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Review

New insights in mucosal vaccine development

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ABSTRACT

Mucosal surfaces are the major entrance for infectious pathogens and therefore mucosal immune responses serve as a first line of defence. Most current immunization procedures are obtained by parenteral injection and only few vaccines are administered by mucosal route, because of its low efficiency. However, targeting of mucosal compartments to induce protective immunity at both mucosal sites and systemic level represents a great challenge. Major efforts are made to develop new mucosal candidate vaccines by selecting appropriate antigens with high immunogenicity, designing new mucosal routes of administration and selecting immune-stimulatory adjuvant molecules. The aim of mucosal vaccines is to induce broad potent protective immunity by specific neutralizing antibodies at mucosal surfaces and by induction of cellular immunity. Moreover, an efficient mucosal vaccine would make immunization procedures easier and be better suited for mass administration. This review focuses on contemporary developments of mucosal vaccination approaches using different routes of administration.

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1. Introduction

The epithelial lining of mucus membranes covers an area of several hundred square metres in an adult. Mucosal surfaces are mainly represented by the gastrointestinal, the respiratory and the urogenital tracts and therefore are vulnerable to infection by pathogenic microorganisms. Mucosal surfaces are protected from external attacks by physicochemical defence mechanisms, innate and adaptive mucosal immune systems which are designed to distinguish antigens that enter the body through mucosal surfaces from those introduced directly into the bloodstream. The mucosal immune system can principally be divided into inductive and effector sites. Antigens are sampled from mucosal surfaces either through collaboration with professional antigen-presenting dendritic cells (APCs), or by producing a specialized epithelial phenotype, the M cell and then stimulate cognate naive T and B lymphocytes [1]. Epithelial barriers on mucosal surfaces at different sites in the body differ dramatically in their cellular organization, and antigen sampling strategies at diverse mucosal sites are adapted accordingly. Multilayered squamous epithelia line the oral cavity, pharynx, esophagus and urethra whereas the intestinal mucosa is covered by only a single cell layer, and the airway and vaginal lining varies from pseudo-stratified to simple epithelium. These diverse epithelia are not impenetrable barriers, but rather are cell assemblies that control cross-talk between the lumen and the lamina propria using multiple antigen sampling strategies. In stratified and pseudo-stratified epithelia, antigen-processing

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dendritic cells serve as motile "scouts" that move into the epithelium, obtain samples of luminal antigens, and migrate back to local or distant organized lymphoid tissues. In simple intestinal and airway epithelia whose intercellular spaces are sealed by tight junctions, specialized epithelial M cells deliver samples of foreign material by transepithelial transport from the lumen to organized lymphoid tissues within the mucosa [2,3].

At the immune effector sites level, such as *Lamina propria* (LP), where the effector cells locally controlling foreign agents, secretory antibodies are induced, especially secretory IgA (SIgA) but also IgM. IgA is the major isotype in secretions, the most important being those of the epithelium lining the intestinal and respiratory tracts whereas IgG is the principal isotype in the blood and extracellular fluid [4]. IgG effectively opsonises pathogens for engulfment by phagocytes and activates the complement system. In contrast IgA is a less potent opsonin and a weak activator of complement. IgA operates mainly on epithelial surfaces where complement and phagocytes are absent, and therefore IgA function chiefly as a neutralizing antibody. Animal viruses or bacteria infect cells or host by binding to a particular cell-surface receptor, often a cell-type-specific protein that determines which cells they can infect (virus) or cell-surface molecules called adhesions that enable them to bind the surface of host cells (bacteria). Antibodies against those patterns can inhibit these adhesive reactions and prevent infection. IgA antibodies secreted onto the mucosal surfaces of the intestinal, respiratory, and reproductive tracts are particularly important in preventing infection by inhibiting the adhesion of bacteria, viruses, or other pathogens to the epithelial cells lining these surfaces [5]. The adhesion of bacteria to cells within tissues can also contribute to pathogenesis, and IgG can protect from damage as IgA protect at mucosal surfaces.

IgA-secreting plasma cells are found predominantly in the LP. At this level, IgA can be transported across the epithelium to its external surface and are secreted as a dimeric IgA molecule associated with a single J chain. This polymeric form of IgA can be endocytosed at the basolateral surface by the poly-Ig receptor (pIgR), then transcytosed and finally secreted into the lumen, where it can combine with antigen to form immune complexes. The extracellular portion of the pIgR still attached to the Fc region of the dimeric IgA may help to protect it from degradation [6]. The neonatal Fc Receptor (FcRn) plays also a role in mucosal immunity during the passive delivery of IgG from mother to young via the placenta or the intestinal route. In adult, it can also transport IgG across mucosal surfaces to confer resistance to intestinal pathogens and therefore use to deliver antigens fused with an IgG Fc fragment through mucosal surfaces [7].

