Development, Regulation, and Function of Secretory Immunity

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The surface area covered by mucosal epithelia probably amounts to more than 200 times that of the skin, thus comprising almost 400 m² in an adult individual. This extensive and generally vulnerable monolayered epithelial barrier is protected by numerous innate chemical and physical mechanisms that cooperate intimately with adaptive, specific mucosal immunity. The main humoral mediators of the local specific immune system are secretory IgA (SIgA) and IgM (SIgM); the former class of antibodies constitutes the largest noninflammatory defense system of the body (1–3). Although the secretory antibody system is mainly directed against colonization of pathogens and penetration of "dangerous" antigens, it is also involved in immune exclusion of innocuous, soluble proteins present in food (Fig. 1). However, the latter types of antigen as well as components of indigenous bacteria generally induce poorly understood suppressive mechanisms collectively called "oral tolerance" when induced through the gut (4,5). This complex phenomenon of mucosally induced tolerance apparently explains why most individuals show no adverse immune reactions to persistent contact with food proteins and the normal microbial flora.

Successful interaction between local innate and specific immunity is a prerequisite for health because the various mucosae are favored as portals of entry by most infectious agents, allergens, and carcinogens. The neonatal period is particularly critical in this respect, as the newborn is immediately exposed to numerous microorganisms, foreign proteins, and chemicals. This chapter contains a summary of how secretory immunity develops, and a discussion of the mechanisms involved in the regulation and function of this adaptive first-line defense system. Its contribution to immune exclusion is virtually lacking during a variable period after birth; breastfeeding, therefore, is important, not only as a natural immunologic "substitution therapy" but most likely also because immune-modulating factors in breast milk may enhance the development of the infant's mucosal immune system (6).
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Soluble antigens

Indigenous microbial flora

Particulate antigens (pathogens)

Uptake: ~10^-5

M

M

Immune exclusion

Epithelial barrier

Suppression of IgG, IgE and DTH (CD4^+ Th1 cells)

Stimulation of IgA (and IgM)

Oral tolerance

Local

Peripheral

FIG. 1. Schematic depiction of two major adaptive immune mechanisms induced in the gut. (1) Immune exclusion limits epithelial colonization of pathogens and inhibits penetration of harmful foreign material. This first line of defense is principally mediated by secretory antibodies of the IgA (and IgM) class in cooperation with various nonspecific, innate protective factors (not shown). Secretory immunity is preferentially stimulated by particulate antigens and pathogenic infectious agents taken up through M cells (M) as indicated (see Fig. 2). (2) Penetrating, soluble dietary antigens (magnitude of uptake indicated) and the normal microbial flora are less stimulatory for secretory immunity (broken arrows) but induce, instead, suppression of proinflammatory humoral immune responses (IgG and IgE antibodies) as well as delayed-type hypersensitivity (DTH) mediated by activated T helper cells (CD4^+) of the γ interferon-producing Th1 subset. This complex and poorly defined phenomenon is called "oral tolerance"; it may exert downregulatory effects both locally and in the periphery.

IMMUNE EXCLUSION AND MUCOSAL HOMEOSTASIS

Induction and Dissemination of Local Specific Immunity

After the first period of passive humoral immunity, survival depends on the infant's specific immune responses. In this respect, the SIgA system is the best defined part of adaptive mucosal immunity (7,8). The relative resistance of SIgA against many microbial and endogenous gastrointestinal proteases makes it well suited for surface protection (3,9). In addition, SIgM may have a protective effect, particularly in early infancy and in selective IgA deficiency, but antibodies of this isotype are more easily degraded in the gut lumen than SIgA (10,11).

Primary B-cell responses that give rise to secretory antibodies seem to be elicited mainly in specialized lymphoepithelial structures where antigens are sampled from the mucosal surface (1,8,11–13). Such organized gut-associated lymphoid tissue (GALT) includes aggregated (Peyer's patches) and scattered secondary B-cell follicles (Fig. 2). In the human, Peyer's patches are mainly found in the distal ileum, whereas most of the solitary lymphoid follicles occur in the appendix and distal large bowel. All these components of GALT appear functionally similar; they contain a characteristic follicle-associated epithelium (FAE) with "membrane" (M) cells...
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Salivary, lacrimal, and mammary glands

FIG. 2. Schematic depiction of homing in the integrated or so-called “common” human mucosal immune system. Naive B and T lymphocytes are recruited to organized gut-associated lymphoid tissue (GALT) through high endothelial venules (HEV) in the parafollicular T-cell zone. Such lymphoepithelial inductive sites contain activated B-cell follicles with follicular dendritic cells (FDC) and the domes are covered with a specialized epithelium where “membrane” (M) cells transport luminal antigens (Ag) inward. Antigen-primed B and T lymphocytes migrate through lymph and reach peripheral blood for subsequent homing to mucosal effector sites. Their extravasation is particularly efficient in the gut lamina propria (heavy arrow) but dissemination also takes place from GALT to more distant effector sites in an integrated manner as indicated (thin arrows).

capable of transporting live and dead antigens into the underlying lymphoid tissue (11–13).

