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Age-Associated B Cells

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Abstract

The age-associated B cell subset has been the focus of increasing interest over the last decade. These cells have a unique cell surface phenotype and transcriptional signature, and they rely on TLR7 or TLR9 signals in the context of Th1 cytokines for their formation and activation. Most are antigen-experienced memory B cells that arise during responses to microbial infections and are key to pathogen clearance and control. Their increasing prevalence with age contributes to several well-established features of immunosenescence, including reduced B cell genesis and damped immune responses. In addition, they are elevated in autoimmune and autoinflammatory diseases, and in these settings they are enriched for characteristic autoantibody specificities. Together, these features identify age-associated B cells as a subset with pivotal roles in immunological health, disease, and aging. Accordingly, a detailed understanding of their origins, functions, and physiology should make them tractable translational targets in each of these settings.

1. INTRODUCTION

Separating hematopoietic cells into subsets based on phenotypic criteria is a useful approach to study their origins, lineage relationships, and functional attributes. Thus, establishing phenotypically defined developmental subsets in the B lymphoid lineage has facilitated analyses of the cellular and molecular events key to lineage specification, B cell antigen receptor (BCR) expression, negative and positive selection, and triage into different mature subsets (reviewed in 1–3). Similarly, applying analogous subset approaches to newly formed and mature B cell pools has revealed distinct developmental requisites, anatomic compartments, and functional capacities for transitional (TR), follicular (FO), marginal zone (MZ), and B1 B cell subsets (reviewed in 4, 5). Likewise, parsing antigen-activated B cells has afforded an understanding of the events and migration patterns associated with establishing germinal centers (GCs), memory B (Bmem) cells and plasma cells (reviewed in 6–8). Until recently, functional subsets within persistent, antigen-experienced B lineage cells—primarily Bmem and plasma cells—were less well-discriminated for several reasons. First, with the exception of transgenic models, the frequency of lymphocytes responsive to a particular antigen within preimmune pools is very low: in the range of 1/20,000 cells for small haptenic determinants (reviewed in 9), and only 1/100,000 cells for more complex protein antigens such as influenza hemagglutinin (10). Thus, even after the characteristic 10- to 50-fold expansion associated with immunization, the number of antigen-specific memory cells remains a small proportion of total B cells. Second, until recently the paucity of reagents that detect antigen-specific B cells—with the exception of some haptenic determinants—precluded tracking responding cells through their activation upon primary challenge and subsequent triage into memory pools. Nevertheless, several Bmem and plasma cell subsets have been defined based on their persistence following immunization, surface marker combinations, and turnover properties (11–15; reviewed in 7, 16).

Against this backdrop, a novel B lymphocyte subset termed age-associated B cells (ABCs) was described (17, 18) and has been scrutinized with growing interest. Although there is heterogeneity within the ABC population, it is largely an antigen-experienced pool that arises under particular signaling circumstances. Further, in both mice and humans, ABCs are a common feature of microbe-specific immune responses and B cell memory (19–26), but they are also strongly associated with autoimmune disease (27–31). Each of these features has prompted intense investigation, as well as an expanding body of commentary, review, and topical volumes devoted to understanding the origins and roles of ABCs in health and disease (32–38).

Herein, the initial characterization and key features of the ABC subset are reviewed, followed by in-depth considerations of their progenitors and the signaling requirements that drive their formation. Finally, their roles in aging, microbial immunity, and autoimmunity are considered.

2. THE ABC SUBSET

The ABC subset was first defined in companion publications by Hao et al. (17) and Rubtsov et al. (18), from the Cancro and Marrack groups, respectively. Hao et al. defined ABCs as $B220^+CD19^+$ splenic cells that lack CD21, CD23, CD95, and CD43, whereas Rubtsov et al. used the expression of Cd11c on $B220^+CD19^+$ splenocytes as their primary criterion. Although the exact markers used to delineate ABCs differed between the two reports, these were largely overlapping populations and shared several key features.

2.1. The Splenic ABC Pool Enlarges Continuously with Age

Both reports showed that, as suggested by their moniker, splenic ABCs increase in number and proportional representation continuously with age. Thus, these naturally occurring ABCs are

nearly undetectable in the spleens of juvenile and young adult mice, are present at a low frequency by 3–6 months of age, and by 12–18 months of age comprise a readily distinguishable and steadily enlarging pool in all strains and F1 combinations examined (17). This gradual accretion continues for the lifetime of the individual, such that ABCs may comprise as much as half of all splenic B cells in mice at 24 to 30 months of age (39). Importantly, even in age-matched, cohoused cohorts, there is considerable individual variation in the onset and kinetics of splenic ABC increases.

These studies, as well as subsequent findings discussed in detail below, reported unique tissue distributions for ABCs compared to other B cell subsets. Thus, once apparent, ABCs are consistently observed in the spleen, and they become steadily more numerous with age. In contrast, there is a paucity of ABCs in most lymph nodes and lymphatics regardless of age, distinguishing them from FO B cells. Hao et al. (17) reported that ABCs are present in the bone marrow and peripheral blood, but with substantial variability both between mice and within an individual at different time points. Notably, the proportional representation of ABCs in the blood does not necessarily parallel that observed in the spleen, suggesting that blood and splenic ABC pools are not in equilibrium. This finding, coupled with the lack of ABCs in the lymphatics, indicates unique trafficking patterns and possibly splenic residency. Regardless of the basis, this lack of equilibrium between splenic and blood ABCs sounds a cautionary interpretive note for studies that track ABCs only in peripheral blood, since the steady increase in representation with age seen in the spleen is not reflected in the blood.

2.2. ABCs Have Unique Activation Requirements and Transcriptional Signatures

Initial functional characterizations established several key features of ABCs, particularly in terms of the signals that drive their activation. These features distinguished them from previously defined B cell pools. Notably, ABCs do not divide in response to BCR cross-linking, again distinguishing them from FO B cells, which proliferate robustly to anti-BCR stimuli. However, while ABCs do not divide after BCR cross-linking, they nonetheless remain viable under these conditions, distinguishing them from MZ and TR B cells, which die rapidly following BCR cross-linking (17, 40–43; for reviews of MZ and TR subset characteristics see 3, 4). ABCs differ from splenic B1 B cells in several respects, including the lack of CD43 and CD5 expression, as well as their absence in neonates and young adults, which is opposite to what is observed in the B1 cell compartment.

Although refractory to BCR cross-linking, ABCs proliferate robustly to stimuli that engage the endosomal nucleic acid–sensing Toll-like receptors TLR7 or TLR9. This propensity for activation via endosomal nucleic acid–sensing TLRs presaged findings in subsequent analyses that examined the signaling requirements for ABC generation, as well as the dichotomous roles played by this subset in both microbe-specific immunity and autoimmunity. Finally, despite having no effect when applied alone, BCR cross-linking can synergize with either of these TLR signals, yielding increased rounds of division compared to either TLR ligand alone (17). Thus, the BCR remains an active signaling system in ABCs, albeit uncoupled from direct mitogenic activity.

