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Commensal bacteria, timing and barrier function in the context of allergic disease

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Abstract

Allergic disease affects millions. Despite many advances in our understanding of the immune system in the past century, the physiologic underpinning for the existence of allergy remains largely mysterious. Food allergies, in particular, have increased dramatically in recent years, adding a new sense of urgency to unraveling this mystery. The concurrence of significant lifestyle changes in Western societies with increasing disease prevalence implies a causal link. Demographic variables that influence the composition and function of the commensal microbiota early in life seem to be most important. Identifying the evolutionary and physiologic foundations of allergic disease and defining what about our modern environment is responsible for its increased incidence will provide insights critical to the development of new approaches to prevention and treatment.

Introduction

The prevalence of allergic disease has climbed steadily during the past fifty years (Asher et al., 2006; Okada et al., 2010). Its clinical presentation often follows an ordered developmental progression (atopic dermatitis, food allergy, asthma, allergic rhinitis) referred to as the allergic march (Alduraywish et al., 2015). Epidemic increases in asthma prevalence were the first to gain notice (Eder et al., 2006; Masoli et al., 2004). More recently, potentially life-threatening allergic responses to food have become an important public health concern (Prescott and Allen, 2011; Sicherer and Sampson, 2014). Nut-free classrooms, virtually unheard of in earlier generations, are now commonplace. In developed countries worldwide, as many as 10% of preschool children currently suffer from food allergies (Prescott et al., 2013). Recent reports estimate that there are 15 million children and adults with food allergies in the United States alone (Branum and Lukacs, 2008; Jackson et al., 2013). While hundreds of foods can elicit an allergic response (Hefle et al., 1996), eight in particular—namely, milk, eggs, peanuts, tree nuts, wheat, soy, fish and shellfish—account

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for most cases (Sicherer and Sampson, 2014). Genetic susceptibility cannot explain a marked increase in prevalence in such a short time frame, suggesting that something about our modern environment is promoting allergic disease.

We begin this review with a brief history of allergy, focusing on IgE-mediated hypersensitivity, and describe emerging concepts regarding its potential physiologic roles. We then discuss recent data that point to early life as an important time where environmental context—particularly with regard to the microbiota—influences susceptibility to allergic disease. We end with some remarks on how recent findings will inform the development of novel therapeutic strategies to prevent or treat food allergy and other allergic diseases.

Why does allergy exist?

The term “allergy” was coined in 1906 by the Viennese pediatrician Baron Clemens von Pirquet from the Greek “allos” meaning other or altered and “ergon” meaning works or reaction. Von Pirquet observed that changes in reactivity occurred on subsequent exposures to an antigen. In some instances, re-exposure resulted in diminished reactivity whereas in others reactivity increased (Igea, 2013). A major advance in the understanding of food allergy occurred in 1921, when Prausnitz and Kustner determined that hypersensitivity to an antigen could be passively transferred with serum from one individual—namely, Kustner, who was allergic to fish—to another—namely, Prausnitz, who was not allergic to fish—by intradermal injection. When Prausnitz subsequently ate fish, the injection site became hot, red, and swollen (Prausnitz and Kustner, 1921). The antigens responsible for this reaction were called atopens, and the plasma factor that conferred sensitivity was known as atopic reagin (Paul, 2013). In 1966, Teruko and Kimishige Ishizaka showed that a novel class of immunoglobulin (Ig), which they designated γE-globulin, or IgE, was responsible for reaginic activity (Ishizaka and Ishizaka, 1966).

Since the discovery of IgE, much about the molecular and cellular mechanisms by which allergens elicit clinical symptoms has been elucidated. However, little insight has been gained into why allergies exist in the first place. One hypothesis prevalent in the current literature suggests that Th2 immunity evolved to facilitate the elimination of worms and parasites and that allergy represents a misfiring of this response to otherwise innocuous substances (Fitzsimmons and Dunne, 2009; Pulendran and Artis, 2012; Stetson et al., 2004). The overwhelming majority of allergens are not, however, helminths or their products. The major allergens are a diverse group for which no one structural or biological activity appears to dominate, and include proteins associated with shellfish, nuts, venoms, pollens, animal dander, and penicillin (Erwin and Platts-Mills, 2005). It is difficult to conceptualize, that within an immune system with both innate and adaptive components that can respond with astonishing precision, a subprogram exists where nearly all of the reactivity is off-target or misguided to common environmental substances. Profet articulated a more comprehensive and intriguing hypothesis that she termed the toxin hypothesis of allergy (Profet, 1991); a similar perspective was offered by Medzhitov and colleagues in 2012 (Palm et al., 2012). This hypothesis states that allergic responses are targeted to expel, and/or reduce the potential damage incurred by noxious substances, such as toxins and venoms, in addition to parasites and worms. In this view, many, if not all, allergens have noxious potential, either

directly or by proxy (Palm et al., 2012). This theory predicts that the manifestation of allergy in some individuals involves excessive, and potentially tissue damaging, immune reactivity against the host. Allergic hypersensitivity may represent the immunologic cost of protection from noxious damage similar to the way that the pathology associated with severe pneumonia or sepsis is a consequence of immunologic responses against infection with microbial pathogens—both are intended to protect their hosts, but may be deadly if excessive. In each case, symptoms occur when subclinical mechanisms of pathogen or noxious substance avoidance or clearance mechanisms fail. Studies demonstrating that phospholipase A2 in bee venom assists in orchestrating Th2 immunity (Palm et al., 2013), and that anti-venom IgE provides a survival advantage against subsequent venom exposures (Marichal et al., 2013) have provided recent support for the toxin hypothesis of allergy.