Although GALT constitutes the major part of mucosa-associated lymphoid tissue (MALT), induction of mucosal immune responses can probably also take place in the palatine tonsils and other lymphoepithelial structures of the Waldeyer pharyngeal ring, including nasal-associated lymphoid tissue (NALT) such as the adenoids (14). Accumulating evidence suggests that a certain regionalization exists in the mucosal immune system, especially a dichotomy between the gut and the upper aerodigestive tract with regard to homing properties and terminal differentiation of B cells (11,12). This disparity may be explained by microenvironmental differences in the antigenic repertoire as well as in the lymphoid and vascular adhesion molecules involved in local leukocyte extravasation (13). It appears that primed immune cells preferentially home to effector sites corresponding to the inductive sites where they initially responded to an antigen. Because bronchus-associated lymphoid tissue (BALT) is lacking in normal lungs of newborns and adults (15), the Waldeyer ring with the palatine tonsils and adenoids probably represents a significant component of MALT in humans, providing primed B cells for secretory effector sites of the upper aerodigestive tract (11,12,14).
Critical Role of Secretory Immunity in Infancy

The appearance of secretory antibodies in breast milk, directed against both intestinal and respiratory infectious agents, is a reflection of the MALT–mammary gland axis of B-cell migration (3,6,12), and the protective value of breastfeeding is highlighted in relationship to infections in the newborn period, particularly in developing countries. Mucosal pathogens are now a major killer of children below the age of 5 years, being responsible for more than 14 million deaths annually. Diarrheal disease alone claims a toll of 5 million children a year, or approximately 500 deaths every hour. These figures document the need for mucosal vaccines to enhance surface defense against common infectious agents, in addition to advocating breastfeeding. Convincing epidemiologic documentation suggests that the risk of dying from diarrhea is reduced 14 to 24 times in breastfed children (16,17). Indeed, exclusively breastfed infants are better protected against a variety of infections (16,18), atopic allergy (19), and celiac disease (20). Moreover, recent experiments in neonatal rabbits strongly suggest that SlgA is a crucial protective component of breast milk (21). The role of secretory antibodies for mucosal homeostasis is furthermore supported by the fact that knockout mice lacking SlgA and SlgM show increased mucosal leakiness (22).

Efficiency of Receptor-Mediated Secretory Antibody Transport

The remarkable magnitude of GALT as an inductive site for B cells is documented by the fact that at least 80% of all immunoglobulin (Ig)-producing blasts and plasma cells (collectively called immunocytes) in an adult are located in the intestinal lamina propria (11). Some 90% of these terminally differentiated mucosal B cells normally produce mainly dimers or larger polymers of IgA, collectively called “plgA” (7,8). Such polymers (as well as pentameric IgM) are efficiently transported externally as SlgA and SlgM antibodies by a transmembrane epithelial glycoprotein of approximately 100 kd called “secretory component” (SC), or the polymeric Ig receptor (plgR), which is constitutively expressed basolaterally on intestinal crypt cells and other serous types of glandular cell (7,11). After transcytosis to the apical surface, SlgA and SlgM are released to the lumen by cleavage of the plgR, and only the C terminal smaller receptor domain remains for degradation in the epithelial cell; the 80-kd extracellular part is incorporated into the Slg molecules as bound SC, thereby conferring protection against proteolytic degradation—particularly to SlgA in which SC becomes covalently linked (Fig. 3). In adult humans, more plgA (40 mg/kg body weight) is translocated to the intestinal secretions by this receptor-mediated mechanism every day than the total daily IgG production (~30 mg/kg) (23). Therefore, the gut is quantitatively the most important effector organ of adaptive humoral immunity.

Excess of unoccupied plgR is released apically to the lumen by proteolytic cleavage in the same manner as SlgA and SlgM to form the so-called free SC (Fig. 3). This 80-kd fragment (identical to bound SC) occurs in most secretions and, by equilibrium with the bound component, it exerts a stabilizing effect on the quaternary
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FIG. 3. Model for external transport of J-chain-containing dimeric IgA and pentameric IgM by the transmembrane secretory component (SC) or polymeric Ig receptor (plgR) expressed basolaterally on glandular epithelial cells. The polymeric Ig molecules are produced with incorporated J chain (IgA + J and IgM + J) by mucosal plasma cells. The resulting secretory Ig molecules (SlgA and SlgM) act in a first line of defense by performing immune exclusion of antigens in the mucus layer on the epithelial surface (to the right). Although J chain is often produced by mucosal IgG plasma cells, it does not combine with this Ig class but is degraded intracellularly (J). Locally produced (and serum-derived) IgG, therefore, is not subjected to active external transport. Free SC (depicted in the mucus) is generated when unoccupied plgR (at the top) is cleaved at the apical face of the epithelial cell.

Constitutive and Cytokine-Induced Regulation of the plg Receptor

As first proposed by our laboratory in the early 1970s, the receptor-mediated epithelial transport mechanism is shared by plgA and pentameric IgM because they contain a common 15-kd polypeptide called J (joining) chain produced by the mucosal immunocytes (7,11,24). The J chain constitutes an essential part of the plgR binding site in the Ig polymers (25). The plgR belongs to the Ig superfamily (8) and binds the two ligands noncovalently in a somewhat different manner through the first of its five extracellular Ig-like domains (26). Although the receptor expression is constitutively regulated, it can be upregulated synergistically by the immunoregulatory cytokines γ interferon (IFN-γ) and interleukin 4 (IL-4) (8,26,27). Also, the proinflammatory cytokines tumor necrosis factor-α (TNF-α) and IL-1 can enhance plgR expression in the human HT-29 adenocarcinoma cell line (26,27).

