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The Mucosal Immune System for the Development of New Generation Vaccine

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Introduction

To understand the intricacies involved in the development of a mucosal vaccine, it is necessary to appreciate the anatomical and functional uniqueness of the mucosal immune system. Foreign antigens and pathogens are generally encountered through normal physiological functions such as ingestion, inhalation and sexual contact and the host thus has evolved organized lymphoid tissue in the regions which facilitate the initiation of antigen-specific immune responses following exposure to these mucosal antigens and pathogens. These organized mucosa-associated lymphoid tissues, including Peyer's patch (PP) and nasopharyngeal associated lymphoid tissue (NALT), are known as IgA inductive sites and contain all of the immunocompetent cells, including B cells, Th cells and CTLs, necessary for the development of effector and memory B cells and T cells upon antigen presentation by dendritic cells (DCs) and macrophages. These antigen-specific lymphocytes then emigrate from the inductive site via lymphatic drainage, circulate through the bloodstream and home to distant mucosal effector sites including the lamina propria (LP) regions of the intestinal, respiratory and reproductive tissues. Thus, these more diffuse tissues where antigen-specific IgA-committed B cells, Th1 and Th2 cells interact and carry out their respective functions for the generation of secretory IgA (S-IgA) antibody responses are a type of mucosal effector site. At such sites, these Th1 and Th2 cells, and B cells communicate with one another through an array of regulatory cytokines, and the generation of S-IgA antibody responses together with epithelial cells which provides a first line of protection at mucosal surfaces [1, 2]. Thus, the common mucosal immune system (CMIS), which consists both of IgA inductive (e.g., organized lymphoid tissues such as PP and NALT) and effector (e.g. diffused LP region) sites is integral to the development of mucosal vaccine (Figure 1).

CMIS: A Basis for the Development of a Mucosal Vaccine

1. Inductive sites for the initiation of mucosal immunity

PP, appendix and solitary lymphoid nodules, collectively termed gut-associated lymphoid tissues (GALT), serve as the mucosal inductive sites for the gastrointestinal tract (GI), while the tonsils and adenoids, collectively identified as NALT, do the same for the upper respiratory tract and the nasal/oral cavity [3]. In addition, a recent study has provided new evidence that isolated lymphoid follicles (ILF) are equipped with immunological chacteristics which are in some respects similar to those of PP [4] and thus should be considered as a part of GALT.

1-1. Presence of professional antigen sampling cells

Among different GALT, characterizations of murine PP are most extensively carried out as an example of mucosal inductive tissues [5, 6]. The gut lumen side of PP is covered by a unique epithelial layer known as

