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Age-Associated B Cells: A T-bet–Dependent Effector with Roles in Protective and Pathogenic Immunity

Kira Rubtsova,*†‡ Anatoly V. Rubtsov,*†‡ Michael P. Cancro,§ and Philippa Marrack*†‡¶

A newly discovered B cell subset, age-associated B cells, expresses the transcription factor T-bet, has a unique surface phenotype, and accumulates progressively with age. Moreover, B cells with these general features are associated with viral infections and autoimmunity in both mice and humans. In this article, we review current understanding of the characteristics, origins, and functions of these cells. We also suggest that the protective versus pathogenic actions of these cells reflect appropriate versus aberrant engagement of regulatory mechanisms that control the Ab responses to nucleic acid–containing Ags. The Journal of Immunology, 2015, 195: 1933–1937.

Advancing age is accompanied by shifts in many qualitative and quantitative aspects of immune function. These changes, collectively termed immune senescence (1, 2), include blunted primary and memory immune responses, reduced vaccine efficacy, and increases in the prevalence of inflammatory and autoimmune pathologies (2–6). Although the underlying mechanisms remain unclear, a growing literature documents contributions from age-associated changes at the systemic, molecular, and cellular levels. Systemically, serum and local concentrations of inflammatory cytokines are progressively elevated in both mice and humans, yielding an overall phenomenon described as inflammaging (7, 8). In addition, monoclonal gammopathies, as well as Abs reactive with chromatin and dsDNA, frequently emerge with increasing age (9–12). Finally, with a few exceptions, such as type 1 diabetes and juvenile rheumatoid arthritis, the frequency of autoimmune disease increases with age. These pathologies may reflect causal relationships with overall changes in the immune system, the cumulative impact of environmental insults, or combinations of these factors. Alternatively, some of these features may instead initiate in young individuals and stem from normal immune activity, but their pathogenic actions may only become manifest once the underlying effectors reach a minimum threshold with advancing age.

At a cellular level, the output of primary lymphoid organs wanes, reflecting a shift toward myeloid lineage preference in hematopoietic stem cell specification (13, 14), reductions in key developmental gene expression (15–17), and altered microenvironmental and homeostatic feedback mechanisms (18, 19). Despite this dwindling lymphocyte production, total numbers of mature B and T cells remain relatively unchanged. Nevertheless, nearly all peripheral lymphoid pools exhibit altered dynamics, shifts in functional subset representation, and changes in clonal composition. Thus, the renewal rates of both T and B cell pools decline (15, 20, 21), in part explaining how overall numbers can be maintained in the absence of newly generated cells. Further, inordinate clonal expansions are observed in both T and B cell compartments. Although some of these age-associated changes may result from immune dysregulation, others may simply reflect the cumulative influence of antigenic experiences and normal homeostatic processes. In accord with this notion, the ratio of T cells displaying naive versus memory phenotype inverts with age. Similarly, a substantial shift in the composition of peripheral B cell pools accompanies advancing age, reflecting the gradual appearance of a novel B cell subset whose properties and origins are the focus of this review.

The emergence and characteristics of age-associated B cells

Recently, our laboratories described a phenotypically and functionally unique B cell subset that accumulates with age that we named age-associated B cells (ABCs) (22, 23). These cells display a characteristic transcriptional profile, compete homeostatically with naive follicular (FO) and marginal zone (MZ) B cells, and bear hallmark features of Ag-experienced cells. ABCs are detected in the spleen, blood, and bone marrow and less frequently in the peritoneal cavity or lymph nodes. Detailed understanding of their locale relative to splenic folicles and MZs is lacking, but recently reported age-associated changes in the cells occupying MZs make these sites a potential candidate (24). Finally, ABCs are associated with appropriate humoral responses to certain classes of infectious and inflammatory stimuli, arise prematurely in autoimmune-prone mouse strains, and display a characteristic transcriptional profile, compete homeostatically with naive follicular (FO) and marginal zone (MZ) B cells, and bear hallmark features of Ag-experienced cells. ABCs are detected in the spleen, blood, and bone marrow and less frequently in the peritoneal cavity or lymph nodes. Detailed understanding of their locale relative to splenic folicles and MZs is lacking, but recently reported age-associated changes in the cells occupying MZs make these sites a potential candidate (24). Finally, ABCs are associated with appropriate humoral responses to certain classes of infectious and inflammatory stimuli, arise prematurely in autoimmune-prone mouse strains,

