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Primary immunoglobulin repertoire development: time and space matter

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The primary immunoglobulin repertoire develops via opposing forces of expanding diversification balanced by contracting selection mechanisms. The resulting shape is essential for host health and immune fitness. While the molecular mechanisms of Ig diversification have largely been defined, selection forces shaping emerging Ig repertoires are poorly understood. During lifetime, human and mouse early B cell development occurs at distinct locations — beginning in fetal liver before transferring to bone marrow and spleen by the end of gestation. During an early life window of time, the murine gut lamina propria harbors developing immature B cells in proximity to intestinal contents such as commensal microbes and dietary antigens. Location and timing of early B cell development may thus endow neighboring antigens with primary repertoire-shaping capabilities.

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Introduction

Vertebrates have evolved sophisticated mechanisms to adaptively respond to virtually any potential infectious insult. A critical component of this adaptive immune system is the generation of an immunoglobulin (Ig) repertoire of great diversity, which can recognize a broad range of antigens. Primary Ig diversity in mice and humans occurs via V(D)J recombination in developing progenitor (pro-) and precursor (pre-) B cells by way of DNA recombination events that assemble variable (V), diversity (D), and joining (J) gene segments together to form variable region exons encoding a vast array of Ig specificities [1–3]. Most of the B cells expressing freshly assembled IgM are removed from the repertoire early in B cell development through selection mechanisms [4–6]. Why certain Ig specificities remain and why others are removed from the primary repertoire is not fully understood.

Fetal liver and post-natal bone marrow (BM) are the two major sites of primary B cell development in mice and humans. Self-antigens present in these microenvironments influence immature B lymphocyte selection checkpoints by way of encounter with freshly expressed IgM on the B cell surface, thus restricting Ig repertoire-shaping influences at this stage of development to antigens present in primary lymphoid tissues [7–10]. In light of recent findings showing that early B cell development can also occur in the mouse gut lamina propria (LP) during weaning age [11••], early B cell developmental events — together with concomitant selection processes — can be positioned in the context of self-antigens unique to the intestine and in proximity to gut luminal contents early in life. This suggests that factors such as *where* and *when* early B cell maturation can take place may be required to fully grasp how the primary Ig repertoire is processed and formed, as antigens available to effect early selection processes may differ substantially in time and space.

Overview of B cell development and primary Ig repertoire

The RAG1/RAG2 endonuclease initiates the V(D)J recombination reaction that assembles variable region exons from germline gene segments at both Ig heavy (IgH) and Ig light (IgL) chain loci to generate primary antibody repertoires [12]. Assembly of the IgH variable region exon occurs in pro-B cells followed by that of IgL in pre-B cells. Expression of IgH μ and IgL (κ or λ) chains generates IgM, which is expressed on immature B cells as the B cell receptor (BCR). RAG expression can continue in immature B cells [13], allowing continued IgL V(D)J recombination that replaces the initially assembled IgL exon with one that generates a new specificity [14–16]. Receptor editing, together with other selection processes such as deletion or induction of anergy [4,17], provide mechanisms whereby antigen-encounter at the immature and transitional B cell stages help shape pre-immune Ig repertoires.

The Ig repertoire can be divided into three subgroups — namely, *emerging*, *available* and *actual* repertoires [18]. The *emerging* repertoire consists of newly formed B cells in the primary lymphoid organs undergoing selection

processes before reaching the peripheral naïve mature B cell pool. The *available* repertoire constitutes the mature naïve follicular, marginal zone, or B-1 B cells populating the peripheral lymphoid organs and tissues (reviewed in Ref [19]). The *emerging* and *available* repertoires exist largely in the context of surface-bound Ig on immature and mature naïve B cells, while the *actual* repertoire contributes to the pool of soluble antibody and memory B cells. While V(D)J recombination is responsible for the primary Ig diversification from which the *emerging*, *available* and *actual* repertoires are derived, secondary Ig diversification processes contribute to the *actual* Ig repertoire. In this regard, mature naïve B cells can participate in further Ig diversification reactions including somatic hypermutation (SHM) and IgH class switch recombination (CSR), which are both dependent upon the enzyme activation induced cytidine deaminase (AID) [20]. In addition to specificities derived from post-GC cells, the actual repertoire contains innate-like natural antibodies secreted by B-1a B cells [21].

