ISSN 1466-2523

Animal Health Research Reviews **5**(2); 209–217
DOI: 10.1079/AHR200470

Advances in mucosal vaccination

Els N. T. Meeusen,[1]* Jean-Pierre Y. Scheerlinck,[1] Sean Wattegedera[2] and Gary Entrican[2]

[1]Centre for Animal Biotechnology, School of Veterinary Science, The University of Melbourne, Australia and [2]Moredun Research Institute, Pentlands Science Park, Edinburgh, UK

Abstract

Pathogens that enter the body via mucosal surfaces face unique defense mechanisms that combine the innate barrier provided by the mucus layer with an adaptive response typified by the production and transepithelial secretion of pathogen-specific IgA. Both the measurement and induction of mucosal responses pose significant challenges for experimental and practical application and may need to be adapted to the species under study. In particular, for livestock, immunization procedures developed in small rodent models are not always effective in large animals or compatible with management practices. This paper reviews the latest advances in our understanding of the processes that lead to secretory IgA responses and how this relates to the development of mucosal immunization procedures and adjuvants for veterinary vaccines. In addition, it highlights the complex interactions that can take place between the pathogen and the host's immune response, with specific reference to *Chlamydia/Chlamydophila* infections in sheep.

Keywords: vaccination; mucus; mucosal immunity; *Chlamydia*; *Chlamydophila*; dendritic cells

Introduction

Mucosal immunity refers literally to the immunity induced at the mucus-covered epithelial surfaces typically present in the gastrointestinal, upper respiratory and lower reproductive tracts. The mucus that covers these epithelial surfaces is produced by specialized high-mucus-producing goblet cells interspersed at varying densities within the epithelial layer and by epithelial cells themselves. This mucus layer consists of an extensive network of secreted polymers (mucins) and provides a first and important barrier against the external environment from physical (e.g. sharp objects) and chemical (e.g. stomach acids) injury as well as infectious injury. Mucus exists in a dynamic state, so that particles, including pathogens, can be physically removed by peristaltic movement (gut) or coughing and cilia movements (airways). Pathogens that enter the mucus layer are further confronted by innate defense molecules secreted by epithelial cells, including trefoil peptides (Podolsky, 1999) and antimicrobial peptides (defensins) (Ayabe *et al*., 2000). In addition, the mucus layer of the intestinal tract, which is in continuous contact with a rich broth of commensal bacteria, contains IgA specific for the bacterial cell walls, produced in a primitive, T-cell-independent manner by plasma cells scattered throughout the lamina propria (Macpherson *et al*., 2001).

While the mucus layer forms an important environment in which both innate and adaptive immune effector mechanisms take place, not all tissues that form part of the mucosal immune system secrete or are covered by mucus. 'Mucosal', non-mucus producing tissues include the epithelium of the mammary gland and upper reproductive and the lower respiratory tract. The absence of mucus in these tissue compartments probably reflects a less frequent contact with the external environment, combined with functional necessities (e.g. gas exchange) and energy preservation. The characteristic feature of mucosal immunity is therefore not necessarily the presence of mucus-covered epithelium but may be more accurately defined by the presence of an epithelial layer that has the property of actively promoting the secretion of immune defense molecules, in particular IgA, into the luminal environment.

*Corresponding author: Centre for Animal Biotechnology, School of Veterinary Science, The University of Melbourne, Victoria 3010, Australia

Despite these defense mechanisms, many pathogens still cause disease by colonizing mucosal surfaces and/or disseminating from the initial site of infection and invading other tissues and organs. A clear understanding of immune regulation at mucosal surfaces, coupled with knowledge of the protective mechanisms to a particular pathogen, underlies rational vaccine design for disease control. Here we discuss advances in mucosal vaccination and highlight some of the problems that have hindered the development of safe and effective vaccines to mucosal pathogens, using *Chlamydia/Chlamydophila* infections of sheep as a specific example.

