Development of Normal Tolerance


‘ABC’ of Mucosal Immunology

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Abstract

Two adaptive homeostatic mechanisms normally preserve mucosal integrity: (i) immune exclusion mediated by secretory antibodies to inhibit penetration of potentially dangerous microorganisms and proteins, and (ii) immunosuppression to counteract hypersensitivity against innocuous antigens. The latter mechanism is called ‘oral tolerance’ when induced via the gut. Similar mechanisms are suppressive against commensal bacteria. Such two-layered anti-inflammatory defense explains why persistent allergy to dietary proteins is not more common, with the exception of gluten intolerance (celiac disease) where abrogation of mucosal homeostasis is overt. Thus, mucosally induced tolerance is generally a robust adaptive mechanism in view of the fact that a ton of food may pass annually through the gut of an adult – regularly giving rise to uptake of intact dietary antigens in the nanogram range after a meal. However, the immunoregulatory network and the epithelial barrier are poorly developed in the neonatal period, which therefore is critical with regard to priming for allergy. Notably, the postnatal development of mucosal immune homeostasis depends on appropriate microbial colonization. In this process, antigen-presenting cells are ‘decision makers’, linking innate and adaptive immunity. Their microbe-sensing function is influenced by both microbial products and dietary constituents, including vitamin A and lipids such as polyunsaturated n-3 fatty acids.

The body is under constant threat of attack by viruses, bacteria and parasites. Evolution has therefore provided mammals with a multilayer immunological defense system. Microorganisms have inhabited earth for at least 2.5 billion years, and the power of immunity is a result of co-evolution in which especially commensal bacteria and parasites have shaped the body’s defense with its two general arms: innate (natural or nonspecific) and adaptive (acquired or specific) immunity. Notably, the adaptive immune system developed rather late in the phylogeny, and most species survive without it.
Mammalians, however, have an extremely sophisticated adaptive immune system of both systemic and mucosal type. There is great redundancy of mechanisms in both systems, providing robustness to preserve homeostasis.

**Innate and Adaptive Immunity**

The attempt of a microbe to invade the body will immediately be counteracted by the innate immune system, which comprises surface barriers, soluble factors, professional phagocytes (e.g., granulocytes and macrophages), and dendritic cells (DCs). These mechanisms restrict invasion of the body by foreign components and inhibit their persistence within the tissue: (a) physical/structural hindrance and clearance (epithelial linings, mucus, ciliary function, peristalsis); (b) chemical factors (pH of body fluids, antimicrobial peptides and proteins), and (c) phagocytic cells contributing to inflammation. Challenge of the innate system often leads to activation of the adaptive system, which by its effector and regulatory T and B cells aids substantially the recovery from a noxious impact.

In contrast to the antigen-specific surface receptors of T and B cells, which show a random and highly diverse repertoire, the recognition molecules of innate immunity are encoded in the germ line. This system is therefore quite similar among healthy individuals but receptor mutations may give rise to disease-promoting polymorphism. Innate responses show no classical memory – that is, re-exposure to a pathogen will normally elicit the same type of proinflammatory reaction, although a subsequent downregulation has been observed, probably to preserve tissue integrity [1].

The innate receptors sense conserved structures that are essential for microbial survival and present in a broad range of microbes including endotoxin (or lipopolysaccharide, LPS), teichoic acids, and unmethylated CpG motifs of DNA [2]. Such structures are generally referred to as pathogen-associated molecular patterns (PAMPs), but they also occur in commensal bacteria and should preferably be called microbe-associated molecular patterns (MAMPs). The intestinal microbiota induces distinct programming of the innate immune system which could partly explain tolerance by the host [3]. This is especially apparent for mucosal epithelia (see below).

The cellular receptors that recognize PAMPs/MAMPs are called pattern recognition receptors (PRRs) – many of them belonging to the so-called Toll-like receptors (TLRs). They are expressed by macrophages and DCs, and also by a variety of other cell types such as T and B cells and epithelial cells. The engagement of PRRs causes cellular activation (mainly via the transcription factor NF-κB), which in the case of antigen-presenting cells (APCs) such as DCs, leads to maturation accompanied by production of cytokines and upregulation or downregulation of cell-surface molecules according to strictly defined kinetics [2].
Cytokines are small hormone-like signaling molecules which stimulate cellular growth, differentiation and functional development via specific receptors on the producer cell itself (autocrine function) or on other cells (paracrine function). Cytokine action is not confined to the immune system; such peptides may also influence the central nervous system and the neuroendocrine system. Some of the first identified cytokines derived from lymphocytes are still designated by the prefix interleukin (IL). An important autocrine and paracrine growth factor secreted by stimulated T helper (Th) cells early in the immune response is IL-2; it is recognized by the heterodimeric IL-2 receptor – one of the two chains designated CD25, which is a useful activation marker.

Altogether, cytokines will critically influence induction of both innate and adaptive immunity with regard to effector potency, particularly the polarization of Th-cell responses in terms of differentiation pathway and further polarized cytokine production. Microorganisms may via PRRs imprint their ‘signatures’ on subsequent immune responses (fig. 1). Thus, the plasticity of the innate immune system prepares the ground for a targeted and powerful protective function of the adaptive immune system.

