CHAPTER FIVE

Microbes and B Cell Development

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Abstract

Animals and many of their chronic microbial inhabitants form relationships of symbiotic mutualism, which occurs when coexisting life-forms derive mutual benefit from stable associations. While microorganisms receive a secure habitat and constant food source from vertebrate hosts, they are required for optimal immune system development and occupy niches otherwise abused by pathogens. Microbes have also been shown to provide vertebrate hosts with metabolic capabilities that enhance energy and nutrient uptake from the diet. The immune system plays a central role in the establishment and maintenance of host–microbe homeostasis, and B lineage cells play a key role in this regulation. Here, I reviewed the structure and function of the microbiota and the known mechanisms of how nonpathogenic microbes influence B cell biology and immunoglobulin repertoire development early in life. I also discuss what is known about how B lineage cells contribute to the process of shaping the composition of commensal/mutualistic microbe membership.
1. MICROBIOTA

1.1. Overview of microbiota and microbiome

Evolutionary pressures over millennia have resulted in complex interrelationships between animals and the microorganisms that inhabit them. Mutualistic and commensal microorganisms are particularly abundant in the gut lumen, where an estimated $10^{14}$ bacteria reside (Savage, 1977). In humans, these microbes represent over 1000 different prokaryotic species belonging to a limited set of a dozen taxa and are dominated by Gram-positive anaerobes (Ley, Peterson, & Gordon, 2006). The term “microbiota” is defined as the collection of microorganisms in a distinct location, such as the human gut. The term “microbiome” generally refers to the collective genes contained within the microbiota, which in the case of the human gut includes some 3 million unique genes (Qin et al., 2010) mostly from bacteria. Of the relatively few known bacterial phyla in the human gut, the two most prominent are the Firmicutes and Bacteroidetes (Human Microbiome Project Consortium, 2012; Ley et al., 2006; Qin et al., 2010). In terms of their relationship to their animal habitats, the microbiota in general have been shown to be stable, resilient, and specific (Palmer, Bik, DiGiulio, Relman, & Brown, 2007; Zaretsky et al., 2012). The microbiota that inhabit animal hosts are frequently described as commensal (one partner benefits while the other is unaffected), yet, as more light has been shed on animal/microbe interdependence, the term mutualist (both partners derive benefit) has been suggested to provide a more accurate description for the bulk of the nonpathogenic cohabitating microorganisms (Ley et al., 2006; McFall-Ngai et al., 2013). Sequencing efforts have produced large data sets revealing a diverse array of bacterial communities across various human body sites, diets, and populations and delineate the range of functional and structural configurations in healthy populations (Human Microbiome Project Consortium, 2012; Qin et al., 2010; Turnbaugh et al., 2007; Yatsunenko et al., 2012). These studies show substantial variability in microbial community structure between individuals in terms of microbial taxa, but a remarkable similarity between individuals in terms of the metagenomic makeup of imputed metabolic functions (Lozupone, Stombaugh, Gordon, Jansson, & Knight, 2012). In this regard, the overall contribution of imputed metabolic capabilities, rather than taxonomy, appears to influence the composition of microbial membership among the gut microbiota.
1.2. Commensal/mutualistic microbes in health and disease

The trillions of commensal bacteria that make the human body their home are required for optimal host physiology. Microorganisms provide defense functionalities to hosts by delivering signals that lead to optimal host immune system development in addition to crowding out potential pathogens (Ferriere, Bronstein, Rinaldi, Law, & Gauduchon, 2002). Mutualistic bacteria have also been shown to provide vertebrate hosts with metabolic capabilities that enhance energy and nutrient uptake from the diet (Ley et al., 2006). In addition, based on the strong associations of the specific intestinal microbiota with diseases such as allergy, inflammatory bowel disease, diabetes, and obesity (de Vos & de Vos, 2012), microbial composition may be a key regulator of human health. In this regard, animal hosts have a vested interest in exerting control over the composition of their microbial partners (Ley et al., 2006). In this regard, microbes have evolved species-specific physical interactions with the host that mediate stable bacterial colonization (Lee et al., 2013), and optimal host immune system development may require specific groups of commensal/mutualist species. For example, despite containing similar phyla and microbe abundance, a human microbiota was not as effective as mouse microbiota in providing the signals required for optimal mouse immune maturation as measured by T cell proliferation, dendritic cell numbers, and antimicrobial peptide expression (Chung et al., 2012). Although the mechanisms underlying this effect remain to be worked out, it appears that specific host species anticipate a specific microbiota, and that optimal immune development does not occur without it. In this context, a fundamental goal in modern immunology is to understand the factors that contribute to the development and maintenance of the homeostatic balance between commensal microbes and the host immune system.

