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BRIEF REVIEWS

B-1 B Cell Development[1]

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CD5$^+$ B cells have attracted considerable interest because of their association with self-reactivity, autoimmunity, and leukemia. In mice, CD5$^+$ B cells are readily generated from fetal/neonatal precursors, but inefficiently from precursors in adult. One model proposed to explain this difference is that their production occurs through a distinctive developmental process, termed B-1, that enriches pre-B cells with novel germline VDJs and that requires positive selection of newly formed B cells by self-Ag. In contrast, follicular B cells are generated throughout adult life in a developmental process termed B-2, selecting VDJs that pair well with surrogate L chain, and whose maturation appears relatively independent of antigenic selection. In the present study, I focus on processes that shape the repertoire of mouse CD5$^+$ B cells, describing the differences between B-1 and B-2 development, and propose a model encompassing both in the generation of functional B cell subpopulations. The Journal of Immunology,* 2006, 176: 2749–2754.

The CD5$^+$ B cell was first identified in mouse and man more than 20 years ago (1, 2), attracting considerable attention as a possible normal counterpart of CD5$^+$ B chronic lymphocytic leukemia (3) and also because of its association with autoantibody production (2, 4, 5) and even autoimmune pathology (6). In normal physiology, these CD5$^+$ B cells have been shown to be responsible for producing Abs to bacterial cell wall components, including T15$^+$ anti-phosphorylcholine (7). The rapid production of Ab by these cells when stimulated (8) can thus serve to limit bacterial spread before induction of adaptive immune responses (9, 10). One model for the role these cells play in the immune system is to express a repertoire of specificities to common pathogens, selected into the germline over evolutionary time, constituting a sort of protective immune "memory" to pathogens that the organism has yet to encounter (11).

Any discussion of B cell subsets, including those that are CD5$^+$, is complicated by the fact that cell surface phenotype can alter depending on microenvironment (cytokine, chemokine, and BCR signaling) and that expression of any particular cell surface protein is unlikely to provide an absolutely unambiguous indicator of membership in a functional subpopulation. Initially, we identified CD5$^+$ B cells by a distinctive combination of cell surface "markers," specifically high expression of IgM, low expression of IgD, and detectable expression of CD5. However, further work with B cell populations in the peritoneal cavity suggested that at least some related cells lacked CD5 (12). This led to the proposal that B cells with a cell surface phenotype associated with CD5$^+$ B cells be termed "B-1," a distinction from more conventional (i.e., predominantly follicular) CD5$^-$ B cells that were termed "B-2" (13). This nomenclature, based purely on cell surface phenotype, allowed inclusion of the presumably related CD5$^-$ subset by introducing the distinction "B-1a" for CD5$^+$ and "B-1b" for CD5$^-$ (14). However, this absolute identification of B-1 with cell surface phenotype has, in our view, led to serious confusion since, as mentioned above, cell surface phenotype can be fluid. Instead, we have proposed that B-1 be used to describe a distinctive type of B cell development, which is predominant in fetal life and infrequent in the adult, with mechanisms that restrict and bias the BCR specificities expressed and involving Ag-dependent selection of B cells with these specificities into a long-lived B cell pool (15). In contrast, B-2 refers to a more "conventional" type of B cell development, which is predominant in the adult, where an extremely diverse BCR repertoire is expressed, producing B cells that mediate adaptive immunity in collaboration with T cells, and where progression to the mature naive B cell pool requires little if any Ag-dependent selection (16). In this review, I focus on a long-standing issue concerning B-1 B cells: whether they arise from discrete precursors through distinctive development.

