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A comparative review of aging and B cell function in mice and humans

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Abstract

Immune system function declines with age. Here we review and compare age-associated changes in murine and human B cell pools and humoral immune responses. We summarize changes in B cell generation and homeostasis, as well as notable changes at the sub-cellular level; then discuss how these changes help to explain alterations in immune responses across the adult lifespan of the animal. In each section we compare and contrast findings in the mouse, arguably the best animal model of the aging immune system, with current understanding of B cell immunity in humans.

Introduction

Immune system function declines with age, as evidenced by blunted primary and recall responses, weakened vaccine efficacy, and increased prevalence of inflammatory pathologies. Reflecting their prominence as experimental models for mammalian immune system function, inbred mice have been the principal tools used to study age-associated molecular, cellular and organismal changes of immune system function. As new methods, tools and opportunities have emerged enabling equivalent studies in humans, the question arises as to whether the concepts established in mice reflect the immunobiology of human aging. Here, we review the similarities and differences between mice and humans regarding age-associated changes in B cell development, homeostasis, and function. Based on experimental and observational evidence, there is a high degree of congruence at the molecular and cellular levels, with some divergence at the population level, nevertheless leading to comparable outcomes in both species: decreased B cell generation, emergence of functionally distinct peripheral B cell subsets, dampened humoral and anamnestic responses, and increased propensity for inflammatory mediator production.

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B cell generation and subset shifts

B cell generation in young adults yields a peripheral compartment held at a steady-state size

In mammals, B cells are generated in the bone marrow (BM) from multipotent hematopoietic stem cells (HSCs). Immunoglobulin (Ig) heavy-chain gene rearrangement occurs during the pro-B cell developmental stage. This is followed by surface expression of the pre-B cell receptor (pre-BCR), containing the μ heavy-chain/surrogate light chain complex associated with BCR signaling molecules, at the early pre-B cell stage [1]. Early pre-B cells whose pre-BCR provides sufficient signaling undergo a short proliferative burst followed by Ig light-chain gene rearrangement. Expression of a complete BCR characterizes the immature (IMM) developmental stage in the BM. The IMM stage is a key central tolerance checkpoint: receptor editing and the deletion of self-reactive clones occur in both mice and humans at this stage [2]. Labeling studies in young adult mice suggest that only ~10% of IMM B cells formed survive this checkpoint.

Developing B cells that survive the IMM checkpoint exit the BM, and pass through transitional (TR) developmental stages before joining the mature pre-immune follicular (FO) or marginal zone (MZ) B cell pools [2]. Recent BM émigrés represent another major selective checkpoint: TR B cells continue to undergo negative selection, and must also meet BCR signaling thresholds and successfully compete for the survival factor, B lymphocyte stimulator (BLyS, also termed BAFF). Studies of B cell repertoire and function in autoimmune disorders and chronic GVHD further support the notion of the TR developmental stage as a major selective checkpoint [3,4]. Moreover, it has been demonstrated in mice that sub-threshold “tonic” BCR signaling and BLyS are both required for continued survival of mature FO and MZ B cells [5].

Although cells of the B lineage are continuously generated in the BM, the TR, FO, and MZ B cell pools – once filled – remain relatively constant in size, especially in inbred mice. Cell labeling and tracking experiments in mice reveal that these pools are dynamic populations held at a steady-state size: influx and egress rates are roughly equal (and the cells are not in cycle), so average cellular lifespan is the major determinant of pool size. The level of systemic BLyS determines the set-point for total TR, FO, and MZ B cell numbers (reviewed in [6]).

Reconstitution of the peripheral B cell pool in humans has been examined following bone marrow transplantation and B cell depletion therapy for lymphoma or autoimmune disorders [7,8]. Results reveal general similarities with peripheral B cell maturation in mice in the early emergence of transitional subsets followed by gradually increasing proportions and numbers of mature naïve B cells. Human B cells also show increased responsiveness to BCR stimulation and increasing sensitivity to BLyS as they mature in the periphery [8,9]. Siblings with a homozygous deletion of BLyS receptor 3 (BR3, also termed BAFF-R) show B cell developmental arrest at the transitional stages and very few mature B cells, as is the case for mouse BR3 mutants or BLyS knockouts [10]. Nevertheless, BLyS neutralization therapy does not ablate mature human peripheral blood B cell pools to the same degree as seen in mouse blood or splenocytes, indicating that the former may be less sensitive to BLyS by comparison [11,12].