1.3. The immune system in host–microbe homeostasis

The mucosal immune system carries out the complex function of containing the enormous microbial load, while at the same time limiting the potential harmful effects of chronic activation of an inflammatory response to otherwise nonpathogenic microbes inhabiting mucosal surfaces (Hooper, Littman, & Macpherson, 2012). In this regard, there is a bidirectional dialogue between microbes and host immune system—commensals/mutualists play a key role in shaping the host immune system, while the host immune system plays a role in shaping the ecological structure of luminal microbes.
content (Hooper et al., 2012). In terms of microbes shaping the immune system, this has been studied a great deal recently in the context of mucosal T cell subsets. Colonization of mice with a specific bacterial species—namely, segmented filamentous bacteria (SFB)—induces the accumulation of proinflammatory \( T_H^{17} \) cells (Ivanov et al., 2009), which have been shown to play a role in host systemic inflammatory diseases (Wu et al., 2010). In contrast to the SFB-\( T_H^{17} \) axis, colonization of mice with clostridial strains induces the expansion of anti-inflammatory, IL-10-secreting regulatory T cells (\( T_{reg} \)) in the gut lamina propria (LP) (Atarashi et al., 2011), which promotes an environment of immune quiescence and tolerance. Additionally, polysaccharide A of \textit{Bacteroides fragilis} leads to T cell IL-10 production, which limits the expansion of proinflammatory \( T_H^{17} \) cells (Round et al., 2011)—a property likely enabling this species to coexist with the host as a commensal (Hooper et al., 2012). Therefore, gut microbes impart both pro- and anti-inflammatory signals and shape the host T cell profile. As discussed below, microbial effects on early B cell development and function were implicated decades ago with the discovery of a chicken hindgut structure functioning as an organ of primary B cell lymphopoiesis, but understanding of the physiologic importance of this connection remains limited over a half of a century later (Shrestha & Wesemann, 2015). Herein, I review recent work that has provided insights into this decades-long mystery.

## 2. EARLY-LIFE B CELL DEVELOPMENT AND THE GUT

### 2.1. Links between the gut and primary immunoglobulin diversification

A potential link between the microbiota and primary B cell development was implicated when it was first demonstrated that the bursa of Fabricius is key for Ig production in chickens (Glick, Chang, & Jaap, 1956). The bursa of Fabricius is an outpouching of the avian hindgut that involutes during puberty and its surgical removal early in life results in the absence of Ig and B cells (Glick et al., 1956; Reynolds & Morris, 1983). The discovery of related gut-associated follicular structures only in prepubertal rabbits and sheep leads to suggestions of a mammalian equivalent of the avian bursa (Cooper, 2010; Reynolds & Morris, 1983). Early B cell development and primary Ig diversification in rabbits are particularly similar to that in chickens in that they both generate B cells with a very limited RAG-mediated V(D)J repertoire in primary lymphoid tissue that subsequently migrate to hindgut
structures where further primary diversification occurs through activation-
induced cytidine deaminase (AID)-mediated gene conversion and somatic
mutation (Perey, Frommel, Hong, & Good, 1970; Vajdy, Sethupathi, &
Knight, 1998). In this context, the gut environment early in life may be ben-
eficial in shaping nascent Ig repertoires. While mechanisms differ between
organisms, many vertebrates appear to also share a link between the gut,
microbes, and early B cell selection events associated with windows of time
early in life. Known examples of these associations are discussed here.

2.2. Ig diversification and selection in rabbits
In rabbits, V(D)J recombination generates a very limited repertoire due
to heavily biased VH gene segment usage from preferential rearrangement
of D-proximal VH gene segments (Knight, 1992). Shortly after birth, B cells
with this limited repertoire migrate to gut-associated lymphoid tissues—
namely, the sacculus rotundus and the appendix—where they undergo
proliferation and AID-mediated preimmune Ig repertoire diversification
to develop a full primary repertoire in the first 1–2 months of postnatal
life (Crane, Kingzette, & Knight, 1996). These hindgut structures subse-
quently undergo changes to resemble secondary lymphoid tissue where
conventional immune responses occur (Weinstein, Mage, & Anderson,
1994). In addition, based on the striking reduction of B cell progenitors
and B cell recombination excision circles in the bone marrow after 16 weeks
of age (Jasper, Zhai, Kalis, Kingzette, & Knight, 2003), the rabbit bone
marrow likely does not contribute substantially to adult B lymphopoiesis.
In this context, primary B cell development and Ig diversification in
rabbits are thought to take place primarily in the postnatal gut in a time-
frame limited to the first 2 months of life (Fig. 1). The role of microbe
exposure in primary Ig diversification in newborn rabbits has been studied
in germ-free animals (Lanning, Sethupathi, Rhee, Zhai, & Knight,
2000; Stepankova & Kovaru, 1985; Stepankova, Kovaru, & Kruml,
1980; Tlaskalova-Hogenova & Stepankova, 1980) as well as in model sys-
tems in which exposure to microbiota was blocked due to removal of the
sacculus rotundus and microsurgical closure of the appendix at birth
(Lanning et al., 2000; Perey & Good, 1968; Stramignoni, Mollo,
Rua, & Palestro, 1969). Prevention of microbial exposure reduced gut-
associated follicle development, B cell numbers, and peripheral Ig diversity
in young rabbits, arguing that access to gut microbes may be required for
optimal B cell development.
The extent to which Ig specificity and potential B cell encounter with contents of the intestinal lumen during primary rabbit B cell diversification was addressed in studies using mutant Alicia rabbits. Alicia rabbit B cells express IgM of the V_Hn allotype almost exclusively at birth. However, during the first weeks of life, the V_Hn representation diminishes in favor of increased V_Hd allotype utilization. When interactions with luminal content were prevented by surgical sequestration of the sacculus rotundus and appendix, the peripheral repertoire throughout the body remained largely of the V_Hn allotype (Rhee et al., 2005), indicating that the presence of