Primary Ig diversification generates an enormous number of possible Ig specificities, theoretically reaching beyond 10^{13} unique combinations in mouse and humans [22]. V(D)J recombination often results in the addition and deletion of nucleotides at the junctions between ligated gene segments and most of the diversity of the primary antibody repertoire is concentrated at the junctions where the V, D, and J segments join together (reviewed in Ref [23]). The terminal deoxytidylnucleotide transferase (TdT) adds non-templated nucleotides at random in the V(D)J junctions resulting in increased diversity [24]. The segment spanning the D segment and its two flanking junctional sequences encodes for the IgH complementarily determining region 3 (CDR-H3). Because of the combinatorial and non-templated nature of the mechanisms that generate the CDR-H3, it is the most diverse component of the preimmune Ig repertoire and is a principal determinant of antibody specificity [25,26].

Selection of emerging B cells and the role of antigen

Under physiological conditions, adult mice produce around $2–5 \times 10^7$ newly formed B cells every day, but only 1–10% end up contributing to the long-lived B cell pool [27]. Thus, the majority of the freshly formed B cells in adults are counter-selected before reaching maturity. In contrast, the available niches and resources in newborns allow developing lymphocyte populations to contribute to the long-lived mature pool until they attain steady-state numbers around weaning age [28,29] (Figure 1) — when the relationship between external environment and the host is being established. In a young mouse, most of the B cells show an immature or naïve phenotype and Ig gene somatic mutations are virtually absent [30], indicating that the newly formed primary Ig pool — together with the antibodies inherited from the immunological experienced mother — are the only Ig repertoire available at this age. Somatically mutated IgG, IgA and IgM first appear after weaning and accumulate in aging, suggesting that continuous encounter with antigens induces an ontogenetic learning process in the B lineage system.

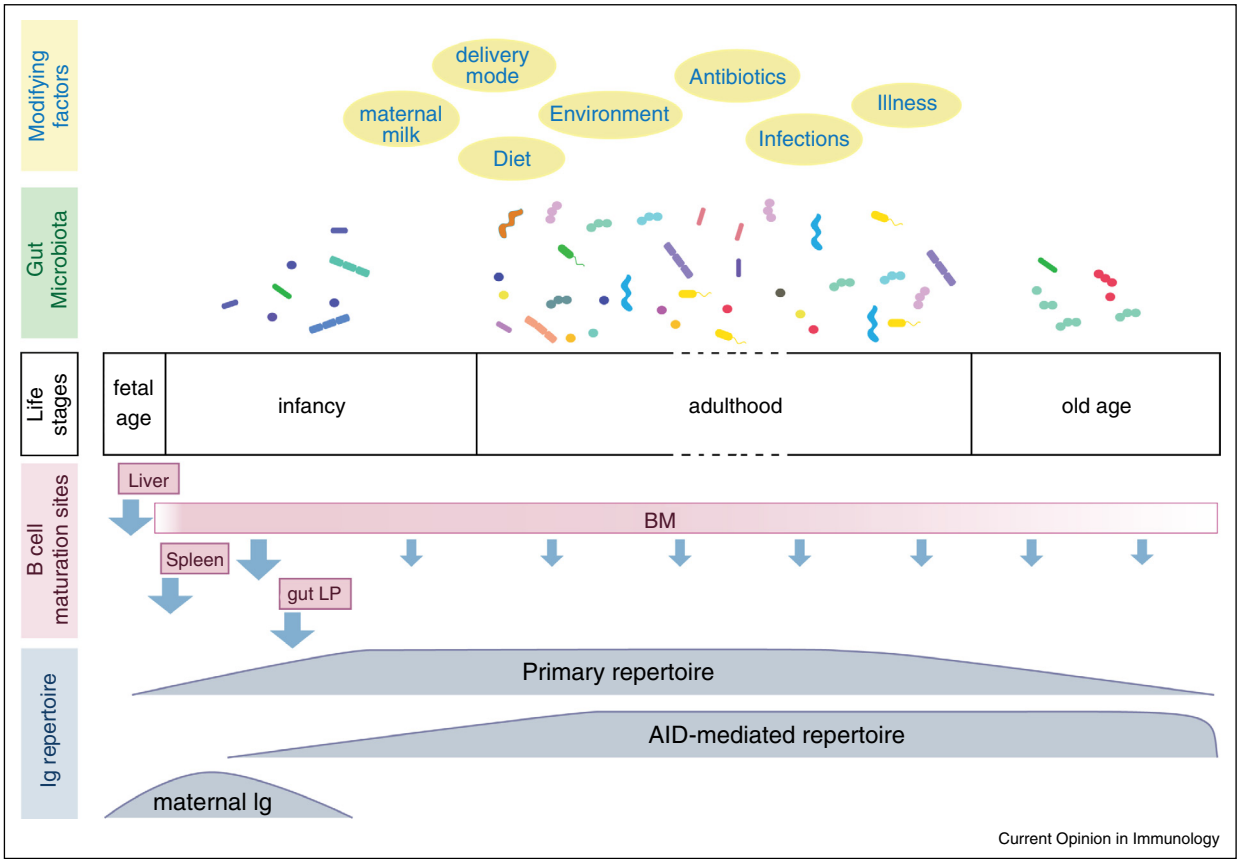
Control of peripheral B cell numbers and selection of clonotypes limits the size and diversity of each B cell population in the primary repertoire [31,32]. Primary Ig repertoire selection can be exerted by competition of B cells for resources, including survival factors [32], and BCR-derived signals [33–38]. In both fetal liver and postnatal BM, B cell development occurs in a stepwise process that involves a series of selection checkpoints that test the stability and binding characteristics of the newly generated IgH and IgL chains (reviewed in Ref [39]). The initial Ig-associated requirement for the survival of a developing B cell is the ability of the new IgH chain to form with a surrogate light chain a functional pre-BCR. B cell precursors that survive this checkpoint clonally expand and initiate rearrangement of the IgL chain loci. While pre-BCR signaling is essential at this checkpoint (reviewed in Ref [40]), the role of potential engagement of pre-BCR to antigen is not clear.

