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Review Article

Sublingual mucosa: A new vaccination route for systemic and mucosal immunity ☆

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ABSTRACT

Needle-free vaccine delivery has become a global priority, both to eliminate the risk of improper and unsafe needle use and to simplify vaccination procedures. In pursuit of greater ease of vaccination, a number of needle-free delivery routes have been explored, with mucosal routes being perhaps the most prominent. Since the vaccine administration route significantly affects immune responses, numerous researchers are attempting to develop alternative vaccine delivery methods including a mucosal route. My group's recent studies demonstrate the potential of the sublingual (s.l.) route for delivering vaccines capable of inducing mucosal as well as systemic immune responses. Sublingual administration conferred effective protection against a lethal challenge with influenza virus (H1N1) or genital papillomavirus. Moreover, CCR7-CCL19/CCL21-regulated dendritic cells are responsible for activation of T and B cells following s.l. administration. This review highlights current knowledge about the safety and effectiveness of s.l. vaccination and describes how s.l. vaccination can induce both systemic and mucosal immunity.

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1. Introduction

As the main entry site of most environmental pathogens, mucosal surfaces such as those of the respiratory, gastrointestinal, and genital tracts act as the first line of defense against pathogenic antigens [1]. Many recent studies have focused on developing mucosal vaccines capable of effectively inducing both mucosal and systemic immune responses, thereby resulting in two layers of host protection [2]. Mucosal vaccination, in contrast to parenteral vaccination, is of particular interest since it can elicit immune responses, mainly secretory IgA (SIgA) antibodies, which are located at the portal of entry of most infectious pathogens [3,4]. Further, since the route of vaccine administration has a significant effect on the outcome of immune responses, much effort has focused on the development of novel mucosal vaccine delivery routes [5–10].

Mucosal and skin surfaces, both boundaries with the exterior environment, are covered with special epithelial layers that act as barriers against exogenous challenges by pathogens and soluble antigens. Functionally independent of the systemic immune apparatus, the mucosal immune system has developed its own highly organized immunological tissues [11]. These tissues maintain homeostasis in the vast mucosa by mounting specialized anti-inflammatory immune defenses such as the production of SIgA antibodies and the induction of tolerance against innocuous soluble substances as well as commensal bacteria [12]. Moreover, when administered with appropriate adjuvants, mucosal vaccination can induce not only protective antigen-specific SIgA antibodies and cytotoxic lymphocyte (CTL) responses against pathogens invading via a mucosal surface, but also IgG responses in the systemic compartment [13]. Furthermore, due to the migration of IgA antibody-secreting cells (ASCs), local mucosal immunization leads to antigen-specific IgA production at distant mucosal sites [14].

In human studies, the strongest responses were elicited in mucosal tissues directly exposed to antigen and in the adjacent mucosa, respectively [15]. Oral immunization can induce strong IgA responses in the small intestine, proximal colon, and mammary glands but is relatively less efficient in the respiratory and reproductive tracts [6]. In contrast, intranasal (i.n.) immunization induces SIgA antibodies in the respiratory system and reproductive tract but is less effective for gut immune responses [16]. However, i.n. administration of certain vaccine antigens requires special delivery devices (nebulizers) that have raised safety concerns. In a clinical trial in Switzerland, i.n. administration of inactivated

Abbreviation: APCs, antigen-presenting cells; ASCs, antibody-secreting cells; BAL, bronchoalveolar lavage; CNS, central nervous system; CTL, cytotoxic lymphocytes; DCs, dendritic cells; HPV, human papillomavirus; i.n., intranasal; LN, lymph node; LP, lamina propria; LT, heat-labile enterotoxigenic *Escherichia coli*; nCT, native cholera toxin; OVA, ovalbumin; s.l., sublingual; SIgA, secretory IgA; VLP, virus-like particles .

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influenza vaccine with heat-labile enterotoxin from enterotoxigenic *Escherichia coli* (LT) as mucosal adjuvant proved capable of eliciting brisk levels of systemic and mucosal immunity but some study participants developed Bell's palsy [17]. Murine studies have demonstrated that i.n. administration of native cholera toxin (nCT) can redirect co-administered vaccine antigen into the central nervous system (CNS), e.g., the olfactory nerves/epithelium, olfactory bulbs, and brain [18,19]. Possibly facial nerve fibers adsorb the adjuvant, which is followed by retrograde transport and neuronal damage. To date, only one nasal vaccine against seasonal influenza virus has been licensed, and only for use in a specific age group.

Oral mucosa, including buccal (the cheek lining), sublingual (s.l.) (underside of the tongue), and gingival mucosa, have recently received much attention as novel delivery sites for therapeutic drugs because they do not subject proteins and/or peptides to the degradation associated with gastrointestinal administration. Among oral mucosal routes, the s.l. route is commonly used for immunotherapeutic treatment of allergies because it quickly absorbs antigen, allowing direct entry into the bloodstream without passing through the intestine or liver and thereby eliciting allergen-specific tolerance [20–23]. No cases of anaphylactic shock were observed in recent human studies of s.l. administered immunotherapy targeting allergies [24–26]. On the basis of these findings, International Vaccine Institute researchers assumed that the s.l. route might be promising for delivery of vaccines targeting infectious diseases.