Engagement of other types of receptors on phagocytic cells such as immunoglobulin (Ig) Fc receptors and complement receptors, triggers phagocytosis and elimination of invading microorganisms. Although pathogens have evolved mechanisms to evade innate immunity (e.g., bacterial capsules), they can usually not persist within the body when an adaptive immune response reinforces innate immunity by providing specific antibodies directed against the invading pathogen or its toxins. Thus, innate immunity influences the character of the adaptive response, and the effector arms of adaptive immunity support several innate defense mechanisms. The nonspecific biological amplification collectively triggered by them is referred to as immune reaction – or hypersensitivity/allergy if clinical harm is observed as variations on the theme of inflammation (fig. 2).

**Mucosal Immune Strategies**

Mucosal immunity provides a first defense line that reduces the need for elimination of penetrating exogenous antigens by proinflammatory systemic immunity. The mucosal immune system operates by two adaptive noninflammatory mechanisms: (a) immune exclusion performed by secretory antibodies to inhibit surface colonization of microorganisms and dampen penetration of potentially dangerous exogenous proteins, and (b) immunosuppressive mechanisms to avoid local and peripheral hypersensitivity to innocuous antigens (fig. 3). The latter strategy is referred to as ‘oral tolerance’ when induced via the gut [4], and probably explains why overt and persistent hypersensitivity to food proteins is relatively rare, with the exception of celiac disease. A
similar downregulatory tone of the immune system normally exists against indigenous or commensal bacteria [5].

Mucosally induced tolerance appears rather robust in view of the fact that more than a ton of food may pass through the gut of an adult every year. After a meal intact antigens are taken up in the nanogram range, usually without...
causing harm. However, the neonatal period is critical, both with regard to infections and priming for allergic disease, because the epithelial barrier and the immunoregulatory network are poorly developed [6]. Notably, the development of immune homeostasis depends on the establishment of a balanced mucosal microbiota as well as on adequate timing and dose of dietary antigens when first introduced [4, 7].

Experiments have demonstrated the crucial role of microbial colonization in establishing [8] and regulating [9] the epithelial barrier. At least in mice, the beneficial effects of commensal bacteria are significantly mediated via PRRs expressed by the gut epithelium, particularly TLRs [10]. Polarized epithelial cells have the ability to dampen the proinflammatory effect of PRR-mediated signals coming from the luminal side [11]. However, after bacterial invasion,
PRR signaling from the basolateral side results in a high level of NF-κB activation with release of defensins to combat the infection [11].

Altogether, accumulating evidence suggests that barrier-related mucosal homeostasis is largely influenced by ‘cross-talk’ between epithelial cells and the underlying lamina propria cells, including macrophages, DCs and T cells [12]. In fetal life, murine gut epithelial cells are sensitive to microbial factors such as LPS because they intracellularly express the receptor for this MAMP, namely TLR4 [13]. Therefore, exposure to LPS in the vaginal tract during birth temporarily activates the neonatal epithelium so that it subsequently

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**Fig. 3.** Schematic depiction of two major anti-inflammatory immune mechanisms operating at mucosal surfaces. (1) Productive immunity providing immune exclusion limits epithelial colonization of pathogens and inhibits penetration of harmful foreign material. This first line of defense is principally mediated by secretory antibodies of the IgA (and IgM) class in cooperation with various nonspecific innate protective factors (not shown). The secretory antibodies are actively exported by the epithelial polymeric Ig receptor (pIgR), also called membrane secretory component. Secretory immunity is preferentially stimulated by pathogens and other particulate antigens taken up through thin M cells (M) located in the dome epithelium covering inductive mucosa-associated lymphoid tissue (fig. 4). (2) Innocuous soluble antigens (e.g., food proteins; magnitude of uptake indicated) and the indigenous microbiota are also stimulatory for secretory immunity (graded arrows), but induce mainly suppression of proinflammatory humoral immune responses (IgG and Th2 cytokine-dependent IgE antibodies) as well as Th1 cytokine-dependent delayed-type hypersensitivity (DTH) and Th17-dependent granulocytic reactions. This homeostatic Th-cell balance is regulated by a complex phenomenon called ‘oral tolerance’ when induced via the gut, in which induction of regulatory T cells is important. Their suppressive effects can be observed both locally and in the periphery.
becomes tolerant to MAMPs because of suppressed TLR signaling. In remarkable contrast, such epithelial tolerance does not develop in mice delivered by cesarean section [13]. These experimental observations may be related to the fact that children delivered by cesarean section are particularly prone to developing food allergy if they have a genetic predisposition [14].

**Secretory Antibodies Reinforce the Epithelial Barrier**

There are many structural, cellular, molecular and functional differences between the mucosal and the systemic immune system (table 1). Mucosal immunity is most abundantly expressed in the gut, and the intestinal mucosa of an adult contains at least 80% of the body's activated B cells – terminally differentiated to plasmablasts and plasma cells (PCs) [15]. Most mucosal PCs produce dimeric IgA which, along with pentameric IgM that likewise contains a polypeptide called 'joining' (J) chain, can be actively exported by secretory epithelia [15]. This external transport is mediated by the polymeric Ig receptor (pIgR), also known as membrane secretory component (SC). Immune exclusion is performed mainly by secretory (S)IgA, and to a lesser extent SIgM, in cooperation with innate nonspecific defenses (fig. 3). In newborns and subjects with selective IgA deficiency, SIgM antibodies are of greater importance than in healthy adults [16].