Figure 1  Overview of known associations between early B cell developmental activities and the gut. Schematic representation of the relationship between B cell developmental activities and age in specific animal examples. Animals shown on the left from top to bottom are chicken, rabbit, sheep, pig, mouse, and human. The length of the rectangular boxes to the right of each animal representation signifies an approximation of life span divided in either 1- or 10-year units as indicated. The top yellow (off-white in the print version) portion of each rectangle indicates the gut or gut-associated tissue, the bottom green (light gray in the print version) portion of each rectangle signifies primary lymphoid tissues such as the bone marrow, fetal liver, or spleen. The blue (dark gray in the print version) coloring indicates active primary Ig diversification events. The orange (gray in the print version) color signifies B cell selection events that occur in unique lymphoid structures that appear to be specialized for B cell selection early in life. The blue and orange colors are meant to emphasize distinctions raised in the text and are not necessarily mutually exclusive.
microbes and/or other luminal components may select for particular Ig specificity during primary development in young rabbits. Based on the finding of conserved amino acids in $\text{V}_{\text{H}}\alpha$ framework regions positioned in areas that could react with a putative antigen, it was proposed that a microbial superantigen may be positively selecting for the $\text{V}_{\text{H}}\alpha$-expressing B cells in the young rabbit intestine (Rhee et al., 2005). In addition, more $\text{V}_{\text{H}}\alpha$ cells were found to proliferate and fewer die compared to $\text{V}_{\text{H}}\beta\gamma$ cells in the gut-associated lymphoid tissue (Pospisil, Young-Cooper, & Mage, 1995) in agreement with a potential microbe-dependent positive selection process. Thus, early-life gut-associated B cell diversification in the rabbit appears to be associated with selection forces influenced by gut luminal contents. The nature of how luminal contents are made available and presented to B cells in the young rabbit gut, and the physiologic roles of these processes are poorly understood.

2.3. Ig diversification and selection in the bursa of Fabricius

Insights into the potential role of microbiota in selection processes during primary Ig diversification have been obtained from studies in chickens, where Ig diversification largely occurs in the bursa of Fabricius, an outpouching of epithelium connected to the distal intestine in birds that is required for B cell development and Ig production (McCormack, Tjoelker, & Thompson, 1991; Ratcliffe, 1989; Ratcliffe & Jacobsen, 1994; Weill & Reynaud, 1987). The chicken IgH locus contains a single functional $\text{V}_{\text{H}}$ segment and several related $\text{V}_{\text{H}}$ pseudogenes (McCormack et al., 1991; Reynaud, Anquez, Dahan, & Weill, 1985). Before hatching, B lineage cells with limited V(D)J repertoire migrate to the bursa primordium in a single wave during embryonic life (Houssaint, Belo, & Le Douarin, 1976; Houssaint, Torano, & Ivanyi, 1983). There, they undergo both expansion and diversification of their Ig genes via AID-mediated gene conversion, using pseudogene V segments as donor sequences (Ratcliffe, 1989; Reynaud, Anquez, Grimal, & Weill, 1987). Despite the discovery of bursa-dependent (B) lymphocytes in chickens decades ago, the physiologic relevance of the connection between primary Ig diversification and the chicken hindgut remains largely mysterious.

In contrast to rabbits, where gut-associated Ig diversification occurs almost exclusively after birth, chicken Ig diversification occurs to a substantial degree during embryonic development, arguing against the role of microbiota in the prehatch phase of chicken Ig diversification. However,
luminal antigens appear to influence early repertoires soon after hatching. In this regard, primary Ig diversification likely continues until bursa involution during sexual maturity at approximately 6 months of age. During this time, transportation of antigen into bursal follicles occurs through M cell–like follicle–associated epithelium (Bockman & Cooper, 1973), thus providing antigen to diversifying B cells during the first few months of life.

