

## **Mucosal adaptive immunity: impact of exogenous stimuli and feeding**

Per Brandtzaeg

Laboratory for Immunohistochemistry and Immunopathology (LIIPAT), Institute of Pathology, Rikshospitalet University Hospital, N-0027 Oslo, Norway.

The vast majority of antigenic challenges confronting the body make contact with mucosal surfaces. To maintain homeostasis in the extensive and vulnerable mucosae, they are protected by specialized anti-inflammatory immune defences such as secretory IgA (SIgA) antibodies and tolerance against innocuous antigens, including dietary proteins and components of commensal bacteria. The induction of mucosal immunity is highly dependent on exogenous stimuli and the neonatal period is critical. Both the mucosal barrier with its reinforcement by SIgA and the immunoregulatory network require successful adaptation after birth, depending on appropriate microbial colonization as well as adequate timing and dose of food antigens when first introduced. Breastfeeding can in several ways have a beneficial effect on the developing immunophenotype of the infant.

### **Introduction**

Numerous genes are involved in the regulation of innate and adaptive immunity, with a variety of modifications introduced over million of years. In this evolutionary process the mucosal immune system has generated two layers of noninflammatory defence: (i) immune exclusion performed by secretory antibodies to modulate or inhibit surface colonization of microorganisms and dampen penetration of potentially dangerous soluble factors; and (ii) suppressive mechanisms to avoid local and peripheral hypersensitivity to innocuous antigens. When induced via the gut, the latter arm is referred to as 'oral tolerance' and probably explains why overt and persistent allergy to food proteins is relatively rare. Similar downregulatory mechanisms operate against antigens from the commensal microbial flora.

Mucosal tolerance appears to be a rather robust adaptive immune function in view of the fact that more than a ton of food may pass through the gut of an adult every year, resulting in a substantial uptake of intact antigens. However, the neonatal period is particularly critical, both with regard to infections and priming for allergic disease. This is so because the mucosal barrier function and the immunoregulatory network are poorly developed for a variable period after birth. Notably, the postnatal development of mucosal immune homeostasis depends on the establishment of a normal bacterial flora as well as on adequate timing and dose of dietary antigens when first introduced.

### **Antibody-mediated mucosal defence**

The intestinal mucosa contains at least 80% of the body's activated B cells, terminally differentiated to Ig-producing blasts and plasma cells. Most of these cells produce dimeric IgA which, along with pentameric IgM, can be actively transported through secretory epithelia by the polymeric Ig receptor (pIgR), also known as membrane secretory component. Immune exclusion is mediated mainly by secretory IgA (SIgA) and secretory IgM (SIgM) in cooperation with innate non-specific defence.

IgA-producing cells are normally undetectable in the mucosa before 10 days of age but thereafter a rapid increase takes place, although IgM usually remains predominant up to 1 month. Little increase of intestinal IgA production usually takes place after 1 year and a faster establishment of secretory immunity is often seen in developing countries.

In the first postnatal period, only occasional traces of SIgA and SIgM occur in intestinal juice, whereas some IgG is often present, reflecting paracellular 'leakage' from the lamina propria that after 34 weeks of gestation contains readily detectable maternal IgG. Neonatal mucosal uptake of SIgA antibodies from breast milk is of no importance for systemic immunity, except perhaps in the preterm infant. Although gut closure normally occurs in humans mainly before birth, an appropriate mucosal barrier function may not be established until after 2 years of age; the different variables involved in

this process are poorly defined. Interestingly, the postnatal colonization of commensal bacteria is important both to establish and regulate the epithelial barrier.

### **Immune induction in gut-associated lymphoid tissue**

**Immune regulation.** Intestinal lymphoid cells are located in three compartments: organized gut-associated lymphoid tissue (GALT); the lamina propria; and the surface epithelium. GALT comprises Peyer's patches, the appendix and numerous isolated lymphoid follicles. All these structures are believed to represent inductive sites for intestinal immune responses, while the lamina propria and epithelial compartment principally constitute effector sites. The domes of GALT are covered by a characteristic epithelium that contains membrane (M) cells, which effectively transport antigens from the gut lumen.

