10
IgA and Intestinal Homeostasis

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10.1. Introduction

The body is under constant threat of attack by viruses, bacteria, and parasites, and most pathogens use the mucosae as portals of entry. Evolution has therefore provided mammalians with several complex and potent layers of defense. Microorganisms have inhabited Earth for at least 2.5 billion years, and the power of immunity is a result of coevolution in which particularly the commensal bacteria have shaped the body's defense functions in a state of mutualism (Bäckhed et al., 2005; Hooper and Gordon, 2001). In humans, the critical role of the immune system becomes clinically apparent when it is defective. Thus, inherited and acquired immunodeficiency states, or more subtle immunoregulatory defects, are characterized by increased susceptibility to infectious diseases—sometimes caused by the commensal microbiota that is normally considered to be nonpathogenic (Haller and Jobin, 2004; Sansonetti, 2004; Yan and Polk, 2004).

The immune system can be divided into two general arms: innate ("natural" or "nonspecific") immunity and adaptive ("acquired" or "specific") immunity, which work together synergistically (Chaplin, 2003). Notably, the adaptive immune system developed rather late in the phylogeny, and most species survive without it. However, this is not true for mammalians, which have an extremely sophisticated adaptive immune system of both systemic and mucosal type.

There appears to be a great redundancy of mechanisms in both systems—providing robustness to ensure that essential defense functions are preserved.

The success of such a complex overall strategy is evident because most humans have a normal gut despite the fact that its mucosa is covered only by a monolayered and therefore quite vulnerable epithelium. The prevailing mucosal homeostasis is indeed remarkable because the large intestinal surface area—approaching 300 m^2 in an adult—is exposed to an enormous load of commensal bacteria, comprising perhaps up to 800 different species. The human gut microbiota includes ~10^{14} bacteria (i.e., ~10 times the number of body cells), making up a weight of 1–2 kg. In addition, ~1000 kg of food is passing through the gut of an adult every year, and intact food proteins in the amount of 3–10 ng/mL reach the blood circulation after meals (Husby et al., 1985; Paganelli and Levinsky, 1980). In the face of these persistent antigenic challenges, tightly regulated local immune mechanisms are clearly needed to preserve a healthy intestinal mucosa (MacDonald and Monteleone, 2005).

10.2. Nature and Triggering of Innate Immunity

10.2.1. Innate Defense Mechanisms

It is increasingly being appreciated that innate immune mechanisms play a key role for the tuning of adaptive immunity in the gut and maintenance of mucosal homeostasis. Innate responses use preformed or rapidly synthesized effectors and sensors that lead to spontaneous activation or modulation of cellular functions (Chaplin, 2003). In addition to soluble components, the innate defense system comprises surface barriers, professional phagocytes, and dendritic cells (DCs). Together, these functions constitute a primary layer of natural protection against invading microorganisms, with the common goal of restricting their entry into the body by providing (1) physical/structural hindrance and clearance mechanisms (epithelial linings of skin and mucosae, mucus, ciliary function, peristalsis), (2) chemical factors (pH of body fluids, numerous antimicrobial proteins and peptides such as lysozyme, lactoferrin, peroxidase, and defensins), and (3) phagocytic cells (e.g. neutrophils, eosinophils, monocytes/macrophages, and immature DCs). As discussed later in this chapter, challenges of the innate system often lead to activation or modulation of adaptive immunity, including the secretory immune system; such a development might enhance substantially the resistance against, and recovery from, mucosal infections.

10.2.2. Pattern Recognition Receptors

The recognition molecules of the innate immune system are encoded in the germline (Chaplin, 2003). Basically, this system is therefore quite similar among healthy individuals and shows no apparent memory effect; that is,

reexposure to the same pathogen will normally elicit more or less the same type of response. The actual cellular receptors sense microbial molecular structures that are conserved and often essential for survival of the microorganisms. Such structures include, for instance, endotoxin or lipopolysaccharide (LPS), teichoic acid, peptidoglycan, and unmethylated CpG motifs of DNA (Beutler and Rietschel, 2003); together, they are traditionally called pathogen-associated molecular patterns (PAMPs), but they also occur in commensal bacteria (Medzhitov, 2001) and are therefore preferably called microbe-associated molecular patterns (MAMPs).

It remains unclear whether the intestinal microbiota through its MAMPs induces a distinct molecular program in the innate immune system, which could explain why commensal bacteria are normally tolerated by the host (Nagler-Anderson, 2001; Philpott et al., 2001). The relative absence of microbial recognition structures from the apical surface of normal gut epithelium (see later) as well as potential attenuation of their signaling by certain bacteria [e.g., modulation of nuclear factor (NF)-κB-mediated responses] also appears of crucial importance to such tolerance (Haller and Jobin, 2004; Kelly et al., 2004, 2005; Kobayashi et al., 2002; Moynagh, 2005; Neish et al., 2000; Rakoff-Nahoum et al., 2004).

The receptors of the innate immune system that recognize PAMPs or MAMPs as cellular triggers are collectively called pattern recognition receptors (PRRs)—many of which belong to the so-called Toll-like receptors (TLRs), which will be discussed later. PRRs are expressed mainly by macrophages and DCs but also by a variety of other cell types, including T- and B-cells as well as epithelial cells (see later). Engagement of PRRs with their intracellular signaling pathways causes controlled activation of the cells; in the case of DCs, this leads to maturation accompanied by production of various cytokines and upregulation or downregulation of surface molecules according to strictly defined kinetics (Ricciardi-Castagnoli and Granucci, 2002). Such cellular modulation will critically influence further development of both innate and adaptive immunity.

Importantly, there are both stereotypical and selective responses of innate host cells to different types of microorganism. In this manner, exogenous triggers can imprint their "signatures" on the immune system. Thus, the plasticity of the innate system prepares the ground for a targeted and enhanced function of adaptive immunity (Liew, 2002).

10.3. Nature and Function of the Intestinal Immune System

10.3.1. Homeostasis-Promoting Mechanisms

During evolutionary modulation, the mucosal immune system has generated two anti-inflammatory layers of defense (Fig. 10.1): (1) immune exclusion performed mainly by secretory immunoglobulin A (SIgA) antibodies to restrict

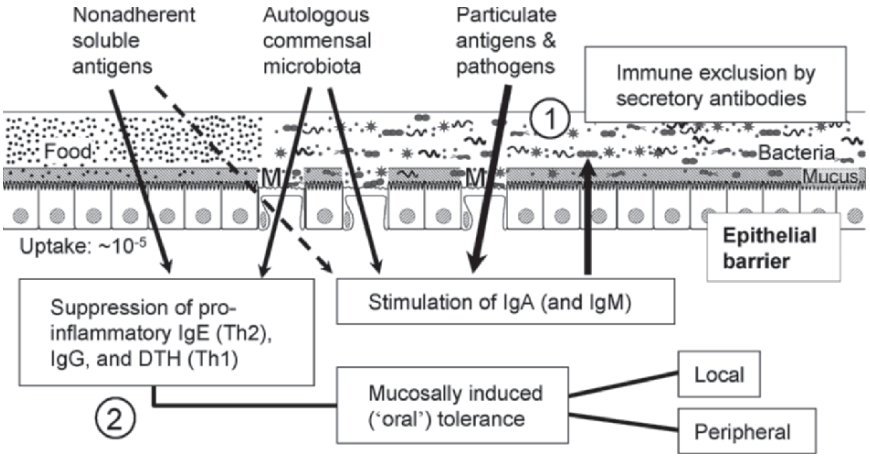

FIG. 10.1. Schematic representation of two major homeostatic immune mechanisms in the gut. **(1)** Immune exclusion limits epithelial colonization of microbes and inhibits penetration of foreign material (magnitude of normal food protein uptake indicated) into the gut mucosa. In cooperation with various innate immune mechanisms (not shown), this first line of defense is principally mediated by pIgR-dependent secretory antibodies of the IgA (and IgM) class. Mucosal production of IgA (and IgM) is strongly stimulated by pathogens and other particulate antigens taken up through thin M-cells (M) in the domes of gut-associated lymphoid tissue (thick solid arrow). The commensal microbiota and penetrating food proteins (magnitude of normal uptake indicated by arrow thickness) are also stimulatory for secretory immunity, but to a lesser extent (thin solid and broken arrows, respectively). **(2)** The latter types of exogenous impact induce, in addition, suppression of proinflammatory humoral immune responses (IgG and Th2 cytokine-dependent IgE antibodies) as well as downregulation of Th1 cytokine-dependent delayed-type hypersensitivity (DTH). Such local and peripheral homeostatic control of Th1 and Th2 responses induced via the gut is collectively termed "oral tolerance."

epithelial penetration and host invasion of microorganisms as well as other potentially dangerous antigens and (2) immunosuppressive mechanisms to avoid local and peripheral hypersensitivity to innocuous luminal antigens. Such mucosally induced inhibitory mechanisms are collectively referred to as "oral tolerance" when stimulated via the gut (Brandtzaeg, 1996; Mowat, 2003) and they probably explain why overt and persistent allergy to food proteins is relatively rare (Bischoff et al., 2000). A similar downregulatory tone of the immune system normally develops against antigenic components of the commensal intestinal microbiota (Duchmann et al., 1997; Helgeland and Brandtzaeg, 2000; Moreau and Gaboriau-Routhiau, 2000).

Secretory immunity can thus be considered as a first line of defense that aims at reducing the need for systemic immunity, because the latter will engage forceful proinflammatory mechanisms to enable elimination of microorganisms when required to save life. Systemic immunity therefore represents

a "two-edged sword" prone to cause immunopathology, in contrast to the noninflammatory properties of SIgA antibodies. Oral tolerance appears to be a rather robust backup system for secretory immunity in view of the fact that substantial amounts of intact dietary antigens enter the body after a meal, usually without causing harm (Husby et al., 1985; Paganelli and Levinsky, 1980). However, because both the mucosal barrier function and the immunoregulatory network are poorly developed for a variable period after birth, the neonatal period is particularly critical with regard to infections and priming for allergic disease (Brandtzaeg, 2002a ; Holt, 1995). It is important in this context that the postnatal development of mucosal homeostasis depends on the establishment of a normal microbiota as well as on an adequate timing and the dose of dietary antigens when first introduced (Brandtzaeg, 1998; Moreau and Gaboriau-Routhiau, 2000).

