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Understanding the Heterogenous Population of Age-Associated B cells and Their Contribution to Autoimmunity and Immune Response to Pathogens

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Abstract

In humans and mice, susceptibility to infections and autoimmunity increases with age, due to age-associated changes in innate and adaptive immune responses. Aged innate cells also have reduced activity, which leads to decreased naïve T cell and B cell responses. Aging innate cells also contribute to an overall heightened inflammatory environment. Naïve T cell and B cells undergo cell intrinsic age-related changes that lead to reduced effector and memory responses. However, previously established B and T cell memory responses persist with age. One dramatic change is the appearance of a newly recognized population of age-associated B cells (ABC) with a unique $CD21^{-}CD23^{-}$ phenotype. Here we discuss the discovery and origins of the naïve phenotype IgD^{+} versus activated $CD11c^{+}Tbet^{+}$ ABC, with a focus on their protective and pathogenic properties. In humans and mice, antigen-experienced $CD11c^{+}Tbet^{+}$ ABC increase with autoimmunity and also appear in response to bacterial and viral infections. However, our analyses indicate that $CD21^{-}CD23^{-}$ ABC include resting naïve progenitor ABC expressing IgD. Like generation of $CD11c^{+}Tbet^{+}$ ABC, the naïve ABC response to pathogens depends on TLR stimulation, making this a key feature of ABC activation. We put forward a potential map of the development of distinct subsets from the putative naïve ABC. We suggest defining the signals that can harness the response of naïve ABC may contribute to protection against pathogens in the elderly, while $CD11c^{+}Tbet^{+}$ ABC may be useful targets for therapeutic strategies to counter autoimmunity.

Keywords

age-associated B cells; B lymphocytes; autoimmunity; memory B cells; aging

I. Immune Responses to Respiratory Infections in the Aged

With age, humans become more susceptible to infections, leading to increased global morbidity and mortality.[1,2] Lower respiratory tract infections (LRTI) are the most common

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AUTHOR CONTRIBUTIONS

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cause for hospitalizations in people over the age of 65 and 90% LRTI-related deaths are within the elderly population.[1,3] Many viruses and bacteria cause LRTI such as *Streptococcus pneumoniae,* influenza and more recently SARS-CoV-2.[1,3,4] According to the CDC, over half of influenza-related hospitalizations and deaths in the US, are in patients over the age of 65.[5] Unfortunately, current vaccination strategies often fail to induce sufficient immunity in aged populations leaving them vulnerable to future outbreaks.[6–8] Aged males and females often show distinct susceptibility to infections, tumors and autoimmunity.[8] Susceptibility to autoimmune disorders increases more among aged females, while there is a higher incidence of tumors in aged males[8]. Aged males are more highly susceptible to respiratory infections such as influenza and in the current COVID pandemic.[4,8] Understanding the mechanisms involved in responses of the aged immune system should provide insights that can inform development of new strategies to improve vaccine efficacy and therapeutic intervention in the elderly.

Previous studies have established that age-related changes in human and murine immune systems increase susceptibility towards infections.[8] Aged innate cells express lower levels of toll-like receptors (TLR) which are one class of pathogen recognition receptors (PRR), and thus, in the aged, infections that stimulate these PRR produce reduced levels of type I interferon and other inflammatory mediators during responses to pathogens and reduce ability to clear the infection.[9,10] Aged neutrophils and macrophages exhibit decreased migration and phagocytosis.[8,9,11] As animals age, immature monocytes constitutively secrete higher levels of inflammatory cytokines in the absence of infection increasing basal levels of inflammation during aging.[8] Macrophages from aged animals express lower MHC-II and costimulatory molecule levels leading to a decreased ability to presentation antigen to T cells.[6] Although aged dendritic cells (DC) secrete proinflammatory cytokines, they have defects in migration, phagocytosis, and antigen presentation to T cell during infection.[12–14] Therefore, unchecked inflammatory responses by immature monocytes and DC and poor function of neutrophils, macrophages and DC leads to an increase in tissue injury and impaired response of T cells, and hence less effective pathogen clearance.[8,9] The increased proinflammatory environment that develops in the absence of infection may also contribute to age-related autoimmune disorders.[8]

In the adaptive immunity, T and B cells undergo multiple changes with age that play an even greater role in impaired immune responses. The aged thymus produces less naïve CD4 T cells, leading to a decrease in the CD4 TCR repertoire and a progressive reduction of the naïve CD4 T cell compartment (Figure 1).[15–18] When stimulated the aged naïve CD4 T cells proliferate less and produce less IL-2 contributing to a decrease in CD4 T cell effector responses, especially reduced formation of T follicular helper cells (T_{FH}).[19] One key change is a reduced response of CD4 T cells to IL-6, produced by interacting APC, which acts as a signal 3 for T_{FH} and memory CD4 T cell generation.[20,21] The limited T_{FH} generation causes a dramatic decrease in germinal center B cell (GCB) responses, which leads to fewer Ab-secreting cells (AbSC), long-lived PC (LLPC) and memory B cells (B_{mem}) (Figure 1).[6,16,17,20,22–24] Unlike young naïve CD4 T cells, aged naïve CD4 T cells are poor at developing into new T cell memory cells.[25] Naïve CD8 T cells have similar defects and their poor response impairs development of cytotoxic effectors that kill infected targets. As with

CD4 cells, memory CD8 T cells generated early in life remain more competent.[26] Thus, it is response to new or previously unseen pathogens that is mostly hampered by age.