While the evolutionary selection pressures at the root of allergic hypersensitivity remain to be fully explained, a deeper understanding of the physiologic role of allergy may provide insight into underlying variations in disease susceptibility on both the genetic and environmental levels. A *barrier regulation hypothesis* of allergy suggests that diverse barrier mechanisms including allergen exclusion and deactivation may underlie this variance. In the context of food allergy, non-food-allergic individuals may have relatively more effectual barrier immunity—thus leaving the allergic response untriggered. Allergen penetration of barriers may induce perturbations that lead to epithelial stress, which can set the stage for a Th2 response (Pulendran and Artis, 2012; Strid et al., 2011). That Th2 immunity, in turn, sets tissue repair pathways in motion provides further evidence for its tight coupling to barrier integrity (Pulendran and Artis, 2012). While it is not clear what barrier mechanisms may be defective in allergic individuals, our modern environment harbors clues. In this context, the rising prevalence of food allergies in westernized societies parallels that of other allergic and inflammatory disorders such as asthma, inflammatory bowel disease and diabetes (Thorburn et al., 2014). These diseases, and others, are increasingly associated with demographic variables uncommon in earlier generations that have become widespread in the 21st century including Caesarean birth, formula feeding, repeated exposure to antibiotics and consumption of a diet of processed foods high in fat and sugar, and low in dietary fiber (Cho and Blaser, 2012; Feehley et al., 2012). What these lifestyle practices have in common is their ability to alter the composition of the bacterial populations that live in and on our bodies.

Acquisition of a commensal microbiota

Changes that occur during infancy and early childhood seem to be particularly important. The last ten years has witnessed an explosion of information about microbial symbionts, which outnumber the cells of their eukaryotic hosts by at least ten fold (Savage, 1977). Commensal bacteria are among the best characterized, but other microbial populations including viruses, bacteriophage and fungi abound. It is clear that the influence of these microbial inhabitants on their hosts' physiology is profound. Nowhere is this more apparent than in the regulation of immunity at the mucosal barriers that form the body's interface with the external environment (Cho and Blaser, 2012). Characteristic commensal bacteria occupy different anatomic sites (Cho and Blaser, 2012; The Human Microbiome Project Consortium, 2012). Maintenance of homeostasis is a particular challenge in the intestines,

the lumen of which contains, in addition to the many potential antigens present in food, trillions of bacteria that increase in density from its proximal to distal end (Donaldson et al., 2015). Initial colonization occurs at birth and, during natural delivery, bacteria derived from the vagina and feces provide the founder population for the neonate (Koenig et al., 2011; Pantoja-Feliciano et al., 2013). Breast milk further shapes the diversification of the microbiota by providing a rich source of secretory IgA, as well as prebiotic glycans that promote the expansion of species adapted to utilize this food source (Barile and Rastall, 2013; Coppa et al., 2004; Rogier et al., 2014). Caesarean birth and formula feeding disturb this co-evolved host–microbe developmental strategy. In infants born by Caesarean section, bacterial populations derived from the skin of the mother or caregiver predominate (Dominguez-Bello et al., 2010; Mueller et al., 2015). Formula feeding may exacerbate this effect; since our knowledge of all of the factors that influence the emergence of the neonatal microbiota is incomplete, pre or probiotic supplemented infant formulas may not fully replicate the effect of breast milk on neonatal microbial succession and stability (Guaraldi and Salvatori, 2012). The composition of intestinal bacterial communities is plastic during infancy and early childhood and continues to change rapidly in response to environmental interventions, including invasion by pathogenic microorganisms, antibiotic treatment and diet (Dominguez-Bello et al., 2011), which may, in turn, affect immune homeostasis. Not surprisingly, antibiotic use profoundly impacts these developing microbial communities; emerging epidemiological data links pre-natal and early post-natal antibiotic use to the subsequent development of atopic dermatitis (Lee et al., 2014) and cow's milk allergy (Metsala et al., 2013).