10.3.2. Stimulation of Gut-Associated Lymphoid Tissue

10.3.2.1. Immune-Inductive Tissue Structures

The intestinal IgA system is the best understood component of mucosal immunity. In fact, the gut mucosa contains at least 80% of the body's activated B-cells (Brandtzaeg et al., 1989)—mostly differentiated to IgA-producing blasts and plasma cells (PCs). Their generation depends on complex mechanisms of B-cell induction in gut-associated lymphoid tissue (GALT), which comprises Peyer's patches (PPs), the appendix, and numerous solitary or isolated lymphoid follicles (Brandtzaeg et al., 1987, 1999a; Brandtzaeg and Johansen, 2005 Brandtzaeg and Pabst, 2004). All of these structures are believed to represent inductive sites contributing to intestinal immune responses, whereas the lamina propria and epithelial compartment principally constitute effector sites receiving primed memory/effector B- and T-cells from GALT.

The domes of GALT are covered by a characteristic follicle-associated epithelium, which contains antigen-sampling M-cells. These very thin and bell-shaped specialized epithelial cells transport effectively both living and nonproliferating antigens (especially particles) from the gut lumen into the organized lymphoid tissue (Neutra et al., 2001). Many enteropathogenic infectious agents use the M-cells as portals of entry, so they represent extremely vulnerable parts of the surface epithelium. However, such "gaps" in the epithelial barrier are needed to facilitate efficient induction of mucosal immunity (Fig. 10.1).

Gut-associated lymphoid tissue structures resemble lymph nodes with B-cell follicles, intervening T-cell zones, and a variety of antigen-presenting cells (APCs) such as macrophages and DCs, but there are no afferent lymphatics. Therefore, microbial stimuli must come directly from the epithelial surfaces—mainly via the M-cells but also aided by DCs, which might penetrate the surface epithelium with their processes (Brandtzaeg and Johansen, 2005; Milling et al., 2005; Rescigno et al., 2001). Hence, induction and regulation of mucosal

immunity takes place primarily in GALT but also to a lesser extent at the effector sites (Brandtzaeg and Johansen, 2005; Brandtzaeg and Pabst, 2004).

10.3.2.2. Priming and Dispersion of Mucosal B-Cells

Antigens are presented to naïve T-cells in GALT by APCs after intracellular processing to immunogenic peptides. In addition, luminal peptides might be taken up and presented by B-lymphocytes and epithelial cells to subsets of intraepithelial and subepithelial T-lymphocytes (Brandtzaeg et al., 1999a). Both professional mucosal APCs, B-cells, and the small intestinal villous epithelium as well as the follicle-associated epithelium of GALT surrounding the M-cells express antigen-presenting MHC class II molecules— in humans particularly HLA-DR—in addition to classical and nonclassical MHC class I molecules (Brandtzaeg et al., 1987, 1999a; Christ and Blumberg, 1997). Interestingly, MHC class II-expressing naïve and memory B-lymphocytes abound juxtaposed to the M-cells. Such B-cells might present antigens efficiently to T-cells in cognate downregulatory or immunostimulatory interactions (Brandtzaeg et al., 1999a). $CD4^+$ helper T (Th) activated in GALT release cytokines such as transforming growth factor (TGF)-β and interleukin (IL)-10, which might drive the class switch and differentiation of mucosal B-cells to predominantly IgA-committed plasmablasts, although their regulation still remains unclear (Brandtzaeg and Johansen, 2005; Brandtzaeg et al., 2001; Fagarasan and Honjo, 2003).

B-Cells activated in GALT structures migrate rapidly via draining lymphatics to mesenteric lymph nodes, where they might be further stimulated; they next reach thoracic duct lymph and peripheral blood to become seeded by preferential homing mechanisms into distant mucosal effector sites, particularly the intestinal lamina propria, where they finally develop to Ig-producing PCs (Brandtzaeg et al., 1999b; Kunkel and Butcher, 2002). This terminal differentiation is modulated by "second signals" from local antigen-sampling DCs, lamina propria $CD4^+$ T- cells, and available cytokines (Brandtzaeg and Johansen, 2005; Brandtzaeg and Pabst, 2004).

10.3.2.3. Role of SIgA in Exclusion and Inclusion of Antigens

Most B-cells that home to mucosal effector sites apparently belong to clones of an early maturation stage, as indicated by their high level of J- chain regardless of concomitant isotype—although the IgA class normally predominates (Brandtzaeg et al., 1999a, 1999b). Locally produced J-chain-containing dimeric IgA and pentameric IgM are exported to the lumen by the polymeric Ig receptor (pIgR) to provide SIgA and secretory IgM (SIgM) antibodies, as discussed later. In the lumen, SIgA will coat commensal bacteria—demonstrated both in saliva (Brandtzaeg et al., 1968) and in feces (van der Waaij et al., 1996). This coating reduces bacterial access to the epithelial surface and protects against

bacterial overgrowth and invasion (Macpherson et al., 2005) and it provides herd defense against the horizontal spread of infectious agents by the fecal-oral route (Wijburg et al., 2006). After the induction of a secretory immune response in the newborn's gut, GALT will temporarily be "shielded" by SIgA—apparently reflecting an antibody-mediated negative feedback mechanism for homeostatic regulation of the mucosal B-cell system (Shroff et al., 1995).

Uptake of SIgA-coated bacteria by M-cells mighty nevertheless be enhanced because an apical receptor specific for IgA/SIgA has been identified on these cells (Mantis *et al.,* 2002; Roy and Varvayanis, 1987). SIgA might exploit this M-cell receptor for targeting of antigens to DCs in the domes of PPs (Rey et al., 2004). This putative positive feedback mechanism might particularly target relevant environmental antigens to GALT of breast-fed infants by means of cognate maternal SIgA antibodies. In this manner, SIgA could act as an instructive immunological enhancer accompanied by a balanced cytokine pattern that promotes homeostasis (Favre et al., 2005). Many reports show that, over time, breast-fed babies develop enhanced secretory immunity (Brandtzaeg, 2002b).

Whether SIgA-coated or not, commensal bacteria sampled by the M-cells mainly become destroyed by subepithelial macrophages, but they are also taken up by DCs in tiny amounts (~0.0001%); this is sufficient to induce an immune response in GALT and mesenteric lymph nodes (Macpherson and Uhr, 2004). In conventional (CVN) clean mice, however, the systemic immune system remains untriggered—a compartmentalization that might contribute to peripheral tolerance or, rather, ignorance of the indigenous microbiota under normal conditions, although this might not be the case in humans (Macpherson et al., 2005).

10.3.3. Stimulation of GALT by Commensal Bacteria

10.3.3.1. Different Ways of Microbial B-Cell Activation

Stimulation of mucosal B-cells might occur by various mechanisms. Some microbial substances are superantigens, which interact with the B-cell receptor (BCR) outside the antigen-binding site—the prototype being *Staphylococcus aureus* protein A. B-Cells exposed to cross-linked protein A show a strong polyclonal response. B-Cells in GALT could be expanded in a similar manner both by exogenous and microbe-induced endogenous superantigens (Lanning et al., 2005).

Other microbial substances called Type 1 T-independent (TI-1) antigens are directly mitogenic for B-cells—including sugars, lipid structures, and certain nucleic acids. Type 2 T-independent (TI-2) antigens, on the other hand, are not by themselves mitogenic but cause extensive cross-linking of BCRc by repeating epitopes; their B-cell activation is exerted through synergy with soluble factors (e.g., cytokines) and interaction with various types of accessory cell. There are notable species differences in the mechanisms involved; for instance, wherease LPS acts as a TI-1 antigen on mouse B-cells, it acts as a TI-2 antigen on human B-cells. Interestingly, microbial polysaccharides might

also function as T- cell-dependent antigens and exert profound homeostatic effects on the immune system by stimulating $CD4^+$ T-cells after presentation by DCs on MHC class II molecules. Thus, it was recently shown in germ-free (GF) mice monocolonized with the ubiquitous gut commensal *Bacteroides fragilis* that a single bacterial polysaccharide had a striking impact both on lymphoid organogenesis and immune modulation (Mazmanian et al., 2005).

Although PPs containing organized primary follicles with naïve B-cells are present at birth, it takes some days before activated secondary follicles with germinal centers (GCs) appear as a result of stimulation from the environment. Studies in GF and CVN specific pathogen-free rats have demonstrated that bacterial colonization drives intraepithelial T- and B-cell accumulation with formation of M-cell pockets after an initial GC reaction—apparently induced by DC-mediated antigen transport from the lumen (Yamanaka et al., 2003). Whereas TI antibody responses are restricted with regard to affinity and memory, the GC reaction is generally believed to be driven by BCR competition for a limited amount of T-cell-dependent antigens, resulting in memory/effector B-cells that undergo BCR affinity maturation and class switch (Manser, 2004). As reportedly shown for GALT of experimental animals, however, IgA differentiation stimulated by commensal gut bacteria might, under certain conditions, bypass the usual BCR requirement (Macpherson et al., 2001, 2005). Nevertheless, there always appears to be a dependency on some folliclelike aggregates of B-cells that, interestingly, might lack antigen-retaining follicular DCs and GCs.

In line with this observation, recent studies have demonstrated that if there is a sufficient innate drive of the immune system, B- cells might survive with a restricted repertoire and rather low affinity. Thus, in the GF-appendix model in rabbits, it was shown that certain commensal bacteria are quite efficient in promoting GALT development and that such ability depends on certain stress responses in the same bacteria, suggesting a nonspecific (superantigen?) impact on GALT (Rhee et al., 2004). Another study reported independency of BCR engagement for GALT development in mice where the Epstein-Barr virus (EBV) protein LMP2A was transgenically introduced as a constitutive BCR surrogate providing a weak signaling pathway (Casola et al., 2004). It was concluded that commensal bacteria—by interacting with innate immune receptors—can promote the GC reaction in GALT independently of BCR-mediated antigen recognition.

