuals. Although T cell alterations play a significant role in age-related humoral immune changes, alterations in B cells also occur. We here provide an overview of age-related changes in B cell markers and functions. Our studies have shown that intrinsic changes in B cells with age contribute to reduced antibody responses such as those to the influenza vaccine.

### Keywords

Aging; B cells; immunoglobulin class switch; vaccination

## Introduction

Aging in humans is associated with decreased immune functions which lead to greater susceptibility to infectious diseases and reduced responses to vaccination [1]. In elderly individuals, diseases are more severe than in young individuals and have a greater impact on health outcomes such as morbidity, disability, and mortality [2].

Immunosenescence indicates age-associated immune dysfunctions which occur in both innate and adaptive systems [3–6] and include reduced natural killer cell cytotoxicity on a per cell basis [7], reduced number and/or function of dendritic cells in blood [6, 8–10], decreased total naive and increased memory T cells [10–13], decreased naïve and memory B cells [14–17].

The decrease in naïve T cell numbers with age is believed to be a consequence of thymic involution in combination with ongoing differentiation of naïve T cells into memory or effector cells. A significant age-dependent loss of naïve T cells has also been reported in the lymph node [18]. One of the most dramatic qualitative changes in the memory T cell population during aging is the appearance of CD8+ clonal expansion and loss in CD28 expression [11, 19], which is associated with an increase in cytomegalovirus (CMV) positivity [20, 21]. Analysis of telomere length and proliferative capacity has suggested that these CD8+CD28− T cells have reached replicative senescence [22]. Unsuccessful vaccination has been positively correlated with the expansion of these T cells. The presence

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of high proportions of CD8+CD28− T cells would reduce an effective antiviral response and impact homeostatic mechanisms regulating the size of the memory and naïve T cell pools. Therefore, frequencies of CD8+ CD28− T cells are useful biological markers of immunosenescence. Cytokine production and T cell proliferation are also affected with age [23], mainly due to signal transduction defects, lipid raft formation, and intracellular effectors [24, 25].

Aging affects the humoral immune response quantitatively and qualitatively, as both the amount and the specificity of the isotypes produced are changed [26, 27]. The changes in the humoral immune response during aging significantly contribute to the increased susceptibility of the elderly to infectious diseases and reduce the protective effects of vaccination [28]. Not only decreased antibody production but also reduced duration of protective immunity following immunization has been reported [29]. Although T cell alterations play a significant role in age-related humoral immune changes, intrinsic alterations in B cells also occur and help to explain reduced humoral responses observed in aging.

## Effects of Aging on Mature B Cell Subsets

Both the percentages and the numbers of mature human B cells in blood significantly decrease with age, as we [15, 16] and others [14, 17, 30–32] have shown. An age-related decline in mature B cells has also been reported in the tonsils [33]. Conversely, the percentages and numbers of human B cell precursors in the bone marrow decline only moderately [34], or they do not decline significantly with age [35]. The human memory B cell population consists of two distinct populations: IgM memory (IgG-IgA-CD27+) and switch memory (IgG+IgA+) B cells [36, 37]. It has been shown [37] that in the IgG+IgA+ B cell subset, not only CD27+ cells but also CD27− cells express mutated IgV region genes, high levels of the costimulatory molecules CD80 and CD86, and high in vitro Ig secretion as compared with naïve B cells and therefore can be considered as switch memory B cells. Also, IgM memory B cells carry somatically hypermutated IgV region genes [38]. These cells are generated in the spleen and control *Streptococcus pneumoniae* infections, which is the main cause of pneumonia in older adults [39, 40].

We have evaluated the percentages and the absolute numbers of B cells and B cell subsets by staining 100 μl of blood from individuals of different ages (20–90 years). Our published results [15, 16] indicate that naïve B cell numbers, but not percentages, and switch memory (both CD27+ and CD27−) B cell percentages and numbers were significantly decreased by age. The percentages of IgM memory B cells were unchanged by age, but the absolute numbers were significantly decreased due to the decrease in total B cell numbers. In the literature, two groups have reported an increase in the percentage of total memory B cells, identified as CD19+CD27+. However, in one report [41], this was not significant whereas in the other report, it was significant, but the analysis was carried out on a limited number, five, of elderly subjects [42]. Additionally, these results are not necessarily conflicting with ours [15, 16], as we also reported that the percentage of IgM memory B cells (IgG–IgA–CD27+), which represents the major memory B cell subset, is not significantly affected by age. We

suggest that the switch memory B cell subset (IgG+ IgA+CD27+) should be measured, as it can predict the vaccine responsiveness (see below).

