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REVIEW

Non-Reflex Defense Mechanisms of Upper Airway Mucosa: Possible Clinical Application

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Summary

The sinonasal mucosa has an essential role in defense mechanisms of the upper respiratory tract. The innate immune system presents the primary defense against noxious microorganisms followed by induction of the adaptive immune mechanisms as a consequence of the presence of pathogens. This well-known activation of adaptive immune system in response to presence of the antigen on mucosal surfaces is now broadly applicated in vaccinology research. Prevention of infectious diseases belongs to substantial challenges in maintaining the population health. Non-invasive, easily applicable mucosal vaccination purposes various research opportunities that could be usable in daily practice. However, the existence of multiple limitations such as rapid clearance of vaccine from nasal mucosa by means of mucociliary transport represents a great challenge in development of safe and efficient vaccines. Here we give an updated view on nasal functions with focus on nasal mucosal immunity and its potential application in vaccination in nearly future.

Key words

Nasal immunity • Nasal-associated lymphoid tissue • Mucosal vaccination

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Introduction

Nose is the first organ of respiratory tract, which is in contact with inhaled air. Presence of air-born pollutants of chemical or biological character and presence of microorganisms, which could be inhaled, represent a risk for the respiratory tract. This is the reason why upper airways possess very important functions regarding the protection and defense of entire respiratory tract. These functions are however, frequently neglected with the main attention directed to cough as the major airway defensive process (Ji *et al.* 2018, Chen *et al.* 2019).

In this review we point to non-reflex defensive processes of sinonasal mucosa with special focus on local immune mechanisms and their role in the respiratory defense.

Anatomy and histology of nasal cavity

The inner surface area of the nasal cavity is about 150 cm^2 and its volume is about 15 ml (Sahin-Yilmaz and Naclerio 2011). The great surface area of the nasal cavity is conditioned by a typical slit-like form of the cavity, which is shaped with the help of inferior, middle and superior nasal turbinates. Thereby mucosa of the turbinates facilitate humidification and regulation of temperature of the inhaled air (Elwany *et al.* 2009).

The most superficial layer of nasal mucosa, which is in initial contact with outer environment is the epithelium. The superior part of the nasal cavity has an

olfactory epithelium, while the rest of nasal cavity has typical respiratory epithelium that is ciliated, pseudostratified and columnar. This respiratory epithelium consists of three main cell types (Herbert *et al.* 2018). First type are basal cells, which serve as progenitors of other cell types and also assist in the adhesion of columnar cells to the basement membrane (Harkema *et al.* 2012). Second type are columnar cells. There are two types of these cells - ciliated and non-ciliated. Ciliated cells possess about 100 cilia and they begin to appear just behind the anterior edge of the inferior turbinate (Ferkol and Leigh 2012).

The last type are goblet cells. The average goblet cell density is similar to that found in the trachea and major bronchi (Wine and Joo 2004, Wine 2007). Goblet cells contribute a little to secretion of the nose. However, the major volume of total nasal secretion is produced by submucosal glands.

Nasal functions

Two main functions are related to the nasal cavity. First is smell that is not further described in this article, second is heating, humidification and cleaning of inspired air (Keir 2008). Nasal breathing is important for the survival of most species, including almost all newborns in the first weeks of life. The width of the nasal cavity is regulated actively through sympathetic innervation and tone of the venous plexuses. These changes in width happen regularly at intervals of 2-4 hrs (Lang *et al.* 2003, Kim *et al.* 2006).

*Heating and humidification: t*he nose is adapted for air-conditioning function by several means. 1) the slit-shaped cross-section of the nasal cavity provides close contact between inhaled air and mucous membrane, 2) the cross-section of cavity can adapt efficiently and rapidly to changing needs by regulating the tone in venous plexuses, 3) heat exchange is mediated by a large amount of arterial blood flow in arteriovenous anastomoses, 4) the nasal mucosa has significant secretory capacity (Elad *et al.* 2008). The nose also serves as a water reservoir. This feature refers to the fact, that the body saves about 100 ml of water a day as a result of condensation of exhaled water in the ventral part of the nose, which has about 3-4 ° C lower temperature than the lung. This water contributes to the nose's excessive discharge in cold environment (Sahin-Yilmaz and Naclerio 2011).

