Impaired B lymphopoiesis in old age: a role for inflammatory B cells?

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Published online: 8 November 2013
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Abstract Continued generation of new B cells within the bone marrow is required throughout life. However, in old age, B lymphopoiesis is inhibited at multiple developmental stages from hematopoietic stem cells through the late stages of new B cell generation. While changes in B cell precursor subsets, as well as alterations in the supporting bone marrow microenvironment, in old age have been known for the last 20 years, only more recently have insights into the cellular and molecular mechanisms responsible become clarified. Our recent discovery that B cells in aged mice are pro-inflammatory and can diminish B cell generation within the bone marrow suggests a potential mechanism of inappropriate “B cell feedback” which contributes to a bone marrow microenvironment unfavorable to B lymphopoiesis. We hypothesize that the consequences of a pro-inflammatory microenvironment in old age are (1) reduced B cell generation and (2) alteration in the “read-out” of the antibody repertoire. Both of these likely ensue from reduced expression of the surrogate light chain ($\lambda 5 + V_{preB}$) and consequently reduced expression of the pre-B cell receptor (preBCR), critical to pre-B cell expansion and Vh selection. In old age, B cell development may progressively be diverted into a preBCR-compromised pathway. These abnormalities in B lymphopoiesis likely contribute to the poor humoral immunity seen in old age.

Keywords Aging · Inflammation · Age-associated B cells · B lymphopoiesis

Introduction

Like most of the body’s organ systems, the immune system deteriorates physically and functionally in old age. The demise in antibody-producing B cell functions in elderly mice and humans likely results from a variety of factors, both intrinsic to B cells and due to poor quality of T cell help [1–3]. However, recent analyses now have revealed that changes within the bone marrow in old age also contribute to (1) the abrogation of normal B cell generation, (2) alterations in peripheral B cell homeostasis, and (3) deviation of B cell functions—all of which occur against the backdrop of an increasingly pro-inflammatory “aged microenvironment” [1–5]. The purpose of

this review is to discuss the abnormal B lymphopoiesis seen in old age, primarily in the mouse model, and connect the altered development of new B cells in old age to the dysfunctions seen in B cell immunity. We will focus on recent studies by ourselves and others that show the potential of pro-inflammatory B cells to promote changes within the bone marrow microenvironment that affect both B cell production and function [6–8]. We hypothesize that, as a consequence of increased inflammation locally within the bone marrow, the pathways of B lymphopoiesis are deviated from normal in old age.

Old age interferes with multiple stages in B lymphopoiesis

Declines in B cell precursors accompany old age

Studies in inbred mice of several strains at ~2 years of age led to the first realization, ~20 years ago, that B

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Fig. 1 Old age impairs multiple stages in B lymphopoiesis. The relative effects of old age on the sizes of the myeloid-biased hematopoietic stem cells (My-HSC), lymphoid-biased hematopoietic stem cells (Ly-HSC), common lymphoid progenitor cells (CLP), pro-B cells, and pre-B cells are illustrated. The expression of the SLC proteins $\lambda 5$ and VpreB is reduced in aged mice and likely compromises the preBCR checkpoint. Two types of newly formed

immature B cells are shown: CD43/S7⁻, the conventional immature B cell, and CD43/S7⁺ immature B cells which exhibit characteristics of activation, anergy, and are speculated to express autoreactive specificities. In old age, B lymphopoiesis is diminished overall, but the production of CD43/S7⁺ new B cells, favored when preBCR is poorly expressed, is maintained [63]

lymphocyte development was compromised in old age [9, 10]. As shown in Fig. 1, multiple stages—from hematopoietic stem cells to late stages of immature B cell development—are altered in old individuals. Whether interference at each of these cellular checkpoints in old age results from a common mechanism or, alternatively, whether unique defects in regulation affect each stage is not known. The effects of old age at the pre-B cell stage are probably best understood. Pre-B cells are the immediate precursors of new B cells; they express a μ Ig heavy chain, but not conventional κ or λ Ig light chains, and comprise the largest subset of B cell precursors within the bone marrow. However, pre-B cells are variably reduced in the majority of old mice ≥ 2 years of age and can be diminished by $>80\%$ in some individuals [11]. Pre-B cell loss in mice occurs progressively during the second year of life with some loss of pre-B cells seen as early as 15 months old [9, 11]. In old mice, pre-B cell loss is often accompanied by reductions in earlier B cell precursors (pro-B cells) where μ heavy chain gene rearrangement is in process. Pro-B cells are reduced in at least one-third of ~ 2 -year-old mice [11]. Therefore, both pre-B and pro-B cells are adversely affected by old age in mice, with pre-B cells being generally more extensively reduced.

The transition at the pro-B $\rightarrow$ pre-B cell stage is dependent upon productive μ Ig heavy chain variable region gene rearrangements to produce a functional μ heavy chain protein. This gene rearrangement process, mediated by the recombinase enzymes RAG-1 and RAG-2, is impaired in aged pro-B cells due to lower levels of RAG expression [12]. This contributes to diminished pre-B cells in old mice. Early pre-B cells, at the time of μ Ig heavy chain protein expression, undergo extensive proliferation directed by the pre-B cell receptor (preBCR), a complex of μ Ig heavy chain and the surrogate light chain (SLC) proteins $\lambda 5$ and VpreB [13]. In B cell precursors from aged mice, the loss of the $\lambda 5$ surrogate light chain protein correlates with the reductions seen in pre-B cells [14, 15]. Therefore, it is likely that poor pre-B cell production in aged mice is also due to reduced preBCR expression and function as a consequence of low SLC.

