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Review

Harnessing Nasal Immunity with Iga to Prevent Respiratory Infections

John Joseph[1,2] *

[1] Center for Nanomedicine, Department of Anesthesiology, Perioperative and Pain Medicine, Brigham and Women's Hospital, Boston, MA 02115, USA.
[2] Harvard Medical School, Boston, MA 02115, USA.
* Correspondence: jjoseph39@bwh.harvard.edu

Abstract: Nasal cavity is a primary checkpoint for the invasion of several respiratory pathogens. Numerous pathogens including SARS-CoV-2, *S. pneumonia*, *S. aureus*, etc., adhere to the nasal epithelium or mucus to invade and trigger an infection. IgA serves as the first line of defense against foreign antigens and pathogens. They exhibit cross-reactivity against a diverse variety of antigens through immune exclusion, which intercepts the invasion of pathogens through the mucosal lining. Advances in intranasal immunization technology underscore the elevated neutralizing IgA levels at local and distal mucosa in contrast to the parenteral vaccines. This review highlights the adjuvants that induce IgA class switching and the challenges of maintaining nominal IgA levels at the mucosal surface. Finally, the review features the paradigm-shifting of conventional immunization techniques to IgA-inducing vaccines to enhance protection against homologous and heterologous pathogens.

Keywords: Secretory IgA; IgA class switching; SARS-CoV-2; respiratory pathogens;nasal vaccines; vaccine adjuvants

1. Introduction

Nasal cavity plays a protective role in trapping air-bone particles and pathogens owing to the intricate anatomy and adhesive property of mucus. The foreign body captured in nasal mucosa is cleared via the mucociliary clearance mechanism. Respiratory pathogens have entry checkpoints in the nasal cavity[1,2]. The apical surface of the ciliated and goblet cells in the nasal lining promotes the binding of SARS-CoV-2 through the interaction between spike protein and surface receptors, ACE2, of the epithelial cells. Host cell proteases such as TMPRSS2 trigger the activation and pathogenesis of SARS-CoV-2[3]. Other viruses, including RSV, adenovirus, parainfluenza, and influenza virus, aggravate inflammation and compromise host cell functions, facilitating the invasion of the mucosal epithelial cells and destruction of their ciliary activity [4]. Along similar lines, bacteria like *S. pneumonia* and *S. aureus* colonize the nasal cavity and cause a wide range of respiratory infections. *S aureus* adheres and invades epithelial cells through the surface protein SasG [5,6]. Immunoglobulin A (IgA) plays a pivotal role in the forefront defense to intercept the binding of respiratory pathogens, including viruses and bacteria, to nasal and lung epithelium [7,8]. Class switching of IgA-secreting B cells occurs in the lymphoid organs, specifically in the MALT (Mucosal Associated Lymphoid Tissue)[9]. However, deficiency of IgA secretion can lead to an increased risk of allergies and respiratory infections, including SARS-CoV-2, influenza, *Streptococcus pneumoniae,* etc. [10–12]. The nominal level of IgA in humans ranges from 61 to 365mg/dL [13]. Studies have shown that impaired levels of IgA are caused by factors such as age, drug-induced, autoimmune diseases, immunodeficiency, etc. [14]. Airborne infections such as COVID-19 produce early neutralizing IgA antibodies, implying the protective role of IgA in reducing the severity or elimination of viral infections[15].

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Similar findings were associated with antigen-specific IgA antibodies against influenza, which had more profound protection in animals and humans than neutralizing IgG[16]. Likewise, the efficacy of influenza and COVID-19 vaccines showed a linear correlation with neutralizing IgA levels. However, a timely IgA production by *de novo* or immunization is essential to protect the host from respiratory infections. In this review, we discuss the protective role of IgA in the nasal cavity and advances in IgA-inducing vaccine technologies.

