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Mucosal vaccines: Strategies and challenges: A brief overview

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Abstract
Mucosal surfaces are a major portal of entry for many human pathogens that are the cause of infectious diseases worldwide. Mucosal immunization has potential benefits over conventional parenteral immunization, eliciting immune defence in both mucosal and systemic tissue for protecting from pathogen invasion at mucosal surfaces. However, numerous challenges remain in the way of creating a viable mucosal vaccination, including weak mucosal surface adhesion, insufficient uptake to penetrate the mucus, and challenges in avoiding potent gastrointestinal system breakdown. Recently, increasing efforts to overcome these issues have been made, and we herein summarize the latest findings on these strategies to develop mucosa-targeting vaccines, including different routes of administration, mucosa-targeting route, the development of mucosa-targeting vectors, the use of mucosal adjuvants, nanoparticle formulations, encapsulating vaccines into nanoparticle formulations, and M cell and Dendritic cell (DC) targeting vaccines. Here, I discuss the expanding knowledge on strategies and challenges used in the development of mucosal vaccines.

Keywords: Mucosal vaccines, pathogens, brief, mucosa-targeting

Introduction
Vaccination is an efficient and cost-effective form of infectious disease prevention that can lead to global eradication. However, there is an urgent and growing need for the development of new and improved vaccines to further reduce the global burden of infectious disease morbidity and mortality, particularly against those targeting the respiratory and gastrointestinal (GI) tract. In veterinary medicine, there are also severe lacks of vaccines that are effective, which is made worse by rising antibiotic and multi-drug resistance [1]. The need for zoonoses vaccines is especially important because at least 60% of viruses that might damage humans have their origins in animals [2]. Numerous pathogens, including the rotavirus, rotavirus, influenza, *salmonella enterica*, ETEC, *mycobacterium tuberculosis* and HIV, invade and infect the body at the mucosal surfaces of the digestive, respiratory and reproductive tracts. These pathogens significantly increase morbidity and mortality in both humans and animals [4, 5]. Injected vaccinations also provide a modest or non-existent level of protection at mucosal locations. Injectable vaccines provide little to no protection at mucosal sites due to the fact that mucosal locations account for >90% of all infections that enter the body, whereas mucosal vaccines trigger both mucosal and systemic immune responses [14, 15]. Vaccines are advantageous compared with systemic vaccines from a production and regulatory perspective [6, 7]. For instance, mucosal immunisation is non-invasive and needle-free. By avoiding problems with blood-borne illnesses caused by contaminated needles, mucosal vaccination helps to increase vaccine uptake and safety, especially in underdeveloped countries.

1. Mucosal vaccination prevents harmful effects like inflammation at the injection site.
2. Mucosal vaccines allow for frequent boosting.
3. Pre-existing systemic immunity usually does not obstruct the entry of vaccine into mucosal inductive sites, increasing the rate of vaccination "take," for [8].
4. The possibility for delivery by people with no medical training, increased compliance, and convenience of administration, particularly for preventing the pandemic spread of diseases like influenza virus infections [9-13].

Indeed, the long-term B and T memory responses are strongly induced by mucosal vaccinations. So, directing memory and effector immune cells to the mucosal membranes via tissue-specific homing receptors can successfully provide protection against infections.

Specialized dendritic cells (DCs), which move from the mucosal tissue to these lymph nodes, give B and T cells mucosal homing capabilities only in the draining lymph nodes Antigen-triggered B and T lymphocytes leave the draining lymph nodes after mucosal immunisation, travel through the lymphatic system, enter the bloodstream, and then "seed" the mucosal tissues [14-18]. Although constraints such mucosal barriers, mucosal tolerance and commensal bacteria are tough aspects for the production of mucosal vaccine design, mucosal vaccines are more effective than parenteral vaccines when taking into account the aforementioned advantages. In this review, we talked about the significance of mucosal vaccines as well as their tactics and difficulties in vaccine development.

What is the Mucosal vaccination?

Administration of vaccines at one or more mucosal sites leading to induction of local and systematic immune response at mucosal site of administration and other mucosal sites.