Other mechanisms likely also contribute to the losses seen in both pro-B and pre-B cells. Changes in the balance between pro- and anti-apoptotic signals may affect survival of B cell precursors in old mice. For example, pre-B cells in old bone marrow have reduced levels of the pro-survival protein Bcl-xL [16]. In any event, the generation of B cell precursors declines in old mice, as shown both

experimentally and by mathematical modeling of BrdU kinetic data [12, 17, 18].

Whether similar compromise of B cell precursors occurs in the elderly human remains controversial. While some reports indicate continued maintenance of B cell precursors with old age in humans [19, 20], an analysis of B cell precursors in a large sampling of patients indicated that there is a decline in B cell precursors in adults with increasing age [21].

Declines in B cell precursors in old age are associated with preferential loss of lymphoid-biased hematopoietic stem cells

In recent years, much attention has been directed to assessing the capacity of pluripotent hematopoietic stem cells (HSC) to generate new lymphoid cells in aged mice. These studies consistently have demonstrated that HSC are maintained in normal to elevated numbers in old murine bone marrow; however, HSC in old bone marrow are less capable of generating lymphoid cells while myeloid cell generative potential is unaffected [22]. Recently, HSC have been shown to be heterogeneous and have been subdivided based on surface phenotype, with CD86⁺ CD150⁺ HSC exhibiting greater lymphoid potential [23]. Of interest, the CD86⁺ CD150⁺ HSC subset with high lymphoid potential is reduced in old bone marrow, and the majority of HSC in aged bone marrow express the CD86[−] CD150⁺ phenotype characteristic of myeloid cell-biased HSC [23]. It is worth mentioning that inflammation induced in young adult mice by repetitive injections of low amounts of LPS resulted in a “shift” in HSC phenotypes with depletion of the lymphoid-biased HSC pool [24]. Since, as will be discussed below, old age results in a pro-inflammatory bone marrow microenvironment, it is tempting to speculate that inflammation may promote the decline in lymphoid-biased HSC seen in aged mice.

Compromised B lymphopoiesis in old age reflects degradation of the B lineage transcription factor network

Downstream of the HSC, the common lymphoid progenitor cell (CLP) represents a key stage, since most CLP within the bone marrow is destined to become B lineage cells. In old bone marrow, CLP numbers decline; this coincides with poor expression of the transcription factor EBF1 [25, 26]. In pro-B cells from aged mice, both E2A and EBF are down-regulated [[15, 26–29]; Fig. 2]. E2A and EBF1 regulate transcription of the SLC genes [30] and reduced E2A levels correlate significantly with diminished levels of the $\lambda 5$ SLC protein in aged pro-B cells [15].

E47/E2A is down-regulated in aged pro-B cells primarily through increased phosphorylation, ubiquitination, and,

consequently, more rapid protein turnover [28, 29]. Not surprisingly, given the low expression of both E47/E2A and EBF1, the transcription factor Pax-5 is also reduced in aged pro-B cells [[28]; Fig. 2]. This is likely due to both diminished transcription, regulated by E2A and EBF1, as well as increased Pax-5 protein turnover in pro-B cells [28].

Retroviral expression of EBF1 in aged CLP restored their capacity to generate B lineage cells [26]. Consequently, the transcriptional program, involving E2A, EBF1, and Pax-5, which governs the formation of B cell precursors [31, 32], is significantly impaired in old age. However, whether these changes are caused by alterations in the environmental milieu supporting development of B lineage cells or, alternatively, are the result of intrinsic changes in HSC remains unclear.

It is of particular significance that the E2A, EBF1, and Pax-5 transcription factors, while certainly comprising a diversity of functional capabilities, all overlap in regulating the expression and/or function of the preBCR. E2A and EBF1 promote transcription of RAG genes [31], important for μ heavy chain expression, as well as the SLC genes [30]; Pax-5 promotes transcription of the adaptor BLNK/Slp65 which is crucial for B cell development and “links” preBCR-dependent activation of kinases with downstream signaling processes [33].

Another protein associated with preBCR signaling is Bob-1 (OcaB; Obf-1), which, although normally a transcription factor, also associates with and stabilizes activated Syk and enhances preBCR signaling in pre-B cells [34]. EBF1 induces Bob-1 expression [35] and Bob-1 protein levels are reduced in pro-B cells cultured from aged mice [[28]; Fig. 2]. Common to E2A, EBF1, Pax-5, and Bob-1 proteins is their accelerated degradation and turnover in aged pro-B cells [28]. We have shown that increased Erk/MAPK activity in B cell precursors from aged mice leads to increased phosphorylation of E47/E2A (Thr³⁵⁵) and this targets E47/E2A for ubiquitination and proteasome-dependent degradation [29]. While information regarding EBF1 phosphorylation is not available, Pax-5 does undergo Erk/MAPK-induced phosphorylation [36] and Bob-1 is phosphorylated on Ser/Thr residues making it a possible target for Erk/MAPK [37]. These findings suggest that a common mechanism, e.g., increased Erk/MAPK activity, results in phosphorylation, ubiquitination, rapid turnover, and lower steady-state levels of multiple key factors (E2A, EBF1, Pax-5, Bob-1), important to preBCR function in particular and B cell development in general.