The early life window of opportunity

Perturbations in the composition of the commensal microbiota during a neonatal window of opportunity around the time of weaning have been associated with the development of atopy in various murine models (Bashir et al., 2004; Hill et al., 2012; Olszak et al., 2012; Russell et al., 2012; Stefka et al., 2014). Recent analysis of a large prospective birth cohort supports the concept that gut dysbiosis during the first 100 days of life can influence the subsequent development of allergic disease (Arrieta et al., 2015). Bacteria in the genera *Lachnospira*, *Veillonella*, *Faecalibacterium* and *Rothia*, normally abundant, were selectively depleted in samples obtained at three months of age in infants who exhibited wheezing and positive allergen skin prick testing at twelve months when compared to controls that didn't exhibit this clinical phenotype. This dysbiosis was no longer detectable in fecal samples obtained at the twelve-month time point (Arrieta et al., 2015; Dominguez-Bello and Blaser, 2015). Studies that predate the advent of high throughput sequencing methodologies have associated commensal dysbiosis with the pathogenesis of allergic disease (Bjorksten et al., 1999; Bjorksten et al., 2001; Kalliomaki et al., 2001). More recent work has confirmed and extended these earlier findings (Nakayama et al., 2011; Thompson-Chagoyan et al., 2011; Thompson-Chagoyan et al., 2010) (Abrahamsson et al., 2012; Azad et al., 2015; Ling et al., 2014; Nylund et al., 2015; Penders et al., 2013). Berni Canani et al showed that the intestinal microbiota of infants allergic to cow's milk is significantly more diverse than that of healthy age-matched controls when obtained at the time of diagnosis at 4–5 months of age (Berni Canani et al., 2015). Early life changes in fecal bacterial composition and diversity present a

"chicken and egg" conundrum. Do environmentally induced changes in the intestinal microbiota drive allergic disease by limiting microbial diversity and depleting populations of bacteria with barrier-protective function? Or is intestinal dysbiosis itself the consequence of allergic inflammation, as some murine model work seems to suggest (Noval Rivas et al., 2013)? These two scenarios are not mutually exclusive as they may operate at different points in space and time. In this context, the intestinal microbiota is likely to have multiple complex roles in initiating, regulating and promoting allergic sensitization.

The skin microbiome and epicutaneous sensitization

The allergic march typically manifests first in the skin, suggesting that this site may have either a particular role in initiating allergic sensitization or the lowest threshold to manifest symptoms. The skin has a site-specific microbiome that varies from region to region; different niche-specific communities populate moist or dry areas (Belkaid and Segre, 2014). In patients with atopic dermatitis, marked reductions in skin microbial diversity occur during disease flares; effective treatment restores diversity to the skin bacterial community (Kong et al., 2012). Flares are characterized by an increased abundance of pathogenic *S. aureus* (which accounts for the overall drop in microbial diversity). In mice, the release of δ-toxin by *S. aureus* induces mast cell degranulation and exacerbates allergic sensitization to a model antigen applied to tape-stripped skin (Nakamura et al., 2013). Mutations in filaggrin —a gene product required for skin epithelial barrier function—are strongly associated with atopic dermatitis in genome wide association studies (Baurecht et al., 2007; Brown et al., 2008; Filipiak-Pittroff et al., 2011; McAleer and Irvine, 2013; Palmer et al., 2006; Palmer et al., 2007; Weidinger et al., 2006). Accordingly, atopic-like skin lesions develop, and allergen priming is facilitated, in mice homozygous for a spontaneous deactivating mutation in the gene encoding filaggrin (designated $Flg^{ft/ft}$ for flaky tail mutant) (Fallon et al., 2009). In addition, while spontaneous dermatitis appears to develop to the same degree in germ free (GF) and specific pathogen-free (SPF) $Flg^{ft/ft}$ mice, commensal bacteria are important for IL17A expression as well as eosinophil and neutrophil recruitment to sites of inflammation in these animals (Hoff et al., 2015). In the setting of impaired epithelial barrier function, environmentally induced perturbations in skin microbial communities (particularly those that increase *S. aureus* abundance) may influence allergic sensitization. Clinical sensitization to peanut may be primed via the skin through the use of peanut-containing oils (Lack et al., 2003) or environmental exposure to peanut allergens in house dust (Brough et al., 2015; Brough et al., 2013). In this regard, future work promises to determine the degree to which molecular and clinical sensitization (Box 1) occurs parenterally, and is expected to identify the extent to which microbial communities influence this process.

Mechanisms regulating non-responsiveness to dietary antigen

Oral tolerance can be defined as the induction of mucosal and systemic non-responsiveness to dietary antigen (Iweala and Nagler, 2006; Pabst and Mowat, 2012). In experimental models of oral tolerance, the ability of intragastric administration of soluble protein antigens to induce systemic non-responsiveness is revealed by subsequent peripheral immunization with the same antigen in adjuvant. As such, these experimental models are imperfect surrogates for the physiologic process that induces tolerance to food that they are intended to

represent. The accepted paradigm has been that oral tolerance is mediated primarily by the induction of antigen specific Foxp3$^+$ regulatory T cells (Tregs) (Pabst and Mowat, 2012). Specialized populations of dendritic cells (DC) are thought to carry antigens that are able to cross the epithelial barrier to the mesenteric lymph nodes (MLN) that drain the gut associated lymphoid tissue (GALT). In the MLN, antigen presentation to naïve T cells in the presence of TGF–β and retinoic acid induces upregulation of Foxp3 and expression of the gut homing receptors α4β7 and CCR9, allowing these newly differentiated Tregs to home back to the intestinal lamina propria (LP) to protect against subsequent allergen challenge (Hadis et al., 2011).