Our recent molecular experiments with the same cell line have shown that IFN-γ, IL-4, and TNF-α enhance plgR expression by transcriptional activation (28), and,
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FIG. 4. Schematic representation of regulatory aspects of secretory immunity that take place in inductive compartment (on the left) and at mucosal effector site (on the right). Various cytokines are apparently involved in isotype switching and terminal differentiation of mucosal B cells to promote the striking generation of plasma cells that produce dimeric IgA with J chain (IgA + J) at secretory effector sites. Some of these cytokines may be derived from antigen-presenting cells (APC), T helper cells (Th), or the epithelium, as indicated. In the gut, both transforming growth factor β (TGF-β) and vasoactive intestinal peptide (VIP) may act as IgA switch factors. Certain Th- and APC-derived cytokines also upregulate epithelial expression of the transmembrane secretory component or polymeric Ig receptor (plgR), thereby providing a regulatory link between the immunologic activity at the effector site and the magnitude of local external transport of secretory IgA (SlgA).

thus, they can provide an immunoregulatory link between the level of a local immune response and the antibody transport function of the receptor (Fig. 4). We and others have cloned and characterized the promoter region of the receptor gene; DNA elements that bind transcription factors belonging to the basic helix-loop-helix leucine zipper family and the interferon regulatory factor family seem to be most important for the constitutive and IFN-γ-enhanced plgR transcription, respectively (26).

Immunohistochemical observations on celiac disease, chronic gastritis, and Sjögren’s syndrome all show an immune response–associated enhancement of secretory immunity; signs of upregulated plgR expression and increased uptake of IgA are seen in glandular epithelia in all these immunologically active lesions (27). Nevertheless, the plgR-mediated epithelial transport capacity may be insufficient in certain patients with an unusual intestinal IgA cell expansion, resulting in excessive amounts of plgA in serum (29).

Regulation of the plg Receptor by Other Mediators

In addition to cytokines, other soluble factors can modulate the expression of plgR both in vitro and in vivo. Butyrate, which is an abundant fermentation product
in the colon, can enhance the stimulatory effect of IL-1 and TNF-α in HT-29 cells, particularly in the presence of IFN-γ, but decreases the stimulatory effect of IL-4, even in the presence of IFN-γ (30). It is currently not known whether this reflects the in vivo response of the large bowel epithelium. Furthermore, both constitutive and cytokine-enhanced plgR expression appear to depend on adequate presence of vitamin A (retinoic acid) and the nutritional state of the subject (31,32). In the rat kidney, plgR mRNA levels were found to be upregulated by a vasopressin-coupled pathway in response to variations in water intake (33). Moreover, parasympathetic and sympathetic autonomic nerve stimulation of rat submandibular glands increased the output SlgA significantly (2.6 and 6 times, respectively); this might reflect an effect of neurotransmitters on the secretory epithelial cells, although an influence of nerves on the local immunocytes could be a contributing factor (34).

Protective Function of SlgA Antibodies and Free Secretory Component

The main purpose of the secretory antibody system is, in cooperation with innate mucosal defense mechanisms, to perform immune exclusion (Fig. 1). Most importantly, SlgA inhibits colonization and invasion by pathogens, and plgR transported plgA and pentameric IgM antibodies may even inactivate viruses (e.g., rotavirus and influenza virus) inside secretory epithelial cells and carry the pathogens and their products back to the lumen (35–39), thus avoiding any cytolytic damage to the epithelium (Fig. 5). Both the agglutinating and virus-neutralizing antibody effects of plgA are superior to those of monomeric antibodies (1), and SlgA antibodies may block microbial invasion efficiently (40). Thus, individuals negative for human immunodeficiency virus (HIV) who live with HIV-positive partners for several years often appear to be protected by specific SlgA antibodies in their genital tract (41). A potentially important additional anti-infectious defense function is the ability of IgA antibodies to induce loss of bacterial plasmids that code for adherence-associated molecules and resistance to antibiotics (42).

Induction of SlgA responses has also been shown to interfere significantly with mucosal uptake of soluble macromolecules in experimental animals (1). Collectively, therefore, the functions of locally produced plgA would be to inhibit mucosal colonization of microorganisms as well as the penetration of antigens, and this effect is most probably enhanced by the relatively high levels of polyreactive SlgA antibodies (43,44). In the gut, interaction of SlgA with the intestinal superantigen protein Fv (Fv fragment binding protein) may, moreover, build an immune fortress by forming large complexes of intact or degraded antibodies with different specificities (45), thereby probably reinforcing immune exclusion.

It has been claimed that SlgA is capable of antibody-dependent, cell-mediated cytotoxicity and can promote phagocytosis through FcαRI (CD89) present on macrophages and granulocytes, enhance sticking of certain bacteria to mucus, interfere with growth factors (e.g., iron) and enzymes necessary for pathogenic bacteria and parasites, and exert positive influences on the inductive phase of mucosal immunity by promoting antigen uptake in GALT (1,3). The latter possibility adds to the importance of breastfeeding in providing a supply of relevant SlgA antibodies to the infant’s gut.
FIG. 5. Schematic representation of three levels at which dimeric IgA or secretory IgA (SIgA) may provide immune protection after being produced with J chain (IgA + J) by plasma cells in the lamina propria. Left: Dimeric IgA is transported by the transmembrane secretory component or polymeric Ig receptor (plgR) across epithelial cells and released into the lumen as SIgA antibodies that perform immune exclusion by interaction with luminal antigens (■). Middle: Dimeric IgA antibodies interact with viral antigens within epithelial cells during plgR-mediated transport, thereby performing intracellular virus neutralization and removal of viral products. Right: Dimeric IgA antibodies interact with antigens in the mucosal lamina propria and shuttle them back to the lumen by plgR-mediated transport.

Interestingly, potentially tissue-damaging eosinophils possess not only FcεRI but apparently also a receptor for free or bound SC (46). This probably explains why SIgA has particularly strong eosinophil-degranulating properties (47,48) and, in this respect, appears to be more potent than cross-linked IgE antibodies, which instead may be more involved in the recruitment and priming of these cells (49). Also, SIgA has been shown to induce degranulation of IL-3 primed basophils (50). Thus, by interacting with eosinophils and basophils (and perhaps also mast cells), SIgA provides a proinflammatory potential for the secretory immune system when immune exclusion fails, such as in parasitic mucosal infestations.