Like naïve T cells, generation of pre and pro-B cells in the bone marrow (BM) decreases with age due to changes in the BM microenvironment.[27,28] Although the total number of peripheral B cell remain the consistent, follicular B cells (FOB), which are the B cells that initiate GCB, decrease in the spleen.[28] Due to a diminishing immature B cell pool in aged mice, splenic B cells are not replenished with new mature B cells and aged FOB consists of longer-lived, older naïve B cells, which express a reduced antibody repertoire (Figure 1).[28–30] Like FOB, marginal zone B cells (MZB), which are usually T-independent, decrease with age due to changes in anatomical structure in the marginal zone.[28,31] These structural changes also prevent MZB from interacting with macrophages in the marginal zone decreasing the MZB response to blood-borne pathogens.[31]

Intrinsic changes in B cells also contribute to decrease in GCB responses, class switch recombination (CSR) and somatic hypermutation (SHM) (Figure 1).[28] Studies show that human and murine aged B cells express less E47, a transcription factor that plays a role in *Aicda* transcription.[32,33] *Aicda* gene encodes for activation-induced cytidine deaminase (AID), an enzyme required for CSR and SHM in B cells.[33] Therefore, lower E47 expression in aged B cells leads to lower expression of AID, which decreases isotype-switching and affinity maturation (Figure 1).[33] Decrease in CSR and SHM negatively impact production of antigen-specific GCB, antibodies, and memory B cells, which all play critical roles in protection clearing infections and providing long-term immunity to prevent re-infection.[16,28] Since naïve T and B cells are most impaired by aging, it is unsurprising that vaccines given to the elderly, including that for influenza, are often ineffective and provide only short-term protection, requiring yearly immunization.[6]

II. Discovery of Aged Associated B cells (ABC)

Although the numbers of splenic B cells remain roughly constant between youth and old age in mice, the subset composition changes.[28] In young humans and mice, naïve splenic B cells are composed mostly of naïve FOB and MZB subpopulations, but these decline with age while isotype-switched IgG^+ memory B cells accumulate.[8,23,34–36] This shift from naïve to antigen-experienced B cells contributes to a decreasing immunoglobulin repertoire with age, and leads to poor responses to new pathogens.[28–30] In 2011, studies by Michael Cancro and colleagues, identified a novel subset of splenic B cells that emerges in unimmunized aged mice.[34] Unlike FOB ($CD23^+$) and MZB ($CD21^+$), this new B cell subset lacks CD23 and CD21. They were called “age associated B cells” (ABC) (Figure 2).[34,37] Flow cytometry analysis revealed that $CD21^-CD23^-$ ABC maintain surface IgD expression, but do not express CD86 and MHC II (Table 1).[23,28,34,38] We find that $CD21^-CD23^-$ ABC are a heterogenous population composed IgD^+ and IgD^- $CD21^-$ $CD23^-$ ABC.[23] In the absence of intentional immunization, the IgD^+ B cells have a phenotype consistent with unswitched naïve B cells, while the IgD^- B cells have a phenotype of more antigen-experienced B cells.[28,36,39] Therefore, we proposed that $CD21^-CD23^-$ ABC are composed of resting, unswitched naïve B cells (IgD^+) and isotype-switched memory B cells (IgD).[23] We believe these 2 subsets comprise the resting compartment of ABC (Table 1).

Philippa Marrack and her colleagues described another distinct ABC population that lacked CD21 expression but expressed CD11c and CD11b and accumulated in young autoimmune-prone mice.[40] Unlike the resting $CD21^{-}CD23^{-}$ ABC, $CD11c^{+}CD11b^{+}$ ABC express elevated CD86, MHC-II and Fas, suggesting that they are likely activated and/or atypical memory B cells (Table 1).[40,41] Further gene array analysis has revealed that this population expresses high levels of T-bet, a transcription factor associated with $IgG2a/c^{+}$ memory B cells during viral infections.[40–45] Later studies revealed that $CD21^{-}CD23^{-}$ABC also express CD11c and T-bet mRNA suggesting that this population is possibly related to the more activated $CD11c^{+}CD11b^{+}$ ABC subset.[46] However, whether $CD11c^{+}CD11b^{+}$ ABC arise from $CD21^{-}CD23^{-}$ABC and if they represent a small or larger fraction of the presumed ABC subset remains unclear (Figure 3).

III. Requirements for ABC development and/or differentiation

Several studies from Cancro's group suggest that $CD21^{-}CD23^{-}$ABC arise from antigen-experienced FOB that accumulate with age.[34,46] When young $CD23^{+}$-enriched FOB were adoptively transferred into unimmunized WT hosts they divided more compared to FOB transferred into CD40 $^{-/-}$ and MHC-II $^{-/-}$ hosts.[34,46] Moreover, the highly divided fraction of donor FOB cells lacked CD21 and CD23 expression, but expressed high levels of T-bet.[46] In addition, aged CD154 $^{-/-}$ mice, which lack CD40L on T cells did not form $CD21^{-}CD23^{-}$ABC. Since CD4 T cell activation requires MHC II-bound peptides on antigen presenting cells, and CD40 binding to CD40L mediates T and B cell interactions, this data suggests that generation of $CD21^{-}CD23^{-}Tbet^{+}$ ABC is T-cell dependent.[37,46] Further studies to evaluate if ABC are antigen-experienced quantified the number of somatic mutations in resting ABC in unimmunized aged mice and compared it to aged FOB, MZB, and GCB. This study revealed that $CD21^{-}CD23^{-}$ABC contain more somatic mutations in their heavy and light chains compared to aged FOB and MZB, but not as many as GCB, which undergo multiple rounds of affinity maturation.[46] This indicates that $CD21^{-}CD23^{-}$ABC have undergone at least some affinity maturation consistent with the hypothesis they are antigen-experienced memory B cells. However, this ABC subset has a diverse B cell receptor (BCR) repertoire similar to aged FOB and MZB, suggesting that the subset in aged mice, housed in SPF conditions, had undergone minimal clonal selection indicating a more naïve like status.[46] Moreover, reports indicate that $CD21^{-}CD23^{-}$ABC mostly express IgD, and contain a separate population of IgD^{-} B cells and they occur equally in germ-free vs. specific pathogen-free (SPF) aged mice (Kugler-Umana and Swain, unpublished results), implying there may be multiple subsets of ABC.[23] We propose that the majority of resting ABC in SPF mice are indeed naïve and that they develop by an intrinsic pathway, independent of foreign and commensal microbe exposure. We suggest that the $CD21^{-}CD23^{-}$ABC may also contain a fraction of memory B cells that accumulate in T-cell dependent manner. Thus, we propose, in very old mice (over 18 months) resting ABC are dominant naïve B cell population.