Mucosal immune system

The mucosal immune system, which makes up the majority of the immune system, evolved to give defence at the mucosae, which are the primary sites of infectious danger [20]. It is component of the immune system that reacts to and defends the body from pathogens that come in contact with mucosal surfaces, like those of the gastrointestinal and respiratory tracts, while also preserving tolerance to commensal organisms that reside on the mucosal epithelium's exterior. The mucosal immune system is composed of organized mucosa associated lymphoid tissues, such as Peyer's patches, as well as diffusely distributed cells within the lamina propria. (cellular immunology-Abbas). This immune system can be classified into inductive and effector sites based on morphological and functional characteristics. Mucosal effector sites are diffuse lamina propria regions, which are the effector sites for antibody production (IgA) and T cell responses. Mucosal inductive sites are the areas where antigen-specific immune responses are first triggered.

Mucosal vaccination induces immune responses in distant, multiple mucosal effector sites because of transport of the B and T cells from inductive sites to effector sites which is a cellular basis for the common mucosal immune system [16, 21-24]. An extensive network of mucosal inductive sites, including the gut-associated lymph reticular tissue (GALT) and nasopharyngeal-associated lymph reticular tissue (NALT), serves as a continual source of memory B and T lymphocytes for mucosal effector sites [16, 21-24]. MALTs are a complex immunological network structure which includre mucosal tissues such as the gut-associated lymphoid tissues (GALT), also known as Peyer's Patches (PPs), the nasopharynx-associated lymphoid tissue (NALT), the bronchus-associated lymphoid tissue (BALT), the conjunctiva-associated lymphoid tissue (CALT) and the vaginal-associated lymphoid tissue (VALT) [25]. The MALT consists of T-cell zones, B cell enriched regions with an abundance of surface IgA-positive (sIgA+) B cells, and a subepithelial region with APCs enabling the initiation of specific immunological responses.

The lymphoid cells, columnar epithelial cells and a subpopulation of developed microfold (M) epithelial cells that make up the follicle-associated epithelium that covers the MALT, are all essential for the beginning of mucosal immune responses. M cells take up antigens (Ags) from the nasal and intestinal mucosa (DCs) and transport them to the underlying APCs, such as dendritic cells [25].

Induction of the mucosal immune response: MALT is an extremely compartmentalised immunological system that operates mostly independently of the systemic immune system [26]. Active antigen-sampling starts mucosal immune responses at inductive sites and uses a few specialised and unique mechanisms since mucosal barrier capabilities vary [27]. Specialized APCs (Dendritic cells, Macrophages) that transport exterior antigens to deeper lymphoid tissues are part of the mucosal immune system [28, 29].

M cells: Short, truncated microvilli, a thin glycocalyx, and an invaginated basolateral pocket with lymphocytes and a diminished amount of intracellular lysosomes are the typical characteristics of M cells [30]. The M cell's short microvilli facilitate the antigen sampling process, allow particles to reach the apical membrane, and allow transcellular transit to the underlying lymphoid tissues across the basolateral membrane. Microfold cells use receptor-dependent transport systems to take in bacterial and viral pathogens.

M cells express a variety of surface pattern recognition receptors for this reason, which recognise and bind with the pathogen-associated molecular patterns released by bacteria. TLR-2, PAF-R, TLR-4, and TLR-5 are all part of the PRRs [31, 32, 33]. PAMPS includes bacterial lipopolysaccharide, phosphotidylcholine and CpG oligodeoxynucleotides [ODN] etc…According to Tyrer *et al*. the study, the platelet-activating factor receptor, TLR-2, TLR-4 and a5b1 integrin all aid in the transcytosis and absorption of bacteria. These receptors are expressed differently by M cells and enterocytes. M cells exhibit higher levels of TLR-4 and a5-b1 integrin expression than enterocytes, although PAF-R is equally expressed on both types of cells. While suppression of the apically expressed a5b1 integrin greatly reduced the ability of M cells to translocate bacteria, inhibition of TLR-4 and platelet-activating factor receptor reduced Gram-negative bacteria uptake by both cell types [34]. Many bacteria, including *Mycobacterium avium*, contain fibronectin binding protein, which the a5 b1 integrin receptors bind through to mediate the absorption and trancytosis of pathogens [35].

For the first time, Tyrer *et al*. study revealed that a5 b1 integrin receptors are only found on the apical surfaces of M cells and only on the lateral and basolateral walls of enterocytes, facilitating the uptake of pathogen from the lumen [36]. The carbohydrate residues on the M-cell surface known as lectin receptors are essential for pathogen invasion through M cells because they can bind to glycoprotein or glycolipid molecules on the surface of pathogens. The mouse M cells' a l-fucose carbohydrate moiety is positioned on the apical membrane by the lectin Ulex europaeus agglutinin-1 (UEA-1) protein [37, 38].

