Journal Articles

2015

B cells in the aging immune system: time to consider B-1 cells

N. E. Holodick
Northwell Health

T. L. Rothstein
Zucker School of Medicine at Hofstra/Northwell

B cells in the aging immune system: time to consider B-1 cells

Nichol E. Holodick[1] and **Thomas L. Rothstein**[1,2]

[1]Center for Oncology and Cell Biology, The Feinstein Institute for Medical Research, Manhasset, New York

[2]Departments of Medicine and Molecular Medicine, The Hofstra North Shore–LIJ School of Medicine, Manhasset, New York

Abstract

The investigation of immune senescence has uncovered many changes in B cell development, maintenance, and function with increasing age. However, most of these studies have focused on conventional B cell subsets in the spleen. The B-1 cell subset is an essential arm of the innate immune system, which in general has been understudied in terms of immune senescence. Here, we review what is currently known about B cells during aging and go on to describe why B-1 cell biology is an important component of the aging immune system in the context of diseases that most affect the aged population.

Keywords

B cells; aging; B-1 cells; senescence

Introduction

The global life expectancy is rapidly increasing. In the year 2000, there were 605 million people aged 60 years and over, and by the year 2050 the World Health Organization (WHO) predicts that this age group will reach 2 billion. Numerous diseases are associated with increased age, including heart disease, cancer, stroke, Alzheimer's disease, diabetes, influenza, and pneumococcal infection.[1] The immune system plays a central role in many of these diseases. In order to decrease the burden of disease as our population ages, it is necessary to understand changes within the immune system with increasing age. In particular, B cells play both adaptive and innate roles in diseases of the aged, which is the focus of this article. Herein we will first review what is known about conventional B-2 cells in the aged immune system, and then discuss the importance of widening our understanding of the role B-1 cells play in two primary diseases of the elderly, *Streptococcus pneumoniae* infection and atherosclerosis.

S. pneumoniae is the most common cause of pneumonia.[2] In the United States alone, 40,000 people die each year from pneumococcal infection.[2] The incidence of pneumococcal

Address for correspondence: Nichol E. Holodick, The Feinstein Institute for Medical Research, 350 Community Drive, Manhasset, NY 11030. nholodic@gmail.com.

infection and the mortality rate increase dramatically in people over the age of 65, moving from an average mortality rate of 0.92 in ages 5–49 to 6.7 in people over the age of 65.[3] These numbers clearly reveal an alarming disparity between the two age groups, despite the availability of a vaccine, PPSV23, approved for adults 65 years of age and older since 1983.[2] Postvaccination antibody titers are the same in young adults (under the age of 45) and elderly adults (over the age of 65); however, the elderly population contains antibodies less effective at clearing bacteria.[4] These data clearly demonstrate that pneumococcal infections still pose a great challenge in prevention and treatment, particularly in the elderly population, which immunization and B-2 cell adaptive immunity have not been able to overcome.

Atherosclerosis is the number one cause of death globally, and its incidence increases greatly in persons aged 65 and over.[5] Blockage of arterial walls and subsequent rupture of plaques causes heart attacks and strokes. Inflammation of the arterial wall endothelium leads to arterial wall thickening and consequently plaque formation.[6] The main cause of this inflammation is increased levels of modified serum low-density lipoproteins (LDL), which become trapped in the arterial wall.[6] LDL is more antigenic after it becomes oxidized (OxLDL), and this occurs once in the arterial wall.[6] Remarkably, many studies have shown that anti-OxLDL antibodies, B-1 cells, and B-1 cell–derived natural IgM are protective against atherosclerosis.[7,8] However, it is not completely understood how these antibodies and/or B-1 cells are maintained throughout adult life. In order to maintain protection against atherosclerosis, it is essential to understand how to maintain these protective antibodies with increasing age.

When optimizing vaccination strategies, enhancing passive protection, and/or developing other treatments for mitigating pneumococcal infection and/or providing protection against atherosclerosis, considering the roles of B lymphocytes is of great importance. B cells produce antibodies that fight infection by (1) binding pathogens, thereby preventing them from infecting host cells; (2) neutralizing toxins; (3) opsonizing pathogens; or (4) activating complement, which coats pathogens and leads to opsonization and/or lysis. B cells also function as antigen-presenting cells.[9] Various subsets of B cells have been defined in both mice and humans by their distinct phenotypic and functional characteristics. This brief review will focus on murine B cells during aging, as aging of human B cells has been reviewed elsewhere.[10] In the mouse, these subsets include B-2 cells, which comprise follicular (FO) and marginal zone (MZ) B cells found mainly in the spleen, and B-1 cells, which include B-1a ($CD5^+$) and B-1b ($CD5^-$) cells found in the spleen, peritoneal cavity, bone marrow (BM), and pleural cavity. Together, these B cell subsets provide immediate (B-1 cells) and long-lasting (B-2 cells) protection against infection, whereas natural IgM and B-1 cells provide protection from atherosclerosis.

Numerous studies have elucidated the age-related changes affecting conventional B-2 cells. These changes occur from the earliest developmental stages throughout maturity. Going forward, this extensive knowledge about B-2 senescence will be extremely valuable in advancing the knowledge about B-1 cell senescence, which is currently limited.

B cells in the aging immune system: development, maintenance, and function

B cell development in the aging immune system

B cell development begins with hematopoietic stem cells (HSCs). HSCs are self-renewing pluripotent cells found in fetal liver and adult BM, which have the ability to give rise to all blood cells.[11] B cell development continues through a series of differentiation steps dictated by expression of transcription factors, cytokines, and cell surface receptors. Proper immunoglobulin rearrangement allows the B cell to progress through critical stages of differentiation, culminating in a naive B cell expressing a B cell receptor (BCR), which is necessary for B cell survival and response to antigen[12] (Fig. 1). Each stage of B cell development is marked by specific gene and surface marker expression [13, 14]. During immunoglobulin rearrangement, non-templated (N) nucleotides can be added to the joins of gene segments, which increases diversity of the receptor.[15] B-2 cell development continues throughout life in the BM of adult mice from specific $B220^+CD19^-$ B cell progenitors.[16] Conversely, the B-1 cell population originates mainly during fetal life and was thought to persist throughout adult life only by self-renewal.[17]. Only recently, Dorshkind and colleagues identified a B-1 cell–specific progenitor, $Lin^-CD45^{lo/-}CD19^+$, found in low numbers in the adult BM and abundantly in the fetal liver.[18] Due to the recent understanding that B-1 cell progenitors are present in adult BM, little is known about how aging affects B-1a cell development and/or maintenance. However, B-2 cell development in the aged immune system has been shown to be impaired as a result of both intrinsic and extrinsic factors.

First, we will discuss the effects of aging upon HSCs, which is where B cell development begins. The number of HSCs has been shown to increase with age, do functional defects including self-renewal and homing defects.[19] Despite the increase in number, transfer experiments demonstrate that HSCs from aged mice are less effective at generating both B and T cells as compared to young mice, (reviewed in Ref. 20). Interestingly, transferring aged whole bone marrow (WBM) into young recipients generates the same frequency of B cells as transferring WBM from young donors.[21] However, when HSCs were purified from BM of aged donors and then transplanted into young recipients, B cells are not readily detected.[22,23] These seemingly contradictory results have been hypothesized to be due to age-related changes in the surface phenotype of HSCs[23] and cell-intrinsic defects related to homing.[21] Different phenotypic subsets of HSCs have been described (reviewed in Ref. 19). It is possible that the different subsets' surface phenotypes change with age, which could alter reconstitution results upon purification of certain HSC subsets owing to loss or exclusion. WBM, on the other hand, would contain all subsets of HSCs regardless of changes in surface phenotype. Interestingly, these studies also revealed an increase in myeloid differentiation from aged WBM[21] and purified HSCs.[22] It was subsequently shown that HSC potential for myeloid or lymphoid differentiation can be divided by CD86 expression ($CD86^-CD150^+$ and $CD86^+CD150^+$, respectively), and the HSCs with myeloid potential were increased in aged BM whereas the HSCs with lymphoid potential were reduced.[24] Together, these studies demonstrate changes in aged HSCs, which could lead to further alterations seen in B cell development in the BM.