While FOB are T-cell dependent, MZB. B1b, and B1a cells are T-cell independent B cell subsets that rapidly become antibody-secreting cells in response to BCR crosslinking and TLR signaling[39]. However, these subsets decrease in number and activity.[8,27,31] Studies shows that $CD21^{-}CD23^{-}$ ABC divide more with *in vitro* with TLR7 and TLR9 stimulation

compared to aged FOB and MZB.[34] Our own studies indicate that in aged mice, the resting ABC are the major B cell population that responds to influenza infection independent of T cells (Figure 3). We transferred FACS-sorted resting phenotype ABC into influenza-infected $RAG^{-/-}$ host and studied their differentiation and activity.[23] We found infection caused naïve ABC to express Fas and become influenza-specific Ab-secreting cells (AbSC).[23] We termed this subset as induced age-associated B cells (iABC). Further studies show that iABC formation from donor ABC depends on TLR7 and/or other endosomal TLR expressed by host cells (Kugler-Umana and Swain, unpublished results). TLR7 is an endosomal sensor capable of detecting ssRNA which we believe is how influenza infection stimulates the ABC response. This suggests that resting ABC can mount an influenza-specific response, without T cells, but dependent on TLR pathways.

Like activation of $CD21^{-}CD23^{-}$ABC, MZB and B1b subsets, initial $CD11c^{+}CD11b^{+}$ ABC generation also requires TLR signaling (Figure 3).[40] Studies indicate that unimmunized aged $TLR7^{-/-}$ and $Myd88^{-/-}$ aged mice do not accumulate the $CD11c^{+}CD11b^{+}$ ABC subset compared to aged WT, $TLR4^{-/-}$ and $TLR3^{-/-}$ mice. Moreover, repeated administration of TLR7 agonists increases the number of ABC in young mice compared to repeated administration of TLR3, TLR4 and TLR9 agonists suggesting that chronic TLR7 stimulation is particularly important for $CD11c^{+}CD11b^{+}$ ABC generation.[40] Further *in vitro* and *in vivo* studies also shows that, in both cases, TLR7 stimulation synergizes with BCR signaling to generate $CD11c^{+}$ B cells from young B cells that express T-bet which are presumably the Ag-experienced B cells.[42] Additionally, overexpression of T-bet also leads to an increase of CD11c and CD11b expression in B cells.[42] Although TLR7 stimulation plays an important role in generating these active ABC, $CD11c^{+}CD11b^{+}$ ABC also require IL-21.[47,48] Adding IL-21 *in vitro* in addition to TLR7 or 9 stimulation also led to an increase in $CD11c^{+}$T-bet^{+} B cells from cultured FOB, compared to TLR7 or 9 stimulation alone.[47] Later studies investigated the molecular pathways that contribute to IL-21 dependent $CD11c^{+}CD11b^{+}$ ABC generation. Their studies revealed that young SWAP-70 and DEF6 double knockout (DKO) mice develop lupus and prematurely accumulate $CD11c^{+}CD11b^{+}$T-bet^{+} ABC which lack CD21 and CD23 expression.[48] Additionally, they show that this increase in ABC is due to the absence of regulation, by the SWAP-70 and DEF6 proteins, of IL-21 mediated IRF5 accessibility to key targets involved in ABC formation.[48] This suggests that accumulation of $CD11c^{+}CD11b^{+}$T-bet^{+} ABC also depends on IRF5 signaling.[48] Altogether these studies support the idea that the ABC with the activated $CD11c^{+/}CD11b^{+}$, T-bet^{hi} phenotype arise from a combination of Ag or auto-Ag recognition, IL-21 and TLR7 stimulation. Although ABC generation depends on IL-21 in the context of autoimmunity, whether IL-21 dependence holds true in aged mice where T cell help is deficient and ABC activation is T-cell independent, remains unclear.[16,23]

IV. Role of ABC in autoimmunity (mouse and human)

Multiple studies suggest that both ABC populations play a role autoimmune pathogenesis (Table 2).[38,47,49,50] Prior to becoming mature B cells, pro-B cells express a surrogate light chain (SLC), which along with μ heavy chain form a functional pre-BCR, that is required for B cell selection.[51,52] Without SLC, the pro-B cell pools shift to SLC^{low} pro-B cells and allows for the introduction of autoreactive B cells into the peripheral B cell pool.[51,52]

Studies show that CD21^{-}CD23^{-}ABC secrete TNF-α that reduces SLC^{high} pro-B cells in aged mice, which increases the pool of SLC^{low} pro-B cells and autoantibodies.[35,51,52] Therefore, this ABC subset may aid the development of autoreactive B cells and thus contribute to rising autoimmunity with age.

