

## Review

# Ageing, autoimmunity and arthritis: Senescence of the B cell compartment – implications for humoral immunity

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## Abstract

Immunosenescence is associated with a decline in both T and B lymphocyte function. Although aged individuals have normal numbers of B cells in the periphery and are capable of mounting robust humoral responses, the antibodies produced are generally of lower affinity and are less protective than those produced by young animals. Here we review multiple studies that address the mechanisms that contribute to this decline. Taken together, these studies suggest that age-associated loss of the ability to generate protective humoral immunity results in part from reduced B lymphopoiesis. As the output of new, naïve B cells declines, homeostatic pressures presumably force the filling of the peripheral B cell pool by long-lived antigen-experienced cells. Because the antibody repertoire of these cells is restricted by previous antigenic experience, they make poor quality responses to new immunologic insults.

**Keywords:** aging, B cells, homeostasis, immunosenescence, lymphopoiesis

## Introduction

During the past decade the number of laboratories investigating immune senescence has increased dramatically, rapidly advancing our understanding of how the immune systems of higher organisms change with age. Historically, aging has been thought of as a state of immune deficiency. Elderly individuals present with increased susceptibility to, and severity of, infectious diseases and decreased vaccine efficacy. More recently, however, the status of the aged-immune system has been described as dysregulated [1] or remodeled [2]. Age-associated changes in both phenotype and function have been reported for many cell types, including T cells, B cells, natural killer (NK) cells, and follicular dendritic cells (FDCs; for review see [3]). The consequences of these changes are seen in all phases of immunity – cellular, humoral, and innate.

Not surprisingly, with this wave of new information has come controversy, as conflicting reports have emerged in

quick succession. Close examination of this literature, however, reveals that many apparent discrepancies can be reconciled when trends, rather than specific details, are analyzed. With this in mind, our review focuses on age-associated alterations in the B cell compartment in both mice and humans. Specifically, we believe that on balance the literature indicates that B lymphopoiesis declines with age, and that this decline 'drives' the selection of antigen-experienced B cells in the peripheral B cell compartment. Over time large numbers of antigen-experienced B cells, including poly/self-reactive subtypes such as marginal zone (MZ) and CD5<sup>+</sup> B1-like cells, accumulate and eventually dominate the periphery. Finally, we discuss how this antigen-experienced repertoire is maintained and what role it may play in the deterioration of humoral immunity that is evident in many aged individuals.

## Age-associated impairment in B lymphopoiesis

Most available evidence indicates that aging is associated with a decline in B lymphopoiesis. For the purpose of the

BAFF = B cell activating factor; BCR = B cell receptor; BM = bone marrow; BrdU = bromodeoxyuridine; FDC = follicular dendritic cell; GC = germinal center; HSC = hematopoietic stem cell; IFN = interferon; Ig<sub>H</sub> = immunoglobulin heavy chain; Ig<sub>L</sub> = immunoglobulin light chain; IL = interleukin; MZ = marginal zone; NK = natural killer; RAG = recombinase activating gene; TNF = tumor necrosis factor.

present review we consider B lymphopoiesis in terms both of the complex process of mature B cell development from committed bone marrow (BM) progenitors, and of the rate at which new cells are produced and progress from one developmental stage to another.

In adult mice, development of B cells occurs in the BM in a series of steps that are definable by changes in cell surface expression of a variety of molecules (for detailed reviews, see [4–7]), and is dependent on IL-7 and other factors made by stromal cells [8]. Current models hold that the first lineage committed B cell precursors derive from common lymphoid precursors. Among the earliest definable B lineage committed cells are pro-B cells. Pro-B cells express very low levels of cell surface Ig- $\alpha$  and Ig- $\beta$ , which transduce signals, supporting immunoglobulin heavy chain (Ig<sub>H</sub>) gene rearrangement and differentiation into pre-B cells. In turn, pre-B cells express on their surfaces low levels of rearranged Ig<sub>H</sub> in association with Ig- $\alpha/\beta$  and surrogate light chains  $\lambda 5$  and VpreB. These cells/clones expand, and then undergo immunoglobulin light chain (Ig<sub>L</sub>) rearrangement. Expression of rearranged light chains in association with  $\mu$  heavy chains and Ig- $\alpha/\beta$  marks the transition to the immature B cell stage.