Filtration: the more proximally the inspired particles are trapped, the faster they are removed (nose - minutes, bronchi - hours, alveoli - days to weeks). Therefore, the capture and deposition of inhaled particles in the nasal cavity is beneficial and significant. The effectiveness of the nasal filter depends largely on the size of the inhaled particles. Almost all particles larger than 10 μm are trapped in the nose, while particles smaller than 2 μm can get around the nasal filter (Garcia *et al.* 2009).

The role of sinonasal tract in immunity

The sinonasal tract has an important role in respiratory immunity, because it is the place of first contact with inhaled pathogens. Nasal mucosa stores all potentially harmful, toxic or infectious inhaled particles (Sato and Kiyono 2012, Uhliarova *et al.* 2015). The effectiveness of nasal secretions by neutralizing or eliminating potentially harmful pathogens is evident from the fact, that most of people enjoy relative health as a normal part of life (Ooi *et al.* 2008). The characteristics of innate and adaptive immune mechanisms are summarized in Table 1.

Table 1. Differences between innate and adaptive immunity

Innate Immunity	Adaptive Immunity
Responses evolved early, present from birth	Responses acquired with exposure to pathogens
Immediate response	Slowlier response (3–5 days)
Germline encoded receptors (nonspecific, hundreds of receptors only)	T- and B-cell receptors specific to the antigen (1014–1018 receptors)
Not dependent on prior exposure	Has memory
Immune response the same regardless of exposure to antigen	More effective immune response on subsequent encounter with the antigen

Innate immunity

The innate immune system presents the primary defense against infection and damage caused by microorganisms, with the consequent activation of the adaptive immune system in response to the presence of pathogens (Lane 2009).

The respiratory epithelium is actively involved in innate immunity. This happens by means of mucociliary clearance that mediates the physical removal of inspired pathogens (Wiesmiller *et al.* 2003, Bennett *et al.* 2008), recognition of microbes by pattern recognition receptors, that are expressed by epithelial cells (Janeway and Medzhitov 2002, Basu and Fenton 2004), secretion of various mediators of inflammation (Lane *et al.* 2006), antimicrobial peptides (Ganz 2003, Kim *et al.* 2006), and interaction with acquired immune system. Failure of nasal innate immunity can lead to colonization by pathogenic microorganisms and thus to the development of recurrent infections (Lane 2009, Uhliarova *et al.* 2014).

Barrier function, mucociliary transport

The ciliated cells are surrounded by an inner layer of serous fluid with an upper lying viscous-elastic mucus layer, generated by goblet cells and submucosal glands. The movement of the mucus layer with adhered foreign material plays an important role among defense mechanisms. The thickness of both layers is about 5 μm. The surface layer of mucus is substituted every 10 to 20 minutes and this rapid replacement contributes to mucosal protective features (Antunes *et al.* 2009). Unlike the mucus layer, the periciliary layer is more stable and contains many defensive substances. Because the mucosa does not have a keratin layer to protect it against various exogenous factors, the mucus layer provides many of the protective properties (Thornton *et al.* 2008).

With regard to the size of the glycoprotein molecule (200 kDa to 400 kDa) and their ability to polymerize into molecule larger than 2000 kDa (Antunes *et al.* 2009), glycoproteins represent a renewable, flexible, persistent extracellular surface, that covers and protects the mucosa. This gelatin layer isolates the epithelium, protects the aqueous layer underneath, lubricates the mucosal surface and moisturizes the inhaled air. Each glycoprotein molecule binds water molecules to itself, providing a source for moisturizing the inhaled air (Thornton *et al.* 2008). The thickness and compound of bilayer is substantial for mucociliary transport. If the serous layer is too thin, the cilia movement will be decelerated by the surface mucus layer. If it is too thick, the mucus layer loses contact with the cilia and the mucociliary clearance is slowed down (Braiman and Priel 2008).