The CD11c^{+}CD11b^{+}T-bet^{+} ABC accumulate at a faster rate in several young mouse models for SLE such as Mer $^{-/-}$ mice, NZBxWF1, and SWAP-70 $^{-/-}$DEF6 $^{-/-}$ double knock out mice compared to young wild-type mice[40,53]. These ABC can make more autoantibodies against chromatin, nuclear ribonuclear proteins (nRNP) and cardiolipin compared to FOB, suggesting they directly contribute to autoimmunity[40,48,53]. One possibility is that the autoimmune environment may mimic the aging environment leading to the generation of this subset even in the young. Many studies on CD11c^{+}CD11b^{+}T-bet^{+} ABC function have been done in mouse models of autoimmunity. Several indicate that CD11c^{hi} T-bet^{hi} ABC secrete IFNγ and can present self-antigen to T cells.[40,42,44,50,54] Furthermore, the presence of CD11c^{hi} T-bet^{hi} ABC correlate with kidney damage and early mortality in SLE-prone mice.[50]

According to a study by Marrack, healthy human patients across different ages and sexes did not have CD11c^{hi}CD21^{low} ABC cells.[40] However, this ABC population increased with age in peripheral blood among aged RA (Rheumatoid Arthritis) patients (Table 2).[40] In contrast, CD21^{-}CD23^{-} ABC and CD11b^{+}CD11c^{+}T-bet^{+} ABC develop in healthy WT aged mice and CD21^{-}CD23^{-} ABC develop in germ-free mice (Kugler-Umana and Swain, unpublished results).[34,40,41] Human ABC are a CD11c^{hi}CD21^{low} subset that express activation markers such as CD80 and CD86, lack IgD and IgM expression, and express IgG.[40,55] Given this phenotype and human exposure to multiple infections and non-infectious Ag, it makes sense that the human ABC populations are most likely isotype-switched activated memory B cells.[39,40] Like murine CD11b^{+}CD11c^{+}T-bet^{+} ABC, CD11c^{+}T-bet^{+} ABC accumulate in the blood of young SLE patients and correlate with SLE symptoms (Table 2).[40,50,56] Also like human ABC found in aged RA patients, this autoimmune-associated B cell population lacks surface IgD and IgM expression and a fraction express IgG.[40,56] The CD11c^{+} autoimmune B cells express IL-21 inducible genes suggesting this subset is IL-21 dependent in human SLE as well.[48,54] Recent studies have also identified CD11c^{+}T-bet^{+} ABC in inflamed synovial tissues of aged RA patients and CD11c^{+} B cells in nephritic kidneys in young SLE patients suggesting that this subset may contribute to local inflammation (Table 2).[56,57] Although human ABC lack CD21 expression, they are not equivalent to the murine CD21^{-}CD23^{-} naïve predominant ABC population since the human ABC do not express surface IgD and do express CD80 and CD86.[23,34] Unlike human ABC increase in aged RA patients, in MS patients this population decreases with age.[40,55] Furthermore, unlike the 2011 RA study, in this 2016 MS study there is a significant increase of CD11c^{hi}CD21^{low} ABC in aged healthy patients compared to young patients.[40,55] This suggests that human ABC are not associated with all autoimmune conditions and there is a need to collect more data from healthy aged patients. Furthermore, the progenitor of the different human subsets of distinct phenotype is unclear and they may not all be derived from bone fide ABC. Studies in mice show that the activated ABC subset in human autoimmune disease may be a good therapeutic target. B cell specific T-bet deletion in young SLE model mice reduced development of splenic CD11c^{hi}T-bet^{hi} ABC, and led to decreased spontaneous GCB formation, which reduced autoantibody

production and kidney damage. T-bet targeting or the depletion of the activated ABC subset could be a useful therapy for autoimmunity.[50]

V. Role of ABC in Bacterial and Viral infections (mouse and human)

Several ABC-like populations have been identified in bacterial and viral infections. Initial studies identified a $CD11c^{+}IgM^{+}$ B cell population in *E. muris*-infected young mice, which were described as unswitched memory B cells, which express T-bet.[58–60] To study their secondary responses without the interference of neutralizing antibodies, this subset was sorted and transferred into *E. muris* challenged vs. naive hosts.[60] With *E. muris* challenge, this $T\text{-}bet^{+}$ memory B cell subset differentiated into AbSC, suggesting they could provide protection against *E. muris* reinfection.[60] Similar to *E. muris* infection, $CD11c^{+}T\text{-}bet^{+}$ ABC accumulate in young mice infected with gammaherpesvirus 68 (gHV68), mouse CMV, lymphocyte choriomeningitis virus, and vaccinia.[42] To determine if such ABC contributed to anti-ghV68 antibody production and ensure that T-bet deletion is restricted to B cells, they made mixed bone chimeras containing a mixture of bone marrow cells from μMT mice and either T-bet $^{-/-}$ mice or WT mice.[42] Results showed that the B cell $T\text{-}bet^{-/-}$ chimeras had higher ghV68 viral titers and less ghV68-specific antibodies, suggesting that $T\text{-}bet^{+}$ B cells play a role in viral clearance.[42] This data coincides with reports that show that T-bet drives NP-specific $IgG2a^{+}$ memory B cells and influenza-specific AbSC cells formation.[43,47,61] Meanwhile, a recent study indicates that $T\text{-}bet^{hi}$ influenza-specific memory B cells are restricted to the spleen, blood and bone marrow in humans and mice and that they produce anti-HA stalk antibodies in mice, indicating that they may have potential to contribute to protective responses.[62]

In humans, $T\text{-}bet^{+}$ B cells accumulate in chronically-infected HIV-positive patients, chronically-infected aged Hepatitis C (HCV) patients and patients immunized with live yellow fever or vaccinia vaccine.[63,64] Antiviral treatment of chronic HCV infection reduced the number $T\text{-}bet^{+}$ B cells, suggesting that chronic infection is needed to sustain this subset.[64] Like the $CD11c^{+}T\text{-}bet^{+}$ ABC in autoimmune patients and autoimmune-prone mice, this subset lacks CD21, IgD, and IgM expression, but does express IgG, CD86 and CD95 indicating these are isotyped-switched memory B cells.[63,64] Furthermore, in HIV-positive patients, a large frequency of HIV-specific B cells expressed T-bet, which correlates with the production of HIV-specific IgG1 antibodies in the serum.[63] More recently, studies indicate that rhinovirus infection produces $T\text{-}bet^{+}$ memory B cell in human subjects, which infiltrate nasal tissue and make heterotypic IgG responses.[65] This suggests that $T\text{-}bet^{+}$ B cells may play a role in inducing and/or mediating human antigen-specific B cell responses. One major challenge to understanding the role of different subsets, is that T-bet can be expressed by conventional B cells as well as ABC B cells and so great care must to taking in designing experiments that ascribe functions to ABC.