Restoration of tolerance has been the guiding principle behind oral (Wood and Sampson, 2014) and epicutaneous (Mondoulet et al., 2015) approaches to immunotherapy, in which small increasing doses of allergen are administered via ingestion or skin patch to patients with food allergies. The clinical efficacy of either approach is variable, for unknown reasons. Moreover there is little evidence for the ability of either mode of immunotherapy to induce long-lasting tolerance (as opposed to transient desensitization) in the absence of ongoing allergen administration (Wood and Sampson, 2014). The timing of antigen administration is clearly important; introduction of allergenic foods early in life seems to be optimal. An increasing prevalence of peanut allergy was already apparent by the year 2000, prompting the American Academy of Pediatrics to caution parents to withhold peanuts from children with a family history of atopy until age 3 (Gruchalla and Sampson, 2015). Yet, the prevalence of peanut allergy continued to rise (Branum and Lukacs, 2008; Jackson et al., 2013). A recent large-scale randomized trial (LEAP study) showed that early introduction of peanuts to high-risk infants who were not yet clinically reactive greatly *reduced* the incidence of peanut allergy in this population as determined by oral food challenge at five years of age (Du Toit et al., 2015). Allergy prevention by early life dietary exposure may be mechanistically distinct from oral or epicutaneous desensitization protocols administered to patients who are already clinically sensitized. While recent peanut avoidance practices in certain countries may have contributed to increased clinical sensitization to peanut, it is important to note that the delayed introduction of potentially allergenic foods cannot explain the full magnitude of a generational increase in food allergies overall.

Recent work has proposed that, in addition to the induction of antigen specific Tregs, the maintenance of tolerance to dietary antigen requires an epithelial barrier-protective response mediated by a class of mucosa-associated commensal anaerobes (Cao et al., 2014; Stefka et al., 2014). Atarashi et al originally reported that anaerobic Firmicutes, in particular the Clostridia class, are critical for the induction of Foxp3$^+$Tregs in the intestinal LP (Atarashi et al., 2011). Both immunological and physical adaptations are required to fortify the single-cell layered intestinal epithelium from continuous bombardment by the wide variety of dietary and microbial antigens continuously present in its lumen (Cao et al., 2014; Iweala and Nagler, 2006; Nagler-Anderson, 2001). Mouse studies suggest that many of these specialized physical adaptations are regulated by IL-22; its production by RORγt$^+$ innate lymphoid cells (ILC3) controls enterocyte proliferation, the production of mucus and secretion of the anti-microbial peptides (AMPs), such as RegIIIβ and RegIIIγ (Sabat et al., 2014). Microarray analysis of epithelial cells isolated from differentially colonized gnotobiotic mice demonstrated that Clostridia colonization selectively induces the

expression of the anti-microbial peptide *Reg3b*, implicating activation of the IL-22/IL-23 axis (Stefka et al., 2014). Treatment of mice with neutralizing antibodies to IL-22 reduced the expression of *Reg3b/g* and increased Clostridia abundance (Stefka et al., 2014).

How does innate immune signaling by Clostridia regulate allergic sensitization to food? When dysbiosis was induced by antibiotic treatment of neonatal mice high concentrations of the peanut allergens Ara h 2 and Ara h 6 were detected in serum upon oral food challenge at one week post weaning (Stefka et al., 2014). Clostridia-induced IL-22 was necessary and sufficient to significantly reduce serum peanut allergen levels. Detection of Ara h 2 and Ara h 6 by capture ELISA indicated that these proteins reached the systemic circulation with their secondary structure (B cell epitopes) intact. Resistance to degradation may, in fact, be a distinguishing feature of food allergens (Valenta et al., 2015). The relative roles of antigen specific and bacteria induced Foxp3^{+}Tregs in the intestinal LP has not yet been examined. The repertoire of T cell receptors detectable on Tregs in the gut is distinct from that found in peripheral sites; at least some bacteria induced Tregs are likely to recognize bacterial antigens (Lathrop et al., 2011). Other mucosa-associated bacterial populations may also stimulate innate immune signaling pathways in the host through direct cell-to-cell contact or by their secretion of metabolites. Several reports have shown that bacteria-produced short chain fatty acids (SCFAs) regulate both the proportions and functional capabilities of intestinal Tregs (Arpaia et al., 2013; Furusawa et al., 2013; Smith et al., 2013). In this context, both antigen-specific and bacteria-induced Tregs cooperate with ILC-produced IL-22 to maintain barrier homeostasis.