2. Cross-reactivity and immune exclusion of IgA against respiratory pathogens

A major challenge associated with the respiratory tract is the susceptibility to pathogen entry despite the epithelial and mucosal barrier. In this context, IgA plays a crucial role in modulating mucosal immunity and conserving homeostasis. Contrary to other immunoglobulins, IgA mediates the clearance of toxins and pathogens from the mucosal tissue by immune exclusion, receptor blockade, and steric hindrance [17]. Secretion of IgA is orchestrated in MALT through the crosstalk between innate and adaptive immune cells, mainly macrophages, dendritic cells (DCs), and B and T lymphocytes. MALT is essentially the primary site for IgA class switching and production of IgA-secreting B cell population. Pathogen entry to the nasal lining is detected by DCs residing underneath the nasal epithelium and presents antigen and production of cytokines in MALT [18]. Upon specific immunomodulatory cues, IgA class switching occurs along with affinity maturation of B cells leading to the increased transportation of antigen-specific IgA-producing B and T cells to the effector site to mount an immune response against pathogens [19].

IgA occurs in monomeric and dimeric isoforms. The dimeric IgA comprises two IgA covalently linked by15 kDa polypeptide, known as the J chain, and a secretory component (SC), which are essential for immune exclusion. IgA produced in lamina propria undergoes transcytosis through the epithelial layer with the aid of polymeric Ig receptor (pIgR) and is secreted into the mucus layer as SIgA. It is important to note that pIgR is needed for antibody stabilization and facilitates efficient binding to pathogenic proteins. SIgA can recognize a diverse variety of epitopes of pathogens or toxins and impede their affinity or entry towards epithelium by a phenomenon called 'immune exclusion' (Figure 1a) [20]. Thus, the IgA-pIgR complex inhibits the virus proliferation in infected cells and eliminates the virus. Pathogens that breach the mucosal barrier are neutralized mainly by polymeric IgA in the lamina propria and are cleared into the luminal surface (Figure 1 b). Moreover, SC in both free and IgA-bound states neutralizes microbes to protect the epithelial layer [21]. SIgA also binds to antigenic domains of bacteria and viruses to induce agglutination. These processes have been shown to disrupt the microbial membranes, affect their motility, and cause detrimental alteration of their gene expression, thus interfering with their virulence (Figure 1c). In addition, pathogens are entrapped in the mucus layer and eliminated by the natural mucociliary movements[22]. Although these processes have been researched for decades, there is more to comprehend and warrants further research. Various experiments have been conducted to study the immunological functions of the mucus layer, SC, and the role of polysaccharide chains in SC. Studies carried out in the absence of SC showed poor adherence and retention of IgA molecules in the epithelia or mucus layer, significantly lowering neutralization efficiency. It was concluded that the mucus layer, along with SC glycosylation, is essential to augment and maintain the functions of SIgA. Murine models of lung infection with shigella flexneri revealed that the binding of SIgA with mucus is necessary for providing first-line defense against the invasion of bacteria. Likewise, the deletion of carbohydrate moieties of SC in the SIgA complex hindered the anchoring to mucin and led to the failure of its protective role [23]. In another instance, the intranasal challenge of respiratory syncytial virus (RSV) along with neutralizing IgA treatment in mice showed a significant reduction in lung viral titer and, subsequently, mitigated pneumonia[24]. Similar findings on IgA protection were reported in animals

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infected with influenza and reovirus. Interestingly, intravenous administration of antigen-specific IgA against influenza, specifically in the polymeric isoform, protected the mice from RSV infection owing to the nasal secretion of IgA from serum[22]. However, the monomeric IgA was not effective in preventing infection.