Immature B cells are the earliest cells in the lineage that express a *bona fide* antigen specific B cell receptor (BCR), and therefore they are the first population to be vetted for self-reactivity. Immature B cells that express autoreactive BCRs are functionally silenced or deleted; a subset of these cells that exhibit autoreactivity of low affinity are driven by self-antigen to enter the B1 compartment. Emigration of immature B cells to the periphery and their acquisition of membrane-bound (m)IgD antigen receptors indicates entry into the transitional B cell compartment. Fully mature B cells subsequently move to the follicle and can be delineated from other peripheral B cell populations by a variety of cell surface markers, including reduced expression of mIgM.

Many groups have documented age-associated changes in B lymphopoiesis in variety of mouse strains [9–16]. A common finding of those studies is the decline in absolute numbers of pre-B cells, as measured by flow cytometry. The reported severity of this decline varied from study to study and from animal to animal, ranging from moderate (but statistically significant) to extreme, depending on the strain, sex and age of the mice studied, and on the particular methods used to generate and analyze the data. Some studies further correlated reduced pre-B cell numbers with reduced numbers of immature and/or transitional B cells [11,16,17]. Several mechanisms, including failure to progress in development, and increased apoptosis of both pro-B and pre-B cells, have been purported to limit the pre-B cell pool in aged mice. It has been shown in these animals that a proportion of pro-

B cells fail to progress in development to the pre-B cell stage. This has been attributed to impaired expression of pre-BCR components, including rearranged Ig<sub>H</sub> and  $\lambda 5$ /VpreB surrogate light chains [16,18]. Age-related reductions in pre-BCR components at the level of surface expression are highly correlated with reduced transcription of the molecules; reduced expression and activity of E2A transcription factors have been specifically implicated in the case of  $\lambda 5$ /VpreB [19]. Notably, levels of expression of recombinase activating gene (RAG) proteins in individual pro-B and pre-B cells are similar between aged and young mice, but total BM RAG expression is reduced in aged animals because of reduced numbers of pre-B cells [18].

Nevertheless, the relative importance of these impairments is called into question by experimental evidence from our laboratory, which demonstrates that aged immunoglobulin transgenic mice also fail to generate new B cells efficiently [12]. These immunoglobulin transgenic mice express a mature, fully rearranged BCR very early in development, thus obviating the need for endogenous Ig<sub>H</sub>,  $\lambda 5$ , and VpreB. These data indicate minimally that factors in addition to expression of pre-BCR must limit B cell production in older animals. If Ig<sub>H</sub>,  $\lambda 5$ , or VpreB was solely limiting, then production should have been rescued by the immunoglobulin transgenes. These data do not exclude the possibility that signal transduction downstream from the pre-BCR or transgenic BCR is impaired. Additionally, both mRNA and protein levels of the survival molecule Bcl- $x_L$  are reduced in pro-B and pre-B cells harvested from aged as compared with young mice, and this may result in the increased apoptosis observed in these cell populations [15,20].

The possibility also exists that pre-B cells may be fewer in number in aged mice because the numbers and/or activity of their progenitors are limited. This explanation has not been rigorously examined, but at least one group has claimed that absolute numbers of pro-B cells remain constant with aging [10]. Nonetheless, recent advancements in cell sorting technologies have allowed more detailed discrimination of rare BM subpopulations, and it is now clear that absolute numbers of early B cell progenitors also decline with age, including pro-B cells and early B cell precursors/common lymphoid precursors. Furthermore, diminished IL-7 responsiveness is correlated with these reductions in cell numbers [21]. *In vitro* studies also show that cultured pro-B/pre-B cells from aged mice proliferate poorly in response to exogenous IL-7, but surface expression of IL-7 receptor remains unchanged [21–23]. Taken together, these findings suggest that signal transduction via the IL-7 receptor may be impaired, or that the crosstalk that occurs between the IL-7 receptor and other receptors (e.g. pre-BCR), and is necessary for development, is impaired.