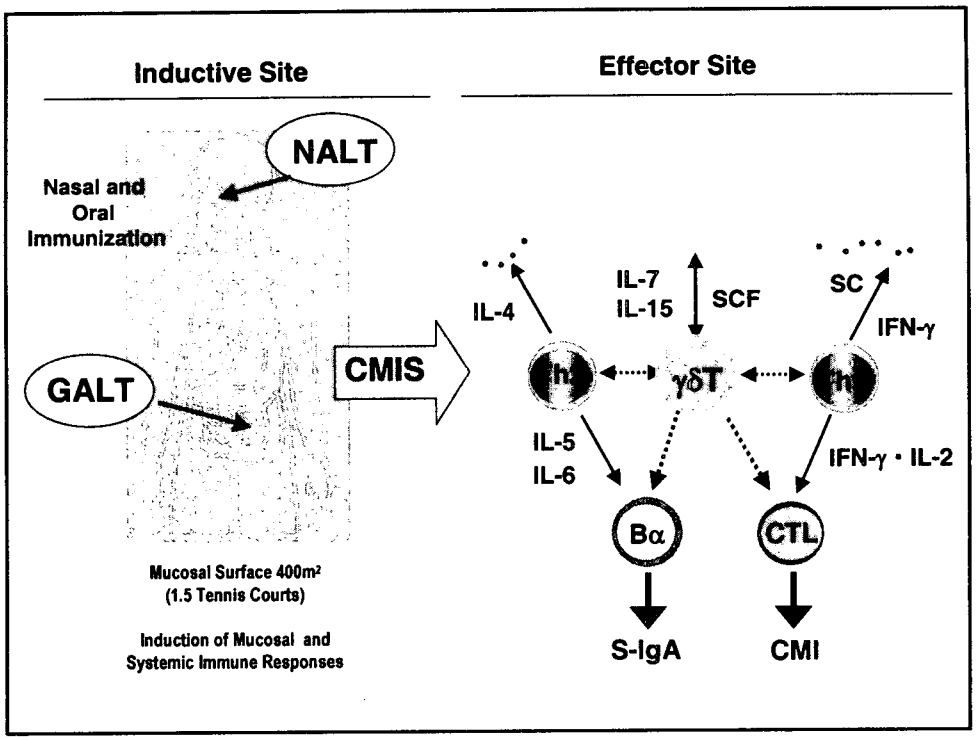

Fig. 1. A concept of the common mucosal immune system (CMIS). The CMIS consist with the organized mucosa-associated lymphoid tissue (e.g., PP) and the diffused effector tissues (e.g., i-LP) for the induction of antigen-specific IgA response

follicle-associated epithelium (FAE). The FAE is enriched with specialized antigen-sampling cells known as microfold (M) cells (Table 1), so named because of their unique characteristics of irregular and shortened microvilli [7]. M cells are equiped with an apparent pocket at the basal membrane site, which contains T cells, B cells, macrophages or DCs. The M cells also have small cytoplasmic vesicles and few lysosomes, and are adept at the uptake and transport of lumenal antigens [8]. It is believed that antigen uptake by M cells does not result in the processing and presentation of the antigen, but rather in the delivery of the intact antigen to the underlying antigen presenting cells (APC) including macrophages and DCs [9]. In addition to the transport of lumenal antigens, M cells serve as a port of entry for pathogens. Invasive strains of *Salmonella* and reovirus initiate the infection by invading the M cells of the PP [10, 11]. Therefore, M cells are considered as a gateway to the outside environments for the mucosal immune system.

1-2. Unique characteristics of mucosal DC

PP are anatomically characterized as possessing a dome configuration, with the area just below the FAE known to be enriched with IgA-committed B cells, Th1 and Th2 lymphocytes, macrophages and DCs, all necessary for the induction of antigen-specific immune responses. After the uptake and delivery of antigen via M cells, the antigens are immediately processed and presented by DCs [12]. At least three DC subpopulations have been identified in PP: myeloid DCs ($CD11b^+$), lymphoid DCs ($CD8\alpha^+$) and double-negative DCs [13]. Myeloid DCs in the subepithelial dome (SED) appear to be immature in that they do not express maturation markers such as DEC-205. Lymphoid and double negative DCs are capable of supporting Th1 cell development for the subsequent generation of cell-mediated immunity (CMI), while myeloid DCs induce Th2 cells for the induction of IgA antibody immune responses in mucosal effector sites.

Table 1. Uniqueness of the Mucosal Immune System

Common Mucosal Immune System	
Inductive Site	Effector Site
Organized lymphoid tissue	Diffused tissue
GALT Peyer's patch Isolated lymphoid follicle	Intestinal lamina propria (LP)
NALT	Respiratory LP
	Genitourinary LP
M Cells : Antigen sampling	Columnar epithelial cells
B cells : μ to α isotype switching	B cells : Differentiation of $sIgA^+$ B cells To IgA plasma cells
$CD4^+$ T cells : Differentiation of Th0 to Th1 or Th 2 cells	$CD4^+$ T cells : Effector Th1 or Th2 cells
$CD8^+$ T cells : Precursor CTL	$CD8^+$ T cells : CTL
APC : Three subsets of DC	APC : Intraepithelial DC

Lymphoid and double negative DCs are classfied as being of the DC1 subtype, while myeloid DCs are classified as being of the DC2 subtype [14, 15]. Further, the myeloid DCs produce inhibitory cytokines, IL-10 and TGF-β following exposure to innocuous food antigens and may mediate the T cell differentiation of Th3 or T regulatory (Tr) cells for the induction of systemic unresponsiveness to orally administered antigen known as oral tolerance or mucosally-induced tolerance. Myeloid DCs in the SED region express the chemokine receptor CCR6, whose ligand, CCL20, is expressed by the FAE. In mature myeloid DCs, the chemokine receptor CCR7 exhibits an enhanced expression and migrates from SED to interfollicular region (IFR), where it then interacts with T cells [13].