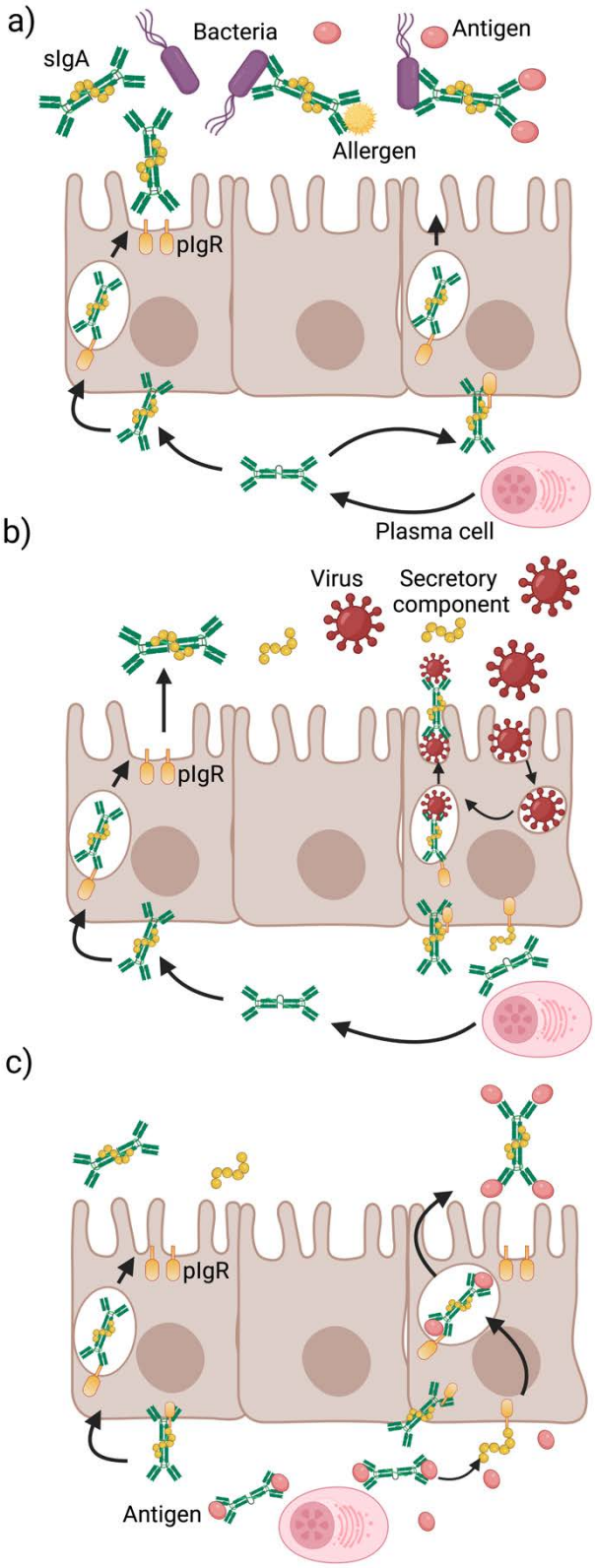

Figure 1. Immune exclusion of SIgA to facilitate pathogen neutralization or antigen clearance. Protective role of SIgA at the mucosal surface a) Cross-protection of IgA against diverse pathogens and antigens b) intracellular virus neutralization

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in infected epithelial cells c) Antigen clearance from lamina propria to mucosal lumen through the complex formation of antigen with IgA-pIgR complex.

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

B lymphocytes primarily induce IgA secretion in the MALT in response to endogenous antigens from commensal flora and exogenous invasion of pathogens or immunization. Foreign antigens induce T cell-dependent pathways to produce high-affinity IgA antibodies. On the other hand, antigens from commensal flora generate low-affinity antibody molecules through T cell-independent pathway. Dendritic cells (DCs) underlying mucosal epithelia sample luminal antigens through its extended dendrites to capture and present antigen to B cells in MALT (Figure 2)[25]. Consequently, T cells are activated, leading to the IgA class switching recombination (Ig CSR)[19]. The Ig CSR will result in IgA production in a T cell-dependent and independent manner. Activation of high-affinity IgA in T cell-dependent pathway demands the interaction between CD 40 of B cell with CD 40L of T cell [26]. This interaction upregulates the activation of T follicular helper cells (Tfh cells), Th17 cells, and FOxp3+Treg cells. Subsequently, this will lead to the aggravated release of pro-inflammatory cytokines like IL4, IL5, IL6, IL10, IL13, IL17, IL21, and TGF β that trigger the Ig CSR and release of high-affinity IgA molecules [27–29]. However, studies have shown IgA production in CD40 deficient mice and some human cells, indicating the T cell-independent mechanism. In T-cell independent pathway, the capture of commensal antigen enhances the production of TNF family subtypes, BAFF (B cell activating factor) and APRIL (A proliferation-inducing ligand) that in turn activates ILC1, ILC2, RORϒt, pDCs and the production of IL5, IL6, IL10, IL17, and TGFβ. This results in the stimulation of Ig CSR and the production of low-affinity IgG and IgA molecules[20].

