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Perspectives on fetal-derived CD5+ B1 B cells

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Abstract

CD5$^+$ B cell origins and their pre-disposition to lymphoma are long-standing issues. Transfer of fetal and adult liver bone marrow Pro-B cells generates B cells with distinct phenotypes: fetal cells generate IgM^{high}IgD^{low}CD5$^+$, whereas adult cells IgM^{low}IgD^{high}CD5$^-$. This suggests a developmental switch in B lymphopoiesis, similar to the switch in erythropoiesis. Comparison of mRNA and miRNA expression in fetal and adult Pro-B cells revealed differential expression of Lin28b mRNA and Let-7 miRNA, providing evidence that this regulatory axis functions in the switch. Recent work has shown that Arid3a is a key transcription factor mediating fetal-type B-cell development. Lin28b-promoted fetal development generates CD5$^+$ B cells as a consequence of positively selected self-reactivity. CD5$^+$ B cells play important roles in clearance of apoptotic cells and in protective immune responses, but also pose a risk of progression to leukemia/lymphoma. Differential Lin28b expression in fetal and adult human B-cell precursors showed that human B-cell development may resemble mouse, with self-reactive “innate-like” B cells generated early in life. It remains to be determined whether such human B cells have a higher propensity to leukemic progression. This review describes our recent research with CD5$^+$ B cells and presents our perspective on their role in disease.

Keywords

B-cell development; stem cells; B1a cells; Transgenic mouse models; B-cell leukemia; CD5$^+$ B cells

Introduction

CD5$^+$ B cells were originally identified in the autoimmune mouse strain, NZB, and were shown to produce certain autoantibodies in mice [1, 2]. Later, we found that CD5$^+$ B-cell numbers were enriched in the peritoneal cavity [3] and this enabled monitoring of CD5$^+$ B-cell generation in mice after precursors cell transfer. Unexpectedly, we found inefficient generation of CD5$^+$ B cells by precursors in bone marrow from adult mice (>2 months old), in contrast with CD5$^+$ B-cell generation by hematopoietic precursors in neonatal liver [3]. Such biased production, along with the fact that CD5$^+$ B cells self-renew and persist

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Conflict of interest

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throughout life [4], prompted to the hypothesis that a large fraction of $CD5^+$ B cells present in adult mice are generated early in life [5].

Later, we subdivide B lineage cells in bone marrow B-cell development, identifying a stage where cells have immunoglobulin (Ig) heavy chain DJ rearrangements on both chromosomes, but lack productively rearranged VDJs [6]. We called these Pro-B cells, to distinguish them from Pre-B cells that express heavy chain protein in their cytoplasm. When Pro-B cells were transferred into immunodeficient SCID mice that lack lymphoid populations, they generated mature B cells, but not other lymphoid cells and not a self-renewing precursor pool [6]. Pro-B cells with a phenotype similar to those in bone marrow have been identified in fetal liver, and they possessed partial IgH rearrangements, similar to bone marrow [7].

We performed cell transfer experiments of these Pro-B cells into SCID mouse recipients, comparing the B cells generated from these committed B-cell precursors isolated from fetal and adult sources [7]. Flow cytometry analysis showed that the B cells generated by these Pro-B cells at two different stages in the animal's life are very different, with fetal precursors generating cells with a B1 phenotype ($IgM^{high}IgD^{low}$, many $CD5^+$) and adult precursors generating cells with a B2 phenotype ($IgM^{low}IgD^{high}$, predominantly $CD5^-$) (Fig. 1). Thus, we proposed that a "developmental switch" may occur in B lymphopoiesis, similar to that long-recognized in erythropoiesis [8], with fetal (B-1) development generating mostly B1 B cells and adult development (B-2) generating mostly B2 B cells.