In addition to these shared findings, each initial report contained further basic observations. Hao et al. (17) pursued experiments aimed at understanding ABC progenitors and the homeostatic niche that they occupy. They found that while most peripheral B cell pools, including ABCs, are ablated by sublethal irradiation (5 Gy), the ABC pool does not return rapidly, despite full autoreconstitution of the FO and MZ B cell compartments. This observation strongly suggests that most naturally arising ABCs do not represent de novo production of a unique preimmune B cell type by progenitors prevalent in the aged bone marrow but are instead members of a slowly accumulating population, probably derived from the preimmune peripheral B cell compartments. This possibility was directly addressed by adoptive transfer studies, which showed that ABCs can arise from

splenic FO B cells within 30 days after transfer to replete recipients. Moreover, the transferred cells had been labeled to track division, and the donor-derived ABCs were found only among the most extensively divided cells. Interestingly, neither donor nor recipient ages influenced this result; FO B cells from either young or aged donors were equally efficient at ABC generation in either aged or young recipients. Finally, Hao et al. also showed that, similar to FO and MZ B cells, ABCs express two of the BAFF (a.k.a. BLyS) family receptors, BAFFr (a.k.a. BR3) and TACI. However, unlike FO and MZ B cells, which rely on BAFF for survival in vivo (44; reviewed in 45–47), ABC numbers were unchanged following administration of a BAFF-blocking antibody that eliminated the FO and MZ pools (17, 48). This observation suggests that ABCs more closely resemble Bmem cells in their homeostatic survival requirements, inasmuch as prior studies had shown that most Bmem cells are BAFF independent (48). Nonetheless, because they express the BAFFr and TACI receptors, they can consume or sequester BAFF, implying that ABCs occupy homeostatic space at the expense of the preimmune FO and MZ pools.

Rubtsov et al. (18) focused on the ABC transcriptional signature and their potential roles in autoimmune diseases. A striking finding, now reproduced in multiple laboratories regardless of the phenotyping strategy used, was that most ABCs express T-bet (49), a master transcriptional regulator encoded by the *Tbx21* gene that had been previously associated with lineage specification among activated T cells (reviewed in 50). Although T-bet had been reported in B cells as a transiently expressed antibody isotype switch factor favoring IgG2a/c, as well as in some B lymphoproliferative disorders (51, 52), it had not previously been associated with a durable B cell differentiation subset.

2.3. ABCs Are Associated with Humoral Autoimmunity

Rubtsov et al. (18) also reported that ABCs arose earlier and more prominently in several autoimmune-prone mouse strains, particularly among females. Through a variety of approaches, these authors implicated TLR7 as the prime mediator of ABC expansion in these strains. These studies also reported the characteristic features of $CD11c^+$ B cells in human blood, revealing many similarities and some differences from murine splenic ABCs. For example, while mouse splenic ABCs contain both isotype-switched and unswitched cells regardless of the phenotyping criteria applied, most $CD11c^+$ cells in human peripheral blood are isotype switched. Finally, in small cohorts of several autoimmune diseases surveyed, including scleroderma and rheumatoid arthritis (RA), ABCs were significantly elevated in patients compared to healthy controls. This observation presaged many of the subsequent findings linking ABCs to autoimmunity. Moreover, it foreshadowed the multifaceted roles of TLR9 and TLR7 in both specifying ABC fate and promoting autoimmunity versus tolerance.

2.4. Heterogeneity Within the ABC Subset

Based on the collective phenotyping criteria applied by different groups, at least three populations are contained within the ABC subset. Thus, within the $CD21^-CD23^-$ splenic B cell pool, only about two-thirds of the cells are T-bet^+, and among these, roughly half are $CD11c^+$. It is not yet clear whether these represent stable, unrelated pools of cells or whether they are instead at different stages of differentiation or activation within a single lineage. This is not only an important fundamental question from the standpoint of ABC origins, but it also raises questions concerning differentiative chronology and plasticity, discussed in greater detail below.

In toto, these initial reports defined a B cell subset with unique phenotypic characteristics, transcriptional regulators, and activation requirements that accumulates with age and that is

correlated with autoimmune disease. These features have prompted questions as to their origins—both in terms of the signals that drive their formation and in terms of their progenitor/successor relationships—as well as their functional roles, particularly in the immunobiology of aging, microbe-specific immunity, and autoimmunity.

3. REQUISITES FOR ABC DIFFERENTIATION AND PERSISTENCE

The signals that specify and drive ABC fate among activated B cells, as well as the downstream events that maintain ABCs as a stable functional subset, have been increasingly interrogated (53). Early studies had associated ABCs or ABC-like cells with an inflammatory microenvironment, including canonical Th1 cytokines, particularly IFN-γ. Recently, more comprehensive analyses have been undertaken to parse the nature and order of signals required to drive B cells to ABC fate. The aggregate of these findings is summarized in **Figure 1**.