Early work

Initial characterization of B cells coexpressing surface Ig and CD5 indicated a number of novel features, including production of natural autoantibodies, reduced numbers in xid (*btk* deficient; Ref. 17) mutant mice, and relative enrichment in the peritoneal cavity (2, 18–20). The relative abundance of CD5$^+$ B cells in the peritoneal cavity suggested this site as a potential source for precursors, but surprisingly, fractionation of Ig$^-$ and Ig$^+$ peritoneal cavity cells and transfer into irradiated hosts revealed that these cells could be engrafted by Ig$^+$ but not Ig$^-$ cells (21), leading to the concept that the CD5$^+$ B cell population could be maintained by self-renewal, and so did not necessarily depend on replenishment by Ig$^-$ precursors. Even more unexpectedly, an attempt to engraft CD5$^+$ B cells in lethally irradiated mice reconstituted with bone marrow precursors was relatively ineffective (19). This cell transfer work

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supported the model of $CD5^+$/B-1 B cells arising from distinct precursors that are abundant in fetal liver but relatively rare in bone marrow of adult animals, where most development gives rise to cells that occupy the B cell follicles in spleen, B-2 B cells. Solvason et al. (22) also identified a source of B-1 B cell precursors in the fetal omentum.

Another thread of research with $CD5^+$/B-1 B cells focused on the distinctive specificities of the Abs produced by these cells (18). A key finding was the connection between a series of B cell lymphomas generated by Haughton et al. (23) and this normal subset. His laboratory had reported that this "CH" series of B lymphomas expressed a restricted set of B cell receptors, initially identified by anti-Id reagents and later verified by sequencing (24). Many of these lymphomas were found to be $CD5^+$ (25) and to use V_HV_L combinations that were frequently seen in the normal $CD5^+$/B-1 B cell subset (26, 27). Thus, in addition to chronic lymphocytic leukemia, there was another connection between $CD5^+$ B cells and transformed B cells, this time in the mouse. Furthermore, the findings of limited BCR diversity in the CH lymphomas highlighted the important role that BCR-mediated selection for distinctive specificities was likely to play in the generation of these cells.

Developmental origins: the lineage vs activation models

The close association of $CD5^+$/B-1 B cells with autoreactivity and with a relatively limited set of BCRs led some to ask whether the specificity of these B cells might be a determining feature (24). In fact, the induction of CD5 on human B cells by treatment with the activating agent PMA had been reported years earlier (28), so the idea that CD5 expression on B cells might simply reflect "activated B cells" was a very attractive explanation (29). Yet, analysis of mouse B cells activated with the B cell mitogen LPS did not reveal induction of CD5. However, a few years later, Wortis and coworkers (30) reported that activation of B cells with certain anti-IgM Abs could result in up-regulation of CD5 on cells that previously showed a "B-2" cell surface phenotype. Furthermore, in addition to the xid naturally occurring *btk* mutation (20), a series of engineered mutations affecting BCR signaling were found to alter the frequency of B-1 B cells (31–36). Finally, Lam and Rajewsky (37) performed an elegant experiment, replacing a "B-2 type" BCR by a "B-1 type" (derived from a CH lymphoma) in splenic B cells using an inducible system and demonstrated the alteration of B-2 cell surface phenotype to B-1 type. This led many to conclude, together with Haughton et al. (38), that "B-1 cells are made, not born." These investigators discounted the early transfer data as simply indicating a different BCR repertoire in fetal cells due to the absence of N-nucleotide addition (see following).

Yet, early committed D-J rearranged B cell precursors, isolated from fetal liver and bone marrow and transferred into adult scid mice, showed that most fetal pro-B cells became B-1 B cells, whereas most bone marrow pro-B became B-2 (39). This indicated that it was not simply a distinctive fetal microenvironment that selected B-1 B cells based on BCR because the pro-B cells did not yet express a complete BCR. Clearly, to understand B-1 development it is important to determine the differences between fetal liver and bone marrow precursors. A well-recognized distinction is expression of TdT, an enzyme responsible for inserting nontemplated nucleotides at the D-J_H and VD junctions of the IgH chain that is very low or absent during fetal/neonatal B cell development in mice (40). This has very significant implications for restriction of CDR3 diversity, resulting in decreased CDR3 length and yielding junctional sequences that are essentially germline encoded. In addition, VDJ recombination in the absence of TdT results in preferential joining of certain DJs and VDs, facilitated by homologies at these junctions (41). Thus, VDJ diversity will be more restricted as a consequence of the absence of TdT, and some have suggested that this alone will lead to a specialized repertoire that preferentially generates B-1 B cells.