A word on nomenclature

Many years ago, shortly after the first meeting devoted to $CD5^+$ B cells in Miami in 1991, the terms B-1 and B-2 were proposed to describe these phenotypically distinct B cells [9]. This was a compromise among scientists with two different views of these cells: 1) $CD5^+$ B cells arise from distinctive B-cell development ("lineage hypothesis"); and 2) $CD5^+$ B cells arise by distinctive activation ("activation hypothesis"). Recent work provides the underlying mechanism for some of the distinctions between fetal and adult B-cell development, providing strong support for the lineage hypothesis [10, 11]. Furthermore, it is clear that $CD5^+$ B cells are selected by antigen [12] and that BCR signaling upregulates CD5 expression [13], so in a sense, both views are correct. We propose the nomenclature suggested above, using B-1 and B-2 to describe distinctive fetal and adult development, and use B1a ($B220^{lower}CD5^+$), B1b ($B220^{lower}CD5^-CD11b^+$) and B2 ($B220^{higher}CD5^-$) to describe distinctive B-cell phenotypes. In general, most $CD5^+$ B cells are generated by B-1 development and most B2/follicular B cells by B-2 development [7, 11]. We think that this terminology is important, since some B-1 development occurs in adults, particularly giving rise to $CD5^-$ B1b cells [14–16]. Such B-1 development in adult bone marrow may represent the progeny of infrequent stem cells that continue to express low levels of the fetal-type regulator, Lin28b [10]. Moreover, it is clear that certain types of activation and tolerance can generate cells from B-2 development with a $CD5^+$ phenotype [17, 18], even if such cells do not persist for an extended period.

Aspects of what we discuss below have also been previously reviewed as part of two presentations at a recent meeting devoted to B1 B cells [19, 20].

Distinctions between fetal and adult B lymphopoiesis

Obvious questions raised by the Pro-B transfer experiments were for example how such a switch is mediated and what it consists of. We first attempted to analyze gene expression differences in fetal and adult Pro-B cells by performing global mRNA analysis [17]. Microarray analysis revealed a striking difference in the expression of terminal deoxynucleotidyl transferase (TdT), a gene that encodes the enzyme mediating non-germline nucleotide addition (N-addition) at the Ig heavy chain junctions [21]. A paucity of such N-addition limits CDR3 diversity, a critical component of Ig antigen recognition [22]. Furthermore, low TdT favors rearrangement of gene segments that share short regions of homology, limiting diversity [23]. Thus differential expression of TdT is one reason for the difference between B-1 and B-2 development, since the IgH repertoires generated in their progeny will differ. However, this is not the complete explanation, since a TdT null mouse generated by gene targeting developed a mature pool of B cells that resembles B2/follicular B cells [24].

Development of B-cell pools with distinctive heavy chains could also occur by altered dependence on pre-BCR signaling [25]. Bone marrow B-cell development depends critically on assembly of a newly-rearranged VDJ-μ heavy chain with pre-existing B-lineage specific proteins encoded by the genes λ5 and VpreB that together make up the surrogate light chain (SLC) [26, 27]. Assembly of the IgH chain with SLC produces the pre-BCR, a complex that mediates a tonic signal indicating that a productive Ig heavy chain has been generated [28]. The pre-BCR also induces a number of changes in developing pre-B cells, including down-regulation of the Rag proteins [29] and rapid proliferation of pre-B cells. An IgH rearrangement that is frequent in B1 B cells, but rare in B2, utilizes a gene from the V_H11 family [30, 31]. These heavy chains are nearly always paired with a specific light chain, a member of the Vκ9 family; this BCR recognizes the haptenic group phosphatidyl choline, exposed on aged erythrocytes [32]. Analysis of B-cell development in transgenic mice bearing this heavy chain revealed poor expression of the V_H11-μ transgene and extensive endogenous IgH expression in spleen B cells of adult mice [25].