Immune-inductive mucosa-associated lymphoid tissue (MALT) resembles lymph nodes with B-cell follicles, intervening T-cell zones and a variety of APCs such as macrophages and DCs, but there are no afferent lymphatics (table 1). Exogenous stimuli therefore come directly from the mucosal surfaces via a follicle-associated epithelium containing specialized M cells, probably aided by DCs which may penetrate the epithelium with their processes [11]. In the intestine, induction and regulation of mucosal immunity hence takes place primarily in gut-associated lymphoid tissue (GALT) and mesenteric lymph nodes, and also to some extent at the effector sites to which activated T and B cells home (fig. 4). Retinoic acid derived from vitamin A in the diet appears to have a positive impact both on IgA PC differentiation and gut homing [17].

IgA-producing PCs are generally undetectable in the mucosa before 10 days of age, but thereafter a rapid increase takes place, although IgM-producing PCs often remain predominant up to 1 month [16]. Little increase in intestinal IgA usually takes place after 1 year. A much faster establishment of secretory immunity is often seen in developing countries with a heavy microbial load. The mucosal PC development reflects the progressive microbial stimulation of GALT [15]. Accordingly, only occasional traces of SlgA and SlgM occur in intestinal juice during the first postnatal period, whereas some IgG is often present – reflecting paracellular 'leakage' from the lamina propria that after 34 weeks of gestation contains readily detectable maternal IgG [16]. In addition, some IgG may be actively exported by epithelial FcRn.
Table 1. Characteristics of the systemic versus the mucosal immune system

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MALT = Mucosa-associated lymphoid tissue; GALT = gut-associated lymphoid tissue; PNAd = peripheral lymph node addressin; SLC = secondary lymphoid-tissue chemokine; ELC = Epstein Barr virus-induced molecule 1 ligand chemokine; CCR = CC chemokine receptor, MAdCAM-1 = mucosal addressin cell adhesion molecule 1; TECK = thymus-expressed chemokine; MEC = mucosae-associated epithelial chemokine.
**Fig. 4.** Schematic depiction of the human mucosal immune system. Inductive sites for mucosal T and B cells are constituted by regional mucosa-associated lymphoid tissue (MALT) with its B-cell follicles and M cell (M)-containing follicle-associated epithelium through which exogenous luminal antigens are actively transported to reach professional antigen-presenting cells (APCs), including dendritic cells (DCs), macrophages, B cells and follicular dendritic cells (FDCs). In addition, intra- or subepithelial DCs may capture antigens and migrate via draining lymph to regional lymph nodes where they become active APCs, which stimulate T cells for productive or downregulatory (suppressive) immune responses. Naive T and B cells enter MALT (and lymph nodes) via high endothelial venules (HEVs). After being primed to become memory/effector T and B cells, they migrate from MALT and regional lymph nodes via lymph and peripheral blood for subsequent extravasation at mucosal effector sites. This process is directed by the profile of adhesion molecules and chemokines expressed on the microvasculature, the endothelial cells thus exerting a local gatekeeper function for mucosal immunity (table 1). The mucosal lamina propria (effector site) is illustrated with its various immune cells, including B lymphocytes, J chain-expressing IgA and IgM plasma cells, IgG plasma cells with a variable J-chain level (J), and CD4+ T cells with effector (Teff) or regulatory (Treg) function. Additional features are the generation of secretory IgA (SIgA) and secretory IgM (SIgM) via plgR (SC)-mediated epithelial export, as well as paracellular leakage of smaller amounts (dashed arrow) of both locally produced and serum-derived IgG antibodies into the lumen. Note that IgG cannot interact with J chain to form a binding site for plgR. The distribution of intraepithelial lymphocytes (mainly T-cell receptor α/β+CD8+ and some γ/δ+ T cells) is also schematically depicted. Insert (lower left corner) shows details of an M cell and its ‘pocket’ containing various cell types.
Uptake of SIgA antibodies from breast milk via the neonatal gut mucosa is negligible and of no immunological importance in humans, except perhaps in preterm infants [18]. So-called ‘gut closure’ normally occurs mainly before birth, but the mucosal barrier may be inadequate up to 2 years of age; although the mechanisms involved remain poorly defined, the development of SIgA is one decisive variable. Importantly in this context, pIgR-deficient mice that lack secretory antibodies show reduced epithelial barrier function and increased uptake of antigens from food and commensal bacteria [19]. Also importantly, oral tolerance could not be induced in patients with inflammatory bowel disease where the epithelial barrier is deteriorated [20].