Novel fetal/adult distinctions in B cell development

In addition to the absence of TdT, fetal precursors have also been shown to lack PLRLC, a lymphocyte-restricted myosin L chain (40, 42), and to show distinctive delayed expression of MHC class II (43, 44). Furthermore, the precise lineage restriction of early developing hemopoietic cells in fetal liver may differ from that in bone marrow; many years ago a novel B/myeloid bipotential precursor was reported in fetal liver (45), and more recently, similar cells, with a novel $CD19^+B220^-$ phenotype, have been described in mouse bone marrow (46). These cells clearly do not fit into the current conventional picture of progressive lineage restriction, where common lymphoid progenitors generate B, T, and NK cells but not myeloid cells, and where later-stage $CD19^+$ pro-B cells become B-lineage restricted due to expression of Pax-5, a transcription factor suppressing non-B lineage cell fates and activating CD19 transcription (47). In fact, there are reports (48, 49) in the literature of an association between B-1 B cells and myeloid cells, so this classical developmental picture needs further investigation, searching both for alternate pathways in bone marrow and determining whether the details established for bone marrow hold for fetal liver.

Microenvironmental interactions may determine the B-1/B-2 development choice in very early precursors and there is evidence that altered levels of Notch-2 signaling may bias the production of B-1 B cells, conceivably contributing to differences between fetal and adult development (50, 51). Also, there are recent reports that an alternate growth factor to IL-7, thymic stromal-derived lymphopoietin (TSLP[3]; Ref. 52), shows different capacity to mediate proliferation of early B lineage cells from fetal liver and adult bone marrow (53). That is, while IL-7 can mediate the proliferation of both pro-B (μ^-) and pre-B (μ^+) cells from bone marrow, TSLP can only support the proliferation of bone marrow pre-B cells (54). Curiously, this distinction does not hold for fetal precursors, where TSLP induces proliferation of both μ^- and μ^+ precursors. As a consequence, in mice lacking IL-7, TSLP substitutes for IL-7 in fetal development, generating long-lived populations of B-1 and marginal zone (MZ) B cells, but does not rescue bone marrow development, so that these animals completely lack follicular B-2 B cells. Obviously a complete picture of the basis of B-1 vs B-2 development remains to be determined.

Selection in B-1 B cell development

A key feature of B cell development that appears to differ between fetal and adult precursors is the response to pre-BCR signaling. A key checkpoint in the production of B cells is completion of a productive Ig H chain rearrangement; in its absence, development is blocked at the $CD43^+$ pro-B stage, as

[3] Abbreviations used in this paper: TSLP, thymic stromal-derived lymphopoietin; MZ, marginal zone; SLC, surrogate light chain; HEL, hen egg white lysozyme; ATA, anti-thymocyte/Thy-1 autoantibody; PtC, phosphatidylcholine.

seen in scid and Rag-null bone marrow (55, 56). Other mutations that interfere with normal BCR signaling, such as μ-mt (μ H chain membrane exon targeted), Syk, and Igα/Igβ knockout, all result in a block at the same developmental stage (57–59). Importantly, this block at the μ H chain checkpoint can be overcome by complementing the scid or Rag-null defect with a rearranged H chain transgene. Two key pre-B cell specific proteins, λ5 and VpreB, are also required to pass this checkpoint (60, 61). These peptides together substitute for IgL chain before conventional L chain rearrangement and so are termed "surrogate light chain" (SLC). The complex formed with H chain, by analogy with the BCR, is referred to as the "pre-BCR." Upon pre-BCR assembly, cells undergo a burst of proliferation, expanding clones of pre-B cells that then independently rearrange L chain, generating a large BCR repertoire by combinatorial diversity (62).