*Howard Hughes Medical Institute, National Jewish Health, Denver, CO 80206; †Department of Biomedical Science, National Jewish Health, Denver, CO 80206; ‡Department of Immunology and Microbiology, School of Medicine, University of Colorado Denver, Anschutz Medical Campus, Aurora, CO 80045; †Department of Pathology and Laboratory Medicine, Perelman School of Medicine, University of Pennsylvania, Philadelphia, PA 19104; ¶Department of Medicine, School of Medicine, University of Colorado Denver, Anschutz Medical Campus, Aurora, CO 80045; and §Department of Biochemistry and Molecular Genetics, School of Medicine, University of Colorado Denver, Anschutz Medical Campus, Aurora, CO 80045

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Address correspondence and reprint requests to Dr. Kira Rubtsova, National Jewish Health, 1400 Jackson Street, Denver, CO 80206. E-mail address: rubtsovak@njhealth.org.

Abbreviations used in this article: ABC, age-associated B cell; FO, follicular; MZ, marginal zone; TFH, T follicular helper, ...

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and may be enriched for autoreactive Ab specificities (23, 25). The origins and roles of ABCs in normal immune responses, as well as in immune senescence and autoimmunity, remain areas of intense investigation.

Although sharing many features, some heterogeneity exists among ABCs. Hao et al. (22) identified ABCs by the lack of both CD21 and CD23 expression. The frequency and the numbers of these B cells increased with the age, accounting for as much as 30% of splenic B cells in 22- to 30-month-old mice. Further phenotypic analysis of this CD23<sup>-</sup>/CD21<sup>-</sup> ABC population revealed that they differ from MZ, FO, or B1 B cells and showed that they express several markers shared with exhausted memory B cells (26). Simultaneously, Rubtsov et al. (23) reported a population of CD11c<sup>+</sup>/CD11b<sup>+</sup> B cells that appears in healthy aged female mice and in autoimmune-prone animals (23). These cells clearly overlapped with those reported by Hao et al. (22), because they expressed low levels of CD21 and CD23 and elevated levels of CD5, Fas, and CD138. However, in contrast to the more broadly defined cells described by Hao et al. (22), the CD11c<sup>+</sup>/CD11b<sup>+</sup> B cells described by Rubtsov et al. (23) uniformly expressed high levels of the activation markers CD80, CD86, and MHC class II. A comparison of surface markers among the ABCs defined by Hao et al. (22) and Rubtsov et al. (23) is shown in Table I. Importantly, both groups found that ABCs accumulate with age and tend to arise earlier and more consistently in female mice. Although this surface phenotype heterogeneity remains to be fully resolved, it likely reflects alternative routes of ABC generation.

A key feature of ABCs is that they express and depend upon B cell–intrinsic expression of the transcription factor T-bet (25). Consistent with this notion, T-bet overexpression induces acquisition of the ABC phenotype (25), indicating that it acts as a master regulator of ABC character. The exact mechanism whereby T-bet promotes and maintains the ABC phenotype remains unclear, but ongoing chromatin immunoprecipitation and deep sequencing studies will likely reveal both direct and indirect effects of T-bet on characteristic ABC gene expression patterns.

As might be anticipated from their unique T-bet driven transcriptional program, ABCs differ substantially from other B cell subsets in their activation requisites, functional capacities, and survival requirements. In contrast to FO or MZ B cells, ABCs survive but respond poorly to BCR engagement. However, they proliferate robustly to stimulation with either TLR9 or TLR7 agonists, either alone or in combination with BCR ligation. Moreover, following TLR stimulation in vitro ABCs elaborate a unique spectrum of regulatory cytokines, with notably robust production of both IL-10 and IFN-γ. Recent in vivo studies have suggested that they are also an abundant source of TNF-α in vivo (27).

While most murine ABCs express IgM, they rapidly switch to IgG production after stimulation with TLR ligands (23, 25). Regardless of their source—autoimmunity, age or viral infection—ABCs are prone to IgG2a/c production (23, 25), consistent with the established role of T-bet in switching to this IgH isotype (28–32). However, the specificity of the IgG produced by ABCs differs depending on their source; ABCs obtained from autoimmune or aged mice produce autoreactive IgG, whereas ABCs from virally infected mice produce predominantly antiviral IgG (Fig. 1) (23, 25). Together, these observations imply involvement of BCR signaling during the differentiation and recruitment of B cells into the ABC subset, despite their apparently dampened response to BCR ligation alone.