Education of B cells in the intestinal mucosa

IgE specific for food allergens is required (though not sufficient, see Box 1) for the development of an anaphylactic response to food. But the mechanisms for development of Ig against dietary substances, and the morphing of food protein-reactive Ig into IgE, are poorly understood. The pre-immune Ig repertoire is generated in developing bone marrow (BM) B cells through RAG-dependent DNA assembly of V, (D), and J gene segments resulting in production of IgM, which serves as the B cell receptor (BCR). Immature B cells undergo receptor editing, where continued RAG expression and V(D)J recombination leads to changes in BCR specificity, which are thought to facilitate the development of B cell tolerance (Nemazee, 2000; Yurasov and Nussenzweig, 2007). As essentially all studies examining BCR editing have utilized BCR transgenic or knock in mice—which artificially and severely limit the Ig repertoire—its role in regulating the natural repertoire is not clear. Several other poorly defined selection events also play a significant role in shaping the primary Ig repertoire during maturation through the transitional-to-mature B cell stages (Levine et al., 2000). Identifying the extent to which luminal antigens (both microbial and food) regulate these primary Ig repertoire-modifying B cell selection events may help shed light on mechanisms of molecular sensitization.

After the primary Ig repertoire is established, activated mature B cells can undergo secondary Ig diversification events, namely, IgH class switch recombination (CSR), and Ig variable region exon somatic hypermutation (SHM). The IgH constant region exons (C_Hs) are arranged in tandem, beginning with Cμ (that encodes the IgM constant region) at the 5′

end. CSR replaces initially expressed IgM with other isotypes such as IgG, IgE, or IgA by targeted repositioning of the alternative IgH locus constant region exons (e.g. Cγ, Cε, Cα), which results in deletion of intervening C_Hs. SHM can lead to affinity maturation through iterative rounds of mutation and selection in germinal centers (Tong and Wesemann, 2015). Allergen-reactive B cells that undergo IgH CSR to IgE have the potential to elicit symptomatic allergy. However, if these B cells remain unswitched, or undergo IgH CSR to IgG or IgA instead of IgE, molecular sensitization does not occur. IgG^+ B cells may therefore house a reservoir of memory B cell specificities which can sequentially switch to IgE (He et al., 2015). Unlike IgG, CSR to IgA in mice (or IgA2 in humans), precludes that particular clone from future switching to IgE, or any other IgH isotype, because the exons encoding IgA (mice) and IgA2 (humans) occupy the 3′ most part of the IgH locus.

In terms of food allergy, the degree to which SHM is required for the development of allergen reactive B cells undergoing CSR to IgE is not known. While allergen sensitization may occur by cutaneous exposure as mentioned above, most food allergen contact likely occurs in the gut mucosa where commensal microbes are required to generate an environment that favors B cell CSR to IgA. Accordingly, GF mice have minimal IgA production, and this appears to license abnormally high levels of mucosal B cell CSR to IgE (Cahenzli et al., 2013). In this regard, an environment permissive for IgE may potentially result from insufficient stimuli from gut microbes. A non-mutually exclusive alternative is that defective barrier function may allow increased allergen access to peripheral lymphoid tissue where cognate B cell CSR to IgE may take place.

In addition to the lack of clarity regarding where IgE CSR occurs, how food allergen-reactive specificities appear within the Ig repertoire in the first place is not understood. As mentioned above, recent work supports the idea that the intestinal microbiota influences lymphocytes and their receptor repertoires (Chai et al., 2014; Lathrop et al., 2011; Wesemann et al., 2013). The first hint that contents of the gut lumen influence the primary Ig repertoire came from studies in chickens, where B cell development and diversification takes place in the bursa of Fabricius, an outpouching of epithelium within the avian hindgut (Weill and Reynaud, 1987). More recent work in chicks (Davani et al., 2014), newborn rabbits (Rhee et al., 2005), and lambs (Mutwiri et al., 1999) shows that the B cell repertoire in the GALT is positively selected toward gut luminal content early in life. The mouse small intestinal LP harbors early developing B cells that peak around weaning age, when V(D)J recombination and receptor editing processes modulate primary LP Ig repertoires (Wesemann et al., 2013). Consistent with LP-specific receptor editing, RAG-expressing LP B-lineage cells have similar V_H repertoires, but significantly different Vκ repertoires, when compared to those of Rag2-expressing BM counterparts. Colonization of GF mice results in an increased ratio of Igλ- versus Igκ-expressing B cells specifically in the LP, indicating that microbial antigens and/or their products may drive early mucosal B cell selection (Wesemann et al., 2013). The degree to which positive versus negative selection takes place at the receptor editing stage in the setting of a polyclonal repertoire remains to be determined. In addition, the role of dietary versus microbial antigens in influencing selection events awaits future elucidation. In humans, B cell processes in neonatal intestine have not yet been examined, but precursor B cells have been observed in fetal intestine (Golby et al.,

2002), and transitional B cells have also been seen in human adult gut tissue (Vossenkamper et al., 2013).