Yet, not all H chains associate equally well with SLC. For example, Klinman and coworkers (63) found that most H chains with a V_H81X rearrangement do not pass the pre-BCR H chain checkpoint due to poor association with SLC. This explained a longstanding paradox because the J_H-proximal V_H81X gene was observed to be very frequently rearranged in transformed early B cell lines but much less abundant in analyses of V_H gene usage in peripheral B cells (64). We have observed similar behavior with V_H11 (65), a V gene strikingly enriched in CD5$^+$/B-1 B cells, where it encodes a large fraction of the anti-phosphatidycholine (PtC)/anti-bromelainized mouse RBC specificity (26). As with V_H81X, V_H11 is handicapped in bone marrow development, yet it contributes very significantly to the CD5$^+$/B-1 B cell repertoire, constituting 5–10% of the pool (66). This distinctive feature of V_H11 (and V_H81X) may be shared by other V_H genes, as Melchers' group (67) has reported that, unexpectedly, many rearrangements of V_H7183/Q52 family genes generate H chain proteins that fail to associate with SLC.

How might one account for overexpression of a "handicapped" H chain? A potential explanation comes from the observation that fetal precursors may respond differently to pre-BCR signaling (Fig. 1). Comparison of the proliferation of fetal liver and bone marrow B cell precursors bearing an Ig transgene capable of strong pre-BCR signaling showed an inhibition of fetal precursors, when compared with those isolated from bone marrow (65). In contrast, a V_H11 transgene, assembling relatively low amounts of pre-BCR, did not inhibit pre-B cell expansion and so had an advantage relative to more "conventional" (SLC strongly associating) H chains. It may be that the maintenance of apparently "nonfunctional" (SLC weakly associating) V_H genes in the germline serves a useful purpose, targeting them to fetal development where they may be selected into the B-1 repertoire. More speculatively, if the function of the classical pre-BCR checkpoint is to screen IgH chains for their capacity to assemble with L chain, where an "average L chain" is templated by SLC (68), then poor association with SLC may imply the converse: preference for distinctive L chains. If this is the case, then expression of IgH chains with certain V_Hs will require a restricted set of corresponding V_L L chains, i.e., V_H11 with Vκ9. This expression of a limited set of germline-encoded receptors sounds suspiciously similar to receptors of the innate immune system, and so the fetal B cell system might be considered intermediate between a true innate immune system and the conventional adaptive B cell immune system.

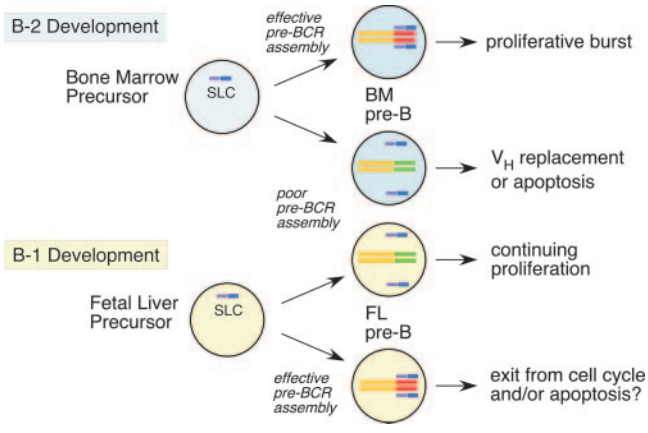

FIGURE 1. Contrasting responses to pre-BCR assembly: B-2 proliferative burst vs B-1 exit from cycle. In B-2 development, a characteristic of B-2 precursors that are abundant in bone marrow of adult mice, weak/ineffective pre-BCR signaling also fails to down-regulate Rag expression, resulting in continued potential for H chain rearrangement and the possibility of generating a strongly signaling pre-BCR, rescuing the cell from apoptosis. In B-1 development, a characteristic of B-1 precursors that are abundant in fetal liver, weak/ineffective pre-BCR signaling allows ongoing proliferation that is terminated by L chain rearrangement and expression of a functional BCR.

