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Inducing mucosal IgA: A challenge for vaccine adjuvants and delivery systems

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Abstract

Mucosal IgA or secretory IgA (SIgA) are structurally equipped to resist chemical degradation in the harsh environment of mucosal surfaces and the enzymes of host or microbial origin. Production of SIgA is finely regulated and distinct T-independent and T-dependent mechanisms orchestrate immunoglobulin heavy chain α class switching and SIgA responses against commensal and pathogenic microbes. Most infectious pathogens enter the host via mucosal surfaces. To provide a first line of protection at these entry ports, vaccines are being developed to induce pathogen-specific SIgA in addition to systemic immunity achieved by injected vaccines. Mucosal or epicutaneous delivery of vaccines helps target the inductive sites for IgA responses. The efficacy of such vaccines relies on the identification/engineering of vaccine adjuvants capable of supporting the development of SIgA alongside systemic immunity and delivery systems that improve vaccine delivery to the targeted anatomic sites and immune cells.

Mucosal surfaces provide a physical barrier to the entry of foreign factors and microbes into the host while allowing important functions; such as uptake of air and food, reproduction, and vision to occur. The protection of these surfaces is ensured by the mucosal immune system, which consists of a complex network of cells and molecules designated as the Mucosal-Associated Lymphoid Tissues (MALT). The role of the MALT is clearly distinct from that of the systemic immune system, which primarily maintains the inner body sterile and free of microbes, foreign antigens, and altered or dead cells. The mucosal immune system is designed to tolerate commensal microbes and food, but also to initiate adaptive immune responses against invading pathogens and provide a first line of defense at their portal of entry. The SIgA represent the hallmark of immune response at mucosal sites and contribute to homeostasis via a variety of mechanisms (1). SIgA Abs are induced by postnatal exposure to commensal microorganisms indicating that these Abs play a role in sensing commensal microbes and limiting their overgrowth (2, 3). SIgA Abs also protect the host by binding to the surface of luminal microbes and toxins to prevent them from attaching to epithelial cells (Figure 1) (4). This exclusion mechanism limits the ability of microbial pathogen-associated molecular patterns (PAMPs) to trigger inflammatory responses and therefore contributes to the anti-inflammatory effect of SIgA. While induction of SIgA is desirable for optimum protection of mucosal surfaces and limiting systemic infections, production of these antibodies (Abs) is differently regulated than systemic IgG and IgA Abs

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and they are poorly induced by conventional injected vaccines. This review will discuss regulation of IgA responses and ongoing efforts toward the development of vaccines capable of promoting both systemic immunity and SIgA responses in mucosal tissues.

1. IgA and secretory IgA

Immunoglobulin A (IgA) is the most abundant immunoglobulin in mucosal tissues and represents the hallmark of mucosal immune response. This immunoglobulin isotype is also the second most abundant immunoglobulin isotype in the circulation. Structurally, IgA present in the circulation are either monomeric IgA or polymeric (pIgA) immunoglobulins, where monomers are grouped together by a joining chain (J-chain) (Figure 1). Secretory IgA (SIgA) are exclusively present at mucosal surfaces and consist of dimeric IgA linked via the J-chain to the secretory components (SC) (Figure 1). The latter is a portion of polymeric immunoglobulin receptor (pIgR) expressed at the basolateral surface of the epithelial cells, which is acquired during the release of IgA molecules in the lumen after transepithelial transport. The secretory component protects the SIgA from degradation by microbial and host proteolytic enzymes in the gastrointestinal tract and body secretions. Only one type of IgA molecule exists in mice. In contrast, human IgA molecules are divided into the IgA1 and IgA2 subclasses. The IgA1 are dominant in the serum. The IgA2, which are more resistant to proteolytic degradation are the main isoform found in mucosal secretions.