Mucosal Tolerance Induction and Abrogation

Oral tolerance, as defined by experimental feeding in mice, exhibits extensive biological complexity. Variables include genetics, age, dose and timing of postnatal oral antigen administration, antigenic structure and composition, epithelial barrier integrity, and the degree of concurrent local immune activation as reflected by local cytokine profiles and expression of co-stimulatory molecules on APCs [4]. In addition, there is an increasing awareness of the suppressive effects of various regulatory T (Treg)-cell subsets induced by conditioned APCs, particularly mucosal DCs as discussed later.

By dampening early immune activation (e.g., expression of co-stimulatory molecules), the shielding effect of maternal SIgA on the breastfed infant’s GALT may contribute to hyporesponsiveness not only against commensal bacteria, but also against dietary antigens such as cow’s milk proteins and gluten [6, 21]. Antibodies to food constituents are present in breast milk, and breastfeeding protects against the development of celiac disease in children, unrelated to the time of solid food introduction. The balance of evidence suggests that exclusive breastfeeding also protects against allergic disorders such as atopic dermatitis and asthma, particularly in genetically predisposed children [22]. On the basis of these observations, it can tentatively be concluded that mixed feeding, rather than abrupt weaning, promotes tolerance to food proteins.

This notion is supported by studies reporting that cow’s milk allergy is more likely to develop in infants whose mothers have relatively low levels SIgA antibodies to bovine proteins in their milk [6]. The presence in breast milk of the immunosuppressive cytokine transforming growth factor (TGF)-β might further contribute to oral tolerance because of its downregulatory effect on GALT and enhancing effect on the epithelial barrier [6, 23]. Also, small amounts of food antigens in breast milk may in fact enhance tolerance induction [24].

Understanding the immunological mechanisms involved in development of mucosal homeostasis versus abrogation of tolerance to dietary antigens is of
fundamental interest. Food allergy clearly reflects a break in oral tolerance and may be the starting point for an ‘allergic march’ – that is, the development of subsequent allergic diseases in the airways which particularly takes place in atopic individuals with a genetic predisposition [25].

Resident APCs from healthy human gut mucosa are quite inert in terms of productive stimulatory properties, and they do not express detectable surface levels of TLR2 or TLR4 [19]. Furthermore, only negligible levels of the LPS receptor CD14 are normally present on these cells, and their proinflammatory cytokine response is usually low [19]. Thus, in a quiescent steady state intestinal DCs carry penetrating dietary and innocuous microbial antigens away from the mucosa to the mesenteric lymph nodes [25] where the same cells, in a normal maturation process, become conditioned for tolerance induction and drive the expansion of Treg cells [26]. Hyperactivation of immunological effector cells in the mucosa, with accompanying inflammation, is thereby avoided both initially and also subsequently because of homeostatic control when the Treg cells home to the lamina propria (fig. 4).

Conditioning for tolerance in mucosa-draining lymph nodes (fig. 4) appears to depend on stimulation of the migrating APCs by MAMPs of certain commensals (fig. 1), or by components of parasites such as helminths [27], in concert with TGF-β, IL-10, IL-2 and retinoic acid from vitamin A [28]. Several studies suggest that LPS plays a central role in such early programming of the immune system [27], and allergy is associated with functional mutations in PRRs recognizing this and other MAMPs, including CD14, TLR2, TLR4 and NOD [29]. The ‘extended hygiene hypothesis’ therefore suggests that suboptimal PRR stimulation and delayed maturation of the mucosal immune system contribute significantly to the increasing incidence of not only allergy, but also other immune-mediated inflammatory disorders [27].

This emerging concept has been tested in several clinical studies evaluating the beneficial effect on immune homeostasis exerted by probiotic preparations derived from intestinal commensals and eggs of the porcine helminth (whipworm) *Trichuris suis* [27]. In this context, viable strains of lactobacilli and bifidobacteria have been reported to enhance IgA responses, both in humans and experimental animals [25]. In a double-blind study of infants with a family history of atopy, the prevalence of atopic dermatitis was reportedly reduced by 50% at the age of 2 years when the probiotic *Lactobacillus* GG strain was administered to the mothers before birth and to the babies daily for 6 months; the positive effect was found to be maintained after 4 years [30].

It is unclear whether such results are explained by SIgA-mediated reinforcement of the intestinal barrier, expansion of Treg cells, or the involvement of both these anti-inflammatory mechanism – perhaps combined with direct strengthening of epithelial integrity (fig. 3). Notably, atopic dermatitis is associated with loss-of-function mutations in the filagrin gene which contributes to the epidermal barrier; similar mutations appear to predispose for the combination of asthma and atopic dermatitis [29]. These striking
findings suggest that a leaky epithelium is an important variable in allergy induction.

**Innate Decision-Making Directing Adaptive Immunity**

The power of the immune system is a result of co-evolution of the host with microorganisms that have shaped its defenses in a state of mutualism [27]. The generally prevailing mucosal homeostasis is remarkable because of the large surface area to be defended – continuously being exposed to at least 800 different bacterial species. It has been estimated that the human gut microbiota is composed of \( \sim 10^{14} \) bacteria, or approximately 10 times the number of body cells, making up a weight of 1–2 kg [27].