Properties and function of secretory IgA

IgA is the most prominent antibody present at mucosal surfaces and plays a major role in protection against invading pathogens. In contrast to monomeric IgA produced in peripheral tissues, plasma cells in the lamina propria of mucosal surfaces produce IgA in the form of two or more (polymeric) IgA units linked together by an additional polypeptide, the J chain. This J chain is critical in targeting the polymeric IgA to the polymeric IgA receptor (pIgR) expressed on the basolateral surface of epithelial cells, its subsequent transport through the epithelial cells and its secretion at the luminal surface. Before secretion, the pIgR is enzymatically cleaved and the remaining fraction bound to the IgA-J chain complex is called the secretory component. IgA secreted into the lumen (secretory IgA or SIgA) therefore consists of a complex of dimeric or polymeric IgA molecules linked with two additional polypeptides: a J chain and an epithelial-derived secretory component. This configuration of the antibody confers additional properties on the complex, including protease resistance (Crottet and Corthesy, 1998), anchoring antibody to mucus (Phalipon and Corthesy, 2003) and activating effector cells such as eosinophils (Lamkhioued *et al*., 1995). Expression of J chain by B cells and pIgR by epithelial cells is therefore a distinguishing characteristic of 'mucosal' or 'secretory' immunity. Polymeric Ig receptor expression has also been observed in the epithelium of the upper reproductive tract (Kutteh *et al*., 1988) and mammary glands (Rincheval-Arnold *et al*., 2002) and is therefore not linked to mucus production.

As well as protecting mucosal surfaces against colonization and invasion by pathogens, SIgA also functions in eliminating antigens from tissues via immune complex formation (Robinson *et al*., 2001) and intraepithelial neutralization of virus replication (Fujioka *et al*., 1998). In addition, as IgA is a non-inflammatory antibody that binds complement only weakly, it protects the tissues from excessive immune-mediated damage.

Transforming growth factor-β (TGF-β) produced by non-bone marrow-derived stromal cells in the lamina propria is abundantly expressed in mucosal tissues and is critical for switching the antibody isotype to IgA through TGF-βRII expressed by B cells. Except for the T-cell independent, early switch to IgA by locally generated lamina propria B cells, the T-cell dependent differentiation of IgM^+ to IgA^+ B cells that takes place in Peyer's patches and other organized lymphoid tissues also requires Th2 cytokines and cellular signals (Fagarasan and Honjo, 2003). The final differentiation of IgA^+ B cells to IgA producing plasma cells takes place once they migrate back into mucosal tissues, under the influence of locally produced factors including interleukin (IL)-6.

Mechanisms and sites of induction of mucosal immune responses

It has been a long-standing paradigm of mucosal immunology that mucosal immune responses are generated in restricted, specialized areas of the mucosal tissues. In the intestinal tract, the most studied mucosal compartment, the inductive sites for mucosal immunity were thought to reside solely in the Peyer's patches and many vaccination studies aim to specifically target these structures. Peyer's patches are distinctive lymphoid aggregates in the intestinal tract with defined B- and T-cell areas similar to lymph nodes. The epithelial layer overlying the Peyer's patches is distinct from the surrounding mucosal epithelium in that the enterocytes produce less mucus and digestive enzymes and are interspersed by microfold or membranous cells (M cells) that lack a brush border glycocalyx or microvilli. M cells display increased pinocytic activity and can transport antigen and microbes, via a transepithelial vesicular transport system, to underlying antigen-presenting cells (APC). Lymphocytes primed in the Peyer's patches migrate to the mesenteric lymph nodes, where they undergo further proliferation and differentiation before exiting via the blood to the thoracic duct and extravasating into the mucosa. IgA^+ B cells and $CD4^+$ T cells stimulated at mucosal sites such as the Peyer's patches preferentially migrate back to mucosal tissue and less effectively to systemic sites, where they can respond rapidly to the next antigenic challenge (Meeusen *et al*., 1996; Reinhardt *et al*., 2001; McSorley *et al*., 2002). Peyer's patch-like structures have also been identified in the other epithelial tissues of many species, including the rectal and nasal tissues of sheep (Stanley *et al*., 2001; Sedgmen *et al*., 2002)