The role of Ig specificity in chicken B cell development and Ig diversification was addressed in experiments that utilized a retroviral gene transfer model employing a truncated Igμ heavy chain (Tμ) construct lacking the antigen–binding VDJ and C1 segments (Sayegh, Demaries, Iacampo, & Ratcliffe, 1999). A variation of this construct in which Tμ is fused to a lamprey variable leucine-rich repeat (VLR) protein segment that recognizes either fluorescent phycoerythrin (PE) (Tμ-VLRPE) or hen egg lysozyme (Tμ-VLRHEL) was used more recently (Davani, Pancer, Cheroutre, & Ratcliffe, 2014; Davani, Pancer, & Ratcliffe, 2014). Tμ does not require the presence of light chains for its surface expression on B cell precursors and can thus be distinguished from endogenous IgM (Sayegh et al., 1999). After induction of Tμ expression in chicken embryos, B cell expansion and primary Ig diversification was observed in Tμ+ B cells in the absence of endogenous IgM. Expression of the Tμ in the absence of antigen–binding capability supported rapid cell division and Ig diversification via gene conversion during embryonic life similar to that observed with endogenous IgM expression (Reynolds, 1987). However, bursal cells expressing Tμ demonstrated substantially reduced rates of cell division and increased levels of apoptosis after hatching (Sayegh & Ratcliffe, 2000), indicating that the antigen–binding region—and by extension, perhaps antigen encounter—may be important for early bursal B cell survival. Bursal B cells expressing Tμ-VLRPE similarly diminished after birth. However, in the presence of experimentally provided gut luminal PE, Tμ-VLRPE-expressing B cells survive and are exported to the periphery at enhanced levels compared to controls without PE. Thus, although antigen encounter does not seem to be required for the early stages of bursal B lineage cell diversification, luminal antigens appear to mediate positive selection of B cells after hatching. As primary antibody diversification through AID–mediated gene conversion likely continues in the chicken until the bursa involutes at 3–6 months of age (Arakawa & Buerstedde, 2004), gut–resident antigen–dependent positive selection processes appear to be occurring simultaneously with primary Ig diversification.

Negative selection also appears to play a role as B cells expressing Tμ-VLRHEL, a construct reactive against constitutively produced HEL results...
in the disappearance of systemic and bursal T\(\mu\)-VLR\(^{\text{HEL}}\)-expressing B cells (Davani, Pancer, Cheroutre, et al., 2014). Additionally, in contrast to what is seen during luminal PE administration, intra-ovo injection of PE in the T\(\mu\)-VLR\(^{\text{PE}}\) model leads to a mild reduction of T\(\mu\)-VLR\(^{\text{PE}}\)-producing B cells in the spleen, but no change in the percentage of bursal T\(\mu\)-VLR\(^{\text{PE}}\)-producing B cells (Davani, Pancer, Cheroutre, et al., 2014). Thus, the timing, location, context of presentation, as well as biophysical properties of the antigen may all be involved in influencing early B cell selection outcomes. The mechanisms and contributions of negative versus positive selection to the B cell development process in the chicken remain to be fully elucidated.

2.4. Lessons from sheep and pigs

Sheep intestines harbor two distinct types of Peyer’s patches that differ in their ontogeny, cell composition, and physiology (Griebel & Hein, 1996). Ileal Peyer’s patches resemble the young rabbit sacculus rotundus and chick bursa in that they are made up of \(~95\%\) B cells that are essentially all IgM\(^+\) and \(<0.5\%\) of CD4\(^+\) T cells (Griebel & Ferrari, 1995; Hein, Dudler, & Mackay, 1989; Larsen & Landsverk, 1986). In contrast, jejunal Peyer’s patches consist of \(~40\%\) IgM\(^+\) B cells, 10–15\% CD4\(^+\) T cells, 4–6\% plasma cells, and 35–40\% isotype switched B cells (Griebel & Ferrari, 1995; Hein et al., 1989; Larsen & Landsverk, 1986). In addition, ileal Peyer’s patches are present at birth and involute between 6 and 15 months of age (Fig. 1), whereas jejunal Peyer’s patches are present throughout the entire life of the animal (Reynolds, 1986; Reynolds & Morris, 1983). These observations stimulated the hypothesis that jejunal Peyer’s patches may be a significant site for the induction of mucosal immunity in the form of secondary lymphoid tissue, similar to Peyer’s patches in rodents and primates, whereas ileal Peyer’s patches in neonates and young lambs may serve a function more in line with primary Ig repertoire modulation, perhaps similar to young rabbits and chicks (Reynaud, Garcia, Hein, & Weill, 1995; Reynaud, Mackay, Muller, & Weill, 1991). In this regard, the observation that only \(~5\%\) of ileal Peyer’s patch B lineage cells survive, with the remainder dying \textit{in situ} by apoptosis (Motyka & Reynolds, 1991; Pabst & Reynolds, 1987; Reynolds, 1986), indicates that a substantial selection process occurs before ileal Peyer’s patch involution early in life. The localization of the ileal Peyer’s patches just above the ileal–cecal junction, where the highest concentrations of bacteria accumulate, suggests that luminal microbes likely influence this selection process.
The potential role of microbes in the development of the primary Ig diversification in sheep ileal Peyer’s patches was addressed in experiments where B cell somatic mutation of the V segment of lambda light chain was analyzed from sterile fragments of the ileal loops surgically sequestered from microbes and other luminal contents during fetal life, as well as in germ-free sheep 6 and 8 weeks after birth (Reynaud et al., 1995, 1991). The size of ileal Peyer’s patches was decreased in surgically sterile ileal loops as well as in germ-free sheep after birth—indicating that luminal microbes play a role in ileal follicle size. Earlier studies reported high levels of somatic mutation in the surgical loops and germ-free animals, suggesting that microbes may not be required to induce primary Ig diversification in sheep (Reynaud et al., 1995). However, the discovery of more Vλ gene segments revealed that many of the unique nucleotide patterns initially thought to be a result of mutation in early studies were actually hard-coded germline variants (Jenne, Kennedy, McCullagh, & Reynolds, 2003). In this context, these newer studies clarified that somatic mutation occurs almost exclusively after birth (Jenne et al., 2003), consistent with a potential role for microbes and/or dietary substances as potential influencers of this process. Ileal and jejunal Peyer’s patches in piglets are structurally very similar to those of lambs, and it has been shown that piglet ileal Peyer’s patch B cell somatic mutation is dependent upon microbial exposure early in life as well (Butler & Sinkora, 2013).

