IntraNasal BRSV and PI3 vaccination:
Innovation for the prevention of respiratory disease in cattle

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Introduction

It is well established that the immune system that operates at mucosal surfaces does so largely independently of that which is found systemically. Moreover the strategies for defence at mucosal sites differ and there is clear evidence that protective responses at mucosal surfaces are most effectively stimulated by local application of antigen. Despite this it remains surprisingly difficult to stimulate a protective effect at these sites and the aim of this review is to briefly outline mechanisms whereby the immune system may provide protection in the respiratory tract, to highlight the difficulties in generating such responses and to illustrate how these may be overcome by the development of novel delivery systems.

Mucosal Associated Lymphoid Tissue (MALT)

In order to understand the mechanisms responsible for providing protection in the respiratory tract, and how these responses may be stimulated by immunisation it is necessary first to consider the lymphoid architecture present at different levels of the tract. Organised mucosa-associated lymphoid tissue (MALT) is widely distributed in mucosal tissues and is the initial inductive site for mucosal immunity. MALT is located at strategic sites in order to most efficiently sample antigens presented at mucosal surfaces. Those of particular importance to the respiratory tract are the nose or nasopharynx-associate lymphoid tissue (NALT), the lymphoid tissues of Waldeyer’s ring, the larynx-associated lymphoid tissue (LTALT), and the bronchus-associated lymphoid tissue (BALT).

Figure 1


Whereas in small rodents, NALT is present as paired lymphoid aggregates in the floor of the nasal cavity in the entrance to the pharyngeal duct4 no comparable aggregates are found at this site in farm species. However single isolated lymphoid nodules have been described in horses and sheep7,8,11 and it is likely that similar structures will be found in other farm species. The lymphoid tissues of Waldeyer’s ring are well developed in farm species, and guard the nasal, oral and auditory passages into the pharynx. They are formed by large aggregates of lymphoid nodules (tonsils) and are the main targets for nasal vaccines.
Immune Responses in the Respiratory Tract

Defence mechanisms that operate in the respiratory tract are multi-faceted involving both non-specific (innate) and specific acquired elements. Whereas historically most studies of non-specific defence mechanisms focussed upon the anatomical properties of the tract that led to deposition of particles of particular sizes to defined areas, subsequent and more recent studies have identified the importance of soluble factors and receptor mediated processes. Of the latter much attention is now directed towards Toll-like receptors (TLR’s). These are a highly conserved group of proteins that are found in such diverse species as insects and cattle. The TLR family contains greater than ten members and they are pivotally involved in distinguishing between host and foreign antigens. Recognition of foreign molecules is based on the detection of molecular patterns that are indicative of entities likely to cause harm. These have been termed pathogen associated molecular patterns (PAMP’s) and include bacterial LPS, flagellin and different nucleic acids. To date there have been relatively few studies in the bovine respiratory tract but it has been recently reported that BHV-1 infection results in increased expression of TLR2 and TLR4. The significance of this in the induction of mucosal responses has yet to be determined.

Immunoglobulins, G, A, M and E have all been detected in respiratory tract secretions, but quantitatively IgA and IgG are the most important. In contrast to most other species the bovine is relatively deficient of IgA, the only exception being the respiratory secretions of adult animals. However in young calves IgG1 greatly predominates in respiratory tract secretions. The predominance of IgG1 in respiratory secretion is shown in Figure 3. By 6 weeks of age the IgG1:IgA ratio has fallen but this still differs markedly from the ratio of 13:1 found in adult animals.

Figure 2
(A) Section of tonsil from a 6 month old pig labelled with antibodies to CD3 (red), CD45RC (green), and MHC class II (blue), magnification x20; (B) Section of tonsil from a 6 month old pig labelled with antibodies to CD8 (red) and CD4 (green), magnification x20.

Lymphoid tissue that is associated with the larynx has been described on the epiglottis in the vestibulum laryngis and on the plica aryepiglottica (LTALT) of cattle, but studies relating to the effect of age upon development have yet to be reported. In contrast is has been shown that the development of BALT is antigen dependent and that it may be severely enlarged following respiratory infection. At birth BALT can not be detected but increasing numbers of lymphoid nodules and aggregates appear from 4 to 18 months of age.

By convention only lymphoid tissues in the mucosa that fulfil certain morphological and functional properties are called MALT. Cells of the immune system are however not restricted to these areas in the mucosa and may in total number be more numerous than those within MALT. Whereas MALT is located strategically at sites to allow efficient sampling of antigens presented at mucosal surfaces and are primarily involved in the inductive phase of the immune response, cells responsible for effector function are found outside MALT in the lamina propria or lumen of the respiratory tract. MALT consists of organised lymphoid tissue with lymphoid follicles and T cell dependant interfollicular areas. The lymphoid follicles are predominately made up of B lymphocytes embedded in a network of follicular dendritic cells, and smaller T cells. In the conventional model, these organised structures are the inductive sites populated by naive cells, which following priming migrate via the lymphatics before homing back to the respiratory tract to populate “non-MALT” areas, and where they differentiate into effector cells.

Figure 3
Ratio of IgG1:IgA in nasal and tracheal washings of calves aged from 1-6 weeks.