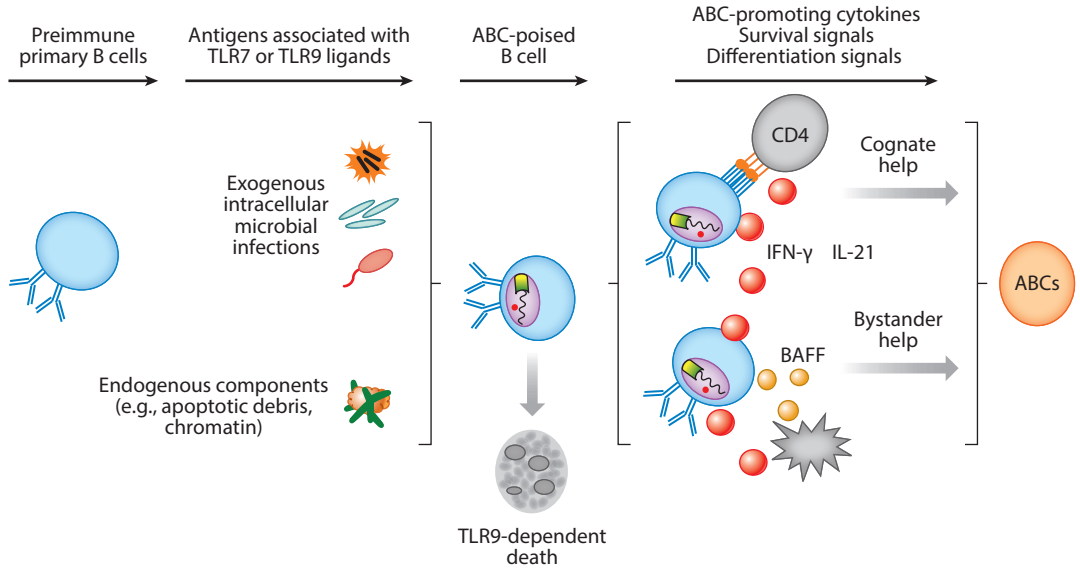

Figure 1

Routes of ABC generation. Preimmune B cells, including follicular and possibly marginal zone, transitional, and B1 B cells, may serve as progenitors for ABCs. Poising these cells for ABC fate requires that antigens bound and internalized via the BCR include TLR9 or TLR7 agonists. These may include microbial pathogens such as viruses and intracellular bacteria or parasites, as well as endogenous antigens, such as chromatin and apoptotic debris. These interactions are schematized within the endosome (*lavender intracellular organelle*). Following this key poising event, several alternative scenarios may ensue. If TLR9 has been engaged and no further signals or interactions occur, the cells will be triaged to a programmed cell death fate, reflecting an intrinsic TLR9-dependent peripheral tolerance mechanism. In contrast, further signals may promote survival and engender a variety of ABC-associated fates. Central to adopting a T-bet$^+$ ABC fate is receipt of IFN-γ or IL-21 signals. These ABC-promoting cytokine signals may occur in the context of cognate T cell help and CD40 costimulation, yielding either germinal center formation or extrafollicular differentiation with somatic hypermutation and class switch recombination. Either of these will result in plasmablast and T-bet$^+$ ABC Bmem cell formation. Alternatively, these cytokine signals may be received as bystander events from T cells or other accessory cell types, with accompanying affinity maturation, yielding unswitched memory or rapid plasmablast differentiation. Finally (not shown), some ABC differentiation may occur through homeostatic proliferation of naive B cells or development from bone marrow progenitors. Abbreviations: ABC, age-associated B cell; BAFF, B cell–activating factor belonging to the TNF family (a.k.a. BLyS); BCR, B cell antigen receptor; Bmem, memory B.

DO OTHER INNATE SENSOR SYSTEMS FOSTER ABC FATE?

Although TLR7 and TLR9 are presently the only signals shown to poise cells for ABC fate, other innate signaling systems might also do this. For example, alternative intracellular nucleic acid–sensing systems (STING, HMGB1–3, etc.), or sensors for other exogenous or intrinsic non–nucleic acid danger-associated molecular pattern receptor ligands (e.g., heat shock protein receptors), might have similar abilities. It will be worthwhile to examine these possibilities, especially in the context of autoimmune or autoinflammatory diseases where sensors and ligands other than TLR9 or TLR7 may have been implicated.

3.1. TLR7 or TLR9 Signals Are Necessary to Poise Naive B Cells for ABC Fate

Detailed in vitro and in vivo investigations of the signaling requisites that control adoption of the ABC fate were first reported by Naradikian et al. (54). These studies used the upregulation of T-bet expression in FO B cells under different activating conditions in vitro to monitor ABC differentiation. A fundamental observation from this work was that TLR9 or TLR7 signals are necessary to poise B cells for adopting the ABC fate. Thus, regardless of subsequent cytokine exposure, BCR ligation—either alone or with CD40 costimulation—does not enable ABC fate. In contrast, when the activating stimuli include either TLR9 or TLR7 agonists, subsequent exposure to IFN-γ or IL-21 results in ABC differentiation. Coculture experiments in these studies established that both the TLR and cytokine signaling requisites are cell intrinsic. This requisite for TLR stimulation confirmed and extended prior observations that TLR7 was a prime driver of ABCs in autoimmune-prone mice and fit well with previous studies showing that mice deficient for MyD88, an adaptor molecule necessary for TLR signaling, lacked ABCs (see the sidebar titled Do Other Innate Sensor Systems Foster ABC Fate?).

3.2. Interplay Between IFN-γ, IL-21, and IL-4 Regulates ABC Fate in TLR-Poised B Cells

While TLR7 or TLR9 signals are necessary to poise activated FO B cells for ABC differentiation, they are not sufficient. Instead, these signals must be followed by either IFN-γ or IL-21. Moreover, IL-4 negatively regulates T-bet$^+$ ABC fate in the context of IL-21, but it does not block ABC fate driven by IFN-γ. These observations are in agreement with the view that ABCs tend to be associated with Th1 immune responses.

These regulatory relationships were confirmed in vivo through experiments that monitored T-bet$^+$ ABC formation in various knockout mice following influenza or *Heligmosomoides polygyrus* infections, examples of Th1- versus Th2-skewed responses, respectively. Thus, during influenza infection, ABCs formed in wild-type strain C57BL mice, failed to appear in IFN-γ-deficient mice, yet arose in mice doubly deficient for IFN-γ and IL-4. Conversely, ABCs do not arise during *H. polygyrus* infection of wild-type mice but emerge robustly in IL-4-deficient mice under the same conditions. Thus, IL-4 actively blocks ABC differentiation in the absence of IFN-γ, but without IL-4 ABCs can be generated independent of IFN-γ, presumably via IL-21 signals.

Naradikian et al. also examined human peripheral blood B cells for these signaling requisites. While the same general relationships were observed, some activated human CD27$^-$ B cells exhibited T-bet expression directly induced by IFN-γ without concomitant TLR ligands. The basis for this apparent discrepancy has not been explored, but it may reflect intrinsic differences between mouse and human B cells or, more likely, may indicate that some B cells within the CD27- peripheral blood pool have already experienced ABC-poising signals.

The intracellular events underlying these signaling requisites remain unclear. For example, how signals from endosomal nucleic acid sensing TLRs poise B cells to assume ABC fate is puzzling. A potential clue is that these effects are rapid; T-bet expression begins within 12 h of TLR ligand and cytokine exposure, which is well before the first division event. Thus, while epigenetic modifications downstream of TLR signaling may play a role by altering transcriptional targets, these observations seem more consistent with immediate shifts in the intracellular signaling systems or metabolic status of the cell. Both TLR7 and TLR9 can rapidly influence broad aspects of B cell phenotype and physiology, ranging from cytokine receptors and calcium flux to mitochondrial metabolism (55, 56), providing fertile ground to explore the basis for this rapid shift to ABC specification. In terms of the instructive cytokine signals that must follow TLR7 or TLR9 ligation, it seems likely that interplay within the JAK-STAT family will be involved, given the relationships between IFN-γ, IL-21, and IL-4 in driving or blocking ABC fate. However, whether this is at the level of STAT phosphorylation or multimerization per se, or reflects more complex downstream events that dictate transcriptional regulatory activity and targets, is yet to be determined.

There is a similar lack of information regarding the requirements to sustain ABC character, as well as the relative roles played by T-bet per se in either driving or sustaining the ABC phenotype. Transcriptional analyses of IFN-γ- or IL-21-treated cells from wild-type or T-bet-deficient cells revealed that while some aspects of ABC phenotype relied strongly on T-bet per se, others—such as CD11c expression—were largely direct effects of each cytokine rather than downstream targets of T-bet (54). Whether this is the case in vivo remains somewhat controversial, since T-bet has been implicated as necessary in some systems but not others to drive CD11c induction (57). It is likely that these discrepancies reflect differing routes of ABC formation, in terms of progenitor B cell pools and the TLRs and cytokines involved in these different models, but definitive resolution of these apparent discrepancies awaits further investigation.

4. ABC ORIGINS IN VIVO

Although B cells with the phenotypic and functional characteristics of ABCs likely originate via several mechanisms, substantial evidence indicates that many, if not most, naturally occurring ABCs are antigen-experienced B cells that arise in T cell–dependent immune responses and then persist as a distinct Bmem population. Nonetheless, some cells within the ABC phenotypic window may reflect a unique preimmune subset or homeostatically expanded pool driven by endogenous ligands.