Altogether, it is well established that the gut microbiota is required for activation of GALT with normal intestinal PC development (Crabbé et al., 1970) and that commensal bacteria can shape the BCR repertoire of the host (Lanning et al., 2005). Perhaps the GC reaction during the evolution of GALT to generate a protective antibody repertoire was not antigen-specific but, rather, cross-reactive. The indigenous microbiota might in this context act as polyclonal B-cell activators through several mechanisms, including PRR signaling (Beisner et al., 2005). TLRs expressed by DCs and B-cells are likely molecular candidates to mediate such an innate drive of GALT development (Pulendran and Ahmed, 2006).

10.3.3.2. Cross-reactive SIgA Antibodies as a Homeostatic Mechanism

The peritoneal cavity is recognized as an important source of intestinal B-cells in normal mice, perhaps providing up to 40–50% of the IgA^+ PCs in lamina propria (Kroese et al., 1989). The precursors are self-renewing IgM^+ B1 ($CD5^+$) cells, which give rise to "natural" cross-reactive SIgA antibodies directed mainly against microbial TI antigens with no clear dependency on a GC reaction (Macpherson et al., 2000). Notably, however, rather than being encoded in germline, murine B1-cells often show hypermutation of Ig heavy-chain V-region (V_H) genes as a sign of selection (Bos et al., 1996). It remains controversial where the B1-cells switch to the IgA phenotype and how they reach the gut mucosa (Brandtzaeg et al., 2001; Brandtzaeg and Pabst, 2004), but their egress from the peritoneal cavity appears to depend on downregulation of their adhesion molecules by MAMP signaling via TLRs (Ha et al., 2006).

To clarify this complex issue, several studies have analyzed the intestinal immune system of GF mice after monoassociation with a variety of noninvasive, commensal bacteria (Shroff et al., 1995; Umesaki et al., 1995). It was generally found that these microbes induce a GC reaction in GALT with generation of IgA^+ plasmablasts that accumulate in the lamina propria and produce both "natural" and specific IgA. Individual bacterial species were shown to differ, however, with regard to the maximal amount of total "natural" IgA that they induced and the fraction that could be shown to be specific for antigens of the colonizer (Bos et al., 2001). All tested bacteria apparently elicited a waxing followed by a long-term waning IgA response, which was accompanied by a GC reaction that notably showed a much more rapid development as well as decline. This could be attributed to the "shielding" of GALT from microbial antigens by the production of specific SIgA antibodies, because of the relatively long-term persistence of both specific and "natural" IgA^+ PCs in the lamina propria.

Such homeostatic immune modulation has been particularly well documented with segmented filamentous bacteria (SFB, related to *Clostridia*), which become a major gut colonizer of the distal ileum of mice after weaning. Colonization of formerly GF weanlings results in a transient GC reaction in GALT and seeding of the lamina propria with IgA^+ plasmablasts producing an SIgA level comprising 50–70% of that seen normally in CVN mice; only about 1% of this IgA has been found to show specificity for the SFB (Jiang et al., 2001; Talham et al., 1999). Interestingly, supercolonization with *Morganella morganii* 100 days following monoassociation with SFB induced little change in production of total intestinal IgA, although the specific response to *M. morganii* increased 20-fold compared to that against SFB (Talham et al., 1999). The chronic GC reaction observed in GALT of CVN mice is therefore likely caused by continuous exposure of the gut to novel microbial antigens. The sustained colonization of commensal gut bacteria might thus provide the necessary chronic stimulation of previously induced "natural" and specific IgA production.

10.3.3.3. Contribution of B1- and B2-Cells to Intestinal IgA Production

The relationship between B1-cells and conventional bone-marrow-derived B2-cells in murine IgA responses induced by the indigenous microbiota remains elusive (Macpherson et al., 2005); when eliminating one of these subsets in genetically manipulated mice, the other subset is likely to occupy the whole intestinal B-cell compartment. Both the B1 and the B2 subset of intestinal IgA^+ PCs showed very restricted (oligodisperse) usage of V_H genes and multiple clonally related sequences when the repertoire was analyzed by complementarity-determining region (CDR)3 spectrotyping, cloning, and sequencing (Stoel et al., 2005). Out of 15–20 sequences examined from various types of mouse, there were 2 or more likely clonal relatives based on identical V/D/J junctional sequences. Such restricted V_H-gene usage was seen whether monoassociated immunodeficient recipients of B-cells or CVN immunocompetent mice of several common strains were analyzed. It could have been expected that polyclonal microbial stimuli would rather have induced polydisperse B-cell responses in the gut. Therefore, perhaps both B1 and B2 intestinal cells–stimulated specifically by TI-1 and/or TI-2 bacterial antigens via their BCRs–will generate an oligodisperse population of IgA^+ PCs that produce "natural" cross-reactive antibodies. In this process, B-cells with randomly recombined V/D/J segments– but without appreciable N-additions or point mutations in the V_H regions–might be selected by relatively few TI antigens in the gut.

Despite many remaining questions, these murine studies constitute useful information to explain the enormous IgA drive provided by the intestinal microbiota in the absence of a high-affinity BCR development. It appears that the production of large amounts of IgA with a restricted or oligodisperse repertoire provides antibody capacity to bind with low affinity to the numerous redundant epitopes of commensal bacteria to maintain mutualism with the indigenous microbiota; this homeostatic mechanism therefore relies mainly on large quantities of cross-reactive SIgA (Macpherson et al., 2005). Altogether, the induction of IgA by commensal bacteria seems to be a rather primitive system that limits their local colonization and penetration through the epithelial barrier without eliminating them from the gut. Superimposed on this "innate-like" defense, the B2 system has the property to undergo GC-driven high-affinity BCR selection for particular virulence factors of pathogens to clear ongoing infections.

10.3.3.4. Intestinal B-Cell Responses Differ Between Humans and Mice

It remains unclear whether such a two-layered SIgA defense also operates in humans, where there is no evidence to suggest that peritoneal B1-cells contribute to intestinal IgA production (Boursier et al., 2002; Brandtzaeg et al., 2001). In the human gut, both IgA^+ and IgM^+ PCs have highly mutated V_H-region genes even from childhood—consistent with precursor selection in GCs (Boursier et al., 1999; Dunn-Walters et al., 1997a; Fischer and Kuppers, 1998).

The level of mutations is significantly higher in human intestinal B-cells than that seen in splenic PCs (Dunn-Walters et al., 2000). Moreover, spectrotyping of CDR3 variability shows a rather restricted repertoire for circulating IgA^+ cells compared with V_H transcripts from the colon, which are quite diverse, particularly for the V_H1–V_H5 regions (Holtmeier et al., 2000). The IgM V_H-region genes in the human peritoneal cavity likewise exhibit fewer mutations than the corresponding genes from intestinal B-cells (Boursier et al., 2002). Further, the V_H4–34 genes used by IgG and IgA in human peritoneal B-cells show significantly lower numbers of mutations than their mucosal counterparts. Also notably, V_H-gene sequences from human PP B-cells are clonally related to ileal lamina propria PCs, in accordance with a predominant GC derivation from GALT (Dunn-Walters et al., 1997b). Finally, clonally related V_H transcripts are widely distributed along the colon, likewise suggesting seeding from GALT mainly via peripheral blood (Holtmeier et al., 2000).

Altogether, the balance of evidence suggests that human intestinal IgA^+ PCs are generated by GC reactions in GALT and that derivation from the peritoneal cavity, or a putative lamina propria switch process, is negligible or absent (Bousier et al., 2005; Brandtzaeg and Johansen, 2005; Brandtzaeg and Pabst, 2004). Nevertheless, considerable levels of cross-reactive "natural" SIgA antibodies directed against self as well as microbial antigens do occur in human external secretions (Bouvet and Fischetti, 1999). The reason for this could be microbial polyclonal activation of GALT independently of BCR-mediated antigen recognition or, alternatively, stimulation via BCR by microbial TI antigens, as discussed earlier.

10.3.4. Antibody-Mediated Mucosal Defense

10.3.4.1. Secretory Immunity and Immune Exclusion

As briefly mentioned earlier, the unique and efficient export of SIgA and SIgM antibodies to the epithelial surface is mediated by pIgR– also known as membrane secretory component (SC)–which is cleaved and sacrificed as bound SC to stabilize the secretory antibodies in the lumen (Brandtzaeg, 2003; Phalipon and Corthésy, 2003). The ligand structure specific for this receptor depends on the J-chain, which is incorporated selectively into dimeric IgA and pentameric IgM (Brandtzaeg, 1974a, 1974b; Brandtzaeg and Prydz, 1984; Johansen et al., 2000, 2001). It has been estimated that such pIgR-mediated transport of dimeric IgA, on average, results in the remarkable daily delivery of ~3 g of SIgA into the intestinal lumen of an adult human (Conley and Delacroix, 1987). Because of this efficient epithelial transport—which basically is constitutive—SIgA can act as a sustained first line of antibody-mediated defense against microorganisms and other antigens; it thereby performs homeostatic modulation of the host response elicited both by commensal bacteria and overt pathogens. Notably, with its high level of cross-reactivity even in humans (Bouvet and Fischetti, 1999), the mucosal IgA system is well

designed to provide antibody-mediated immune exclusion of the extremely diverse intestinal microbiota, which expresses a multitude of redundant epitopes, while allowing a persistent host–commensal mutualism. Such production of "natural" SIgA antibodies might at least, in part, reflect the innate drive of the intestinal immune system, as discussed earliere, whereas affinity maturation of B-cells in the GCs of GALT provides a more powerful defense to expel pathogens from the host.

10.3.4.2. Homeostatic Defense Functions of SC and SIgA

Free SC is generated by apical cleavage and epithelial release of unoccupied pIgR (Mostov and Blobel, 1982). Interestingly, free SC has been shown to possess several innate immune properties such as binding enterotoxigenic *Escherichia coli* and reducing the effect of *Clostridium difficile* toxin (Phalipon and Corthésy, 2003). These observations suggest that SC has phylogenetically originated from the innate defense system, like many other proteins involved in adaptive immunity. Importantly, membrane SC has been exploited as the only identifiable epithelial receptor involved in secretory immunity, as documented by the phenotype of pIgR knockout mice, which have no active external transport of dimeric IgA and pentameric IgM (Johansen et al., 1999).