GALT structures resemble lymph nodes with B-cell follicles, intervening T-cell zones and a variety of antigen-presenting cells (APCs) such as macrophages and dendritic cells, but there are no afferent lymphatics. Exogenous stimuli therefore come directly from the gut lumen via M cells. Induction and regulation of mucosal immunity hence takes place primarily in GALT and the draining mesenteric lymph nodes, while differentiation of B cells to plasma cells takes place mainly in the lamina propria where second signals are provided by antigens taken up by local dendritic cells.

**Stimulation and homing of B cells.** Antigens are presented to naïve T lymphocytes in GALT by APCs after intracellular processing. The primed T cells in GALT release immunological mediator substances (cytokines), and transforming growth factor (TGF)- $\beta$  together with interleukin (IL)-10 drive the switching of B cells to IgA expression. Activated T and B cells home rapidly via lymphatics to mesenteric lymph nodes where they are further stimulated; they then may reach peripheral blood and finally become seeded into distant mucosal effector sites, particularly the intestinal lamina propria but also lactating mammary glands. Their local extravasation is facilitated by 'homing receptors' interacting with ligands on the microvascular endothelium ('addressins'), with an additional fine-tuned navigation conducted by chemokines.

**Perinatal development of GALT.** Very few B cells with IgA expression circulate in the blood of newborns (< 8 per million mononuclear cells), although this number is remarkably increased (~ 600 per million mononuclear cells) already after 1 month, reflecting the progressive stimulation of GALT. An initial early elevation of Ig-producing cells (mainly of the IgM class) can be seen in preterm infants, especially in those with intrauterine infections. Thus, mucosal immune cells are competent at least during the final trimester, but APCs need to be activated by exogenous 'danger signals' that enable them to provide appropriate costimulatory signals to prime naïve T cells. The indigenous microbiota is very important in this context as shown by the fact that the intestinal IgA system of germ-free mice is normalized after 4 weeks of conventionalization. *Bacteroides* and *Escherichia coli* strains seem to be particularly immunostimulatory, but also lactic acid-producing bacteria.

The retarded postnatal immune activation of GALT parallels the functionally decreased systemic immunocompetence in the newborn period. Thus, peripheral CD4<sup>+</sup> T cells of infants show reduced capacity for production of interferon (IFN)- $\gamma$  and IL-4, as well as for B-cell help. One reason might be that there are relatively few circulating memory (CD45R0<sup>+</sup>) T cells in infancy. Interestingly, the responsiveness of neonatal naive (CD45RA<sup>+</sup>) T cells does not differ significantly from that of virgin counterparts in adults, and animal studies suggest that the chief explanation for the apparent immunological immaturity is to be found in a deficient APC function. Thus, neonatal macrophages, dendritic cells and B cells are all unable to deliver adequate costimulatory signals to naive T cells.

It is possible that suboptimal development of mucosal immune functions contributes to the increased frequency of certain diseases in industrialized countries, particularly allergies and other inflammatory disorders. This 'hygiene hypothesis' has been tested in several studies by evaluating the beneficial effect of probiotic bacterial preparations. Especially viable strains of the commensal intestinal microflora, such as lactobacilli and bifidobacteria, have been reported to enhance IgA responses, both in humans and experimental animals, apparently in a T cell-dependent manner. A recent double-blind study of infants with a family history of atopic (IgE-mediated) allergy, reported the prevalence of atopic eczema to be reduced by 50% at the age of 2 years in those receiving the probiotic *Lactobacillus* GG strain daily for 6 months. It remains to be shown whether this beneficial effect was mediated via a reinforced SIgA-mediated barrier function or by promotion of oral tolerance.