4.1. ABCs Display Characteristics of Antigen-Experienced B Cells

While the adoptive transfer studies by Hao et al. (17) had established that FO B cells can serve as progenitors for ABCs, these experiments did not directly address whether this represented antigen-driven differentiation per se. More recent studies have examined aspects of this question in detail. Work by Russell Knode et al. (58) yielded several observations implying that naturally accumulating ABCs are products of antigen-driven B cell responses, probably involving cognate T cell help. Sequence analyses of heavy and light chain genes from ABCs sorted according to the criteria of Hao et al. revealed that they utilize a diverse array of germ line V_H and V_κ genes, largely congruent with the distribution seen in FO B cell pools, ruling out the possibility that they are analogous to the age-associated T cell clonal expansions previously described (59, 60). Further, many ABC immunoglobulin heavy and light chain variable region sequences are somatically mutated, implying a GC origin and, by extension, a requisite for cognate T cell help and costimulation. Consistent with this idea, these authors used the adoptive transfer approach developed

WHICH PREIMMUNE B CELL SUBSETS ARE ABC PROGENITORS?

Although both in vitro assays and adoptive transfer studies show that FO B cells can adopt the ABC phenotype under appropriate conditions (17, 43, 54), whether these are the major reservoir of in vivo ABC progenitors, as well as whether other mature B cell subsets can assume this fate, has not yet been directly addressed. Thus, TR, MZ or B1 B cells may also contribute to ABC pools. Further, because each of these preimmune subsets undergoes selective steps during its differentiation that may involve innate sensor signals, it may have already undergone poising for ABC generation. In addition, these three subsets tend to be enriched for autoreactive and/or polyreactive antibody specificities, making them attractive potential candidates for autoimmune ABC progenitors. Finally, it is tempting to speculate that intrinsic features that distinguish these potential progenitor subsets might underlie some of the heterogeneity seen in the overall ABC pool.

by Hao et al. and showed that neither MHC-II-deficient nor CD40-deficient FO B cells could yield ABCs. Further, they found that CD154-deficient mice fail to develop natural ABCs with advancing age.

Together, these findings are consistent with the notion that ABCs arise following antigen-driven activation, cognate T help and assumption of GC characteristics. This view is also consistent with the emergence of antigen-specific ABCs in a growing list of viral, bacterial, and parasitic infections discussed in detail below. Nonetheless, a cautionary note to these interpretations is that both the requisite for cognate T cell interactions and the assertion that ABCs have entered GCs are based on inference, but have not yet been directly demonstrated. Thus, while MHC class II and CD40/CD154 interactions may enable ABC generation in vivo, the in vitro observations of Naradikian et al. (54), as well as recent studies from the Lund group (61), show that the ABC phenotype can be achieved without CD40 ligation. Thus, under certain circumstances, bystander cytokine production—particularly IFN-γ—may suffice to drive ABC differentiation. Similarly, while recent studies have shown that T-bet$^+$ B cells express GC-associated surface markers such as PNA and CD95 during the first several weeks of an immune response, anatomic localization of ABCs in GCs has not been directly demonstrated, and somatic hypermutation can occur independent of GC formation (62). Thus, it remains possible that ABCs arise and undergo somatic hypermutation in extrafollicular or other GC-independent niches. These possibilities are not mutually exclusive, and ABCs may arise via each of these routes depending upon the initiating signals and context (see the sidebar titled Which Preimmune B Cell Subsets Are ABC Progenitors?).

4.2. ABCs May Arise Through Alternative Routes

An alternative view of ABC generation is that, at least in some instances, they may arise without the engagement of exogenous antigen. This prospect is supported by findings from the Swain group indicating that cells with ABC characteristics emerge under conditions of limited exogenous antigen availability, and that with advancing age an increasing proportion of primary responses to influenza infection involves ABCs (63). Although a formal possibility, the lack of ABC emergence following ablation and autoreconstitution (17) argues against an ABC-skewed B lineage progenitor pool that dominates B cell genesis with advancing age. However, an attractive route to reconciling these findings with observations indicating that ABCs are the product of prior BCR-driven activation is that cells with the ABC phenotype might be established through homeostatic proliferation. This process has been well established for T lymphocytes, whereby endogenous ligands involved in T cell positive selection and peripheral survival drive gradual proliferation and

turnover within naive pools, resulting in cells that bear hallmarks of prior activation and memory (64; reviewed in 65, 66). Further, homeostatic expansion has been implicated both in the inversion of memory to naive T cell ratios and in the T cell replicative senescence observed with age (67, 68). Together, these suggest a provocative parallel to ABCs, which similarly increase relative to naive B cell pools with age, bear evidence of prior ligand-driven expansion, and are refractory to antigen-receptor-driven proliferation. Indeed, B cells rely on analogous positive selection via BCR signaling and cytokine availability for their maturation and continued survival (69–75; reviewed in 76, 77). Moreover, evidence for B cell homeostatic expansion, especially under lymphopenic conditions, has been described (78–80). Although the phenotypes of the expanded cells were not assessed for defining ABC markers in these studies, the generation of ABCs through this mechanism seems likely, especially as bone marrow B cell genesis wanes with age, imposing homeostatic pressure to sustain the peripheral B cell pools through proliferation rather than genesis (81–83). Importantly, these two paths of ABC differentiation are neither mutually exclusive nor mechanistically disparate, inasmuch as both rely on alternative forms of ligand-driven expansion. Nonetheless, a remaining question is whether ABCs derived from homeostatic expansion also require the participation of innate sensor signals, either independent of or in addition to BCR signals. Alternatively, these might make up the minority subcategory of $CD21^{-}CD23^{-}$ ABCs that lack T-bet and CD11c expression.

In summary, currently available data are most consistent with the idea that the ABC pool reflects a combination of formative routes, whose relative contributions may vary with age, antigenic load, and other variables. It is tempting to speculate that more detailed and deliberate parsing of ABC phenotypic heterogeneity will separate cells corresponding to these pathways. For example, the ABCs defined by Hao et al. (17) include IgM^{-} and IgM^{+} cells, and within the IgM^{+} pool a range of sIgD expression is observed. It is tempting to speculate that these might represent switched ($IgM^{-}IgD^{-}$) and unswitched ($IgM^{+}IgD^{-}$) memory from antigen-driven immune responses, versus homeostatically expanded primary cells ($IgM^{+}IgD^{+}$), respectively. Analogously, ABCs include cells with both somatically mutated and germ line configuration immunoglobulin genes, which might also help to resolve cells of different origins.

5. ABCs ARISE AND PERSIST IN MICROBE-SPECIFIC IMMUNITY

A steadily growing literature indicates that ABCs arise and persist as a durable Bmem subset in immune responses to viral, bacterial, and parasitic infections in mice and humans. In addition to tracking surface-marker criteria for the ABC phenotype, the majority of these studies have employed T-bet expression as a defining characteristic. Together, these findings directly establish $T\text{-bet}^{+}$ ABCs as a Bmem pool that plays distinct roles in terms of effector function and protective immunity. Moreover, they are the first to associate a Bmem subset with a characteristic transcriptional regulatory program, rather than surface markers alone.