Failure of effective pre-BCR signaling results in continued rearrangement, explaining why an IgH-μ transgene may not block endogenous IgH locus rearrangement [33]. Analysis of assembly of the V_H11-μ transgene with SLC, by immunoprecipitation of SLC components followed by western blotting with IgH-μ in transfected Pro-B cells, revealed weak assembly of the Pre-BCR [25]. This was unexpected, since this heavy chain was isolated from the $CD5^+$ B-cell pool, whereV_H11 rearrangements constitute 5–15% of the BCR repertoire [25]. This finding suggested a potential difference in dependence on pre-BCR signaling between fetal B-1 and adult B-2 development. Analysis of pre-B-cell proliferation in several IgH-μ transgenic mice, comparing cells isolated from fetal liver with those isolated from adult bone marrow (all on a Rag-1 null background to block endogenous V_H gene expression) revealed a clear result: a human IgH-μ and the high-copy V_H11 Tg lines inhibited fetal proliferation, whereas a low-copy V_H11 Tg line allowed ongoing proliferation to continue

[25]. This means that optimal fetal B-1 development occurs with lower pre-BCR expression compared to bone marrow B-2 development, so different sets of VDJ segments will be expanded in the pre-B pool from fetal liver and bone marrow.

Mechanism underlying distinctions between fetal and adult B-cell development

Analysis using the anti-Thymocyte/Thy-1 autoreactive (ATA) transgenic system (see *BCR signaling, CD5+ B cells, and chronic leukemia*) showed that BCR signaling is critical for B-1 development and is less important for B-2 development [13]; we investigated expression of microRNAs, since these tune TCR responses during T cell development [34]. A clear result emerged, with expression of Let-7 family members higher in bone marrow and lower in fetal liver [34]. This prompted us to examine the RNA-binding protein Lin28b, since it functions in a kind of binary switch with Let-7: Lin28b sequesters precursor Let-7, preventing its processing into functional microRNA and, in turn, Let-7 targets Lin28b mRNA for degradation [35].

Recently the Muljo laboratory showed that retroviral expression of human Lin28b in mouse bone marrow hematopoietic stem cells could induce the generation of B1a B cells in cell transfer experiments [10]. They also found that innate-like γδ T cells were induced by Lin28b. This was not completely unexpected since Ikuta and Weissman showed many years ago that certain innate-like γδ T cells were only generated from fetal stem cells [36]. Thus the fetal/adult switch in B (and T) cell development is regulated by the Lin28b/Let-7 axis. The Lin28b/Let-7 axis functions to regulate a variety of metabolic, proliferation, and differentiation pathways [37, 38]. For example, the Lin28b blocking of Let-7 function releases repression of a network of proto-oncogenes, including the insulin-PI3K-mTOR pathway, Ras, Myc, Hmga2, and the Igf2bps, resulting in increased metabolism and growth [39, 40]. Such reprogramming of metabolism also enhances tissue repair [41]. Let-7 targets genes involved in cellular proliferation, so expression of the Lin28b protein can result in increased proliferation and even malignancy [42–44].

In order to understand how Lin28b was regulating fetal B-1 development, we determined the genes reciprocally perturbed by mis-expression of Lin28b in bone marrow and Let-7 in fetal liver B lineage progenitors using microarray analysis [37]. We identified a short list of genes that included a transcription factor, Arid3a, also known as BRIGHT. The 3′ untranslated region of this gene includes target sites for miRNAs that are found at higher levels in bone marrow relative to fetal precursors, Let-7 and miR-125b. Following this lead, we cloned Arid3a into a retrovirus and expressed it in BM Pro-B stage cells. We found that Arid3a over-expression in BM Pro-B was sufficient to generate large numbers of B1a B cells, similar to that obtained by transduction of Lin28b. Next we generated Arid3a knockdown constructs and transduced fetal Pro-B cells, finding that we could reciprocally decrease the production of B1a cells. Therefore we concluded that Arid3a is a key mediator of the developmental switch in B lymphopoiesis [11].

Arid3a was originally identified by its capacity to bind to IgH V segments, increasing their expression level in a cell line treated with antigen and IL-5 [45]. Later work showed that

Arid3a was not restricted to activated B cells, but was also expressed in B-cell progenitors [46]. Further analysis showed that Arid3a binding site motifs were common in the promoter regions of both human and mouse V_H genes [47]. Thus Arid3a may be altering accessibility of V_H genes to rearrangement in fetal development. Furthermore, Arid3a has been found to shuttle between the cytoplasm and the nucleus [48]. In the cytoplasm, Arid3a has been shown to interact with the Tec kinase Btk [49] and, in fact, functional Btk is required for Arid3a activity [50], by phosphorylation of TFII-I which then forms a tripartite complex with Arid3a and Btk. Importantly, Arid3a has also been found to alter BCR signaling, due to its association with BCR-containing lipid rafts [51]. Thus, altered expression of Arid3a may change the initial V_H repertoire and also impact selection at the newly-formed B-cell stage. A model for Lin28b/Let-7/Arid3a regulation of the developmental switch in B lymphopoiesis is shown in Figure 2.