From the pre-B cell stage and beyond, B lineage cells need to constantly receive some type of BCR-mediated signal for both clonal selection and survival [33,37,41]. The nature of these BCR signals, and the contribution of antigen engagement, versus 'tonic' signaling [42–45] are not completely understood. Several studies using monoclonal BCR transgenic and knock-in animals have demonstrated that self-antigens can negatively select B cell clones in the BM and periphery through Ig light chain editing or B cell clonal deletion [7–10]. However, the degree to which this kind of negative selection occurs in the setting of a natural polyclonal Ig repertoire is unknown. In addition, the relative contribution of positive selection — which has been shown to occur at the immature B [46] and transitional B cell stages [47] — to the available Ig repertoire similarly remains to be fully defined. As B cell subsets can harbor distinct Ig repertoires [33,35,48], antigens present in the microenvironment where B cell development takes place may exert defining roles in B cell selection and fate.

B cell development in the gut

The gut constitutes a dynamic environment constantly exposed to foreign components such as food, commensal/mutualistic microbes, and potential pathogens. The first evidence of the link between gut and early B cell development was shown in chickens. Glick and colleagues demonstrated in 1956 that bursa of Fabricius, which is a diverticulum of avian hindgut during puberty, plays key roles in Ig production and diversification [49]. The primary Ig diversification and/or early B cell selection events

Figure 1



Relationships between the timing and location of B cell development and establishment of the immunoglobulin repertoire. During fetal life, primary Ig repertoire generation takes place in the liver before migrating to fetal BM and spleen at the end of the gestation period. B cell development and selection at this stage occurs in the absence of gut microbes and other environmental antigens. After birth, commensal/mutualistic microbial colonization is marked by low diversity, low abundance and instability before weaning [58,68]. In this context, the neonatal Ig repertoire is comprised of maternal Ig and nascent primary Ig repertoire in BM and spleen. At weanling age in mice, B cell development also takes place in the gut LP, alongside a dramatic change in the gut microbiota with the introduction of solid food [58,68]. In this limited window of time, B cell niches and resources are not limiting and it is suggested that most of the emerging B cells greatly contribute to the primary Ig repertoire [29,31] (size of the blue arrows represent the putative relative contribution of each site to the Ig repertoire). Late-infancy and adult gut microbiota are marked by anaerobic species, along with higher diversity, abundance and stability. Modifying factors such as diet, environment, infections, use of antibiotics, and disease conditions influence gut microbiota composition throughout life. BM is the principal site of B cell development in adults, and with aging, BM input of immature B cells decreases and antigen-experienced B cells that have undergone SHM (AID-mediated repertoire) accumulate [69].