4. IgA deficiencies and IgA1 proteases: threats to nasal vaccines?

Normal serum IgA levels are inevitable for homeostasis between proinflammatory and anti-inflammatory factors to control infections, allergies, and auto-immune disorders. Selective IgA deficiency is predominantly marked by low levels of IgA in serum, without altering other immunoglobulins[30]. Patients with IgA deficiency are reported to have a serum IgA less than 7mg/dL [31]. This condition increases the susceptibility to recurrent respiratory and gastrointestinal infections, autoimmune disorders, and allergies [32]. Many factors, including impaired B cell maturation, calcium modulator, and transmembrane activator, contribute to this condition. On the contrary, common variable immunodeficiency (CVID) impairs the immunological functions of IgA, IgG, and IgM [33]. Therefore, it is important to monitor the levels of IgA antibodies and study the factors that could lead to the deleterious clinical manifestations caused by IgA deficiency. Significantly low levels of IgA production in respiratory and gut epithelium are observed in vitamin A deficiency (VAD) models in response to viruses and vaccines [34,35]. However, the levels and function of other immunoglobulin remained unaffected by the retinol deficiency, leading to an increased IgG to IgA ratio. Hence, VAD makes it difficult to confer IgA-mediated protection via respiratory infections and vaccines. Numerous studies have shown that single intranasal administration of vitamin A palmitate or retinyl palmitate, an ester of retinol and palmitate, helps in the improved protection from infections and viral antigens/vaccines in VAD populations. This protection was further strengthened with the administration of intranasal vitamin A supplements, which is an established IgA-class switching factor [36].

IgA1 proteases have been widely reported to interfere with IgA's host defense mechanisms by cleaving IgA1 antibodies and hampering the structural integrity and function[37]. These proteolytic autotransporter proteins are produced by various pathogenic bacterial species such as *Haemophilus influenzae, Neisseria meningitidis, Neisseria gonorrhoeae, and S. pneumoniae* [38]. They specifically recognize and cleave certain proline-threonine and

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proline-serine peptide bonds in the IgA1 hinge region sequence TPPTPSPSTPPTPSPS in the IgA1 molecule generating intact Fcα and Fabα fragments. As a result, the recognition of bacterial epitopes by the IgA1 is hindered [39].