While some or even most CD5$^+$/B-1 B cells may arise through this type of development, biasing production to fetal life, there are clear examples of B-1 specificities that are exceptions. Thus, another prototype anti-PtC/anti-bromelainized mouse RBC BCR uses V_H12 in combination with a specific L chain and this BCR has been studied extensively by Clarke and coworkers (69–72). His group has found that the development of B lineage cells with V_H12 rearrangements is constricted at several points, such that B cells with this H chain become progressively restricted to the prototypic anti-PtC BCR, even though the most frequently found V_H12 rearrangement generates a H chain capable of associating with SLC. Thus, at the pre-B stage, rearrangements containing CDR3 segments of 10 aa and with a glycine residue in the fourth position ("10/G4") are much more likely to be found among productive V_H12 sequences (70). A likely explanation for this preference is pre-BCR selection, such that most V_H12 rearrangements generate H chains that fail to associate with SLC, whereas those with the 10/G4 segment do and so can mediate progression to the late pre-B stage where L chain rearrangement takes place.

Interestingly, at the late pre-B stage, a second bottleneck in V_H12 development occurs, as it appears that only a restricted subset of L chains can pair effectively with the 10/G4 V_H12 H chain and mediate progression to the newly formed surface BCR$^+$ stage (73). In 10/G4 V_H12 H chain transgenic mice, most Tg$^+$ B cells in spleen lacking the prototypic Vκ4/5H L chain become arrested at a transitional stage and do not enter any long-lived mature B cell pool. When a different L chain, expressed as a transgene is paired with the 10/G4 V_H12 H chain, these cells become arrested at an immature transitional stage, but this arrest can be overcome by low-level BCR cross-linking, whereupon the B cells progress to a "B-2" mature (CD23$^+$) phenotype (73). These authors interpret their results as evidence for a type of "positive selection." Obviously, another explanation is that the transgene L chain fails to pair very effectively with V_H12 H chain but achieves sufficient BCR surface expression to enable the B cells to persist until the transitional stage in spleen, where a modest increase in BCR signaling results in a level of tonic signaling comparable to that on a normal follicular B-2 B cell BCR.

Forcing expression of the V_H12-Vκ4/5H (71) or V_H11-Vκ9 (74) BCR by transgenesis tends to increase the numbers of $CD5^+$/B-1 B cells in such mice, sometimes to a striking extent, leading some to conclude that antigenic specificity is the only criteria for generating B cells with the B-1 cell surface phenotype. However, we have observed that lower expression of the $V_H11V\kappa9$ BCR can lead to cells from bone marrow development becoming arrested at the immature transitional ($AA4^+$) B cell stage, suggesting that a certain level of self-reactive BCR signaling can serve to block much of bone marrow development at a short-lived $CD5^+$ stage (my unpublished observation). Furthermore, it is clear that numerous BCR transgenes, including those to self-Ags such as DNA (75), anti-MHC class I (76), and hen egg white lysozyme (HEL) as a "neo-self" Ag in Tg-HEL mice (77), do not mediate B-1 B cell development. In such mice, the B-1 B cells that do develop express BCRs encoded by endogenous V genes, even when such endogenous BCRs are exceedingly rare in other B cell populations. The strong selection for certain specificities in B-1 development needs to be taken into account when analyzing mice transferred with precursor populations long after the initial engraftment because clonal selection and/or expansion can appear to indicate an incorrectly high precursor frequency.

An attractive model for B cell subset determination relates it to strength of signal transduced by the BCR (78, 79). The role of BCR signaling in B cell development has been directly tested using BCR transgenic mice bearing a different B-1 B cell specificity, where the Ag level can be regulated or even eliminated. Among natural autoantibody specificities, IgM binding to thymocytes has been found to be very significantly enriched in the B-1 B cell population, and an analysis of B cell hybridomas generated from sorted $CD5^+$ B cells allowed determination of BCRs used and the determinants recognized (80). Several antithymocyte autoantibodies recognize glycosylation epitopes present on the Thy-1 cell surface protein (anti-thymocyte/Thy-1 autoantibody; ATA) and the H and L chains from one such prototypic Ab were cloned and used for generating transgenic mice (81). Importantly, while many natural autoantibodies recognize determinants whose expression cannot be easily regulated, such as PtC or glycolipids, this specific ATA glycosylation-dependent epitope was only detected on Thy-1, which had been inactivated by gene targeting. This allowed analysis of B cell development of transgenic B cells where the BCR either was autoreactive (wild-type Thy-1) or not (in Thy-1 null mice). Using transgenic mice expressing only the H chain, Hayakawa et al. (81) showed that the presence of Thy-1 was necessary for the accumulation of ATA Ig in the serum and generation of a $CD5^+$ ATA B cell subset in the peritoneal cavity, concluding that ATA B-1 B cells developed through a process of positive selection.