While the extent to which postnatal ileal Peyer’s patch B cell activities are physiologically and functionally distinct from jejunal Peyer’s patches in lambs remains to be fully resolved, insightful experiments have shown interesting distinctions. In this regard, a model antigen system utilizing glycoprotein-D (gD) of bovine herpesvirus-1 was employed to assess anti-gD antibody responses after gD challenge in sterile loops of jejunum versus ileum in young lambs. Anti-gD antibodies and B cell proliferation were strongly elicited in the jejunal Peyer’s patches, but not those in the ileum (Mutwiri et al., 1999). However, gD-reactive antibodies were discovered in the spleens of ileum loop-injected sheep, and not in loops that did not contain follicles, suggesting that the gD antigen may have had a positive selection effect on emigrant lymphocytes from the ileal Peyer’s patches (Mutwiri et al., 1999), reminiscent of the model antigen–dependent positive selection in chickens (Davani, Pancer, & Ratcliffe, 2014) and the putative microbe–dependent positive selection activities that may occur in young rabbits (Rhee et al., 2005). Additionally, involution of ileal lymphoid follicles was delayed following gD antigen exposure, supporting the notion that ileal B cells sense and respond to the luminal environment.
2.5. Perspectives on the role of the gut in B cell biology early in life

Due to distinct diversification and selection mechanisms, the hindgut B cell structures in hatchling birds, young rabbits, lambs, and piglets may not allow a simple categorization of the B cell activities occurring in these species as the same process (Butler & Sinkora, 2013). However, there are three aspects that are common among gut B cell activities in the young of these species. 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, these 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 (Fig. 1). 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—is not fully understood. In this regard, mice and humans do not appear to have gut–associated B cell follicles that involute a few months after birth as is found in the avian bursa, rabbit sacculus rotundus, or artiodactyl ileal Peyer’s patches. However, early developmental activities and unique selection processes may exist through other mechanisms. In this regard, while very little is known about B cells in the newborn human intestine, the human fetal small intestinal LP has been shown to harbor pre–B cells expressing Vpre–B (Golby et al., 2002) (Fig. 1). As Rag-dependent V(D)J recombination is essentially the sole driver of the primary human Ig repertoire, the presence of pre–B cells in the human fetus raises the question whether early B cell development may serve a similar purpose as the gut-resident primary Ig diversification in chickens and rabbits. The extent to which early B cell development and primary Ig diversification occur in the intestines of human infants and children has not been addressed. However, as discussed below, recent studies have examined this in mice.

2.6. B cell development in the mouse lamina propria early in life

Similar to human, RAG–mediated V(D)J recombination during early B cell development is the major driver of preimmune diversification in mice. Consistent with the notion that the gut may provide some benefit to the process of early B cell selection and preimmune Ig diversification early in life,
RAG-expressing early developing B lineage cells—undergoing active V(D)J recombination—have been observed in the small intestinal LP of weanling mice (Wesemann et al., 2013). Using a RAG2-GFP reporter model wherein a RAG2-GFP fusion protein is produced from the endogenous RAG2 locus, it was shown that RAG2-expressing CD19⁺ B220low cells could make up approximately 4% of total CD19⁺ cells at weaning age (18–24 days). In contrast, RAG2⁺ cells are essentially nondetectable in the first week after birth and decrease to background levels at approximately 5–6 weeks of age. The coincident timing of early gut B cell accumulation with weaning age hinted at a potential role for the gut microbiota in this process, given that gut microbes expand markedly as mice are weaned off of IgA found in maternal milk (Mackie, Sghir, & Gaskins, 1999). In this context, cohousing mice that were initially germ free with conventionally raised mice from age 3 to 4 weeks of age lead to increased levels of pro-B cell levels in the gut LP (Wesemann et al., 2013).