According to the original hygiene hypothesis, the increasing incidence of allergy in Westernized societies might be explained by reduced or aberrant microbial exposure early in infancy, resulting in too little Th1-cell activity and therefore an insufficient IFN-\( \gamma \) level to cross-regulate optimally IgE-inducing Th2-cell responses (fig. 2). In this context, an appropriate composition of the commensal microbiota and exposure to food-borne and fecal microbes probably exert an important homeostatic impact [27], both by enhancing the SIgA-mediated barrier function and by promoting mucosally induced tolerance through a shift from a predominant Th2-cell activity in the newborn period to a more balanced cytokine profile later on [25].

The extended hygiene hypothesis postulates that induction of Treg cells (fig. 1) is an important part of this microbe-driven homeostasis [27]. Naturally occurring Treg cells are present in large numbers in fetal mesenteric lymph nodes, probably as part of a peripheral tolerance mechanism keeping auto-reactive effector T cells in check to avoid inflammation and tissue damage. These Treg cells are apparently induced in the thymus [31]. After birth, the decision between induction of a potentially harmful systemic-type productive immunity against innocuous environmental and dietary proteins, versus hyporesponsiveness to such antigens, may be largely instructed by MAMPs in mucosa-draining lymph nodes as discussed above (fig. 3).

Altogether, MAMPs do not only modulate the epithelial barrier of neonates [11, 13, 19], but also the activation profiles of innate and adaptive immune cells. Appropriate balancing of the immune system therefore appears to depend on a fine-tuned ‘cross-talk’ between APCs/innate immunity and T cells/adaptive immunity early in the newborn period [25, 27]. A relatively narrow postnatal window apparently exists for such early programming – starting when the infant’s gut mucosa is colonized with commensal vaginal and intestinal bacteria from the mother’s birth canal [25, 31]. In healthy individuals, the Th2-skewed cytokine profile of the newborn is then deviated towards a Th1 profile as a sign of immunological maturation. However, in the atopic child the Th2 skewing will continue and thus predispose to allergy. IgE sensi-
tization may start even in utero, although this possibility remains elusive and does not necessarily suggest that the infant is atopic and will become allergic [32]. Instead, the infant may later develop a balanced immune system. Thus, most children with food allergy will out-grow their disorder before the age of 2 years, and we have shown that such a positive development is associated with expansion of CD25+ Treg cells [25].

It is important to learn more about the maturation of the immune system in early life to exploit such knowledge for future prevention and treatment of allergy. Fortunately, some plasticity of immunoregulatory pathways persists even after the newborn period so APCs can later on be conditioned to induce Treg cells by environmental factors such as LPS and cell wall lipids from parasites [27]. Immunological homeostasis is, in addition, influenced by nutrition; especially lipid intake such as fish oil enriched in polyunsaturated n-3 fatty acids may protect against the onset of allergy, but apparently not against established allergic disease [25]. Prevention strategies should therefore be targeted early, perhaps even in utero. Animal experiments have suggested that the ratio of n-6:n-3 fatty acids is of special importance for neonatal tolerance induction [25]. This ratio varies in breast milk from different parts of the world, which may contribute to the reportedly variable effects of breastfeeding on allergy prevention. A derivative of n-3 fatty acids termed Resolvin E1 (RvE1) binds with high affinity to a receptor (ChemR23) on APCs, thereby attenuating NF-κB activation [25]. This may explain the apparent anti-inflammatory effect of n-3 fatty acids.

Conclusions

Many variables influence mucosally induced tolerance and productive IgA-dependent secretory immunity. Some of these variables are reciprocally modulated to achieve homeostasis (fig. 5). Increased epithelial permeability is an important primary or secondary event in the pathogenesis of many diseases, including allergy, celiac disease and inflammatory bowel disease. The barrier function is determined by the individual’s age (e.g., preterm versus term infant), genetics, mucus, interactions between mast cells, nerves and neuropeptides, concurrent infection, and the mucosa-shielding effect of SIgA provided by breast milk or produced by the infant’s gut. The integrity of the epithelium furthermore depends on different immune-suppressive mechanisms, including Treg cells (fig. 1, 3).

Many studies have suggested that allergy is associated with delayed or impaired development of the IgA system, and an underlying deficiency of antigen-specific SIgA has been documented in a mouse model of food allergy – implying the involvement of secretory immunity in oral tolerance [19]. Indeed, multiple minor dysregulations of both innate and adaptive immunity are reportedly associated with food allergy in children [19]. It is
therefore not surprising that epidemiological reports suggest that breast-feeding protects against allergy, especially in families with allergic heredity [22]. The same is true for celiac disease [21]. The remarkable output of SIgA during feeding serves as an optimally targeted passive immunization of the breastfed infant’s gut, and may also serve as a positive homeostatic feedback loop [19].

Secretory immunity is of great importance for the intestinal epithelial barrier because SIgA not only maintains mutualism with the indigenous microbiota but also forms the first line of defense against commensals and pathogens as well as other harmful agents [19]. In addition, the epithelial barrier depends on interaction with microbial factors (MAMPs) from the environment and particularly from the indigenous microbiota, both by direct interaction with PRRs of the epithelium [11, 19] and for induction of mucosal tolerance via tolerogenic APCs and Treg cells (fig. 1). In mouse experiments it has been shown that a single molecule from a commensal gut bacterium can induce crucial modulation and homeostasis of the host’s immune system [19].