- Regulation of Ig class switch for production of IgA

B cells can undergo Ig class switch recombination (CSR) and acquire the ability to produce IgA after CD40-CD40L ligation in the presence of TGF-β with contribution from other cytokines including IL-4, IL-5, IL-6, IL-10, and IL-21 (5–8) (Figure 1). Other costimulatory signals such as B cell-activating factor of the TNF family (BAFF), a proliferation-inducing ligand (APRIL), retinoic acid (RA), and nitric oxide also facilitate CSR for the production of IgA (7, 9, 10). BAFF and APRIL further enhance IgA responses by providing survival signals, and/or inducing plasma cell differentiation and IgA secretion (10, 11). Retinoic acid, a metabolite of vitamin A, plays an important role in the production of mucosal IgA since in addition to acting synergistically with IL-5 and IL-6 to induce IgA secretion (12), it also induces expression of gut-homing receptors on B cells (12) (Figure 1). Additional cytosolic factors and transcription factors contribute to the regulation of IgA class switching. For example, the noncanonical IκB kinases TANK-binding kinase 1 (TBK1) in B cells was shown to negatively regulate IgA class switching by attenuating noncanonical signaling via the transcription factor NF-κB (13). The cytosolic protein clathrin, which controls receptor-mediated endocytosis and internalization of receptors, also influences antibody isotype switching and production of IgA (14). Thus, absence of clathrin light chain in B cells enhances immunoglobulin switch to IgA, an effect consistent with a defective endocytosis of TGFβR2 and subsequent increased TGFβR2 signaling (14).

- T-dependent and T-independent mechanisms of IgA induction

IgA can be produced by naïve B cells in gut-associated lymphoid tissues (GALT) or nasopharyngeal-associated lymphoid tissues (NALT) in response to stimulation by commensal microbes, microbial pathogens, or after vaccination. Comparison of naïve B

cells in the intestinal Peyer's patches with IgA producing cells in the intestinal lamina propria have shown that a change of energy metabolism occurs during differentiation of B cells into IgA-producing cells (15). Hence, while both naïve and IgA-producing cells use the tricarboxylic acid cycle and fatty acid for energy production, glycolysis preferentially occur in IgA-producing cells (15). Immunoglobulin CSR and production of IgA can occur via T-independent or T-dependent mechanisms depending on the nature of antigen and the cellular origin of help received by B cells (Figure 1). High affinity IgA are generated in a T-dependent fashion, while IgA produced in a T-independent manner lack or only have limited antigen specificity due to limited somatic hypermutation. In the gut, IgA-producing cells are generated in germinal centers of the Peyer's patches, but also in other secondary lymphoid tissues (cryptopatch and cryptopatch-derived isolated lymphoid follicle) and microbe-induced tertiary lymphoid tissues. This process is finely regulated and it has now been shown to require prolonged interaction of B cells with dendritic cells in the subepithelial dome (SED) of the Peyer's patches and DC-mediated production of TGF-β via integrin αVβ8-mediated activation of TGF-β (16). Innate lymphoid cells play a role upstream of these interactions as they facilitate the maintenance of DCs in the SED (16).

Several T cell subsets are involved in the induction of IgA. Among these cells, follicular T helper (Tfh) cells (17) play a key role in both immunoglobulin CSR and somatic hypermutation in antigen recognizing regions and subsequent generation of high-affinity antibody producing plasma cells and memory B cells. The expression of CXCR5 by Tfh cells allow them to locate into B cell follicles, where they produce IL-21, an IgA promoting cytokine (5). Tfh were reported to differentiate from other Th cell subsets suggesting a plasticity of mucosal T cells and potential for rapid adaptation to the luminal environment and ultimately produce the mucosal IgA Abs needed to maintain homeostasis.