The bone marrow microenvironment deters B lymphopoiesis in old age

Several lines of investigation indicate that the bone marrow microenvironment is altered in old age and contributes to

Fig. 2 Old age impairs the transcriptional network necessary for B lineage development and limits preBCR expression. **a** The hierarchical interrelationships among the E2A, EBF1, and Pax-5 transcription factors are shown. In addition, the roles of these transcription factors in the production/function of proteins key to the proper assembly and function of the preBCR are indicated. **b** The relative levels of total EBF proteins (EBF 1, 2, 3) in representative young (Y) and old

(O) pro-B cells, after expansion in vitro in IL-7 supported cultures, are shown as determined by Western blot analysis. The protein Ubc9 served as a control. Similarly, the relative levels of EBF, E47/E2A, Pax-5, and Bob-1 proteins in old pro-B cells are shown as a percentage of their values in young pro-B cells. Data are summarized from [28]

reductions in new B cell output. In vivo, reciprocal adoptive transfers of either young or aged bone marrow cells into irradiated young or aged recipients indicated that reconstitution of B lymphopoiesis was influenced by the host microenvironment [12].

Functionally, bone marrow stromal cells from aged mice continue to synthesize normal quantities of the growth promoting cytokine IL-7; however, the levels of bioactive IL-7 available for stimulation of B cell precursors is reduced [38]. B cell precursors from aged mice, while expressing both IL-7 receptor α and common γ chains, show diminished proliferation in response to IL-7 as well [11, 25, 39].

Of interest, the chemokine RANTES (CCL5) has been shown to increase myeloid production from HSC and inhibit lymphopoiesis [40]. Notably, RANTES is produced by bone marrow stromal cells, and the levels of RANTES increase within the bone marrow in old age [40]. Therefore, it has been suggested [41] that the cytokine environment may be altered within the bone marrow in old age and bias

the capacity of HSC to develop along myeloid, as opposed to lymphoid, pathways.

Old age alters the B cell specificity repertoire and can impair protective immunity

Does deficient B lymphopoiesis cause the antibody repertoire to be less protective in old age?

Reduced quantities of antibodies result in impaired immunity in old age; however, alterations in the quality of the antibody response may also have a detrimental impact on humoral immunity in the elderly. Mature follicular (FO) B cells function less effectively in mounting immune responses in old age due to a combination of diminished T cell help as well as B cell-specific defects [42, 43]. The formation of germinal centers in response to T cell-dependent antigens is reduced in old mice [44]; moreover, both Ig isotype (class) switching [45–47] and somatic

hypermutation [48] are impaired, and consequently, affinity maturation of the antibody response is reduced. These features of reduced T cell-dependent immunity generally apply to both old mice and elderly humans [45–49]. While diminished T cell function in old age certainly contributes to poor immune responses, B cell intrinsic deficiencies also occur in old age. In old B cells, somatic hypermutation of Ig V regions in response to antigen stimulation was decreased even when young adult T cell help was provided [48].

Both Ig isotype switching and somatic hypermutation rely on the actions of activation-induced cytidine deaminase (AID) [50]. In old B cells, LPS stimulation led to reduced synthesis of E47/E2A and this, in turn, resulted in lower transcription of AID and reduced isotype switch [45–47]. In old age, mature FO B cells gain the capacity to produce the pro-inflammatory cytokine TNF α and E47/E2A and AID expression are reduced by TNF α [51]. Systemic treatment of old mice with neutralizing anti-TNF α antibodies effectively corrected the deficient Ig isotype switch capacity in old FO B cells [51].

In addition to impaired B cell functions in old age, there are also alterations in the “pre-immune” repertoire of available B cell receptor specificities for antigen that can have a profound detrimental effect on protective immune responses. The most well-studied example is the antibody response in aged BALB/c mice to the phosphorylcholine (PC) antigenic determinant expressed on the bacterium, *S. pneumoniae*, as well as by various filariae. In the BALB/c mouse, protective immunity to *S. pneumoniae* is critically dependent on the predominance of a particular anti-PC-specific antibody which utilizes the germ line-encoded T15 idiotype and provides high affinity antibodies for clearance of this pathogen [52, 53].

In old mice, titers of anti-PC antibodies elicited by *S. pneumoniae* are robust; however, the low affinity and lack of the T15 idiotype exhibited by these antibodies impair their efficacy. Surprisingly, the frequency of T15⁺ splenic B cells responsive to PC actually increases by ~ threefold in aged mice [52]. However, in old mice, the splenic B cells responsive to PC that are T15[−] show a ~ fivefold increase in frequency [52]. Consequently, while a majority of PC responsive B cells in young adult spleen are T15⁺, in old mice, a majority of splenic anti-PC-specific B cells are lower affinity T15[−] clonotypes.

The alterations seen in the splenic PC responsive B cell repertoire in old mice appear to have their origins in the old bone marrow [54]. Similar increases in T15[−] anti-PC B cell clonotypes are also observed at very early immature B cell stages within the bone marrow of aged mice. This strongly suggests that the alterations in the antibody repertoire to PC in old mice occur as a consequence of abnormalities in B cell development within the bone marrow.