Despite such expanding knowledge about immune regulation, it remains elusive how the allergic march is driven from food allergy to respiratory

Fig. 5. Summary of variables with an impact on the developing immunophenotype of the infant. Immunological homeostasis depends on a controlled balance between mucosally induced tolerance and productive immunity. Several of the components acting on this balance are reciprocally modulated as indicated by bidirectional arrows. Shaded panels represent components that may be subjected to intervention modalities as discussed in the text. The importance of epithelial barrier function is highlighted by a frame.
allergy on an individual basis [25]. However, clinical problems with certain dietary antigens including gluten are not surprising because husbandry and agriculture were introduced relatively recently. Evolutionary adaptation is slow when the pressure on the genome results from disorders that generally are not directly deadly on their own. Also, it should be realized that the current epidemic of allergy is a small price to pay for the remarkable reduction in infant mortality caused by improved hygiene.

Hopefully, novel strategies will emerge to compensate for the missing microbial stimuli apparently needed to induce immune homeostasis. Molecular refinement of probiotic and prebiotic intervention is an exciting avenue for further research [27].

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References

Discussion

**Dr. Sartor:** With the dysbiosis concept you have a relative balance of good and bad bacteria, but they all tend to stimulate the same pattern recognition receptors. How does the innate immune cell recognize different TLR ligands from good and bad bacteria? We heard that perhaps polysaccharide A could stimulate TLR2, but then Yang et al. [1] say that inducing TLR2 is a detrimental thing if you don’t have appropriate NOD2 regulation. How do these innate cell differentiate all of this?

**Dr. Brandtzaeg:** It’s an endless discussion, however, because we don’t know enough about TLR2 and NOD2. We know that the epithelium has certain ways of controlling signaling via its pattern recognition receptors, whether the signals come from the apical side or the basolateral side. The latter is more dangerous meaning invasion, while the epithelium can downregulate NF-κB when the signals come from the apical side which represents the natural exposure to commensals. You would agree with this, I suppose. So that means that as long as the bacteria don’t invade the epithelium...
it seems to be alright to give signals to the pattern recognition receptors. That stimulates defensin secretion which we need to preserve a tight barrier to keep mucosal homeostasis.

**Dr. Sartor:** Just to emphasize, multiple studies have in fact demonstrated that induction of epithelial TLR is absolutely essential for mucosal homeostasis. Yet if you get though that and stimulate macrophage TLRs you actually can get detrimental effects. Although plasmacytoid dendritic cell activation is protective, the question remains how do you determine whether you get IL-10 production from the dendritic cells versus IL-12? That’s the complexity.

**Dr. Brandtzaeg:** The complexity of dendritic cells was shown in a study we published in 2007. The plasmacytoid dendritic cells are more clever at inducing IL-10 in the T-effector cells in a way which could be very important. We were studying cytomegalovirus infection [2]. I think some sort of permeability is needed to get enhanced stimulation of dendritic cells. Our knockout mice, which lack secretory IgA, are hyper-reactive as they are bombarded all the time by too many bacterial products from the environment; when they are also systemically sensitized, they are likely to get anaphylaxis. But there is one good thing about these mice with their leaky epithelial membrane; using the traditional oral tolerization model, it is actually much easier to achieve mucosally induced tolerance both in the T-effector cells and in humoral immunity like IgG1. This is something we are working on right now. In this mouse model we have knocked out the polymeric Ig receptor which brings dimeric IgA and pentameric IgM to the surface. So it’s both a matter of keeping things away and a matter of inducing tolerance; both mechanisms will preserve the tiny epithelial layer which is so vulnerable. I think that perhaps explains much of the complexity of the epidemiological studies with regard to allergy and Th1 diseases, where we know too little about what is going on with regard to membrane permeability. I think it’s very interesting now that more and more genes which have to do with the tightness of the epithelium are being found to be associated with these diseases as polymorphism. We can’t do anything about the genes but we can perhaps impact on the membrane from the outside. Prebiotics have not been mentioned in the previous talks, and that is one of the questions I would like to ask Dr. Björkstén, whether that could solve some of the problems with probiotics. If you could have prebiotics to get the beneficial bacteria to proliferate, perhaps that’s a better way to go. I think the industry now is more focused on prebiotics than on probiotics.

**Dr. Motala:** One of the observations with allergies is that food allergy obviously comes on early in life, however we know that some people develop allergies, including food allergies, later in life. Can you speculate on whether you think that this might be an alteration of barrier function or it has got anything to do with autoimmune function in these individuals who develop allergies later in life?