5.1. ABCs Arise in Mouse Models of Infectious Disease

Studies from the Winslow group were the first to associate $T\text{-bet}^{+}$ B cells with microbial immunity using an *Ehrlichia muris* infection model. This group described both IgM^{+} $CD11c^{+}$ plasmablasts (84) and similar $T\text{-bet}^{+}$ memory B cells (85, 86) that were capable of protecting mice from fatal challenge (87). In addition, recent reports from this group showed that $T\text{-bet}^{+}$ Bmem cells are multipotential, since they could give rise to multiple effector B cell lineages upon serial adoptive transfers (19).

Concomitant studies from the Marrack and Wherry groups were the first to associate T-bet$^+$ ABCs with viral infections in mice (20, 21). Rubtsova et al. (20) showed that gammaherpesvirus 68 infection was correlated with the expansion of CD11c$^+$ T-bet$^+$ ABCs. Moreover, these cells secreted virus-specific IgG2a antibody ex vivo and were in part responsible for sustained reductions in viral load. Analogous studies by Barnett et al. (21) employed the lymphocytic choriomeningitis virus (LCMV) model. Using a variety of knockout and passive transfer approaches, they showed that T-bet$^+$ B cells were required for control of chronic LCMV infection, and that the IgG2a produced by these cells was only partially responsible for reduced viral titers. Subsequent studies have associated T-bet$^+$ ABCs or cells with similar characteristics in additional mouse infection models, including influenza (22, 61).

5.2. ABCs Are Generated in Human Infections and Immunizations

Several early histological observations in humans detected cells with ABC-like features. However, while T-bet$^+$ B cells were observed in certain conditions, they were considered an aberrant differentiation stage arising in unusual inflammatory conditions or neoplasia (52, 88–90). Among the first reports to identify a cell with ABC characteristics in an antiviral response were those describing an atypical memory B cell population in individuals with HIV. Moir et al. (91) termed these cells tissue-like memory B cells, as they shared some characteristics of a previously described tonsillar population (92); they lacked CD21 and CD27 and expressed several inhibitory receptors. In addition, they were hyporesponsive to BCR cross-linking, leading to the suggestion that they might represent an exhausted Bmem population. However, they nonetheless proliferated robustly to TLR9 stimulation, similar to murine ABCs. Although not assessed in these studies, T-bet expression has been demonstrated in these cells in subsequent analyses by Knox et al. (24, 93).

The expansion of a similar atypical memory cell subset among individuals living in malaria-endemic areas was reported by Weiss et al. (94) in 2009 (reviewed in 95). Further findings from this group revealed that these cells are driven by IFN-γ (25) and display a diverse V_H gene usage (96), consistent with the Th1 cytokine requirements and broad V_H usage characteristic of ABCs (54, 58).

Since these early observations, a steadily growing catalog of acute and chronic infections that are associated with T-bet$^+$ ABCs has emerged. These include transient increases in ABCs following either live virus vaccinations or infections, including influenza, yellow fever, and vaccinia (23, 24). Further, sustained T-bet$^+$ ABC pools are observed in chronic infections, including HIV, hepatitis C virus, and tuberculosis (24, 26, 93, 97). Interestingly, peripheral blood ABC frequencies in HIV-infected individuals are reduced during antiretroviral therapy. This raises the intriguing possibility that T-bet$^+$ ABCs found in the blood may represent an activated or mobilized differentiation state that wanes upon viral clearance, despite their retention in the spleen or other tissues as T-bet$^+$ ABC memory cells. Such a model might explain why the blood and splenic ABC pools are not in equilibrium and would be consistent with observations made in autoimmune diseases, where the ABC frequency in blood tracks with clinical disease activity (30).

5.3. The Induction and Function of ABCs in Humoral Immunity

Considered together, the emergence and persistence of T-bet$^+$ ABCs in pathogen-specific immune responses raise several questions. The first revolves around whether particular antigen characteristics are necessary for inducing and maintaining T-bet$^+$ ABC Bmem cells. An intriguing commonality among the diseases studied so far is that, in nearly all cases, these involve intracellular infections. It is tempting to speculate that this feature is necessary to meet all criteria required

ABCs IN THE CONTEXT OF VACCINES AND VACCINE DESIGN

Since T-bet$^+$ ABCs in the peripheral blood wax and wane with vaccinations, this may prove a fruitful avenue of investigation—both in terms of what formulations engender, impede, or reactivate ABCs and in terms of whether preexisting ABC pools might predict the efficacy or durability of particular vaccine formulations. Inasmuch as nucleic acid–containing adjuvants and delivery platforms are garnering increased attention, whether these either foster or impede ABCs over other memory cell fates may prove an important consideration. Moreover, such considerations may have particular import for vaccines aimed toward efficacy in elderly individuals, since their antigen-responsive B cell pool may be increasingly dominated by ABCs.

for ABC generation, including abundant IFN-γ and internalization of TLR7 or TLR9 ligands. Nearly all of these infections indeed drive Th1-dominant responses, favoring IFN-γ and discouraging IL-4 production. In addition, these are likely to fulfill the need for TLR7 or TLR9 agonists from apoptotic debris either associated with cytolytic cell death or associated with pathogen components internalized via BCR ligation. Establishing the relative roles of these and other parameters will be key to translational manipulation of the ABC Bmem cells in vaccine design or other settings (see the sidebar titled ABCs in the Context of Vaccines and Vaccine Design).

A related but separate question is the functional roles played by ABCs in humoral immune memory, particularly compared to their classical T-bet$^-$ Bmem counterparts. One difference already established is the skewing of isotype switching toward IgG2a/c in mice and IgG1 in humans. This will tend to favor effector functions associated with inflammatory processes and drive antibody-dependent cellular cytotoxicity as a major effector mechanism. However, as demonstrated in mouse studies, viral control was only partially restored upon adoptive transfer of virus-specific IgG2a/c, indicating that additional functional distinctions likely exist (21). These probably include differences in anatomic localization, regulatory cytokine production, antigen presentation, and the establishment or maintenance of plasma cell pools.

Growing evidence suggests that T-bet$^+$ ABCs may differ substantially in recirculation and tissue residency properties. This is implied by the clear disparity in molecules involved in homing and trafficking, as well as in direct assessments of ABC location. In fact, this may reflect an intriguing parallel with T-bet-expressing T cells, suggesting this transcriptional program is in part generic to effector memory cells with proscribed tissue residency characteristics.

There is ample evidence that ABCs produce unique and characteristic arrays of inflammatory and regulatory cytokines. Upon activation with TLR7 or TLR9 ligands, naturally occurring ABCs differ from FO B cells in this regard; they produce somewhat higher levels of IFN-γ and exceptionally high levels of IL-10, and these patterns are upheld by transcriptional array and ELISPOT analyses of ABCs ex vivo (17, 18, 58). Moreover, when used as antigen-presenting cells in vitro, they skew naive CD4 T cells toward Th17 differentiation (17). Thus, either during their formation or in recall responses ABCs may play a role in shaping the quality of the overall response.