In this review, I provide an overview of the effectiveness and safety of the s.l. route for vaccine delivery, the protective mucosal immune responses achieved, and the immunological aspects of the s.l. mucosa that need consideration for future mucosal vaccine design.

1.1. Localization of antigen-presenting cells in the sublingual mucosa

Histologically, the murine s.l. mucosa shows a superficial keratinized pluristratified epithelium overlying a thin lamina propria (LP) with capillary vessels, scattered mononuclear cells, and numerous fibroblasts (Fig. 1a) [27]. Beneath the epithelial layer the LP contains mononuclear cells devoid of organized lymphoid structures. In an immuno-histochemical study, MHC class II$^+$ and/or CD11b$^+$ cells were detected in both epithelium and LP of mice at steady state (Fig. 1b); the numbers were much increased within 2 h after nCT administration via the s.l. mucosa (Fig. 1c and d). CD11c$^+$ and Langerin$^+$ cells were also found, although the expression intensities were moderate [28]. To trace the antigen uptake pattern in s.l. mucosa, mice were administered FITC-ovalbumin (OVA) alone or with nCT by the s.l. route in a time-dependent manner [27]. The FITC-conjugated OVA adhered to the s.l. mucosa for 2 h after s.l. administration without nCT (Fig. 1e) and with nCT (Fig. 1f); most FITC-OVA disappeared within 4 h of administration. The histological traits of the s.l. mucosa resemble other mucosal tissues (e.g., skin and buccal and vaginal mucosa): They possess stratified epithelium, lack mucosa-associated lymphoid tissues, and show ability to adhere to and penetrate antigen with or without mucosal adjuvant.

1.2. Role of draining lymph node in sublingual vaccination

It is important to identify the site of primary antigen presentation after vaccination. By day 3 after s.l. administration of OVA plus nCT [27], adoptively transferred OVA-specific CD4$^+$ T cells isolated from DO11.10 mice had proliferated mainly in the cervical and mediastinal lymph nodes (LN) and spleen of recipient BALB/c mice. After s.l. vaccination of mice treated with FTY 720, which prevents the egress of T cells from secondary lymphoid organs [29], proliferating OVA-specific CD4$^+$ T cells were found only in the cervical LN, not in mediastinal LN or spleen [27]. The cervical LN is the primary draining LN for priming of CD4$^+$ T cells after s.l. vaccination and s.l. administration of protein antigen with mucosal adjuvant enhances the antigen-specific CD4$^+$ T cell activation that initially takes place in cervical LN.

1.3. Mechanism for induction of CD4$^+$ T cell activation following sublingual vaccination

After identifying the site of primary antigen presentation, my group assessed mRNA expression levels of chemokines and chemokine receptors in s.l. mucosa and cervical LN in a time-dependent manner. CCL19 (ligand of CCR7) and CCL27 and CCL28 (ligands of CCR10) in the s.l. mucosa were substantially expressed at steady state [27]. mRNA expression levels of CCL19 were higher at 0.5 and 2 h but were considerably lower by 6 h after s.l. administration with nCT. Sublingual mucosa possessed mature professional antigen-presenting cells (APCs) and had unique chemokine expression patterns at both steady state and after nCT administration. mRNA expression levels of CCL21 in cervical LN were approximately 30 times higher 24 h following s.l. vaccination with nCT than at steady state and other time points. In addition, predominant enhanced

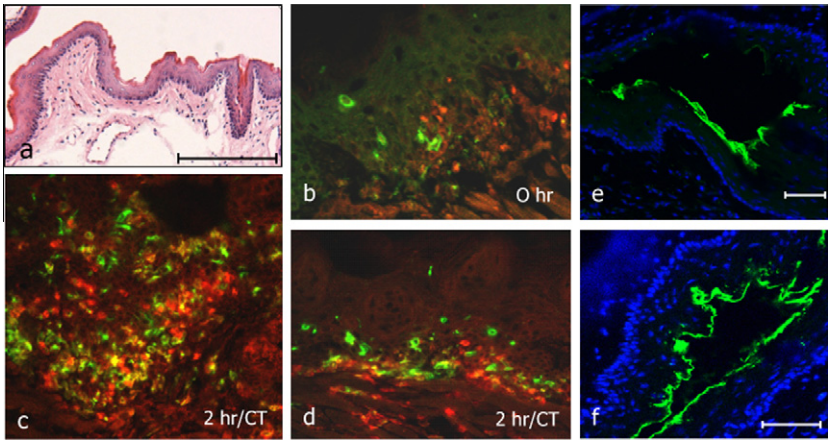

Fig. 1. Morphology of the s.l. mucosa and *in vivo* antigen uptake patterns following s.l. immunization. Small pieces of s.l. mucosal tissue of naïve BALB/c mice were fixed with paraffin, cut longitudinally (length of the tongue), and stained with H&E (**A-a**). Sublingual mucosa was isolated at 0 h (**A-b**) or 2 h following s.l. immunization with OVA plus nCT (**A-c, d**). Tissue sections were stained with FITC-conjugated anti-MHC class II and PE-conjugated anti-CD11b mAbs. Naïve BALB/c mice were immunized with FITC-OVA (**A-e**) and FITC-OVA plus nCT (**A-f**) by the s.l. route and the s.l. mucosa was harvested 2 h later. DAPI was used for counterstaining.