## Importance of homeostatic immune regulation

**Oral tolerance.** It is believed that mucosal homeostasis depends not only on the SIgA system, but also on suppressive mechanisms collectively called oral tolerance when generated via the gut. Rodent studies suggest that the commensal microbiota is important both for induction of oral tolerance and for reconstitution of this function after its experimental abrogation. It seems justified to believe that oral tolerance normally operates also in humans. Thus, in the healthy state the vulnerable gut mucosa exhibits virtually no proinflammatory IgG response and contains very few hyperactivated T cells. Moreover, the systemic IgG response to dietary antigens tends to decrease with increasing age, and a hyporesponsive state to bovine serum albumin has been demonstrated by intradermal testing in adults. This homeostatic state is abrogated in inflammatory bowel disease.

The fact that resident APCs from normal human gut mucosa are quite inert in terms of immune-productive stimulatory properties, supports the notion that they play a central role in oral tolerance. One possibility is that they carry penetrating dietary and innocuous microbial antigens away from the mucosa to the mesenteric lymph nodes where they become conditioned to induce tolerance, thereby avoiding local hyperactivation of immune cells.

**Decision-making in the immune system.** 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. In this context, an appropriate composition of the commensal microbiota and exposure to foodborne and orofecal microbes most likely exert an important homeostatic impact, both by enhancing the SIgA-mediated barrier function and by promoting oral tolerance through a shift from a predominant Th2-cell activity in the newborn period to a more balanced cytokine profile later on. The extended hygiene hypothesis postulates that induction of regulatory T (Treg) cells is part of this homeostatic mechanism. The decision making between induction of productive systemic-type immunity, with the potential for tissue damage and inflammation, *versus* a tolerogenic response seems to be largely instructed by the microbial impact conditioning APCs and T cells. Altogether, appropriate balancing of the immune system apparently depends on adequate 'cross-talk' between innate and adaptive immunity early in the newborn period, and APCs may also subsequently be conditioned to induce Treg cells by environmental factors such as cell wall lipids from parasites, particularly helminths.

Circumstantial evidence is accumulating to support the extended hygiene hypothesis. Thus, the intestinal microbiota of young children in Sweden was found to contain a relatively large number of *Clostridium* spp., whereas high levels of *Lactobacillus* spp. and *Eubacterium* spp. were detected in an age-matched population from Estonia; this difference could be taken to explain the lower incidence of allergy in the Baltic countries compared with Scandinavia. A recent Finnish study likewise reported that atopic infants had more Clostridia and tended to have fewer bifidobacteria in their stools than non-atopic controls. Absence of early postnatal gut colonization with a normal commensal microbiota dominated by lactic acid-producing bacteria might likewise explain a more than 8-fold risk for food allergy in genetically predisposed children delivered by caesarean section. Altogether, such observations make a good case for studying the potential clinical benefits of prebiotics and probiotic bacterial strains from indigenous gut bacteria to aid immune regulation.

The feeding and treatment regimen (e.g., antibiotics) to which the newborn is subjected, as well as the nutritional state, have a significant impact on the indigenous microbiota and on gut integrity, and may hence disturb the conditioning of the developing mucosal immune system. Intestinal colonization of lactobacilli and bifidobacteria is promoted by breast milk because it acts as prebiotics through its large amounts of oligosaccharides; these microorganisms may directly enhance the Th1 profile in the gut by inducing IL-12, IL-18 and IFN- $\gamma$ . Also notably, *E. coli* is a strong inducer of IL-10 secretion, apparently derived both from APCs and Treg cells. IL-10 has indeed been shown to be an important suppressive cytokine in the murine gut. Thus, the indigenous microbiota may have an impact on mucosal homeostasis beyond that of enhancing the SIgA system and promoting a Th1-cytokine profile that counterbalances Th2-cell responsiveness. However, there are probably also other factors than microbial stimuli contributing to the induction of the various Treg subsets, for instance hormones. Children who outgrow their cow's milk allergy develop CD25<sup>+</sup> Treg cells and may therefore serve as an interesting clinical model to explore the complex phenomenon of oral tolerance.