Microbes are generally more closely associated with the Peyer's patch epithelium than the surrounding mucosal membranes, and this is usually interpreted to indicate that Peyer's patches are the predominant site of entry for pathogens and the induction of immune responses. However, few M cell-specific receptors have been identified and preferential microbial adhesion to M cells may be more a consequence of their increased

https://doi.org/10.1079/AHR200470 Published online by Cambridge University Press

accessibility through the lack of a protective filamentous brush border glycocalyx. It would seem counterintuitive that the mucosal tissues have specifically created vulnerable spots that attract pathogens so they can then induce a protective immune response. In addition, several epithelial surfaces lack Peyer's patches or Peyer's patch-like structures, including the stomach, lungs and reproductive tract, and most parasites do not specifically target Peyer's patches but can still induce vigorous mucosal immune responses. It may therefore be that Peyer's patches have evolved primarily to sample the intestinal microflora and induce tolerance to continuous stimulation by non-pathogenic organisms. This may also be the case for the isolated lymphoid follicles (ILF) that have similar structure (including M cells) and function to Peyer's patches and are induced after birth in parallel with colonization of the gut (Mowat, 2003). If infection can occur at any mucosal tissue site, how can mucosal immunity, commonly induced by antigen-presenting dendritic cells, be initiated? It has often been observed that dendritic-like cells are located beneath the epithelium of mucosal tissues, and if the pathogen breaks the epithelial barrier it can be assumed that antigens released by the pathogen in the tissue can be taken up by these subepithelial dendritic cells and transported via the afferent lymph to the draining lymph nodes for initiation of an immune response. Very recently, it has been shown that dendritic cells present throughout the mucosa can play a more active role by extending cellular processes through epithelial tight junctions into the lumen, take up bacteria and retreat back into the tissues (Rescigno *et al*., 2001). Both pathogenic and non-pathogenic organisms can be sampled in this way, but only pathogens seem to induce the migration of dendritic cells to the mucosal lymph nodes (MLN), while dendritic cells that take up non-pathogens remain in the lamina propria. This differential activation of dendritic cells may be controlled by signaling through innate response receptors, such as toll-like receptors (TLRs). Within the lymph nodes, mucosal dendritic cells can present antigen to naive T cells and thereby govern immunity as well as tolerance (Nagler-Anderson, 2001). A schematic representation of the different antigen presentation pathways is shown in Figure 1.

Mucosal adjuvants and other factors affecting the generation of a secretory immune response

The mucosal tissues constitute an environment conducive to the induction of tolerance, IgA and anti-inflammatory Th2-type responses. This Th2-biased microenvironment can be seen in (i) the abundant production of TGF-β and IL-10 by epithelial and mesenchymal cells (Mowat, 2003), (ii) a cytokine profile dominated by IL-4 and IL-10 in mucosal but not peripheral lymph nodes (Premier and Meeusen, 1998) and (iii) the presence in most mucosal tissues of distinct subsets of dendritic cells that produce IL-10 or TGF-β instead of IL-12 (Nagler-Anderson, 2001).