The next steps in development from HSCs to a committed B cell progenitor are also associated with changes during aging. HSCs first develop into non–self-renewing multipotent progenitors (MMP), followed by lymphoid-primed multipotent progenitors (LMPP), early lymphoid progenitors (ELP), common lymphoid progenitors (CLP) (which are also called early B cell precursors (EBP)), and then progress to pre-pro B cells[23,25] (Fig. 1). Commitment to the B cell lineage is dependent upon expression of a number of transcription factors, including Ikaros, Pu.1, E2A (E12/E47), EBF (early B cell factor), and PAX-5.[23] In particular, E2A and EBF act together to direct CLPs toward B cell commitment, and, as will be described herein, also have other significant roles throughout B cell development.[26] E2A plays a role in the commitment of both MPPs and LMPPs to lymphoid versus myeloid lineages;[26] however, the effect of aging upon E2A expression and function in MMPs or LMPPs has not yet been studied despite the age-related decrease in these populations.[27] A decrease in the number of CLPs has also been observed in aged mice,[27,28] as well as a decrease in the number of pro-B[28] and pre-B cells[29] found in the BM of aged mice. It was proposed that the decrease in early B cell precursors results from defects in transcription regulated by E2A/EBF proteins,[27,30,31] which aid in early B cell specification and differentiation from MMPs, LMPPs, and CLPs to pro-B cells.[26] This hypothesis is supported by the findings that both stem cells and CLPs from aged mice have reduced potential to produce B cells, and Ebf1 is reduced in CLPs from aged mice, which, if restored, can reestablish B cell generation from aged CLPs.[27] These results demonstrate that cell-intrinsic defects affect aged CLPs and early stages of B cell differentiation. However, it has also been shown that cell-extrinsic defects or BM microenvironment defects also play roles in age-related changes leading to a decrease in pro- and pre-B cell numbers in the aged.

Interestingly, both cell intrinsic and extrinsic defects affect the early stages of B cell development. IL-7 receptor α chain (IL-7Rα) expression is first seen in the CLP stage. IL-7 is secreted by BM stromal cells and is necessary for the survival and continued development of FO B-2 cell progenitors. However, IL-7 is not necessary for MZ B cells or B-1 cell progenitors, whereas the IL-7Rα chain is necessary for all B cell subset progenitors.[32] Early in the CLP stage, IL-7 signaling is necessary for the induction of the transcription factor EBF1, which is required for initiation of immunoglobulin gene rearrangement and therefore differentiation into the pre-pro-B cell stage of development.[26,33] It has been shown that aged pro-B cells have an impaired ability to respond to IL-7 despite the normal expression level of IL-7R.[34] Interestingly, transfer of aged BM into young recipients yielded numbers of pre-B cells seen in young mice; however, transfer of young BM into aged recipients yielded a decrease in the numbers of pre-B cells, which are normally observed in aged mice.[35] These results point to a defect in the BM microenvironment. Along these lines, it has been demonstrated that BM stromal cells from aged mice are defective in releasing IL-7.[34] Furthermore, BM stromal cells from aged mice increase the production of the chemokine RANTES, which promotes myeloid differentiation and inhibits lymphoid differentiation.[36] These studies clearly demonstrate that extrinsic factors play a role in age-related changes in B lymphopoiesis.

The roles that cell-intrinsic defects play in the loss of pro-B and pre-B cells in the aged have been studied extensively. The pro-B cell stage of development is initiated by the assembly of the BCR, which begins with homologous recombination of germline heavy chain genes to

form the variable region and continues through the pre-BII stage where homologous recombination of the light chain genes occur (reviewed in Ref. 37). The variable region of the heavy chain comprises three gene segments: variable (V), diversity (D), and joining (J), whereas the light chain comprises two: (V) and (J). Rearrangements of these segments are regulated by recombination signal sequences (RSS), which flank the segments. In addition, the RSS serves as the binding site for the proteins RAG-1 and RAG-2, which once bound to each RSS cleave the DNA between the coding sequence and RSS. After cleavage by RAG-1 and RAG-2, the coding ends remain as hairpin loops that are opened by the Artemis–DNA-PK complex. The opening of the coding ends by Artemis–DNA-PK sometimes results in palindromic DNA sequences, referred to as P-nucleotides. Next, non-templated nucleotides (N-nucleotides) are added to the single stranded ends of the hairpins by the enzyme terminal deoxynucleotidyl transferase (TdT). Finally, DNA repair enzymes remove unpaired nucleotides and the two single stranded coding ends are ligated. It has been shown that both *Rag2* and recombinase activity are decreased in pro-B cells of aged mice,[35] which would decrease the pre-B cell pool. E2A expression is also decreased in pro-B cells from aged mice,[27,30,31] which contributes to decreased expression of Pax-5 and increased turnover of Pax-5.[38] Pax-5 expression is required for B cell progenitors to remain committed to the B cell lineage throughout development through repression of non-B lineage genes in pro-B cells.[39] These results demonstrate that, in addition to the cell-extrinsic defects described above, cell-intrinsic defects present in aged pro-B cells also lead to decreased numbers of pro-B cells in aged mice. Similar results have been shown for pre-B cells.

Once pro-B cells finish assembly of the heavy chain, they enter the pre-B cell stage. At this stage, the rearranged heavy chain pairs with VpreB and lambda 5, which together make up the surrogate light chain and is regulated by E2A and EBF1.[40] Rearrangement of the light chain (V) and (J) regions occurs in the pre-BII stage. In light of the fact that E2A is decreased in aged pro-B cells and regulates transcription of the surrogate light chain genes, it is not surprising that aged pro-B/early pre-B cells show reduced surrogate light chain expression, which consequently leads to reduced pre-BCR expression.[31] It has been suggested that low surrogate light chain expression can lead to a more autoreactive repertoire, due to a less stringent pre-BCR checkpoint.[41] In fact, serum autoantibodies have been shown to increase with age.[42] E2A and EBF1 also promote transcription of RAG genes, which are required during both pro-B and pre-B cell development.[26] Overall, these studies demonstrate that E2A, EBF1, and Pax-5 play central roles in the reduced number of pro and pre-B cells in aged mice.

It is not known to what extent the aged BM environment contributes to these observed defects; however, IL-7 signaling is necessary for *Ebf1* induction, yet BM stromal cells are defective in releasing IL-7. Furthermore, mature B cells in the periphery of aged mice have been shown to affect B lymphopoiesis. Increased longevity of peripheral mature B cells in aged mice[43] has recently been shown to play a role in the reduced numbers of B cell progenitors. This study eloquently demonstrated that depletion of mature B cells lead to an increase in pro-B cells, pre-B cells, MMPs, and CLPs.[44] It has been further hypothesized that, owing to the increased longevity of mature peripheral B cells, there is a decrease in the need for B cell generation, which HSCs sense and which leads to their increased myeloid

potential.[45] Together, these studies indicate that aging has far-reaching effects upon B cell development; from the earliest stem cells, to committed lymphoid progenitors, to committed B cell progenitors, and even to the longevity of mature B cells, which in turn has consequences for early B cell development (Fig. 2). However, further investigation is required to elucidate how the interplay between cell-extrinsic and cell-intrinsic changes influence the aged immune system.