Recently, a new IgA receptor was found in mouse M cells, which may help with the transport of secretory IgA from luminal secretions into lymphoid tissue associated with the stomach [39]. Smith *et al*. studied the role of antibodies in vaccine M-cell targeting and discovered that coating micro particles with IgG or IgA or even the antigen-antibody complex improves its uptake by Peyer's patches M cells [40]. opsonization of Vibrio cholerae with IgA or IgG (isolated from healthy human colostrum and serum) increases its

absorption via M cells, according to Blanco *et al*. The expression of adherent junction protein has been changed in M cells [42]. These cells exhibit enhanced expression of proteins such as polymerized actin, b-catenin, E-cadherin, and a-actinin, which are crucial for maintaining tight junctions and for the function of endocytic processes in cells.

Gebert *et al*. examined FAE and non-FAE intestinal epithelia in rabbits in gut-associated lymphoid tissue, and they found that FAE tight junctions differ from non-FAE intestinal epithelia in that they seem to have a greater number of junctional strands linked to their *Zonula occludens* [43]. The discovery showed that M cells have exceptionally well-differentiated tight junctions under physiologically appropriate conditions, which would only allow pathogen entry via an endocytic absorption process. Translocation may also be aided by other "nonreceptor" dependent M cell apical surface specialisation. These include reduced mucous gel on PP epithelia [45] and a thin glycoprotein coat on M cells at the location of the filamentous brush boundary glycocalyx on enterocytes [44], which may help M cells transport antigenic substances even more easily.

Mucosal DCs

DCs are crucial regulators in the production and regulation of immune responses and are significant adaptive immune response regulators [46]. Antigens can penetrate the epithelial barrier for gut mucosal immunity through paracellular routes. Transcellular routes, or tight connections between epithelial cells, are the principal factors limiting the pace of paracellular pathways [47]. In order to direct immune responses to a specific tissue, T cells and B cells can be imprinted with homing characteristics by DCs.

Particularly, DCs are at the centre of almost all multicellular signalling networks that support immunological homeostasis. DCs are typically recognised for their ability to serve as CD4+ regulatory T cells' antigen-presenting cells (APCs). According to Rescigno *et al*. study, DCs produce tight junction proteins and extend their dendrites into the lumen between intestinal epithelial cells to collect antigen for lymphocyte presentation. Niess *et al*. proceeded to explain this intricate antigen sampling mechanism *in vivo*, where it was discovered to be an effective method of antigen uptake that may prime T cells to fight *Salmonella typhimurium* infection. Since the function of DCs was discovered to be compromised in CX3 CR1-negative DCs, the intraepithelial extension of DCs to the lumen and sampling of bacteria is dependent upon the chemokine receptor CX3CR1-mediated contact with intraepithelial cells.

The chemokines claudin-1 and occludin produced by mucosal epithelial cells also aid in the recruitment of DCs. Claudin-1 aids DCs expand their probing into the lumen by allowing them to enter the tight junction of epithelial cells [48, 49, 50]. DCs go to the lymphoid follicles after antigen sampling, where processed antigen is given to B and T cells to start a humoral and T cell-mediated immune response [51]. The primary APCs that start the initial immune response are the DCs. The lymphoid organs and peripheral tissues both include various subsets of these cells [52, 53]. The anatomic distribution of the DC population, the differential expression of certain cell-surface markers, and the DCs' function as innate immunity effector cells or in the generation of adaptive immunological responses are used to characterise the subsets of DCs (reviewed by Rescigno *et al*.) [54].

Lymphoid cells

Immunity of the intestinal mucosa is significantly influenced by T and B cells. Following internalisation and transport by M cells, the foreign antigen is released into the underlying lymphoid tissues where it is processed by APCs before being presented to CD4+ cells. Naive B and T cells go through high endothelial venules to the underlying lymphoid tissue (MALT) in response to antigenic stimulation. Immune response is triggered in lymphoid tissue based on the nature of the antigen, the type of APCs, and the local cytokine environment. APCs process and deliver antigens to CD4+ T cells, which are stimulated and develop into effector T helper cells that produce effector cytokines. These cells then divide into typical Th1 and Th2 cells as a result of stimulation. Through CD8+ T cells, the Th1 cells promote CMI production and participate in the defence against intracellular infections. In this instance, the memory cells could be CD4+ or CD8+ [55].