BCR signaling, CD5$^+$ B cells, and chronic leukemia

The ATA BCR system enabled us to test the role of BCR signaling in B1a generation, and discover differences in B-cell fate arising from different levels of BCR signaling in B-1 and B-2 development [12, 52, 53]. This unmutated ATA IgM (V_H3609/V_k21) was identified as a B1a BCR in the SM/J mice [54] that are non-autoimmune but that have an elevated level of ATA IgM autoantibody in serum [55]. The BCR binds thymocyte membrane and was later shown to recognize highly glycosylated Thy-1 (CD90), abundantly expressed on immature thymocyte membranes [56], including dying thymocytes. We generated V_H3609μTg mice, and also VDJ knock-in mice, finding that mature B1a cells with this BCR accumulated in the presence of Thy-1 self-antigen, but not in the absence of Thy-1, demonstrating for the first time that positive selection occurs in mouse B cells [12, 53], as has long been known for T cells.

Analysis of "V_HV_L double-transgenic" ATAμκ mice on mouse backgrounds where *lck*-Thy-1 transgenes resulted in different levels of Thy-1, resulting in different intensities of BCR signaling, revealed that high BCR signaling during early/neonatal B-1 development led to positive selection, whereas comparable signaling led to maturation arrest during B-2 development in BM [52]. However, during B-2 development a weaker BCR signal (lower Thy-1 background) promoted MZ B-cell generation and very low or absent BCR signaling allowed FO B-cell generation [13], as diagrammed in Figure 3A. Thus, positive selection occurs in B-2 cell development, generating different mature subsets depending on differences in BCR signaling, although strong signaling in adult BM did not yield mature B1a cells. Instead some CD5$^+$ B cells were generated that failed to mature, revealed by continued expression of CD93/AA4.1[52]. This indicates that the threshold for BCR tolerance and requirement for BCR signaling differ between fetal B-1 and adult B-2 development.

B-cell chronic lymphocytic leukemia (B-CLL) is the most common form of adult leukemia in Western countries, with an incidence that increases with advancing age [57]. In the early 1980s, expression of the CD5 pan-T cell surface glycoprotein on B-CLL was noted as a unique feature of human B-CLL, in contrast with other B-cell lymphomas [58, 59]. This was a major impetus prompting us to test for Ly-1/CD5 expression on normal mouse B cells.

Importantly, a year after transfer of purified peritoneal B1 cells into young irradiated mice, together with bone marrow stem cells, B1a of peritoneal B1 origin (IgM^b allele$^+$) were clearly detectable in both spleen and peritoneal cavity, together with BM derived $CD5^-$ B cells (IgM^a allele$^+$) constituting the predominant B-cell pool. Using B1a B cells from spleen of 1–2 week old neonatal mice for transfer also revealed persistence of B1a [60]. Thus B1a B cells persist for life and potentially could originate B-CLL. Importantly, BCRs on B-CLLs have been shown to encode self-reactivity [61].

In a physiologically normal self-antigen environment, we found that during B-2 development in bone marrow, autoreactive marginal zone (MZ) B cells can be generated from $V_H3609\mu^+$ immature B cells, by pairing this heavy chain with a V_k19-17 light chain (Figure 3B). This MZ B associated V_H3609/V_k19-17 BCR IgM has autoreactivity to intestinal goblet cell granules (AGcA), predominantly recognizing the highly glycosylated polymatrix form of intact mucin 2 glycoprotein [53]. In such mice, $CD5^+$ B1a B cells are generated by pairing the transgenic heavy chain with a V_k21-5 light chain, yielding ATA B cells from B-1 development [12]. Thus, autoreactive B cells can be generated in different B-cell subsets with different autoreactivities and/or different affinities [13, 53]. By crossing μκTg mice with these autoreactive BCRs [52, 53] with the CLL-promoting Eμ-hTCL1 transgenic mouse [62], we are now asking whether these different autoreactive B-cell subsets (and FO B cells generated in the same mice) pose similar risks for progression to CLL, or whether only certain B cells behave in this fashion.