IgA at the Mucosal Barrier

While IgE and the IgG isotype subclasses largely function within host circulation and tissues, both IgA and IgM can bind to immunoglobulin J (joining) chain, which enables transport across the epithelium and release into the lumen (Macpherson et al., 2008). IgA is dominant when sheer amount is taken into consideration. More IgA is produced than all other IgH isotypes combined (Conley and Delacroix, 1987), and most of this IgA is secreted from mucosal tissues. Until relatively recently IgA was thought to function primarily by binding to luminal content and limiting antigen access to host tissues (referred to as immune exclusion (Pabst, 2012)). IgA may also play a role in shaping the composition of the commensal microbiota and governing the establishment of its mutualistic relationship with its host (Macpherson et al., 2015; Peterson et al., 2007). The aberrant expansion of pathobionts in mice deficient in activation induced activation-induced cytidine deaminase (which cannot undergo CSR) provided the first clue that IgA and SHM may be required to maintain commensal homeostasis (Fagarasan et al., 2002; Suzuki et al., 2004; Wei et al., 2011). Interestingly, $Foxp3^+$ Tregs contribute to IgA selection, forming a coordinated axis to regulate and potentially diversify the composition of the microbiota in the gut (Cong et al., 2009; Kawamoto et al., 2014; Tsuji et al., 2009).

The physiologic advantage of early life B cell selection in the proximity of gut content, as discussed in the animal examples above, is not yet clear. It is possible that gut microbe-selected primary (IgM) repertoires may remain localized in the GALT and serve as a foundation for both CD4 T cell (T) independent and T dependent IgA production. Commensal microbes can elicit T independent IgA production with antibacterial specificity (Macpherson et al., 2000; Shroff et al., 1995) with contributions from both conventional (B2) and innate (B1b) B cell lineages (Bunker et al., 2015). T independent production of innate-like, polyreactive IgA may facilitate non-invasive commensal bacterial uptake into Peyer's patches in the small intestine, enabling the production of T dependent, commensal specific IgA (Fransen et al., 2015). T independent IgA responses are also elicited by ILC3-derived lymphotoxin (LT)-α3, linking ILC3 to both innate and adaptive barrier responses (Kruglov et al., 2013). Gut microbe-primed primary Ig repertoires may thus provide a reservoir of luminal content-reactive B lineage cells as T-independent IgA precursors, distinct from the T-dependent IgA induced by toxins and invasive organisms (Pabst, 2012; Palm et al., 2014).

In terms of dietary allergen reactivity, non-IgE isotypes may also have a regulatory function. This could be particularly relevant for IgA against food proteins that resist degradation in early digestion processes. IgA coating may reduce the chance that allergens gain access to the epithelium, slowing movement in the mucus layer (Lai et al., 2009; Olmsted et al., 2001), thus inhibiting allergen contact and entry. Clinically, however, selective IgA-deficiency is a relatively common immune deficiency and is rarely associated with symptomatic disease (Hammarstrom et al., 2000), possibly due to compensating secreted IgM (Macpherson and McCoy, 2015). Although several studies do not find an association of

IgA deficiency and atopy (Franco et al., 2011; Kanok et al., 1978; Plebani et al., 1987), a study of 2423 Swedish children, reported that 33% of those identified with IgA deficiency had food allergy when compared to 12% of their IgA-sufficient counterparts at 4 years of age (Janzi et al., 2009). Larger studies will be required to identify the potential role of mucosal IgA and IgM in the regulation of food allergen sensitization.

The barrier regulation hypothesis of allergic sensitization to food

The immune system can be viewed broadly as an essential component of our ability to co-exist with an environment that is abundant in both innocuous exposures and infectious or noxious threats. A complicated, multiply redundant, system with both innate and adaptive components is required to maintain homeostasis at barrier surfaces to eliminate potentially harmful foreign substances while avoiding pathological responses to commensal microbes or food. Emerging evidence is consistent with a *barrier regulation hypothesis* of allergic sensitization (Figure 1) which views allergy as an additional layer of protection against alien elements that somehow penetrate other layers of this system. In the healthy state, a microbiome replete with mucosa-associated taxa (like the Clostridia) that regulate the production of IL-22 stimulates the intestinal mucosa to produce a protective mucous layer and AMPs that titrate the abundance of this bacterial community. These bacteria-induced barrier protective functions reduce the ability of food allergens to cross the epithelial barrier and gain access to the systemic circulation. Early life luminal content induces an IgM and IgA response that shapes the repertoire to aid in immune exclusion. Perhaps IgA targeting of mucin degrading bacteria is also important early in life to protect the developing mucus barrier of the small intestine and limit uptake of dietary allergens. Potentially noxious (or noxious by proxy) food proteins that evade digestion may be excluded by either (or both) of these barrier mechanisms. Defects in innate or adaptive bacteria-induced barrier protective responses may exacerbate genetic predispositions (e.g. mutations in filaggrin) that render the host susceptible to allergen contact and entry and elicit direct or indirect stress in the epithelial barrier, particularly in the skin or intestinal mucosa.