Whether alterations occur in more clonally diverse antibody responses in old age is not known. The antibody repertoires specific for the influenza PR8 hemagglutinin protein as well as to the hapten (4-hydroxy-3-nitrophenyl) acetyl (NP) are similarly diverse in young and old mice [55, 56]. However, in humans, the diversity of the antibody repertoire contracts in the elderly and this correlates significantly with poor health [57].

Compromise of the pre-B cell receptor contributes to B cell repertoire “reshaping” in old age

As discussed above, the expression of the SLC proteins, which together with μ heavy chain comprise the preBCR, is substantially reduced in pro-B cells from aged mice. The role of the preBCR in selecting the μ heavy chain repertoire, based on the differential binding of individual μ heavy chains to SLC, has been investigated using mice deficient in SLC expression. Young adult mice which lack the SLC generate early pre-B cells; however, since these early pre-B cells fail to express the preBCR, proliferation is curtailed at this developmental stage, and numbers of late-stage pre-B cells are substantially reduced [13]. Moreover, pre-B cells from SLC-deficient mice are enriched for μ heavy chains that cannot associate with SLC [58–61]. Whether the SLC-low B cell precursors in aged bone marrow now show a “relaxed” preBCR selection of μ heavy chains remains to be directly tested.

The preBCR checkpoint has been shown to function in tolerance to self-antigens [62]. Studies of the newly generated, immature B cell populations in the bone marrow of old mice exhibit unique characteristics suggestive of self-reactivity. These include an increase in the proportion of bone marrow immature B cells that expressed the surface antigen CD43/S7, often co-expressed with CD5, CD11b, and/or PD-1—all surface proteins associated with dampening B cell activation and in maintaining anergy and B cell tolerance [63].

Further studies established that the remaining pre-B cell pool in aged mice retained the capacity to generate CD43/S7⁺ new B cells, but were deficient in precursors of the more conventional CD43/S7[−] immature B cells [63]. Similar disparities in the production of CD43/S7⁺ versus CD43/S7[−] immature B cells were seen in experiments using B cell precursors from young adult λ 5 gene knockout mice [63]. This suggests that the CD43/S7⁺ immature B cells are generated predominantly when preBCR signaling is diminished. Both new B cells derived from precursors from either aged mice or λ 5-deficient young mice had relatively high expression of “dual κ/λ ” light chain isotypes indicative of light chain receptor editing or receptor dilution [63]. Moreover, the majority of CD43/S7⁺ immature bone marrow B cells, in either young or aged

mice, were anergic as evidenced by poor mobilization of calcium upon BCR ligation (Alter et. al., manuscript in preparation).

Taken together, these findings indicate that when SLC is low and preBCR function compromised, disproportionately the new B cells generated express a phenotype associated with self-reactivity and B cell anergy. In old mice, due to low SLC expression, not only are the numbers of pre-B cells reduced dramatically, but the repertoire of B cell specificities among new B cells may be biased toward autoreactive clonotypes.

B cells contribute to pro-inflammatory microenvironments and “feed-back” inhibition of B lymphopoiesis in old age

B cells become biased toward “antigen-experienced B cells” in old age

While B cell development within the bone marrow is compromised in old age, the total number of mature B cells is maintained in the peripheral lymphoid tissues. Notably, the immediate precursors of mature FO and marginal zone (MZ) B cells, the transitional B cell stages, are also highly reduced in old age [4] and the migration of newly formed B cells within the bone marrow into the spleen is impaired [64]. Kinetic studies indicate that the turnover of mature B cells within the spleen is much slower in old mice [65]; this alteration in homeostasis largely accounts for the maintenance of normal B cell numbers in the spleen in old age even as development of new B cells and their migration into the spleen are diminished.

It is not surprising that subpopulations of B cells that are chronically exposed to antigens are expanded in old age. These “antigen experienced B cells,” which can include a variety of B cell phenotypes [66], include a newly recognized subset termed “age-associated B cells” or “ABC” [6–8]. While the phenotype and functions of ABC vary somewhat among laboratories, and research into the properties of ABC is only beginning, several features of ABC are notable: (1) ABC are increased in the spleens of old mice of several strains with the CD23⁺ CD21/35⁺ mature ABC pool increased by 5–10-fold at 2 years of age [6–8]; (2) ABC are derived, at least in part, from FO B cells, possibly via antigen-driven selection [6–8], (3) in old age, B cells with the ABC phenotype are pro-inflammatory and secrete TNF α while B cells with the ABC phenotype from young mice do not [8], and (4) ABC respond strongly to TLR stimulation, but relatively weakly to typical T cell-dependent signals [6].

Aged-associated B cells: a key contributor to deficient B lymphopoiesis in old age?

Recently, it has been suggested that B cells play an important role in the down-regulation of B lymphopoiesis in old age [67–69]. Repeated depletion of B cells in vivo, followed by autoreconstitution of the bone marrow, “corrected” the poor B lymphopoiesis seen in old mice [67] and mice with genetic defects which caused a lack of mature B cells did not exhibit impaired B lymphopoiesis in old age [68]. ABC are an attractive candidate to negatively regulate B cell development: (1) ABC are increased, both in number and proportion, not only in the spleens of old mice, but also in the bone marrow [6–8]; (2) ABC in old mice produce TNF α and are pro-inflammatory [8, 51]; (3) recently, we have reported that the decline in B cell precursors seen in the bone marrow of aged mice is significantly correlated with increases in bone marrow ABC and, more precisely, with increases in the ABC to FO-like/recirculating B cell ratio within the bone marrow [8]; and 4) adoptive transfer of old ABC into RAG-2 knockout recipients resulted in a significant decline in bone marrow pro-B cells in vivo [8].