Human $CD5^+$ B cells

An obvious question arises whether these findings apply to human B-cell development. Many years ago we investigated the frequency of human B cells expressing CD5 and found that normal donors could be classified into $CD5^+$ B low and $CD5^+$ B high, with rheumatoid arthritis patients enriched for the $CD5^+$ B high pattern[63]. Furthermore, sorted $CD5^+$ B cells were enriched for production of rheumatoid factor, an autoantibody. Finally, examination of B-cell in cord blood [63], the progeny of fetal development, showed a very high level of $CD5^+$ B cells. Considering the similarities to mouse B1 B cells, it is reasonable to ask whether there is differential expression of Lin28b in human B lineage progenitors. Intriguingly, there are recent reports showing that Lin28b mRNA expression is high in fetal and low in adult hematopoietic stem cells [10, 64]. Thus it is reasonable to hypothesize that this regulatory axis is functioning in human lymphoid development, similar to mouse.

Following the differentiation of $Lin28b^+$ progenitor cells to mature B cells in humanized mice may give insights into the selection, phenotype, and specificities of human B1 B cells. We raise this issue because CD5 can be induced by BCR signaling and humans are exposed to a wide array of pathogens that mice maintained in clean animal facilities are not. Furthermore, there is a report that CD5 is transiently expressed on most immature human B cells [65]. Recently there has been considerable controversy over the identification and characterization of human B1 B cells [66–69]. Additional analysis by the same group has described a $CD11b^+$ subset of this population that is increased in patients with systemic lupus erythematosus and stimulates T cells [70]; this analysis has generated more controversy [71]. The analysis of B cells differentiated from $Lin28b^+$ progenitors, either

natural or transduced, may help to settle this issue, at least in the context of a human equivalent of a pool of fetal-generated B cells that persist into adults, where the phenotype of such cells may not precisely mirror that identified in mice.

Conclusions

What is the significance of fetal-generated B cells in mouse or man? Distinctive features of this cell pool in mouse are autoreactive BCRs and self-renewal throughout life. As mice age, B1 B cells can generate clonal expansions, as detected by distinctive bands on a southern blot with a J_H probe [72]. Such clones can also progress to a frank leukemia or lymphoma stage [73]. This process is accelerated by B-lineage targeted transgenic expression of the human TCL1 gene [62]. Thus B1 B cells clearly pose a risk in aged mice of dysregulated growth and even leukemia.

We wonder if a similar process takes place in humans, where a self-reactive population of B cells, possibly generated during distinctive B-1 development, may generate an expanded clone. This may be the origin of monoclonal B-cell lymphocytosis (MBL), detected in relatives of chronic lymphocytic leukemia patients [74]. Such clonal expansions likely have a low probability of progressing to B-CLL depending on antigen stimulation and second hits, either genetic or epigenetic. Significantly, in B-CLL stereotyped (recurrent) V_H or V_L segments have been described, suggesting antigen selection [75]. Approximately 50% of B-CLLs have BCRs that are unmutated and these cases have a poorer prognosis compared to those with hypermutated BCRs [76]. BCRs from B-CLLs have been shown to include several different types of self-reactivity [77] and recently there has been a report that many B-CLLs may be self-binding [78]. Possibly such unmutated B-CLL cases arise from a germline encoded self-reactive B cell, similar to B1 B cells in mice. This issue clearly merits further investigation and examination of the role of the Lin28b/Let-7 axis (and Arid3a) in human B-cell development may help to shed light on the origin of a B-cell pool with a potential risk for progression to B-CLL.