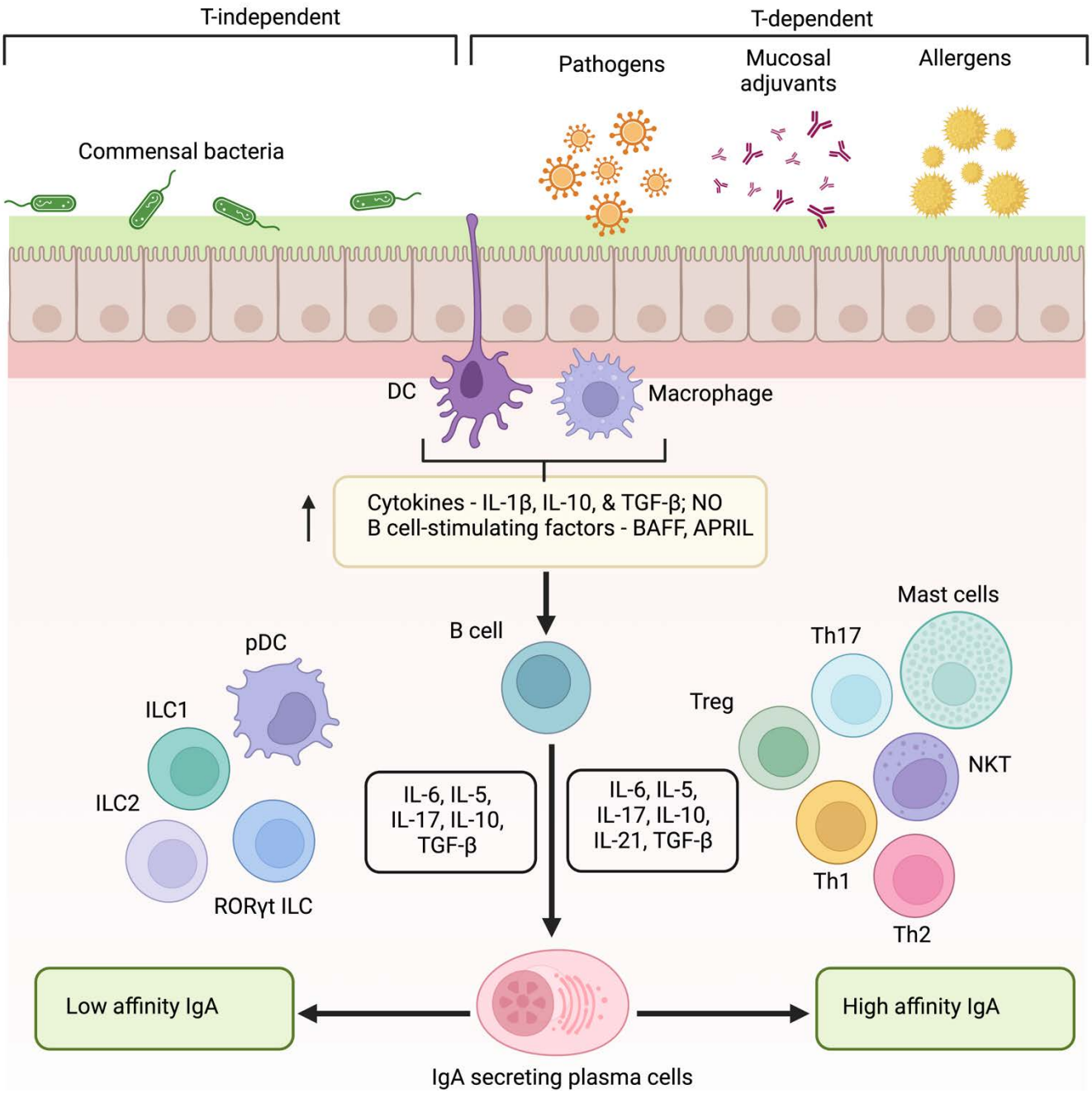

Figure 2. Mechanism of low and high-affinity IgA production. Induction of IgA occurs in a T-dependent and independent manner. Mucosal surface exposure to allergens, pathogens, or vaccines triggers epithelial cells and antigen-presenting cells (DCs and macrophages) to elicit the production of cytokines, nitric oxide (NO), and BAFF and APRIL to activate B lymphocytes. Class switching to high-affinity IgA occurs with the aid of Th cells through a T cell-dependent manner. On the contrary, plasmacytoid DCs and innate lymphoid cells (ILCs) favor the induction of low-affinity IgA.

Since Fcα is required for the agglutination process and opsonophagocytic activity, the IgA1 protease-mediated cleavage paves the way for bacterial survival and colonization. Few studies have shown that the Fabα fragment is known to retain its surface antigen binding capacity termed fabulation even after the cleavage [40]. Several studies revealed

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circulating antibodies in serum and nasal secretions that can neutralize these proteolytic enzymes [41]. These antibodies regulate the proteases secreted by the commensal flora. Hence, nasal immunity depends on the balance between the level of these neutralizing antibodies and the secreted enzymes. This is the underlying reason why children with a history of atopic disease encounter recurring immunological dysfunctions that could be attributed to the cleavage of IgA molecules by the IgA1 proteases in the absence of protease-neutralizing antibodies [42].

5. Recent advances in IgA induction with intranasal vaccines

Vaccines are aimed to elicit a long-lasting immune response against pathogens. Adjuvants are immunostimulants to trigger adequate innate and adaptive immunity. These components alter the kinetics, longevity, and robustness of the host immune response [43]. The addition of adjuvants in vaccines is beneficial in decreasing the dose of antigens and frequency of vaccine administration. They have proven to bolster immune activation in immunocompromised, elderly, and neonates[44–46].