Both ABC and FO/recirculating B cells gain the capacity to produce TNF α during old age [8, 51], and ABC-derived TNF α induces apoptosis of pro-B cells in vitro [8]. While FO/recirculating B cells also produce TNF α , some B cells with an FO phenotype secrete the anti-inflammatory cytokine IL-10 [8]. These may represent a regulatory B cell (“Breg”) population within the FO pool [70]. IL-10 effectively blocks the induction of apoptosis in pro-B cells induced by TNF α stimulation [8]. Unlike old FO B cells, ABC fail to produce IL-10, but do secrete TNF α [8]. Therefore, as the ABC (TNF α -secreting cells) to FO/recirculating B cells (TNF α /IL-10-secreting cells) ratio increases within the bone marrow of aged mice, and ABC becomes the majority mature B cell subset, we hypothesize that the local microenvironment becomes increasingly pro-inflammatory and detrimental to B lymphopoiesis (Fig. 3).

Although TNF α derived from old ABC within the bone marrow may directly induce apoptosis of pro-B cells, arguably the greater effect of ABC-derived TNF α is to activate macrophages (or possibly other bone marrow cells) to secrete cytokines (in addition to TNF α) that induce pro-B cell apoptosis. Non-B bone marrow cells from RAG-2 KO mice, after adoptive transfer of old ABC, induce apoptosis in pro-B cells [8]. Moreover, young bone marrow cells were induced to suppress B cell precursor growth after co-culture with old ABC, and this was dependent on ABC-derived TNF α [8]. Therefore, we propose that expansion of ABC in old mice induces the activation of macrophages that inhibit B cell precursor growth and survival. In old age, a pro-inflammatory microenvironment

Fig. 3 Age-associated B cells (ABC) promote a pro-inflammatory bone marrow environment which causes apoptosis in pro-B cells. Age-associated B cells (ABC) from aged mice are capable of $\text{TNF}\alpha$ secretion; $\text{TNF}\alpha$ is a potent inducer of apoptosis in pro-B cells [8]. $\text{TNF}\alpha$ also activates bone marrow macrophages, likely leading to production of a variety of cytokines and biomediators, in addition to $\text{TNF}\alpha$, which cause apoptosis in B cell precursors [8]. The follicular (FO) and recirculating mature B cells in old mice also produce $\text{TNF}\alpha$, but a subset produces IL-10 as well [8, 70]. IL-10 “protects” pro-B cells from $\text{TNF}\alpha$ -induced apoptosis [8]. As the numbers of FO B cells within the bone marrow decline and ABC become the predominant mature B cell population, the pro-inflammatory effects of ABC are hypothesized to cause extensive pro-B cell apoptosis and compromise late stages of B lymphopoiesis

within the bone marrow likely contributes to the loss of pro-B/pre-B cells and possibly earlier progenitors.

We have previously reported that $\text{TNF}\alpha$ treatment of pro-B cells leads to lower expression of $\lambda 5$ SLC [71]. Consequently, ABC from old mice may be capable, via $\text{TNF}\alpha$, of lowering levels of $\lambda 5$ SLC in the residual pro-B cells. ABC may promote the down-regulation of the typical B cell developmental pathway which is dependent upon the preBCR, leaving the remaining small pro-B cell pool, with low SLC, to generate a reduced population of pre-B cells characterized by limited preBCR function (Fig. 1).

It is tempting to speculate that the pro-inflammatory bone marrow microenvironment postulated for old mice also contributes to the loss of CLP and possibly the bias of hematopoietic stem cells to myeloid rather than lymphoid differentiation. That this may be feasible is suggested by the loss of lymphoid-biased HSC in young mice given low levels of LPS over time to simulate chronic inflammation [24].

Summary and conclusion

Old age leads to diminished immune responses to infectious diseases and this contributes to increased morbidity

and mortality in the elderly. The development of immune cells, including B lymphocytes, is compromised in old age. Deficits in B lymphopoiesis occur as a consequence of alterations at multiple stages in the B cell developmental program, including at the preBCR checkpoint. Whether the alterations seen in HSC specification to the lymphocyte lineages and changes in later stages of B cell development stem from a common set of mechanisms associated with old age is not known. Inflammation is capable of causing bias in favor of myeloid vs. lymphoid HSC; inflammatory cytokines, in part derived from ABC, also cause apoptosis in late-stage B cell precursors as discussed above. Therefore, it may be that chronic inflammation, typical of old mice and humans, provides a common denominator for understanding the changes seen during B lymphopoiesis in senescence. The down-regulation of B lymphopoiesis in old age may involve reduced preBCR function. This not only affects the normal homeostasis of B cells, but also likely the diversity of the B cell repertoire. The latter alterations may promote autoreactivity, while reducing humoral immunity to pathogens, and contribute to the immune dysfunctions seen in old age.

Acknowledgments We thank all members of the Riley laboratory past and present and our collaborators, Dr. Bonnie Blomberg and Dr. Daniela Frasca and their laboratories, for reading of the manuscript, stimulating discussions, and assistance in the performance of our studies described herein. Support was provided by the National Institutes of Health, National Institute on Aging Grant R01 AG025256 to RLR.