1-3. Generation of antigen-specific lymphocytes

Distinct follicles (B cell zones), which contain germinal centers where significant B cell division occurs, are located beneath the dome area of PP. The PP germinal centers are considered to be sites where affinity maturation along with frequent B cell isotype switches of IgM to IgA occur [16]. Thus, the tissue contains the high frequency of surface IgA positive ($sIgA^+$) B cells. All major T cell subsets are found adjacent to the B cell zones (T cell-dependent zones). These T cells are of the mature variety and almost all of them belong to αβ TCR^+ T cells (αβT cells). Approximately 65% of the αβT cells are $CD4^+$ $CD8^-$ and exhibit properties of Th cells. After presentation of processed antigen by the distinctive subset of DCs described above, these Th cells can become Th1 and/or Th2 type cells for the induction and regulation of antigen-specific CMI and IgA responses, respectively (Tqble 1). Approximately 30 % of αβ T cells are $CD4^-$ $CD8^+$ and contain precursors of CTL [17]. Thus, the organized inductive tissues (e.g. PP) are equipped with all of the immunocompetent cells necessary for the initiation of antigen-specific mucosal immune responses.

2. Effector sites for mucosal immunity

After the initial activation by antigen at mucosal inductive sites such as PP, mucosal lymphocytes, including $sIgA^+$ B cells, $CD4^+$ Th1/Th2 cells and $CD8^+$ T cells, leave those sites to home to distant mucosal effector tissues via the CMIS of mesenteric lymph nodes, thoracic ducts and the bloodstream (Figure 1). The IgA isotype, especially S-IgA is the primary immunoglobulin involved in the protection of mucosal surfaces and is locally produced in the gastrointestinal and upper respiratory tracts, as well as the nose, middle ear, uterine and reproductive mucosa, and glandular tissues (e.g., salivary, lactating mammary and prostate) by the immunocompetent cells originating in the organized mucosa-associated lymphoid inductive tissues such as PP and NALT. The observation of these two distinct sites, i.e., the inductive site where antigen uptake initially occurs and the effector site at the mucosal surface where the formation of S-IgA takes place, has lead to the concept of CMIS [1-3, 5, 6].

The LP region of the GI tract is the most studied mucosal effector tissue. At the effector sites, $sIgA^+$ B cells terminally differentiate into IgA plasma cells under the influence of Th2 cell derived IgA enhancing cytokines for the generation of S-IgA, a process that is facilitated by the mucosal intranet formed by antigen-specific Th1 and Th2 cells, $sIgA^+$ B cells and epithelial cells. These mucosal effector sites are characterized by an array of Th1 and Th2 cytokines and so provide a suitable environment for the promotion of the unique B-cell differentiation pathways needed to supply high numbers of IgA antibody-producing cells To this end, Th1-derived IL-2 and Th2-derived IL-5, IL-6 and IL-10 are important IgA-enhancing cytokines for the preferential activation and clonal expansion of $sIgA^+$ B cells and their terminal differentiation into IgA plasma cell [18]. These plasma cells produce dimeric or polymeric forms of IgA which become S-IgA following binding with secretory component (SC) synthesized by epithelial cells. The production of SC was shown to be up-regulated by both Th1 (IFN-γ) and Th2 (IL-4) cytokines [2]. This important mucosal IgA binding SC was recognized as a part of the polymeric Ig receptor, which mediates the active transport of the dimeric or polymeric forms of IgA produced by intestinal LP IgA plasma cells across the epithelium [19].