However, currently, it is difficult to know how the balance between SIgA and free SC can influence eosinophil activation, because free SC in solution (but presumably not on a surface) may be blocking in this respect (46,48). Free SC, moreover, may block epithelial adhesion of *Escherichia coli* (51), and a pneumococcal surface protein (SpsA) has recently been shown to interact with both free and bound SC (52). Such observations suggest that, phylogenetically, SC has originated from the innate defense system.
Noninflammatory Mucosal Clearance of Antigens

Intact antigens have been shown to cross the normal gut barrier and enter the blood stream, even in adults, particularly after food intake, although the actual level of uptake remains uncertain (1). Work performed in experimental animals with mucosal application of $^{125}$I-labeled albumin has been difficult to interpret because of marker instability; both degradation of the carrier molecule and release of the label can result in considerable overestimation of protein penetrability as determined by scintillation counting compared with data based on immunologic quantification (53).

Several routes can be visualized for the penetration of intact soluble antigen through the normal intestinal epithelium: passive paracellular diffusion or uptake through epithelial discontinuities (e.g., the cell extrusion zones of the villus tips); translocation through enterocytes by endocytosis and subsequent exocytosis; or transport by M cells in GALT. The relative importance of these different mechanisms remains unknown; and the consequences in terms of sensitization or induction of oral tolerance probably depend on the route of uptake as well as on the nature of the antigen (soluble, lectinlike, or particulate). These possibilities have been reviewed in detail elsewhere (1,4,54).

Quantitative studies performed on peripheral blood have provided some information about the absorption of intact dietary antigens in healthy human adults. Paganelli and Levinsky (55) found up to 3 ng/ml of $\beta$-lactoglobulin in peripheral blood after an intake of 1.2 L of bovine milk. Kilshaw and Cant (56) often detected both $\beta$-lactoglobulin and ovalbumin in peripheral blood as well as in breast milk of lactating women, the levels ranging from 110 pg/ml to 6.4 ng/ml in the latter fluid. Husby et al. (57) reported a serum level up to 10 ng/ml of ovalbumin, which corresponds to approximately $10^{-5}$ of the amount consumed. Most of the antigen appeared in the circulation after 2 to 5 hours and it was present partly in immune complexes.

Receptor-mediated clearance of immune complexes from the gut lamina propria to the lumen may take place because plgR expressing epithelial monolayers have been shown to translocate undegraded antigens bound to plgA antibodies from the basolateral side to the apical culture medium (58). Interestingly, monomeric IgA or IgG antibodies, when cross linked through antigen to plgA of the same specificity, also contributed to this plgR-mediated epithelial transport of immune complexes. Secretory epithelia, thus, may participate in noninflammatory removal from the mucosa of locally trapped antigens (Fig. 5).

Additional IgA-Mediated Putative Homeostatic Mechanisms

Experimental evidence also suggests that IgA in gut mucosa may influence local homeostasis by its binding to FcαRI on mucosal leukocytes. First, monomeric IgA—and particularly plgA or IgA containing immune complexes—is able to suppress the attraction of neutrophils, eosinophils, and monocytes, thereby reducing the availability of the numerous potent inflammatory mediators that may be released from these cells (1). Second, IgA can downregulate the secretion of proinflammatory
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cytokines such as TNF-α from activated monocytes (59), and perhaps also from mucosal macrophages. Third, activation of neutrophils and monocytes that results in generation of reactive oxygen intermediates (respiratory burst) may also be inhibited by IgA (60). On the other hand, plgA or aggregated monomeric IgA can trigger resting monocytes to show increased activity such as TNF-α secretion (61) and can also cause eosinophil degranulation (see above). This proinflammatory potential of IgA probably reflects the need for additional local antigen elimination mechanisms when mucosal immune exclusion fails, such as in intestinal parasitic infestations. Altogether, these divergent, experimental in vitro results emphasize that the contribution of IgA to normal mucosal homeostasis must be remarkably fine-tuned.

Advantage of IgA Over IgM in the Secretory Immune System

In normal adults, external secretions contain much more SIgA than SIgM, which is mainly explained by the striking predominance of local plgA-producing cells (11). However, in well-controlled, quantitative studies of jejunal fluid and parotid saliva, the concentration ratio of IgA to IgM is found to be 2.4 to 4.9 times greater than the corresponding local immunocyte class production ratio (62–65). This estimate is based on the observation of a fairly similar synthetic rate in IgA- and IgM-producing cells (66). Notably, mucosal IgA plasma cells release a variable amount of monomeric IgA in addition to plgA (7), whereas IgM-producing cells are virtually restricted to polymer secretion (67). On a molar basis, therefore, these calculations mean that the external transfer of plgA is favored at least 6 to 12 times over that of pentameric IgM, suggesting the existence of significant biological variables of secretory immunity other than the local immunocyte distribution. Such variables could reflect differences in diffusion properties across the stromal matrix and basement membrane, in the affinity of the Ig polymers for plgR, or in the efficiency of the plgR-mediated epithelial transcytosis of the two ligands.