Barrier surfaces are necessarily poised in an anti-inflammatory state. Upon allergen-mediated activation epithelial cells secrete cytokines including TSLP, IL-33, and IL-25 that educate dendritic cells and ILC2 to promote Th2 immunity (Peterson and Artis, 2014). In this context, Tregs are “reprogrammed” to the Th2 lineage (Noval Rivas et al., 2015) and ILC2 secrete IL-5 and IL-13 (Lee et al., 2015), promoting the induction of an allergic response—including B cell IgH CSR to IgE, which may occur in situ (Cahenzli et al., 2013) in addition to organized lymphoid tissue, where most CSR to IgE likely takes place (Figure 1). Allergic disease can be viewed, in this context, as a consequence of environmentally induced dysregulation of the epithelial mucosal barrier (Cahenzli et al., 2013; Hill et al., 2012; Prioult and Nagler-Anderson, 2005).

The *barrier regulation hypothesis* suggests that the increased amounts of food-allergen specific IgG and IgA (in addition to IgE) detectable in atopic individuals is a reflection of reduced barrier exclusion that results in increased allergen availability in the systemic circulation (Shek et al., 2005). In the absence of commensal dependent barrier regulation, GF mice have reduced mucosal production of IgA and concomitant increases in IgE

production (Cahenzli et al., 2013; Herbst et al., 2011; Hill et al., 2012; McCoy et al., 2006; Stefka et al., 2014) and exhibit exaggerated responses to both aero (Herbst et al., 2011) and food (Stefka et al., 2014) allergens. Reducing the intestinal bacterial load in wild-type mice by treatment with broad-spectrum antibiotics raises the concentration of serum IgE (Hill et al., 2012). Microbial exposure early, but not late, in life is required for regulating IgE levels (Cahenzil et al., 2013) consistent with an early life window of time when luminal content maximally exerts its influences on the immune system.

Concluding Remarks and Future Perspectives

The dramatically increased incidence of allergic disease over a few decades suggests that allergic susceptibility is influenced by environmental factors—which offers new hope for effective treatments. This environmental component to sensitization indicates that the allergic state is plastic and can thus be modulated. As the factors that contribute to sensitization are discovered, innovative modifications can be engineered to reduce the burden of allergic disease. Some of the outstanding questions and challenges include the identification of the factors that license molecular sensitization to become clinical sensitization, how to achieve sustained unresponsiveness rather than transient desensitization, the effect of the environment on Ig selection, as well as IgE production, maintenance, and memory, and what regulates the allergic march.

The microbiota is one target already identified that has the potential to modulate responses at multiple points along the continuum of allergic disease. Microbiome-modulating strategies may be efficacious as either a preventive therapy (to restore functionality in the context of environmentally induced dysbiosis) or as an adjunctive treatment co-administered with orally administered allergens. Ingestion of health-promoting bacterial preparations, or probiotics, has a long history of use and study in the context of treatment of both allergic and inflammatory disease (Hill et al., 2014). Evidence for efficacy has largely been confined to early infancy; a meta-analysis of clinical trials determined that prenatal, or early post-natal, administration of probiotics reduced total IgE levels and the risk of atopic sensitization, but not asthma or wheezing (Elazab et al., 2013; Fiocchi et al., 2015). Counteracting the dysbiosis-associated dysregulation and other potential barrier-related defects that have contributed to recent increases in allergic disease is an attractive prospect for the development of a new class of microbiome-modulating therapeutic approaches (Olle, 2013).

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Box 1

What is Sensitization?

Treating and curing food allergy will likely require experts in multiple fields of clinical and basic sciences. A clear language can enhance communication and facilitate scientific advances. Over the past decades, words such as sensitization, desensitization and tolerance have become confusing in the setting of dialogue between clinicians and scientists. As an attempt to align language in a mechanistic context and to foster clarity between fields, we propose that the following clarifications to the nomenclature be considered.

Molecular sensitization

The presence of IgE specific for a food allergen. Molecular sensitization is not sufficient for clinical sensitization.

Clinical sensitization

A state where allergic symptoms are expected to appear upon exposure to the allergen based on history or testing. This is established by a clinical history of unambiguous association and/or by oral food challenge in allergist's office. This term is similar to what some refer to as being clinically reactive.

Clinical desensitization

The loss of clinical reactivity to an allergen for which one was previously clinically sensitized. In food allergy, this often occurs in the setting of an experimental desensitization program with escalating doses of the particular food. Clinical desensitization often occurs without molecular desensitization, but the desensitized state, if achieved, may require continued allergen exposure.

Molecular desensitization

The loss of measureable allergen-specific IgE.