In addition to affecting the amount of early B cell progenitors in the gut mucosa, conventionalization of germ-free mice resulted in an increase in the Igλ/Igκ ratio specifically in the LP of the small intestine, while other tissues such as the spleen or bone marrow remained unchanged (Wesemann et al., 2013). As increased Igλ usage in the B cell repertoire is a marker for increased receptor editing (Hertz & Nemazee, 1997; Retter & Nemazee, 1998; Tiegs, Russell, & Nemazee, 1993), these observations are consistent with the notion that commensal microflora influence the Ig repertoire by affecting BCR editing of immature gut B cells in the gut LP. Additional evidence suggesting that receptor editing can occur in the gut includes the finding of B lineage cells with phenotypic characteristics of editing cells—namely, RAG2⁺B220lowIgMlow—in the small intestinal LP of weanling mice. Furthermore, comparisons of the emerging Ig repertoire in RAG2⁺ gut LP cells compared to RAG2⁺ cells from the bone marrow reveal significant differences inVk gene segment usage despite similar VH usage (Wesemann et al., 2013). The observation of different Vk gene segment usages between these populations is consistent with BCR editing or a BCR editing-like selection process in the gut as early Ig repertoires in the BM and LP would be exposed to distinct antigens.

As B cell receptor editing is a mechanism of antigen-mediated shaping of primary Ig repertoires, these findings suggest a model wherein luminal contents play a role in shaping preimmune repertoires during a window of opportunity early in life (Bird, 2013). Although mechanistically distinct, early B cell development and microbe-dependent primary Ig repertoire modification in weanling mice may be functionally related to the process
of gut-resident B cell receptor selection/diversification that occurs in young chicks, rabbits, piglets, and lambs. The mechanism underlying these effects as well as the physiological relevance of the early-life timing and gut locale awaits further elucidation.

3. MICROBIAL INFLUENCE ON IMMUNOGLOBULIN PRODUCTION

3.1. Microbial influence on IgA production

IgA is the largest IgH isotype produced by humans and represents over three-quarters of IgH isotype produced by the body as a whole (Conley & Delacroix, 1987; Delacroix, Hodgson, McPherson, Dive, & Vaerman, 1982). The gut mucosal system is home to the largest activated B cell pool, containing at least 80% of all plasmablasts and plasma cells, mostly producing dimers of IgA (Brandtzaeg et al., 1989). IgA production has been shown to be dependent upon the presence of microbes as germ-free animals have extremely low levels of IgA (Benveniste, Lespinats, Adam, & Salomon, 1971; Benveniste, Lespinats, & Salomon, 1971; Shroff, Meslin, & Cebra, 1995). In addition, most intestinal IgA is directed against intestinal flora (Hooper & Macpherson, 2010).

While mechanistic details underlying how microbes influence IgA production have yet to be fully uncovered, unique advances have been made in studies using a mutant *Escherichia coli* strain (HA107) engineered to allow reversible colonization of germ-free mice. The HA107 bacteria are alive, but unable to divide in mice, and become undetectable 72 h after installation into the mouse gut by gavage. This enables animals to receive known quantities of live organisms, become germ free again, and be rechallenged to assess memory responses (Hapfelmeier et al., 2010). Gut challenge with differing amounts of HA107 revealed that the intestinal barrier appears to sample only a tiny fraction of the live luminal bacteria as up to a hundred million (10⁸) CFU of HA107 bacteria produced no measurable IgA responses, whereas greater amounts (10⁹ and 10¹⁰ CFU) provide robust, specific IgA responses (Hapfelmeier et al., 2010). While highly specific, the IgA response against HA107 demonstrated an additive effect in response to sequential bacterial exposures as opposed to a synergistic effect seen with classical systemic immune memory responses (Hapfelmeier et al., 2010). In addition, although specific IgA responses can last over 16 weeks after initial challenge with HA107, exposure of HA107-treated, germ-free mice to other commensal bacterial species resulted in rapid abrogation of HA107-specific IgA and
generation of an IgA response that matched the existing commensal content (Hapfelmeier et al., 2010), thus indicating that IgA responses can continually evolve to react against the most prominent luminal species.

The extent to which IgA responses to E. coli/HA107 colonization reflect general IgA responses to bacteria in general remains to be elucidated, as different bacterial species are known to have varying effects on mucosal immune responses. For example, colonization with SFB has been shown to be exceptionally effective at producing large amounts of IgA (Talham, Jiang, Bos, & Cebra, 1999). Notably, stimulation with these bacteria is also associated with induction of T<sub>H</sub>17 cells (Ivanov et al., 2009). With the use of IL-17 fate reporter mice, it was shown that T<sub>H</sub>17 cells that expand and home to the small intestine due to SFB colonization migrate into the Peyer’s patches and take on a T follicular helper (TFH) phenotype to assist B cells undergo activation toward IgA-producing cells (Hirota et al., 2013). In addition, mice that lacked T<sub>H</sub>17 cells were deficient in their ability to mount an antigen-specific IgA response when immunized with cholera toxin. Although the mechanism of how SFB specifically leads to T<sub>H</sub>17 accumulation in the gut is not fully understood, these studies provide a link between how a specific microbe, namely SFB, is connected to T cell-dependent IgA production.