In addition to Ab secretion, ABCs can serve as Ag presenters; following activation, they can produce regulatory cytokines capable of skewing the differentiation of other adaptive and innate cell subsets. For example, early studies showed that ABCs obtained from aged animals can present Ag and tend to induce Th17 polarization (22). More recent findings extend this idea and suggest that ABCs obtained from aged or autoimmune mice process and present Ag more efficiently than do other B cells (33).

The accumulation of ABCs has profound effects on the dynamics and homeostasis of peripheral B cell pools. Interestingly, ABCs express the canonical BAFF receptors BR3 and TACI, but unlike FO and MZ B cells, they do not rely on BAFF for survival. Thus, as ABCs accumulate they engage reciprocal decreases in FO B cell numbers through competition for BAFF (22). Moreover, recent studies from Riley and colleagues (27) suggest that ABCs negatively influence B-lineage commitment or development of bone marrow progenitors, implying a causal role for ABCs in the decline of B cell lymphopoiesis with age. These observations may bear on reports that B lymphocyte ablation can rejuvenate B lymphopoiesis in aged individuals (34), in as much as ABCs do not reappear quickly during self-reconstitution.

It is tempting to speculate that the progressive dominance of ABCs at the expense of FO B cells impacts adaptive humoral responses, and a growing body of evidence suggests that this may be the case. For example, adoptive-transfer experiments showed that multiple aspects of T follicular helper (T<sub>FH</sub>) cell differentiation—including those that depend upon B cell Ag presentation, such as the upregulation of IL-4 and IL-21 production—are profoundly compromised in aged mice, regardless of T cell donor age (35, 36). Thus, the outcome of cognate presentation by ABCs may differ from other APCs, failing to reinforce the T<sub>FH</sub> cell program or directing pre-T<sub>FH</sub> cells to alternative effector fates. In agreement with this idea, ABC presenters skew primed T cells to a Th17 fate in vitro (22).

### Table I. Comparison of the expression of surface markers by mouse and human ABCs and exhausted human B cells

<table>
<thead>
<tr>
<th></th>
<th>Mice (Ref. 22)</th>
<th>Mice (Ref. 23)</th>
<th>Humans (Ref. 23)</th>
<th>Exhausted Human B Cells</th>
</tr>
</thead>
<tbody>
<tr>
<td>CD19</td>
<td>N/A</td>
<td>High</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>B220</td>
<td>+</td>
<td>+</td>
<td>N/A</td>
<td></td>
</tr>
<tr>
<td>CD11c</td>
<td>+/−</td>
<td>+</td>
<td>+</td>
<td>N/A</td>
</tr>
<tr>
<td>CD11b</td>
<td>N/A</td>
<td>+</td>
<td>N/A</td>
<td>+</td>
</tr>
<tr>
<td>CD21</td>
<td>−</td>
<td>−</td>
<td>−</td>
<td>Low</td>
</tr>
<tr>
<td>CD23</td>
<td>−</td>
<td>−</td>
<td>−</td>
<td>+</td>
</tr>
<tr>
<td>Fas</td>
<td>N/A</td>
<td>+</td>
<td>+</td>
<td>N/A</td>
</tr>
<tr>
<td>CD138</td>
<td>N/A</td>
<td>Int</td>
<td>N/A</td>
<td>Low</td>
</tr>
<tr>
<td>CD5</td>
<td>−</td>
<td>Int</td>
<td>+</td>
<td>N/A</td>
</tr>
<tr>
<td>CD80/86</td>
<td>Low</td>
<td>High</td>
<td>High</td>
<td>High</td>
</tr>
<tr>
<td>MHC class II</td>
<td>Low</td>
<td>High</td>
<td>High</td>
<td>High</td>
</tr>
<tr>
<td>T-bet</td>
<td>N/A</td>
<td>+</td>
<td>+/−</td>
<td>N/A</td>
</tr>
<tr>
<td>Surface IgM</td>
<td>+</td>
<td>+/−</td>
<td>−</td>
<td>N/A</td>
</tr>
<tr>
<td>Surface IgD</td>
<td>Low</td>
<td>+/−</td>
<td>−</td>
<td>−</td>
</tr>
</tbody>
</table>

Int, intermediate; N/A, not available; −, negative; +, positive.
ABC generation in health and disease

ABCs probably arise from activation-driven differentiation. Early work ruled out the possibility that ABCs represent the product of B cell genesis in the aged microenvironment, because they do not reappear after irradiation and autoreconstitution. Instead, multiple lines of evidence now suggest that they are a normal differentiative alternative taken by naive B cells when responding to certain classes of exogenous and endogenous stimuli. Initial evidence that ABCs can arise from naive B cells was suggested by experiments in which FO B cells from young donors were transferred to replete young or old congenic hosts. One month later, the recovered donor cells that had undergone extensive division had adopted an ABC phenotype, regardless of host age (22). Although these findings showed that ABC-like cells could be derived from quiescent preimmune B cells, the activating stimuli were unclear, and the paucity of recovered cells prevented detailed functional analyses.