A crucial protective role of secretory antibodies is supported by the fact that such $pIgR^{-/-}$ mice have "leaky" mucosal epithelia and excessive intestinal uptake of antigens from commensal bacteria such as *E. coli,* thereby eliciting a systemic antimicrobial IgG response (Johansen et al., 1999). Also, similarly to J-chain knockout mice that likewise lack both SIgA and SIgM, $pIgR^{-/-}$ mice are less resistant than wild-type mice against cholera toxin and early colonization of the gut epithelium by pathogens (Lycke et al., 1999; Uren et al., 2005). Due to absent secretory immunity, $pIgR^{-/-}$ mice further show reduced protection as well as cross-protection against mucosal challenge in an influenza model with the live A/PR8 strain after intranasal immunization with inactivated influenza vaccines of various types (Asahi et al., 2002).

Altogether, animal and cell culture experiments have suggested that SIgA antibodies promote intestinal homeostasis by neutralizing viruses and bacterial products through noninflammatory mechanisms in various mucosal compartments (Table 10.1 and Fig. 10.2). In addition to traditional luminal neutralization (Davids et al., 2006), it has been demonstrated that dimeric IgA antibodies—when exported by pIgR—can remove antigens from the lamina propria and neutralize viruses within the epithelium or block their transcytosis through polarized epithelial cells (Alfsen et al., 2001; Bomsel et al., 1998; Burns et al., 1996; Feng et al., 2002; Huang et al., 2005; Mazanec et al., 1993, 1995; Robinson et al., 2001). It has also been reported that dimeric IgA can neutralize bacterial LPS within intestinal epithelial cells (Fernandez et al., 2003)—suggesting a novel intracellular, noncytotoxic, and anti-inflammatory role for this antibody class during its transport to the lumen.

TABLE 10.1. Antimicrobial effects of SIgA antibodies.

- Dimeric IgA provides efficient microbial agglutination and virus neutralization.
- Performs noninflammatory extracellular and intracellular immune exclusion by inhibiting epithelial adherence and invasion.
- Exhibits cross-reactive ("innatelike") activity and provides cross-protection.
- SIgA (particularly SIgA2) is quite stable (bound SC stabilizes both isotypes of IgA).
- SIgA is endowed with mucophilic and lectin-binding properties (via bound SC in both isotypes and mannose in IgA2).

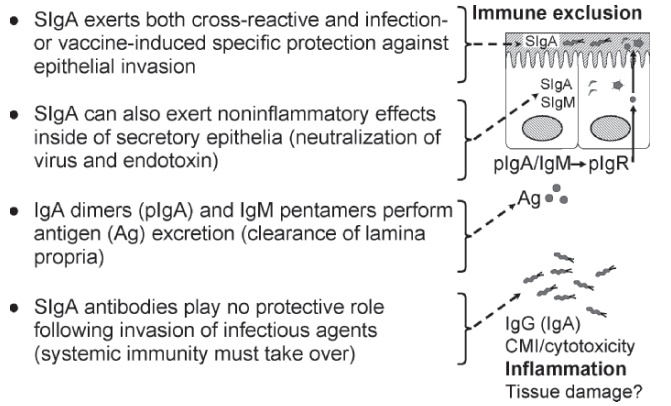

FIG. 10.2. Different principles of SIgA-mediated contribution to mucosal homeostasis. In addition to immune exclusion at the epithelial surface, the pIgR-mediated external transport of dimeric IgA and pentameric IgM (pIgA/IgM) might be exploited for intraepithelial pathogen neutralization and antigen excretion. However, when infection with pathogen invasion takes place, systemic immunity must take over to save life. This involves potent proinflammatory mechanisms such as complement activation by IgG antibodies, cell-mediated immunity (CMI), and cytotoxicity, all of which might cause tissue damage.

10.3.4.3. Role of SIgM and IgG Antibodies

Although SIgA is the chief effector of immune exclusion, SIgM also contributes—particularly in the newborn period and in IgA deficiency (Brandtzaeg et al., 1987, 1991; Brandtzaeg and Nilssen, 1995). In addition, there might be a significant contribution to immune exclusion by serum-derived or locally produced IgG antibodies transferred passively to the lumen by paracellular leakage (Persson et al., 1998)—or perhaps to some extent exported actively by the neonatal Fc receptor (FcRn) expressed on the gut epithelium (Yoshida et al., 2004). Interestingly, monomeric IgA or IgG antibodies, when cross-linked via antigen with dimeric IgA of the same specificity, might contribute to pIgR-mediated epithelial excretion of foreign material from the intestinal lamina propria (Mazanec et al., 1993). Notably, however, because IgG is complement activating, its contribution to surface defense is potentially proinflammatory,

which could jeopardize the epithelial barrier function (Brandtzaeg and Tolo, 1977). Such deterioration of local homeostasis is most likely counteracted by a variety of complement regulatory factors produced by mucosal epithelia (Berstad and Brandtzaeg, 1998).

It should finally be noted that when overt infection with microbial invasion occurs, SIgA antibodies will no longer determine the fate of the host, as experimentally documented in pIgR$^{-/-}$ compared with wild-type mice (Sun et al., 2004; Uren et al., 2005). Moreover, studies in mucosally vaccinated wild-type mice challenged with live influenza virus intranasally have suggested that whereas SIgA antibodies are essential to control virus replication locally, serum IgG antibodies protect against clinical illness (Bižanov et al., 2005). However, although systemic immunity might be considered a life-saving layer of defense, it operates at the risk of causing inflammation and tissue damage (Fig. 10.2). Thus, it has been experimentally documented that SIgA antibodies prevent virally induced pathology in the upper airways, whereas IgG antibodies neutralize newly replicated virus after the initiation of infection (Renegar et al., 2004). The lung parenchyma, which lacks a SIgA system, fully depends on serum-derived IgG and monomeric IgA for antibody protection (Daniele, 1990).

10.3.4.4. Interactions Between SIgA and Innate Defense Factors

Several studies have shown that mucosal immunity can be enhanced by cooperation between SIgA and innate defense factors. Immune reactions that take place at epithelial surfaces might stimulate the release of mucus from goblet cells and thereby reinforce the mucosal barrier against penetration of soluble molecules and microorganims (Walker et al., 1982; Walker and Bloch, 1983). Through the affinity of SC to mucus, the mucous barrier is endowed with a "flypaper" effect by topically retaining SIgA antibodies, which can trap antigens (Lim and Rowley, 1982; Phalipon and Corthésy, 2003).

Antigens of immune complexes in the mucous layer are more rapidly degraded by proteolytic enzymes than free antigens (Walker et al., 1975). It has been proposed that IgA1 is especially miscible with mucus, whereas the antibody function of IgA2 might take place mainly in the external secretory fluid (Clamp, 1980). This might be particularly relevant in the distal gut, where there is a predominance of local IgA2 production (Kett et al., 1986); this subclass is also more resistant to degradation by certain proteases other than IgA1 (Kilian et al., 1996). Although the subclass dependency is not determined, it has been proposed that SIgA-coated bacteria retained in mucus form a biofilm on the colonic epithelium (Bollinger et al., 2003); microbial immune exclusion might thus be enhanced by the rapid turnover of the gut epithelium.

In vitro data suggest that SIgA antibodies significantly promote the bacteriostatic effect of lactoferrin— probably by inhibiting bacterial production of iron-chelating agents, which interfere with its function (Rogers and Synge, 1978). SIgA can also enhance the broad antimicrobial spectrum of

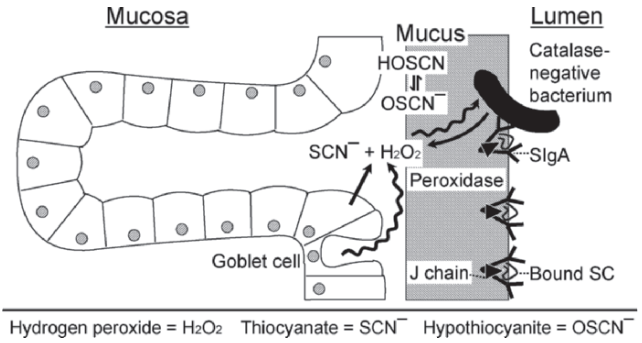

FIG. 10.3. Cooperation between SIgA and the peroxidase defense system. Schematic illustration of postulated microbial immune interactions taking place in the mucous layer of colonic mucosa. Details are discussed in the text.

the peroxidase defense system, apparently with no dependency on antibody specificity (Tenovuo et al., 1982). This effect might be explained by the mucophilic properties of bound SC in SIgA; cross-reactive SIgA antibodies could thus retain bacteria for prolonged and focused action of the enzyme with its biocidal product hypothiocyanate (Fig. 10.3). Human milk is rich in both lactoperoxidase and leukocyte-derived myeloperoxidase (Moldoveanu et al., 1982), which survives enzymatically active in gastric juice (Gothefors and Marklund, 1975). Moreover, peroxidase is produced by colonic goblet cells and is therefore most likely part of the innate gut defense (Venkatachalam et al., 1970).

There is a possibility that catalase-positive bacteria might resist the oxidizing effects derived from H_2O_2 (Fig. 10.3). However, bacterial catalase is restricted to the cytoplasm or periplasm of microorganisms and would therefore not be expected to alter substantially the availability of H_2O_2 as the substrate for peroxidase. Some bacteria might even produce H_2O_2 and, importantly, catalase-positive pathogens have been shown to be cleared efficiently from the airways *in vivo* by the peroxidase defense system (Gerson et al., 2000).

Cooperation between SIgA and the natural antimicrobial actions of gastric acid and intestinal peristalsis was demonstrated in a study of bacterial overgrowth in the jejunum of vagotomized patients; mucosal protection deteriorated when IgA deficiency was combined with a suboptimal function of these two innate defense mechanisms (McLoughlin et al., 1978). As always when IgA is selectively lacking, the study subjects necessarily had compensatory SIgM in their gut fluid (Brandtzaeg and Johansen, 2005). However, although several antimicrobial activities have been identified for this secretory antibody class (Brandtzaeg *et al.,* 1987), it clearly cannot adequately replace SIgA (Table 10.2).

TABLE 10.2. Effect of cooperation between SIgA antibodies and natural defense mechanisms on bacterial overgrowth in the jejunum.

