Mini Review

The mucosal immune system for secretory IgA responses and mucosal vaccine development

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Immunoglobulin A (IgA) is the body's predominant antibody isotype, and the bulk of the body's IgA-producing cells reside in various mucosal and exocrine tissues. Production of IgA at mucosal surfaces is strictly regulated through coordinated communication among mucosal B and T cells, mucosal dendritic cells (DCs), and epithelial cells. Although organized lymphoid tissues such as Peyer's patches (PP) have long been recognized as key sources of IgA plasma cells at mucosal surfaces, IgA-mediated mucosal immune response is maintained even in PP-null conditions. Mucosal DCs likely are specialized to provide help to B cells to promote IgA-producing plasma cells. Intriguingly, induction of the proliferation of microbiota-specific IgA^+ B cells is independent of T helper activity but may depend on lamina propria DCs expressing iNOS, CX_3CR_1, and TLR5.

In the quest to develop an oral vaccine to boost mucosal immunity, rice was genetically engineered to express the B subunit of cholera toxin (CT-B). The recombinant CT-B that accumulates in the protein bodies of rice is resistant to the harsh gastrointestinal environment, and immunization of mice with the vaccine provoked a protective mucosal IgA response against CT. Furthermore, the vaccine is stable and maintains immunogenicity at room temperature for at least 24 months. Because they are easy to administer and requires neither refrigeration nor needles, rice-based mucosal vaccines are highly practical for global immunization against infectious diseases.

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Discovery of the mucosal immune system

For decades, vaccine researchers have been fighting infectious diseases without much help from one of the body's leading defensive weapons, the mucosal immune system. Most successful vaccines to date, such as those for childhood measles, mumps, and rubella, have been aimed at stimulating host systemic immunity to produce serum antibodies against disease-causing organisms. Therefore, most vaccines have been given by injection for induction of effective systemic immunity. However, our present molecular and cellular understanding of the mucosal immune

system allows us to consider the use of oral and nasal immunization for induction of antigen-specific immune responses at the mucosal surface as well as systemically. It is now plausible to propose that the current injection-type vaccines be advanced to mucosal forms of vaccine.

The part of the immune system that produces 70% of the body's antibodies (IgA) has been virtually ignored, because little has previously been known about how the mucosal immune system operates. Mucous membranes line the airways, reproductive system, and gastrointestinal tract, and many pathogens first encounter the body through the mucosal epithelium. The mucosae of the aerodigestive and reproductive tracts have a combined surface area of at least 400 m^2, which is exposed continuously to harsh external environments and is the first port of entry of many pathogens[1].

During the past 40 years of study, we have recognized that a distinct system of immunity manifested by production of the IgA antibody isotype exists at mucosal surfaces[1,2]. Mucosal tissues are heavily populated with immune cells: the intestinal lining is estimated to contain more lymphoid cells and produce more antibodies than any other organ or tissue in the body[3,4]. The mucosal immune system consists of specialized local inducer sites, the organized mucosa-associated lymphoid tissue (MALT), and widespread effector sites, both of which are separated from the mucosal surface by epithelial barriers[5]. Some mucosal tissues, such as the vagina, have no local organized MALT but rely on antigen uptake by intraepithelial dendritic cells and transport into lymph nodes that drain the mucosa[6].

In either case, the first step in the induction of a mucosal immune response is the transport (or uptake) of antigens across the epithelial barrier. After antigen processing and presentation in the organized MALT, antigen-specific IgA-committed B cells proliferate locally and then migrate through the bloodstream to distant mucosal and secretory tissues, such as the lamina propria and salivary glands. There, the IgA-committed B cells differentiate primarily into polymeric IgA-producing plasma cells. Dimeric or polymeric IgA antibodies are transported across epithelial cells into glandular and mucosal secretions through polymeric Ig-receptor-mediated transcytosis.

Classic experiments[7] have suggested that Peyer's patches (PPs) contain IgA precursor B cells that can populate the lamina propria (LP) of the gastrointestinal tract and become IgA-producing plasma cells. This study was the first to suggest the presence of a mucosal migratory pathway from the organized MALT to widespread effector sites for induction of the IgA response. Subsequent studies have shown that oral administration of antigens leads to the production of secretory IgA (S-IgA) antibodies in the gut as well as in secretions at distant mucosal sites such as salivary and mammary glands[7]. In addition, human colostrum and milk are well known to contain antibodies to gastrointestinal bacteria, including *Escherichia coli*[8], and to the oral bacterium *Streptococcus mutans*[9]. Another study[10] provided strong evidence that after antigen exposure of intestinal tissue with PPs, IgA precursor B cells migrate from the patches through the lymphatics to the bloodstream and thereby reach the lamina propria of other regions of the gastrointestinal tract, where they become mature IgA-synthesizing plasma cells. These elegant studies have made important contributions to the concept of a mucosal immune migration system comprising inducer (e.g., PP) and effector (e.g., lamina propria) sites (Figure 1A).