Mature B cells in the aging immune system

In addition to developmental impairment, mature peripheral B cells display altered maintenance and function in the aged immune system. Aged mice display a decrease in B cell diversity[46,47] and an increase in long-lived B cells,[43] which, in a transgenic model favoring selection, have been shown to be the result of an increase in antigen-experienced B cells.[48] Despite the decrease in pro-B, pre-B, and immature B cell subsets, the number of FO B cells is only slightly decreased in aged animals.[49] In the case of MZ B cells, a significant decrease has been demonstrated in aged mice as compared to the young.[50] Furthermore, the aged splenic environment causes a decrease in migration of immature and MZ B cells.[51] However, with advancing age, there is an increase in a subset of B cells termed age-associated B cells (ABCs), which are found in both the spleen and BM.[52] These B cells are shown to arise from exhaustively expanded mature B cells. Interestingly, ABCs produce TNF-α, which has been shown to induce apoptosis in pro-B cells.[53] It is still not clear if the cell-intrinsic and cell-extrinsic changes occurring during early B development are the initiating factors leading to the increase in peripheral B cell longevity, which then propagates the decrease in B cell development, or if the long-lived peripheral B cells initiated the decrease in B lymphopoiesis. Regardless, the changes in B cell development (described above) and peripheral B cell characteristics may lead to the decreased repertoire diversity observed in aged B cells.

Responses to antigens and the ability to produce effective antibodies are defective in the aged immune system. B cells produce high-affinity antibodies within the germinal center (GC), where antibodies undergo affinity maturation and somatic hypermutation. However, there is a decrease in the number and volume of GCs formed in aged mice.[54] In addition, aged mice display reduced somatic hypermutation (SHM), reduced class-switch recombination (CSR), and decreased AID expression, which is required for both SHM and CSR.[30] Interestingly, E2A expression is also decreased in aged mature splenic B cells and is required for AID expression.[30] Therefore, mature B cells from aged mice have defects in the mechanisms used to generate effective antibodies. These defects may have consequences upon generation of antibodies for diseases affecting the elderly such as *S. pneumoniae* infection.

After immunization of old mice, the amount of antibody produced is not always changed, but the affinity to the specific antigen is severely decreased.[55] This has been shown to be the case for the pneumoccal vaccine in the elderly.[56] The B cell repertoire pre- and postimmunization is also different in aged mice. In the case of antibodies to phosphorylcholine (PC), a cell wall epitope of *S. pneumoniae*, older mice show usage of a variety of VH genes other than the restricted VH1 utilized by the T15 idiotype of younger

mice.[46] Significantly, these anti-PC antibodies found in older mice have been shown to be less protective than the T15 idiotype found in young mice.[57,58] Together, these studies suggest that immunization in the aged does not elicit the generation of protective antibodies. Consequently, the aged individual is dependent upon natural IgM and/or previously formed memory for protection against *S. pneumoniae* infection. At the time of these reports, much of B-1 cell biology was unknown. Therefore, many of these differences observed in aged mice are still not fully understood. Today, we are able to precisely collect and study various subsets of B-1 cells, and it has been clearly demonstrated that B-1a cells are required to clear *S. pneumonia.*[59] Thus, although we now know B-1a cells are essential for the maintenance of health, the role B-1a cells play in the aging immune system has yet to be fully investigated.

B-1 cells: essential protective role in diseases of the elderly

Role of B-1–derived natural IgM

B-1a cells are phenotypically characterized by cell surface markers: $CD5^+ IgM^{high} IgD^{low}$ $B220^{low} MAC-1^+ CD23^- CD43^+$.[60] B-1 cells residing in the BM and spleen produce 80% of the natural, non-immune serum IgM in mice.[17] Natural IgM is non-immune, polyreactive, low-affinity immunoglobulin (Ig) that functions in infection, atherosclerosis, B cell homeostasis, inflammation, and autoimmunity (reviewed in Ref. 61). It is able to bind conserved structures, sometimes more than one, on organisms, and thereby neutralize and facilitate the removal of such infectious agents. The unique ability of natural antibodies to play this essential role in the immune system is afforded by their structure, both in the constant and variable regions. IgM is mainly in the pentameric form, which effectively binds the complement component C1q and is important for both the clearance of pathogens and removal of apoptotic cells.[61] Natural IgM produced by B-1 cells is also germ line like, as evidenced by the relative lack of N-region additions, and has a more restricted repertoire.[62] B-1 cell–derived natural IgM is essential for (1) controlling bacterial and viral infections,[17] (2) aiding in the elimination of excess autoantigens that are increased in the elderly through removal of apoptotic cells,[61] and (3) binding oxidized low-density lipoprotein (OxLDL), in particular by antibodies bearing the T15 idiotype, which helps control the inflammatory process leading to atherosclerotic plaques.[7] Given these functions, B-1 cell–derived natural antibody has been shown to play essential protective roles in two diseases that significantly affect the elderly, *S. pneumoniae* infection and atherosclerosis.

Due to the germ line–like Ig and restricted repertoire, B-1 cell–derived natural IgM is evolutionarily conserved, which affords its ability to control bacterial infections. IgM produced by B-1 cells recognizes phosphatidylcholine (PtC) and other discrete microbial cell wall determinates such as PC from *S. pneumoniae*. In particular, anti-PC antibody derived from B-1a cells has been well characterized and shown to be protective against *S. pneumoniae* infection.[59,63] The importance of anti-PC and anti-pneumococcal capsular polysaccharide 3 (PPS3) germ line–like natural antibodies was shown in mice lacking B-1a cells, as they were unable to survive acute infection with *S. pneumonia.*[59] N-addition has been shown to play a key role in antigen receptor diversity and antibody effectiveness to certain pathogens. The prototypical B-1a anti-PC antibody, T15, has no N-addition.[63,64]

Remarkably, TdT transgenic mice vaccinated with heat-killed *S. pneumoniae* generated an anti-PC response, but these anti-PC antibodies were not protective against *S. pneumoniae* infection.[65] This study demonstrates that the increased diversity generated by N-addition can be detrimental for microbial protection, and highlights the importance of structure in the protection provided by evolutionarily conserved natural antibody.

Anti-PC natural antibody, in particular T15, has also been shown to be protective in atherosclerosis. Natural antibodies to OxLDL have been observed in healthy individuals over the age of 100[66] and in healthy mice, and were subsequently shown to be identical to the anti-PC T15 clone.[67] Interestingly, when atherosclerosis-prone mice were injected with heat-killed *S. pneumonia*, they exhibited high levels of serum anti-OxLDL IgM and reduced atherosclerosis.[68] More recently, it has been demonstrated that mice deficient in secretory IgM or C1q develop increased atherosclerotic lesions as compared to mice with serum IgM or C1q.[61,69] It has also been shown that treatment of mice that have advanced atherosclerosis with natural IgM reduces disease and increases anti-OxLDL levels.[70] In accordance with these studies, it has been demonstrated that B-1a cells and B-1a cell–derived natural IgM are protective against atherosclerosis.[8] Together, these studies establish a protective role for natural antibodies and B-1a cells in atherosclerosis.