Interestingly, Morrison and coworkers [24] have shown that multipotent hematopoietic stem cells (HSCs) increase in numbers by as much as fivefold with age. Importantly, however, in that study HSCs sorted from aged animals and transferred to young irradiated recipients were defective in their ability to reconstitute the B cell compartment, but they retained their ability to reconstitute both the T cell and myeloid compartments effectively. From these data, the authors concluded that B lineage progenitor activity declines with age, ultimately resulting in decreased generation of mature B cells. Two other groups investigating HSCs recently corroborated those findings [25,26]. Further studies conducted both in our laboratory [12] and in that of Weksler [27], in which the rate of new B cell production was determined in aged as compared with young mice following lymphopenia induced by  $\gamma$ -irradiation or cyclophosphamide, demonstrated that the absolute numbers of B cells generated per unit time in both the BM and spleen are markedly reduced.

In addition to the reports outlined above, B lymphopoiesis in aged animals has been studied as a function of production rate to determine whether the described defect in generative (or regenerative) capacity is confounded by cells that progress through development more slowly. Determination of production rate is most frequently measured as rate of incorporation of bromodeoxyuridine (BrdU) into dividing cells. Using this method, Kline and coworkers [11] demonstrated that both pre-B and immature B cell subsets incorporate BrdU more slowly in aged than in young animals, concluding that B cell maturation is retarded in aged mice. Recently, however, investigators from the laboratory of Witte [17] contested this notion, concluding that despite reduced numbers of pre-B cells the rate of BrdU incorporation, and hence the rate of new B cell production, does not change with age. Furthermore, the authors of that report contend that total numbers of immature and transitional B cells do not decline with age, maintaining that 'the major defect in B cell development of old mice is the inability of newly made cells to join the peripheral B cell compartment.' They hypothesize that new B cells may be unable to home to the spleen efficiently. However, experimental evidence from Albright and coworkers [28] demonstrates that mature, splenic B cells transferred from aged or young mice to young recipients localize in the spleen with comparable efficiency. The discrepancies between the findings of Johnson, Owen and Witte [17] and those of other groups quite possibly reflect differences in experimental protocol and/or mouse colonies.

Finally, one must also consider the influence of the aged BM microenvironment on B lymphopoiesis as it occurs in aged animals. Normal B cell development is critically dependent on the BM microenvironment, with stromal cells providing specialized niches that nurture

lymphopoiesis through coordinated expression of various chemokines (e.g. SDF-1/CXCL12) and cytokines (e.g. IL-7). Very few studies have explored molecular changes in the BM microenvironment as a function of age. Stephan and coworkers [22] reported that stroma derived from aged animals is defective in its ability to release IL-7 and support B lymphopoiesis in culture. Furthermore, Li and colleagues [27] showed that when BM cells derived from young mice are transferred to lethally irradiated recipients, absolute numbers of splenic B cells (measured at 3 weeks after transfer) are reduced in aged as compared with young recipients. Therefore, these data suggest that both B lineage intrinsic and extrinsic factors may limit B lymphopoiesis in aged animals.

Most investigators agree that in humans, like mice, some B lymphopoiesis continues for the lifetime of the organism. It is also generally agreed that pathways of B cell development change and progenitor activity declines as humans mature from fetus to adult. In contrast, it is still a matter of debate whether adult humans undergo the further reductions in B cell output described in aged mice. As one can easily imagine, experiments using human BM are exceptionally challenging for a variety of reasons. Adult marrow specimens are often of limited availability and rarely come from normal donors. In addition, the precise surface characteristics of BM B cell developmental intermediaries are not fully defined in humans, but they clearly differ from those defined in mice. Ultimately, variations in human genotype and environmental experience, which are not found in inbred mouse strains housed under controlled conditions, confound results and potentially mask differences in B lymphopoiesis due to aging.