VI. Positive ABC Role in Overcoming Aged Defects and Shared Strategies of T and B Aging

Based on the literature in the field and our data discussed above, we propose that ABC, defined as the lineage not expressing CD21 and CD23, are a lineage of B cells with subsets

at different differentiation stages. We find that in unimmunized aged mice, the majority of ABC are resting naive B cells expressing sIgD, and/or IgM. This population increases with age and is more prominent in females (Figure 2).[34] In contrast, in autoimmunity and chronic infection, activated memory ABC population(s) develop and accumulate.[38] The activated populations are likely diverse and functionally heterogenous, having developed under different conditions. Although many studies have focused on the activated, T-bet^{hi} ABC as mediators of autoimmunity, the activated ABC induced by infections may play critical positive roles in protective immunity. At least in aged mice they are the major naïve B cell subset that responds to new pathogens.[23] Furthermore, recent studies indicate that a T-bet^{hi} B cells may play a role in producing anti-HA stalk antibodies during influenza infection.[62] This coincides with reports that shows that broadly-neutralizing antibodies are maintained as we age and accumulate with each subsequent vaccination.[66–68] Since ABC accumulate with age, we propose that this age-dependent subset may be an important source of these broadly neutralizing antibodies. This may be particularly important given that aged immune systems do not generate effective Ab responses from naïve FOB cells and naïve CD4 do not develop T_{FH} in aged mice, unless pathogen recognition (PR) signals are high.[17,20,21] Therefore, understanding ABC generation and activation may provide a strategy for a universal influenza vaccine in aged populations.

We should also consider that T_{FH} that drive T-cell dependent B cell responses, decrease with age leading to poor primary memory B cell formation and antibody responses towards new pathogens. However, our studies show that ABC can respond in the absence of T_{FH} and CD4 T cells to produce AbSC.[20] Unlike FOB, *in vitro* TLR7 and TLR9 stimulation activates CD21^{-}CD23^{-}ABC and *in vivo* chronic TLR7 stimulation induces CD11c^{+}Tbet^{+}ABC.[34,40] This suggests that primary aged B cells responses become less T-cell dependent and depend more on PR pathways. This may be a useful strategy to circumvent the poor T cell responses in the aged (Figure 4). By defining the factors required for protective ABC responses, we may learn how to harness the positive potential of ABC in anti-pathogen immunity.

Like aged B cells, aged T cells also become more dependent on PR pathways.[20,21] Our early studies showed that addition of IL-2 or addition of pro-inflammatory cytokines [IL-1, IL-6, TNF] increased aged CD4 T cell responses.[19,69] Indeed we found that APC stimulated with TLR agonists make IL-6 and interact with the aged naïve CD4 T cells, restoring much of the aged naïve CD4 T cells response.[17,20,21] We suggest this heightened dependence on PR signaling limits the naïve T cell response to circumstances where a pathogen presents a compelling danger, most likely to prevent un-necessary inflammation and limit development of autoimmunity. This change is thus analogous to the change in the B cells with aging, where the responsive naïve ABC have a strict dependence on TLR7/9, indicating they are restricted by the need for PR signaling. We suggest the increasing dependence on PR pathways is a shared beneficial strategy, for the animal and a key feature of T and B cell aging.

Unsurprisingly most aging studies imply that all aging is harmful and leads to harmful loss of immune functions. However, we argue that loss of certain aspects in adaptive immunity in aging may be part of a beneficial strategy.[70,71] The loss of naïve T and B cells with age is largely caused by homeostatic shifts. This is particularly clear for naïve T cells because the

thymus undergoes gradual involution and makes less T cells. We propose that this may be an evolutionary strategy to make room for memory T and B cells, which accumulate with exposure to pathogens throughout young life.[6] Unlike naïve T cells, memory CD4 cells generated in young mice retain their function with age.[72] Our research shows that development of naïve CD4 defects is also a programmed process driven by the increased chronological age of the naïve CD4 population that becomes longer-lived.[73,74] Additional studies, where CD4 T cells are depleted from aged mice, indicated that newly generated CD4 T cells do not express key age-related defects and function like young cells.[22,75] Furthermore, without a thymus and heterogenous expression of the pro-apoptotic protein BIM, the naïve CD4 T cell population develop aging defects more quickly and become older.[73,74] We recently found that loss of naïve CD4 T cells and functional reduction in IL-6 response, occur in germ-free mice just as in conventional mice even though they mice lack exposure to pathogens and to commensal microbes (Swain et al. unpublished). These results suggest that the changes in naive T cells are part of a developmental program and that they have helpful effects.

We suggest that identifying the changes in the aged immune system and strategies to overcome those changes, may provide alternative vaccination strategies in the elderly (Figure 4). Since T and B cell memory cells accumulate and remain responsive with age, one strategy would be to immunize young and middle-aged populations with key vaccines to increase the T and B cell memory pool.[6,66,72] Based on our studies of aged naïve T cells, another strategy would be to develop vaccines that provide higher levels of antigen and TLR-signaling and thus drive more optimal CD4 T cells responses including new memory responses to emerging or altered pathogens.[17,20,21] Not only should increases in antigen dose and TLR signaling improve naïve CD4 T cell responses, but it may also enhance the naïve ABC responses. Targeting both aged naïve T cells and ABC may lead to establishing new memory cells to emerging or altered pathogens in aged populations.