The particles captured in the superficial mucus layer are transported by mucociliary clearance into the posterior pharynx at the rate of 1 cm/min. The superficial mucus layer is then swallowed and replaced every 10 to 20 min. Thus, the surface mucinous layer is constantly formed and subsequently removed and replaces the original layer. This mechanism presents an important barrier function of the nasal mucosa. Microorganisms and various exogenous particles are trapped in the mucus and subsequently eliminated by these processes. However, this mucus bilayer serves only as a selective filter, since large particles never reach epithelial cells, while smaller molecules absorb easily. Below the superficial mucus layer there is a periciliary layer which serves as a surrounding for cilia. The serous layer is more stable and does not undergo such rapid exchange as superficial layer. The stability of this layer provides many of the protective secretory functions (Antunes *et al.* 2009).

Mucociliary transport rate measurement

Mucus is displaced from the respiratory tract into the pharynx by mucociliary clearance, where it undergoes deglutition or is eliminated by coughing. Mucociliary clearance in the respiratory tract is led by the coordinated beating of cilia in the respiratory epithelium (Tarran *et al.* 2006). Cilia beat with frequency about 10-14 Hz. The constant clearance of the mucus into the pharynx is the most important protective mechanism in the upper and lower airways. The rate of mucus clearance is 10-24 mm/min in the trachea, in the nose 4.5-7 mm/min, and in the bronchioli 0.5-2 mm/min. There is large interindividual variability, but intraindividually the clearance rates are fairly constant (Önerci 2013). Wide spectrum of methods to measure mucociliary transport includes e.g. saccharin test, the use of either 99m Tc-labelled particles (rhinoscintigraphy) or 99m Tc-labelled resin particles, radiopaque teflon dicks, dyes and combination of dye and saccharin. For measurement of total nasal clearance of a deposited dose the gamma scintigraphy method with total clearance of 99m Tc-labelled solutions is often used (Sherly and Prathibha 2014).

Other clearance mechanisms

Sneezing and sniffing have to be mentioned as

reflex or partially reflex clearance mechanisms (Javorka *et al.* 1994). They also greatly contribute to the movement and removal of nasal secretions (Sahin-Yilmaz and Naclerio 2011). Despite the fact, that sniffing physiologically does not belong primary among clearance mechanisms, it belongs to mechanisms, which participate on the quicker motion of nasal secretions with captured foreign particles.

Regulation of secretion

Processes leading to higher microvascular permeability cause the leak of plasma proteins into the nasal mucosa and subsequently into nasal secretions. The leak of plasma proteins is suggested to be the first human defense line on mucosal surfaces (Baraniuk and Kim 2007). The afferent nerve fibers that conduct the bursts from the epithelium run through the trigeminal nerve. Efferent innervation of nasal mucosa involves parasympathetic nerves running in the Vidian nerve (innervating glands and vascular plexus), and sympathetic nerves that innervate the vascular bed. Parasympathetic nerves are the major motor nerves in the nose. Parasympathetic nerve stimulation leads to increased nasal secretion (Sarin *et al.* 2006).

Recognition of pathogens with sinonasal cells

Antigen recognition is noticeably different between innate and adaptive immune systems. T-cell and B-cell receptors of the adaptive immune system are formed during their development, so that each lymphocyte has one antigen-specific receptor (Ooi *et al.* 2010, Meylan *et al.* 2006). These T-cell and B-cell receptors are not coded by the germ cells, so they cannot be transferred to the next generation, even when they provide an evolutionary benefit to survival (Ooi *et al.* 2008, Lane *et al.* 2004).

However, there are only a few hundreds of innate immune receptors (Ooi *et al.* 2010). The innate immune system recognizes several patterned structures present on microorganisms, and does not recognize all possible antigens. These structures are called pathogen-associated molecular patterns (PAMPs). Examples of PAMP present lipopolysaccharides, lipoteichoic acid, peptidoglycan, mannans, bacterial DNA and double-stranded RNA. These molecular patterns are crucial for the innate immune system for several reasons: 1) PAMPs are expressed by microbial pathogens, but are not produced by host cells - this allows the innate immune system to distinguish the body's own molecules from others, 2) PAMPs represent encoded molecular patterns necessary for the survival or pathogenicity of microbes, 3) PAMPs are usually common to all types of pathogens. For example, all gram-negative bacteria have lipopolysaccharides and all gram-positive bacteria have lipoteichoic acid (Iwasaki and Medzhitov 2004, Ooi *et al.* 2008).