Conflict of interest The author declares that he has no conflict of interest.

References

1. Linton PJ, Dorshkind K. Age-related changes in lymphocyte development and function. *Nat Immunol.* 2004;5:133–9.
2. Cancro M, Hao Y, Scholz J, Riley R, Frasca D, Dunn-Walters D, Blomberg BB. B cells and aging: molecules and mechanisms. *Trends Immunol.* 2009;30:313–8.
3. Scholz JL, Diaz A, Riley RL, Cancro MP, Frasca D. A comparative review of aging and B cell function in mice and humans. *Curr Opin Immunol.* 2013;25:504–10.
4. Miller JP, Cancro MP. B cells and aging: balancing the homeostatic equation. *Exp Gerontol.* 2007;42:396–9.
5. Vasto S, Candore G, Balistreri CR, Caruso M, Colonna-Romano G, Grimaldi MP, Listi F, Nuzzo D, Lio D, Caruso C. Inflammatory networks in ageing, age-related diseases and longevity. *Mech Ageing Dev.* 2007;128:83–91.
6. Hao Y, O'Neill P, Naradikian M, Scholz J, Cancro MP. A B-cell subset uniquely responsive to innate stimuli accumulates in aged mice. *Blood.* 2011;118:1294–304.
7. Rubtsov A, Rubtsova K, Fischer A, Meehan R, Gillis J, Kappler J, Marrack P. Toll-like receptor 7 (TLR7)-driven accumulation of a novel CD11c(+) B-cell population is important for the development of autoimmunity. *Blood.* 2011;118:1305–15.

