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Unraveling B-1 progenitors

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B-1 cells comprise a small percentage of the B lymphocytes that reside in multiple tissues in the mouse, including the peritoneal and pleural cavities. Functionally, B-1 cells participate in innate immunity by producing the majority of the natural IgM in serum, which protects against invading pathogens before the onset of the adaptive immune response. B-1 cells arise from fetal and neonatal progenitors and are distinct from the adult bone marrow progenitors that give rise to follicular and marginal zone B-2 cells. Recent studies have attempted to delineate the progenitors of B-1 cells from those of B-2 cells. Notably, the identification of $CD45R^{-/lo}CD19^{+}$ B-1 progenitors and expression of two surface determinants, CD138 and major histocompatibility class II antigens, distinguish developing B-1 cells from B-2 cells.

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Introduction

B-1 cells were originally identified as $CD5^{+}$ B cells that participate in autoimmunity, and share similarities with those responsible for human chronic lymphocytic leukaemia [1,2]. Normally, B-1 cells make up about 1–5% of the total B cells in the mouse. B-1 cells are found in a variety of tissues including the spleen, peritoneal cavity, pleural cavity and intestines [3]. The distinctive surface phenotype of B-1 cells features expression of higher levels of IgM ($sIgM^{hi}$) and lower levels of sIgD ($sIgD^{lo}$) than that of B-2 cells and, unlike marginal zone B cells, B-1 cells do not express CD21 in the peritoneal cavity but express CD21 at low levels in the spleen. Most of the B-1 cells, but not all, in the peritoneal and pleural cavities express CD11b — a macrophage/granulocyte marker that is part of the CR3 complement receptor. However, the majority of the B-1 cells found in the spleen do not express this marker [3]. The B-1 cells in the peritoneal cavity are divided between a majority subset (B-1a) and a minority subset (B-1b), distinguished by the expression of CD5 (i.e. B-1a cells are $CD5^{+}$ whereas B-1b cells are $CD5^{-}$) [4].

Functionally, B-1 cells differ from B-2 cells in several ways. The B-1 antibody repertoire tends to be more restricted than the B-2 repertoire [5]. Furthermore, B-2 cells participate in the adaptive response by undergoing somatic hypermutation of Ig genes, which leads to affinity maturation of the antibody response. In contrast, B-1 (both B-1a and B-1b) cells are largely responsible for the innate immune response and respond readily to a variety of T-independent antigens [6,7]. In lipopolysaccharide-stimulated animals, peritoneal B-1a cells respond rapidly by migrating to the spleen, where they divide and differentiate into IgM-producing plasma cells. B-1a cells that are resident in the spleen, in contrast, differentiate immediately to plasma cells without undergoing cell division [8•]. B-1 cells normally express higher basal levels of B lymphocyte induced maturation protein-1 (Blimp-1) than B-2 cells, which show roughly the same Blimp-1 level as T cells (perhaps reflecting background staining levels). However, similar to B-2-derived plasma cells, Blimp-1 expression is highly upregulated in the antibody-secreting B-1 plasma cells ($CD138^{+}$) [8•,9,10].

In allotype chimeras in which B-2 cells derive from progenitors marked with one IgM allotype (IgMb) and B-1a cells derive from progenitors marked with a different IgM allotype (IgMa), B-1a cells have been shown to be the major producers of serum IgM natural antibodies that provide the first line of defense against influenza virus [11,12]. Similarly, B-1a cells have been shown to provide the initial protection against *Streptococcus pneumoniae* [13,14•]. B-1b cells have been shown to provide long-term protection against *S. pneumoniae* in mice pre-immunized with pneumococcal polysaccharide [13,14•] and to be required for protection against *Borrelia hermsii* [15].

Numerous cell transfer studies have demonstrated that fetal liver and adult bone marrow have different abilities to reconstitute B-1 versus B-2 cells in irradiated recipients. Hence, it was concluded that B-1 and B-2 cells constitute distinct cell lineages. Co-transfers of $B220^{-}$ cells sorted from fetal liver with $B220^{-}$ cells sorted from adult bone marrow showed that, in the same adoptive recipients, >90% of the B-1 are derived from the fetal liver source whereas B-2 cells are derived from both sources [4]. Furthermore, pro-B cells ($B220^{+}CD43^{+}IgM^{-}$) sorted from fetal liver mainly gave rise to $CD5^{+}$ (B-1a) cells, whereas FACS-sorted (fluorescence-activated cell sorter) pro-B cells from adult bone marrow gave rise to $CD5^{-}$ (B-2) cells [16]. Thus, by the time B-cell progenitors (either fetal

or adult) express B220 and initiate immunoglobulin (Ig) heavy chain rearrangement, they are already intrinsically committed to become either B-1 or B-2 cells.