Figure 4
Ratio of IgG1:IgA in respiratory tract secretions of calves.
The relative levels of IgA, IgG & IgM differ according to the level of the respiratory tract. Studies in horse have shown that relative to albumin there is significantly more IgA and IgM, and significantly less IgG in the nasal cavity than in the trachea. IgA and IgM levels were greatest in the nasal cavity and decreased progressively to the bronchi, whereas IgG levels showed the reverse trend. Results such as these have led to the following conclusions: that there is significant local production of immunoglobulins into the respiratory tract, that the proportion of immunoglobulin derived from local production is greatest in the nose and least in the lung, that whilst the levels of IgA are greatest in the nose and least in the lung the reverse is true for IgG.

**Figure 4**

*Immunoglobulin (IgA blue; IgG red; IgM green) to albumin ratio in horse respiratory tract secretions and serum. Respiratory tract secretions were collected by nasal, tracheal and bronchial lavage.*

The changes in profile of immunoglobulins at different levels of the respiratory tract reflect the level of microbial challenge and the strategy the immune system has evolved to provide protection. Within the nose there is a resident microflora and IgA acts to prevent potentially invading bacteria & viruses from binding to the epithelial cells and so gaining entry. The process does not involve inflammation and is not associated with an impairment of barrier function. In contrast the lumen of the lung is relatively sterile and any invading micro-organisms are actively eliminated by IgG mediated by inflammatory processes that involve the activation of complement and the recruitment of neutrophils. These mechanisms are associated with a transient impairment of barrier and respiratory function. Neutrophils play an important role in clearing infection in the lung and the process of emigration from capillaries and post-capillary venules to the alveoli and bronchi involves a number of cellular adhesion molecules. Recently it has been shown that the level of expression of these is enhanced during episodes of infection.

Interferons (IFNo/ß) play a key role in the innate control of virus replication. For example following bovine respiratory syncytial virus (BRSV) infection there is increased production of IFNo/ß from both nasal fibroblasts and alveolar macrophages. IFNo/ß can significantly enhance immune responses and viruses that inhibit their production may inhibit control by the adaptive immune response. Following infection BRSV replicates in the luminal part of epithelial cells and any virus excreted into the lumen of the respiratory tract is cleared by neutrophils. Virally infected epithelial cells are induced to undergo apoptosis and are subsequently phagocytosed. The number of both CD4+ and CD8+ T cells is increased during infection but their precise role has not been determined.

**Towards vaccination**

The primary portal of entry of many viral pathogens of cattle is at the respiratory mucosal surface. Numerous studies have demonstrated that immunisation via the mucosal route not only is most effective at providing a barrier against infection, it also generates a significant systemic response. Given the relative ease of antigen delivery and the organisation of the local lymphoid tissue (MALT), intranasal immunisation offers attractive possibilities. However to successfully achieve this there are a number of obstacles that have to be overcome.

Intranasal immunisation with a number of peptide antigens has been shown to result in the induction of tolerance rather than active immunity. Mucosal tolerance is a well established process that has been most widely studied with respect to fed antigens. It can be defined as a specific acquired response whereby prior mucosal presentation (eg feeding) can reduce or prevent the subsequent response to further challenge. Whilst it is a beneficial process with respect to the prevention of damaging allergic reaction to food and other harmless environmental antigens, it is clearly highly undesirable with respect to stimulating protective responses following vaccination against potential pathogens. Many factors can influence the induction of tolerance, but most importantly in the present context it can be prevented by the use of replicating antigens, antigens that target TLR’s and certain adjuvants.

The use of mucosal vaccines in domestic species has an impressively long history. For example a live cold-adapted feline herpesvirus type 1 (FHV-1) intranasal vaccine that mimics the “natural method of infection” was first described in 1976. The vaccine provided a rapid onset of protection with partial protection from challenge after two days and complete protection by day 4. Despite these early successes the development of novel mucosal vaccines that are safe, effective and cost effective remains a major challenge.
A wide range of different strategies have been evaluated and to date around twenty vaccines have been licensed for mucosal delivery. Approaches that have been followed can be broadly divided into two types, based on non-replicating or replicating antigens. Considering first the non-replicating group, particulate antigens have been shown to be better oral immunogens than soluble antigens. A number particle based systems have been used including microparticles, liposomes, cocheates and ISCOMS. An alternative approach to stimulate the response to non-replicating antigens is through the use of mucosal adjuvants. Of these the most widely used are based on cholera toxin and the closely related heat labile enterotoxin of *E.coli*.

Live multivalent vaccines using attenuated, recombinant viral or bacterial species have several potential advantages over killed or subunit vaccines. These include immunogenicity, antigen persistence and cost. Attenuated viruses have been isolated and cloned by a variety of methods, including: isolation of naturally occurring strains; sequential passage in cell culture; mutagenesis; and the creation of organisms with specific genetically defined mutations. A number of live attenuated viral vaccines have been in use for several years and in humans they are directed towards the control of poliovirus, adenovirus, varicella virus and vaccinia virus. Clearly for a number of these there is a long history of continued use and safety, but the challenge that remains is ensuring a proper balance between attenuation and immunogenicity.

**References**


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