Mucosal associated lymphoid tissue (MALT) is the principal mucosal inductive site where immune responses are initiated. LP is considered to be an effector site which is also important for expansion and terminal differentiation of B cells. MALT comprises approximately 80% of immune cells in the body and is the largest lymphoid system in mammals [8]. It has three major functions: (1) the protection of mucosal surfaces against colonization and invasion by microbial pathogens, (2) the prevention of the internalization of commensal bacteria or antigens as non-degraded proteins derived from food and environment, and (3) induction of tolerance against innocuous soluble substances, as well as commensal bacteria. Mucosal effector sites are formed by a surface epithelium with a concentration of intraepithelial T lymphocytes (IEL) and secretory antibodies (especially SIgA). Sub-epithelial compartment, or chorion, is an effector site where pile up effector cells (NK-like cells, macrophages, B and T cells). Antigen presenting cells (APC) including dendritic cells (DCs), sentinels of the immune system, are also present in the mucosal lymphoid tissue, to detect foreign agents.

It should be notified that mucosa are naturally highly exposed to huge amount of antigens every days so different regulation mechanisms exist in a manner that does not result in untoward immune reactions. Those mechanisms are called immune tolerance and depend of the dose of antigen: anergy/deletion (high dose) or regulatory T-cell (Treg) induction (low dose). It has been shown in mice that tolerance to oral antigen requires CD8+ T cells for local suppression of IgA responses [9]. In contrast, recognition of foreign agents as pathogens requires the recognition of pathogens-associated molecular patterns (PAMPs), like LPS or flagellin. PAMPs are danger signals which are recognized by pathogen recognition receptors (PRRs) like Toll-like receptors (TLR) or Nod-like receptors (NLR) expressed by cells of the innate immune system and present in quantities at mucosal sites both in animal models and in humans. The role and the high expression of NOD1 receptor have been described in lungs during asthma [10] and in intestinal mucosa for inflammatory bowel diseases (IBD) [11]. NOD2 receptor is also involved in IBD and expressed on the intestinal mucosa of Crohn patients [12]. TLR7 expression has been described in the human intestinal mucosa as TLR4 has been shown to be expressed at the genital level.

It's now well described that local mucosal immune responses are important for protection against diseases which occur mainly by those routes. Topical application of a vaccine may be necessary to induce a protective immunity. In some cases, systemic IgG are sufficient to protect against occurring mucosal infections such as poliovirus. Mucosal vaccines could induce in certain conditions with the use of appropriate adjuvants, both systemic IgG, protective SIgA and CTL responses against pathogens [13]. By the migration of IgA antibody-secreting cells (ASCs), local mucosal immunization could lead to antigen-specific IgA production at distant mucosal sites [14]. In contrast, traditional injected vaccines are generally poor inducers of mucosal immunity and are therefore less effective against infections at mucosal sites [4,15]. In a practical way, they are easily administered (e.g. oral route), and therefore more accessible to developing countries. As soluble antigens are not efficiently uptake when administered by mucosal routes, and generally induce immune tolerance, mucosal immunization requires adjuvants and/or efficient carrying vehicles as delivery systems. The ideal mucosal vaccine should: (1) preserve vaccine antigens from enzymatic or chemical degradation (2) limit their elimination or excessive dilution in organism, (3) facilitate the preferential uptake of antigen by specialized NALT/GALT/BALT M cells in order to target APC, dendritic cells or epithelial cells, (4) facilitate the co-uptake of both antigen and adjuvant to APCs in order to stimulate appropriate specific immunity as neutralizing SIgA and/or helper and cytotoxic T lymphocytes. Secretory antibodies may block the colonization of the mucosal epithelium by pathogens or prevent attachment of microbial toxins on epithelial cells, and then cytotoxic T cells could eliminate infected cells and prevent microbial invasion.

This review focuses on new vaccinal approaches using different mucosal routes to induce appropriate mucosal and systemic immune responses. At first, we describe the different mucosal vaccines that are currently used in clinical practice and then we will develop different approaches to improve the effectiveness of mucosal vaccination.

1.1. Registered human mucosal vaccines (Table 1)

Only seven vaccines are routinely administered mucosally to humans. They target five of the main enteric pathogens (Table 1): poliomyelitis, *Vibrio cholerae*, *Salmonella typhi*, rotavirus, and influenza whereas vaccines are still lacking against the two other most important causes of enteric diseases, enterotoxigenic *Escherichia coli* (ETEC) and Shigella.

Poliomyelitis is due to poliovirus which enters the organism through oral route and cross the intestinal epithelial lining through M cells and enterocytes. In approximately 0.1–2% of cases, the virus