VII. Conclusions

We conclude that the process of aging leads to major changes in innate and adaptive, T and B cell immunity. We focus on changes in naive CD4 and naïve B cells, that make it difficult to readily vaccinate the elderly to new pathogens and propose different vaccine strategies for aged populations (Figure 4). We discuss that for B cells, there is a shift of naïve B cells from conventional FOB responses which become limited, to a recently described ABC subset (age-associated B cells) and point out the naïve ABC can respond only if sufficient pathogen recognition is present. We draw parallel to the cell intrinsic changes in naïve CD4 T cells, which also become dependent on PR activation of APC. We suggest this may reveal a basic strategy to avoid un-necessary responses with age which can be pathogenic and possibly to avoid dangerous autoimmunity, but to retain sufficient potential to respond to pathogen infection. These new hypotheses need further evaluation but provide a novel framework to think about some of the aging changes that most impact immunity.

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Abbreviations:

ABC	age-associated B cells
AbSC	antibody-secreting cells
AID	activation-induced cytidine deaminase
APC	Antigen-presenting cell
BCR	B cell receptor
BM	bone marrow
CSR	Class switch recombination
DC	dendritic cells
FOB	follicular B cells
GCB	germinal center B cells
iABC	induced age-associated B cells
MZB	marginal-zone b cells
LLPC	long-lived plasma cell
LRTI	lower respiratory infection
nRNP	nuclear ribonuclear proteins
PR	pathogen-recognition pathway
PRR	pathogen recognition receptor
RA	Rheumatoid Arthritis
SHM	somatic hypermutation
SLE	systemic lupus
SPF	Specific pathogen-free
SLC	surrogate light chain
TCR	T cell receptor
Tfh	T follicular cell
TLR	toll-like receptor

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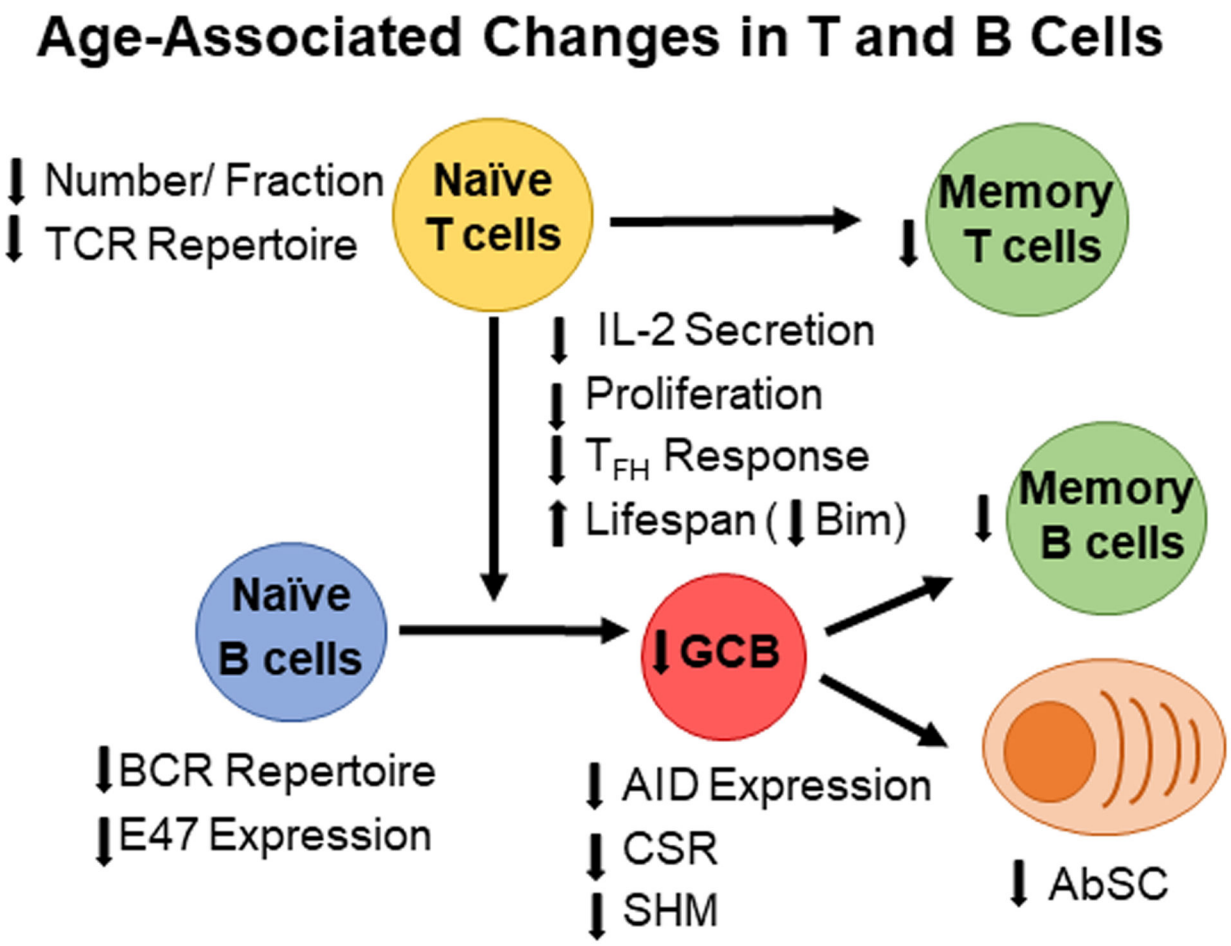

Figure 1: Age-Associated Changes in T cell and B cells.

With age, the number of naïve B cells and T cells decreases leading to a smaller TCR and BCR populations and repertoires. Upon pathogen exposure, naïve CD4 T cells secrete less IL-2 leading to lower effector T cell responses including T follicular helper cells (Tfh). Lower Tfh responses decrease GCB responses, which leads to fewer memory B cells and antibody-secreting cells towards newly encountered pathogens. However, memory B cells from previous immune responses accumulate with age leading to a larger memory B cells pool in the aged.