	Clinical conditions without (+) or with (−) defective adaptive or innate immunity				
Variables					
SIgA	+	+	−	−	−
Peristalsis	+	−[a]	+	+	−[a]
Gastric acid	+	−[b]	+	−[b]	−[b]
Effect on jejunal colonization	−	−	−	−	+

[a]Complete vagotomy.
[b]Gastric drainage (gastroenterostomy) or pernicious anemia.
Source: Modified from McLoughlin et al. (1978).

10.3.4.5. Homeostatic Backup Mechanisms in SIgA Deficiency

Despite their compensatory intestinal SIgM, IgA-deficient subjects have raised levels of serum IgG antibodies to dietary antigens and show an increased frequency of infections, allergy, and autoimmune disorders, including celiac disease (Brandtzaeg and Nilssen, 1995; Burrows and Cooper, 1997). The same is claimed to be true for Crohn's disease, with some 20-fold increased incidence associated with IgA deficiency (Hammarström, personal communication). However, observations in IgA knockout mice have questioned the role of SIgA in mucosal defense (Brandtzaeg, 2003); explanations might be underestimation of the impact of compensatory SIgM and the fact that these mice show reduced inflammatory potential at mucosal sites due to decreased APC and Th1-cell functions (Arnaboldi et al., 2005).

As discussed earlier, knockout mice that lack both SIgA and SIgM ($pIgR^{-/-}$ or J $chain^{-/-}$) exhibit decreased resistance to cholera toxin and pathogen colonization in the gut; the leaky epithelial barrier results in a threefold increased intestinal generation of IgA^+ PCs (Johansen et al., 1999; Uren et al., 2003). These mice further appear to compensate for their lack of secretory immunity by increasing the number and cytotoxic activity of intraepithelial lymphocytes (IELs) in the gut (Yamazaki et al., 2005). Notably, IELs might not only be involved in antimicrobial defense but also exert a positive effect in the induction of oral tolerance (Grdic et al., 1998; Mennechet et al., 2004). Interestingly, $pIgR^{-/-}$ mice show enhanced ability to mount a tolerogenic response against fed antigen compared with wild-type mice—most likely representing an activated homeostatic mechanism in the face of a decreased mucosal barrier function (Karlsson et al., 2005). Basolateral release of MHC class II-positive exosomelike vesicles ("tolerosomes") from antigen-pulsed gut epithelium induces oral tolerance (Östman et al., 2005); perhaps this mechanism is enhanced in $pIgR^{-/-}$ mice because more antigen is taken up by unshielded epithelial cells.

It has likewise been observed that humans with hypogammaglobulinemia or selective IgA deficiency can have raised numbers of $\alpha\beta^+CD8^+$ and $\gamma\delta^+$ IELs

in their gut; possibly, enhanced induction of oral tolerance—in addition to compensatory SIgM—might contribute to the fact that they suffer from relatively little intestinal pathology compared with their frequent clinical airway problems (Brandtzaeg and Nilssen, 1995).

10.4. Mucosal Immunity in Neonatal Defense

10.4.1. Marked Species Differences

In contrast to rodents and ungulates, the newborn human has high levels of circulating maternal IgG because of FcRn-dependent placental transfer in fetal life, and not as a result of breast-feeding. Also unlike ungulates, intestinal uptake of SIgA antibodies from breast milk is of no importance for systemic immunity in infants, except perhaps in the preterm neonate (Brandtzaeg, 2002a). This is so because "gut closure" normally occurs in humans mainly before birth; but the mucosal barrier function might nevertheless be inadequate up to about 2 years of age. Although many variables influencing gut closure remain poorly defined, maturation of the intestinal immune system plays a major role.

Only occasional traces of SIgA and SIgM are exported from the human intestinal mucosa to the gut lumen during the first postnatal period, whereas some IgG is often present—probably reflecting both paracellular and FcRn-mediated transfer from the lamina propria (Harris et al., 2006; Persson et al., 1998), which after 34 weeks of gestation contains readily detectable maternal IgG (Brandtzaeg et al., 1991). IgA^+ PCs are normally undetectable in the mucosa before the infant is 10 days of age, but thereafter a rapid increase takes place—IgM^+ PCs often remaining predominant up to 1 month. On average, little increase of intestinal IgA production usually takes place after 1 year. A much faster establishment of secretory immunity can be seen in developing countries because of a more massive mucosal exposure to microorganisms (Brandtzaeg et al., 1991).

10.4.2. Individual IgA Variations Affecting Immunological Homeostasis

The postnatal mucosal B-cell development shows large individual variations, even within the same population (Brandtzaeg et al., 1991). This disparity might reflect a genetically determined effect on the establishment of the mucosal barrier function—perhaps in part exerted via diversity of the intestinal microbiota among individuals (Zoetendal et al., 2001). It has been proposed on the basis of serum IgA levels that a hereditary risk of atopy is related to a retarded postnatal development of the IgA system (Taylor et al., 1973). This notion was later supported by showing significantly reduced numbers of IgA^+ PCs (with no compensatory increase of IgM^+ PCs) in the jejunal

mucosa of atopic children (Sloper et al., 1981). Also, an inverse relationship was found between the serum IgE level and the jejunal IgA^+ PC population in children with food-induced atopic eczema (Perkkiö, 1980).

It was subsequently reported that infants born to atopic parents show a significantly higher prevalence of salivary IgA deficiency than age-matched control infants (van Asperen et al., 1985). Interestingly, Kilian et al. (1995) found that 18-month-old infants with presumably IgE-mediated allergic problems had significantly higher proportions of IgA1 protease-producing bacteria in their throats than age-matched healthy controls. This was in keeping with a previous report that showed much less intact SIgA in nasopharyngeal secretions of children with an allergic history than controls with episodes of acute otitis (Sørensen and Kilian, 1984). In this context, it is important to note that it takes up to 3 months after birth before the $IgA2^+$-to-$IgA1^+$ PC ratio in salivary glands has increased to the adult level, with ~33% $IgA2^+$ PCs (Thrane et al., 1991).

Altogether, a poorly developed or enzymatically reduced SIgA-dependent barrier function as part of dysregulated mucosal homeostasis—most likely often combined with hereditary atopic predisposition—might explain the pathogenesis of allergy. This notion accords with a recent study reporting multiple dysregulations of both innate and adaptive immune functions—including reduced IgA production—variably expressed in children with food allergy (Latcham et al., 2003).

Support for such clinical observations has been provided by an experimental model of anaphylaxis in which mice were either sensitized or tolerized to β-lactoglobulin (β-LG) via the gut (Frossard et al., 2004). Compared with anaphylactic mice, the tolerant mice were found to have more β-LG-specific IgA-secreting B-cells in PPs and higher levels of IgA antibodies to β-LG in feces. An increase of β-LG-induced IL-10 and TGF-β production by PP T-cells was also observed in the tolerant mice. This could be a cytokine link to the enhanced IgA–cell differentiation (see earlier). Notably, reduced expression of TGF-β has been found in the gut of food-allergic children, suggesting a deficiency of regulatory T (Treg) cells secreting this suppressive cytokine (Pérez-Machado et al., 2003). A deficiency was recently documented also for the $CD4^+CD25^+$ Treg -cell phenotype in peripheral blood of persistently cow's milk-allergic children in contrast to those who outgrew their milk allergy (Karlsson et al., 2004).

10.4.3. Critical Role of Breast-Feeding in Mucosal Defense

The lactating breast is a remarkable production site for SIgA, with a daily output of 0.5-1.0 g (Brandtzaeg, 1983). Experiments in neonatal rabbits have convincingly demonstrated that SIgA is a crucial antimicrobial component of breast milk (Dickinson et al., 1998), in addition to a variety of other factors that might enhance mucosal homeostasis (Brandtzaeg, 2002a).

Mucosal infections are a major killer below the age of 5 years—being responsible for more than 10 million deaths of children annually—mainly in developing countries where infants are highly dependent on SIgA antibodies from breast milk to protect their mucosae; epidemiological data do suggest that the risk of dying from diarrhea is reduced 14–24 times in breast-fed children (Anonymous, 1994). In fact, it appears that breast-feeding is the most efficient feasible intervention measure, with the potential of preventing 13% of all deaths below 5 years of age (Black et al., 2003; Jones et al., 2003).

Although the value of breast-feeding in Westernized countries is clinically most apparent in preterm infants, population studies show that exclusively breast-fed infants are generally better protected against a variety of infections and probably also against allergy, asthma, and celiac disease (Brandtzaeg, 2002a; Ivarsson et al., 2002; Kull et al., 2002; Schoetzau et al., 2002, van Odijk et al., 2003). This strongly suggests that the mucosal barrier function in newborns can be reinforced by breast-feeding, which appears to be particularly important in the face of a parental history of allergic diseases (Benn et al., 2004).

10.4.4. SIgA Antibodies and Induction of Oral Tolerance

Through avoidance of too early intestinal immune activation—for instance, by limiting the upregulation of the costimulatory B7 (CD80/CD86) molecules on APCs (Brandtzaeg, 1998; Chen et al., 2000)—the shielding effect exerted by SIgA from breast milk on the suckling's GALT (see earlier) might 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 (Juto and Holm, 1992), and breast-feeding has been shown to protect significantly against the development of celiac disease in children (Ivarsson et al., 2002)—an effect that appears to be unrelated to the time of solid food introduction (Brandtzaeg, 1997). Therefore, mixed feeding rather than abrupt weaning seems to promote tolerance to food proteins (Ivarsson et al., 2002). This notion is also supported by reports suggesting that cow's milk allergy is more likely to develop in infants whose mothers have relatively low levels of milk SIgA antibodies to bovine proteins (Savilahti et al., 1991; Järvinen et al., 2000). It is noteworthy in this context that allergic mothers reportedly have relatively low levels of ovalbumin-specific milk SIgA (Casas et al., 2000).

The presence of TGF-β and IL-10 in breast milk might contribute to its tolerogenic properties because these cytokines exert pronounced immunosuppressive effects in the gut (Ishizaka et al., 1994; Steidler et al., 2000) and TGF-β enhances the epithelial barrier function (Planchon et al., 1994). Also, these two cytokines are important switch and differentiation factors in the development of IgA^+ B-cells (Brandtzaeg and Johansen, 2005).