In a recent in vitro study, we examined the impact of these variables on the epithelial transport of plgA and pentameric IgM (68). In vivo observations suggest that monomeric IgA, as is the case with IgG, diffuses more easily across basement membranes than pentameric IgM; the latter is mainly found intravascularly (78%), whereas most of the former (60%) is abundantly distributed in interstitial tissue fluid. In addition, some SIgA (2%) present in intestinal juice appears to be derived from serum plgA, whereas much less (<1%) SIgM originates from serum IgM (69). Therefore, we compared the diffusion properties of plgA and pentameric IgM as well as their binding to, and translocation by, polarized Madin–Darby canine kidney (MDCK) cells transfected to express the human plgR. Not unexpectedly, plgA diffused more readily than pentameric IgM through various filters used to support the MDCK monolayers. This result was supported by in situ immunofluorescence staining of the intestinal mucosa that showed preferential retention of pentameric IgM, both in lamina propria vessel walls and in epithelial basement membrane zones (68). The jejunal basement membrane is composed of a fine collagen network, with an estimated pore size of 13.5 nm (70). Radiographic analyses of the Fc region of pentameric IgM, combined with computerized molecular modeling (71), and electron
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microscopical analysis (72), suggest a molecular diameter in the range of 30 to 40 nm. Thus, the size of pentameric IgM most probably imposes severe restriction on its diffusion across basement membranes in vivo and, hence, its access to the plgR. Also, it is possible that some of the retained pentameric IgM is broken down along with the proteolytic turnover of basement membranes, although this ligand was transcytosed as efficiently as plgA by the human plgR (68).

Altogether, the plgA molecule appears to be particularly well designed for efficient protection of mucosal surfaces by possessing molecular characteristics that make it well-suited, both for efficient external transfer and for survival in exocrine secretions. Also, plgA serves to maintain local immunologic homeostasis, both by performing immune exclusion as SlgA antibodies and by its anti-inflammatory action within the mucosa. These properties of plgA may collectively have represented an evolutionary advantage over IgM in selecting SlgA as the dominant secretory antibody class in humans. Therefore, plgA appears to be a "smaller and smarter" antibody than pentameric IgM in terms of mucosal defense.

POSTNATAL DEVELOPMENT OF SECRETORY IMMUNITY

Mucosal B-Cell System

Peyer’s patches and other GALT structures are well-developed at birth, discrete T- and B-cell areas being apparent as early as 19 weeks of gestation (73). However, secondary follicles with germinal centers signifying B-cell activation do not occur until some weeks after birth; this reflects their dependency on exogenous environmental stimulation. The germinal center B cells of murine Peyer’s patches express small amounts of surface IgA along with less IgM or IgG (74). Such isotype skewing reflects B-cell switching in the course of clonal differentiation to precursors for IgA-producing immunocytes, the preferential induction of which is the hallmark of GALT (13).

The fact that the postnatal immune activation of GALT is retarded parallels the temporary immaturity of systemic immunocompetence observed in the newborn period (5,75,76). Thus, very few B cells with IgA-producing capacity (presumably GALT derived) are present in the peripheral blood of newborns (< 8/10^6 mononuclear cells); after 1 month, however, this number is remarkably increased (~500/10^6 mononuclear cells), reflecting the progressive environmental stimulation of GALT (77). An initial early increase in positive cells can be seen in preterm infants, especially in those with intrauterine infections, although IgM production predominates in these cases. In agreement with these observations in peripheral blood, only occasional IgM- and IgG-producing intestinal immunocytes are present at birth, and local IgA immunocytes are either absent or extremely rare even until after 10 days of age (10). The numbers of mucosal IgM- and IgA-producing cells increase rapidly after 2 to 4 weeks, the latter class becoming predominant at 1 to 2 months and usually peaking at about 12 months.

An early SlgM antibody response is probably of protective value, but it is known that specific immunity to certain bacterial capsular polysaccharides is poor or lacking
before 2 years of age. This creates a window of susceptibility at the time of disappearance of protective maternal IgG antibodies together with weaning and deprivation of passively acquired SlgA from breast milk. The basis for the impaired immune response to polysaccharides is unclear, but reduced levels of complement receptor 2 (CR2, CD21) expression on B cells and follicular dendritic cells, together with low complement activity in newborns, may result in lack of CR2/B-cell receptor synergy, thereby contributing to defective B-cell activation (78). Compelling evidence indicates that the interaction of the complement split product C3d with CR2 is an extremely important link between innate immunity and specific B-cell responses (79).

Individual Variations in the Development of Mucosal Homeostasis

It should be noted that the postnatal mucosal B-cell development shows large individual variation (10). To some extent this variability might be genetically determined and could exert an important impact on children’s health when their SlgA-mediated immune exclusion and other noninflammatory mucosal antigen-handling mechanisms are transiently inefficient.

On the basis of IgA measurements in serum, it has been suggested that infants and children at hereditary risk of atopy have a retarded postnatal development of their IgA system (80,81). Perhaps their SlgA-mediated immune exclusion and other noninflammatory mucosal antigen-handling mechanisms are transiently deficient. This notion was later supported by quantitation of jejunal immunocytes; a significantly reduced IgA response to luminal antigens, without any IgM compensation, was found in the mucosa of atopic children (82). Another study showed an inverse relationship between the serum IgE concentration and the number of IgA-producing cells in jejunal mucosa of children with food allergy (83). More recently, it was reported that infants born to atopic parents have a significantly higher prevalence of salivary IgA deficiency than age-matched control infants (84). Interestingly, Kilian et al. (85) found that the throats of infants aged 18 months with presumably IgE-mediated clinical problems contained significantly higher proportions of IgA1 protease-producing bacteria than age-matched healthy controls.