Fox3+ T cells were the first T cell subset shown to undergo differentiation into Tfh cells in the GALT to support IgA responses. Thus, following the adoptive transfer of Fox3+ T cells to T cell deficient mice, these cells could trigger formation of germinal centers in the Peyer's patches (18). Formation of germinal centers was limited to Peyer's patches and, to a lesser extend mesenteric lymph nodes, and the process was initiated by Fox3+ T cells that have lost expression of Foxp3. The re-differentiation of Fox3+ T cells appears to be gut-specific, since evidence of this process was not seen in the spleens or peripheral lymph nodes. Mechanisms leading to this re-differentiation of Foxp3 cells remains to be elucidated, but it is possible that gut-derived signals down regulate Foxp3 allowing the expression of the transcription factor Bcl6 and other characteristics of Tfh cells. The re-differentiation of GALT Fox3+ T cells into Tfh could not be confirmed in other studies (19). Nonetheless, a couple of reports suggest that a small subset of mucosal resident Treg can respond to T-dependent antigens and co-express Bcl6, upregulate CXCR5 and migrate into germinal centers to function as regulatory Tfh cells (20, 21).

Th17 cells are preferentially found in mucosal tissues and their presence in the intestine has been shown to depend on stimulation from commensal bacteria, such as *Segmented filamentous* bacteria (22, 23). Adaptive transfer studies have confirmed the tropism Th17 cells for mucosal tissues of the gut, but also demonstrated that in recipient host, these cells down-regulate their expression of Th17 characteristics (i.e., RORγt and IL-17A expression)

to acquire characteristic of Tfh cells, including the transcription factor Bcl6, the surface molecules CxCR5, PD-1, and the ability to produce the IgA-promoting cytokine IL-21 (19). In another study, adoptive transfer of T cells lacking MyD88 into germ-free Rag−/− mice helped demonstrate that gut microbiota-mediated signaling through MyD88 in $CD4^+$ T cells induce their differentiation into Tfh cells and promote the production of high-affinity SIgA (24).

A number of studies have now shown that immunoglobulin CSR and production of IgA can be induced in a T-independent manner in lymphoid structures of the GALT (i.e., Peyer's patches, isolated lymphoid follicles, cryptopatches and mesenteric lymph nodes) (7, 25, 26). It was also suggested that IgA production could take place in the intestinal lamina propria independently of cognate T cell help following interactions of APRIL with the transmembrane activator and CALM interactor (TACI) on B cells (27, 28). It is important to indicate that a major difference between T-dependent and T-independent production of IgA is the source of cytokines that provide help to B cells. Innate lymphoid cells, which are present in high number in mucosal tissues, can produce the same pattern of cytokines than T helper cells and thus may play a central role in the T-independent production mucosal IgA (29). The plasmacytoid dendritic cells (pDCs) are a source of APRIL, BAFF, IL-10 and IL-6 (30), and thus, could be key players in this process. In addition to soluble cytokines, membrane-bound lymphotoxin β (LTα1β2) produced by $ROR\gamma t^+$ ILCs was shown to be critical for T cell-independent IgA induction in the lamina propria (31). The list of cells potentially involved in the T-independent production of IgA continues to grow and now includes eosinophils, which are widely considered as proinflammatory and associated with allergy and eosinophilic gastrointestinal disorders (32). Eosinophils promote CSR and production of IgA by providing active TGFβ (32).

T-dependent IgA responses predominantly involved B2 cells, while T-independent IgA responses involve both follicular B2 cells and innate B1 cells that reside in the peritoneal cavity. A study that addressed the relative contribution of the B cell subsets to IgA responses toward commensal microbes has shown that most commensals elicit T-independent IgA responses by the orphan B1b and B2 cells, while only atypical commensal such as segmented filamentous bacteria elicits T-dependent IgA responses (33).

- Role of epithelial cells in production of SIgA

SIgA Abs contain a secretory component synthesized by epithelial cells. Host and microbial factors, which stimulate production of IgA-promoting cytokines (i.e., BAFF and TGF-β) by epithelial cells enhance production of SIgA (34). The pIgR which mediate the transepithelial transport of polymeric IgA and secretion of SIgA is expressed by different type of epithelial cells and the resolution of its crystal structure provides new information about multiple conformations used by the secretory component to protect mammals from pathogens (35). The transepithelial trafficking of the pIgR was shown to involve both the transcytotic pathway and one arm of the regulated secretory pathway (36). Factors which participate in the complex regulation of pIgR expression and transcytosis also contribute to optimizing SIgA production and mucosal immunity (37, 38). Both the classical and the alternative NF-κB pathway regulate pIgR expression (39, 40). Inflammatory (IL-1), Th1 (IFN-γ, TNF) and

Th2 (IL-4) cytokines were shown to stimulate pIgR expression by epithelial cells (37), The fact that these cytokines, as well as those produced by Th17 cells, stimulate pIgR expression (41) highlights the central key role of SIgA in protection of mucosal tissues against intracellular pathogens, extracellular pathogens, and other toxins and foreign products.