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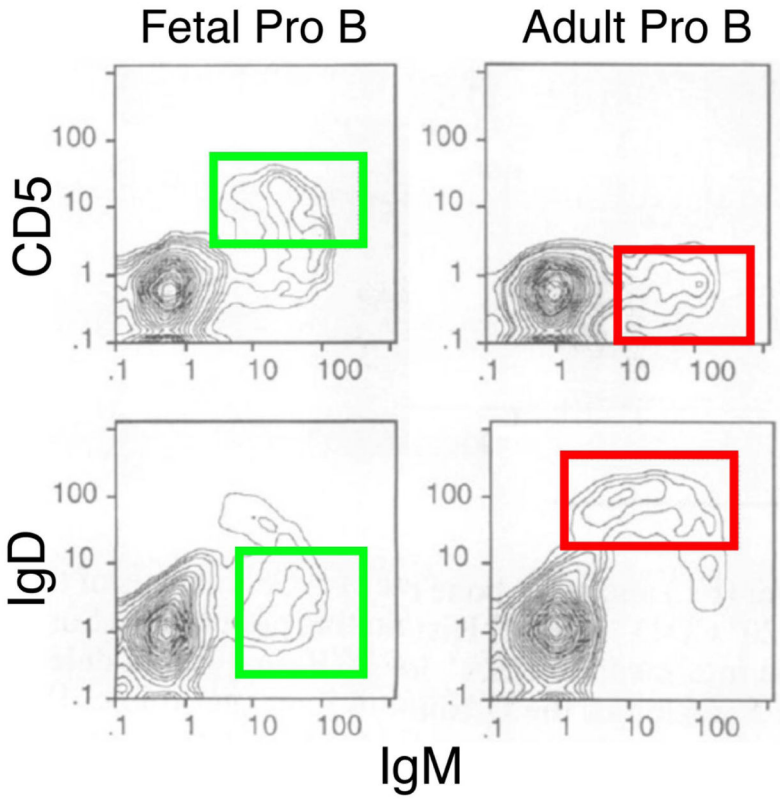

Figure 1. A developmental switch in B lymphopoiesis

Fetal and adult Pro-B cells were transferred to SCID mice (n=4 of each type). Three weeks after, CD5/IgM and IgD/IgM profiles of spleen B cells were analyzed. Fetal phenotype (B1) marked by green box, adult phenotype (B2) marked by red box. Data shown are representative of 4 independent experiments.