However, McKenna and colleagues [29] conducted an elegant and very thorough study of the aging human B cell compartment in 2001, examining a total of 662 BM specimens derived from 598 patients ranging in age from 2 months to 92 years. In that report the percentage of B lymphocyte precursors was determined as a function of age, and data from each patient were depicted as an individual dot on a composite scatter plot. Although a broad range was found at all ages, linear regression analysis showed a statistically significant decline in B lymphocyte precursors with increasing age. In contrast, two other studies [30,31] concluded that production of B cells in humans remains relatively constant throughout adult life. Interestingly, both studies presented some data that indicate that B lymphopoiesis declines with age but these trends were not statistically significant. It should be noted, however, that this lack of statistical significance is probably due to the low numbers of patients examined and/or the use of data presentation in which means were calculated for groups containing individuals that differed in age by as much as 26 years. Because aging is a gradual process that is asynchronous within the population, a

group design is inappropriate for full evaluation of changes that occur over time. Further investigation, in which large numbers of individuals are analyzed separately, preferably in terms of absolute numbers of B cell precursors, is needed to resolve these discrepancies.

As discussed above, many factors may contribute to reduced B cell production in aged mice, including possible defects in levels/function of both IL-7 and its receptor. Rossi and coworkers [30] state that IL-7 is unnecessary for B cell development in humans, and suggest that this may account for the species related differences reported by some investigators. Indeed, two studies [32,33] concluded that human B cell development is IL-7 independent, whereas two others demonstrate that IL-7 is required [34,35]; the former utilized fetal derived tissue and the latter used adult BM. It is well documented that human B cell development differs significantly between fetus and adult. Moreover, researchers in the laboratory of Vieira [36] recently demonstrated that deletions of IL-7 or IL-7 receptor permit B cell development in fetal but not adult mice. Taken together, these studies indicate that IL-7/IL-7 receptor may in fact be essential for B lymphopoiesis in adult humans and, importantly, may play a role in aging.

### **The aged peripheral B cell repertoire: what does it look like and how did it get there?**

Because the number of functional B cell progenitors decreases with age, it is logical to expect that the numbers of mature B cells in the periphery would also decrease. Experimental evidence from several groups, however, demonstrates that mature B cell numbers are roughly equivalent in aged and young mice [12,17]. This apparent paradox can be explained in part by the increase in lifespan (measured using BrdU incorporation) of mature B cells in the periphery of aged mice [11]. Careful dissection of splenic B cell subsets by our laboratory and others also revealed significant alterations in sub-population distribution as mice age [12,37]. Specifically, the percentage of naïve follicular B cells declines dramatically, whereas subsets of antigen-experienced cells increase. Importantly, the type of antigen-experienced cells that accumulate varies from aged mouse to aged mouse (even among cohabiting animals), and can include increased numbers of one or more of the following B cell subsets [12]: MZ, CD5<sup>+</sup> B1-like, and memory. Experiments conducted in our laboratory show that within the spleens of aged mice it is only these antigen-experienced subpopulations that incorporate BrdU very slowly, and hence have an extended lifespan (Johnson SA, Cambier JC, unpublished observation). These data are consistent with a previous report that activated B cells and their clonal descendants have a longer lifespan than do resting B cells [38]. Importantly, elevated total serum immunoglobulin concentrations,

including elevation in autoantibodies, distinguish mouse strains with increased numbers of MZ, B1, and memory B cell subsets, and not surprisingly aged mice [12,39–41].

Finally, stable B cell expansions with clonal Ig<sub>H</sub> have been detected in aged, unimmunized mice [37,42]. These clonal B cell populations tend to be CD5<sup>+</sup>, and in some instances they are thought to be precursors of two B cell derived cancers, namely chronic lymphocytic leukemia and multiple myeloma [37]. The origin of CD5<sup>+</sup> B1 cells in young, adult mice is a controversial matter. Some investigators maintain that B1 and B2 cells derive from distinct progenitors (for review see [43]), whereas others believe that they derive from a common progenitor or 'B-0' cell (for review see [44]). In the latter case, surface expression of CD5 and commitment to the B1 pathway requires antigen receptor engagement under specific conditions (e.g. the absence of T cell help) [45]. This requirement for entry into the B1 pathway selects for cells that bear receptors that have low affinity for environmental/self antigens. Importantly, the CD5<sup>+</sup> B cell expansions found in the periphery of aged animals are not found among B cell precursors in the BM [37]. Thus, it has been hypothesized that these cells develop in the periphery, probably as a result of encounters with environmental antigens.