The receptors of innate immune system are expressed on many effector cells, such as macrophages, dendritic cells, B cells and respiratory epithelial cells (Diamond *et al.* 2000, Ooi *et al.* 2010). Mature dendritic cells belong also to significant antigen presenting cells. Pattern recognition receptors on the surface of these cells recognize different PAMP on the surface of organisms that induce T - cell activation (Von Bubnoff *et al.* 2001, Himi *et al.* 2011).

Three categories of pattern recognition receptors are distinguished: secreted, endocytic, and signalling (Iwasaki and Medzhitov 2004). Secretory pattern recognition receptors function as opsonins to activate phagocytosis or complement. The best described receptors of this category are mannan-binding lectin, and surfactant proteins SP-A and SP-D (Crouch *et al.* 2000). Endocytic pattern recognition receptors are expressed on the surface of phagocytes. These receptors mediate the phagocytosis of pathogens by phagocytes and dendritic cells, where they are deactivated and presented to lymphocytes afterwards. An example is the mannose receptor of the macrophages (Gazi and Martinez-Pomares 2009). Signalling receptors activate signalling pathways that lead to the transcription of various inflammatory cytokines and antimicrobial peptides (Delves and Roitt 2000). Nasal epithelial cells also belong to antigen presenting cells. They are capable of amplifying an inflammatory response at local tissue level (Lane *et al.* 2004).

Secreted factors in innate immune response

Complement is a system of 30 plasma proteins and proteins on cell surfaces in which the basic physiological activities involve firstly protection of the host from infection by opsonization, chemotaxis, and activation of leukocyte lysis, secondly the connection between innate and adaptive immunity (by enhancing antibody responses and enhancing immunological memory), and thirdly the destruction of immune complexes and inflammatory products (Lane 2009).

IL-1b, IL-6 and IL-8 are thought to be important factors in the innate immune response (Lu *et al.* 2009, Nishi *et al.* 2009). Cytokines are proteins with low molecular weight that regulate differentiation, proliferation, growth, inflammation, immunity, and function of immune cells (Van Wetering *et al.* 2005, Lu *et al.* 2009). Inflammation caused by microorganisms is associated with enhanced influx of neutrophils and levels of cytokines IL-1b and IL-6 in paranasal sinus tissue as a result of response to presence of bacteria or viruses (Van Wetering *et al.* 2005). Active inflammation in nasal tissues is linked with changes in nitric oxide concentration as well. There is an evidence of increased nasal nitric oxide concentration in exspired air in various inflammatory diseases of upper respiratory tract (Antosova *et al.* 2017a, Antosova *et al.* 2017b, Kosutova *et al.* 2019).

Human nasal antimicrobial peptides

The action of endotoxin in the respiratory system is complex. In the epithelial cells, it induces the formation of antimicrobial peptides (AMPs) which in addition to antimicrobial properties act as inflammatory mediators (Scott *et al.* 2011). AMPs act synergistically with lysozyme and lactoferrin, but their relationship with surfactant is not known (Bals *et al.* 2000). In addition to respiratory epithelium, endotoxin has negative influence also on endothelial cells, e.g. by increasing their permeability (Zhou *et al.* 2017). The two major groups of cationic antimicrobial peptides that are integrated in innate immunity on mucosal surfaces are cathelicidins and defensins (Ganz 2004, De Smet and Contreras 2005).

Defensins, an important class of antimicrobial peptides, are a significant factor of the innate immune response, especially on mucosal surfaces that are sensitive to colonization by potential pathogens (Lee *et al.* 2002, Doss *et al.* 2010, Wilson *et al.* 2013). Human defensins belong to the family of antimicrobial peptides, which can be further divided into two classes, a- and b-defensins, characterized by different chemical structure. Defensins are small (29-40 aminoacids) cationic peptides involving six cysteine residues linked by three disulfide bonds (Ooi *et al.* 2008). A-defensins are found in neutrophils (human neutrophil peptides, HNP 1-4) (Gudmundsson and Agerberth 1999), Paneth cells of intestine (HD-5 and -6) (Cunliffe and Mahida 2004), and nasal epithelial cells (Frye *et al.* 2000, Schneider *et al.* 2005). Cathelicidins are generated as prepropeptides, characterized by a highly specific signal peptide (29-30 aminoacids), an N-terminal sequence called cathelin (100 aminoacids) and a very heterogeneous C-terminal domain (10-40 aminoacids). The first and only known human cathelicidin hCAP18 (human cationic antimicrobial peptide, 18 kDa) or CAMP was originally identified in specific granules of neutrophils (Doss *et al.* 2010).