10.5. Microbial and Nutritional Impact on Mucosal Immune Regulation

10.5.1. Effects of Antigen Exposure and Nutrition

The degree of antigenic and mitogenic GALT exposure is decisive for the development of secretory immunity. As discussed earlier, the commensal microbiota is of crucial importance to this end. Thus, the intestinal IgA system of GF mice is normalized after about 4 weeks with CVN microbiota exposure in an ordinary animal facility (Crabbé et al., 1970; Horsfall et al., 1978).

Bacteroides and *E. coli* strains appear to be particularly stimulatory for the development of intestinal IgA^+ PCs (Lodinová et al., 1973; Moreau et al., 1978; Moreau and Gaboriau-Routhiau, 2000). Antigenic constituents of food also exert a significant effect, as suggested by the occurrence of fewer intestinal IgA^+ PCs both in mice fed on hydrolyzed milk proteins (Sagie et al., 1974) and in parenterally fed babies (Knox, 1986). Likewise, mice given total parenteral (intravenous) nutrition have reduced numbers of B- and T-cells in the gut, as well as decreased SIgA levels (Li et al., 1995a, 1995b; Janu et al., 1997), and they show impaired SIgA-dependent influenza-specific immunity (Renegar et al., 2001). The effect of food in the gut lumen could be direct immune stimulation or indirectly mediated by changes in the microbiota or release of gastrointestinal neuropeptides.

In a study of whole-gut lavage obtained from healthy adult volunteers in Dhaka (Bangladesh), the intestinal concentration of IgA was found to be almost 50% higher than in comparable samples obtained from Edinburgh (UK); even more notable, the intestinal IgA antibody titer against LPS core types of *E. coli* was almost seven times higher in the former group of subjects, whereas the levels of ovalbumin antibodies were relatively lower (Hoque et al., 2000). Altogether, therefore, it can be concluded that the large bacterial and dietary antigen load in the gut explains why the greatest density of IgA^+ PCs is seen in the intestinal lamina propria—amounting to some 10^{10} cells per meter of adult intestine, fairly equally distributed in the proximal and distal segments (Brandtzaeg et al., 1989).

In human lactating mammary glands, the PC density is much less—one gland showing an IgA-producing capacity similar to only 1 meter of intestine (Brandtzaeg, 1983). Thus, the daily output of IgA per kilogram wet weight of tissue (minus fat) is not more for lactating mammary glands than for salivary glands. In fact, it remains an enigma how some terminal PC differentiation is accomplished at such concealed secretory effector organs situated at considerable distances from antigen-exposed mucosal surfaces (Brandtzaeg and Johansen, 2005). One possibility is that the response of a subset of mucosal memory B-cells is more dependent on cytokine stimulation than on BCR ligation by antigen (Ehrhardt et al., 2005). Anyhow, the large capacity for storage of dimeric IgA/SIgA in the mammary gland epithelium and duct system—

rather than a high stromal PC density—explains the remarkable output of SIgA during breast-feeding (Brandtzaeg, 1983).

In keeping with the crucial stimulatory effect of luminal antigens on mucosal B-cell differentiation, defunctioning colostomies in children caused a 50% numeric reduction of intestinal lamina propria IgA^+ and IgM^+ PCs after 2–11 months (Wijesinha and Steer, 1982). Prolonged studies of defunctioned ileal segments in lambs revealed an even more striking scarcity of mucosal PCs; this was explained by decreased local proliferation and differentiation of plasmablasts and perhaps reduced B-cell homing from GALT (Reynolds and Morris, 1984). Accordingly, the postnatal establishment of the mucosal IgA system is usually much faster in developing countries than in the industrialized part of the world–a difference that appears to hold true even in undernourished children (Nagao et al., 1993).

Severe vitamin A deficiency, however, reportedly affects adversely mucosal IgA antibody responses in rodents (Wiedermann et al., 1993) but with no consistent disturbance of the epithelial IgA transport (Stephensen et al., 1996); rather, the deficiency might involve lack of retinoic acid-dependent DC-mediated imprinting of gut homing molecules on memory/effector cells in GALT (Iwata et al., 2004; Mora et al., 2006). Nevertheless, undernourished children respond to bacterial overgrowth in the gut with enhanced IgA production and upregulated export of IgA (Beatty et al., 1983); it is of great clinical importance that detrimental effects imposed on the SIgA system by severe malnutrition might be reversed with nutritional rehabilitation (Watson et al., 1985).

10.5.2. Homeostatic Effects of Probiotics

It is possible that a suboptimal development of the SIgA-dependent mucosal barrier function together with inadequate tolerance mechanisms explains the increasing frequency of certain diseases in industrialized countries—particularly allergies and autoimmune/inflammatory disorders (Brandtzaeg, 2002a; Yazdanbakhsh et al., 2002). On the basis of the so-called extended hygiene hypothesis (Rautava et al., 2004), several studies have evaluated the beneficial clinical effect of probiotic preparations derived from commensal gut bacteria (Collins and Gibson, 1999; Isolauri et al., 2001; Kirjavainen and Gibson, 1999). Especially, certain strains of intestinal lactic acid bacteria (LABs)—particularly lactobacilli and bifidobacteria—have been reported to enhance IgA responses, both in humans and experimental animals—apparently in a T-cell-dependent manner (Malin et al., 1996; Prokešová et al., 1998, 1999; Moreau and Gaboriau-Routhiau, 2000; Yasui et al., 1995). A double-blind study of children with a family history of atopy reported the prevalence of atopic dermatitis to be reduced by 50% at the age of 2 years after receiving the probiotic *Lactobacillus* GG strain daily for 6 months after birth (Kalliomäki et al., 2001). It remains unknown whether this beneficial effect was mediated via SIgA enhancement, promotion of oral tolerance, or both.

Similarly, there is some hope that immunization with mycobacterial antigens or bacterial CpG oligonucleotides might skew the cytokine profile toward Th1 and thereby—through cross-regulation and/or Treg-cell induction—dampen Th2-dependent allergic symptoms (Hopkin et al., 1998; von Reyn et al., 1997; Wohlleben and Erb, 2001). Newborns are in fact able to mount a Th1-type immune response when appropriately stimulated (Marchant et al., 1999), similarly to the early mucosal IgA response seen in heavy stimulatory conditions; neonatal expansion of $CD25^+$ Treg-cells can apparently be driven by antigens in the presence of LPS unless counteracted by a strong genetic predisposition for allergy (Haddeland et al., 2005). It is also possible that DNA from probiotic bacteria might induce Treg-cells that enhance mucosal homeostasis because certain strains of LABs appear to act as well by subcutaneous or peritoneal injection as by the oral route (Foligné et al., 2005a, 2005b; Sheil et al., 2004). However, the effect of this approach on the intestinal IgA system has not been studied.

10.5.3. Regulation of pIgR Expression

The postnatal colonization of commensal bacteria is important both to establish and regulate an appropriate barrier function in the gut—including enhanced epithelial expression of pIgR (Table 10.3)—as clearly demonstrated in mouse experiments (Hooper et al., 2001; Neish et al., 2000). Synthesis of pIgR/SC starts in human fetal life around 20 weeks of gestation, but there is a peak of epithelial expression during the first couple of postnatal months—best revealed in salivary glands (Brandtzaeg et al., 1991); this most likely reflects a direct or indirect effect of microbial products following commensal colonization of the mucosae, which starts in the birth canal.

Collectively, the above observations show that the expression of pIgR is both constitutive and subjected to inductive transcriptional upregulation, as first documented at the molecular level in our laboratory (Johansen and Brandtzaeg, 2004). Because one molecule of pIgR is consumed for every ligand

TABLE 10.3. Colonization of germ-free mice with *B. thetaiotaomicron* increases intestinal expression of genes involved in epithelial barrier function and nutritient absorption.

Gene product	Fold Δ over germ-free
Barrier function	
Polymeric Ig receptor (pIgR/SC)	2.6 ± 0.7
Small proline-rich protein 2a (sprr2a)	205 ± 64
Decay-accelerating factor (DAF)	5.7 ± 1.5
Nutrient absorption	
Na^+/glucose cotransporter (SGLT-1)	2.6 ± 0.9
Colipase	6.6 ± 1.9
Liver fatty acid-binding protein (L-FABP)	4.4 ± 1.4

Source: Modified from Hooper et al. (2001).

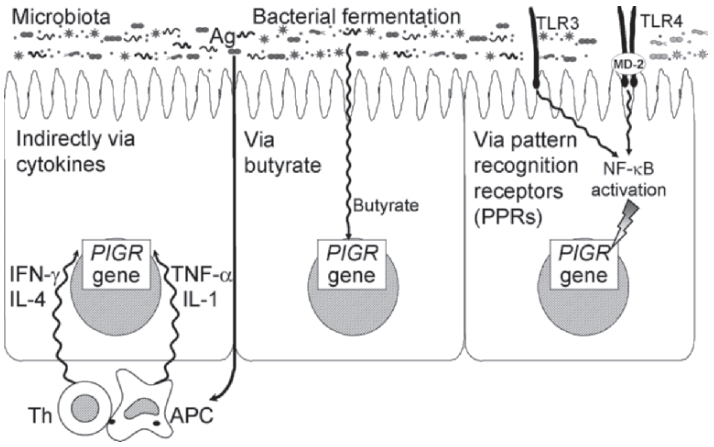

FIG. 10.4. Upregulation of epithelial pIgR expression by microbial activation of the *PIGR* gene locus. Details of the three postulated mechanisms are discussed in the text.

of dimeric IgA or pentameric IgM transported to the lumen, the regulation of pIgR must be crucial for maintenance of intestinal homeostasis.

Celiac disease is a good example of how pIgR expression and SIgA export can be indirectly enhanced via cytokines produced by activated mucosal APCs and T-cells (Fig. 10.4, left panel). There is a remarkable level of interferon (IFN)-γ in the untreated celiac lesion—derived from gluten-specific $CD4^+$ lamina propria T-cells (Nilsen et al., 1995, 1998) and activated intraepithelial $CD8^+$ T-cells (Olaussen et al., 2002). Similar upregulation of pIgR and epithelial transport of dimeric IgA is seen in Sjögren's syndrome and chronic gastritis—supporting the notion that cytokines provide an immunoregulatory link between increased local IgA production and enhanced output of SIgA during infection or low-grade inflammation not causing dysplastic lesions in the secretory epithelium (Brandtzaeg et al., 1992).