B-1 cells' unique ability to produce protective natural IgM: a role for development

As mentioned above, the B-1 cell population originates during fetal life and persists throughout adult life through self-renewal.[71–73] More recently, it was demonstrated that the B-1 cell–specific progenitor (B1P), AA4.1+Lin−CD45R$^{low/-}$CD19+, is found in both the fetal liver (FL) and adult BM.[18] Other groups have also independently shown that total lineage-negative BM can give rise to B-1a cells.[74–76] During fetal development, the enzyme mediating increased junctional diversity by way of N-region additions, TdT, is not expressed.[77] This lack of TdT expression during fetal life allows natural germ line–like antibodies to develop lacking N-additions. Presumably, different selection pressures during fetal life also contribute to the development of these protective natural antibodies. We have recently demonstrated that transfer of fetal liver B1P into adult SCID mice produces B-1a cell IgM with few N-additions; however, transfer of the BM B1P into adult SCID mice produces B-1a cell IgM with abundant N-additions.[78] In accordance with these results, assessment of TdT gene expression revealed high levels of TdT in the B1P from the adult BM and no expression in the B1P from FL. However, upon transfer of AA4.1−Lin−CD45R−CD19− fetal liver cells into adult SCID mice, we found that B-1a cells originating from this population produced IgM with abundant N-additions, despite the lack of TdT expression. The mechanism for this is still unclear, but suggests that AA4.1−Lin−CD45R−CD19− fetal liver cells may contain cells that, once in the BM, differentiate into AA4.1+Lin−CD45R$^{low/-}$CD19+ cells (B1P) and acquire TdT. This hypothesis would fit well with previous reports demonstrating that HSCs and CLPs from adult BM are inefficient at generating B-1a cells.[79,80] Interestingly, fetal liver and neonatal HSCs efficiently generate B-1a cells. The reason for this discrepancy is still not understood, but it is hypothesized that changes in the hematopoietic environment play a role (reviewed in Ref. 81). Together, these results demonstrate that the unique developmental environment in which B-1 cells derive plays a role in the generation of protective natural IgM. However,

much of how this development is maintained throughout adulthood and into old age is unknown.

B-1 cells in the aging immune system

Despite the number of years B-1 cells have been studied, there is a large gap in our knowledge about B-1 cell biology in the aged. The most well-known feature of B-1 cells in the aging immune system is their general expansion.[82] There is an increase in the number of anti-PC B cells in adult mice with age; however, these anti-PC antibodies were shown to originate from newly generated BM B cells and do not have the canonical T15 idiotype.[46] This difference in VH usage would be predicted since these newly generated anti-PC B cells are not being generated during fetal life. Furthermore, studies have demonstrated that non-T15 anti-PC antibodies are less protective.[46,65] Changes in repertoire with age have also been demonstrated for the quintessential B-1 cell specificity, PtC, which utilizes VH11, VH12, and Q52 (VH2). It has been shown that aged splenic B cells utilize the variable heavy chains VH11 and Q52 more often than splenic B cells from young mice.[47] However, another study demonstrated a selective increase in VH12 and not VH11 usage in peritoneal B-1 cells with increasing age.[83] Interestingly, B-1 cells obtained from aged mice have also been shown to produce antibody with abundant N-additions.[74,84] Together, these findings have stimulating implications for B-1 cell maintenance with age.

As described above, BM-derived (BMD) B-1a cells have abundant N-region additions and do not express the same level of repertoire skewing as fetal liver–derived B-1a cells.[74] Furthermore, native B-1a cells from aged mice contain more N-region additions than those from young mice.[74,84] It has also been shown that the number of BM B-1 cell progenitors does not change with age.[85] Together, these results suggest that adult BM progenitors could contribute to the B-1a cell pool over time, thereby changing the spontaneously secreted germ line–like IgM shown to be required for immediate protection against *S. pneumoniae* infection and atherosclerosis. From these studies, we hypothesize that the level of natural IgM protection changes with age as a result of functional and/or repertoire-associated alterations to the B-1a cell pool over time. However, many studies are needed to fill in the large gap in our knowledge of B-1 cell biology with increasing age.

Future focus: innate B cells in aging

Clearly, B-1 cell natural antibody plays an essential role in protection from *S. pneumoniae* infection and atherosclerosis. Nevertheless, our understanding of how this essential arm of the immune system changes with age is greatly lacking. Our knowledge about B-1 cells has grown exponentially to define new subsets and functions and a specific progenitor, and it even extends to defining a human equivalent (reviewed in Ref. 86). In addition, our understanding of how natural IgM is secreted and which subsets secrete natural antibody has been refined.[87–89] However, we are clearly lacking in understanding how B-1a cell development, different subsets, and functions are affected by the aging process. Careful consideration should also be taken of the strain and sex of the mice analyzed. In our own studies, we have found no expansion of B-1 cells in aged male mice, but preliminary results suggest expansion in female mice (unpublished observations). Along these lines, diet and

SPF status might also influence aging studies of mice. Building upon the model set forth by Dorshkind and Montecino-Rodriguez, we suggest possible mechanisms leading to B-1 cell–derived IgM with abundant N-additions with age based on the current literature (Fig. 3). This is just a speculative model, and we anticipate that with more research, especially research focused on maintenance and aging, the precise mechanisms will be worked out. Understanding these mechanisms will most certainly provide the necessary insight to improve the health of the aging population.

Conclusions

The B-1a cell population is capable of producing antibodies that provide immediate protection against *S. pneumoniae* and confer protection against atherosclerosis in young mice. While much is known about conventional B-2 cell subsets, the B-1 cell subset has not been well studied in aged mice to date. Such studies directed at understanding the biology of this unique B cell subset in aged mice will greatly improve our ability to design preventative strategies and/or treatments of *S. pneumoniae* infection in the elderly. In addition, the natural antibodies produced by B-1a cells help reduce the onset of atherosclerosis.[90,91] Therefore, the knowledge gained from further study will not only aid in understanding of this protective B cell subset for preventative strategies and/or treatments of *S. pneumoniae* infection but also will further our knowledge of therapeutic strategies for atherosclerosis.

References

1. Prevention, C.f.D.C.a. The State of Aging and Health in America 2013. 2013

2. Prevention of pneumococcal disease: recommendations of the Advisory Committee on Immunization Practices (ACIP). MMWR Recomm Rep. 1997; 46:1–24.

3. Centers for Disease Control and Prevention. Active Bacterial Core Surveillance Report, Emerging Infections Program Network, Streptococcus pneumoniae, provisional-2009. 2010

4. Iyer AS, et al. Age-related immune response to pneumococcal polysaccharide vaccination: lessons for the clinic. Expert Review of Vaccines. 2015; 14:85–97. [PubMed: 25269650]

5. Organization WH. The top 10 causes of death. 2014

6. Lusis AJ. Atherosclerosis. Nature. 2000; 407:233–241. [PubMed: 11001066]

7. Binder CJ, Silverman GJ. Natural antibodies and the autoimmunity of atherosclerosis. Springer seminars in immunopathology. 2005; 26:385–404. [PubMed: 15609021]

8. Kyaw T, et al. B1a B lymphocytes are atheroprotective by secreting natural IgM that increases IgM deposits and reduces necrotic cores in atherosclerotic lesions. Circulation research. 2011; 109:830–840. [PubMed: 21868694]

9. Chen X, Jensen PE. The role of B lymphocytes as antigen-presenting cells. Archivum Immunologiae et Therapiae Experimentalis. 2008; 56:77–83. [PubMed: 18373241]

10. Scholz JL, et al. A comparative review of aging and B cell function in mice and humans. Current Opinion in Immunology. 2013; 25:504–510. [PubMed: 23932400]

11. Spangrude GJ, et al. Mouse hematopoietic stem cells. Blood. 1991; 78:1395–1402. [PubMed: 1884012]

12. Lam KP, et al. In vivo ablation of surface immunoglobulin on mature B cells by inducible gene targeting results in rapid cell death. Cell. 1997; 90:1073–1083. [PubMed: 9323135]

13. Li YS, et al. The regulated expression of B lineage associated genes during B cell differentiation in bone marrow and fetal liver. J Exp Med. 1993; 178:951–960. [PubMed: 8350062]

14. Li YS, et al. Identification of the earliest B lineage stage in mouse bone marrow. Immunity. 1996; 5:527–535. [PubMed: 8986713]