In a follow-up study, the same authors examined B cell development in transgenic mice expressing both Ig H and L chains, so that all B cells would be made ATA-specific (82). In these mice, most of the newly generated B cells in bone marrow expressed the transgene BCR, as assessed by anti-idiotypic staining, but upon further maturation and migration to the spleen, most B cells were negatively impacted by the presence of Ag: ATA^+ B cells either were arrested before entry into a long-lived pool or else edited the transgenic L chain, eliminating the ATA self-reactivity, and so maturing further. In the absence of Thy-1, developing B cells remained ATA^+ and eventually populated the mature follicular pool. Thus, it appears that only a relatively minor proportion of ATA^+ B cells can be positively selected into the B-1 B cell pool, while others, if forced to express the ATA BCR will experience negative selection, similar to what has been observed with other transgenic models of tolerance, such as the anti-MHC class I system of Nemazee and Burki (76) and the HEL/anti-HEL system of Goodnow et al. (77). Further work needs to be done to determine whether the cells capable of maturing into ATA^+ B-1 B cells derived disproportionately from fetal-type precursors.

Development and function of B-1b ($CD5^-$) B cells

In the past year, there have been several significant reports concerning the development of B-1 B cells that also may bear on their relationship to other mature functional B cell populations. Thus, Graf and coworkers (83) reported that ablation of the PU.1 transcription factor in adult mice results in a gradual loss of B-2 follicular B cells and an accumulation of B cells with a B-1b ($CD5^-$) cell surface phenotype. Importantly, these investigators also showed that normal B-2 B cells had significantly higher levels of PU.1 as compared with B-1 B cells. Dorshkind and coworkers (84) reported that transfer of a novel $CD19^+B220^-$ cell fraction from bone marrow of adult mice could repopulate B-1b but not B-2 (and only inefficiently B-1a) phenotype B cells, prompting them to describe such cells as a B-1-restricted progenitor, a striking finding. An important study from Tedder's group (85) described significant functional differences between B-1a B cells, enriched in huCD19 Tg mice and B-1b B cells, present in CD19-deficient animals. Their analysis of the response to *Streptococcus pneumoniae* indicates that while B-1a B cells produce "innate-type" natural Abs active in the early response, B-1b B cells do not, instead functioning as a component of the adaptive immune system, active later in the response. Considering these data together with a report from Alugupalli et al. (86), showing the key functional role of B-1b ($CD5^-$) B cells in immunity to *Borrelia hermsii*, it seems clear that one must carefully distinguish B-1a and B-1b development, an important area for future investigation.

Reconciling the two models of B-1 B cell origin

Based on the transfer experiments and also on work showing a requirement for Ag in the generation of $CD5^+$ B-1 B cells, we would like to propose a model that takes account of CD5 expression induced by BCR signaling, but also of the common observation that self-Ag encounter results in receptor editing or apoptosis (Fig. 2). We suggest that most newly formed B cell produced in fetal development, and potentially a fraction of those generated in bone marrow, must be selected by self-Ag, whereupon they enter the distinctive B-1 B cell pool. If these cells do not encounter Ag or if BCR signaling is handicapped in some way, then the cell will never mature and instead die. In contrast, most bone marrow B cells experience deleterious consequences upon Ag encounter at the newly formed stage, undergoing apoptosis or remaining blocked in a short-lived unresponsive stage or revising their BCR through L chain editing to eliminate the self-reactivity. Expression of typical B-1 type BCRs by these cells is usually nonproductive because the level of BCR signaling generated exceeds the typical threshold for B cell tolerance, likely set more stringently to screen the much more diverse Ag binding repertoire produced in bone marrow development. Cells with a reactivity intermediate between B1a fetal-type and B-2 follicular-type BCRs may allow/promote entry