Th2 cells, on the other hand, promote the development of memory B cells and plasma cells that secrete antibodies. T17 cells, a newly discovered subtype of T cells, are recognised to be essential for mucosal immunology and B cell activation, nevertheless. Antigen can also enter the mucosa through paracellular pathways or directly through epithelial cells as an alternative to M cell-mediated absorption. Intestinal epithelial cells may possibly contribute to the antigen presentation to mucosal T lymphocytes because they express MHC-II antigen [56, 57].

T cells in epithelial tissues, like tissue-resident epithelial γδ+ T cells, are in a suitable position to assist tissue homeostasis and repair as well as carry out barrier monitoring [58]. The antigen that the mucosal surface absorbs may either be digested or delivered to T cells in the mucosa's lymphoid tissue or it may be transported from the mucosa to systemic tissue via the blood or lymph. The antigen-MHC complex is identified by naive CD4+ T lymphocytes after being presented with the MHC-II molecule on the surface of APC. In MALT, CD4+ T cells become activated and produce cytokines including TGF-b and IL-10, which promote a class switch and the development of IgA-committed mucosal B cells (with J chain expression). Sensitized mucosal lymphocytes quickly move from MALT to mesenteric lymph nodes via draining lymphatics for further differentiation. Then, by expressing Mad-CAM-1 and a4b7 integrin, they disseminate to distant mucosal locations by thoracic duct lymph and peripheral blood. There, they finally develop into plasma cells, where local antigen-sampling DCs, mucosal CD4+ T cells, and accessible cytokines provide the second signal for activation. These pre-activated B immune cells gravitate toward the effector sites that match the inductive sites where they were first activated by antigens [59].

Preferential homing is the movement of individual T and B lymphocytes from lymph nodes to specified distal mucosal locations by expressing tissue-specific receptors. L-selectin (L-sel), the adhesion molecule that enables lymphocytes to interface with high endothelial venules in peripheral lymph nodes, is expressed less by lymphocytes after activation in MALT. On the other hand, blood arteries in the mucosal tissues have increased expression of the a4b7 integrin and its ligand, mucosal address in cell adhesion molecule (MAdCAM-1) [60].

The expression of MAdCAM-1 is increased during intestinal inflammation and is crucial for attracting T and B

lymphocytes to mucosal regions. The recruitment of lymphoid cells into mucosal tissue depends critically on the expression of a4 b7 and its interaction with [61, 62]. The T and B cells that are activated in MALT will cycle back to the mucosal surface, while lymphocytes activated in peripheral organs never enter the mucosa because of the unique interaction between a4 b7 and MAdCAM-1 [63]. As a result, systemic immunisation does not produce a mucosal immune response while mucosal immunisation produces both systemic and mucosal immunity.

Role of microbiota in mucosal immunization: Although the goal of mucosal vaccination is to produce a protective immune response against pathogenic bacteria in the lumen, mucosal tissues are also heavily populated with commensal microbes. Numerous non-pathogenic bacteria that inhabit mucosal tissues have a substantial impact on how the mucosal immune system functions. As a result, they can affect how well mucosal vaccines work. For instance, it has been demonstrated that altering the intestinal flora significantly affects the regulation of T cells [64, 65].

The microbiota is important for the growth and maintenance of the mucosal immune system as well as for preventing pathogen infections, whereas the host immune system is essential for determining the composition of the microorganism [66]. The cooperation of the mucosal immune system and the microbiota, when it is functioning effectively, enables the preservation of regulatory pathways involved in the maintenance of tolerance to harmless antigens and the production of protective responses to causal agents [67]. As a group of active bacteria, probiotics are advantageous to the host because they control both local and systemic immunological reactions to diseases and vaccinations [68]. Different T helper cell subsets, including Th1, Th2, Th17, and regulatory T (Treg) cells, may be induced during colonisation by particular probiotics. It is well established that the intestinal epithelium's identification of microorganisms through TLR2 signalling is essential for maintaining epithelial integrity and homeostasis because it controls the formation of tight junctions [69, 70].