The studies presented above demonstrate that the peripheral B cell compartment in aged mice is 'skewed' in favor of long-lived, antigen-experienced cells, but they do not address the root cause of this shift. Potential causal explanations include the following: BM B cell production is depressed because peripheral B cells live longer; alternatively, peripheral B cells live longer because BM B cell production is depressed. If the former were true then one might predict that ablation of long-lived peripheral B cells in aged animals would restore 'young-like' B lymphopoiesis, and ultimately a young-like peripheral repertoire. To address this hypothesis, Li and coworkers [27] ablated the B cell compartment with cyclophosphamide and found that the subsequently regenerated repertoire was 'old-like', disproving this notion.

In contrast, several lines of evidence support the second alternative described above – that reduced BM B lymphopoiesis may drive the selective increase in antigen-experienced B cell numbers in the periphery. In young adult mice, only a fraction (10%) of newly produced B cells enter the mature B cell compartment and are maintained as part of the naïve preimmune repertoire [46,47]. It has recently become clear that a large proportion of newly produced B cells bear surface immunoglobulin that have some degree of self-reactivity (including environmental and autoantigens), and that these cells are normally eliminated at one of two distinct developmental checkpoints [48]. Whether these cells survive or are eliminated

depends in part on self-antigen induced BCR signal strength and on the presence or absence of non-self-reactive B cells that compete for space (for detailed review, see [49]). Interestingly, in contrived circumstances in which naïve B cells are present, autoreactive B cells from young HEL (Hen Egg Lysozyme)/anti-HEL double transgenic animals are excluded from the follicular niches and die rapidly [50]. In the absence of naïve competitors, however, these same cells enter the follicle and survive. Thus, in normal, young adult animals, competition for limited follicular niches excludes the majority of self-reactive B cells from the peripheral repertoire. Conversely, it has been shown that in aged animals self-reactive B cells gain entry to follicular niches and survive [51]. We postulate that this observed difference (between young and aged animals) reflects the reduction in naïve competitor B cells in the aged environment as a result of reduced B lymphopoiesis. These results resonate with those derived from analysis of the behavior of antigen-experienced B cells in young mice.

Analyses of knockout mice, including those for IL-7, IL-7 receptor,  $\lambda 5$ , and the moth-eaten viable mouse (a naturally occurring hypomorph of SHP-1) in which B lymphopoiesis is impaired and competition is reduced, reveal a skewed peripheral B cell compartment dominated by antigen-experienced cells [39,41,52]. Furthermore, Hao and Rajewsky [53] demonstrate that inducible deletion of RAG-2 in young adult mice results in the gradual loss of naïve follicular B cells, but not of MZ or B1 B cells. Recent studies conducted in our laboratory also suggest that reduced influx of B cells from the BM drives the selection of antigen-experienced cells into the peripheral compartment. Using two different experimental approaches, we found that when B lymphopoiesis is artificially depressed in young animals, either by repeated injection of anti-IL-7 antibodies or by reconstitution of young lethally irradiated recipients with limiting numbers of HSCs from young animals, a skewing of the peripheral compartment results (Johnson SA, Cambier JC, unpublished observations). It is important to note a caveat in the 'limited B lymphopoiesis' model systems described above; unlike in aged mice, total numbers of splenic B cells are reduced in these mice, as compared with controls. This difference in observed cell number may simply reflect a difference in the time (weeks/months versus years) over which cells are allowed to accumulate. However, it may also reflect differences in the splenic microenvironment between young and aged animals. That is, the microenvironment of the old animal may further extend the lifespan of antigen-experienced cells or promote the survival and/or proliferation of antigen-experienced B cells.