Unique characteristics of PPs for mucosal IgA responses

At their bases, murine PPs contain follicles — B-cell zones with germinal centers — and parafollicular regions, which are enriched with T cells. The epithelial dome over the follicles and parafollicular regions is commonly described as the follicle-associated epithelium and includes antigen-sampling M cells[11-13]. M cells were named for their unique microfolds at the topical surface and pocket formation at the basement membrane. M cells have short microvilli, small cytoplasmic vesicles, and few lysosomes. Microorganisms and complex antigens are engulfed by M cells, and the uptake and transcellular passage of luminal antigens, including proteins and small particles, through the cells has been documented[14-16].

PPs contain all the immune-competent cells needed for the induction of an antigen-specific IgA response, including antigen-presenting cells (APCs; dendritic cells and macrophages), IgA-committed B cells, and T helper (Th) lymphocytes types 1 and 2. After their uptake through M cells, antigens are processed and presented by specialized APCs. An important APC population in the subepithelial region of PPs consists of the dendritic cells (DCs)[17]. PPs contain at least three distinct conventional DC subsets, which are characterized by the expression patterns of surface molecules, including CD11b and CD8α[18,19]. The immune response evoked by each DC subset is unique and includes the development of Th2 cells for the IgA response, induction of IL-10-producing regulatory T cells, stimulation of inducible antigen-specific regulatory T cells for oral tolerance, and generation of Th1 cells responsible for microbial clearance.

A large population of DCs isolated from mucosal sites tends to induce Th2 responses and express cytokines, including IL-6

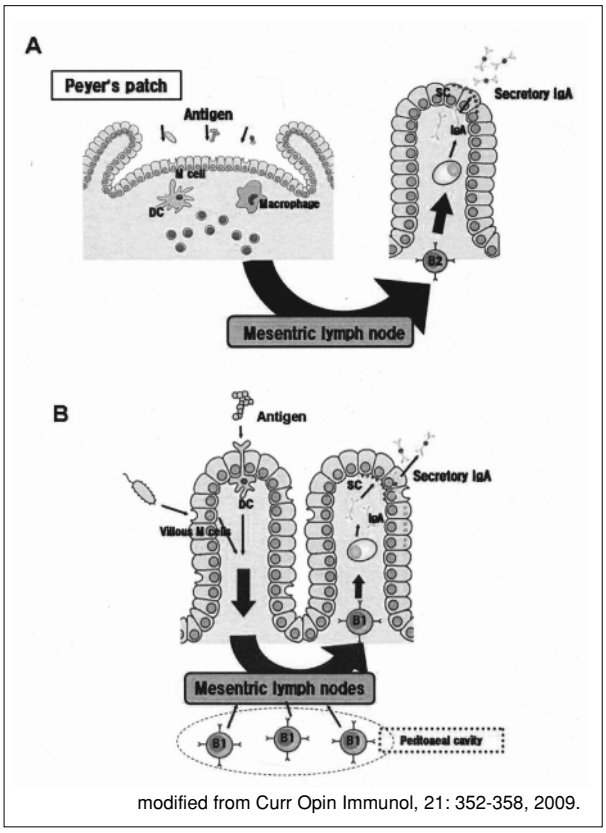

Fig.1 The mucosal immune system. There are two distinctive pathways for the production of secretory IgA (S-IgA)[47]

(A) In the conventional secondary lymphoid follicle such as Peyer's patch (PP) dependent pathway, orally administered antigens are taken up by M cells in follicle associated epithelium of PP and then processed and presented by dendritic cells (DCs) and macrophages for the generation of Th1, Th2, Th17 or regulatory T cells. These antigen-primed Th cells and B-2 lineage of IgA committed B cells migrate to effector sites such as intestinal lamina propria (LP) for terminal differentiation to IgA producing plasma cells.