15. Desiderio SV, et al. Insertion of N regions into heavy-chain genes is correlated with expression of terminal deoxytransferase in B cells. Nature. 1984; 311:752–755. [PubMed: 6092963]

16. Rolink A, Melchers F. B lymphopoiesis in the mouse. Adv Immunol. 1993; 53:123–156. [PubMed: 8512034]

17. Baumgarth N. The double life of a B-1 cell: self-reactivity selects for protective effector functions. Nat Rev Immunol. 2011; 11:34–46. [PubMed: 21151033]

18. Montecino-Rodriguez E, et al. Identification of a B-1 B cell-specified progenitor. Nat Immunol. 2006; 7:293–301. [PubMed: 16429139]

19. Geiger H, et al. The ageing haematopoietic stem cell compartment. Nat Rev Immunol. 2013; 13:376–389. [PubMed: 23584423]

20. Linton PJ, Dorshkind K. Age-related changes in lymphocyte development and function. Nat Immunol. 2004; 5:133–139. [PubMed: 14749784]

21. Morrison SJ, et al. The aging of hematopoietic stem cells. Nat. Med. 1996; 2:1011–1016. [PubMed: 8782459]

22. Sudo K, et al. Age-associated characteristics of murine hematopoietic stem cells. J Exp Med. 2000; 192:1273–1280. [PubMed: 11067876]

23. Miller JP, Allman D. Linking age-related defects in B lymphopoiesis to the aging of hematopoietic stem cells. Seminars in immunology. 2005; 17:321–329. [PubMed: 15979895]

24. Shimazu T, et al. CD86 is expressed on murine hematopoietic stem cells and denotes lymphopoietic potential. Blood. 2012; 119:4889–4897. [PubMed: 22371880]

25. Welner RS, et al. Evolving views on the genealogy of B cells. Nat Rev Immunol. 2008; 8:95–106. [PubMed: 18204470]

26. de Pooter RF, Kee BL. E proteins and the regulation of early lymphocyte development. Immunol Rev. 2010; 238:93–109. [PubMed: 20969587]

27. Lescale C, et al. Reduced EBF expression underlies loss of B-cell potential of hematopoietic progenitors with age. Aging cell. 2010; 9:410–419. [PubMed: 20331442]

28. Miller JP, Allman D. The decline in B lymphopoiesis in aged mice reflects loss of very early B-lineage precursors. J Immunol. 2003; 171:2326–2330. [PubMed: 12928378]

29. Riley RL, et al. B cell precursors are decreased in senescent BALB/c mice, but retain normal mitotic activity in vivo and in vitro. Clinical immunology and immunopathology. 1991; 59:301–313. [PubMed: 2009646]

30. Frasca D, et al. Mechanisms for decreased function of B cells in aged mice and humans. J Immunol. 2008; 180:2741–2746. [PubMed: 18292491]

31. Sherwood EM, et al. 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. [PubMed: 10989124]

32. Jensen CT, et al. FLT3 ligand and not TSLP is the key regulator of IL-7-independent B-1 and B-2 B lymphopoiesis. Blood. 2008; 112:2297–2304. [PubMed: 18566323]

33. Lin H, Grosschedl R. Failure of B-cell differentiation in mice lacking the transcription factor EBF. Nature. 1995; 376:263–267. [PubMed: 7542362]

34. Stephan RP, et al. 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; 158:1598–1609. [PubMed: 9029095]

35. Labrie JE 3rd, et al. Bone marrow microenvironmental changes underlie reduced RAG-mediated recombination and B cell generation in aged mice. J Exp Med. 2004; 200:411–423. [PubMed: 15314072]

36. Ergen AV, et al. Rantes/Ccl5 influences hematopoietic stem cell subtypes and causes myeloid skewing. Blood. 2012; 119:2500–2509. [PubMed: 22289892]

37. Jung D, et al. Mechanism and control of V(D)J recombination at the immunoglobulin heavy chain locus. Annual review of immunology. 2006; 24:541–570.

38. Van der Put E, et al. Decreased E47 in senescent B cell precursors is stage specific and regulated posttranslationally by protein turnover. J Immunol. 2004; 173:818–827. [PubMed: 15240668]

39. Mikkola I, et al. Reversion of B cell commitment upon loss of Pax5 expression. Science. 2002; 297:110–113. [PubMed: 12098702]

40. Sigvardsson M, et al. EBF and E47 collaborate to induce expression of the endogenous immunoglobulin surrogate light chain genes. Immunity. 1997; 7:25–36. [PubMed: 9252117]

41. Riley RL. Impaired B lymphopoiesis in old age: a role for inflammatory B cells? Immunologic research. 2013; 57:361–369. [PubMed: 24203438]

42. Johnson SA, Cambier JC. Ageing, autoimmunity and arthritis: senescence of the B cell compartment - implications for humoral immunity. Arthritis research & therapy. 2004; 6:131–139. [PubMed: 15225355]

43. Kline GH, et al. B cell maintenance in aged mice reflects both increased B cell longevity and decreased B cell generation. J Immunol. 1999; 162:3342–3349. [PubMed: 10092788]

44. Keren Z, et al. B-cell depletion reactivates B lymphopoiesis in the BM and rejuvenates the B lineage in aging. Blood. 2011; 117:3104–3112. [PubMed: 21228330]

45. Melamed D, Scott DW. Aging and neoteny in the B lineage. Blood. 2012; 120:4143–4149. [PubMed: 22936664]

46. Klinman NR, Kline GH. The B-cell biology of aging. Immunol Rev. 1997; 160:103–114. [PubMed: 9476669]

47. Weksler ME. Changes in the B-cell repertoire with age. Vaccine. 2000; 18:1624–1628. [PubMed: 10689139]

48. Johnson SA, et al. Aging-dependent exclusion of antigen-inexperienced cells from the peripheral B cell repertoire. J Immunol. 2002; 168:5014–5023. [PubMed: 11994453]

49. Cancro MP. B cells and aging: gauging the interplay of generative, selective, and homeostatic events. Immunol Rev. 2005; 205:48–59. [PubMed: 15882344]

50. Birjandi SZ, et al. Alterations in marginal zone macrophages and marginal zone B cells in old mice. J Immunol. 2011; 186:3441–3451. [PubMed: 21307289]

51. Wols HA, et al. Migration of immature and mature B cells in the aged microenvironment. Immunology. 2010; 129:278–290. [PubMed: 19845796]

52. Hao Y, et al. A B-cell subset uniquely responsive to innate stimuli accumulates in aged mice. Blood. 2011; 118:1294–1304. [PubMed: 21562046]

53. Ratliff M, et al. In senescence, age-associated B cells secrete TNFalpha and inhibit survival of B-cell precursors. Aging cell. 2013; 12:303–311. [PubMed: 23410004]

54. Zheng B, et al. Immunosenescence and germinal center reaction. Immunol Rev. 1997; 160:63–77. [PubMed: 9476666]

55. LeMaoult J, et al. Effect of age on humoral immunity, selection of the B-cell repertoire and B-cell development. Immunol Rev. 1997; 160:115–126. [PubMed: 9476670]

56. Lee H, et al. The effect of age on the response to the pneumococcal polysaccharide vaccine. BMC Infect Dis. 2010; 10:60. [PubMed: 20219110]

57. Nicoletti C, et al. 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; 147:2750–2755. [PubMed: 1918989]

58. Yang X, et al. Repertoire diversity of antibody response to bacterial antigens in aged mice. IV. Study of VH and VL gene utilization in splenic antibody foci by in situ hybridization. J Immunol. 1994; 152:2214–2221. [PubMed: 8133035]

59. Haas KM, et al. B-1a and B-1b cells exhibit distinct developmental requirements and have unique functional roles in innate and adaptive immunity to S. pneumoniae. Immunity. 2005; 23:7–18. [PubMed: 16039575]

60. Hardy RR, Hayakawa K. B cell development pathways. Annual review of immunology. 2001; 19:595–621.

61. Ehrenstein MR, Notley CA. The importance of natural IgM: scavenger, protector and regulator. Nat Rev Immunol. 2010; 10:778–786. [PubMed: 20948548]

62. Berland R, Wortis HH. Origins and functions of B-1 cells with notes on the role of CD5. Annual review of immunology. 2002; 20:253–300.