The ongoing and intricate interaction between the host immune system and gut microbiota has given rise to the idea of an organs-gut microbiota axis. The development of mucosal vaccines can only be accelerated by comprehending the mechanisms behind the activation of mucosal immunity and the interactions between the mucosal-associated immune system and microbiota.

Strategies to develop mucosal vaccines

The promise of mucosal vaccines is that they can be designed to recapitulate the earliest cellular interactions with local APCs and mucosal follicles to generate local immune responses, conferring Strategies for effective mucosal immunization will Mucosal vaccines provide the possibility of being able to recreate the initial cellular interactions with nearby APCs and mucosal follicles to trigger local immune responses, giving Effective mucosal immunisation strategies include

a) Prevail over physiological barriers at mucosal routes.
b) Targeting mucosal APCs for proper processing of antigens that result in specific T and B cell activation.
c) Managing the kinetics of antigen and adjuvant presentation to encourage long-lasting, protective adaptive immune memory responses.

Mucosal adjuvants: To increase immunogenicity, mucosal vaccinations require strong adjuvants. However, only a few numbers of mucosal adjuvants have sufficient potency without being toxic or reactogenic, and even fewer of them have been given human use approval. Antigen-only mucosal vaccinations frequently fall short of producing a strong immune response capable of offering long-term protection against infection [71]. Aluminum salts and particular varieties of emulsions are the only adjuvants with clinical approval in the United States. For the development and control of the highly vaccine-specific adaptive immune response, adjuvants are essential [72, 73].

Adjuvants are used to defend against pathogens and immune-related disorders, drastically reduce the dose of antigens and boost a wide spectrum of immunological responses. Recently, an adjuvant for use with injectable hepatitis B and HPV vaccinations that combines aluminium salts with a TLR4 agonist (mono phosphoryl lipid A, also known as MPL) received approval [74]. Table 1 lists the categories and targets of primary mucosal adjuvants [75]. While aluminium salts and oil-in-water emulsions rarely produce the CD8+ cytotoxic T cell, the creation of new effective adjuvants and formulations must stimulate not only powerful humoral responses against a variety of infectious diseases but also effectors as well as memory CD4+ and CD8+ T cells. The cholera toxin (CT) and Escherichia coli heat-labile toxin (LT) are the best-studied mucosal adjuvants because they are bacterial enterotoxins that adenosine diphosphate (ADP) ribosylate [76]. Studies demonstrating that B subunits of LT and CT are effective adjuvants that activate the B cell and T17 cell response on RV 2/6-VLP specific antibody through an intrarectal route [77].

Mucosal vaccinations use PRRs and ligands of PRS as adjuvants; the most are used in clinic [72, 78]. According to the study, TLRs ligands (TLR2, 4, 7 and 21) can function as vaccine adjuvants, aiding inactivated avian influenza virus (AIV) vaccines in stimulating chicken immune responses (79). To raise IgG and IgA titers and/or local CTL activity, cytokines are frequently utilised as mucosal adjuvants [80]. A few cytokines, such as interferons (IFNs), granulocyte macrophage-colony stimulating factor (GM-CSF) and interleukins, have been utilised to increase the effectiveness of mucosal vaccinations (ILs). Intranasal immunisation with IL-1 or IL-18 and recombinant adenoviral vectors (rAds) encoding hemagglutinin (HA) and nuclear peroxisome proliferator-activated receptor (NP) increased immunogenicity and offered superior defence against infections with homologous and heterologous influenza virus strains [81]. After intranasal immunisation in mice, the IL-1 family of cytokines can raise HA specific IgG titers in blood and sIgA titers at mucosal surface [82]. Polysaccharides for use as mucosal adjuvants, such as chitosan and curdlan sulphate. These adjuvants have the ability to boost penetration while also acting as strong immunostimulants.

Adjuvants and vaccination components must be balanced. Despite these positive attributes, certain adjuvants are complicated, unstable and poisonous, making it challenging to secure regulatory authority approval to manufacture them. Additionally, the selection process depends heavily on the harmony between the adjuvant characteristics and undesirable effects [83].