(B) Another lineage of B cells, possibly originated from the peritoneal cavity, is considered as precursor for the generation of IgA, without help of Th cells. In the latter case, villous M cells and/or LP DCs directly sample antigen from the lumen and present it to the B cells, which under the influence of cytokines such as BAFF, APRIL, and TGF-β1 derived from LP DCs trigger the process of isotype switching and differentiation to IgA producing plasma cells.

and IL-10 and possibly transforming growth factor-β (TGF-β), retinoic acid, and an enzyme that activates the latent form of TGF-β [18,20-22]. For example, a CD11c$^+$CD11b$^+$CD8α^-DC subset isolated from PPs, which preferentially produces IL-6 and polarizes antigen-specific T cells to produce Th2 cytokines, promotes IgA production by naïve B cells[23]. Furthermore, a novel PP DC subset for mucosal IgA responses has been identified; these PP DCs produce TNF-α and inducible nitric oxide synthase (iNOS), termed Tip-DCs, and function to induce the production of IgA-committed cells[24]. Nitric oxide increases TGF-β receptor expression on PP B cells, leading to the enhancement of class-switching recombination to IgA. However, the relationship between Tip-DCs and other known PP DC subsets remains unclear.

In addition to those located in organized inducer tissue (e.g., PPs), mucosal DCs in the diffuse MALT have been identified and extensively characterized as an important APC subset involved in IgA responses. One subset of CD11c^{high}CD11b^{high} lamina propria (LP) DCs expresses toll-like receptor 5 (TLR5) in the small intestine[25]. When stimulated by the TLR5 ligand bacterial flagellin, TLR5$^+$LP-DCs were able to induce differentiation of IgA$^+$ plasma cells independent of T cells and gut-associated lymphoid tissue (GALT) *in vivo*. Because TLR5$^+$LP-DCs can synthesize retinoic acid and IL-6 themselves, T cell-independent IgA$^+$ plasma cells can be generated.

Mucosal T cells for IgA response

Previous studies have clearly suggested that PPs are the inducer sites for the generation of antigen-specific Th cells, which support the proliferation of surface IgA (sIgA)$^+$ B cells and their differentiation into IgA-producing plasma cells[26]. Although the study preceded the discovery of Th1 and Th2 subsets, these antigen-specific Th cells generated in the cited study, in retrospect, likely would have had the properties of Th2-type cells. Currently it generally is agreed that CD4$^+$ Th cells and derived cytokines from mucosal inducer sites play critical roles in the induction and regulation of the IgA response[27]. For example, depletion of CD4$^+$ T cell subsets *in vivo* by monoclonal antibodies or knockout of the CD4 co-receptor gene markedly affects the induction of mucosal IgA responses[28,29]. In addition, the PP CD4$^+$ T cell subset producing TGF-β is a key element for the gene conversion in isotype switching from sIgM$^+$ B cells to sIgA$^+$ B cells in the germinal centers of PP follicles[30,31].

The Th2-associated IgA-enhancing cytokines IL-5 and (especially) IL-6, possibly in combination with other cytokines (e.g., IL-10) that are produced by Th2 cells in the mucosal effector sites, appear essential for terminal differentiation of sIgA$^+$ B cells to IgA plasma cells[32]. It would be too simplistic to conclude that Th2-type cells and their derived cytokines are the only effectors important in the generation of S-IgA responses[33]. For

example, IL-2 synergistically augments IgA synthesis in B cell cultures in the presence of lipopolysaccharide and TGF-β[31]. Although not directly involved in IgA$^+$ B cell responses, IFN-γ enhances the expression of polymeric Ig receptor (or secretory component, SC), a molecule essential for the formation and transport of S-IgA[34].

In summary, coordinated communication between Th1- and Th2-derived cytokines, B cells, and APCs is central to the induction, regulation, and maintenance of appropriate IgA responses in mucosa-associated tissues.

PP-independent IgA responses

Progress in understanding the molecular basis of PP organogenesis has revealed that the progeny of mice treated with a fusion protein combining the lymphotoxin (LT) β receptor and Ig lack PPs but not mesenteric lymph nodes. Taking advantage of this, we examined whether PPs were essential for the induction of antigen-specific IgA responses *in vivo*[35]. Oral immunization of PP-null mice with chicken ovalbumin (OVA) plus cholera toxin as the mucosal adjuvant resulted in antigen-specific mucosal IgA (and serum IgG) responses. These results suggested that antigen-specific antibody responses can be induced without PPs and that alternative inducer sites are presented in the intestinal tract. In support of this hypothesis, the proliferation of Th2-type OVA-specific CD4$^+$ T cells was induced in the mesenteric lymph nodes (MLN) and spleen of PP-null mice. In contrast, when TNF-LT-α double-knockout mice, which lacked both PPs and MLN, were orally immunized with OVA plus cholera toxin, the production of neither mucosal IgA (nor serum IgG) anti-OVA antibodies was induced[35]. These results clearly show that the MLN play a more important role than had been appreciated in the induction of mucosal IgA responses after oral immunization and suggest that PPs are dispensable for the induction of mucosal IgA responses in the gastrointestinal tract. In addition, these results prompted the notion of additional sites for antigen sampling and induction of PP-independent IgA responses (Figure 1B).