Other antimicrobial peptides as lactoferrin, lysozyme, and secretory leukoprotease inhibitor (SLPI) have been proven in nasal secretions and mucosa of paranasal sinuses (Cole *et al.* 2002). Lysozyme and lactoferrin are bactericidal peptides present in neutrophil granules and epithelial cells (Hager *et al.* 2010). Lysozyme is a 14-kDa large enzyme breaking the beta 134 glycosidic bond between N-acetylglucosamine and N-acetylmuramic acid residues, which together form peptidoglycan, a key component of the bacterial cell membrane, leading to cell lysis. SLPI is a 12-kDa large non-glycosylated protein with only mild antimicrobial and antifungal activity *in vitro* (Tomee *et al.* 1998). Lysozyme content corresponds to 15-30 % of all proteins normally found in nasal secretions. It effectively prevents mucosal infections of most airborne bacteria. Lysozyme is created and excreted by the serous cells of submucosal glands (Wine and Joo 2004).

Lactoferrin is an 80-kDa large protein, which is greatly represented in specific human neutrophil granules. Lactoferrin has a bactericidal and bacteriostatic ability to bind iron, which is necessary for bacterial growth. In addition, lactoferrin assists the adhesion of polymorphonuclear leukocyte (PMN) to endothelial cells and retention of PMN at inflammation spot. Lactoferrin represents about 2 % to 4 % of nasal secretory proteins (Van Wetering *et al.* 2005). Lactoferrin and lysozyme concentrations are highly elevated in nasal lavage in patients with acute or chronic sinusitis (Jeney *et al.* 1990).

Surfactant proteins

Surfactant, a complex of lipids and proteins, is an important component of innate immune system (Calkovska *et al.* 2005, Curstedt *et al.* 2013). It is composed of phospholipids (90 %) and proteins (10 %). There are four main types of surfactant proteins (SPs): SP-A, SP-B, SP-C, and SP-D (Calkovska *et al.* 2008). SP-A and SP-D belong to the collagenous, carbohydrate-binding protein family, which participate in innate immunity. They are also referred to as collectins, because they have collagenous and lectin-binding parts

(Topercerova *et al.* 2019). They participate in immune reactions against wide range of bacteria, fungi, and viruses (Kingma and Whitsett 2006, Woodworth *et al.* 2006, Ooi *et al.* 2007, Kolomaznik *et al.* 2017). In nasal mucosa, SP-A and SP-D are localized in ciliated cells of the surface epithelium and serous acini of the submucosal glands. Stronger expression of surfactant proteins in submucosal glands was observed in patients with chronic rhinosinusitis with and without nasal polyps in comparison with healthy individuals (Uhliarova *et al.* 2016). Moreover, different expression of these proteins in patients with chronic rhinosinusitis in comparison with healthy individuals indicates possible novel target of therapy in these patients and may also have other clinical application. Since antibiotic resistance is significantly increased in the group of people with chronic rhinosinusitis, topical drugs are more and more crucial in optimal, curative treatment. Promising results in the treatment of chronic rhinosinusitis were obtained with surfactants and humanized anti-IL-5 monoclonal antibody applied intranasally (Adappa *et al.* 2012).

Mechanisms of acquired immunity

Group of researchers have succeeded to identify antigen presenting Langerhans cells in normal epithelium and in lamina propria of mucosa. The nasal mucosa is supposed to have a high potential for antigen recognition and for initiation of immune reaction against foreign macromolecules or non-self material inhaled to the nose (Fokkens *et al.* 1989).

There are very few mast cells and eosinophils in the surface epithelium of nasal mucosa, while lymphocytes are more abundant, especially T cells. In the lamina propria mast cells occur more often. However, there is also more lymphocytes and they occur in a T: B cell ratio of 3: 1 and a T-helper cells (CD4) to a T-effector cells (CD8 ratio) 2-3: 1 (Hammad and Lambrecht 2011).