63. Briles DE, et al. Anti-phosphorylcholine antibodies of the T15 idiotype are optimally protective against Streptococcus pneumoniae. J Exp Med. 1982; 156:1177–1185. [PubMed: 7153709]

64. Benedict CL, et al. Terminal deoxynucleotidyl transferase and repertoire development. Immunol Rev. 2000; 175:150–157. [PubMed: 10933600]

65. Benedict CL, Kearney JF. Increased junctional diversity in fetal B cells results in a loss of protective anti-phosphorylcholine antibodies in adult mice. Immunity. 1999; 10:607–617. [PubMed: 10367906]

66. Meroni PL, et al. Anti-beta 2 glycoprotein I antibodies in centenarians. Experimental gerontology. 2004; 39:1459–1465. [PubMed: 15501015]

67. Shaw PX, et al. Natural antibodies with the T15 idiotype may act in atherosclerosis, apoptotic clearance, and protective immunity. The Journal of clinical investigation. 2000; 105:1731–1740. [PubMed: 10862788]

68. Binder CJ, et al. Pneumococcal vaccination decreases atherosclerotic lesion formation: molecular mimicry between Streptococcus pneumoniae and oxidized LDL. Nature medicine. 2003; 9:736–743.

69. Lewis MJ, et al. Immunoglobulin M is required for protection against atherosclerosis in low-density lipoprotein receptor-deficient mice. Circulation. 2009; 120:417–426. [PubMed: 19620499]

70. Cesena FH, et al. Immune-modulation by polyclonal IgM treatment reduces atherosclerosis in hypercholesterolemic apoE$^{-/-}$ mice. Atherosclerosis. 2012; 220:59–65. [PubMed: 22062590]

71. Hayakawa K, et al. Immunoglobulin-bearing B cells reconstitute and maintain the murine Ly-1 B cell lineage. Eur J Immunol. 1986; 16:1313–1316. [PubMed: 3095127]

72. Lalor PA, et al. Feedback regulation of murine Ly-1 B cell development. Eur J Immunol. 1989; 19:507–513. [PubMed: 2785046]

73. Kantor AB. V-gene usage and N-region insertions in B-1a, B-1b and conventional B cells. Seminars in immunology. 1996; 8:29–35. [PubMed: 8850296]

74. Holodick NE, et al. Adult BM generates CD5$^+$ B1 cells containing abundant N-region additions. Eur J Immunol. 2009; 39:2383–2394. [PubMed: 19714574]

75. Duber S, et al. Induction of B-cell development in adult mice reveals the ability of bone marrow to produce B-1a cells. Blood. 2009; 114:4960–4967. [PubMed: 19812384]

76. Esplin BL, et al. A differentiation pathway for B1 cells in adult bone marrow. Proc Natl Acad Sci U S A. 2009; 106:5773–5778. [PubMed: 19307589]

77. Gregoire KE, et al. Ontogeny of terminal deoxynucleotidyl transferase-positive cells in lymphohemopoietic tissues of rat and mouse. J Immunol. 1979; 123:1347–1352. [PubMed: 313953]

78. Holodick NE, et al. B-1a cell diversity: nontemplated addition in B-1a cell Ig is determined by progenitor population and developmental location. J Immunol. 2014; 192:2432–2441. [PubMed: 24477911]

79. Ghosn EE, et al. Distinct B-cell lineage commitment distinguishes adult bone marrow hematopoietic stem cells. Proc Natl Acad Sci U S A. 2012; 109:5394–5398. [PubMed: 22431624]

80. Barber CL, et al. Reduced production of B-1-specified common lymphoid progenitors results in diminished potential of adult marrow to generate B-1 cells. Proc Natl Acad Sci U S A. 2011; 108:13700–13704. [PubMed: 21808010]

81. Montecino-Rodriguez E, Dorshkind K. B-1 B cell development in the fetus and adult. Immunity. 2012; 36:13–21. [PubMed: 22284417]

82. Stall AM, et al. Ly-1 B-cell clones similar to human chronic lymphocytic leukemias routinely develop in older normal mice and young autoimmune (New Zealand Black-related) animals. Proc Natl Acad Sci U S A. 1988; 85:7312–7316. [PubMed: 3262873]

83. Herzenberg LA, et al. B-1 cell origins and VH repertoire determination. Current topics in microbiology and immunology. 2000; 252:3–13. [PubMed: 11125487]

84. Gu H, et al. Sequence homologies, N sequence insertion and JH gene utilization in VHDJH joining: implications for the joining mechanism and the ontogenetic timing of Ly1 B cell and B-CLL progenitor generation. The EMBO journal. 1990; 9:2133–2140. [PubMed: 2113468]

85. Alter-Wolf S, et al. Old mice retain bone marrow B1 progenitors, but lose B2 precursors, and exhibit altered immature B cell phenotype and light chain usage. Mech Ageing Dev. 2009; 130:401–408. [PubMed: 19428460]

86. Rothstein TL, et al. Human B-1 cells take the stage. Ann. N. Y. Acad. Sci. 2013; 1285:97–114. [PubMed: 23692567]

87. Holodick NE, et al. Immunoglobulin secretion by B1 cells: differential intensity and IRF4-dependence of spontaneous IgM secretion by peritoneal and splenic B1 cells. Eur J Immunol. 2010; 40:3007–3016. [PubMed: 21061433]

88. Yang Y, et al. Division and differentiation of natural antibody-producing cells in mouse spleen. Proc Natl Acad Sci U S A. 2007; 104:4542–4546. [PubMed: 17360560]

89. Holodick NE, et al. Splenic B-1a Cells Expressing CD138 Spontaneously Secrete Large Amounts of Immunoglobulin in Naive Mice. Frontiers in immunology. 2014; 5:129. [PubMed: 24734034]

90. Murray CJ, Lopez AD. Mortality by cause for eight regions of the world: Global Burden of Disease Study. Lancet. 1997; 349:1269–1276. [PubMed: 9142060]