### Cytokine networks and aging

The peripheral T cell compartment of aged mice is also skewed toward antigen-experienced cells, including CD4<sup>+</sup>

memory, CD8<sup>+</sup> memory, and NK1.1<sup>+</sup> cells (for review see [54]). In addition, multiple groups have reported changes in cytokine profiles with aging, and it is now clear that age-associated shifts in T cell subset composition are correlated with the progressive decreases in IL-2, and increases in IL-4, IL-5, and IFN- $\gamma$  [55–59]. Importantly, the depressed level of IL-2 found in aged mice may help to sustain the large pool of memory T cells and their cytokine products. In young adult mice a balance between IL-15 and IL-2 provides homeostatic control of CD8<sup>+</sup> memory T cell numbers; IL-15 induces proliferation, and IL-2 induces death [60]. Data from IL-2 or IL-2 receptor knockout mouse models suggest that IL-2 deficiency allows unchecked survival of memory T cells. Perhaps a similar mechanism is at work in the aged spleen.

Aging dependent changes in cytokine networks may also modify the B cell compartment. Spencer and Daynes [61] demonstrated that dysregulated macrophages in the aged spleen are responsible for the overproduction of IL-6, tumor necrosis factor (TNF)- $\alpha$ , and IL-12. *In vitro* data from that group further show that IL-12 stimulates IL-10 production by CD5<sup>+</sup> B cells and IFN- $\gamma$  by NK cells. As noted above, numbers of CD5<sup>+</sup> B cells are increased in the spleens of many aged animals. This overproduction of IL-10, and particularly IFN- $\gamma$ , may strongly influence the ratio of naïve follicular to antigen-experienced B cells in the aged spleen. Both cytokines are known to enhance release of B cell activating factor (BAFF; also known as BlyS, TALL-1, zTNF4, and THANK) by monocytes [62]. BAFF is a member of the TNF superfamily that specifically regulates B cell proliferation and survival. Interestingly from an aging standpoint, transgenic mice that overexpress BAFF have increased numbers of MZ cells and high levels of autoantibodies in their serum, prompting Groom and coworkers [40] to hypothesize that excess BAFF in these animals overrides a critical tolerance checkpoint by providing a survival signal to self-reactive B cells. It is currently unknown whether BAFF becomes dysregulated as a function of aging, but it is an intriguing possibility that warrants investigation.

### The B cell contribution to poor humoral immunity in the aged: defective B cells or defective B cell populations?

As referenced in the Introduction section above, aging is accompanied by a generalized dysregulation of many immune cell types. The studies described above clearly indicate that, in addition to well-documented senescence in the T cell compartment (for review see [63]), senescence in the B cell compartment probably also contributes to the deterioration of humoral immunity that is evident in many aged individuals. The following question then arises; does the B cell contribution to poor humoral immunity in the aged result from functional defects in individual B cells or from shifts in the cellular constitution of peripheral

lymphoid organs from naïve to antigen-experienced cells? We favor the latter hypothesis. It is well documented in both mice and humans that antibody responses in the aged are lacking in quality rather than quantity, indicating minimally that B cells from aged animals are fully competent to produce antibody (for review see [64]). The work of Dailey and coworkers [65] further supports the contention that individual follicular B cells from aged mice function normally. Experiments conducted by this group showed that when equal numbers of follicular B cells were transferred from either aged or young immunoglobulin transgenic donors to young primed recipients, specific thymus-dependent antibody responses generated upon challenge were equivalent, regardless of donor age. Likewise, experiments utilizing antigens that selectively stimulate CD5<sup>+</sup> B cells (e.g. trinitrophenyl-ficolin) or MZ B cells (e.g. native dextran) also show that specific antibody responses are equivalent in young and aged mice, again indicating that the function of these cells is normal [66,67].

So, how do shifts in the B cell constitution of peripheral lymphoid organs from naïve to antigen-experienced translate into the poor quality antibodies generated by aged animals? We propose that because naïve follicular B cells are in short supply, aged immunosenescent animals must rely, in part, on antigen-experienced (MZ, CD5<sup>+</sup> B1-like, and memory) B cells to defend themselves against new immunologic insults. If this is the case, then one would predict that the antibody response of aged mice would bear the hallmarks of antibodies produced by antigen-experienced cells that were initially expanded and selected by cross-reactive antigens or are B1 cells (i.e. it should be of relatively low affinity and poly/self-reactive). A variety of experimental evidence supports this hypothesis.