10.5.4. Microbial Enhancement of pIgR Expression

Upregulation of pIgR expression directly by microbes or their metabolic products is another intriguing possibility to enhance homeostatic immune functions in the gut. As mentioned earlier, a role for commensal bacteria to this end was suggested by the observation that intestinal pIgR mRNA levels were increased almost threefold when GF mice were colonized with *Bacteroides thetaiotaomicron*, a prominent member of the commensal intestinal microbiota (Table 10.3). Also, it was recently observed that infection with reovirus upregulates expression of pIgR in the human colonic epithelial cell line HT-29 (Pal et al., 2005); this might reflect interaction with epithelial PRRs, as discussed later. Changes in the composition of the indigenous microbiota could thus—either directly by

bacterial products or indirectly via cytokines (Fig. 10.4)—explain the reported impact of passive and adaptive immunity on developmental pIgR expression levels in the murine gut (Jenkins et al., 2003).

Altogether, commensal bacteria appear important for maintaining the "tone" of intestinal pIgR expression above its constitutive level. This notion is in keeping with a role for certain strains of commensal gut bacteria to enhance homeostatic immunoregulatory mechanisms—observed directly by administration of probiotics such as LABs (see earlier) or indirectly by adding prebiotics to the diet. Oligosaccharides, for instance, can both promote the growth of LABs and act as a substrate for the intestinal formation of butyrate (C-C-C-COONa), a fermentation product of many anaerobic bacteria in the normal colonic microbiota. Butyrate is an important energy source for colonic epithelial cells and, notably, it can increase gene transcription levels through specific DNA sequences (Glauber et al., 1991).

In this context (Fig. 10.4, middle panel), it is of considerable interest that when we pretreated HT-29 colonic epithelial cells with butyrate, the effect on pIgR/SC expression induced by some cytokines—and particularly various combinations of cytokines—was remarkably enhanced (Fig. 10.5), with the exception of the effect of IL-4 that was reduced (Kvale and Brandtzaeg, 1995). In line with our observations—suggesting an overall positive effect on the pIgR level in the distal gut—Nakamura et al. (2004) reported that mouse pups receiving dietary fructo-oligosaccharides showed significantly enhanced pIgR expression in ileal and colonic epithelium (Fig. 10.6), as well as increased export of SIgA into ileal loops and feces.

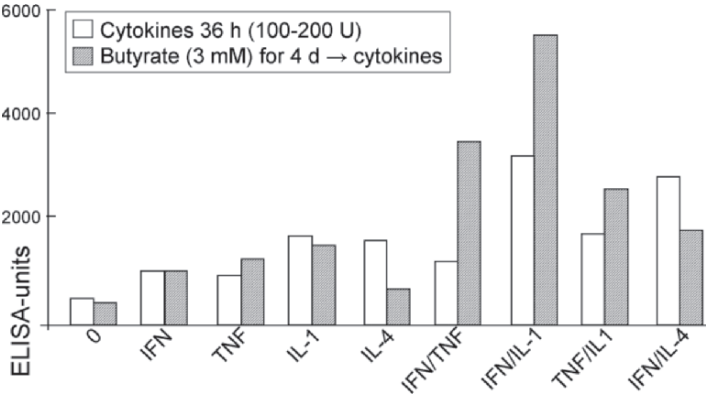

FIG. 10.5. Butyrate enhances cytokine-mediated expression of pIgR in human gut epithelial cells. Preincubation of HT-29 cells for 4 days with butyrate as indicated enhanced the pIgR-inducing effects [shown as enzyme-linked immunosorbent assay (ELISA) units] of TNF-α and IL-1, or these cytokines in combination with IFN-γ, whereas the effect of IL-4 was to reduce pIgR protein expression. Based on data from Kvale and Brandtzaeg (1995).

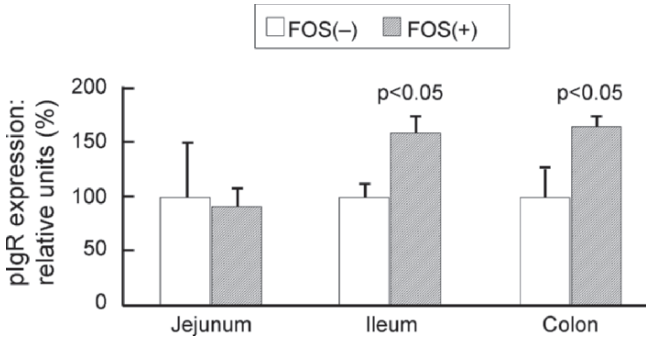

FIG. 10.6. Effect of prebiotics on pIgR expression in murine gut epithelium. Feeding fructooligosaccharides (FOS) to mouse pups enhanced the expression of pIgR in the distal gut (shown as relative units). Based on data from Nakamura et al. (2004).

10.6. Microbial Effects Via Pattern Recognition Receptors

10.6.1. Expression of PRRs in the Human Gut

As previously mentioned, TLRs function as PRRs to sense a variety of microbial constituents or products that trigger innate cellular responses (Medzhitov, 2001). To date, 11 transmembrane TLRs have been identified—acting singly or in combination (Beutler, 2004; Cario, 2005). TLR4 and TLR2 function as the sole conduits for signaling from LPS, which is an integral component of the outer membranes of Gram-negative bacteria. The classical LPS receptor CD14 is anchored in the cell membrane by glycosylphosphatidyl-inositol; in complex with its ligand and TLR4 or TLR2, it represents an important link between innate and adaptive immunity. The same is true for other PRRs that recognize additional PAMPs or MAMPs (Akira, 2003), including TLR9, which binds certain unmethylated CpG motifs of bacterial DNA (Kadowaki et al., 2001; Klinman et al., 1996; Peng et al., 2001). To induce tailored profiles of genes, the TLRs signal partly through "shared" and partly through more "specific" cellular pathways—generally leading to NF-κB activation but with coordinate activation of different transcription factors of the IFN regulatory factor (IRF) family that impose control on the expression pattern (Moynagh, 2005). The result variably includes DC maturation, release of proinflammatory cytokines such as the Th1-inducing IL-12 and IL-18 (Modlin, 2000; Kaisho and Akira, 2001; Medzhitov, 2001), and expression of chemokines and costimulatory molecules (Cario, 2005; Manigold et al., 2000; McInnes et al., 2000).

Although not yet extensively studied in the human gut, subepithelial putative APCs reportedly express certain TLRs. Thus, mRNA for TLR2 and TLR4 has been detected in isolated lamina propria macrophages (Smith et al., 2001), but the proteins were undetectable by immunohistochemistry in

the normal state, although a substantial fraction of the subepithelial macrophage-like cells showed positive staining in inflammatory bowel disease (IBD) lesions (Hausmann et al., 2002). Also of note, only negligible levels of CD14 are normally present on these cells, and their cytokine response is usually poor after LPS stimulation (Rugtveit et al., 1997a; Smith et al., 2001). In IBD, however, CD14 expression is strongly elevated on recently recruited, perivascular monocyte-like macrophages (Rugtveit et al., 1997b). Concomitantly, the costimulatory molecules B7.1 (CD80) and B7.2 (CD86) are markedly upregulated on the subepithelial putative APCs, and the LPS-induced proinflammatory cytokine response of macrophage-like cells isolated from IBD lesions is increased (Rugtveit et al., 1997a).

Altogether, lamina propria APCs are normally in a quiescent state *in situ*, both in the human (Brandtzaeg, 2001) and murine (Chirdo et al., 2005) gut. However, in IBD, the mucosal antigen-presenting potential is presumably increased in parallel with the PRR enhancement because monocyte-derived macrophages can be skewed toward differentiation of activated DCs under the influence of TNF-α (Chomarat et al., 2003).

10.6.2. Epithelial Sensing of Microorganisms

The intestinal epithelium appears to have inherent mechanisms to protect itself against activation from the luminal side—unless production of proinflammatory cytokines, chemokines and defensins is needed to protect against pathogens (Philpott et al., 2001; Sansonetti, 2004; Yan and Polk, 2004). Thus, epithelial cells apparently possess sensing systems that allow discrimination between pathogenic and nonpathogenic bacteria in order to initiate an inflammatory reaction only when elimination of invading microorganisms is needed.

Interestingly in this context, nonpathogenic *Salmonella* strains are able to block the NF-κB transcription pathway in human gut epithelial cells *in vitro* and thereby reduce basolateral IL-8 secretion in response to proinflammatory stimuli, including apical infection with wild-type *S. typhimurium* (Neish et al., 2000). In fetal life, the murine gut epithelium is sensitive to MAMPs such as LPS due to intracellular TLR4 expression (Lotz et al., 2006). LPS exposure during vaginal birth induces epithelial activation, leading to subsequent downregulation of TLR signaling in the neonatal gut epithelium and thereby tolerance to MAMPs.

Studies on the expression of CD14 and TLRs on human intestinal epithelial cells have provided inconsistent results, but the HT-29 adenocarcinoma cell line expresses low levels of TLR3 and TLR4 (Schneeman et al., 2005). Double-stranded RNA (dsRNA), a by-product of viral replication, has been identified as a ligand for TLR3, and epithelial cells can apparently be activated by LPS via TLR4 in a CD14-independent manner (Böcker et al., 2003).

The expression of both TLR3 and TLR4 is reportedly upregulated on epithelial cells in IBD lesions— particularly so for TLR4 (Cario and Podolsky, 2000)—although others have failed to confirm this by immunohistochemistry

(Hausmann et al., 2002). Although the signaling cascades for TLR3 and TLR4 are similar, recent studies have shown that differential usage of adaptor molecules might result in disparate biological responses (Hoebe et al., 2003; Oshiumi et al., 2003; Yamamoto et al., 2002, 2003).