occur in related gut-associated structures of other vertebrate species, including birds [50,51] rabbits [52–54] and artiodactyls [55] early in life. With regard to chickens and rabbits, RAG-mediated V(D)J primary repertoire is very limited and further Ig diversification and selection in gut associated structures through AID-mediated gene conversion and somatic hypermutation resulting in an increase in an expansion of the Ig repertoire [56,57]. Sheep and pigs have unique structures in the small intestine where B cells collect and undergo selection processes early in life [55].

In mice, pro-B, pre-B and immature B cells undergoing BCR editing can be found in the gut LP at weaning age [11••]. Comparisons of the emerging Ig repertoire in RAG2-expressing gut LP cells compared to RAG2-expressing cells from the BM revealed significant differences in Vκ gene segment utilization despite similar V_H usage. The coincident timing of early gut B cell accumulation with weaning age may hint at a role for gut microbes in this process given that gut microbiota diversity and abundance markedly change as mice are weaned off of maternal milk [58]. Further evidence of microbial involvement in early B cell selection events includes a significant change in the Igλ/Igκ usage ratio uniquely in the LP B cells from mice colonized with microbes compared to germ-free littermates [11••].

With regard to these animal examples, three aspects that are common among gut B cell activities. First, some B cell

developmental and selection events occurring in the gut are distinct from B cell activation and clonal expansion events typical of conventional inflammatory responses of secondary lymphoid tissue. Second, selection events appear to be influenced by gut microbes. And finally, there appears to be a window of time early in life when these selection/diversification events take place in each of these species (reviewed in Ref [59]). Together, these aspects raise the notion that interactions with the gut microbiota may be of some value to shape the burgeoning Ig repertoire early in life, but the nature of this benefit — and the degree to which this phenomenon extends to other species — remains to be fully elucidated. In this context, transitional B cells have been observed in human gut associated-lymphoid tissue [60••], but more studies are required to fully understand B cell development and repertoire selection in the human gut.

The notion of a window of time early in life where microbial colonization impacts host immune regulation has been examined recently in other studies. In this regard, microbial exposure early in life — but not in adulthood — appears to prevent the dysregulation of IL-4 and IgE levels seen in germ-free mice [61••]. Additionally, the reduction of mucosal invariant natural killer T (iNKT) cell levels seen upon neonatal colonization of germ-free mice fails to occur if mice are colonized later in life [62••].

Concluding remarks

In addition to being built upon randomness, the primary Ig repertoire appears to include specificities that have adapted on an evolutionary timescale. An example of this is the B-1 lineage T15 clone, which binds to phosphorylcholine (PC) present on the cell wall of *Streptococcus pneumonia* and self oxidized LDL antigen, conferring optimal protection against lethal *S. pneumoniae* bacteremia [63] and atherosclerosis in mice [64]. Another potential example is the human V_H1-69 gene segment that can target a conserved site in the influenza haemagglutinin stem, and with a single somatic mutation, is sufficient to confer high affinity binding and neutralization to the virus antigen [65••]. On the other hand, antibodies that are effective at neutralizing HIV do not appear to be readily available in the primary Ig repertoire [66••] and those that do develop harbor evidence of extensive somatic hypermutation [67]. Although the mutability of HIV is most likely the primary force underlying the difficulty of generating broadly neutralizing antibodies, recent introduction of HIV into humans may in part contribute to the requirement for extensive Ig gene mutation to generate antibodies that effectively confer protection. The notion that evolutionary time may have prepared selection conduits to inject key Ig specificities to ancient threats may be relevant for our understanding of the immune response to pathogens more recently introduced into humans.

Future work will certainly provide more data to advance our understanding of the functional relevance of the primary Ig repertoire in relation to health threats as well as a substrate for secondary diversification mechanisms. In addition, an increased understanding of the positive and negative selective forces responsible for the depth, breadth, and limits of Ig repertoire — particularly modifiable factors such as intestinal content — may provide clues to advance our grasp of health issues relevant to antibody production.

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