## **Effect of breastfeeding on the development of immunity**

**Secretory immunity.** In addition to the remarkable reinforcement of mucosal defence provided by maternal SIgA (and SIgM) antibodies as a natural immunological ‘substitution therapy’ (passive immunization), it is important to emphasize the positive nutritional effect of breastfeeding on immune development. Breast milk also contains a number of immune cells, cytokines, and growth factors that may exert a significant biological effect in the suckling infant’s gut, apparently enhancing in an indirect way even the subsequent health of the individual

Numerous studies of the effect of breastfeeding on secretory immunity have been performed with salivary IgA measurements as a read-out system. Discrepant observations have been made and the influence of contaminating the sample with milk SIgA, shielding of the suckling’s mucosal immune system by maternal SIgA antibodies, and altered growth and composition of the infant’s gut microbiota have been discussed as possible uncontrollable variables. However, evidence does suggest that breastfeeding promotes the postnatal development of secretory immunity, apparently even in the urinary tract; and there are reports on enhanced secretory as well as systemic immune responses to oral and parenteral vaccines in breast-fed babies.

Nevertheless, several prospective studies have reported that the early physiological increase of salivary IgA (and IgM) is more prominent in formula-fed than in solely breast-fed infants, although this difference apparently disappears after weaning. It likewise appears that breastfeeding, in comparison with formula-feeding, reduces the salivary IgA antibody titres to cow’s milk proteins; this decrease was seen after a nursing period of only 3 weeks and appeared also in infants receiving mixed feeding. Altogether, therefore although breastfeeding initially appears to reduce induction of salivary IgA, it will later on in infancy (up to 8 months) boost this response. Experiments in mice have demonstrated that SIgA antibodies from breast milk affect the normal gut flora in the suckling by retarding its contact with the developing GALT. When the host’s mucosal immune response subsequently is successfully elicited, GALT will be further shielded by the SIgA antibodies produced in the gut; local immunostimulation is thereby attenuated despite the continued presence of microorganisms. This could partly explain the hyporesponsiveness that normally exists towards members of the indigenous gut bacteria, resembling some sort of oral tolerance phenomenon both in rodents and in humans.

**Oral tolerance.** This suppressive phenomenon unquestionably involves more than one mechanism, and available data suggest a great complexity. Identifiable experimental variables include: genetics; age, dose and timing of postnatal antigen feeding; antigenic structure and composition; epithelial barrier integrity; and the degree of concurrent local immune activation as reflected by microenvironmental cytokine profiles and the expression of costimulatory molecules on APCs. It has recently been focussed on induction of various phenotypes of Treg cells by conditioned APCs, particularly dendritic cells (see above).

Through avoidance of too early immune activation (e.g., upregulation of the co-stimulatory molecules B7), the shielding effect exerted by SIgA from breast milk on the suckling’s GALT may contribute to the establishment of oral tolerance not only against the indigenous microflora, but also against dietary antigens such as gluten. Antibodies to gluten peptides are present in breast milk, and breastfeeding has in fact been shown to protect significantly against the development of coeliac disease in children unrelated to the time of solid food introduction. On the basis of such studies, it can be tentatively concluded that mixed feeding, rather than abrupt weaning, appears to promote tolerance to food proteins. This notion is supported by a report suggesting that cow’s milk allergy is more likely to develop in infants whose mothers have relatively low levels SIgA antibodies to cow’s milk proteins in their breast milk. The presence of TGF- $\beta$  in breast milk might further contribute to its tolerogenic properties because this cytokine exerts a pronounced immunosuppressive effect on GALT and enhances the epithelial barrier function. Although still a quite controversial issue, the balance of epidemiological studies supports the view that breastfeeding also protects against atopic allergy and asthma.