2. Vaccine adjuvants and delivery systems for induction of mucosal IgA

Alum is the adjuvant the most widely used in injected subunit vaccines for induction of specific T cell responses in the bloodstream and serum IgG responses. Unfortunately, this adjuvant is not effective at triggering the molecular events that support IgA CSR or homing of effector B and T cells in mucosal tissues. Major efforts were dedicated to the development of new vaccine adjuvants for induction of SIgA (Table 1). As mentioned above, the cellular and molecular machinery that supports initiation of SIgA response is located in mucosal tissues and thus can be reached by needle-free vaccines via the oral, nasal, rectal, or ocular routes. The efficacy of such vaccines relies on delivery systems that improve vaccine delivery to the targeted anatomic sites and immune cells (Table 1). Imprinting of mucosal homing potential through induction of homing and chemokine receptors is another important step in the induction of SIgA responses in selected mucosal sites

- Mucosal homing of IgA producing cells and routes of vaccine delivery

Conventional injected vaccines generally induce specific T cell responses in the bloodstream and serum IgG responses. In contrast with injected vaccines, needle-free vaccines administered via the oral, nasal, rectal, or epicutaneous routes have the potential to induce mucosal IgA, in addition to systemic immunity. In this regard, mucosal homing of immune effector cells is finely orchestrated by expression of mucosal addressins and homing receptors, and chemokine receptors. Retinoic acid (RA) produced by antigen-presenting cells in mucosal tissues provides key signals for expression of the gut homing receptor α4β7 (12, 42). Cytokines such as IL-5 and IL-6 enhance the effect of RA on α4β7. The cytokine IL-21 was shown to induce the expression of the gut-homing receptor α4β7, an effect enhanced by TGFβ1 and RA (5). The chemokine receptor CCR9 together with α4β7 represent the best-described factors regulating homing of immune cells into the gut (43). On the other hand, differentiation of IgA-producing cells in the absence of RA leads to induction of α4β1, L-sectin and CCR10, which target B cells to other mucosal compartments including the airways, salivary gland, reproductive mucosa and colon (43). Oral immunization more effectively targets mucosal DCs, which are rich in RA, and this leads to generation of IgA-producing cells capable of migrating into the small intestine. Rectal immunization, which induces expression of both CCR10+ and CCR9+, generates effector cells capable of migrating on both the small and large intestine. Non-cytokines factors such as Sphingosine1-phosphate can fine tune homing of effector B cells to the intestine (44). Similarly, epicutaneous immunization was reported to promote SIgA responses in the gut. It has been suggested that migration of antigen from the skin to Peyer's patches and/or mesenteric lymph nodes could be a reason for induction of effector B cells expressing α4β7 after epicutaneous immunization. However, supplementation of epicutaneous vaccine with RA may be the most effective way to consistently induce α4β7+ cells IgA-producing cells capable of migrating to the gut (42)