The early descriptions of ABCs also indicated that TLR7 and MyD88, but not IFN-αR, were required for the accumulation of ABCs (23), consistent with their being derived from events driven by activating receptors. Subsequent in vitro analyses revealed that T-bet upregulation, the hallmark of ABC generation, was induced most effectively by concomitant receipt of BCR ligation, TLR7 stimulation, and IFN-γ (25) (Fig. 2).

Taken together, these observations suggest that ABCs originate under circumstances involving Ags that engage the BCR and also contain ligands for endosomal nucleic acid sensors, which also induce a promoting cytokine milieu. If this is the case, ABCs would be expected to arise during normal adaptive responses to microbial pathogens, as well as during potentially autoreactive responses to self components, as long as this tripartite set of conditions is established. Indeed, several lines of evidence now indicate that ABCs arise and play key roles in both situations, providing clues about their emergence with age and connection with humoral autoimmunity.

ABCs in infection and immunity. B cells closely resembling ABCs arise during antiviral immune responses (25). These T-bet+ CD11c+ B cells appear at the peak of the humoral immune response during infection with mouse gammaherpesvirus 68, mouse CMV, lymphocyte choriomeningitis virus, and vaccinia. B cells with very similar phenotypic and functional characteristics also were described recently in Ehrlichia muris infection (37). Importantly, ABCs derived during these responses secrete pathogen-specific IgG upon restimulation in vitro more efficiently than FO B cells from the same host, indicating recruitment of Ag-specific B cells into the ABC pool rather than nonspecific enlargement of a bystander ABC pool. Further, ABC differentiation is a critical element of the successful immune response to viral infection. Mixed bone marrow chimeras in which the B cell compartment was T-bet deficient and unable to initiate ABC differentiation displayed dramatically reduced viral-specific IgG2a/c titers, less efficient viral clearance, and higher viral burden (Fig. 1) (25). This is in agreement with prior studies indicating that IgG2a/c most effectively drives viral clearance due to its efficiency in Ab-dependent cell–mediated cytotoxicity and high affinity for activating FcRs (38–41).

These findings also strengthen the idea that ABCs arise via BCR-mediated activation in the context of TLR stimulation and appropriate cytokine milieu; BCR engagement affords virus uptake and trafficking to endosomal nucleic acid sensors, whereas NK cells and T cells secrete abundant IFN-γ in response to the virus to provide the appropriate cytokine microenvironment (Fig. 2).

**FIGURE 1.** ABCs in age, autoimmunity, and infection. The function and outcome of the appearance of ABCs in aged animals are still unknown. In autoimmune animals, ABCs produce high titers of autoantibodies (mostly of IgG2a/c isotype) upon stimulation, which may be the cause of autoimmunity. During the infection, ABCs produce antiviral IgG (mostly IgG2a/c), which is required for efficient viral clearance.

**FIGURE 2.** Model for T-bet induction in B cells and its role in B cell fate. Synergistic signaling via BCR, TLR7, and IFN-γR in B cells leads to the induction of high levels of T-bet expression, which, in turn, drives the expression of an ABC phenotype and class-switching to the production of IgG2a Abs.
**ABCs in autoimmune.** B cells phenotypically similar to ABCs also appear in young autoimmune-prone mice (42). Moreover, the appearance of ABCs is correlated with disease onset in several murine lupus models, including MRL<sup>10</sup>, NZB × WFl, MER<sup>−/−</sup>, and BXSB mice.

The potential relevance of ABCs to human autoimmunity was tested by screening human PBMCs obtained from either healthy or autoimmune donors for the presence of a similar B cell subset. The results show that PBMCs from donors with some autoimmune diseases contained a high percentage of CD11c<sup>+</sup>/CD21<sup>−</sup> B cells. In addition, these human ABC-like cells, similar to their murine counterparts, expressed low levels of CD23 and high levels of CD5 and CD86. However, unlike murine ABCs, the human ABC equivalents were iso-

type switched (Table I) (23). Other investigators observed a similar B cell subset in the peripheral blood of autoimmune patients, but in these studies the cells were identified as CD11<sup>+</sup>CD21<sup>−</sup> (43–46). Together with the more pronounced and reliable emergence of ABCs in female mice, these findings in toto may provide clues as to why the majority of autoimmune diseases are more frequent in females.