Mucosal IgA responses to commensal bacteria are PP-independent

Induction of the production of commensal bacteria-specific IgA is independent of Th cell activity and of organized lymphoid tissues such as PPs, thus reflecting the presence of an evolutionarily primitive specific immune defense system at mucosal surfaces[36]. In this study involving mice, intestinal DCs carrying their live commensal bacteria did not migrate out beyond these lymphoid tissues, preventing systemic infection and ensuring a bacteria-specific IgA response that was restricted to the gut mucosa[36]. In this regard, intestinal LP CD11b$^+$ DCs expressing CX$_3$CR1 form transepithelial dendrites, which enable the cells to directly sample luminal antigens[37]. Thus, a CX$_3$CR1-dependent process, which controls host interactions of specialized DCs with commensal and pathogenic bacteria, may be a key initiator and regulator for mucosally induced tolerance, mucosal inflammation, and humoral immune responses, including mucosal IgA antibody production (Figure 1B).

Mucosal DCs located in the LP are likely to further activate B cells through B cell-activating factor of the TNF family (BAFF) and a proliferating-inducing ligand (APRIL), both of which are B-cell-stimulating factors structurally and functionally related to CD40L[38,39]. Growing evidence indicates that this BAFF-APRIL-mediated signaling pathway supports intestinal IgA production in a T-cell-independent fashion. Recent data indicate that recognition of pathogen-associated molecular patterns (PAMPs) by TLRs at the intestinal epithelial barrier is essential for the production of BAFF and APRIL by LP DCs[40]. TLR signaling not only stimulates DC production of BAFF and APRIL but also elicits DC expression of iNOS, an enzyme that augments BAFF and APRIL synthesis through the generation of nitric oxide[24]. BAFF and APRIL induce IgA class switching by activating B cells in cooperation with cytokines released by DCs or other cells (e.g., macrophages and epithelial cells), including IL-10 and TGF-β1[41].

In addition, an alternative pathway has been proposed in which sIgM$^+$ B cells in the LP can switch to IgA-isotype-bearing B cells without the need for T cell help[42]. The authors showed that cytidine deaminase is induced, and IgA class-switching of B cells occurs, at isolated lymphoid follicles[43]. The formation of these isolated lymphoid follicles is regulated by interactions between lymphoid tissue-inducer cells expressing the nuclear receptor RORγt (RORγt$^+$ LTi cells) and stromal cells. Activation of stromal cells by RORγt$^+$ LTi cells and simultaneously by commensal bacteria through TLRs induces recruitment of DCs, possibly expressing iNOS, and sIgM$^+$ B cells, which eventually switch to sIgA$^+$ B cells[43].

A novel mucosal vaccine delivery strategy for effective mucosal IgA responses

As described previously, IgA is the most abundant immunoglobulin in the body and has an important role in mucosal defense against infectious microbes, including most emerging and reemerging infectious pathogens such as influenza virus and HIV.

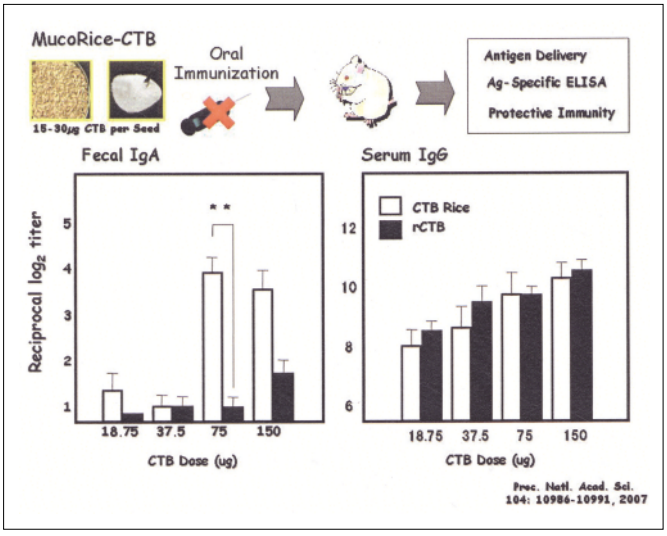

Fig.2 Oral immunization with rice based CT-B induced brisk CT-B-specific serum IgG as well as fecal IgA responses

Table 1 Potential advantages of rice-based vaccine

-Low cost of production
-Rapid scale-up of production
-Multiple vaccines may be produced simultaneously
-Increased resistance to enzyme digestion in gastrointestinal tract
-No need for cold chain during transport and storage
-No need medical assistance in administration/ self administration
-No purification requirement
-Reduced concern over human pathogen contamination during the vaccine preparation
-Eliminate concern over blood borne disease through needle re-use
-Eliminate cost of syringes and needles

S-IgA is constitutively released into the lumen via the polyIg-mediated transport system of the intestinal epithelia, preventing infectious pathogens from binding to mucosal surfaces and neutralizing their toxins. Therefore, mucosal (oral or nasal) vaccines need to be developed that successfully engage the mucosal immune system for the induction of antigen-specific IgA responses at mucosal surfaces.