Therefore, a combination of reduced SlgA-dependent epithelial barrier function and hereditary increased IgE responses might often underlie the pathogenesis of mucosal hypersensitivity, at least in many of the atopic children. This notion accords with the increased frequency of infections, atopic allergies, and gluten-dependent enteropathy (celiac disease) seen in subjects with permanent selective IgA deficiency, although compensatory overproduction of SlgM, to some extent, may counteract the adverse consequences of their absent mucosal IgA responses (10,86).

Role of Antigen Exposure in the Development of IgA-Producing Cells

The antigenic and mitogenic load on the mucosa appears to be a decisive factor for the postnatal development of the secretory immune system. Antigenic constituents of food clearly exert a stimulatory effect, as suggested by fewer lamina propria IgA-producing cells in mice fed on hydrolyzed milk proteins (87) as well as in parenterally
fed babies (88). However, the indigenous microbial flora is of utmost importance, as indicated by the fact that the intestinal IgA system of germ-free or specific pathogen-free mice is normalized after approximately 4 weeks of conventionalization (89,90). Bacteroides and *E. coli* strains seem to be particularly stimulatory for the development of intestinal IgA immunocytes (91,92). Interestingly, early colonization of infants with a nonenteropathogenic strain of *E. coli* has recently been reported to have a long-term beneficial effect in reducing both infections and allergies (93).

Decreased amounts of both dietary and microbial antigens, thus, can explain why the numbers of colonic IgA- and IgM-producing immunocytes were found to be decreased by approximately 50% after 2 to 11 months in children who had been subjected to defunctioning colostomies (94). Postnatal and prolonged observations on defunctioned ileal segments in lambs have even more strikingly revealed a scarcity of immunocytes in the lamina propria; this result was explained by reduced local accumulation of B-cell blasts and might involve both hampered migration from GALT to the mucosa and, subsequently, decreased local proliferation and differentiation (95). It follows that the postnatal development of the mucosal IgA system is usually much faster in developing countries than in the industrialized parts of the world (10). This difference apparently holds true even in malnourished children (96), which reflects the fact that mucosal immunity is highly adaptable to the antigenic load of the environment.

In the light of these findings, a reduced SIgA-dependent barrier function could contribute to the increased frequency of certain diseases in industrialized countries, particularly allergies and other inflammatory disorders. The possible beneficial effect of probiotic preparations, therefore, has been evaluated in several experimental and clinical studies. Interestingly, especially viable preparations containing *Lactobacillus* spp. and *Bifidobacterium* spp. have been reported to enhance the IgA system, both in humans and in experimental animals (97–101), apparently in a T-cell-dependent manner (102).

**Nutrition and Intestinal Immunity**

An early immunohistochemical study of children with low protein-energy intake reported selective reduction of intestinal IgA-producing immunocytes (103). This was supported by experiments in rodents with prolonged and severe malnutrition (104); hampered homing of IgA-expressing B cells from GALT might be involved (105). Severe vitamin A deficiency appears to have a particularly marked adverse effect on mucosal IgA antibody responses (106), but with no consistent downregulation of epithelial IgA transport (107), although cytokine-mediated upregulation of pIgR in vitro appears to depend on this vitamin (31). Interestingly, although energy deficiency reduces intestinal pIgA expression in weanling mice (32), it has been reported that undernourished children respond to bacterial overgrowth in the gut with enhanced synthesis as well as upregulated external transfer of IgA (108). It is of great clinical importance that the detrimental effects of severe malnutrition on the SIgA system can be reversed by nutritional rehabilitation (109).
CONCLUSIONS

Secretory immunity depends on an intimate cooperation between mucosal B cells and the plgR/SC-expressing epithelia. The obvious biological significance of the striking J-chain expression shown by disseminated MALT-derived immunocytes is that IgA and IgM polymers with high affinity for plgR/SC can be produced at secretory effector sites and become readily available for active external transport.

Considerable evidence supports the notion that intestinal immunocytes are largely derived from B cells initially induced in GALT, but insufficient knowledge exists concerning intestinal uptake, processing, and presentation of luminal antigens as a basis for the extensive and continuous priming and expansion of mucosal B cells. Also, it is not clear how the germinal-center reaction in GALT, compared with other parts of MALT (e.g., tonsils), so strikingly promotes preferential isotype switching to IgA and a high level of J-chain expression.

Although the B-cell migration from GALT to the intestinal lamina propria is guided by rather well-characterized adhesion molecules, the chemotactic stimuli involved in extravasation and microcompartmental distribution of various B-cell subsets remain elusive. Importantly, the homing mechanisms of mucosal B cells appear to be remarkably regionalized. Retention and accumulation of the extravasated B cells at secretory effector sites are influenced by antigen-driven local proliferation and differentiation. However, little is known about the necessary stimulatory signals for this process.

The mucosal barrier normally allows some penetration of intact soluble antigens so a need probably always exists for immune elimination in the lamina propria. If immune exclusion is impaired (e.g., in IgA deficiency) or too large an antigen load is found on the epithelial barrier (e.g., in chronic infection), activated nonspecific amplification mechanisms involved in immune elimination may cause hypersensitivity which is observed clinically as mucosal disease. Although the immunopathogenic mechanisms are rather well understood in some of intestinal disorders (e.g., atopic food allergy and celiac disease), the cause of their initiation, possibly involving abrogation of oral tolerance, generally remains unexplained.

Clinical observations in humans, as well as studies of plgR/SC knockout mice, suggest that secretory immunity is not the only important part of intestinal mucosal defense; thus, it is becoming increasingly evident that innate local immunity is crucial and much more complex than previously believed. The cooperation between innate and adaptive mucosal immunity needs exploration to better understand how homeostasis of mucous membranes normally is maintained.