Secretions of nasal mucosa and their proteins originate from epithelial cells (including goblet cells), submucosal glands (involving serous and mucinous cells), blood vessels, and resident secretory cells (involving plasma cells, mast cells, lymphocytes and fibroblasts). Secretions of these cells is composed of a mixture of mucosal glycoproteins, glandular products and plasma proteins (Van Wetering *et al.* 2005). Baseline, resting secretion involves the following main proteins: albumin (about 15 % of all proteins), IgG (2 to 4 %), secretory IgA (SIgA, 15 %), lactoferrin (2 to 4 %), lysozyme (15 to 30 %), non-secretory IgA (about 1 %), IgM (<1 %) secretory leucoprotease inhibitor (low %) and mucosal glycoproteins (about 10 to 15 %) (Raphael *et al.* 1991).

Immunoglobulins

Immunoglobulins are the main specific secreted mediators of host's defense. IgA and IgG are the basic immunoglobulins in nasal secretion, and they seem to act differently. IgG is a plasma protein, that penetrates into the nasal mucosa through capillary wall. IgG is diffusely distributed in the mucosa, but is mainly located near the basement membrane. By analyzing Ig-producing plasma cells, it was found, that approximately 25 % of the plasma cells in the nasal mucosa produce IgG while the rest of the production is IgA (Cerutti *et al.* 2011). SIgA binds microorganisms in the airway lumen and in this form it prevents adherence of these potential pathogens to the mucosa. In contrast, IgGs operate primarily in mucosal tissue itself and prevent the invasion of microorganisms that have already reached epithelium, into deeper layers. IgG levels in secretions may raise up to 125-times by increasing vascular permeability. Nonspecific antimicrobial features of mucus and the presence of normal amounts of IgG in the nasal secretion are capable to sufficiently compensate the function of SIgA, because the majority of patients with selective IgA deficiency do not have disease symptoms and the incidence of nasal infections in these patients does not vary from standard population. In contrast, subjects who have total IgG deficiency or deficiency of some IgG subclasses usually require medical care due to recurrent respiratory infections. Protective functions provided by IgG antibodies are believed to be more important in the prevention of respiratory diseases than SIgA (Kaetzel 2005, Cerutti *et al.* 2011).

NALT

In immunology of mucosa tissues, nasal-associated lymphoid tissue (NALT) represents one of the components of the nasal immune system, the target tissue in local defense strategies, and at the same time a site possibly useful for vaccination induction (Casteleyn *et al.* 2010). In rodents, NALT was solely described as paired lymphatic aggregations found only in choanae (Kuper *et al.* 1990, Kuper *et al.* 1992, Pabst 2015). Lymphatic structures have not been proven in the nose of humans

yet, but functionally equivalent tissue is the Waldeyer's lymphatic ring, which represents an accumulation of oropharyngeal and nasopharyngeal lymphoid structures. The presence of NALT in addition to the structures of the Waldeyer‘s ring in children could present accessory lymphoid tissues. The question remains whether the presence of NALT and its size is equivalent with the size of the tonsils in this age group, or if NALT represents a compensatory lymphoid structure, when the structures of the Waldeyer’s ring are small (Brandtzaeg 2011).

Unlike rodents, human NALT is scattered in the nasal mucosa with specific morphological signs in 38 % of all children, especially in the middle turbinate, with analogous morphology and frequency in the various studied groups (Debertin *et al.* 2003). NALT has been proven to be a highly significant inductive site for activation of immunocompetent cells. NALT induces antigen-specific immune responses in the upper airways and other effector tissues of mucosa (Kurono *et al.* 1999, Shimoda *et al.* 2001, Zuercher *et al.* 2002, Zaman *et al.* 2010).

Because of this finding, NALT might be an anatomical essence for inhalative vaccination strategies in children and have a role in mucosal immune responses (Brandtzaeg 2011).

NALT and vaccination

There is now a great interest in the development of mucosal vaccines against various microbial pathogens. A promising form of immunomodulation for the treatment of different autoimmune diseases and allergies appears to be mucosal-induced tolerance (Holmgren and Czerkinsky 2005, Neyt *et al.* 2012, Kaliner *et al.* 2009).