Sustained unresponsiveness

Acquisition of persistent non-reactivity after clinical desensitization.

Tolerance

Systemic non-responsiveness to dietary antigen mediated by an immunological process that actively maintains mucosal homeostasis. Maintenance dosing is not required.

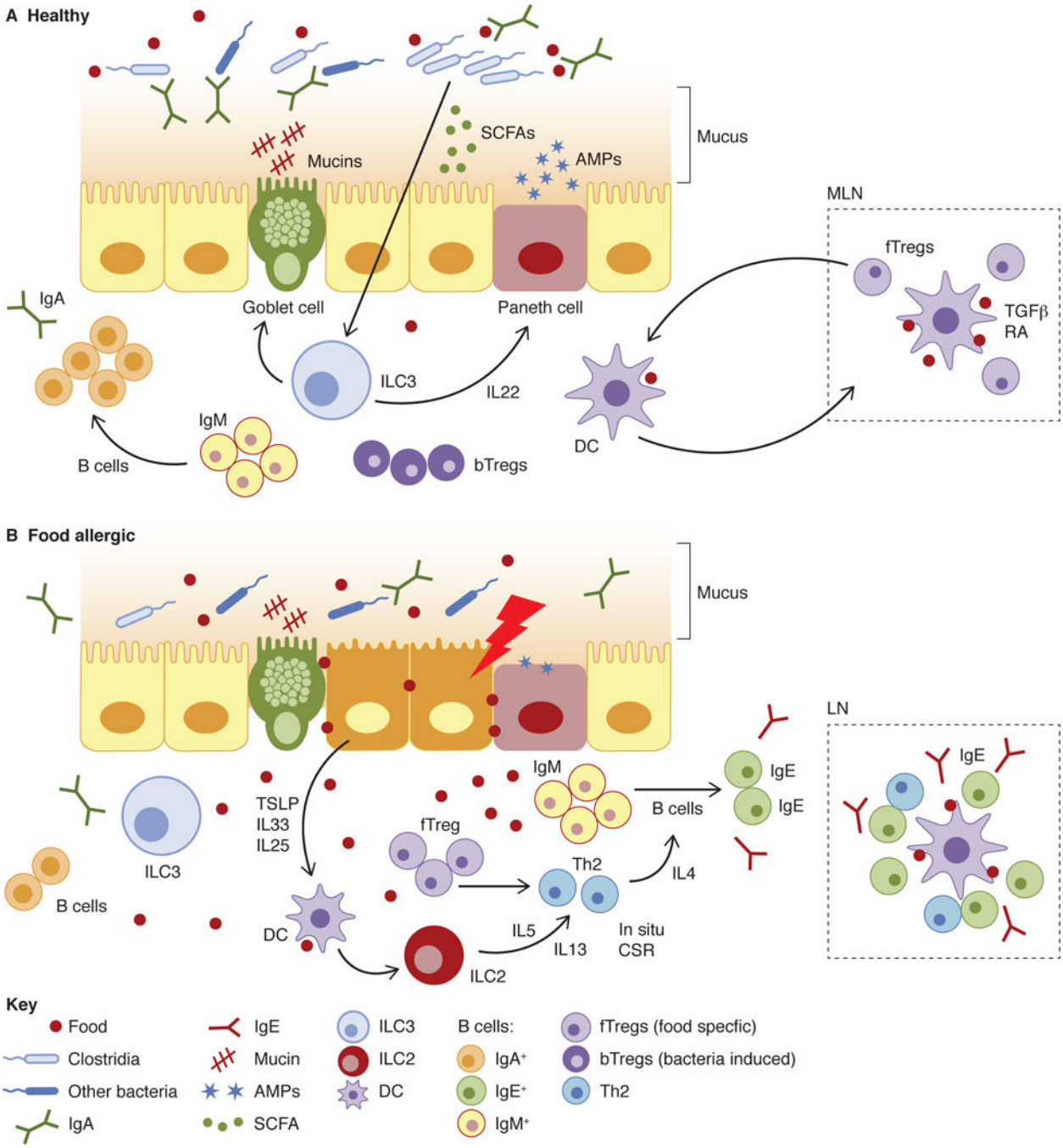

Figure 1. The barrier regulation hypothesis of allergic disease

(**A**) In healthy individuals, both food allergen specific and bacteria-induced Tregs cooperate with ILC-derived IL-22 dependent effector functions (e.g. mucus secretion, induction of AMPs) to maintain barrier integrity and mucosal homeostasis. Allergy protective bacterial populations residing in the proximal colon may secrete metabolites or influence cellular migration from that site to regulate allergen uptake in the small intestine. (**B**) In food allergic subjects, the loss of these allergy protective bacterial populations impairs barrier integrity. Increased allergen contact and depletion of metabolites like SCFAs stress the epithelial layer.

Both DC and ILC2 are primed by epithelial derived cytokines to induce a Th2 response and CSR to IgE, both locally and in peripheral LN.