These findings, together with evidence from a wide range of additional and progenitor studies examining the origins of B-1 and B-2 cells in genetically engineered and unmanipulated mice show that B-1 and B-2 cells arise from distinct progenitors [17,18]. In addition, similar although not as extensive evidence indicates that B-1a and B-1b arise from different progenitors. Thus, these three phenotypically and functionally distinct B-cell subsets meet the standard criterion for assignment into three separate developmental lineages [4,19,20].

This finding led us to propose that the immune system is composed of a series of layers that evolved sequentially in response to complex and evolving antigenic challenges [3,20]. We suggested that B-1a cells constitute the oldest layer, whereas the layer including follicular B cells that participate in high-affinity germinal-center based adaptive immune responses evolved most recently. The existence of distinct progenitors for each of these lineages is implicit in this model. Moreover, phenotypic differences between progenitors and intermediate stages of differentiation leading to each lineage should be detectable.

Recent studies have focused on identifying differences between the fetal liver/neonatal B-cell progenitors that give rise to B-1 cells and the adult bone marrow progenitors that give rise to B-2 cells. Furthermore, because B-1 progenitors (principally B-1b) have been found at low frequencies in adult bone marrow [4], recent studies have also focused on identifying and isolating this small population of B-1 progenitors in adults. In this article, we review the advances in distinguishing B-cell progenitors and in recognizing differences between the B-cell developmental pathways in neonates and adults.

Identification of B-1 progenitors

The potential for B-cell lymphopoiesis can be observed in the intra-embryonic para-aortic-splanchnopleura at day 8.5 of gestation [21]. These B-cell precursors can be found in the para-aortic-splanchnopleura [22,23], yolk sac [24–26], aorta-gonad-mesonephros [27] and placenta [28] in the fetus. However, cytoplasmic IgM has been detected in fetal liver around day 13 of gestation, and $sIgM^+$ B cells can be seen at day 17 of gestation [29–32]. Development of B cells in fetal liver (largely B-1 cells) continues after birth in neonates but gradually switches to adult B-cell development, which becomes predominant at about the time the animals are weaned [33]. For adult cell development (largely B-2 cells), hematopoietic stem cells are seeded in the bone marrow at day 15 of gestation, where they later become the major B-cell development precursors in adult life [34–36].

The B-cell developmental stages are phenotypically similar in fetal liver and adult bone marrow, although some key differences have now been demonstrated. Basically, in both fetal liver and adult BM, B-cell development proceeds through the 'Fr. B–F' stages that Hardy identified some time ago on the basis of the differential expression B220, CD19, CD24, CD43, BP-1, IgM and IgD expression [16,37]. However, current data suggest that the expression of four surface markers (CD45R/B220, CD19, CD138 and MHC class II) and several internal proteins and transcription factors distinguishes the B-cell lineage developments. The data that define the expression of these markers are still in the formative stages, particularly with respect to differences between B-1a and B-1b progenitors. In this regard, most of the information has come from studies focused on comparisons of B-cell progenitors isolated from fetal liver or adult bone marrow. The following section summarizes the current status of these data.

Internal markers

The expression of two genes, terminal deoxynucleotidyl transferase (TdT) and the precursor lymphocyte regulated myosin-light chain (PLRLC), distinguish between fetal and adult pathways of B-cell development [38,39]. The function of PLRLC is not known. However, evidence indicating that PLRLC is induced by IL-7 is consistent with evidence (discussed later) that IL-7 is not absolutely required in B-1 development [40]. TdT, in contrast, is well known to be required for N-region addition during immunoglobulin rearrangement. Thus, expression of TdT has distinct implications for formation of the B-1 versus B-2 repertoire.

Reverse transcription-polymerase chain reaction (RT-PCR) studies detected TdT in pro-B cells in adult bone marrow, but showed that it is only present at low levels, if at all, in fetal pro-B cells [38]. This lack of TdT expression during fetal B-cell development is expected to result in B-1 cells that produce immunoglobulin heavy chains and light chains that have little or no N-region addition. Analysis of the B-1 repertoire of fetal and early neonatal animals is consistent with this absence of TdT. However, results from single-cell RT-PCR studies of FACS-sorted peritoneal B-1 cells from adults demonstrate that ~75% of B-1 cells have N-region addition on either D-J or V-DJ, or both joints in each cell [5]. As B-1 development continues for 2–3 weeks after birth, these findings suggest that, although TdT is not expressed during the fetal period, it is likely to be expressed during neonatal B-1 development. If so, expression of TdT in neonatal B-1 cells is probably low in comparison with its expression in adult bone marrow, because the average size of the N-region in B-1 cells is smaller than that in B-2 cells.