Different routes of mucosal vaccine administration

Mucosal vaccinations can be given in a variety of ways,

including sprays, inhalation, oral administration, scratching, and patching through the skin, genital tract, digestive tract, and respiratory tract. One of them is the oral or nasal route, which is a more convenient administration method, which promotes widespread and dispersed antigen-specific mucosal and systemic immune responses. Through the common mucosal immune system, mucosal inoculation can generate not only local mucosal immune responses at the inoculation site but also comprehensive mucosal immune responses at distant mucosal tissues [84]. Generally, oral or nasal route promotes widespread and diffused mucosal and systemic immune responses to antigens.

Different immunisation methods produce different immunological responses, which can significantly alter the effectiveness of the same vaccine [85]. When it comes to diseases like *Mycoplasma gallisepticum*, the vaccine provided by eye drops is substantially more successful than the vaccine delivered via nasal spray, and the oral route contributes very little to the total success of immunisation [86]. Candidates for the nucleoprotein (NP) and M influenza vaccines performed better when administered intravenously than intramuscularly. A successful example of an intranasal influenza vaccination is Flumist® [87].

Strong mucosal immune responses against HIV mucosal infections were generated after the HIV-1 vaccination was administered intranasal [88, 89]. The administration of COVID-19 vaccinations by aerosol sprays or droplets is a desirable method [90, 91]. Oral vaccines include the well-researched attenuated poliovirus vaccine (OPV), which has been shown to successfully elicit a robust mucosal immunity in the salivary gland, mammary gland, and digestive system [92, 93, 94]. Adenovirus vaccines of types 4 and 7, rotavirus vaccines (RotarixTM, Vivotif), *salmonella typhi* vaccines, and oral cholera vaccines are also included in the list of orally administered vaccines in transdermal vaccination. Micro needle patches have recently undergone substantial development as a unique method of administering several vaccinations to promote mucosal immunity against the influenza virus, malaria and measles virus [95, 96]. It has also been shown that administering eye drops and sublingual (SL) vaccinations successfully stimulate mucosal immune responses [97, 98, 99]. Sexually transmitted illnesses can be avoided by protecting the rectum and genital tract [100, 101].

Nanoparticle based formulations: Insoluble granular vaccine antigen formulations, such as virus-like particles (VLPs), bacterial ghosts, biodegradable nanoparticles and immune-stimulating complexes, protect the antigens from degradation, enhance the attachment and absorption of the antigens onto the mucosal surface and extend the residence time at local mucosal regions [102-106]. The M cells are more effective at absorbing these. Intranasal treatment of a mixture of VLPs each showing the H1, H3, H5 and H7 hemagglutinin (HA) epitopes successfully defended mice against challenges with hetero-variant or hetero-subtypic influenza strains, according to a recent study [107]. Intranasal treatment of a mixture of VLPs each showing the H1, H3, H5 and H7 hemagglutinin (HA) epitopes successfully defended mice against challenges with hetero-variant or hetero-subtypic influenza strains, according to a recent study [107].

In the formation of mucosal vaccines for HIV-1, TB, and malaria, particles encapsulated with mucoadhesive and biodegradable polymer particles, such as chitosan, polyethyleneimine (PEI), poly lactic-co-glycolic acid (PLGA), glycolides, epoxy polymers, hydrogels, and paraffin, have also been used [108]. Animal models for mucosal vaccinations have been explored using lipid-based particles such as liposomes, archaeosomes, niosomes, virosomes, ISCOMs, microbubbles, and emulsions [109, 110]. Feng F *et al*. reported that the adenovirus-vectored HIV vaccine enhanced mucoadhesion to nasal tissues, triggered potent IgA production and induced T-cell immunity in local and distant MALT in mice [111].

Construction of Novel Vectors as Mucosa-Targeting Vaccines

Another important method for producing a potent vaccine is the antigen-delivery method. There has been a lot of research done on numerous vaccines based on inactivated/protein components, recombinant viral vectors, bacterial vectors, DNA vectors, and the mRNA modality. The recombinant Ad5-based vector has been extensively explored as vaccine candidates against SARS-CoV-2, influenza, Ebola, HIV-1, and other infectious diseases. Adenovirus type 5 (Ad5) is a common respiratory virus.