**Dr. Brandtzaeg:** I have no idea, but perhaps an infection could have breached the epithelial membrane; if you have the right genes for allergy you might start the skewing to a Th2 profile, but that’s just speculation. We don’t have any data, but we know that breaching the epithelial membrane by chemicals in various animal models will induce diseases like colitis, etc. So we have plenty of evidence to show that you need an intact membrane to keep homeostasis. For instance in celiac disease we know that gluten is important. In a way the disease is a sort of allergy, and there is some initiating hit which is needed to start that disease, and this may be an infection breaching the epithelial membrane. We see patients presenting with celiac disease all the way up to 80 years of age, although it’s generally considered a pediatric disorder. I think that a subclinical infection may represent the initial hit and then gluten intolerance starts because of the right genetics.

**Dr. Lentze:** You emphasized the barrier of the intestine in the first 6 months of life. The barrier is not very intact and that raises thoughts on strategies in terms of how
to influence the immune system. What do you think is the better way? You already emphasized pre- or probiotics. Is it better to do one or the other or both?

**Dr. Brandtzaeg:** I am not an expert on probiotics and prebiotics; I hope someone will say something about prebiotics. I don’t know how many studies have been done with prebiotics in a real-life situation. I know about experimental studies where oligosaccharides are given and you can see how this would enhance mucosal homeostasis in early life, but in humans I don’t know of any studies at an early age. As I said, a slight permeability of the membrane is not that bad in infancy as it could actually be good for the induction of oral tolerance. That is probably why it is important to give probiotics very early in clinical studies while there is a possibility of getting the right antigens into the mucosa and induce tolerance. That is probably what we are talking about, this window of opportunity just after birth.

**Dr. Vaidya:** As I understand this very complex mucosal immunology, there seem to be two levels of defense: one is of course the barrier, and after the barrier is the immune regulatory response. What exactly determines the immune regulatory response because that is going to differentiate between tolerance and disease? Is it the genetic status or the response of the host?

**Dr. Brandtzaeg:** I am not sure I understood your question. You said there are two levels of defense? Do you mean the sort of keep-away defense by secretory IgA and the mucosally induced tolerance?

**Dr. Vaidya:** Yes, and the second is the immune response. What exactly determines this immune response? Is it genetics or how a host responds to an antigen that has leaked?

**Dr. Brandtzaeg:** The mucosal immune response involves both secretory IgA induction and oral tolerance. Many regulatory factors such as TGF-β, retinoic acid, and IL-10 are common for both processes. I didn’t get to that, but the secretory IgA system depends mainly on antigen induction through what we call M cells in organized mucosa-associated lymphoid tissue. We have a keep-away system that we call immune exclusion performed mainly by secretory IgA, depending on export of locally produced dimeric IgA and pentameric IgM by the polymeric Ig receptor. This mechanism is triggered to some extent by food and the commensal flora but it’s mainly aiming at combating infections. A good thing about the IgA which is actively pumped out is that it’s much more cross-reactive than IgG antibodies, so it can actually react against antigens to which it was not stimulated. So when you get enough of these IgA antibodies out you get a keep-away system with broad reactivity and at the same time the commensal flora and food will stimulate the suppressive mechanism which we call oral tolerance. So these are the two levels of defense which try to keep the barrier intact. This is a complex system where you have inductive sites, lymphoid aggregates with M cells, and mucosal homing — that is, extravasation of mucosal immune cells by receptors, chemokines, adhesion molecules, altogether a gatekeeper function to keep immune cells of systemic immunity out of the mucous membranes.

**Dr. Cerf-Bensussan:** Going further into the importance of this barrier, do you think that the bacteria come in contact with the epithelium? There is a recent paper [3] showing that there is a thick layer of mucus which is sterile in the colon and this is different from what is seen in the ileum. If indeed there is a huge barrier which prevents the bacteria from getting in, and also to show how important the contribution of secretory IgA is, there is a very nice paper by Gordon et al. [4] showing that if you don’t have secretory IgA then you have an innate response of the epithelial cells, which is much higher with a higher production of nitric oxide. Probably if the mucus is crossed, then the flora can elicit the IgA response.

**Dr. Brandtzaeg:** The mucus layer which is depicted here is of course much broader in the large bowel. But above the lymphoid aggregates there is a breach in
the mucus layer so the entrance of antigens into the lymphoid tissue of the gut and other mucous membranes is much easier when you have the M cells. When bacteria are coated by secretory IgA, there is a receptor for IgA on these cells which can take the immune complexes into the local immune system, in a way guide the immune induction especially in the neonatal period when the baby is breastfed. Thus the mothers who have IgA antibodies in their breast milk can guide antigens into their babies’ immune systems and induce the right sort of antibodies and regulatory cells there. So there are lots of complexities which we don’t understand well enough. In their mouse models, Harris et al. [5] showed that when IgA is lacking there is considerable influx of antigens and poor defense against bacteria in the beginning, but after some time the innate immune system will in a way compensate and be much better at defending the epithelial membrane. So this shows that there is a partnership between adaptive and innate immunity; they sort of balance each other and can compensate for each other.

Dr. Du Toit: It strikes me that this induction must happen very early when one looks at premature babies, because in prematurity all these risk factors are present. As Dr. Sinn suggested earlier, these children often get antibiotics and have abnormal colonization or no colonization initially. From the mannitol studies we know that mucosal permeability is raised, we know that through lack of breastfeeding in premature infants the food that they are exposed to is by and large unmodified premature milk formulas, so they would get intact cow’s milk protein formula, and the immune systems are by definition immature. It seems very surprising that we don’t see an epidemic of food allergy among premature babies. I am aware of only one study where in a subsection of at-risk premature babies cow’s milk protein was more prevalent, but it just seems absolutely surprising given the model you have just described.