First, aging is associated with elevation in serum autoantibodies [12,68]. This elevation in autoantibodies has been documented by multiple groups using a variety of mouse strains, and includes antibodies reactive with double-stranded DNA, single-stranded DNA, and histones. In addition, autoantibodies against thymocytes and idiotypic determinants of BCR are detectable. Interestingly, the former have been implicated in impaired T cell poiesis [69], and the latter in suppression of specific B cell responses [70]. Importantly, autoantibodies in the sera of aged animals are rarely accompanied by autoimmune disease, probably because of their low affinity. Furthermore, studies from the laboratory of Weksler [71] demonstrated that aged mice immunized with a classical thymus dependent antigen, namely sheep erythrocytes (SRBC), produce fewer anti-sheep erythrocyte antibody secreting cells than do their young counterparts (probably from follicular B cells), but they produce significant levels of antibody reactive with the classical autoantigen, bromelain-treated mouse erythrocytes, which are not seen

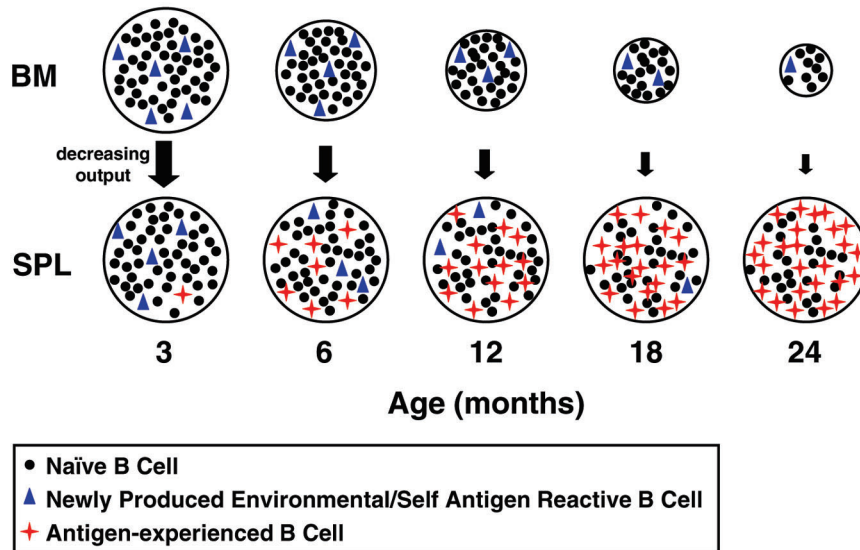
in young mice. This suggests a shift in the cells responding to the antigen from follicular B cells in young mice to antigen-experienced cells in old mice.

Second, studies conducted in the early 1970s [72–74] revealed that antibodies produced by aged as compared with young mice in response to antigenic challenge were of lower affinity and avidity. More recently, Cerny and colleagues [75] have extended these observations by demonstrating that antibodies produced by aged mice immunized with phosphorylcholine immunogens are not only of lower affinity and avidity but are also less protective against infection than those produced by young mice. Thus, the poor quality of the primary humoral response of aged animals probably reflects the mixed response of specific naïve B cells and polyreactive antigen-experienced B cells, rather than some B cell functional defect.

Also contributing to the lower affinity of humoral responses in aged animals may be the recently described impairment of somatic hypermutation [76]. Because germinal centers (GCs) are known to be the primary site of immunoglobulin somatic mutation and affinity maturation, these data point to a defect in GC formation and/or function. Not surprisingly, immunohistologic and flow cytometric analyses show that both the number and volume of GCs decline gradually as a function of age (for review see [77]). Because GCs arise primarily from antigen stimulated follicular B cells, this may simply reflect the reduced number of follicular cells in aged animals. However, precise dissection of the GC reaction shows that in aged mice senescence in both the B cell and T cell compartments contributes to the changes in GC output. Specifically, experiments in which severe combined immunodeficient (*scid*) mice were reconstituted with CD4<sup>+</sup> T cells and unfractionated B cells, from unimmunized young or aged donors in reciprocal combinations, demonstrated that the somatic hypermutation process was severely limited when either B or T cells came from aged donors, and was comparable to that in intact young adult animals only when both cell types were derived from young donors [78]. Importantly, these experiments did not address the role of the aged splenic microenvironment, and it is quite possible that defects in FDC function also contribute to the age-related impairment in the GC reaction [79]. Nonetheless, they indicate that, in addition to the impact of B cell compartment (e.g. follicular to MZ/B1 skewing), 'defective' T cell help may contribute to the poor quality of the humoral response of aged individuals.