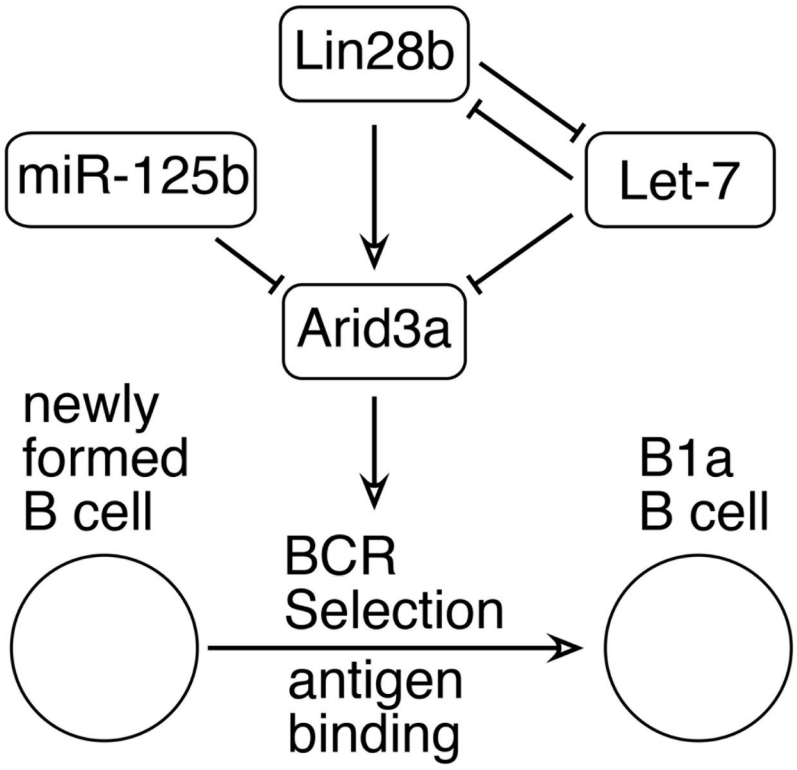

Figure 2. Model for regulation of fetal-type (B-1) development

B-1 development is regulated by the Lin28b/Let-7 axis and is promoted by the Arid3a transcription factor. Lin28b activates transcription of Arid3a, whereas Let-7 targets this mRNA for degradation. Arid3a modulates BCR signaling, altering antigen selection during the transition from newly-formed B to mature B.

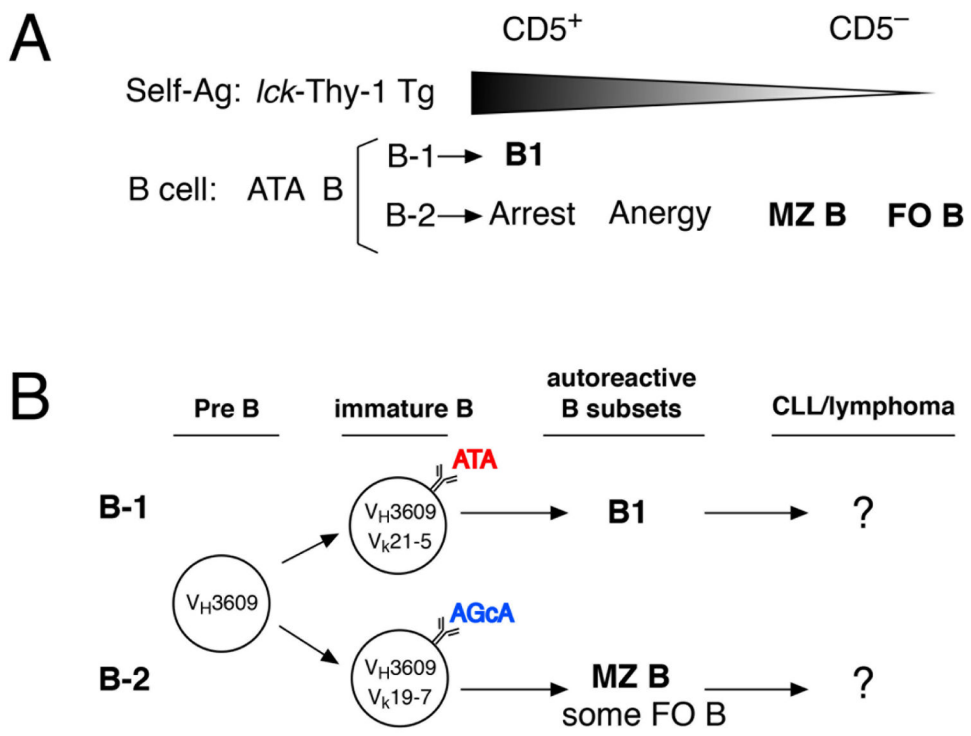

Figure 3. BCR signaling strength governs B-cell subset fate and progression to leukemia

A. Generation of different B-cell subsets governed by BCR signal strength. The ATA μκTg mouse line on a Thy-1 knockout background (ATAμκTg.Thy^{KO}) was crossed with *lck*-Thy-1 transgenic mouse lines expressing different levels of Thy-1 (from higher than normal level, wildtype level, lower, lowest, and completely absent). High levels promote the B1 cell fate from B-1 development, while very low levels generate MZ B cells and FO B-cell maturation occurs in the absence of Thy-1. **B.** In mice expressing $V_H3609\mu$ at the pre-B-cell stage, expression of different light chains results in the generation of different B-cell subsets, either B1 from B-1 development or MZ B (and some FO B cells) from B-2 development. These BCRs have different autoreactivity but both can produce autoantibody, as natural autoreactive B cells: ATA, anti-thymocyte autoantibody, and AGcA, anti-intestinal goblet cell/mucin 2 autoreactivity. We are now asking whether B cells in these different autoreactive subsets carry a similar risk of progression to CLL.