B cell generation rates decline with age

Some of the first analyses of age-associated changes in murine B cell subsets included observations of reduced numbers of cells within the BM pro-B, pre-B, and IMM subsets; indeed all of these subsets are generated at a reduced rate compared to young mice, shown by empirical evidence as well as mathematical modeling [13]. The causes underlying

reduced BM production appear to involve a combination of cell-intrinsic changes, beginning with reduced HSC specification to the B lineage, as well as changes to the BM microenvironment. The bone marrow of aged mice exhibits lower levels of common lymphoid progenitors (CLPs) [14], and HSCs from aged mice as well as humans are biased to produce myeloid, rather than lymphoid, progeny [15]. Alterations in the supportive murine bone marrow microenvironment in old age include reductions in stromal cell-derived bioactive IL-7 [16], an important survival and proliferative factor for B lineage precursors. Mixed bone marrow chimeras show that BM microenvironmental changes control the age-associated reduction in RAG activity in pro-B cells, diminished size of the pro-B subset, and consequent reduction in pre-B cell production [17]. Further changes at the molecular level in aged B lineage precursors include reductions in key B lineage transcription factors (E2A; EBF1) [18,19] and pre-BCR surrogate light chain [19].

Examinations of human bone marrow samples usually involve relatively small numbers of unrelated people, and are necessarily limited to *in vitro* assessments of cell function, so may appear to yield conflicting results. For example, in one study, BM cellularity was largely maintained with age, then showed significant decreases in people 80-100 years old [20]; in another, cellularity decreased significantly in individuals from adolescence to old age (~80 yrs) [21]. The proportion of hematopoietic marrow relative to fat gradually declines in human BM with age, suggesting that B cell output may decrease in humans, as is indicated for mice by most studies; however, some observations suggest that human B lymphopoiesis is maintained well into old age. The proportions of early B cell precursors to immature BM B cells are maintained with age in adults, and there are no age-related changes in mitotic activity [22,23]. In particular, relative percentages of pro-B, pre-B, immature, and mature B cells among CD19+ lymphocytes do not change significantly between 24 and 88 years of age. In contrast, linear regression analysis of the percentage of B cell precursors in BM specimens from several hundred patients yields a statistically significant decrease in B lineage precursors with age either with or without neoplastic involvement [24]. Nevertheless, the number of total B cells in the periphery declines with age (see below).

Another cause underlying reduced BM production may involve compensatory homeostatic mechanisms [25,26]. There is increasing evidence that homeostatic “pressure” imposed by the peripheral B cell compartment suppresses BM generation in aged mice. This may be mediated through recirculating B cells [13]. Aged mice chronically depleted of peripheral B cells, either from birth or from early adulthood, as well as normal aged mice repetitively depleted of B cells by antibody treatment *in vivo*, show evidence of revived BM B cell precursor generation with significant increases in pro-B, pre-B, and IMM B cell numbers [27,28]. In these animals, the peripheral antigen-experienced B cell repertoire is reconstructed to reflect that of a young mouse and the IgG1 response to a chemical antigen is as robust as that of a young animal [28].

In this regard, the recent discovery of a novel peripheral B cell subset termed age-associated B cells (ABC, discussed in greater detail below) may be of particular interest [29,30]. Though the dynamics of this subset have not been measured, it appears to arise from exhaustively expanded FO B cell precursors instead of from the BM [29]. Interestingly, ABCs accumulate early in autoimmune-prone strains of mice, and predominance of an ABC-like subset correlates with gender and age in human autoimmunity [30]. Although murine ABCs express BLyS receptors and bind BLyS, they are not sensitive to BLyS neutralization, and thus appear to be under independent homeostatic regulation in the periphery [29]. Nevertheless, because of their increased prevalence, recirculating ABCs may play a role in regulating BM B lineage generation. ABCs are increased in both number and proportions in the bone marrow of old mice [29,31]. In old mice, ABCs secrete pro-

inflammatory TNF- which promotes pro-B cell apoptosis either directly or indirectly through effects on cells within the bone marrow microenvironment [31].