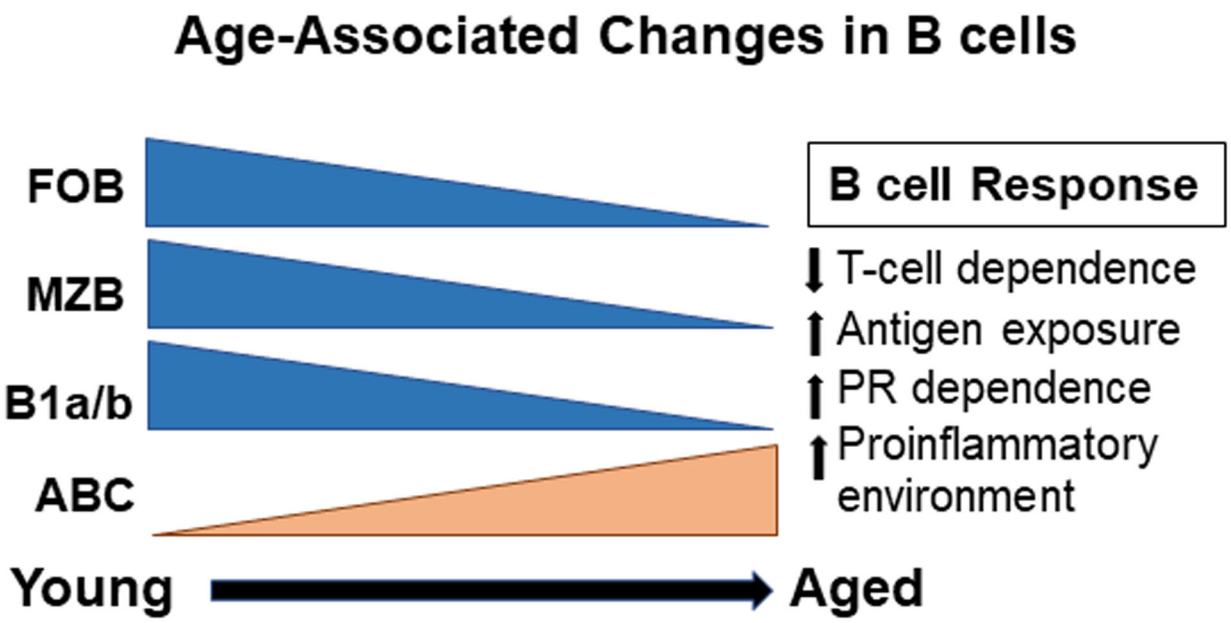

Figure 2: B cell responses shift to PR-dependent mechanisms with age

With age, humans and mice experience higher antigen exposure and an increased proinflammatory environment. Furthermore, the number of FOB, MZB and B1b/a cells decrease as ABC accumulate with age. This can be replicated in chronically infected and autoimmune-prone young mice, which accumulate $CD11c^{+}Tbet^{+}$ ABC. T-cell responses also decrease leading to a higher dependence on PR signals. Unlike, T-cell dependent FOB, ABC can be T-independent, and their activation depends on higher Ag exposure and PR signals. Thus, they can mount a primary response to new pathogens, which may provide protection.

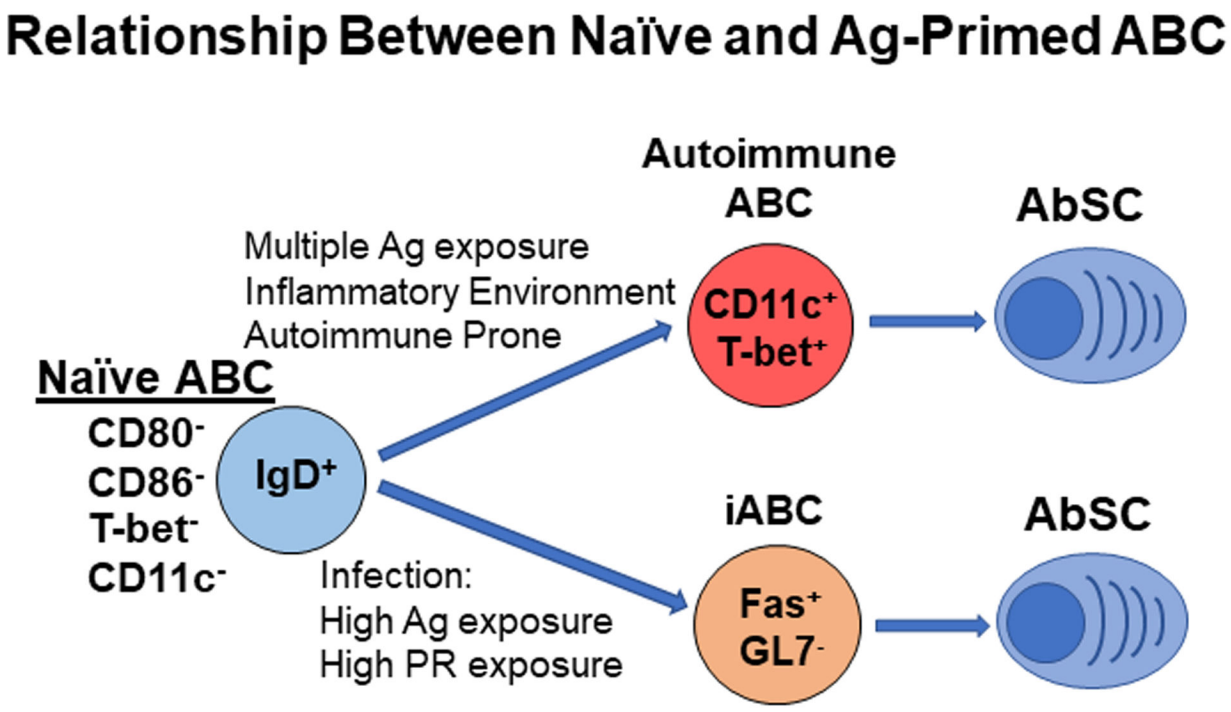

Figure 3: Proposed relationship between naive and primed ABC

Our studies show that with high Ag and PR exposure, naïve ABC can become active ABC which express Fas and differentiate into Ab-producing cells. We termed this subset as induced age associated B cells (iABC). Although autoimmune ABC can accumulate in young mice, we propose that this primed subset may also arise from naïve ABC with multiple Ag exposures and heightened inflammatory environment. Like iABC effectors, this autoimmune ABC differentiates into Ab-producing cells which produces autoantibodies.