10.6.3. Relation Between PRR Signaling and pIgR Upregulation

It was recently reported that pIgR mRNA and protein expression was strongly upregulated in response to both dsRNA/TLR3 and LPS/TLR4 signaling in HT-29 cells (Schneeman et al., 2005). By contrast, dsRNA—but not LPS—increased the mRNA level for TLR3 and TLR4, although the cell surface protein expression of both receptors was enhanced by LPS as well as dsRNA; this suggested that TLR4 could be transported to the cell surface from intracellular stores. A previously characterized binding site for NF-κB in the intron 1 enhancer of the *pIgR* gene (Johansen and Brandtzaeg, 2004; Schjerven et al., 2001) was shown by reporter assays with differently mutated cDNA constructs to be critical for transcriptional activation in response to TLR3 and TLR4 signaling. Analysis of several cytokine/chemokine gene products, including IL-8, demonstrated that TLR3 signaling resulted in a more pronounced proinflammatory response than did TLR4. These data suggested that signaling through TLR4 upregulates pIgR expression while minimizing initiation of inflammation.

Altogether, epithelial TLR engagement by microbes or their products might serve to augment pIgR expression (Fig. 10.4, right panel) and thereby enhance export of SIgA, thus linking the innate and adaptive immune responses to viruses and bacteria. The differential epithelial activation induced via TLR3 and TLR4 implies that particularly the latter receptor might promote SIgA-mediated homeostasis in the presence of commensal Gram-negative bacteria. Experiments in mice have suggested that other epithelial TLRs are also involved in the maintenance of intestinal homeostasis (Bambou et al., 2004; Rakoff-Nahoum *et al.,* 2004). Moreover, evidence is accumulating to suggest that such homeostasis is significantly influenced by cross-talk between epithelial cells and underlying lamina propria cells, particularly macrophages and DCs (Haller et al., 2000; Rimoldi et al., 2005).

10.6.4. Dysregulation of Innate Immunity Might Disturb Mucosal Homeostasis

It follows from the available information that defects in innate immune mechanisms might predispose to abrogated mucosal homeostasis. The best proof of principle in this respect has been provided by the NOD2 family of intracellular sensor molecules carrying a C-terminal leucine-rich repeat domain; its PRR activity recognizes unique muramyl dipeptide (MDP) motifs of peptidoglycans from both Gram-negative and Gram-positive bacteria (Cario, 2005).

The *NOD2 (CARD15)* gene is encoded by the IBD susceptibility locus (*IBD1*) on chromosome 16, and certain mutations of this gene are associated with clinical subsets of Crohn's disease patients—showing a striking dose effect of mutations that affect the PPR function of the molecules (Abreu et al., 2002; Ahmad et al., 2002; Cuthbert et al., 2002; Hampe et al., 2002; Hugot et al., 2001; Mathew and Lewis, 2004; Ogura et al., 2001). These seminal observations provide strong support for the possibility that aberrant sensing of the intestinal microbiota is an early pathogenic event, perhaps involving deficient induction of NF-κB-mediated activation of epithelial Paneth cells leading to insufficient defensin and cryptidin production (Kobayashi et al., 2005; Lala et al., 2003;) and impaired intestinal barrier function (Fig. 10.7). A possible role of SIgA in this context remains to be determined, but membrane targeting of NOD2 is required for NF-κB activation after recognition of MDP in epithelial cells (Barnich et al., 2005). Therefore, one interesting possibility is that membrane-associated pIgR in transcytotic vesicles might provide bacterial products for NOD2 through antigens complexed with receptor-bound dimeric IgA (Fig. 10.2).

Imbalanced triggering of innate immunity could be a common theme for disease initiation on a polygenic susceptibility background in the pathogenesis

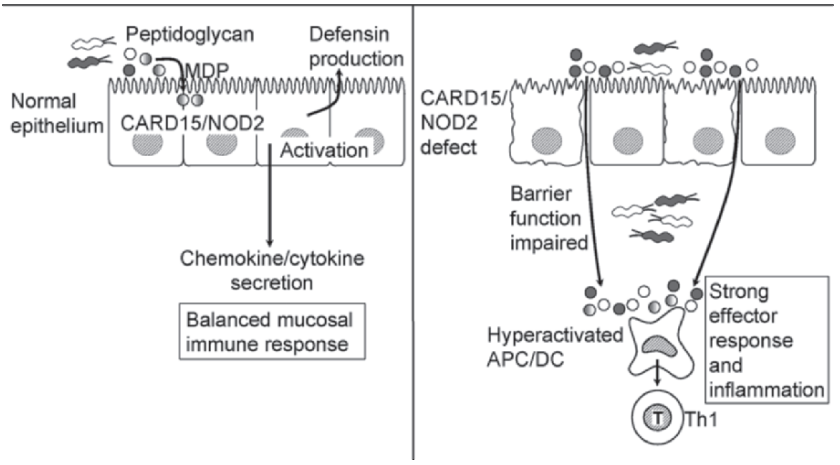

FIG. 10.7. Intestinal homeostasis depends on bacterial reinforcement of the epithelial barrier function. Normal epithelium senses muramyl dipeptide (MDP) motifs from Gram-positive and Gram-negative bacteria via intracellular CARD15/NOD2 molecules. This leads to cellular activation with secretion of protective defensins and cytokines/chemokines, which collaborate with SIgA (not shown) to maintain the barrier function. Mutations of CARD15/NOD2 might result in a leaky epithelium and hyperactivation of antigen-presenting cells (APCs), particularly quiescent lamina propria dendritic cells (DCs). This adverse development might cause strong effector Th1-cell responses with mucosal inflammation.

of a spectrum of clinical IBD entities. In this scenario, it has been suggested that *NOD2*/*CARD15* mutations might cause deficient signaling for downregulatory mechanisms in the gut, which in the normal state maintain lamina propria APCs/DCs in a quiescent state (Bouma and Strober, 2003; Judge and Lichtenstein, 2002). It has been proposed that one function of NOD2 is to limit the proinflammatory effects of TLR2 stimulation by peptidoglycan at the APC surface; mutant NOD2 is unable to sense MDP, which leads to "gain-of-function" for the TLR2 pathway with enhanced production of IL-12 and chronic inflammation (Watanabe et al., 2004).

However, this theory needs substantiation (Cario, 2005). It has alternatively been suggested that mutated NOD2 itself achieves gain-of-function whereby its N-terminal CARD domains become capable of activating Caspase-1 (Maeda et al., 2005). This enzyme might then cleave off the prodomain of IL-1, leading to secretion of mature IL-1, which promotes the inflammatory process. Admittedly, further studies are needed to discern the precise role of NOD2 in MDP recognition and subsequent intracellular signaling, cytokine/chemokine and defensin production, and perhaps upregulation or downregulation of pIgR. However, the near future will undoubtedly see a body of evidence also for other PRR mutations predisposing to intestinal inflammation.

Secondary alterations resulting in defect transcription of the *pIgR* gene might also result in deterioration of the intestinal barrier function, which could appear as patchy lack of pIgR/SC expression with absent SIgA export (Brandtzaeg et al., 1987). In ulcerative colitis, we have observed a relationship between downregulated pIgR/SC expression and the degree of hyperplastic epithelial lesions—overt dysplasia showing the most reduced immunohistochemical staining (Rognum et al., 1982). Expression of pIgR/SC protein and mRNA correlated—both variables being negatively related to the histological grade of dysplasia (Krajci *et al.*, 1996)—suggesting that this defect is a rather late event in the IBD process.

10.7. Concluding Remarks

Several more or less well-defined factors influence the development of productive IgA-dependent intestinal immunity and oral tolerance. Some of these variables are reciprocally modulated by the immune system to achieve homeostasis in the gut. Increased epithelial permeability for luminal antigens is likely an important primary or secondary event in the pathogenesis of mucosal diseases, including food allergy and IBD. This variable is determined by the individual's age (e.g., preterm versus term infant), interactions among mast cells, nerves, and neuropeptides, concurrent infection, and the epithelium-shielding effect of SIgA provided by breast milk or produced in the infant's gut. The pathological consequences will depend on how fast an intact epithelial barrier function can be attained or reestablished, which is influenced both by the age of the individual and by a successful mounting of intestinal IgA responses, as

well as generation of oral tolerance toward innocuous antigens from the diet and commensal bacteria.

Secretory IgA is the best defined effector of the mucosal immune system, and much knowledge has recently been obtained at the molecular level about the constitutive and induced transcriptional regulation of pIgR-mediated secretory antibody export in the gut. Of great importance in infancy is the large capacity for storage of dimeric IgA/SIgA in the mammary gland epithelium and duct system, which explains the remarkable output of SIgA during feeding— serving as passive immunization of the breast-fed baby's gut. Altogether, the secretory immune system is of considerable clinical interest because SIgA not only maintains mutualism with the indigenous microbiota but also forms the first line of immunological defense against infectious agents and other harmful substances. Human studies and characterization of a mouse strain with no secretory immune system ($pIgR^{-/-}$ mice) support the notion that SIgA antibodies are important in reinforcing the intestinal barrier function and promoting mucosal homeostasis.

In summary, it must be emphasized that the vast majority of antigenic challenges confronting the body, including potentially infectious agents, commensal bacteria, and foreign proteins, make contact with mucosal surfaces. Therefore, to maintain homeostasis in the extensive and vulnerable mucosae, they are protected by specialized anti-inflammatory immune defenses in which SIgA antibodies are a prominent effector. The induction of mucosal immunity with its immunoregulatory network is highly dependent on commensal bacteria and the postnatal period is critical. The newborn's adaptation to the environment is significantly modulated by innate ("natural") immune responses, and the SIgA system itself possesses innate features such as production of "natural" cross-reactive antibodies. Through evolution with microbial mutualism, the intestinal immune system has developed two major homeostatic defense layers aiming at control of antigenic challenges and return of the local tissue to a basal state with minimal pathology: immune exclusion provided by noninflammatory SIgA antibodies, which limit epithelial antigen penetration and invasion of microorganisms, and immunosuppressive mechanisms—often referred to as "oral tolerance"—which inhibit overreaction against components of the normal microbiota and other innocuous antigens. Both of these strategies depend on cooperation of adaptive immunity with the innate immune system, including "cross-talk" among commensal bacteria, the intestinal epithelium with its SIgA, and various lamina propria cells. Altogether, it appears that the healthy host not only tolerates the indigenous microbiota but also depends on it for mucosal homeostasis. Dysregulation in one or more of the many homeostatic mechanisms might result in overt intestinal inflammation.

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