91. Weksler ME, et al. Immune therapy for age-related diseases. Trends Immunol. 2009; 30:344–350. [PubMed: 19541533]

92. Yoshimoto M, et al. Embryonic day 9 yolk sac and intra-embryonic hemogenic endothelium independently generate a B-1 and marginal zone progenitor lacking B-2 potential. Proc Natl Acad Sci U S A. 2011; 108:1468–1473. [PubMed: 21209332]
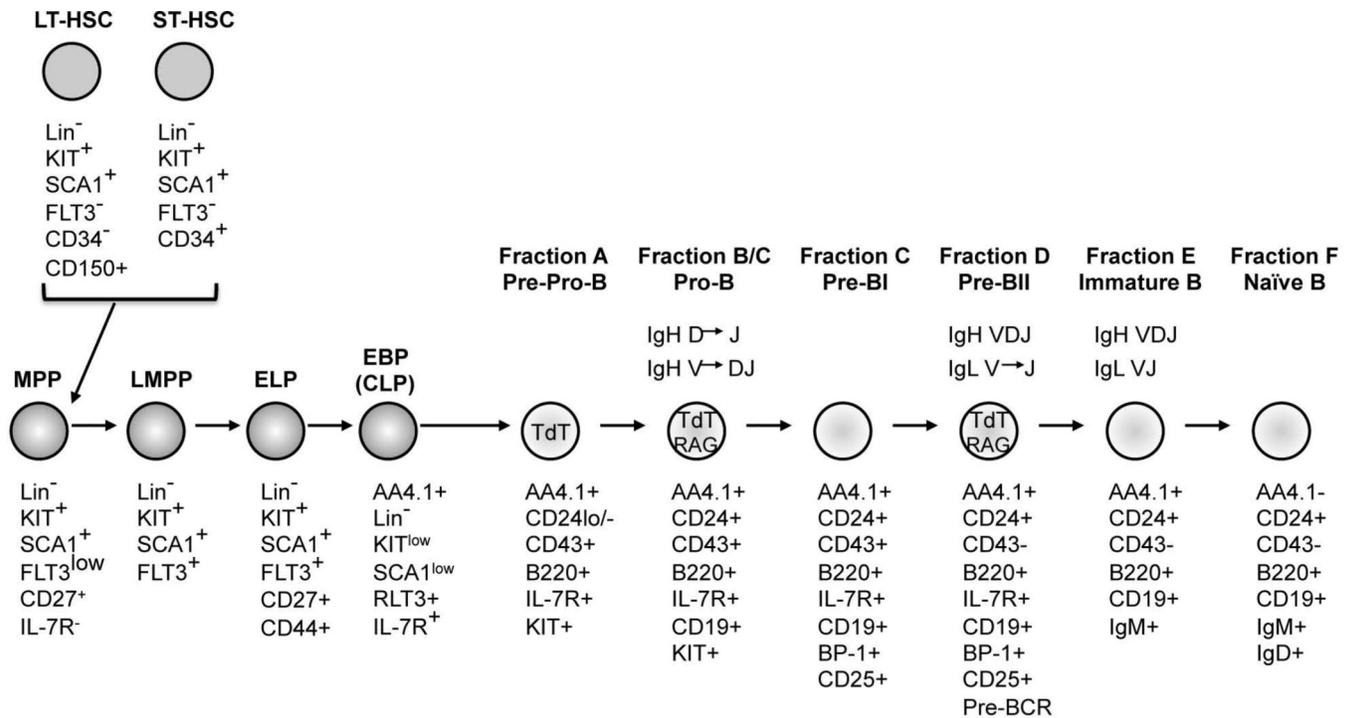
LT-HSC
ST-HSC

Lin⁻
KIT⁺
SCA1⁺
FLT3⁻
CD34⁻
CD150+

Lin⁻
KIT⁺
SCA1⁺
FLT3⁻
CD34⁺

| | | | Fraction A Pre-Pro-B | Fraction B/C Pro-B | Fraction C Pre-BI | Fraction D Pre-BII | Fraction E Immature B | Fraction F Naïve B |

IgH D→J
IgH V→DJ

IgH VDJ
IgL V→J

IgH VDJ
IgL VJ

MPP   LMPP   ELP   EBP (CLP)   TdT   TdT RAG   TdT RAG

Lin^-
KIT^+
$SCA1^+$
$FLT3^{low}$
$CD27^+$
$IL\text{-}7R^-$

Lin^-
KIT^+
$SCA1^+$
$FLT3^+$

Lin^-
KIT^+
$SCA1^+$
$FLT3^+$
$CD27^+$
$CD44^+$

AA4.1+
Lin⁻
KITlow
SCA1low
RLT3+
IL-7R⁺

AA4.1+
CD24lo/-
CD43+
B220+
IL-7R+

AA4.1+
CD24+
CD43+
B220+
IL-7R+
CD19+
KIT+

AA4.1+
CD24+
CD43+
B220+
IL-7R+
CD19+
BP-1+
CD25+

AA4.1+
CD24+
CD43-
B220+
IL-7R+
CD19+
BP-1+
CD25+
Pre-BCR

AA4.1+
CD24+
CD43-
B220+
CD19+
IgM+

AA4.1-
CD24+
CD43-
B220+
CD19+
IgM+
IgD+

Figure 1.

B cell development. B cell development begins with hematopoietic stem cells (HSCs) and continues through a series of differentiation steps dictated by expression of transcription factors, cytokines, and cell surface receptors. Long-term repopulating HSCs (LT-HSCs) and short-term HSCs (ST-HSCs) are named for their capacity to self-renew. The HSCs first differentiate into multipotential progenitors (MPP). Upon signaling through Flt-3, the MPPs differentiate into lymphoid-primed multipotential progenitors (LMPP), which differentiate into common lymphoid progenitors (CLP) upon Flt-3 and IL-7 signaling. Induction of the transcription factors E2A and EBF mark the differentiation of the CLP into a pre-pro B cell, commonly referred to as fraction A. E2A and EBF allow immunoglobulin rearrangement to begin, which is necessary for the development of B cells. Proper immunoglobulin rearrangement allows for the B cell to progress though each stage of differentiation, ending in a naive B cell, which can migrate into the periphery. Each stage of B cell development is marked by specific gene expression and surface marker expression as shown.
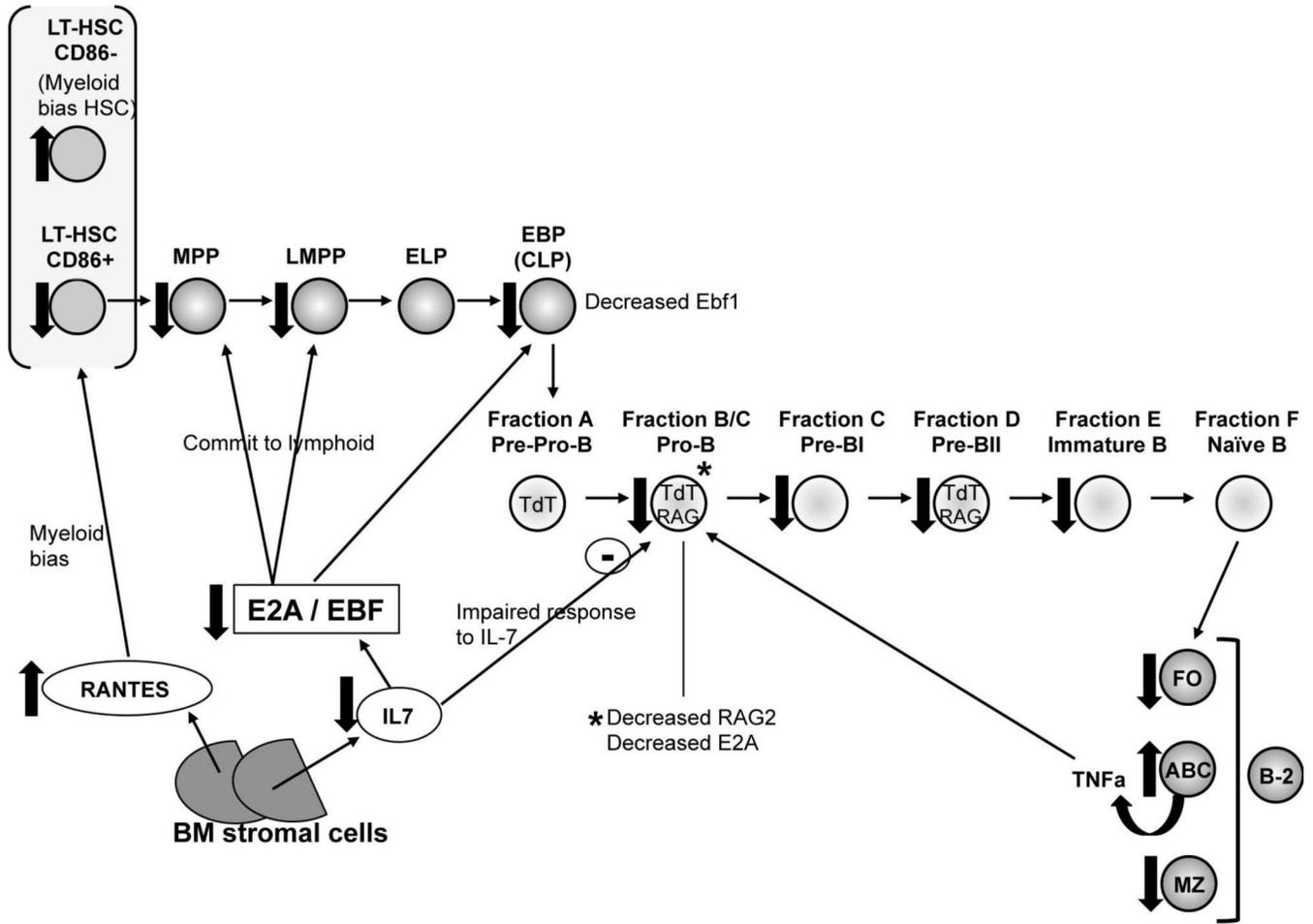
Figure 2.