Despite the clear association of ABCs with IgG2a/c antibody production, how T-bet$^+$ ABCs eventually give rise to antibody-secreting cells remains very poorly understood. Several recent studies have shown that ABCs differentiate rapidly to antibody-secreting plasmablasts upon TLR7 or TLR9 stimulation and exposure to IL-21 (30, 31). However, few if any plasma cells express T-bet, and there is evidence that T-bet represses Blimp-1 expression, suggesting that the formation of plasma cells from ABCs likely involves the loss of T-bet expression. A fascinating possibility is that the splenic ABC Bmem pool is a depot for precursors that continuously feeds plasma cell pools. This might partially resolve the long-standing conundrum of how the bone marrow niche

accommodates life-long plasma cell production without displaying either continuous enlargement or loss of previously generated specificities. Instead, a splenic ABC progenitor pool—which in fact does enlarge continuously with age—might regularly seed the bone marrow with plasma cells derived from a cross section of the ABC Bmem pool. The adoptive transfer studies of the Winslow group are consistent with this possibility (19), as are recent findings from the Allman group showing that a significant proportion of the bone marrow plasma cells display rapid turnover (15). Addressing this important question will require analyses using fate-mapping tools and assessments of turnover in the ABC pool.

A final but related consideration is whether distinct progenitor-successor relationships exist in these pools. Whether there is plasticity within the T-bet$^+$ ABC Bmem pool per se has not been definitively interrogated. The heterogeneity within the ABC pools, particularly in terms of T-bet, CD11c, and immunoglobulin isotype expression, suggests that some of these represent progenitor-successor relationships. For example, are unswitched (IgM$^+$), unmutated ABCs progenitors of class-switched mutated ABCs that arise during antigen challenge? Similarly, are CD11c$^+$ ABCs a differentiation state derived from CD11c$^-$ ABCs based on activation or inflammatory milieu? In addition to potential relationships within the ABC pool being uncertain, it remains unclear whether there is appreciable interchange between the T-bet$^+$ ABC and T-bet$^-$ (non-ABC) Bmem pools.

6. ABCs ARE INVOLVED IN THE IMMUNOBIOLOGY OF AGING

The gradual increase of ABCs with advancing age raises the question of what roles they might play in the immunological changes that accompany aging, collectively termed immune senescence (98–102). These changes include reduced B cell genesis, systemic and local increases in inflammatory mediators, and processes dubbed inflammaging (103–111; see 112, 113 for collected topical volumes), as well as altered or dampened immune responses to primary and recall challenges (104, 106, 111, 114–116). Accruing evidence suggests ABCs have both direct and indirect roles in many of these processes, as schematized in **Figure 2**.

6.1. ABCs Negatively Impact B Cell Genesis and Homeostasis

Both B cell genesis and T cell genesis gradually wane with age, reflecting intrinsic changes in hematopoietic progenitors and their lineage-committed progeny, as well as microenvironmental shifts that thwart progress through developmental stages and maturation (117–124). The Riley laboratory (39, 125) examined whether ABCs are involved in reduced B lymphopoiesis associated with age. Using a combination of in vitro and adoptive transfer approaches, these investigators showed that ABCs impede early B cell developmental steps through the production of TNF-α. These effects were both direct, via the induction of apoptosis in pre-B cells, and indirect, through systemic inflammatory effects on the bone marrow microenvironment. These observations raise the intriguing possibility that, while FO and *MZ* B cell numbers do not seem to play any feedback role in regulating rates of B cell genesis, the ABC pool—more specifically the ABC Bmem pool—may indeed serve such a role. This idea would be consistent with the fact that despite dampened B cell genesis in B lineage–replete aged individuals, the reconstitution of preimmune pools after sublethal irradiation—which ablates all peripheral B cell pools including ABCs—occurs at the same pace in both young and aged mice and yields a rejuvenated B cell compartment (126–129). In light of this interesting possibility it might prove worthwhile to determine whether ablation of the ABC compartment alone would yield similar effects on the rates of bone marrow B cell genesis.

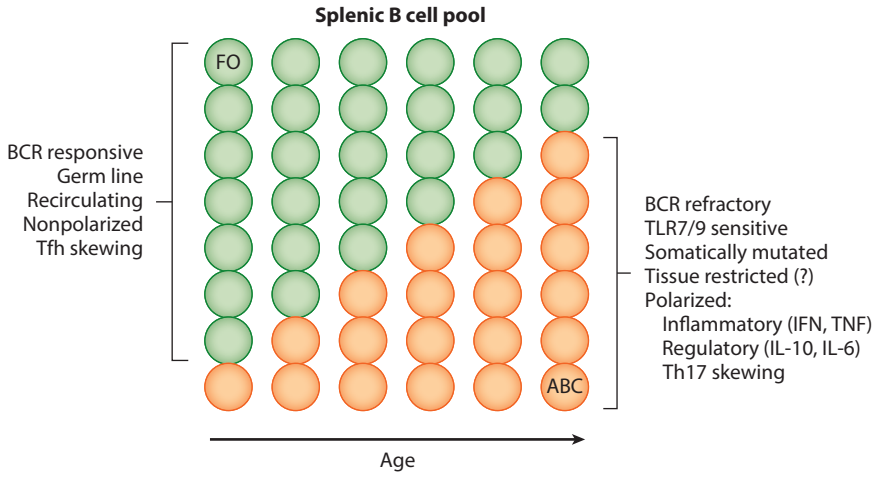

Figure 2

ABCs displace FO B cells with advancing age, contributing to features of immune senescence. ABCs (*orange*) increase in number and proportional representation with age, at the expense of the FO B cells (*green*). This increasingly skews the standing B cell pools toward ABC characteristics, contributing to multiple features of immune senescence. These include depressed bone marrow B cell production, altered B cell activation requisites in primary and recall challenges, and inflammatory or regulatory cytokine production. Abbreviations: ABC, age-associated B cell; BCR, B cell antigen receptor; FO, follicular; Tfh, T follicular helper.

A related impact of ABCs is that they appear to occupy homeostatic space in the primary pool at the expense of the FO subset. Indeed, the sum of ABCs and FO B cells tends to remain constant, indicating that as ABC numbers rise, FO B cell numbers drop correspondingly. This likely reflects expression of the BAFF receptors BAFFr and TACI by ABCs. The BAFF/BAFFr axis is well established as the primary homeostatic regulator of FO and MZ pool numbers; cells in these pools compete for systemic BAFF in order to survive (44, 47) and limiting amounts of BAFF thereby specify steady-state pool sizes. However, despite expressing BAFF receptors, ABCs appear largely BAFF independent and are thus exceptional competitors in this homeostatic space, eventually displacing the FO pool.