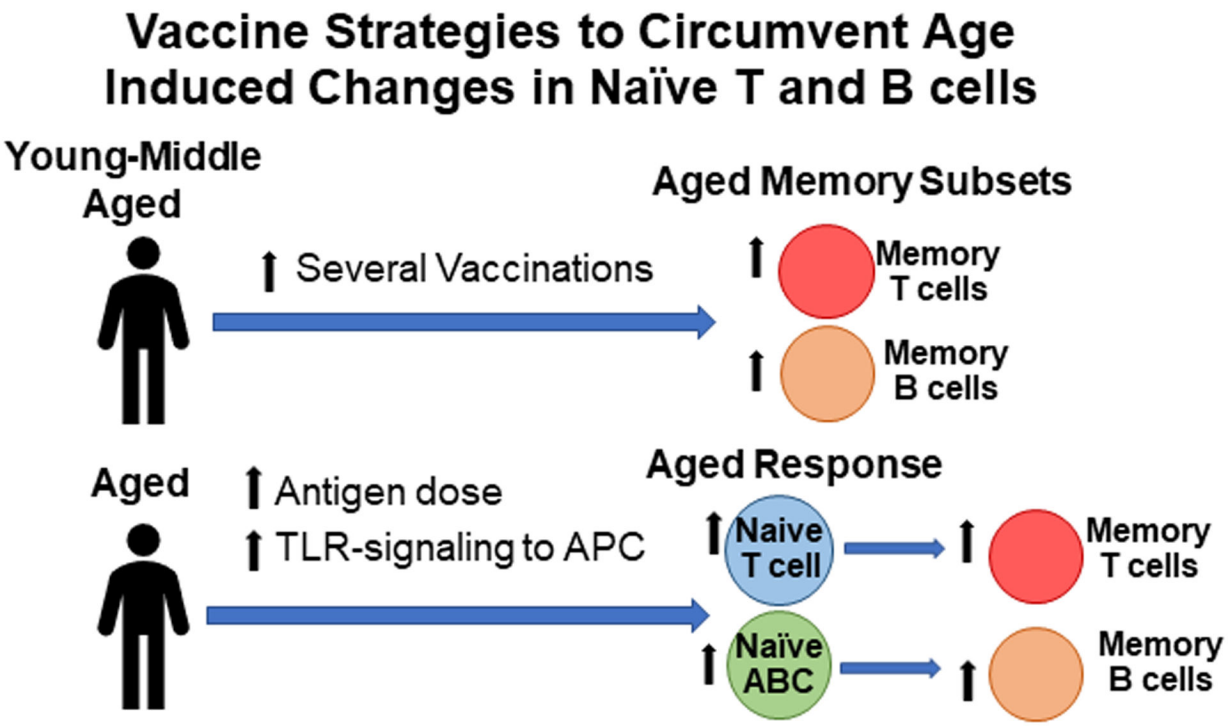

Figure 4: Proposed Vaccine Strategies to Enhance Response in the Elderly

Studies show that memory T and B cell response increase with age and may provide protection against reoccurring infection. One way of enhancing the aged immune system is by increasing exposure to Ag by vaccination in young or middle-aged populations in order to increase the size of the memory repertoire later. Another way of enhancing aged immune responses is by providing higher doses of Ag and TLR-signaling to antigen-presenting cells (APC). These should lead to optimal naïve aged T and B cells responses and help establish new memory T and B cells responses to new pathogens.

Table 1:

Comparison of resting vs. activated ABC phenotypes

Markers	Resting ABC	Activated ABC	References
CD21	−	−	34,40
CD23	−	−	34,40
IgD	+	−	34,40
	(Memory: IgD^-)		
T-bet	+	+	40, 46,48
CD80	N/A	+	40,56
CD86	−	+	34,40
MHC II	−	+	34,40
CD11c	+	+	40,41,46.48
CD11b	N/A	+	40,41,48

The table shows (+)-positive or (−)-negative expression of various markers associated with ABC in resting and activated states, based on the listed references. Resting ABC are composed of IgD^+ and IgD^- (naïve & memory) subsets that lack CD80 and CD86 expression. Meanwhile, active ABC express these two costimulatory markers show decreased IgD expression suggesting they are an active isotype-switched subset.

Table 2:

Comparison of ABC-like subsets in autoimmunity and chronic infections

Condition	Phenotype	Location	Function	References
Rheumatoid Arthritis (RA) (human)	T-bet$^+$CD11c$^+$IgD$^-$IgM$^-$CD21$^-$CD23$^-$CD11c$^+$IgG$^+$	Blood, synovial joint tissues	Local Inflammation, Autoantibody production	40,57
Systemic Lupus (SLE) (human, mouse)	T-bet$^+$CD11c$^+$IgD$^-$IgM$^-$CD21$^-$CD23$^-$CD38$^-$IgG$^+$	Blood, spleen, kidneys	T cell activation, autoantibody production	50,54,56
Multiple Sclerosis (MS) (human)	IgD$^-$IgM$^-$CD21$^-$CD11c$^+$IgG$^+$	Blood	autoantibody production	55
HIV (human)	T-bet$^+$CD85j$^+$CD11c$^+$IgD$^-$IgM$^-$CD21$^-$IgG$^+$	Blood	Antibody production	63
HCV (human)	T-bet$^+$IgD$^-$IgM$^-$CD21$^-$IgG$^+$	Blood	Antibody production	64

Table shows phenotype, distribution, location and function of ABC-like populations in different autoimmune diseases and chronic infections based on the listed references.