IgA class switch occurs in the organized structures of the Nasopharyngeal-Associated Lymphoid Tissues (NALT) (45). However, nasal immunization, which induces α4β1, L-sectin and CCR10, is generally not considered an effective approach for inducing SIgA in the gut. This notion has been challenged by a report indicating that NALT DCs possess the machinery for induction of RA and expression of α4β7+ on effector B cells, but stimulation by microbiota was required for these functions to develop (46). The sublingual route is used effectively for delivery of medication and allergen-specific immune therapy in humans and animals (47–50). This route has recently emerged as a mode of vaccine delivery capable of eliciting both systemic and mucosal immune responses while avoiding many of the side effects and formulation concerns associated with oral or intranasal vaccination (51–53). Perhaps because both routes use cervical lymph nodes as inductive sites, sublingual immunization generally induces IgA responses with similar mucosal tropisms than intranasal immunization. The ocular route has also investigated for delivery of experimental vaccines (54, 55). This route of immunization was found to induce SIgA in the tears, but also in the airways and in the genito-urinary tract (54). The nature of homing receptors imprinted in the Tear-Duct-Associated Lymphoid Tissues (TALT) has not been studies in detail. The fact that nasal immunization also induces SIgA in ocular tissues suggests the existence of TALT-NALT cross-talk via the tear duct bridges between the ocular and nasal cavities (55). Hence, ocular vaccines more likely promote the same homing receptors induced by nasal immunization.

- Vaccine adjuvants for induction of SIgA

Earlier studies with cholera toxin (CT) and heat-labile toxin I (LT-I) from *Escherichia coli* have helped established key principles, which guide current research on mucosal adjuvants. These closely related molecules are AB-type toxins consisting of two structurally and functionally separate enzymatic A subunits and binding B subunits. The B subunit of CT (CT-B) binds to GM1 gangliosides, while the B subunit of LT-I (LT-B) binds to GM1 as well as GM2 asialo-GM1 gangliosides. The A subunits of these toxins are ADP-ribosyl transferases. Binding of the B subunits to gangliosides receptors on target cells allows the A subunits to reach the cytosol where they elevate cAMP via activation of adenylate cyclase. Although the mechanisms underlying the mucosal adjuvant activity of these enterotoxins remain to be fully understood, it is now established that they stimulate APCs to enhance expression of MHC class II and costimulatory molecules, but also IgA-promoting cytokines such as IL-1, IL-6, and IL-10. Their adjuvant activity also induces antigen-specific Th2 and Th17 cells, which support the production of IgA via secretion of IL-4, IL-6, IL-10, and IL-17A (56–58). Despite their efficacy as mucosal adjuvant for needle-free vaccines in experimental animal models, the inherent toxicity of ganglioside-binding bacterial enterotoxins such as CT and LT-I prevent their use in humans.

Approaches used to dissociate the toxicity and the adjuvanticity of the bacterial toxins included partial or complete inactivation of the enzymatic activity of CT (59) and LT (60). Studies with a recombinant derivative of CT, which targets the enzymatic subunit of CT (i.e., CTA1) to B cells (CTA1-DD) have demonstrated that targeting ADP-ribosyl transferase to the right cells could alleviate the unacceptable side effects (CNS targeting and inflammatory responses) of the native CT and LT (61). Our own studies have shown that *Bacillus anthracis*

edema toxin, an adenylate cyclase which targets anthrax toxin receptors and increases cAMP, is a mucosal adjuvant for nasally administered vaccines (62). Edema toxin was found to be potentially safer, as it did not target CNS or promote massive inflammatory responses in airways and lung tissues after intranasal administration (62). We also reported that only minimal (<20%) of edema factor activity was needed for *Bacillus anthracis* edema toxin to act as a mucosal adjuvant and induce mucosal IgA Ab responses (63). Cyclic di-nucleotides that bind Stimulator of Interferon Gamma Genes (STING) were recently shown to be potential alternatives to cAMP-inducing bacterial toxins and derivatives as vaccine adjuvants. For example, STING ligands of bacterial origin, including 3′3′-cGAMP, c-di-AMP, and c-di-GMP, have been shown to effectively elicit mucosal and systemic immune responses following intranasal administration (64–66). More recently, targeting STING with 3′3′-cGAMP was found to be an effective strategy for enhancing the magnitude of immune responses and promoting IgA by sublingual immunization (67). It is worth indicating that induction of Th17 responses is a common feature of all the adjuvants mentioned above, which enhance IgA responses by increasing intracellular levels of the cyclic nucleotide cAMP or directly releasing select cyclic nucleotides (i.e., STING ligands) in the cytosol of immune cells. This point is of importance since Th17 cells are believed to be crucial for production of high-affinity T-dependent IgA (19).