6.2. ABCs Contribute to Inflammaging

The observation that ABCs can contribute to a generalized proinflammatory milieu raises the question of whether they are key players in promoting inflammaging, a conceptual framework that posits that many age-related changes in immune function, metabolism, and other physiological systems can ultimately be traced to increased systemic levels of inflammation-related cytokines (130, 131). Although it has not yet been directly interrogated through gain- or loss-of-function approaches, several observations suggest this is a strong possibility. Initial descriptions showed a propensity for IL-6 and IFN-γ production by ABCs following TLR9- or TLR7-driven activation (17, 18). In addition, ABCs are effective antigen presenters, express comparatively high levels of MHC-II, and tend to skew naive T cells toward Th17 fate when used as presenters in vitro (17, 18, 132). Accordingly, their intrinsic propensity for inflammatory and regulatory cytokine production, coupled with their ability to skew T cell polarization toward inflammatory effector subsets during cognate interactions, strongly implicates ABCs as players in the heightened basal inflammatory states associated with aging.

More recently, the Frasca and Blomberg groups reported provocative findings linking T-bet$^+$ ABC-like cells with an inflammatory axis associated with obesity and visceral adipose tissue (VAT) in aged mice (133–138). In these studies, T-bet$^+$ CD21$^-$ B cells, termed inflammatory B cells or late memory B cells by these authors but largely congruent with the ABC phenotype, were analyzed in VAT across various ages. Major observations included an increase in VAT-resident ABCs with age, and a correlation of VAT with numbers of ABCs. Moreover, splenic B cells from young mice cultured in VAT adipocyte–conditioned medium yielded enrichment for ABCs, and VAT was a source of inflammatory cytokines that promoted ABC migration. This highly provocative set of observations opens a novel avenue of investigation that may eventually provide insight into connections between obesity, inflammation, immunity, and autoimmunity.

6.3. ABCs May Impact the Nature and Composition of Immune Responses with Age

It is tempting to speculate that the combined effects of increasing ABC numbers may underlie some previously intractable observations regarding the ways in which the magnitude and quality of humoral immune responses shift with age. One obvious possibility is that as the ratio of ABCs to FO B cells rises, antigenic challenges will be progressively less likely to draw responses from the naive FO pool but will instead increasingly recruit ABCs, consistent with data from Swain et al. (63). This carries several implications about both the induction and quality of such responses. First, it may suggest that as the potential contributions from the ABC and FO pools wax and wane respectively, robust responses will rely on antigens whose characteristics effectively drive existing ABC activation, such as TLR9 or TLR7 ligands. Second, this implies that with age, the repertoire participating in primary or recall responses will be increasingly colored by previous antigenic exposures, suggesting the ABC pool may be involved in antigenic imprinting characteristic of some sequential viral infections (139, 140) (see the sidebar titled ABCs and Original Antigenic Sin).

7. ABCs ARE ASSOCIATED WITH AUTOIMMUNITY

The association of ABCs with autoimmunity in both mouse models and human disease is now well established and generally accepted. Moreover, observations linking ABCs with autoimmune or autoinflammatory disease point to key roles for TLR7 and TLR9, as well as IFN-γ and IL-21, as key drivers. Finally, they raise the question of what common features, in terms of activation and persistence, contribute to the counterintuitive association of ABCs with both normal antimicrobial immunity and autoimmunity.

ABCs AND ORIGINAL ANTIGENIC SIN

Primary antigenic exposures often imprint individuals such that subsequent responses to heterologous antigens have greater activity against the initially immunizing antigen. First appreciated in the context of influenza vaccination, the basis for this so-called original antigenic sin remains poorly understood. As distinct Bmem subsets such as the T-bet$^+$ ABCs emerge, it will be worthwhile to determine their role in this phenomenon. In the case of influenza this may have particular promise, inasmuch as the dominant antibody isotypes produced against influenza hemagglutinin are those driven by T-bet, IgG2a/c in mice, and IgG1 in humans.

7.1. ABCs Are a Common Feature in Mouse Models of Autoimmunity

ABCs are elevated in nearly all murine models of humoral autoimmunity interrogated to date. The initial reports by Rubtsov et al. (18) had shown that in the NZB/WF1 and *Mer*$^{-/-}$ mouse models of systemic lupus erythematosus (SLE), ABCs were significantly expanded as early as 3 months of age, and comprised 15% of the splenic B cell pools by 6 months of age—levels that are rarely observed in healthy C57BL/6 mice until 12–18 months of age. Importantly, these studies implicated ABCs as sources of antichromatin antibody. This study also showed that ABCs in this model were likely TLR7 dependent, in agreement with the subsequent findings of Naradikian et al. (54).

Subsequent studies have confirmed and extended these findings in multiple mouse models of humoral autoimmunity. Recent studies using NZB/W and surrogate light chain–deficient autoimmune models showed that ABCs in both of these models also bear memory markers, and that hybridomas derived from these ABCs are enriched for anti-Sm and anti-DNA specificities (141). Consistent with these observations, Liu et al. (142) showed that CD11c^{+} T-bet^{+} ABCs are required for antichromatin antibodies in the bm12 chronic GVH SLE model, and studies in the B6.*Sle*-1 model revealed that cells with an ABC phenotype emerge in the IFN-γ–dependent spontaneous GCs associated with disease (143).

Manni et al. (28) used the SWEF protein double knockout model developed by the Pernis group to extend these ideas in several important ways. In addition to reporting the clear elevation of T-bet^{+} ABCs in this model, they used several powerful techniques to probe connections to other signaling systems. Consistent with the observations of Naradikian et al. (54) concerning requisites for ABC generation and activation, these investigators found that the expansion of ABCs in this model was strongly driven by IL-21, since IL-21-deficient SWEF double knockouts developed neither expanded ABCs nor autoantibodies. Importantly, they found that this IL-21-driven ABC expansion relied on IRF5, an established risk factor for SLE in humans.

7.2. ABCs in Human Autoimmune Disease

Rubtsov et al. (18) were the first to employ the term ABC in association with autoimmune diseases, showing that ABCs emerge and expand early in mouse models of autoimmunity and are elevated in the blood of scleroderma and RA patients. However, before the ABC moniker was coined, expansions of cells with similar phenotypic and functional features in SLE patients had been reported (144, 145). Over the last several years, these and subsequent observations have converged, revealing that ABCs, particularly those expressing T-bet and CD11c, are characteristic of a growing list of autoimmune and autoinflammatory disorders (see the sidebar titled ABCs as Diagnostic, Prognostic, or Therapeutic Targets in Autoimmunity).

ABCs AS DIAGNOSTIC, PROGNOSTIC, OR THERAPEUTIC TARGETS IN AUTOIMMUNITY

The growing appreciation that ABCs are associated with a broad spectrum of autoimmune and autoinflammatory diseases raises the possibly that they may be useful translational targets. For example, they might afford diagnostic acuity in diseases where subsets of patients display different spectra of pathologies. This has already been suggested by Warnatz in CVID (153, 154), but it might readily extend to SLE and other diseases of mixed pathology. Similarly, these might provide prognostic tools to predict or monitor the success of planned or ongoing therapies. Finally, ABCs may prove a tractable target for ablative regimes (38), sparing other B lineage subsets and thereby preserving existing non-ABC memory as well as primary antigen-responsive pools.