From a practical standpoint, mucosal immunization, especially by the oral route, has several advantages over the traditional syringe-and-needle form of vaccination. Oral vaccination is less stressful for vaccine recipients and does not require technical skill for administration. Furthermore, the delivery of vaccines through the intestinal tract is considered to be inherently safer than systemic injection, given that diseases such as AIDS and hepatitis could be transmitted if the vaccine delivery device were used inappropriately. An additional advantage is that mucosal vaccination induces both mucosal and systemic immune responses, thus generating two layers of host protection against infectious microbes[1,3,39]. However, several hurdles, including loss of efficacy of the vaccine due to degradation by the acidic gastric pH and intestinal proteolytic enzymes, have to be cleared in the development of mucosal vaccines. The potential for eliciting mucosally induced tolerance by oral immunization with protein antigens must also be considered. Furthermore, regardless of the route of inoculation, our efforts should focus on the development of vaccines that do not require refrigeration (that is, that are 'cold-chain-free'), given the infrastructure characteristics of developing countries.

Currently, transgenic plants have been sought not only as bioreactors but also as potential scaffolds for oral vaccines[44,45]. The advantage of these vaccines is that the plant products — whether leaves, fruit, or seed — can be consumed readily with limited or no processing if the introduced vaccine antigen gene is optimally integrated and expressed without adversely affecting the host plant[44,45]. In this regard, we developed a rice-based oral vaccine with many practical advantages over most traditional and other plant-based oral vaccines[46]. We constructed a rice-based oral vaccine expressing the B subunit of cholera toxin (CT-B) under the control of an endosperm-specific expression promoter and in which codon usage was optimized for rice seed. An average 30 μg CT-B per seed was stored in the protein bodies, which are storage organelles in rice, and the stored antigen was resistant to the harsh gastrointestinal environment[46].

When mice were orally immunized with a powdered form of the rice-based CT-B vaccine, the antigenic protein was effectively taken up by antigen-sampling M cells covering Peyer's patches, and the oral immunization induced protective CT-B-specific serum IgG as well as mucosal S-IgA antibodies (Fig.2). Furthermore, oral administration of the rice-based CT-B vaccine does not stimulate a serum IgG or S-IgA response against rice storage protein[46]. These characteristics should remain key elements of future rice-based oral vaccines.

The rice-based CT-B vaccine possesses the advantage of circumventing other important issues inherent to the most tradi-

tional oral vaccines, including practical concerns of cost and the need for cold storage (Table 1). The vaccine is stable at room temperature for at least 48 months and does not require purification or refrigeration. In addition, because they do not scatter their pollen as widely as do other crops genetically modified to produce vaccines (such as corn, wheat, and tomatoes)[45], rice plants have broader utility and less risk of contaminating normal crops. However, production of the rice-based CT-B vaccine — or any other such plant-based product—should occur in a closed facility adhering to the rules and guidelines of vaccine production and for working with genetically manipulated plants.

Current data suggest that the use of rice-based oral vaccines offers a highly practical global strategy for cold-chain- and needle-free vaccination against many important infectious diseases, including not only cholera but also other emerging and re-emerging infectious diseases such as influenza, botulism, and anthrax.

Summary

Studies in the early 1960s demonstrated the presence of IgA in a unique form in milk and in other external secretions. These studies were followed by the discovery of the secretory component and identification of the J chain; identification and characterization of the gut-associated lymphoid tissue; discovery of mucosal circulation of antigen-sensitized and reactive IgA-committed B cells from the GALT to other mucosal surfaces such as the aerodigestive tract, genital tract, and glandular tissues; and definition of immunologically unique mucosal T cells. Over the past few decades, our concept of the mucosal immune system has been expanded to include M cells, mucosal dendritic cells, and macrophages; Th1, Th2, and Th17 cells; regulatory T cells; and other effector cell networks. The biologic significance of the mucosal immune system increasingly is being understood and appreciated in the context of human infections acquired through mucosal portals of entry; these illnesses include classic as well as newly emerging infectious diseases and have led to the desire for mucosal vaccines to combat the pathogens responsible.

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