SIgA responses can be induced by adjuvants that target specific innate signaling pathways, or specific immune cells and their products. For example, CpG ODNs which target TLR9 can act as mucosal adjuvants for nasal vaccines and promote SIgA in a Th1 environment. Studies with plasmid DNA expressing FLt3L have shown that increasing the number of DCs in mucosal inductive sites is a strategy for inducing SIgA and Th2 responses (68). Mucosal tissues contain high numbers of natural killer T cells, which upon stimulation rapidly secrete large amounts of Th1 (IFN-γ and TNF-α), and Th2 (IL-4, IL-5, and IL-13) cytokines (69, 70). They also express CD40L and induce the expression of costimulatory molecules (i.e., CD40, CD80, and CD86) (71–76). The NKT cell ligand α-Galactosylceramide (α-GalCer), originally isolated from a marine sponge, has now been shown to be an effective adjuvant for induction of SIgA by nasal (77) and oral vaccines (78). Mast cell activators represent another class of mucosal adjuvants described during the last decade (79). These studies have shown that the mast cell activator compound 48/80 promotes SIgA responses by stimulating the migration of DCs into the T-cell areas of the NALT (79), and the development of mixed Th1/Th2/Th17 responses (80). Taken together, these studies suggest that targeting cells present in high numbers in mucosal tissues and capable of instantly release several pro-inflammatory mediators is beneficial for induction of SIgA. This notion is consistent with a recent study that showed that this is not the case for adjuvant targeting/recruiting neutrophils, an immune cell subset primarily found in the systemic compartment. Thus, using sublingual vaccination with Bacillus anthracis edema toxin as adjuvant, we identified an inverse relationship between the ability to recruit neutrophils in sublingual tissues and cervical lymph nodes, and the development of SIgA responses (81). It is worth noting that TGF-β and IL-10, two of the key IgA-promoting cytokines antagonize rather than stimulate neutrophil functions. Furthermore, other have shown that depletion of neutrophils in guinea pigs with primary *C. caviae* ocular infections, reduced rather than enhanced the ocular

pathology and that this correlated with increased titers of C. caviae-specific IgA in the tears (82).

- Delivery systems for SIgA-inducing vaccines

Delivery systems for needle-free vaccines currently used or under development include live recombinant bacterial and viral vectors. These vectors are either attenuated strains of pathogens or microbes that fail to induce infection in the host. Several of such live bacterial and viral vectors induce SIgA via various mechanisms depending on the PAMPs they stimulate and the type of the cargo (for example cytokines) they deliver together with the antigen. Non-living delivery systems such as virus-like particles, nano- and microparticles, liposomes or nanogels do not present the potential safety concerns sometimes associated with the use of live micro-organisms. Many of such non-living delivery systems induced SIgA after immunization by the oral, nasal, ocular, rectal, or vaginal route (55, 83). The overall immune responses to these particulate vaccines and more specifically the magnitude of SIgA responses they promote can be improved by adding surface targeting molecules or peptides to facilitate their uptake by antigen-presenting cells or other innate immune cells (iNK T cells, innate lymphoid cells, mast cells).

Plants were engineered to synthesize and assemble one or more Ags that retain both T- and B-cell epitopes, thereby inducing systemic and mucosal immune responses in both mice and humans (84, 85). Most recently, CT-B subunit has been expressed under the control of the rice seed storage protein glutelin promoter (MucoRice-CT-B). Oral feeding of powdered MucoRice-CT-B to mice and non-human primates resulted in the induction of both systemic and mucosal Ab responses for the protection against CT (86–88). In addition to binding antigen in the lumen, SIgA alone or SIgA in complex with antigen can bind to M cells and be transported from the intestinal lumen to sub-epithelial dendritic cells (DCs) and possibly other APCs. Human SIgA were shown to bind to the DC-specific ICAM-3 grabbing nonintegrin (DC-SIGN) and be internalized suggesting that binding to this receptor is a mechanism used by SIgA to prime adaptive immune responses in mucosal tissues (89). This concept was further confirmed by murine studies, which showed that when used as vaccine antigen delivery system, SIgA interacts specifically with M cells present in the GALT or NALT and delivers antigen to mucosal DC for optimal induction of antigen-specific mucosal and systemic immunity (90, 91).