The relationship between induction of intestinal IgA responses and oral tolerance remains somewhat of an enigma (4). Experiments in CD8 knockout mice have suggested that this phenotype of T lymphocytes (the predominant intraepithelial lymphocyte subset) is crucial for downregulation of the mucosal B-cell system (110). The hyporesponsiveness of the intestinal immune system appears to be robust, because even a strong immunogen such as cholera toxin (CT) is unable to abrogate it, although oral tolerance cannot be induced in the presence of CT (110). On the other hand,
transforming growth factor β has also been shown to be important in promoting IgA switching (Fig. 4) in mice immunized with CT (111), and this cytokine is believed to be one of the major mediators of oral tolerance in murine test systems (112). It is not yet possible to extrapolate such apparently contradictory information to the human mucosal immune system.

It is well established in the human gut that the mucosal immune system responds to infection with local IgA and IgM production (113), and it appears that the level of this secretory antibody response may determine whether clinical symptoms will or will not occur (114). In experimental animals, antibody-dependent immune exclusion has been shown to operate even for small molecules such as chemical carcinogens (115,116). However, further studies are needed because a rational basis for manipulation of local immunity by vaccines is still not satisfactorily established.

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**DISCUSSION**

**Dr. Lentze:** An association is seen between IgA deficiency and autoimmune disease, for instance in celiac disease. Is this because of defective immune protection or immune exclusion in the gut, for instance for gluten molecules, or what is the mechanism?

**Dr. Brandtzaeg:** This could indicate that mucosal tolerance is more easily disrupted in IgA deficiency; but a genetic link might also exist as the HLA-DQ molecules are so important for the association with both celiac disease and some of the autoimmune diseases as well as with IgA deficiency. At present, it is not possible to say which of these two factors is operative, abrogation of tolerance or genetic link. These patients have a very good compensatory secretory IgM response in their gut that uses the same receptor-mediated pump as dimeric IgA. We used to believe that compensation was a paradox because we thought that IgM was an excellent complement activator, and that would mean a phlogistic secretory immune system existed rather than the quiet IgA system that does not activate complement. It turns out that this is wrong, because the only IgM that can use the polymeric Ig receptor (plgR) is a pentameric molecule that has its J chain incorporated in the same way as dimeric IgA. It is the J-chain-deficient hexameric form of IgM that has a fantastic complement-activating capacity, whereas the pentamers have little or no such activity.

**Dr. Black:** I was intrigued by your argument about reduced SIgA barrier function in Western societies. What would you suggest as a technique to improve that?

**Dr. Brandtzaeg:** This involves a controversial probiotic discussion. Several studies are promoting the use of probiotics—lactobacilli and so on—and five or six reports have shown that they will enhance IgA production in the gut (1–5). Another report shows that this effect is T-cell-dependent (6), which fits with what goes on in the Peyer’s patches, wherein we have the whole textbook of immunology with T cells and B cells and so on. A recently reported Czech study (7) showed that this protection is long term, in that several years after the subjects had been fed a nontoxogenic strain of *E. coli* early in childhood they had less food allergy than other children from the same population. So, maybe it reflects a strengthening of the barrier function.

**Dr. Seidman:** You said that IgA is needed to swim in the Ganges. Is the inverse of that statement true? If you do swim in the Ganges, do you have more IgA and, therefore, are you less likely to develop allergy, for example? A clinical suggestion is that perhaps less food allergy and atopy are found in the developing world, because the children are either malnourished and their immune system does not have the luxury of developing an allergic Th2 type
of reaction, or perhaps because their gut immune system is so stimulated from early on in life that they develop a higher level of tolerance. Are there any animal models to prove this? If you induce enteric infection early in life in an animal model, can you stimulate a secretory IgA response and prevent the development of allergy?

Dr. Brandtzaeg: This covers the same ground as the previous question on probiotics. The only clinical study I have seen is that Czech study previously mentioned. The data seemed fairly convincing, although an abstract is always difficult to judge. Nevertheless, it gives some hope for probiotic treatment. I do not know of any reports of animal models. The problem is that we have changed our world so much in such a short time in the industrialized countries, it is horrifying to contemplate the millions of years that genes have evolved to create the mechanisms of immune exclusion and immune tolerance, and then to see that we have changed around everything in our environment in about 100 years. It is no surprise that something must go wrong in the mucosal immune system. Even the high wheat diet involved in the pathogenesis of celiac disease has only been around for 1,000 years or so, and that is nothing compared with the gathering and hunting period of our ancestors.

Dr. Marini: We have done several experiments in preterm babies formula fed giving different kinds of probiotics. We found that in the first few days, we obtain very good colonization. Later on, the infants develop very high specific IgA and IgM and they kill all the administered probiotics (8-11). On the other hand, we were able to achieve persistent colonization with bifidobacteria, when oligosaccharides were added to the formula, similar to that observed in babies fed with human milk. Can you comment about that?

Dr. Brandtzaeg: It is the nature of the secretory IgA system to eliminate the stimulatory bacteria. That is exactly the problem that has plagued people who have been trying to develop live recombinant vaccines. Because when a recombinant organism is made to colonize the Peyer’s patches to induce the production of an immunogen for a vaccine, it takes some time, and then the carrier will be eliminated by the IgA response against the recombinant Salmonella or whatever organism was used. That is exactly what is seen with a new probiotic—the “take,” so to say, will be reduced after some time, because an IgA response has occurred in the gut. Thus, either compensation is made by changes with various bacteria, or one has to be found that is less immunogenic, which is probably what you alluded to with bifidobacteria. The reason that our normal flora survive in the gut is partly that it is changing phenotype all the time. Individual bacterial flora probably changes their immunogenic surface repertoire of antigens every 2 weeks or so, and the immune system develops tolerance against stable and less stimulatory antigenic determinants of the autologous indigenous microbiota.