3.2. Microbial influence on IgE Production

While microbes are required for induction of IgA, it appears that the opposite is true for IgE. In this regard, IgE is elevated at baseline for unknown reasons in germ-free mice and decreases upon colonization with commensal microbes (Cahenzli, Koller, Wyss, Geuking, & McCoy, 2013; Hill et al., 2012). Germ-free mice begin to produce IgE shortly after weaning age to levels 1000–10,000 times that of conventionally raised mice by 64 days of life and maintain this level unless colonized with microbes within the first week of life (Cahenzli et al., 2013). Notably, colonization with one or two species was not sufficient to protect from hyper-IgE despite gavage with high amounts of a single strain of bacteria (multiple rounds of 10<sup>9</sup>–10<sup>10</sup> CFU), but a more diverse set of microbes representing 7–40 different phyla were required to provide a robust protection against hyper-IgE seen at levels observed in germ-free mice (Cahenzli et al., 2013). Based on experiments measuring sterile IgE germline transcripts, it was shown that the IgH class switch recombination was potentially occurring at mucosal sites and not in the spleen or lymph nodes. Additionally, the elevated IgE in germ-free
mice required CD4\(^+\) T cells, IL-4, and the presence of organized lymphoid mucosal tissues, but was not influenced by or dependent upon dietary antigens (Cahenzli et al., 2013). Microbe-dependent depression of IgE levels was abrogated when myd88—a key mediator of innate recognition of microbes—was conditionally deleted in B lineage cells, thus indicating that direct sensing of microbial products by B cells is required in the process of microbial-mediated abrogation of IgE (Hill et al., 2012).

While the potential physiologic rationale underlying elevated IgE in mice lacking signals from a diverse microbiota remains a mystery, germ-free dependent hyper-IgE status preconditions animals for enhanced propensity for T\(_{H2}\) inflammation (Cahenzli et al., 2013; Hill et al., 2012). In this regard, mice with reduced commensal microbial exposure either through antibiotic treatment or through germ-free conditions have an IgE-dependent increase in basal levels of circulating basophils, Th2-activated T cells, and an increased density of FceRI-bound IgE on basophils (Hill et al., 2012). Elevated IgE was also noted to increase the maturation of basophil precursors to mature basophils by increasing their IL-3 responsiveness (Hill et al., 2012). Consistent with previous reports that mast cell homeostasis is regulated by IgE levels (Kitaura et al., 2003), elevated IgE due to germ-free status is also associated with increased amounts of surface-bound IgE on mast cells and exaggerated oral-induced systemic anaphylaxis (Cahenzli et al., 2013). Epidemiologic studies have identified associations between alterations in the composition of communities of commensal bacteria and allergic disease (Kalliomaki et al., 2001; Kummeling et al., 2007; Marra et al., 2009). Why IgE appears to be the default pathway in the absence of a diversity of microbes is a mystery, but work into this question will likely uncover key aspects of Th2-spectrum immunity. In addition, it will be important for future work to determine the extent to which similar mechanisms are at work in affecting susceptibility of human allergic disease.

### 4. B LINEAGE CELL INFLUENCE ON COMMENSEL MICROBE ECOLOGY

While a full understanding of the physiologic roles of B cells and Ig production in the gut is incomplete, it is clear that B cells exert a substantial effect in regulating host–microbe homeostasis through mucosal IgA, which has been shown to have the capacity to carry out a variety of functions. In addition to restricting bacterial access to the epithelium (Cong, Feng, Fujihashi, Schoeb, & Elson, 2009; Hooper & Macpherson, 2010;
Macpherson, Hunziker, McCoy, & Lamarre, 2001), IgA can influence expression of microbial molecules (Peterson, McNulty, Guruge, & Gordon, 2007) and has been shown to promote the survival of specific bacteria (Obata et al., 2010). IgA has also been shown to regulate the metabolic and defense responses of intestinal epithelial cells in a triadue among B cell-produced IgA, intestinal epithelium, and gut luminal microbes (Shulzhenko et al., 2011).

By way of its unique ability to influence microbial communities in the gut lumen, IgA plays an important role to allow host metabolic activities to be carried out by the intestinal epithelium. In this regard, intestinal epithelial cells from B cell-deficient mice upregulate genes related to defense, inflammatory, and interferon-inducible responses while genes involved in metabolic processes such as oxidation and reduction reactions associated with energy generation, and steroid and cholesterol metabolism become significantly downregulated (Shulzhenko et al., 2011). The ~100-fold elevated levels of IgM naturally found in IgA-deficient mice do not appear to protect the intestinal epithelium from these changes in epithelial transcription profiles (Shulzhenko et al., 2011). Gene network analysis has revealed two interacting gene systems within intestinal epithelial cells, one governing lipid metabolism and another governing innate immunity. These two epithelial functions appear to be coordinately regulated and inversely connected via a small number of genes, with the main ones being Gbp6, an IFN-inducible gene suggested to have antibacterial function (Degrandi et al., 2007), and Gata-4, which regulates metabolic functions in intestinal epithelium.