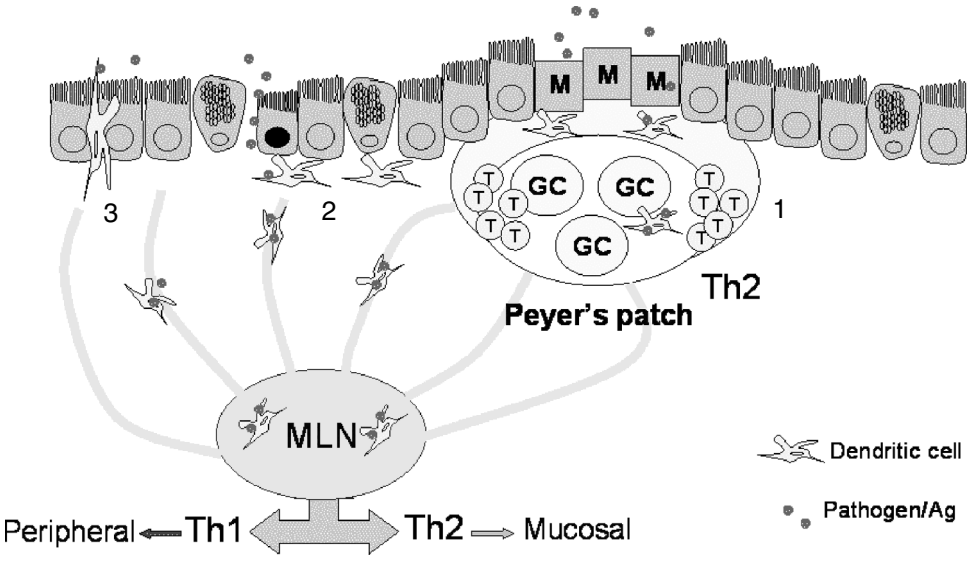

Fig. 1. Pathways for induction of immune responses at mucosal surfaces. The way pathogens or antigens (Ag) come in contact with antigen presenting cells influences the type of immune response that is induced and includes the following steps. (1) Uptake of antigen by M cells in Peyer's patches/isolated lymphoid follicles and transfer to underlying dendritic cells (DC). DC can initiate an immune response in the Peyer's patches, with a biased Th2-type phenotype, or migrate to draining mucosal lymph nodes (MLN), where both Th1 and Th2 responses can be generated. (2) Damage of the epithelial layer and uptake of antigen by underlying DC, which migrate to the MLN. (3) Sampling of luminal antigen by lamina propria DC that extend dendrites through the epithelial junctions and migrate to MLN. GC = germinal center; T = T cell.

https://doi.org/10.1079/AHR200470 Published online by Cambridge University Press

Therefore, introducing antigen into a mucosal site will generally default to a Th2-type response. For example, *Leishmania major* is normally a strong Th1-inducing [high interferon (IFN)-γ] pathogen after subcutaneous delivery in C57BL/6 mice, but induces a Th2 response in the lungs after intranasal delivery (Constant *et al*., 2002). The main property of a 'mucosal adjuvant' may therefore be to direct the antigen for uptake by mucosal tissues and/or to deliver appropriate stimulatory signals to activate APCs. The bacterial products cholera toxin and heat-labile enterotoxin provide both these properties, the B subunits facilitating adherence to epithelial cells and the enzymatically active A subunits increasing antigen presentation through its cyclic AMP-inducing ability. Other bacterial products have recently also been shown to have adjuvant properties through their ability to act as danger signals or pathogen-associated molecular patterns for recognition by innate response receptors (e.g. TLRs) on epithelial cells or APCs. These include bacterial DNA or synthetic oligodeoxynucleotides containing unmethylated cytosine-phosphate-guanosine (CpG) motifs, which bind to TLR-9 to activate APCs and, when delivered at mucosal surfaces, can induce strong mucosal responses (Holmgren *et al*., 2003). In addition, the proinflammatory cytokine IL-1, previously used as an adjuvant for peripheral immunization, has also been shown to be particularly effective for mucosal vaccination when delivered at mucosal surfaces (Staat and Ennis, 1999). In the absence of these molecular stimuli, antigen delivered at mucosal tissues may result in specific systemic and/or local tolerance.

The immune response generated by delivery of antigen via the mucosal route can result in different outcomes depending on the site of priming (Fig. 1). Generation of the immune response in mucosal tissues, including Peyer's patches and ILFs, generally results in typical SIgA responses with little or no systemic response, while initiating of immune responses in the mucosal lymph nodes can induce both mucosal immune responses and systemic immunity or tolerance, depending on the activating signals provided with the antigen.