Several nuclear factors also differ between B-1 and B-2 cells. B-1a development is dependent on the expression

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of NFATc1 [41]. In addition, peritoneal B-1a cells express higher levels of activated STAT3 and lower levels of CREB and PU.1 than B-2 cells [42–44]. Two studies that utilized different methods to eliminate PU.1 expression in genetically engineered mice showed that levels of B-2 cells decrease and 'B-1-like' cells increase dramatically in the absence of PU.1 [45,46]. Interestingly, however, one of these studies [46] concludes that the observed changes in B-cell frequencies are probably caused by ablation of B-2 cells with expansion of B-1-like cells. The other study [45] concluded that these changes are probably caused by forced reprogramming during B-cell development, resulting in the conversion of B-2 cells into B-1-like cells. Further studies are required to resolve this issue.

Surface markers

The developmental stage at which the expression of murine MHC class II (I-A/I-E) can be detected differs markedly between the fetal/neonatal and adult B-cell development pathways. Fetal pro-B and pre-B cells do not express surface MHC class II until the B cells reach maturity and are beginning to express IgD and CD5. In contrast, MHC class II is clearly detectable on adult pro-B and pre-B cells, albeit at lower levels than mature B cells [33,47]. Studies that used this dramatic difference in MHC class II expression to distinguish the fetal/neonatal B-1 progenitors from the MHC class II-expressing B-2 progenitors demonstrated that B-1 development predominates during the neonatal period, but is supplanted by B-2 development by the time the animals are weaned [33].

Recently, our laboratory has shown that a well-known surface protein, CD138 (syndecan-1), also delineates fetal B-cell development from adult B-cell development. CD138 is a heparan sulfate-rich proteoglycan, the expression of which is commonly used to identify plasma cells or antibody-secreting cells [48]. We have shown that pro-B and pre-B cells in adult bone marrow clearly express CD138, but that neonatal pro-B and pre-B cells do not express this marker [49••]. In fact, CD138 is expressed throughout B-cell development in adult bone marrow and only shuts down expression at the end of the development pathway (Fr. E, $B220^+CD43^-IgM^+IgD^{-/lo}$), coincident with high-level expression of sIgM [49••].

CD138 is currently the earliest known marker for distinguishing the adult (B-2) B-cell developmental pathway from the fetal (B-1) developmental pathway. In adults, CD138 is detected on very early B cells (Fr. B, $B220^+CD43^+CD24^+CD19^+BP\text{-}1^-IgM^-IgD^-$), well prior to sIg expression, which initiates at the pre-B cell stage (Fr. D, $B220^+CD43^-IgM^-IgD^-$). In addition, expression of CD138 is highly likely to precede MHC class II expression, as MHC class II is expressed on only a portion of Fr. B cells whereas CD138 is expressed on nearly all Fr. B cells. In any event, the expression of both CD138 and MHC class II on early stage B cells in adult bone marrow clearly distinguishes the (B-2) development pathway in adult bone marrow from the (B-1) pathway that predominates during fetal/neonatal life.

Recent FACS-sorting studies provide direct evidence for the existence of distinct B-1 and B-2 progenitors. B-2 progenitors express $AA4^+CD45R/B220^+CD19^-Lin^-$ (lineage$^-$) and thus, as might be expected, fit within the component of Fr. A that Hardy termed pre-pro-B cells [37,50•]. In contrast, B-1 progenitors are $AA4^+CD45R^{-/lo}CD19^+Lin^-$ [51••]. These cells do not fit into the standard Fr. A definition because they are $CD19^+$ and expression of CD45R/B220 is lower than expected for Fr. A cells. The low expression of CD45R on B-1 progenitors is perhaps not surprising, as its expression on mature B-1 cells is roughly three-fold lower than on B-2 cells. Expression of CD19, which is typically not expressed until Fr. B, suggests a difference in the initial development pattern of B-1 progenitors. The differences in the expression of these markers might account for the previous difficulties in isolating the small number of committed B-1 progenitors in adult bone marrow. As proof that these cells constitute *bona fide* B-1 progenitors, sorted $AA4^+CD45R^{-/lo}CD19^+Lin^-$ cells gave rise in adoptive transfer experiments to a population of B-1 cells that contain both B-1a and B-1b, whereas sorted B-2 progenitors gave rise principally to B-2 cells [51••]. These committed B-1 progenitors, recognizable by the above phenotype, can be seen as early as day 11 in fetal liver and day 15 in fetal bone marrow [51••]. Similar cells are present at low frequencies in adult bone marrow.