The aged developing and mature B cell pools. Both cell-intrinsic and cell-extrinsic changes occur throughout the stages of B cell development ranging from pre-lymphoid commitment (HSCs) to immature B cells. The mature B-2 cell pool in aged mice consists of follicular (FO) B cells, marginal zone (MZ) B cells, and age-associated B cells (ABC). Black arrows depict the relative increase or decrease in the differentiation subsets leading to mature B cell development. The asterisk (*) above fraction B/C denotes additional changes in the cells in the aged, which include decreased RAG and E2A expression.
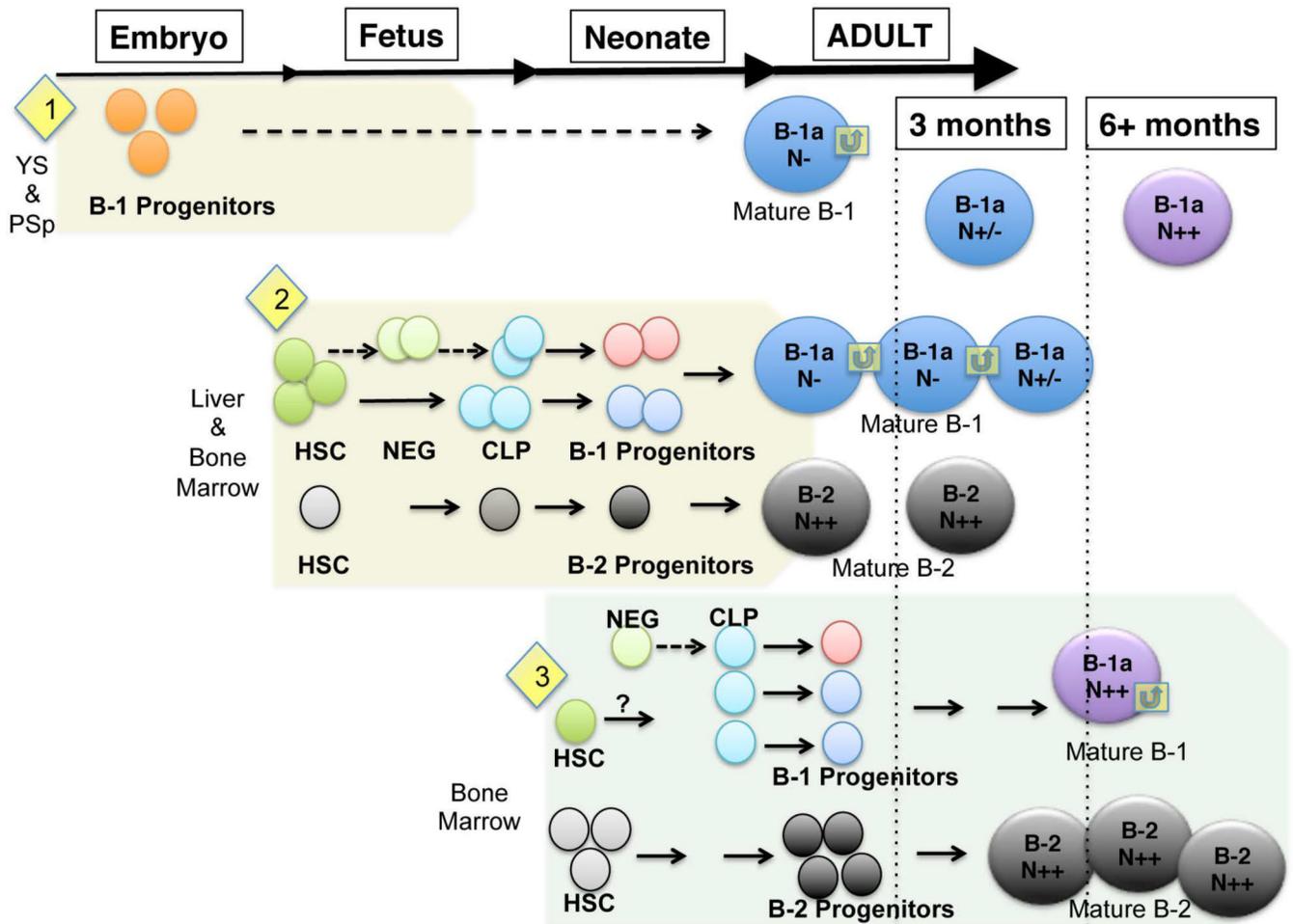
Figure 3.

B-1 cell development and maintenance with age. Building upon the model proposed by
Motecino-Rodriguez and Dorshkind,[81] we add what is currently known about the distinct
waves of B-1 cell development leading to an adult pool of B-1a cells and propose extending
the model to encompass changes with increasing age. (1) During the first wave of
development, B-1 cell progenitors (AA4.1+Lin−CD45R^low/−CD19+) are derived from yolk
sac (YS) and para-aortic splanchnopleura (PSp) endothelium,[92] before the emergence of
HSCs. Whether this first wave of B-1 cells contributes to the adult pool of B-1 cells is still
being examined. (2) The fetal liver and bone marrow is the site of the second wave of
development. Current data has shown that HSCs are capable of giving rise to B-1 cells
during fetal life, whereas HSCs present in the adult bone marrow are significantly less
efficient at producing B-1 cells.[79] It is not yet known whether HSCs have specified
differentiation potential or if they lose their potential to produce B-1 cells in the adult. To
indicate these possibilities, we have chosen a different color for HSCs producing B-1 cells
during fetal life (green) versus those giving rise to B-2 cells (gray), which have limited
development during fetal life. It has been shown that there is commitment to either B-1 or
B-2 lineage at the CLP stage.[81] Our recent results demonstrate that
AA4.1−Lin−CD45R−CD19− fetal liver cells (NEG, light green circles) give rise to B-1 cells

with increased diversity (abundant N-additions) upon transfer into adult SCID mice, whereas transfer of the fetal liver–derived B-1 cell progenitors (AA4.1$^+$Lin$^-$CD45R$^{low/-}$CD19$^+$) into adult SCID mice yields B-1 cells with limited N-region additions. These results suggest that an earlier population may differentiate into B-1 cell progenitors giving rise to more than one type of B-1 cell progenitor (red circle versus light blue circles), although whether this happens physiologically is not known. Either way, B-1 cells that develop during fetal and neonatal life have limited N-additions. (3) The third wave of development occurs in the adult bone marrow and mainly yields B-2 cells. We hypothesize that a possible mechanism of non-HSC–dependent B-1 cell development during adult life might occur via the NEG population of fetal liver cells that have migrated to the adult bone marrow. Furthermore, this adult development of B-1 cells would produce B-1 cells with increased N-additions, contributing to the change in B-1 cell –derived natural IgM with age.