Conclusions and perspectives

Despite major progress made in our understanding of IgA induction and regulation of their functions, much more remains to be elucidated about the contribution of different mucosal cells in these processes and cellular and molecular events that interfere with induction of SIgA in mucosal and non-mucosal sites. In this regards, cells that were previously not known to be involved in IgA responses prevent the development of IgA responses, as suggested by the report linking the presence of neutrophils in a mucosal inductive site with reduced IgA CSR and SIgA responses (81). The opposite may hold truth as suggested by the report that eosinophils also can regulate IgA production via production of IL-1β (92). The development of adjuvants and vaccine delivery systems capable of promoting high titers of

IgA against microbial pathogens has been a major focus of mucosal immunologists. Since children and elderly are the most vulnerable to infectious diseases, the development of future mucosal vaccine should take into account the unique immunologic signatures that may occur in these populations (93). The increasing appreciation of the anti-inflammatory effects of pIgA and SIgA (94) and their potential role in dampening manifestations of allergic disease will certainly broaden the focus of future investigations and could influence future sublingual immunotherapies.

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Abbreviations

APRIL	a proliferation-inducing ligand
BAFF	B cell-activating factor of the TNF family
CSR	Class switch recombination
GALT	gut-associated lymphoid tissues
NALT	nasopharyngeal-associated lymphoid tissues
pIgA	polymeric IgA
pIgR	polymeric immunoglobulin receptor
SIgA	secretory IgA
RA	retinoic acid

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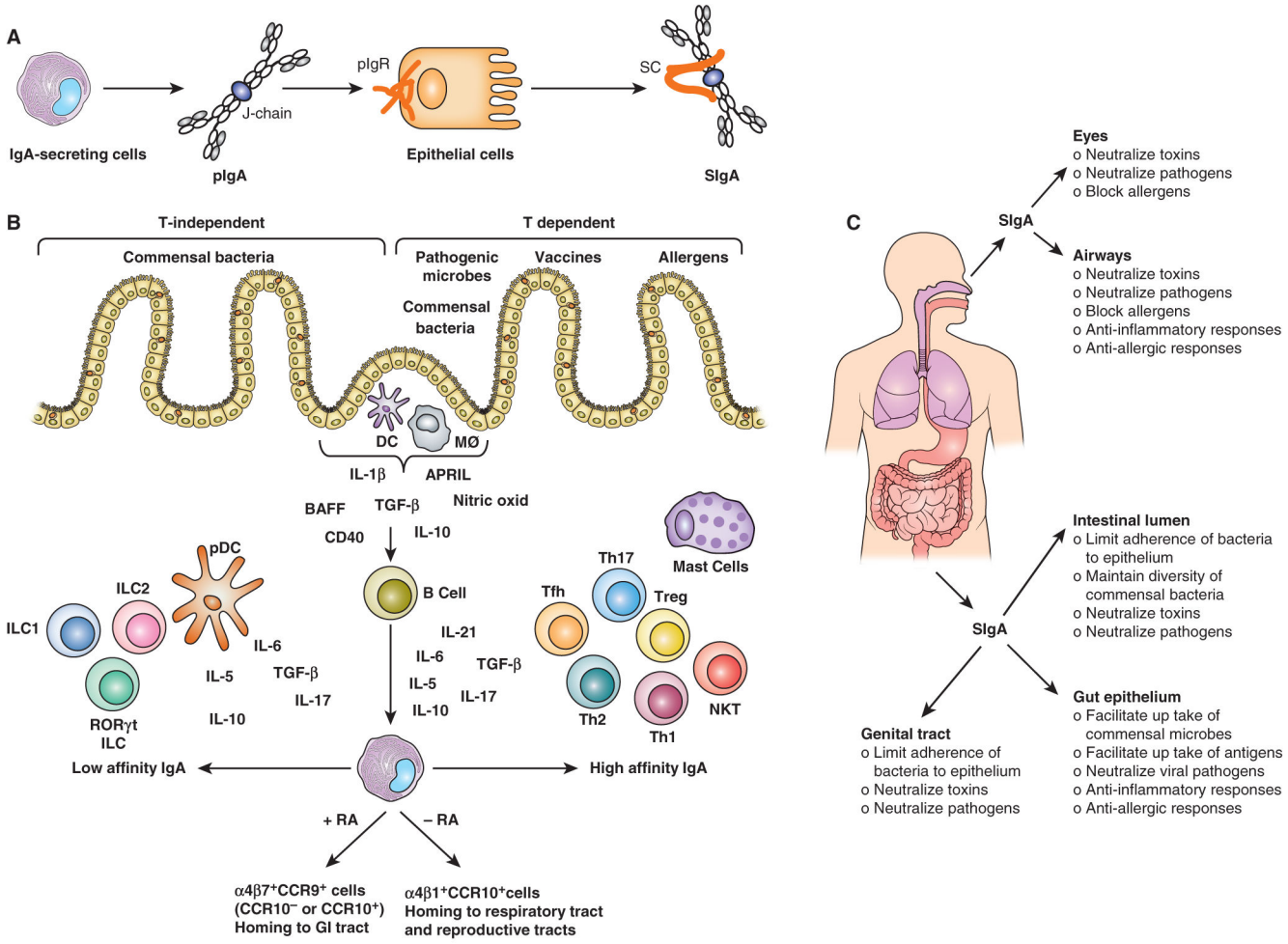