8. Ratliff M, Alter S, Frasca D, Blomberg BB, Riley RL. In senescence, age-associated B cells secrete TNF α and inhibit survival of B-cell precursors. *Aging Cell*. 2013;12:177–332.
9. Riley RL, Kruger MG, Elia J. B cell precursors are decreased in senescent BALB/c mice, but retain normal mitotic activity in vivo and in vitro. *Clin Immunol Immunopathol*. 1991;59:301–13.
10. Stephan RP, Sanders VM, Witte PL. Stage-specific alterations in murine B lymphopoiesis with age. *Int Immunol*. 1996;8:509–18.
11. Van der Put E, Sherwood EM, Blomberg BB, Riley RL. Aged mice exhibit distinct B cell precursor phenotypes differing in activation, proliferation and apoptosis. *Exp Gerontol*. 2003;38:1137–47.
12. Labrie JE, Sah A, Allman D, Cancro M, Gerstein R. Bone marrow microenvironmental changes underlie reduced RAG-mediated recombination and B cell generation in aged mice. *J Exp Med*. 2004;200:411–23.
13. Melchers F, ten Boekel E, Seidl T, Kong XC, Yamagami T, Onishi K, Shimizu T, Rolink AG, Andersson J. Repertoire selection by pre-B-cell receptors and B-cell receptors, and genetic control of B-cell development from immature to mature B cells. *Immunol Rev*. 2000;175:33–46.
14. Sherwood E, Blomberg BB, Xu W, Warner C, Riley R. Senescent BALB/c mice exhibit decreased expression of lambda5 surrogate light chains and reduced development within the pre-B cell compartment. *J Immunol*. 1998;161:4472–5.
15. Sherwood E, Xu W, King A, Blomberg BB, Riley RL. The reduced expression of surrogate light chains in B cell precursors from senescent BALB/c mice is associated with decreased E2A proteins. *Mech Ageing Dev*. 2000;118:45–59.
16. Kirman I, Zhao K, Wang Y, Szabo P, Telford W, Weksler ME. Increased apoptosis of bone marrow pre-B cells in old mice associated with their low number. *Int Immunol*. 1998;10:1385–92.
17. Johnson KM, Owen K, Witte PL. Aging and developmental transitions in the B cell lineage. *Int Immunol*. 2002;14:1313–23.
18. Shahaf G, Johnson K, Mehr R. B cell development in aging mice: lessons from mathematical modeling. *Int Immunol*. 2006;18:31–9.
19. Rossi MI, Yokota T, Medina KL, Garrett KP, Comp PC, Schipul AH Jr, Kincade PW. B lymphopoiesis is active throughout human life, but there are developmental age-related changes. *Blood*. 2003;101:576–84.
20. Nunez C, Nishimoto N, Gartland GL, Billips LG, Burrows PD, Kubagawa H, Cooper MD. B cells are generated throughout life in humans. *J Immunol*. 1996;156:866–72.
21. McKenna RW, Washington LT, Aquino DB, Picker LJ, Kroft SH. Immunophenotypic analysis of hematogones (B-lymphocyte precursors) in 662 consecutive bone marrow specimens by 4-color flow cytometry. *Blood*. 2001;98:2498–507.
22. Muller-Sieburg CE, Sieburg HB, Bernitz JM, Cattarossi G. Stem cell heterogeneity: implications for aging and regenerative medicine. *Blood*. 2012;119:3900–7.
23. Shimazu T, Iida R, Zhang Q, Welner RS, Medina KL, Alberola-Lla J, Kincade PW. CD86 is expressed on murine hematopoietic stem cells and denotes lymphopoietic potential. *Blood*. 2012;119:4889–97.
24. Esplin BL, Shimazu T, Welner RS, Garrett KP, Nie L, Zhang Q, Humphrey MB, Yang Q, Borghesi LA, Kincade PW. Chronic exposure to a TLR ligand injures hematopoietic stem cells. *J Immunol*. 2011;186:5367–75.
25. Miller J, Allman D. The decline in B lymphopoiesis in aged mice reflects loss of very early B-lineage precursors. *J Immunol*. 2003;171:2326–30.
26. Lescale C, Dias S, Maes J, Cumano A, Szabo P, Charron D, Weksler ME, Dosquet C, Vieira P, Goodhardt M. Reduced EBF expression underlies loss of B-cell potential of hematopoietic progenitors with age. *Aging Cell*. 2010;9:410–9.
27. Frasca D, Nguyen D, Riley RL, Blomberg BB. Decreased E12 and/or E47 transcription factor activity in the bone marrow as well as in the spleen of aged mice. *J Immunol*. 2003;170:719–26.
28. Van der Put E, Frasca D, King AM, Blomberg BB, Riley RL. Decreased E47 in senescent B cell precursors is stage specific and regulated posttranslationally by protein turnover. *J Immunol*. 2004;173:818–27.
29. King AM, Van der Put E, Blomberg BB, Riley RL. Accelerated Notch-dependent degradation of E47 proteins in aged B cell precursors is associated with increased ERK MAPK activation. *J Immunol*. 2007;178:3521–9.
30. Sigvardsson M, O'Riordan M, Grosschedl R. EBF and E47 collaborate to induce expression of the endogenous immunoglobulin surrogate light chain genes. *Immunity*. 1997;7:25–36.
31. Medina KL, Singh H. Genetic networks that regulate B lymphopoiesis. *Curr Opin Hematol*. 2005;12:203–9.
32. Rolink AG, Schaniel C, Busslinger M, Nutt SL, Melchers F. Fidelity and infidelity in commitment to B-lymphocyte lineage development. *Immunol Rev*. 2000;175:104–11.
33. Schebesta M, Pfeffer PL, Busslinger M. Control of pre-BCR signaling by Pax5-dependent activation of the BLNK gene. *Immunity*. 2002;17:473–85.
34. Siegel R, Kim U, Patke A, Yu X, Ren X, Tarakhovsky A, Roeder RG. Nontranscriptional regulation of SYK by the coactivator OCA-B is required at multiple stages of B cell development. *Cell*. 2006;125:761–74.
35. Zandi S, Mansson R, Tsapogas P, Zetterblad J, Bryder D, Sigvardsson M. EBF1 is essential for B-lineage priming and establishment of a transcription factor network in common lymphoid progenitors. *J Immunol*. 2008;181:3364–72.
36. Yasuda T, Hayakawa F, Kurahashi S, Sugimoto K, Minami Y, Tomita A, Naoe T. B cell receptor-ERK1/2 signal cancels PAX5-dependent repression of BLIMP1 through PAX5 phosphorylation: a mechanism of antigen-triggering plasma cell differentiation. *J Immunol*. 2012;188:6127–34.
37. Zwilling S, Dieckmann A, Pfisterer P, Angel P, Wirth T. Inducible expression and phosphorylation of coactivator BOB.1/OBF.1 in T cells. *Science*. 1997;277:221–5.
38. Stephan RP, Reilly CR, Witte PL. Impaired ability of bone marrow stromal cells to support B-lymphopoiesis with age. *Blood*. 1998;91:75–88.
39. Stephan RP, Lill-Elghanian DA, Witte PL. Development of B cells in aged mice: decline in the ability of pro-B cells to respond to IL-7 but not to other growth factors. *J Immunol*. 1997;158:1598–609.
40. Ergen AV, Boles NC, Goodel MA. Rantes/Ccl5 influences hematopoietic stem cell subtypes and causes myeloid skewing. *Blood*. 2012;119:2500–9.
41. Su DM, Aw D, Palmer DB. Immunosenescence: a product of the environment? *Curr Opin Immunol*. 2013;25:498–503.
42. Swain S, Clise-Dwyer K, Haynes L. Homeostasis and the age-associated defect of CD4 T cells. *Semin Immunol*. 2005;17:370–7.
43. Song H, Price PW, Cerny J. Age-related changes in antibody repertoire: contribution from T cells. *Immunol Rev*. 1997;160:55–62.
44. Zheng B, Han S, Takahashi Y, Kelsoe G. Immunosenescence and germinal center reaction. *Immunol Rev*. 1997;160:63–77.
45. Frasca D, Landin AM, Riley RL, Blomberg BB. Mechanisms for decreased function of B cells in aged mice and humans. *J Immunol*. 2008;180:2741–6.
46. Frasca D, Riley RL, Blomberg BB. Aging murine B cells have decreased class switch induced by anti-CD40 or BAFF. *Exp Gerontol*. 2007;42:192–203.