B cells with similar phenotype were described in HIV-viremic individuals (47) and identified as FCRL-4-expressing exhausted-like B cells. Moir et al. (47) reported that FCRL-4-expressing B cells have low levels of CD21 and high CD11c expression (refer to Table I for the comparison of exhausted B cells and ABCs). Because FCRL-4-expressing B cells (similar to ABCs) express CD11c and CXCR3, they suggested that this B cell subset is similar to exhausted T cells (48) and can be driven by the persistent viral infection.

The exact combination of events that promote self-reactive ABCs in autoimmune-prone individuals remains unclear. It is tempting to speculate that autoantigen-specific B cells engage and internalize autoantigens via their BCRs and, if these are chromatin or ribonuclear particles, will ligate endosomal TLRs. The third prerequisite for ABC generation, INF-γ or other promoting cytokines, may be derived from TLR7 engagement in NK cells or from bystander TH1 cells. It is noteworthy that TLR7 and IFN-γR signaling are well-established factors in the etiology of humoral autoimmunity (49–55).

Support for this model comes from mixed bone marrow chimerae in which Mer<sup>−/−</sup> mice, which lack receptors for effective clearance of apoptotic debris, were reconstituted with ABCs that could be depleted by diphtheria toxin (23). Notably, ABC depletion reduced autoantibody titers in these animals (23). Also consistent with this idea, TLR7 deficiency in either MER<sup>−/−</sup> or Nba2 mice led to the absence of ABCs and significant reductions in autoantibody titers (Fig. 1) (42). Although these findings all suggest a role for ABCs in humoral autoimmunity, further work is required to fully reveal the underlying causal associations.

**ABCs accumulate with age.** Although the discovery of ABCs arose from studies in aged and autoimmune-prone mice, emerging findings suggest that this unique B cell subset reflects chronic or repeated exposures to stimuli that prompt a T-bet-centered transcriptional program, and that these cells progressively accumulate throughout life, eventually displacing a substantial proportion of the preimmune B cell pool with advancing age. In this context, ABCs may represent a specialized memory B cell subset directed toward chronic or endogenous pathogenic microbes. They might also be the product of B cells that react with nucleic acid–containing autoantigens that, under normal circumstances, are beneficial for housekeeping roles, such as the clearance of apoptotic debris. However, under circumstances in which inflammatory cytokines are persistently elevated, such as in advancing age, they might expand beyond normal homeostatic limits. These possibilities are not mutually exclusive and are amenable to experimental interrogation.

However, it is not clear why the appearance of ABCs is gender biased in aged animals. Sex hormones might contribute, but there is no evidence to support this idea. The X-linked Thr gene might also be involved, because some regions on the lyonized chromosome can escape inactivation and yield to the overexpression of some X-linked genes (30). If Thr is among these, at least in some cells, it might lead to consistently increased numbers of ABCs in females with age.

**Conclusions**

Current findings in toto suggest that ABCs are Ag-experienced B cells that are characterized by a T-bet–driven transcriptional program. Moreover, they play dichotomous roles in health and disease. ABCs are essential for effective immune responses against certain classes of infectious agents, likely reflecting the need for key effector functions mediated by IgG2a/c and inflammatory cytokines. Conversely, the sustained accumulation of ABCs can have detrimental effects, including a propensity for autoinflammatory and autoimmune pathologies. Based on the prerequisite for endosomal TLRs in ABC generation and activation, these seemingly paradoxical outcomes may reflect intricacies of the regulatory mechanisms that have evolved to control Ab responses to nucleic acid–containing Ags. Obviously, sensing pathogen-derived intracellular nucleic acids is critical to inducing immune effectors that eliminate or control such infections. We hypothesize that ABCs evolved, as a product of a specific set of B cell–activating signals, via BCR, TLR7, and IFN-γR. Therefore, we hypothesize that ABCs represent a stage of B cell activation or a differentiated effector stage and, upon further TLR7 triggering, may differentiate into Ab-secreting plasma cells.

We also suggest that evolution selected for the ABC-differentiative pathway, components of which are evident in viral infections, because it leads to effective antiviral humoral immunity. Despite being essential to health, the same mechanism can be triggered in response to self-Ag and thus, in rare individuals, causes damaging disease. Accordingly, interrogating the mechanisms that control ABC formation, activity, and persistence may reveal targets for intervention in both microbial pathogenesis and autoinflammatory diseases.

**Disclosures**

The authors have no financial conflicts of interest.

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