Figure 1. Mechanisms of induction of mucosal IgA and their protection role at mucosal surfaces

(A) Polymeric IgA (pIgA) and secretory IgA (SIgA). Polymeric IgA Abs are made of two monomers of IgA linked by a junction chain (J-chain) and bind to the polymeric immunoglobulin receptor (pIgR) at the basolateral membrane of epithelial cells. After transepithelial transport, they are released into the lumen as SIgA containing the secretory components (SC), a portion of the pIgR, which confers resistance to proteolysis. (B) Induction of IgA class switching and acquisition of mucosal homing capabilities. IgA class switching and production of mucosal IgA can occur in a T-independent or T-dependent fashion. In response to luminal stimulation by microbes, vaccines or allergen, epithelial cells and conventional antigen presenting cells in the Peyer's patches (PP), cryptopatches, isolated lymph follicles or lamina propria will produce cytokines (IL-1β, IL-10, TGF-β), nitric oxid (NO), and the B cell stimulating factors BAFF and APRIL. IgA class switching and production of IgA facilitated through the help of cytokines derived from Th cells and NKT cells (T-dependent) will yield high-affinity IgA. On the other hand, cytokine help from innate lymphoid cells (ILCs) and other innate cells such as plasmacytoid dendritic cells (pDC) (T-independent) will result in low-affinity IgA. The presence of retinoic acid (RA)

imprints IgA-producing cells with homing receptors and chemokine receptors for homing to the gastrointestinal (GI) tract. (C) Mechanisms of protection by SIgA in mucosal surfaces.

Table 1

Main groups of adjuvants and vaccine delivery systems for induction of mucosal IgA.

Adjuvants	Delivery systems
Toxin derivatives	**Live viral vectors**
ADP-ribosyl transferase enterotoxins	Attenuated or adapted viral vectors
Adenylate cyclase toxins	**Live bacterial vectors**
TLR agonists	Attenuated or adapted bacterial vectors
Lipid A-based (MPL)	Lactic acid bacteria
CpG	**Plants**
Flagellin	Rice
Other immune-modulators	**Nanoparticles and other targeting systems**
NKT cell ligands	Virus-like particles (VLP)
Mast cells activators	ISCOMs (Immunogenic immune stimulating complexes)
STING ligands	Nanoparticles
	Nano gels
	SIgA