Table 1: Intranasal vaccine adjuvants of IgA production against respiratory pathogens

Adjuvant	Target	Protection
Type I IFN	Interferon α receptor	Influenza A [50,51]
Flagellin	TLR 5	Influenza A [52]
MV130	TLRs	SARS-CoV-2 [53]
Lipoprotein	TLR 2	SARS-CoV-2 [54]
Poly I:C	TLR3 RIG-I MDA5	MERS-CoV, Influenza A [55,56]
CpG	TLR 9	SARS-CoV-2, Influenza A [56,57]
Cholera toxin	Ganglioside	SARS-CoV-2, Influenza A [56,58]
Enterotoxin B subunit	Ganglioside	Influenza A [59]
Alum	-	Influenza A [60]
Imidazoquinoline	TLR7/8	Influenza A, SARS-CoV-2 [61,62]
Cyclic-di-nucleotide	STING	Influenza A [63]
BDX301	-	SARS-CoV-2 [64]

Adjuvants enhance the presentation of antigens and facilitate the maturation of antigen-presenting cells such as macrophages, dendritic cells, etc. Growing evidence indicates a robust humoral response with intranasal vaccines compared to its parenteral route[44]. Intranasal administration of spike proteins elicited local and systemic mucosal IgA levels more than parenteral administration (Figure 3). Virus-like particles, liposomes, nanogels, etc., are being explored as inducers and delivery platforms[47]. For example, the intranasal COVID-19 vaccine (ChAd-SARS-CoV-2-S) delivered with an adenovirus vector improved

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the levels of antigen-specific IgA in contrast to intramuscular injection. This approach also facilitates the dose reduction of antigens[48]. Live/attenuated viral and bacterial vectors, recombinants of the original pathogen, are reported to trigger SIgA through recognizing PAMPs. It is important to note that these recombinant vectors do not elicit an infection. More interestingly, the early response of neutralizing IgA was observed in blood, BAL, and saliva of patients infected with SARS-CoV-2, followed by IgG and IgM. IgA antibody titer spiked three weeks following the onset of the disease and lasted for months, which was not observed for IgG[15]. Numerous researchers in mice and humans validated early seroconversion. Similar findings on IgA were also validated with MERS vaccines[49].