## Effects of Aging on B Cell Function

**In Vitro Ig Class Switch—**Specific antibody responses in humans vaccinated against tetanus, encephalitis viruses, *Salmonella, S. pneumoniae*, and influenza decrease with age [28, 43–45]. At least one contributing factor in B cells from elderly individuals responsible for their inability to respond well to vaccination is a defect in the molecular events leading to the production of secondary isotypes, known as class switch recombination (CSR). CSR is very important for the humoral immune response because it generates antibodies of the same specificity but with different effector functions. Patients unable to class switch have been described and include those with hyper-IgM (HIGM) syndromes: HIGM1, due to a genetic defect in the CD40L expressed on T cells [46, 47]; HIGM2, characterized by mutations in *aicda*, the gene coding for activation-induced cytidine deaminase, AID, crucial for CSR and somatic hypermutation (SHM) [48]; HIGM3, characterized by mutations of the *cd40* gene [49]; HIGM4, characterized by a defect in CSR downstream of AID which does not affect SHM [50]; and HIGM5, characterized by mutations of the *ung* gene, coding for uracil-DNA glycosylase [51], a DNA repair enzyme involved in early steps of CSR and SHM.

AID, which is required for both CSR and SHM, is expressed by activated B cells, mainly in germinal centers (GCs) of peripheral lymphoid organs [52]. CSR and SHM occur in GCs in response to both T-dependent (CD40L/cytokines) and T-independent stimuli (Toll-like receptors) [52, 53]. We [16, 43] and others [54] have shown that specific B cell defects occur in aging. These include decreases in CSR, AID, and E47 transcription factor [16], which transcriptionally activates AID [55]. We have evaluated the age effects on intrinsic B cell functions in vitro by stimulating total B cells for 1–7 days and measured E47, AID, and CSR. We have shown that in response to a T cell mimic stimulus such as anti-CD40/IL-4, B cells from elderly individuals have significantly reduced capacity to undergo in vitro CSR, and there is an intrinsic defect in the expression of E47, AID, and in the secretion of IgG, suggesting reduced ability to generate a new protective response, e.g., to influenza vaccine [16]. As AID has been shown to be necessary for both CSR and SHM, we would have predicted defects in both with aging. However, as we will discuss below, this does not appear to be the case, although this has yet to be tested in individuals directly measured for decreased AID. But, dependence of these two processes on AID has also been shown in mice to be different by the Nussenzweig and Papavasiliou groups [56, 57].

An increased level of mutations in Ig genes of older people as compared to younger controls has been reported [58–60], but SHM occurs at the same rate in young and old humans, and therefore, this difference has been attributed to a different accumulation rate [61].

There are not many studies that have investigated whether the B cell repertoire changes with age. A recent study which has investigated the B cell repertoire in DNA samples from the peripheral blood of individuals of different ages (19–94 years), using spectratyping analysis of the CDR3 $IgV_H$ region, has shown that elderly individuals had a dramatic reduction in their B cell repertoire diversity, and this loss of diversity was characterized by clonal expansions of B cells in vivo [54]. This reduced B cell diversity could be considered a

predictive marker of poor health status and response to vaccination, as found for a subset of very elderly individuals participating in the Swedish longitudinal study NONA [54].

Both switch memory and IgM memory B cells have been shown to proliferate and differentiate into plasma cells in response to T cell-derived stimuli [52] but this differentiation is not expected to up-regulate AID in the switch memory cell subset when restimulated. To evaluate which B cell subsets are able to undergo CSR in vitro, we sorted naïve, IgM memory, and switch memory B cells from young subjects and stimulated them with anti-CD40/IL-4 for five consecutive days to induce AID. Results demonstrated that naïve and IgM memory B cells, but not switch memory B cells, switched in vitro [16]. We also showed that both sorted naïve (CD19+CD27−) and total memory (CD19+CD27+) B cells from elderly individuals have lower AID response when stimulated in vitro with anti-CD40/IL-4 and anti-IgM to mimic BCR signal because naïve B cells require the activation of the BCR signal transduction to undergo CSR whereas memory B cells do not [62]. Therefore, in the memory B cell population, AID is measuring mainly the function of IgM memory B cells. This also eliminates the possibility that reduced function we see is due to reduced percentage, as both naïve and IgM memory cells are not increased in percentage in the elderly. Therefore, the numbers of switch memory B cells decrease with age, and their generation in vivo is reduced similar to what we see in vitro with naïve and IgM memory B cells from elderly individuals being able to generate less AID.