The peripheral B cell compartment changes in quality with age

Although TR subsets are also reduced in number and production rates in aged mice, the FO pool is either maintained or slightly decreased in size. The MZ subset is maintained or moderately increased in size in C57BL mice, whereas it is decreased in BALB/c mice [32]. Recent work characterizes an anatomical disorganization of the splenic MZ in aged mice, which may affect the ability to clear circulating particulate antigens or respond to blood-borne pathogens [33]. As might be predicted, the FO pool shows decreases in production rate and turnover, with a correspondingly lengthened average cellular lifespan [32]. It is now known that in aging mice, the peripheral B cell compartment includes a steadily increasing number of ABCs, likely at the expense of the FO subset, such that total B cell number does not change appreciably [29,31]. Splenic MZ, B1, and memory B cells also have an extended lifespan in aged mice [32]. Finally, increases in the proportion and number of antigen-experienced subsets – including chronically activated and memory B cells as well as B-1s – are observed with age in mice in most reports.

While FO B cells are derived from the so-called B-2 lineage, and participate in adaptive immune responses, cells of the B-1 B lineage are generally viewed as a component of the innate immune system [34]. B-1 and B-2 cells both use somatically generated Ig antigen recognition receptors, but B-1s predominate in mucosal and coelomic locations and produce “natural” antibody prior to antigen encounter; thus, B-1s are likely responsible for standing titers against commensal and opportunistic bacteria [34]. B-1s comprise a small proportion of the peripheral B cell compartment in young adult mice, but their representation increases significantly with age, partly due to clonal expansions.

In humans, both the proportions and numbers of circulating (blood) B cell subsets shift significantly with age [35,36]. There is consensus that both the percentage and absolute number of total B cells, defined by expression of the lineage marker CD19, decrease with age in humans; however, understanding of specific subsets is complicated by wide variation between individuals as well as the variety of phenotyping approaches employed (reviewed in [35]). For example, using CD19, CD27 and IgD antibodies, it is possible to identify the following major circulating B cell subsets: naïve mature (IgD+CD27–); IgM or unswitched memory (IgD+CD27+), also referred to as natural effector B cells; switched memory (IgD–CD27+), and exhausted or “double negative” memory (IgD–CD27–). With the inclusion of additional markers, a novel CD24– CD38– memory B cell subset that accumulates with age in the elderly has recently been characterized [37]; as have ABCs, defined in humans as CD11b+CD11c+ [30]. Several groups have observed that although the percentage of naïve B cells increases with age [38,39], the absolute number may not differ significantly between young and elderly subjects. Similarly, the proportion of CD19+ B cells decreases to age 35, then remains steady to age 65 in human tonsil; this reflects stable percentages of CD40+ resting B cells throughout the lifespan, but significant decreases in activated (CD38+) and B-1 (CD5+) B cells [40]. In contrast, the percentage of IgM memory B cells is not statistically different between young and elderly subjects, but the absolute number is decreased [36]. Switched memory B cells decrease in both percentage and number with age, suggesting an intrinsic defect in the ability of such B cells from elderly individuals to undergo class switch [36,41]. These cells are responsible for driving the rapid secondary antibody response after re-exposure to the antigen, which is important for the elimination of pathogens not cleared by pre-existing antibodies. They are long-lived and quiescent, express somatically hypermutated Ig V genes, and are able to generate more rapid and robust responses compared to antigen-inexperienced naïve B cells (reviewed in [36]).

In contrast with the above data, two groups have reported an increase in the percentage of memory B cells, identified as CD19+CD27+. This was not statistically significant in the first report [42], whereas in the second it was significant for an analysis carried out on a limited number of elderly subjects [43]. Nevertheless, these results are not necessarily in conflict, as the percentage of IgM (“unswitched”) memory B cells, which represent the predominant memory B cell subset, is maintained with age, but their number is decreased [36,39]. In other reports, IgM memory B cells are reduced in the elderly and this is hypothesized to result in a predisposition to pneumococcal infection [39,44].