Mucosal pathogens that elicit inflammatory responses

The balance of inflammatory and regulatory cytokine production at mucosal surfaces is important not only for the generation of protective immune responses, but also to avoid immunopathology. The predominance of anti-inflammatory, Th2-type cytokines may be one of the reasons that *Chlamydia/Chlamydophila* are successful mucosal pathogens. *Chlamydia/Chlamydophila* are obligate intracellular Gram-negative bacteria that cause a wide variety of diseases in a diverse group of host species (Longbottom and Coulter, 2003). The developmental cycle of the organism is biphasic, consisting of the extracellular, infectious, metabolically inactive elementary body and the intracellular, non-infectious, multiplying reticulate body. The reticulate bodies exist within a specialized intracellular compartment, termed the inclusion body, that actively inhibits fusion with host cell lysosomes.

Chlamydia/Chlamydophila infect their hosts via mucosal surfaces, causing disease in the respiratory, gastrointestinal and reproductive tracts, as well as the eye. In some cases the infection remains limited to epithelial cells; in others the organism disseminates to cause disease at distal anatomical sites. There are two striking features common to these pathogens and the diseases they cause. The first is that persistent infection and/or reinfection is common, suggesting that the host's immune response is potent enough or sufficiently long-lasting to provide sterile immunity. The second is that persistent chlamydial infections are associated with inflammation that leads to tissue damage and pathology (Stephens, 2003). These factors have impeded the design and implementation of vaccines to control *Chlamydia/Chlamydophila* infections in humans. There are two commercially available vaccines for the control of chlamydial abortion in sheep. Both of these vaccines involve immunization with whole organisms. One consists of a live attenuated temperature-sensitive strain developed by chemical mutagenesis; the other comprises inactivated organisms delivered in adjuvant (Entrican *et al*., 2001; Longbottom, 2003). The vaccines are not administered mucosally and little is known about the means by which they elicit protection. Such an empirical approach does not easily lend itself to modification should the vaccine fail. Additionally, the live vaccine poses a risk of zoonotic infection since the temperature mutation permits the attenuated strain to grow normally at 37°C (but not 39°C, hence its application in sheep). The use of whole organisms as chlamydial vaccines has been abandoned in human medicine because of concerns over immunopathology and enhanced tissue damage during infection that followed vaccination (Longbottom and Coulter, 2003). A subunit vaccine is a desirable objective for both human and veterinary application, but to achieve this requires an understanding of protective host immunity and host–pathogen interactions during acute and persistent infection.

Host immune control of *Chlamydia/Chlamydophila* can be mediated through IFN-γ (Entrican *et al*., 1998). The effects of recombinant ovine IFN-γ on the growth of *C. abortus* in the ovine ST-6 cell line can be seen in Figure 2. Restriction of chlamydial growth is a result of IFN-γ-induced expression of indoleamine-2,3 dioxygenase (IDO), an enzyme that degrades tryptophan (Entrican *et al*., 2002). Analysis of the recently-sequenced genome of the S26/3 strain of *Chlamydophila abortus* reveals the absence of a functional *trp* operon, thereby confirming dependence of the organism on host cell tryptophan (D. Longbottom, personal communication). The relationship

https://doi.org/10.1079/AHR200470 Published online by Cambridge University Press

between IFN-γ and control of chlamydial growth is complex, since the cytokine can exert both chlamydiostatic and chlamydiocidal effects (Brown *et al*., 2001). These features are of great interest given the immunopathogenesis of chlamydial abortion. IFN-γ is widely regarded as a cytokine that is detrimental for pregnancy, and tryptophan degradation by placental trophoblast cells is a mechanism by which maternal T cells are tolerized to paternal histocompatibility antigens (Munn *et al*., 1998). There is a paradox that remains unresolved: why is the placenta a site of multiplication for *C. abortus* if Trp is degraded by IDO? It is not yet known if placental IDO expression occurs in sheep, or indeed if such a mechanism for tolerance to paternal antigens is necessary, given the structural differences between the rodent and ruminant placentas (Meeusen *et al*., 2001; Entrican *et al*., 2002). The placenta is a highly specialized organ and the balance between regulatory and proinflammatory cytokines may be altered by *C. abortus* to such an extent that immunopathology and abortion are unavoidable consequences of the host response (Entrican, 2002). The role of IDO in immune regulation has broader implications than tolerance during pregnancy. Expression of IDO by dendritic cells and the ability of IFN-γ to inhibit dendritic cell activation of T cells appear to be important immunoregulatory mechanisms (Grohmann *et al*., 2003). This could impact on the quality of an immune response elicited by vaccines that drive inflammatory cytokine production at mucosal sites.