Uniqueness of Mucosal T Cell Network for the Induction and Regulation of IgA Response

Mucosal immunization induces antigen-specific Th1- and/or Th2-type responses depending on the nature of the antigen, adjuvant and antigen delivery vehicle used. For example, administration of intracellular pathogens such as *Salmonella* led to the formation of Th1 cells producing IFN-γ, IL-2 and TNF-β [20]. *In vivo*, murine Th1 type immune responses are associated with the development of CMI and B-cell responses characterized by IgG2a synthesis. IFN-γ is the major cytokine responsible for the production of IgG2a antibodies the mouse [21]. On the other hand, the use of cholera toxin (CT) as a mucosal adjuvant with soluble protein antigen induces antigen-specific Th2 cells producing IL-4, IL-5, IL-6 and IL-10 [22]. Among these Th2-type cytokines, IL-4 has been shown to switch μ heavy chain to certain γ subclasses (e.g. IgG1) and to ε isotype (IgE) [23]. Further, Th2 cells are considered to be the major helper phenotype for the support of IgA responses via the production of IL-5 and IL-6 in the murine system. Further, Th1 and Th2 cells are both reciprocally regulated by the cytokines they secrete; Th1 cells, by IFN-γ, and Th2 cells, by IL-4 and IL-10 [24]. These cross-regulatory cytokines play an important role in the maintenance of appropriate immunological homeostasis in mucosa-associated compartments.

In addition, recent evidence suggests that the differentiation of Th1 or Th2 type cells from naïve precursors is regulated by distinct subtypes of DCs, termed DC1 and DC2 [14]. In this regard, bacterial infection activates DCs via initial stimulation of the toll-like receptors (TLRs: e.g., TLR2 and TLR4) and induces production of mainly Th1-inducing cytokines such as IL-12 [25]. Therefore, TLR-stimulated DCs tend to direct T cell differentiation to the Th1 cell type. However, it remains unknown whether DCs can induce Th2

cell differentiation by stimulation of certain TLR following exposure to helminthes or selected microbes [26]. Although IL-4 plays a major role in driving the Th2 pathway, the cellular source of this early IL-4 also remains to be fully defined [27]. However, NKT cells have been shown to be an effective population for the production of IL-4 [28].

Recently, immune-suppressive cytokine (e.g. IL-10 and/or TGF-β)-producing T cells, such as Th3 cells and Tr cells were described. These suppressor-type cells can be induced by oral exposure to protein antigen [29, 30]. Th3-produced TGF-β, promotes IgA isotype switching and has suppressive properties for both Th1 and Th2 cells [34]. Tr cells are characterized by their ability to produce IL-10 but not IL-4 and to inhibit Th cell responses [29]. Produced by Tr and Th3 cells, these reglulatory (or suppressive) cytokines may lead to the creation of the polarized quiescent condition characteristic of the mucosal surface.

Recent Strategies for the Enhancement of Mucosal Immunity

New generation of mucosal vaccine must: (1) protect vaccine antigen from physical elimination and enzymatic digestion, (2) effectively target mucosal inductive tissues including M cells, and (3) appropriately stimulate the innate immune system to generate effective adaptive immunity.

Firstly, both oral and nasal administration is a major mucosal immunization routes for the induction of antigen-specific mucosal and systemic immune responses. In fact, many new vaccine candidates have been or being tested by both routes to determine the most effective immunization regimen for the induction of protective immunity via the CMIS. In many cases, nasal immunization has been shown to be more effective than the oral route, since it requires smaller doses of vaccine antigen with a lesser amount of adjuvant [31]. An additional advantage of nasal route is that there is no antigen loss through digestion, as there is with oral immunization. To overcome the obstacles of oral immunization, an interesting and practical approach would be the adaptation of a plant expression system [32]. The use of a plant expression system allows the accumulation of vaccine antigen within the intracellular compartment of the plant cell, which will itself provide with a natural protective shield. The edible vaccine is now considered to be more stable and practical than purified vaccine antigens in the harsh environment of the gastrointestinal tract [32].