47. Frasca D, Van der Put E, Riley RL, Blomberg BB. Reduced Ig class switch in aged mice correlates with decreased E47 and activation-induced cytidine deaminase. *J Immunol.* 2004;172:2155–62.
48. Yang X, Stedra J, Cerny J. Relative contribution of T and B cells to hypermutation and selection of the antibody repertoire in germinal centers of aged mice. *J Exp Med.* 1996;183(3):959–70.
49. Frasca D, Landin AM, Lechner SC, Ryan JG, Schwartz R, Riley RL, Blomberg BB. Aging down-regulates the transcription factor E2A, activation-induced cytidine deaminase, and Ig class switch in human B cells. *J Immunol.* 2008;180:5283–90.
50. Chahwan R, Edelmann W, Scharff MD, Roa S. AIDing antibody diversity by error-prone mismatch repair. *Semin Immunol.* 2012;24:293–300.
51. Frasca D, Romero M, Diaz A, Alter-Wolf S, Ratliff M, Landin A, Riley R, Blomberg B. A molecular mechanism for TNF- α -mediated downregulation of B cell responses. *J Immunol.* 2012;188:279–86.
52. Klinman NR, Kline GH. The B-cell biology of aging. *Immunol Rev.* 1997;160:103–14.
53. Nicoletti C, Borghesi-Nicoletti C, Yang XH, Schulze DH, Cerny J. Repertoire diversity of antibody response to bacterial antigens in aged mice. II. Phosphorylcholine-antibody in young and aged mice differ in both VH/VL gene repertoire and in specificity. *J Immunol.* 1991;147:2750–5.
54. Riley SC, Froscher BG, Linton PJ, Zharhary D, Marcu K, Klinman NR. Altered VH gene segment utilization in the response to phosphorylcholine by aged mice. *J Immunol.* 1989;143:3798–805.
55. Zharhary D, Klinman NR. B cell repertoire diversity to PR8 influenza virus does not decrease with age. *J Immunol.* 1984;133:2285–7.
56. Zharhary D, Klinman NR. The frequency and fine specificity of B cells responsive to (4-hydroxy-3-nitrophenyl)acetyl in aged mice. *Cell Immunol.* 1986;100:452–61.
57. Gibson KL, Wu YC, Barnett Y, Duggan O, Vaughan R, Kondeatis E, Nilsson BO, Wikby A, Kipling D, Dunn-Walters DK. B-cell diversity decreases in old age and is correlated with poor health status. *Aging Cell.* 2009;8:18–25.
58. ten Boekel E, Melchers F, Rolink AG. Changes in the V_H gene repertoire of developing precursor B lymphocytes in mouse bone marrow mediated by the pre-B cell receptor. *Immunity.* 1997;7:357–68.
59. Kline GH, Hartwell L, Beck-Engeser GB, Keyna U, Zaharevitz S, Klinman NR, Jack HM. Pre-B cell receptor-mediated selection of pre-B cells synthesizing functional mu heavy chains. *J Immunol.* 1998;161:1608–18.
60. Keyna U, Beck-Engeser GB, Jongstra J, Applequist SE, Jack HM. Surrogate light chain-dependent selection of Ig heavy chain V regions. *J Immunol.* 1995;155:55365542.
61. Ye J, McCray SK, Clarke SH. The transition of pre-B1 to pre-BII cells is dependent on the VH structure of the μ /surrogate L chain receptor. *EMBO J.* 1996;15:1524–33.
62. Keenan RA, De Riva A, Corleis B, Hepburn L, Licence S, Winkler TH, Mårtensson IL. Censoring of autoreactive B cell development by the pre-B cell receptor. *Science.* 2008;321:696–9.
63. Alter-Wolf S, Blomberg BB, Riley RL. Deviation of the B cell pathway in senescent mice is associated with reduced surrogate light chain expression and altered immature B cell generation, phenotype, and light chain expression. *J Immunol.* 2009;182:138–47.
64. Wols HA, Johnson KM, Ippolito JA, Birjandi SZ, Su Y, Le PT, Witte PL. Migration of immature and mature B cells in the aged microenvironment. *Immunology.* 2010;129:278–90.
65. Kline GH, Hayden TA, Klinman NR. B cell maintenance in aged mice reflects both increased B cell longevity and decreased B cell generation. *J Immunol.* 1999;162:3342–9.
66. Johnson SA, Rozzo SJ, Cambier JC. Aging-dependent exclusion of antigen-inexperienced cells from the peripheral B cell repertoire. *J Immunol.* 2002;168:5014–23.
67. Keren Z, Naor S, Nussbaum S, Golan K, Itkin T, Sasaki Y, Schmidt-Supprian M, Lapidot T, Melamed D. B-cell depletion reactivates B lymphopoiesis in the BM and rejuvenates the B lineage in aging. *Blood.* 2011;117:3104–12.
68. Keren Z, Averbuch D, Shahaf G, Zisman-Rozen S, Golan K, Itkin T, Lapidot T, Mehr R, Melamed D. Chronic B cell deficiency from birth prevents age-related alterations in the B lineage. *J Immunol.* 2011;187:2140–7.
69. Van Den Broeck A, Cambier J. B cells talk to their progenitors. *Blood.* 2011;117:2985–6.
70. Yanaba K, Bouaziz J.-D., Matsushita T, Tsubata T, Tedder TF. The development and function of regulatory B cells expressing IL-10 (B10 cells) requires antigen receptor diversity and TLR signals. *J Immunol.* 2009;182:7459–72.
71. King A, Keating P, Prabhu A, Blomberg BB, Riley RL. NK cells in the CD19[−] B220⁺ bone marrow fraction are increased in senescence and reduce E2A and surrogate light chain proteins in B cell precursors. *Mech Ageing Dev.* 2009;130:384–92.