The association of ABCs or the ABC-like cells termed double negative (DN) with SLE was definitively established in recent publications from the Ettinger and Sanz groups (30, 31). Several features of these cells suggest that they correspond to the ABC subset and are analogous to those described in the mouse models discussed above. Both of these groups provide compelling evidence that both TLR7 and IL-21 play key roles in the generation and activation of these cells, consistent with the requisites for ABC formation and activation described earlier (17, 18, 27, 54, 146), and reminiscent of these features in mouse models of SLE (18, 28, 29). In addition, transcriptional profiles show that these cells differ from other mature peripheral cells, and many of the unique transcriptional characteristics previously shown in murine and human ABCs are shared. Wang et al. (30) also provided direct evidence that elevated peripheral blood ABCs strongly correlate with disease activity (SLEDAI score) and that the ABC pool in these patients is highly enriched for autoantibody specificities. Consistent with the idea that these cells track with disease activity, a recent clinical trial showed that in SLE patients treated with belimumab, the loss of cells consistent with the ABC phenotype—although T-bet was not included in the analysis—was correlated with therapeutic response (147). ABCs have also been implicated in RA patients (148), and in some cases they are found in tissues of affected joints. Moreover, ABCs have been reported in mouse models transgenic for certain risk alleles, such as *PTPN22*, that span several humoral autoimmune diseases (149, 150).

In addition to these increasingly appreciated associations with SLE and RA, an accumulating literature indicates that elevated ABCs are a common feature of additional autoimmune and autoinflammatory disorders, especially those associated with a Th1 cytokine signature, including Crohn disease and Sjögren syndrome (151, 152). Finally, the Warnatz group (153, 154) investigated a subset of common variable immune deficiency (CVID) patients who, in addition to the universal infectious complications of this disease, present with autoimmune cytopenia and interstitial lung disease. This subset of patients (CVIDc), but not those with infectious complications only (CVIDio), had clear elevations of an atypical, T-bet$^+$ B cell population with the phenotypic characteristics of ABCs. In addition, these features tracked with a Th1, IFN-γ-skewed, cytokine milieu.

8. TLR9-MEDIATED B CELL TOLERANCE MAY UNDERLIE THE LINK BETWEEN ABCs AND AUTOIMMUNITY

The constellation of signaling requirements for ABC formation and maintenance has compelling congruency with signals from innate sensors and instructive cytokines that have recognized roles in autoimmunity and tolerance. This concurrence may underlie the association of ABCs with both microbe-specific immunity and humoral autoimmune disease, providing fertile ground for understanding how peripheral B cell tolerance is maintained and broken. In particular, roles for TLR7 and TLR9—the same signaling systems that poise B cells for ABC fate—have now been firmly established in both promoting autoimmunity and maintaining peripheral B cell tolerance. These associations, coupled with findings that show antigens containing TLR9 ligands not only poise cells for ABC fate but also initiate an intrinsic cell death program, suggest peripheral tolerance mechanisms may be based on these signals.

Prevailing concepts of breached B cell tolerance have focused heavily on failures of deletional mechanisms operating during B cell development, followed by the fortuitous activation of escaped autoreactive clones. Deletional and editing mechanisms based on BCR signal strength have been appreciated for nearly 50 years (155, 156) and clearly act to eliminate strongly self-reactive clones during the immature and transitional stages in the bone marrow and periphery, respectively (157–165; reviewed in 159, 166–168). Nonetheless, the preimmune B cell pools contain appreciable numbers of cells with demonstrable polyreactivity or autoreactivity (reviewed in 169). Thus,

further avenues to peripheral tolerance, beyond these well-accepted deletional mechanisms, are likely.

The Marshak-Rothstein group was the first to connect endosomal TLRs with humoral autoimmunity (reviewed in 170, 171). Leadbetter et al. (172) and Lau et al. (173) showed that B cells from AM14 rheumatoid factor transgenic mice respond optimally when the activating immune complexes contain either TLR7 or TLR9 agonists. This has led to an extensive literature documenting roles for innate sensors both in mouse models of autoimmunity and in human autoimmune disease (reviewed in 170, 174). However, recent studies yielded a surprising result: Whereas TLR7 duplications foster disease and TLR7 deficiency ameliorates disease (175, 176), TLR9 knockouts exacerbate autoimmune manifestations, including earlier disease onset and more severe glomerulonephritis (177–182). This finding provides a potential connection between the signals that regulate ABC differentiation and those that can either promote or impede autoimmunity. In addition, they indicate a role for TLR9 in maintaining peripheral B cell tolerance. Several recent studies have probed these relationships (43, 183, 184). In studies using the AM14 model, Nündel et al. (183) showed that TLR9, but not TLR7, mediated a negative regulatory effect on AM 14 B cells both in vitro and in vivo. Sindhava et al. (43) confirmed and extended these findings by showing that when stimulated with antigens that deliver a TLR9 ligand via the BCR, B cells undergo cell cycle arrest and mitochondrial cell death after an initial proliferative burst. TLR9-dependent programmed death was circumvented by survival cytokines or CD40 costimulation. Moreover, in the presence of IFN-γ or IL-21, the rescued cells assume the ABC phenotype, presumably reflecting prior receipt of TLR9 signals necessary to poise them for ABC fate. Thus, the frequent association of ABCs with humoral autoimmunity may reflect failure of or rescue from this peripheral tolerance mechanism. Moreover, it more broadly implies that molecular pattern recognition systems that parse internalized antigen components, rather than BCR epitope specificity per se, underlie peripheral B cell tolerance (185).

SUMMARY POINTS

1. ABCs are a unique and durable B cell subset whose numbers increase continuously with age. They are refractory to BCR ligation but are activated by TLR7 or TLR9 signals.
2. ABCs can be generated from naive follicular B cells and possibly other preimmune B cell subsets, such as marginal zone, B1, and transitional B cells. Their differentiation requires TLR9 or TLR7 signals in the context of Th1 cytokines, particularly IFN-γ and IL-21. In contrast, Th2 cytokines such as IL-4 can impede ABC differentiation.
3. Naturally occurring ABCs likely arise through multiple mechanisms. Most are antigen-experienced, representing memory B cells developed in responses to microbial infections and vaccinations. However, some ABCs may also arise through homeostatic proliferation or other intrinsic developmental pathways.
4. ABCs contribute to immunological features of aging, including damped B cell genesis, altered immune responses to both primary and recall antigen challenges, and an increasing overall inflammatory climate.
5. ABCs are elevated in autoimmune and autoinflammatory diseases, including SLE, RA, Sjögren syndrome, scleroderma, and others. ABCs in these diseases are enriched for autoreactive antibody specificities, and their diminution has been associated with clinical response.

FUTURE ISSUES

1. What are the molecular, metabolic, and epigenetic events that underlie endosomal TLR-mediated specification of ABC fate and TLR9-mediated B cell tolerance?
2. What are the roles of ABCs in protective immunity and recall responses?
3. What are the relative contributions of ABCs to the establishment and maintenance of long- versus short-lived plasma cell pools?
4. How does the bifurcation of humoral memory into ABC and non-ABC arms inform vaccine design, particularly from the standpoint of efficacy in elderly populations where ABCs may represent the major antigen-responsive pool?
5. Can ABCs be targeted for diagnosis, prognosis, or therapeutic manipulation in autoimmune diseases?

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