Another attractive approach would be the identification and development of M cell-targeting molecules, especially those of the mucosal inductive tissue of PP and NALT, since these cells have been shown to be the gateway across the mucosal barrier [2, 3, 8]. A number of pathogens, including *Salmonella* and reovirus, target M cells as a mode of entry into the host [10, 11]. In fact, these bacteria and viruses are being genetically attenuated and engineered to deliver antigens into mucosal inductive sites and to induce antigen-specific immune responses. Identification of bacterial and viral virulence factors associated with invasion or infection of M cells can provide tools to construct more efficiently attenuated bacterial or viral vectors used for the M cell-targeted oral and nasal vaccines. In this regard, conjugates of reovirus protein 1 as a ligand of M cells lack the risk of a live vector system but remain efficient in the induction of antigen-specific immunity by means of nasal immunization with DNA vaccine [33].

Finally, recent accumulating evidence shows that the recognition of pathogen-associated common molecules [e.g., peptidoglycan (PG) and lipopolysaccharide (LPS)] by the pattern recognition receptor family, or TLRs provoke rapid activation of innate immunity leading to the production of proinflammatory cytokines and upregulation of costimulatory molecules on APC. Activated innate immunity subsequently leads to the production of effective adaptive immunity against infectious agents. In other words, the TLRs are considered to be adjuvant receptors [26, 34, 35]. In fact, unmethylated CpG dinucleotides (CpG) of bacterial DNA, as a known ligand for TLR9, can exhibit an immunostimulatory activity on lymphocytes and APCs [36]. This stimulation leads to the creation of Th1-dominant immune responses. This TLR9-mediated immuno-enhancing activity has been utilized as a systemic as well as a mucosal adjuvant [37].

Further, bacterial cell wall components of PG and LPS have been shown to stimulate APC via TLR2 and TLR4, respectively [26]. For the development of a new generation of mucosal adjuvants, ligand molecules, which possess specificity for different TLRs, should be considered. In contrast to these TLR-specific-bacterial associated molecules, CT and heat-labile toxin (LT) produced by *Vibrio cholera* and *Escerichia coli* respectively, do not use the TLRs, but these pathogen-derived immunostimulatory molecules can induce effective antigen-specific Th1 and/or Th2 immune responses as well as antigen-specific mucosal IgA antibody responses to co-administered antigens via oral routes [38, 39]. Since some recent studies have suggested that the toxicity and adjuvanticity of the toxin can be separated by the gene manipulation of the A subunit, nontoxic mutant CT/ LT, which maintain the adjuvant activity but not the toxicity, may pave the way for the development of promising mucosal vaccine adjuvants [40-44].

Summary

The mucosal immune system provides a first line of defense against invasion of infectious agents via inhalation, ingestion and sexual contact. For the induction of protective immunity at these invasion sites, one must consider the use of the CMIS, which interconnects inductive tissues, including PP and NALT, and effector tissues of the intestinal, respiratory and genitourinary tracts. In order for the CMIS to induce maximal protective mucosal immunity, co-administration of mucosal adjuvant or use of mucosal antigen delivery vehicle has been shown to be essential. When vaccine antigen is administered via oral or nasal route, antigen-specific Th 1 and Th2 cells, cytotoxic T lymphocytes (CTLs) and IgA B cell responses are effectively induced by the CMIS. In the early stages of induction of mucosal immune response, the uptake of orally or nasally administered antigens is achieved through a unique set of antigen-sampling cells, M cells located in follicle-associated epithelium (FAE) of inductive sites. After successful uptake, the antigens are immediately processed and presented by the underlying DCs for the generation of antigen-specific T cells and IgA committed B cells. These antigen-specific lymphocytes are then home to the distant mucosal effector tissues for the induction of antigen-specific humoral (e.g., IgA) and cell-mediated (e.g., CTL and Th1) immune responses in order to form the first line of defense. Elucidation of the molecular/cellular characteristics of the immunological sequence of mucosal immune response beginning from the antigen sampling and processing/presentation by M cells and mucosal DCs followed by the effector phase with antigen-specific lymphocytes will greatly facilitate the design of a new generation of effective mucosal adjuvants and of a vaccine delivery vehicle that maximizes the use of the CMIS.

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