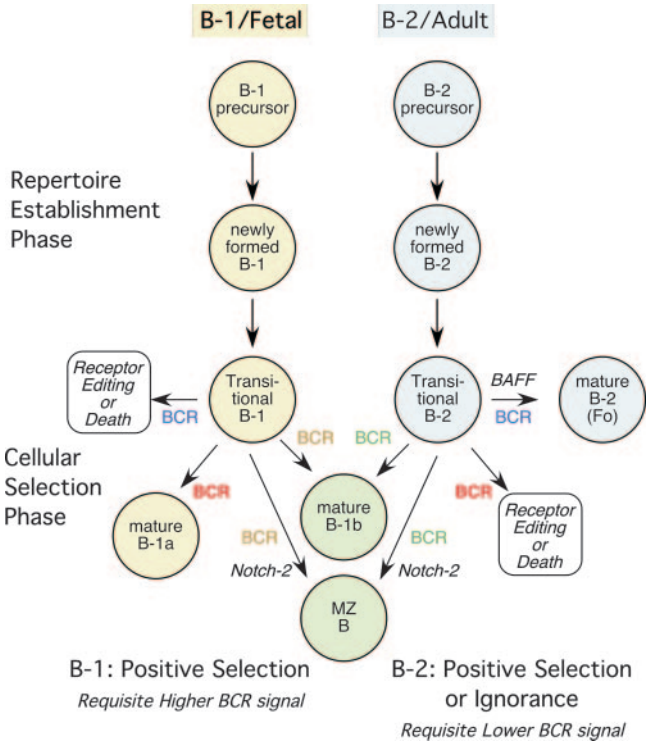

FIGURE 2. A unified model for B cell development: B-1/fetal and B-2/adult development, generating four functionally distinct B cell subsets, B-1a, B-1b, MZ, and B-2/follicular. There is clear evidence of a requirement for antigenic selection of at least some B-1a and MZ B cells. In B-1/fetal development, the strongest BCR-mediated signals generate B-1a cells, whereas intermediate strength signals can produce B-1b and MZ B cells, while the weakest BCR signaling leads to receptor editing or death. In contrast, in B-2/adult development, the strongest BCR-mediated signals mediate receptor editing or death, whereas intermediate strength signals can produce B-1b and MZ B cells, while the weakest BCR signaling generates B-2/follicular B cells. Analysis with an ATA transgenic mouse indicates that self-reactivity with a B-1-type BCR results in CD5 induction on immature/transitional stage cells produced from bone marrow (i.e., in "B-2 development"), many of which either undergo receptor editing to eliminate autoreactivity or remain blocked in development and die. Thus, B-1 type self-reactivity does not foster the maturation of B-2 precursors. In contrast, B cells generated by B-1 development maintain CD5 expression induced during positive selection by Ag while progressing to the mature B cell pool. Certain B cell subsets have additional microenvironmental signaling requirements for their development, such as Notch-2 by MZ and BAFF by B-2/follicular. Different levels of BCR signaling are indicated by color (blue, green, yellow, and red; weak/negligible to strong).

into the B1b and MZ B cell pools, suggesting a hierarchy of selection as proposed recently (16).

Future directions

A number of key issues relevant to B-1 B cell development are areas of active research. Among these are: 1) discovering the underlying mechanisms that separate B-1/fetal from B-2/adult B cell development, including identifying key differences in gene expression, signaling networks, and receptors; 2) determining what role the specialized peritoneal cavity microenvironment may play in the selection or maintenance of these cells; and 3) understanding the relationship between B-1 lymphocytes and plasma cells in the process of Ig production. Finally, in the context of clinical medicine, it will be important to determine the relevance of B-1 development in mouse to potentially related human pathologies, autoimmunity, and B cell leukemia.

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