Dr. Brandtzaeg: Especially the immune exclusion system may be important, and that’s probably why breastfeeding is so essential for premature babies, for instance by protecting against necrotizing enterocolitis. I can’t answer your question, as I haven’t seen any studies in these premature babies with regard to allergy. But definitely the genetic background is important and you probably need a very large cohort to reveal the genetic impact in addition to a deficient barrier function. Perhaps Dr. Björkstén knows something about allergy in premature babies.

Dr. Björkstén: They have a reduced risk.

Dr. Isolauri: Coming back to the same question again, it also depends on how you breach the barrier. For instance rotavirus infection in young infants does breach the gut barrier but they do not have cow’s milk allergy even when they get the rapid refeeding scheme. Coming then finally to probiotics and prebiotics; probiotics have been shown to improve rotavirus infection while prebiotics are less effective due to the fact that the infection or inflammation in the gut always disturbs the microbiota balance, so the infant does not have the good bifidobacteria present to be selectively stimulated by prebiotics.

Dr. Brandtzaeg: You could combine them perhaps; a combination of pre- and probiotics could be good.

Dr. Björkstén: Based on what you said about the immature barrier function in early infancy, and breastfeeding is undoubtedly an optimal feeding, but I would like to question the WHO recommendation of exclusive breastfeeding with nothing added for 6 months. There is no traditional society where you have exclusive breastfeeding because you always have added foods in addition to the breast milk. Would it be possible that you actually induce better immune responses or immune regulation by providing babies with the most important antigens under the protection of breastfeeding? As you know in Sweden, we had an unfortunate experience with total exclusion of
gluten for 6 months and then sudden induction of high amounts. We got a staggering increase in the incidence of celiac disease. So I wonder whether we should give other foods while breastfeeding, although obviously no infant formulae.

**Dr. Brandtzaeg:** This is an endless discussion, but there is a mouse model now which is a very hot topic. It shows that if the mother is inhaling ovalbumin, this gets into her circulation and then into the breast milk. The pups, if they are exposed to the same model of asthma later on, are actually protected [6]. This shows that small amounts of antigens combined with breast milk could be very important for inducing tolerance. In most studies with so-called exclusive breastfeeding, it has not been completely exclusive, but I'm not sure.

**Dr. Björkstén:** The discussion concerning exclusive breastfeeding originally was that you should not give formula, but that's another story. Now we carefully avoid everything and I think that may be wrong.

**Dr. Brandtzaeg:** It could be, but this is a very delicate discussion actually. I think everybody agrees that when you introduce solid food it should be mixed with breastfeeding, that's very important; it could also have something to do with the uptake of IgA-antigen complexes by the M cells to guide the baby's immune system to respond to the antigens to which the mother is exposed. But these again are animal studies although we know that also humans have an IgA receptor on the M cells which probably can guide antigens into the inductive lymphoid tissue of the gut. So we have a lot to learn but I think we see some solid facts.

**Dr. Exl-Preysch:** I go back to what was discussed before in terms of preterm infants and allergies. As I know there is only one study that was conducted years back by Lucas et al. in the UK. They had breast milk delivered in bottles for preterm babies, and showed very nicely that there was no difference between the whole group. But again the infants or preterm infants with an increased risk of allergies were likely to have significantly more allergies when they were given a normal preterm non-hydrolyzed formula compared to those babies who got human milk. This was when we started to have hydrolyzed preterm formula, which is like a human milk fortifier. We should provide something that has the same allergen level provided by human milk. As far as I know this is the only randomized control study in the world that really showed very nicely that those preterm infants at risk were also more often at risk of developing allergies when they got contact proteins.

**Dr. Brandtzaeg:** The genes are difficult to get away from, but we know that breastfeeding is very important for preterm babies. I think this discussion as to whether completely or partially hydrolyzed formula is preferential also relates to the problem which Dr. Björkstén raised: should the newborns be exposed to some antigens early or not, or meet no antigens whatsoever?

**Dr. Smith:** We know a little bit about the genetics of atopic dermatitis and a lot of the epithelium integrity in the filaggrin gene change is a pure structure protein defect which is associated with a rise in IgA. We know very little about the genetics of food allergy, and the only rather repeatedly consistent disease is Netherlands syndrome where there are massive epithelium integrity issues in the skin and gut, and massive IgA levels. The phenotypes that will occur with this look as though integrity is where it's at in atopic disease where intervention might play a role.

**Dr. Brandtzaeg:** The filaggrin gene is really the first gene to be clearly associated with atopic dermatitis, so that again shows the importance of the epithelial barrier. I think more of these genes are being found now in asthma, celiac disease and inflammatory bowel disease, dealing especially with the tight junction complex. Tight junctions consist of so many proteins, and are regulated by so many genes that the polymorphism in these genes will be very diverse, so it will take a lot of time to really work this out.
References