Layers of immunologic redundancy exist to keep a distance between the intestinal mucosal barrier and commensals, mutualists, and pathogens alike, but recruitment of secondary layers of defense does not come without a cost. In the example above, upon failure of the B cell-IgA system, epithelial defense mechanisms sacrifice metabolic functions in favor of defense. In this regard, observations that lack of IgA is responsible for inability to properly process nutrients from the diet in mice are clinically relevant for diseases of immune deficiency, where Ig production is defective such as common variable immune deficiency (CVID). As intestinal epithelium may activate defense programs at the expense of metabolic functions in the absence of IgA, the immune system may essentially function as a shield to allow gut barrier tissue to perform metabolic functions. Biopsies from human small intestine CVID patients have increased sets of interferon-induced genes and a decrease in groups of genes involved in lipid and carbohydrate metabolism and micronutrient transport in agreement with the findings in mice.
(Shulzhenko et al., 2011). Consistent with a role for Ig in allowing for optimal metabolic functions, patients with antibody deficiency syndromes such as CVID indeed have problems with malabsorption and low weight gain (Agarwal & Mayer, 2009; Hughes, Cerda, Holtzapple, & Brooks, 1971; Owens & Greenson, 2007). As current therapeutic Ig replacement protocols for CVID provide only systemic IgG, these findings suggest that there may be some rationale for developing a gut luminal IgA replacement protocol or microbial control treatments for these patients (Shulzhenko et al., 2011).

Studies using mice lacking IgA in the setting of AID deficiency have played a key role to elucidate the role for IgA and somatic hypermutation in the maintenance of commensal bacterial homeostasis (Fagarasan et al., 2002; Suzuki et al., 2004; Wei et al., 2011). AID-deficient mice have an accumulation of intestinal IgM plasma cells and a microbe-dependent enlargement of intestinal follicular structures (Fagarasan et al., 2002). Gut luminal microbes mediate the intestinal follicular hyperplasia as antibiotic treatments normalize the phenotype. In addition, IgA plays a role in shaping the microbial ecology in the intestinal lumen, as AID-deficient mice appear to harbor a large increase in small intestinal anaerobic bacteria (Fagarasan et al., 2002; Suzuki et al., 2004). Notably, the microbial community-shaping function of IgA appears to be restricted to the small intestine.

Because AID is required for both IgH class switch recombination and somatic hypermutation, the contributory roles of these two individual processes cannot be determined in AID-deficient mice alone. These two pathways were dissected in mice carrying a knock-in mutation of AIDG23S. Although mice with this G23S AID mutation have defects in both switch recombination and somatic hypermutation, the defect in somatic hypermutation is much more pronounced in vivo (Wei et al., 2011). In this context, AIDG23S mice are able to compensate for the reduced CSR efficiency in terms of total levels of Ig levels, resulting in normal levels of all Ig isotypes, including IgA, in the serum and feces. However, somatic hypermutation is attenuated, reaching levels only ~10–20% of that found in wild-type mice (Wei et al., 2011). Both the total number of mutations per clone and the number of mutated clones appear to be reduced in both Peyer’s patch germinal center B cells as well as IgA+ plasma cells in the intestinal LP. This mouse model was used to identify the extent to which secretion of non-somatically mutated IgA is sufficient to maintain bacterial homeostasis and to mount protective mucosal immunity. In this respect, despite normal levels of IgA in intestinal secretions, AIDG23S mice manifest phenotypes similar to those of AID-deficient mice. Notably, AIDG23S microbiota was shown
to have overrepresentation of anaerobes such as SFB (Wei et al., 2011). Thus, somatic hypermutation in concert with IgA plays a role in influencing microbial membership of the intestinal microbiota.

5. CONCLUDING REMARKS

Nearly a half of a century has passed since the discovery of the link between Ig development and a chicken hindgut structure—after which the B lymphocyte lineage is named (Glick et al., 1956). With regard to the gut, data generated since that time suggest that the gut environment, in particular the microbiota, plays a special role in shaping the Ig repertoire, but potentially only during a limited window of time early in life. Future work will certainly provide more data to advance our understanding of the functional relevance of early-life microbial exposures in shaping the fitness of humoral immune responses. In addition, as specific ecologies of intestinal microbiota may predispose to disease (de Vos & de Vos, 2012), years to come will assuredly uncover a greater understanding of the role of B lineage cells in establishing and maintaining microbial relationships that are optimal host health.

ACKNOWLEDGMENTS

D.R.W. is supported by NIH grants AI089972 and AI113217, by the Mucosal Immunology Studies Team, and holds a Career Award for Medical Scientists from the Burroughs Wellcome Fund.

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