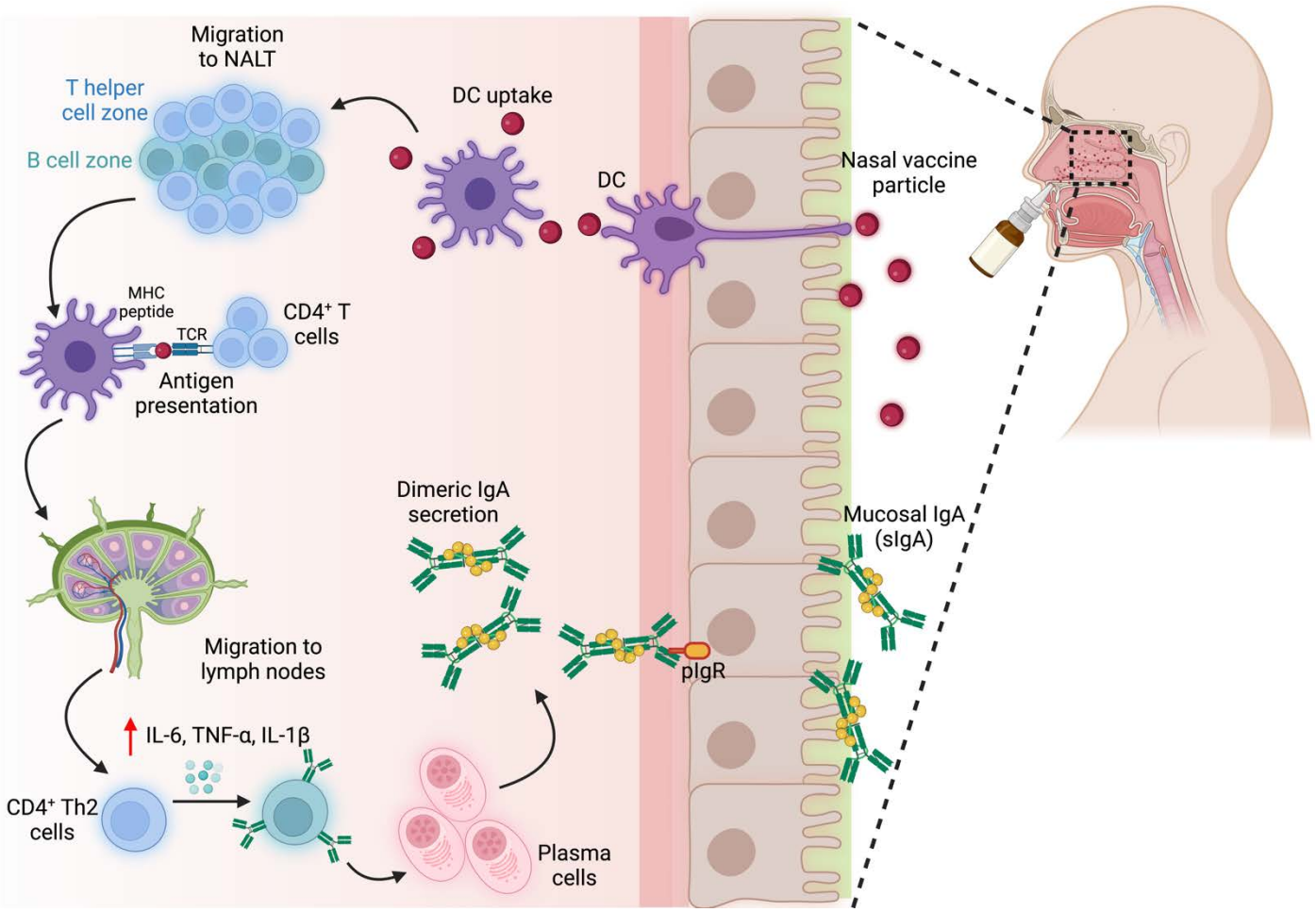

Figure 3. Induction of neutralizing IgA in following intranasal administration of vaccines. An immune response is elicited in NALT, leading to the secretion of IgA on the mucosal surface.

Targeting specific signaling pathways, subsets of immune cells, receptors, and cytokines can shape IgA class switching, hence protecting against antigens. For instance, IL5 and TGF-β1 are associated with the expansion of IgA-secreting B lymphocytes in the murine model[65]. Similarly, intranasal administration of retinyl palmitate resulted in elevated production of IgA in the nasal mucosa and heightened protection against influenza A virus. This unequivocally highlights the role of retinoic acid derivative in correcting IgA levels. Numerous nasal adjuvants have also been identified to induce IgA production (Table 1). Novel delivery systems are currently being explored to facilitate transient permeabilization of the nasal epithelial barrier to transport antigen/adjuvant to MALT[66]. Despite these strong indications, only a single intranasal vaccine technology exists against respiratory infection. The slow advancement in mucosal immunization is due to a lack of safe

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vaccine adjuvants and difficulties associated with quantifying the metrics of neutralizing IgA in nasal secretion. The rapid mucociliary clearance, entrapment of antigens in mucus, enzymatic degradation, and physical barrier of nasal epithelial further worsen the challenges[67]. Next-generation nasal vaccines should target the inductive site of mucosal immune cells to trigger an acute innate and adaptive response rather than tolerance. Additionally, the needless approach provides a safe, cheap, patient-compliant, and efficient alternative for large-scale immunization, especially in the case of a global pandemic.

6. Conclusion

The COVID-19 pandemic underscored the necessity of an effective prophylactic strategy against homologous and heterologous viruses. Numerous reports support the enhanced local and systemic levels of IgA via intranasal vaccines. Current research is advancing to identify novel adjuvants and delivery platforms to overcome the mucosal barrier and prolong vaccine exposure in the nasal cavity. A comprehensive understanding of the mechanism of antigen processing and subsequent orchestrating events of IgA secretion is warranted to improve the efficacy and hasten the regulatory burden on intranasal vaccine approval.

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Abbreviations: PPRs- pathogen recognition receptors, PAMPs – Pathogen Associated Molecular Pattern, LPS- Lipopolysaccharide, TLR-Toll like receptor, STING- stimulator of interferon gene, polyI:C – polyinosine polycytidylic acid, RIG-I – retinoic acid inducible gene, MDA5- melanoma differentiation associated gene, Treg- T- regulatory cells, NALT- nasal associated lymphoid tissue, MALT- mucosal associated lymphoid tissue.

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