An exhausted memory B cell subset has recently been identified and defined as memory B cells which have down-regulated the CD27 marker (CD19+IgD–CD27–). Most of these so-called double negative (DN) memory B cells are IgG+, carry short telomeres, and increase significantly in percentage, but not in number, with age [45]. The proportion of human B-1 B cells in blood, identified as CD20+CD27+CD43+CD70–, declines with age [46] and this may contribute to infection susceptibility.

The peripheral B cell repertoire is increasingly skewed or limited with age, reflecting several molecular and population-based changes. The skewing process may begin during B cell genesis in BM, as indicated by altered light chain and V_H usage, e.g. among B cells responsive to phosphorylcholine in aged inbred mice [47,48]. BM output and TR subsets are reduced with age in mice, and this would predict reduced mature B cell diversity. The proportion and sizes of splenic FO and MZ subsets are (more or less) maintained in mice; nevertheless, there is evidence for homeostatic expansion of constitutively activated / “antigen-poised” cells such as MZ and B1 cells [49,50], as well as clonal expansions in aged mice, which also would be expected to reduce overall repertoire diversity. Murine B cells may be selected and accumulate with age based on reactivity to environmental (self) antigens [49], while antigen-inexperienced cells are gradually excluded from the peripheral repertoire, as an animal ages [29,30,49].

In humans, several studies have investigated changes in the B cell or antibody repertoire with age (reviewed in [50]). One of these has analyzed DNA samples from the peripheral blood of individuals at 19-94 years of age, using spectratyping analysis of the IgV_H CDR3 region [51]. Some elderly individuals showed a significant collapse in repertoire diversity, together with oligoclonal expansions; moreover, the extent of the loss in diversity correlated with frailty [51]. Hyperexpansions of plasma cells yield monoclonal gammopathies of undetermined significance (MGUS) and other monoclonal B cell expansions are also associated with age [52]. In general, multiple clonal expansions have been observed in the elderly, and the resultant skewing of the B cell repertoire correlates with frailty [50,51].

B cell function: humoral responses

B cell-intrinsic changes occur with aging

Humoral immune responses in aged mice and humans are less robust and poorly protective compared with those in young adults. High-affinity antibodies are produced in the germinal centers (GC) of B-cell follicles as a consequence of affinity maturation (reviewed in [53]). A progressive decline in GC formation during aging has been reported in mice and this leads to decreased somatic hypermutation (SHM), antibody affinity maturation, and recirculating long-lived plasma cells in the bone marrow ([54]). Reduced affinity maturation of the antibodies generated in response to an influenza vaccine has recently been reported in humans [55]. These age-related changes include defects not only in T cells and follicular dendritic cells but also in B cells. Aged mice showed qualitative shifts in antibody repertoire diversity, based on V_H and V_L use, as well as expanded cross-reactivity to several self and foreign protein antigens, and reduced average affinity and protective activity of serum

antibody [48]. Studies elucidating intrinsic B lymphocyte defects in class switch recombination (CSR), activation-induced cytidine deaminase (AID) and E47 transcription factor expression have shown that these defects occur in both mice and humans (reviewed in [56]). Briefly, E47, which regulates AID and CSR in murine splenic B cells, is down-regulated with age due to increased E47 mRNA decay. Tristetraprolin (TTP) is a negative regulator of mRNA stability of cytokines and transcription factors including E47. Levels of TTP are higher and phosphorylation of TTP is lower in old compared to young B cells, leading to more E47 mRNA degradation. Protein phosphatase 2A (PP2A) is a serine/threonine protein phosphatase that plays an important role in the regulation of a number of major signaling pathways. Not only the amount but also the activity of PP2A is increased in the splenic B cells of aged mice. As a consequence of increased PP2A activity, TTP is less phosphorylated in comparison to TTP in B cells from young animals. PP2A dephosphorylation of TTP likely results in more binding of hypophosphorylated TTP to E47 mRNA, inducing its degradation. The age-related decrease in AID expression, due to down-regulation of E47, decreases CSR and affinity maturation in the elderly and this significantly contributes to the reduced response of the elderly to infectious diseases and vaccination [38].