Studies using mice deficient in IL-7/IL-7R system (IL-$7^{-/-}$, IL-$7R\alpha^{-/-}$ or $\gamma c^{-/-}$) have shown that the development of B-1 cells is less sensitive to perturbations in the IL-7 cytokine system. In IL-$7^{-/-}$ mice [40], and in mice in which signaling through IL-7 receptor is disrupted ($\gamma c^{-/-}$ mice) [52,53], B-2 development fails drastically but B-1 development remains detectable. In contrast, in IL-$7R\alpha^{-/-}$ mice, all B-cell development is drastically decreased [54]. This difference is explained by the ability of thymic stroma lymphopoietin (TSLP) to support B-1 development in the IL-$7^{-/-}$ mice. This is because the TSLP receptor, which contains the IL-7Rα chain and is active in the IL-$7^{-/-}$ mice [55], enables decreased but effective signaling that permits some, although not all, B-1 development. The ability of TSLP to support B-1 development is confirmed by studies demonstrating that sorted B-1 progenitor cells from the fetal and adult bone marrow expand and differentiate in response to TSLP *in vitro* and provide long-term B-1 reconstitution in severe combined immunedeficiency recipients [51••]. In contrast, sorted adult pro-B cells responded poorly to TSLP *in vitro*. However, TSLP is not an absolute requirement for B-1 development, because B-1 cell numbers are not drastically affected

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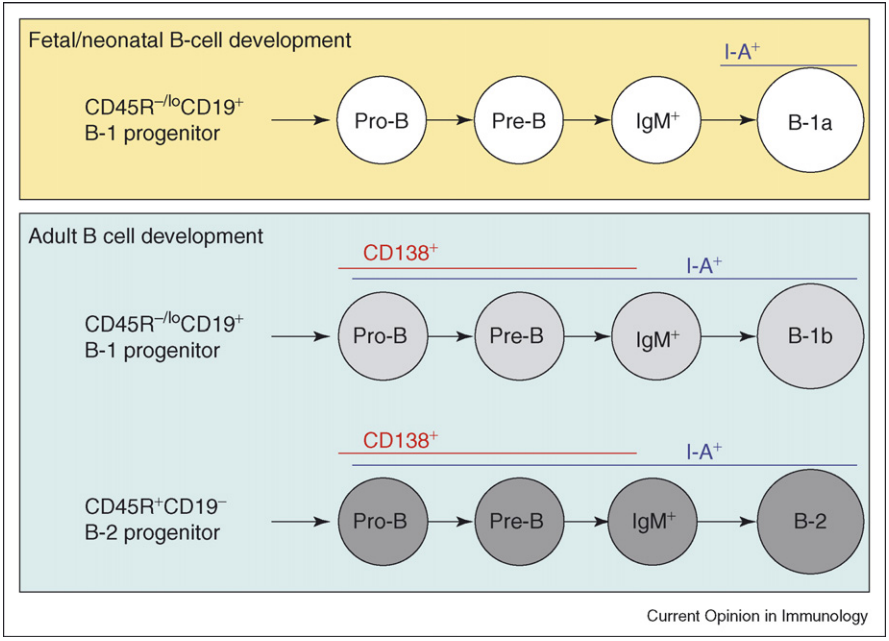
Figure 1



The B cell developmental pathways. The model shows the phenotypes of cells at sequential stages of B-cell development in neonatal versus adult bone marrow. The fetal/neonatal pathway is taken as indicative of the B-1 developmental pathway, because B-1 development predominates in fetus and neonates. Similarly, the major adult bone marrow pathway is taken as indicative of B-2 development, because B-2 cells represent the predominant output of adult bone marrow. The minor B cell developmental pathway shown in adult bone marrow is taken as indicative of B-1b development, because adult bone marrow is known to contain a small number of progenitors for B-1b cells. Progenitor studies in adoptive recipients are consistent with these assignments.

in TSLP$^{-/-}$ mice. It is probable that IL-7 supports B-1 development in the absence of TSLP.

Conclusions

We have proposed that the immune system evolved sequentially in 'layers' that were progressively more responsive to the ever-increasing pathogen challenge. The B-1a, B-1b and B-2 lineages, with their specialized functions to ensure survival of the species, stand at the center of this layered immune system. The functional differences between these lineages are well-established [17]. However, the question of whether they derive from unique progenitors, and thus can be designated as distinct developmental lineages, has been discussed for some time. This question has been resolved by the recent demonstration that phenotypic differences distinguish early B-1 progenitors from early B-2 progenitors. The cell surface proteins CD138 and I-A distinguish early stages in the B-1 developmental pathway from similar stages in the B-2 developmental pathway.

The detection of committed progenitors to generate B-1 or B-2 cells in early B-cell development (Hardy Fr. B or earlier) places the commitment event prior to surface μ expression (Figure 1). Thus, although antigen selection has been evoked as a trigger of differentiation events resulting in B-1 or B-2 cells, the differences between the lineages trace to events that occur well prior to antigen-dependent stages of development. By contrast, antigen-dependent selection can be expected to play a key role in defining the differences in the repertoires that are ultimately established in each of the lineages, particularly at antigen-accessible sites within the spleen and peritoneal cavity. Future studies are needed to determine how and when these differences are established and what role (if any) the differences in the B-cell development pathways play in modulating the eventual lineage repertoires.

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