It should be noted that as compared to systemic immunisation, mucosal vaccination (i.e., nasal) with Ad5-vectored vaccines may provide higher mucosal immunity and protective efficacy. The influenza virus is also a promising mucosal vector, similar to the former. Mice were protected from RSV challenge by recombinant live attenuated influenza expressing an RSV G-protein domain because it elicited a strong G-specific immune response in the lung and bronchoalveolar fluid [112]. Additionally, a baculovirus-vectored human papillomavirus (HPV) vaccine that was administered orally or intravenously provided protection against vaginal HPV infection [113, 114]. The only licenced TB vaccine, *Mycobacterium bovis* Bacillus Calmette-Guérin (BCG), has been further developed as vaccine vectors against HIV-1 and SARS-CoV-2 [115-118].

Mucosal immune cells-targeted strategies

M cells and DC cells in MALT play a key role in antigen uptake and antigen presentation. To improve the mucosal immune responses, it makes sense to design antigens that specifically target these cells. Mucosal vaccinations have a rationale attributed to recent thorough analysis of the M cells or DC subsets and the mechanism of antigen presentation. Based on the specificity of the mucosal immune cell surface receptors, the mucosal immune cells-targeted method.

M cell and DC -targeted mucosal vaccination

M cells as specialized epithelial cells are ability to transport antigens from the lumen to the MALTs [119]. Mucosal immune responses can be delivered to M cells with remarkable efficiency using M-cell ligands. The most extensively studied plant lectin, Ulex europaeus agglutinin 1 (UEA-1), has the ability to bind exclusively to-L-fucose residues on the surface of M cells. A successful oral vaccine delivery strategy was created by Du *et al*. by altering polynanoparticles with UEA-1, which has been shown to significantly increase intestinal and serum IgG and IgA production in animal models [120]. The outer membrane protein H (OmpH) and its ligands are employed as adjuvants to induce mucosal immunity in a variety of bacterial illnesses [121].

Due to their crucial significance in bridging innate and

adaptive immunity against the vaccine antigens, DCs are being recognised as essential immunisation determinants. DCs are advantageous candidates for vaccination and immunological treatment due to their adaptability and specific antigen-presenting capacity [122]. By secreting IL-12, DCs are essential for cell-mediated immunity. They also stimulate adaptive immunity by encouraging the generation of IFN-γ. Over the past ten years, numerous DC receptors and DC subsets have been discovered and used in targeted tactics. These receptors primarily consist of the C-type lectin receptors (CLRs), the TLR family, and the Fc receptors (FcRs). DC receptors such Clec12A, Clec9A, and DEC205 have been shown in multiple studies to be promising targets for antibody-based vaccination [123]. It is investigated how to build mucosal vaccinations that target the receptors langerin, DCIR, dectin-1 and CLEC9A [122, 124].

In general, the local mucosal immunity induced at the vaccination site is stronger than that at the distal mucosal site. The mucosal immune system possesses the ability to express tissue-specific homing receptors. DCs that are specific to the intestinal mucosa act as imprinting cells when vaccine antigens are taken up by those cells. Imprinting cells help to up-regulate the expression of 4-7-integrin and CCR9 molecules on lymphocyte surface as well as MADCAM1 and CCL25 on epithelial cells and epithelial cells, respectively [125]. In order for T cells to preferentially home into the skin via P-and E-selectins and CCL27, respectively, they can be imprinted to express P-and E-selectin ligands and CCR10 [126]. The receptor for CCL28, which is released by epithelial cells in the intestines, salivary glands, tonsils, respiratory tract, and mammary glands, is expressed by IgA-secreting B cells in MALT. In order to control the DC imprinting impact on lymphocytes, antigens might be designed to conjugate with these molecules. This would successfully trigger immune responses at certain mucosal regions.

Challenges in vaccine design

Current methods of vaccination target the systemic immune system and elicit only a weak mucosal immune response. The vaccine must be administered directly to the mucosal locations in order to amplify mucosal responses. Direct mucosal immunisation, however, has been challenging. The difficulty in designing mucosal vaccines is to boost immunogenicity without sacrificing safety. One difficulty with mucosal immunisation is that mucosal fluids tend to dilute mucosal vaccines, and bulk flow may prevent effective deposition onto the mucosal system's epithelium [40]. Mucosal vaccinations also have a tendency to get caught in the mucus gel and then be broken down by proteases. The method of delivery might not be ideal for promoting immunity at mucosal surfaces, the point of entry for the causal agent. Animal models are typically used for evaluations that are very time-consuming and not always successful, such as screening, adjuvant identification and adjuvant identification. The difficulties faced in creating mucosal vaccines are unheard of in the field of vaccination.