Vaccine responses are poor in a majority of aged individuals

In humans, specific antibody responses to vaccines against tetanus toxoid, encephalitis viruses, Salmonella or Pneumococcus decrease with age. Currently available vaccines are protecting only part of the human population because of the age-related decrease in immune functions. Vaccination against Streptococcus pneumoniae and influenza are strongly recommended in children under 2 years of age and individuals over 65 years of age to protect them from infection. However, although commercially available vaccines against these infectious diseases provide protection and ensure lasting immunological memory in children and adults, they are much less effective in elderly individuals.

The immune response to the pneumococcal vaccine is a T-independent type II response likely initiated by MZ B cells in which IgM are key and IgG are skewed towards the IgG2 subclass [57]. Poor antibody responses to pneumococcal challenge observed in elderly people are thought to result from functional alterations in MZ and IgM memory B cells [57]. IgM memory B cells are generated in the spleen, carry somatically hypermutated antibodies specific for polysaccharide antigens and decrease in number with age as do all B cells. The most common vaccine (PPV23) comprises 23 different polysaccharides which are thought to cover serotypes responsible for 90% of infections. However, because the IgM memory B cell compartment is not fully formed in humans until 2 years of age, this vaccine elicits a poor response in the very young. A conjugate vaccine (PCV) has been used to induce T-dependent responses (in which IgG is skewed towards the IgG2 subclass) and has certainly improved efficacy in infants and this is now the recommendation. While there is no doubt that the vaccine does help protect against invasive pneumococcal disease, and is therefore currently recommended for the older population, its protection against community acquired pneumonia is extremely poor. At the present time it seems as if the best way of reducing pneumococcal disease in the elderly is by ensuring effective vaccination of children and thereby providing herd immunity [58].

As to the response to the influenza vaccines, these are T-dependent responses that induce IgG specific antibodies. Many studies have shown that the age-related decrease in the response to influenza vaccination is correlated with the decrease in T cell [59] and dendritic cell [60] function. However, several recent studies have clearly demonstrated that age-related intrinsic defects in B cells also occur. A reduced production of vaccine-specific antibodies has been reported and attributed to a reduced number of responding plasmablasts and reduced secretion of plasmablast-derived polyclonal antibodies in elderly individuals compared with that in young individuals [61]. Moreover, the *in vivo* response to the vaccine

(measured by fold-increase in antibody titers after vaccination) and the in vitro responses of B cells to the influenza vaccine (measured by fold-increase in AID after vaccination) are both decreased in elderly individuals and are correlated [41,62]. Fold-increase in AID after vaccination has also been correlated with fold-increase in antibody affinity maturation [55]. For this reason, AID has been proposed as a biomarker which monitors the in vivo production of good protective antibodies. B cell-specific biomarkers which can predict the in vivo response to the influenza vaccine have also been identified. The percentage of switched memory B cells and CpG-induced AID, both measured before vaccination, are decreased by age and are significantly correlated with the in vivo response to the vaccine [41,62] and therefore have been proposed as predictive markers of the in vivo response.

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Summary

Empirical and observational evidence indicates that mice provide a robust model for understanding mechanisms that underlie age-associated changes in B cell generation and function, based on numerous similarities between mice and humans.

- B cell generation in bone marrow declines in mice but is apparently maintained in humans, although the data supporting this for humans are based on static rather than kinetic analyses. Shifts in naïve and antigen-experienced peripheral B cell subsets are now well documented in both species.
- Antibody responses to both T cell-dependent (TD) and T cell-independent (TI) antigens are generally dampened with age in both mice and humans, although there may be wide variation between individuals, likely reflecting underlying genetic, epigenetic, and environmental differences.
- Further studies in mice and humans are needed to identify predictors of the antibody response to antigens and vaccines, including gene signatures able to identify more accurate correlates of protection. Moreover, the identification of specific B cell defects may be used to target novel intervention strategies able to improve vaccine efficacy in immunodepressed populations.