Mucosal surfaces are constantly in contact with the outer environment. That is why they present the largest lymphoid organ of human body. Mucosal surfaces are responsible for the interactions between host and microbe that influence the formation and maturation of mucosa-associated lymphoid tissues and thereby facilitate the induction and regulation of innate and acquired mucosal immune responses. Because most dangerous pathogens infiltrate the body via mucosal surfaces by inhalation, ingestion or sexual contact, mucosa represents a great possible area for vaccination. Mucosal vaccination has many physiological and practical advantages, such as painlessness, reduced costs and lower risk of bloodborne diseases transmission (Lamichhane *et al.* 2014).

In last years, studies of nasal vaccination have shown highly efficient immune responses without explaining the specific mechanism (Mowat 2003, Lamichhane *et al.* 2014).

Immunization by the nasal route is an interesting opportunity that has been increasingly explored. Nasal or tonsillar immunization in man lead in an antibody reaction in the mucosa of upper airways and local secretions (saliva, nasal secretions) without inducing an immune reaction in the intestines (Johansson *et al.* 2004, Kang *et al.* 2009). With special interest for possible vaccination against HIV and other sexually transmitted diseases, it has been found, that not only vaginal but also nasal immunization leads to significant IgA and IgG responses cervical and vaginal mucosa in humans (Kozlowski *et al.* 1997).

NALT disposes all the immune cells in the body, which facilitate the induction of immune reactions of the mucosa against inhaled antigens. Furthermore, intranasal vaccination is also absorbing for its effective evoking of antigen-specific immune reactions in mucosal and systemic sections of human body as well (Kang *et al.* 2009, Zaman *et al.* 2010).

Despite the multiple well-known benefits of nasal vaccination, there is a presence of important limitations as well. One of the major limitations of intranasal immunization is the speedy clearance of the vaccine substance from nasal mucosa by means of mucociliary apparatus. The need of creating of a highly immunogenic and sufficient vaccine is in vaccinology the primary challenge. In parenteral vaccination, the application of macromolecular carriers as adjuvants seems to be the most promising route (Muzikova and Laga 2016). The use of mucoadhesive adjuvants to enhance the active time of vaccines on the nasal mucosal surface can be also helpful in nasal vaccination and increase their effectiveness. Proteolytic activity of the mucosal enzymes presents another limitation which, consecutively, limits the nasally delivered vaccines (Kang *et al.* 2009).

To induce defensive immune reactions on mucosal, the delivery pathway and adjuvants for vaccination are essential. However, the host immune system tries to maintain homeostasis by tolerating of mucosal antigens, instead of immune activation in the response to their presence. Therefore, activation of nasal immunity by means of vaccination is a challenge. By using of general mucosal immunity, targeting on microfold epithelial cells and nasal dendritic cells could help the activation of effective mucosal immunity. Novel

methods of immunization and antigen delivery schemes also indicate good potential for the development of efficient and harmless mucosal vaccines against different pathogens (Fukuyama *et al.* 2012).

Conclusion

Nasal immunization has a longer history then we may think. The first described experiment to vaccinate humans nasally against smallpox was noticed in the Golden Mirror of Medicine, in Chinese medical text in 1742. Nasal vaccination was performed with the aid of powdered scabs, that were blown into the nose or stuffing the nasal cavity with a smallpox vesicle smeared cotton (Plotkin 2014).

Mucosal vaccination is a non-invasive, medical waste-free and practical vaccine strategy that offers preventive immunity against various pathogens in mucosal and systemic compartments. Unfortunately, the idea of mucosal vaccines struggles with two major challenges: efficiency and safety. Both of them are fairly difficult problems to solve compared with systemic vaccine research thanks to the unique mucosal environment. Global warming may represent various unexpected pathogens, such as the Plasmodia parasites causing malaria, into new locations, where they never before caused pandemic infectious diseases. This knowledge supports the development of newer mucosal vaccines. According to recent facts about mucosal vaccines, a fitting combination of different mucosal vaccine schemes may enable the development of applicable vaccines in the next few years.

Conflict of Interest

There is no conflict of interest.

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