Another study has also shown that not only the numbers but also the function of IgM memory B cells in response to the pneumococcal vaccine is impaired in elderly individuals [17]. In more detail, in vitro specific antibody production and plasma cell differentiation, and in vivo serum anti-pneumococcal IgM production are significantly reduced in elderly as compared to younger individuals. These results help to explain the poor humoral immunity against pneumococcal infection in elderly people.

**Serum Antibody Responses—**We recently started to establish an in vivo biological significance of AID and measured the antibody response to influenza vaccination by the hemagglutination inhibition assay (HI) and associated this with the B cell response to the vaccine in vitro. Infectious pathogenic diseases are fairly common, with influenza alone affecting up to an estimated 50 million people each year in the US. Approximately 40,000 deaths are attributed to influenza, and elderly individuals and those with weak immune systems are the most affected.

The serologic response to the influenza virus vaccine varies with age [63–67]. Successive annual vaccinations increase protection against influenza [68–70], suggesting that cellular and humoral immune mechanisms are important for protection in elderly individuals. Age-related decreases in antibody responses to influenza vaccination have previously been attributed to decreased T cell function with age [11, 71, 72], as a reduction in naive T cells and a concomitant increase in memory/effector T cells [23], loss in CD28 expression [19], and (CMV) positivity [21] have been well documented. Moreover, the decreased protective effect of vaccination in elderly people has been significantly correlated with the presence of CMV latency [21, 73].

There are currently no studies that have evaluated possible age-related intrinsic defects in the B cell-specific response to influenza vaccination. Our study has evaluated the serum response to seasonal influenza vaccination in subjects of different ages and associated this with the specific B cell response to the vaccine in vitro. Our results show that the specific AID response of B cells to the influenza vaccine given in vitro, the in vivo serum HI response to vaccination, and the ex vivo percentage of switch memory B cells and their increase after vaccination are correlated and are decreased with age [43]. Therefore, although antibody responses represent the major defense of the organism by neutralizing virus prior to infection, the antibody response of elderly individuals may not be sufficient to adequately prevent a new virus infection. We also evaluated whether B cell-specific immunological parameters could predict poor anti-influenza virus vaccine responses and therefore can be used as biological markers of immune senescence. Our data demonstrate that the AID response correlates with an optimal HI serum response in most (so far 68%) of the subjects.

**Mucosal Immunity—**Infections of the respiratory and digestive tracts represent a major disease burden of the elderly. These tracts are particularly sensitive to infections and inflammatory diseases, suggesting that mucosal immune defenses are compromised [74–76]. Therefore, the efficacy of oral vaccination diminishes with age [77].

Both gut-associated lymphoreticular tissues (GALT) and nasopharyngeal-associated lymphoreticular tissues (NALT) have been reported to be reduced by age, with GALT declining at an earlier age than NALT. This is evidenced by a reduction in GALT mass, intestinal specific IgA responses, and impaired oral tolerance induction [78]. Age-associated changes in the intestinal microbiota have also been reported. In particular, the numbers and the species of protective anaerobes diminish in the elderly, suggesting that the quality of secretory IgA (sIgA) responses may be altered. This is important considering that pathogen-specific sIgA in the mucosa is the first line of defense in reducing invasion, dissemination, and/or growth of bacteria and viruses [78, 79]. Moreover, age-related differences in the gut microbiota composition may be related to the progression of diseases and frailty in the elderly population. In a recently published study, by using the human intestinal tract chip and quantitative PCR of 16S rRNA genes of bacteria and archaea, a significant difference in the composition of the gut microbiota among individuals of different ages has been demonstrated [80]. Briefly, gut microbiota composition of young individuals and centenarians was found significantly different, showing an enrichment in facultative anaerobes, which is associated with and responsible for an increased inflammatory status in these very old individuals.

Despite evidence of mucosal immunodeficiency, serum IgA levels are elevated in the elderly as compared with younger controls [31, 32], but these IgA are predominantly monomeric IgA which are not transported to the mucosal surface as sIgA [81]. Conversely, no age-related differences in total Ig titers in the intestinal lumen or in the cultures of duodenal biopsies have been reported [82].

## Conclusions

Intrinsic/autonomous B cell changes with age have been recently described in humans. Identification of B cell deficiencies/predictive biomarkers with age will allow targets for design of possible adjuvants, new drugs, small molecules, and/or non-invasive lifestyle changes to improve the immune and effective vaccine responses.

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