Study of the GC reaction in healthy aged humans is impractical for obvious reasons. Nonetheless, the products of the GC reaction, namely antibodies, have been studied. In aged humans, as in mice, antibody affinity is reduced and total levels of serum autoantibodies are increased [80,81].

Figure 1



The B cell compartment changes with age. BM, bone marrow; SPL, spleen.

Again, as in mice, these autoantibodies lack specificity for organs and rarely contribute to autoimmune disease [2]. The demonstration of increased autoantibodies in the serum of elderly humans is of importance, however, because it indicates that a similar state of immune dysregulation exists in aged humans and mice.

Current literature contains many reports describing a shift in T cell subsets from naïve to memory in aged humans (for review see [3]). Unfortunately, a paucity of information exists regarding the nature of the B cell compartment in these same individuals. Available evidence suggests that the total number of B cells declines as human beings age [82]. Although on the surface this seems counter to the situation in mice, one must remember that studies of aged humans are confined to examination of peripheral blood B cells. Certain B cell subsets, including MZ B cells, do not recirculate, and thus would not be accounted for in studies of peripheral blood [52]. As noted previously, total numbers of MZ B cells increase in many aged mice. Moreover, data reported as percentages, rather than as total numbers, indicate that CD27<sup>+</sup> memory B cells increase in the blood of elderly humans [82]. Aged humans further parallel aged mice in dysregulation of measurable cytokines. Several groups reported that aged, as compared with adult, humans have increased levels of IL-4, IFN- $\gamma$ , and IL-12 [83,84]. These cytokines all have strong potential to sustain long-lived antigen-experienced B cells.

## Conclusion

As illustrated in Fig. 1, we believe that aging is associated with decreased B lymphopoiesis in the BM, which

ultimately limits the output of new B cells to the periphery. Under these conditions, lack of competition for space in peripheral niches allows environmental/self-reactive B cells, which would normally be silenced, to enter and survive. Over time, these self-reactive B cells, as well as antigen-experienced B cells (CD5<sup>+</sup> B1-like, MZ, and memory), accumulate and eventually dominate the peripheral B cell compartment. It is likely that cytokine dysregulation helps to maintain this skewing of B cell populations. Furthermore, available data indicate that individual B cells of all subtypes function normally, but that humoral immunity is greatly diminished in many aged animals. We maintain that this decline in humoral immunity reflects the forced reliance on antigen-experienced B cells, rather than on naïve, follicular B cells, to respond to new immunologic insults; lack of appropriate T cell help and 'defective' FDC function probably also play a role.

If one believes, as we do, that a causal link exists between decreased BM production of B cells and decreased humoral immunity, then one might hypothesize that increasing B cell output to 'young-like' levels would improve humoral immunity. In fact, recent experiments conducted in our laboratory demonstrate that reconstitution of aged mice with HSCs from young mice re-establishes a normal, young-like peripheral B cell compartment, consisting primarily of naïve, follicular B cells (SA Johnson and JC Cambier, unpublished observation). We have not yet measured the impact of this treatment on humoral immunity but we have high hopes. We are also investigating other strategies for improving B cell output from the BM of aged individuals. For example,

because decreased B cell production may result from impaired signaling through IL-7 receptors, it might be possible to bypass this defect using a gene therapy approach. Such approaches, while not providing a 'fountain of youth', may someday enhance the quality of life of the aged by increasing their resistance to infectious agents.

### Competing interests

None declared.

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