Dr. Marini: Do you feel that intermittent administration of probiotics is likely to be better than continuous administration?

Dr. Brandtzaeg: It could be for reasons just mentioned, but I have no personal experience in this field.

Dr. Marini: You say that changing the intestinal flora can be good for the prevention of atopy. But so far, no clear demonstration shows that this works. In fact, only one epidemiologic study (from Estonia) looked at the change in lifestyle in that country, and although an increase in allergy was predicted, the opposite was found (12). I have not yet seen any prospective study showing that by changing the flora, the risk of allergy is really reduced in babies from high risk families. We need such studies.

Dr. Brandtzaeg: I agree that epidemiologic studies are difficult to interpret, but I think what has been done by Björkstén et al. in Sweden is pretty convincing (13,14): comparing the Baltic countries with Sweden, it was shown that a change had occurred in the bacterial flora in the gut in Sweden; the Baltic countries now have a gut flora similar to that of
Swedish population just after World War II, and they have less allergic disease. Data also relate to the East German migration to the West after the wall came down, showing that these people have less allergy than a cohort of the same age who lived in West Germany in infancy (15). Thus, several epidemiologic studies indicate that the bacterial flora is important for the maturation of the immune system in the gut, both in terms of tolerance and also of the IgA system.

Dr. Zoppi: From your presentation, I presume that oral vaccines do enhance the secretion of IgG and IgM. Is that correct?

Dr. Brandtzaeg: Yes, that is correct. In many vaccine experiments, it has been shown that giving an oral vaccine increases the titer of the correct IgA antibodies in gut fluid and, to some extent, also in tears, saliva, and the respiratory tract. But, we do not yet understand the molecules involved in homing of primed B cells outside the gut, because the adhesion molecules have not been identified. Some of intestinal type may be expressed in the lactating breast, but not in the glands of the nose, the tear glands, and salivary glands. Other homing molecules must exist that are not yet understood. Some evidence indicates that B cells originating from the tonsils will try to stay in these upper regions—they do not like going into the gut mucosa. So, some sort of regionalization exists in the mucosal homing mechanisms that may be important when designing local vaccines. Importantly, however, nasal vaccines usually increase production of systemic IgG antibodies in addition to stimulating a secretory IgA response.

Dr. Alpers: This is a very interesting scheme, but is IgA really the most important factor? I am unaware of any evidence that IgA-deficient nursing mothers have infection-prone newborn infants. So, if IgA is the major protective factor, that is inconsistent with the evidence. Is there sufficient secretory IgM in these cases?

Dr. Brandtzaeg: Yes, in the gut lots of secretory IgM is produced in IgA deficiency and transported by this same polymeric Ig receptor-dependent mechanism as dimeric IgA, even into breast milk.

Dr. Alpers: So SIgM is enough? SIgA is not needed?

Dr. Brandtzaeg: It is well known that patients with selective IgA deficiency have more problems both with autoimmune disease and with infections and allergy. That has been well established, especially in the upper respiratory tract where most of the problems occur. Secretory IgM, which to a large extent may replace IgA in the gut, does not replace IgA in the upper respiratory tract nearly so consistently, although those patients who could compensate with IgM have been shown to have fewer problems than those who could not. Those who did not compensate with IgM for a lack of IgA had more otitis media, more recurrent tonsillitis, and some even developed pneumonia (16). This shows that IgA deficiency can be a problem, and a need exists for compensation. In the gut is seen fairly consistent compensation with pentameric secretory IgM using the plgR or polymeric Ig receptor. I do believe in the secretory IgA system: the mucosal B-cell induction and homing with access to the mammary gland is such a complex scheme that it would not have developed if it were of no use for the baby.

Dr. Lionetti: The incidence of inflammatory bowel disease is greater in the developed world than in the developing world, and this seems to parallel the decreased number of gastrointestinal infections early in infancy. Would you speculate whether the decreased number of infections play a role in the increased response of the mucosal immune system, especially the T cells?

Dr. Brandtzaeg: That would fit in with what has been discussed in relation to allergy. Early microbial stimulation of the mucosal immune system appears to be important for subsequent immune regulation, the so-called "hygiene" hypothesis. Also, unpublished data from Lennart
Hammarström at Huddinge Hospital in Sweden indicate a 20 times increase of Crohn’s disease in selective IgA deficiency, which is something quite new.

Dr. Maki: Is uptake of antigen always via M cells? What about cases of increased permeability or a disease such as celiac disease?

Dr. Brandtzaeg: M cells are known to be keen to take up particulate antigens and various types of pathogens. No good evidence supports that food antigens use the M cells for entrance, although, in experimental systems, horseradish peroxidase can go via the M cells into the immune system. However, most likely, food antigens will mainly enter the immune system through the large surfaces at the so-called “mucosal effector sites” and primarily induce tolerance there, as in intestinal mucosa.

Dr. Yamashiro: It takes nearly 1 month after birth until plasma cells appear in the intestine. Does the IgA level in the saliva reflect the IgA level in the intestine?

Dr. Brandtzaeg: Actually, a little more IgA is found in saliva than in the gut fluid initially. A few plasma cells are seen in salivary glands earlier than in the gut. That is strange, but it may be related to antigens in the amniotic fluid, and the fetus is swimming in this fluid. Also, the tonsils develop quite early as inductive sites, and plasma cells can be seen below the crypt epithelium only a few days after birth. However, these are minor differences and in both regions it takes months or even years before the IgA system at the mucosal secretory effector sites is fully developed to an adult level (17).

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