Mucosal barriers

Physical and chemical barriers are two different types of mucosal barriers. Innate immunity includes both physical and chemical barriers, such as tight junctions and the mucus that goblet cells in the respiratory, gastrointestinal and reproductive tracts create. In addition, innate immune cells and Toll-like receptors are essential components of the first line of defence. The primary physical barriers that allow the antigenic contents of the vaccine to be promptly cleared during nasal immunisation are mucus and ciliary movements [128]. Because of mucus and enzymes, notably proteases, which break down protein antigens that are pH-sensitive, oral vaccine vaccination is challenging to perform [129].

The complexity of antigens recognition mechanism impairs mucosal vaccine design

For immunisation to be effective, antigenic components in the vaccine must be recognised. When exposed to antigens during immunisation, the mucosal immune response enters the stage for antigen identification via immunoglobulins and T cell receptors, which are exceedingly varied molecules. Mucosal vaccinations powerfully generate long-term B and T cell memory by presenting the antigen in the form of antigenic peptides that are recognised by the diverse T cell receptors (TCR). T and B cells use altered antigen recognition receptors to identify a variety of antigens. T cells can only recognise foreign antigens when their antigen recognition receptors (TCRs), which are expressed on the cell surfaces of host cells, attach to MHC molecules. The inability of antigens, especially recombinant proteins, to adequately activate immune responses for protective immunisation is a barrier to the development of mucosal vaccines. This is largely because the mucosal immune system is unable to identify vaccination antigens. Designing efficient vaccines necessitates a grasp of the mechanics of antigens recognition due to the mucosal immune system's intricacy.

Immunotolerance effect the mucosal vaccination

Immune tolerance is the physiological condition where the immune system is unresponsive to the harmless antigens or the nutrients, where it will protect body from the hyper immune response and avoid the inflammation. T reg cells are a crucial subset of T cells that are crucial for immunological control [130, 131]. Antigen dose, formulation and frequency of exposure are some of the variables that affect mucosal immunotolerance brought on by antigens. Long-term exposure to low doses results in low-zone tolerance, but short-term exposure to large doses results in high-zone tolerance, which overwhelms the immune system [132]. The primary innate component of mucosal tolerance is the mucosal epithelial lining because it is an essential factor in determining whether an immune response would be pro-inflammatory or regulatory [133]. Immunotolerance has been discovered to be an active process that involves the suppression of mucosal immunity and memory that is introduced to the microbe via the mucosal surfaces in the lungs and GI tract. Mucosal vaccination may result in T-and B-cell tolerance if antigens are used without an adjuvant [134]. Antigens taken orally usually cause an immune hypo responsiveness or oral tolerance condition.

Concluding remarks and prospects

For many years, mucosal immunity and mucosal vaccines have attracted less than their due share of research and development, considering that most infections and environmental allergies have a mucosal portal of entry. However, methodological advancements that have made it possible to study mucosal immune responses more closely in recent years have increased interest in both trying to

understand the unique characteristics of mucosal immunity in comparison to systemic immunity and in developing mucosal vaccines to treat allergic or autoimmune diseases as well as to prevent mucosal infections. In this review, we discuss emerging strategies that are expected to be instrumental for developing a new-generation of mucosal vaccines. Significant improvements in vector design, antigen selection and expression, as well as antigen stability and localization need to be achieved before mucosal vaccines can be commercialized.

The development of mucosa-targeting vaccines has been greatly limited due to the physical, chemical, and biological barriers of MALTs. The difficulties of mucosal tissues' sampling and lack of surrogate biomarkers with which to assess mucosal immune responses also restrict the development of mucosal vaccines. To overcome these challenges, various strategies to improve the efficacy of mucosal vaccines have been rapidly developing in recent years, though their effectiveness should be further evaluated in clinical studies. It is of great significance to develop novel mucosa-targeting vaccines as the next generation of vaccine technology against emerging infectious diseases. Among them, intranasal vaccination is extensively thought of as a promising approach to eliciting mucosal immunity against respiratory pathogens, such as influenza and SARS-CoV-2. In the next few years, the clinical trial of new mucosal vaccines will be pivotal. In the next few years, the clinical trial of new mucosal vaccines will be pivotal. The improved formulations and better delivery technologies will be main part for the continued enhancement of mucosal vaccines development platform.

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