Although cell-mediated immunity is crucial for resolving an established chlamydial infection, experimental data indicate that antibody can inhibit infection, particularly at mucosal surfaces. Delivery of IgA or IgG monoclonal antibodies directed against the major outer membrane protein (MOMP) partially protect mice against genital *C. trachomatis* infection if the monoclonal antibodies are delivered by injecting the hybridoma cells directly into mice (Cotter *et al*., 1995). The monoclonal antibodies are found in both serum and vaginal secretions, but protection occurs only at low challenge doses of *C. trachomatis*. It may be that serum antibody to MOMP has limited efficacy during infection. This is borne out by the observation that ewes infected with *Chlamydia abortus* during pregnancy seroconvert to MOMP and to the polymorphic outer membrane proteins, but still go on to abort (Fig. 3), demonstrating that antibodies to these molecules cannot protect against abortion once infection is established. This correlates with many studies of chlamydial infection in humans, in which seroconversion has been used for diagnosis, yet reinfections and/or persistent infections are common (Stephens, 2003).

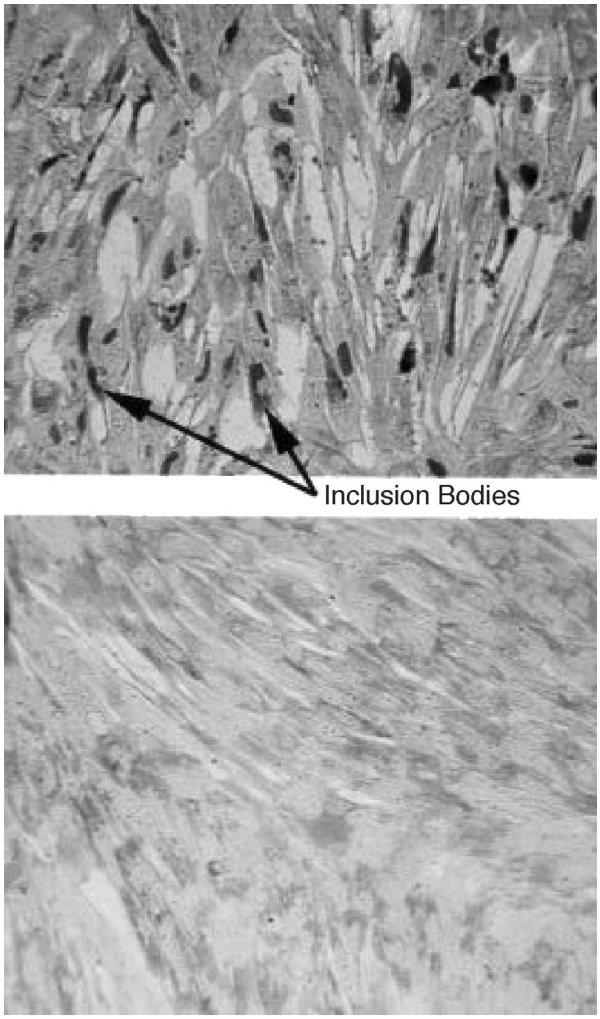

Fig. 2. Inhibition of the growth of *Chlamydophila abortus* by IFN-γ. Ovine ST-6 adenocarcinoma cells were cultured in medium alone (top panel) or medium containing 250U/ml recombinant ovine IFN-γ (bottom panel) for 24 h then infected with *C. abortus* elementary bodies at a multiplicity of infection of 0.5. The cells were fixed in methanol 72 h later and stained with Ziehl-Neelsen's carbol fuchsin to visualize the chlamydial inclusions, and counterstained with hematoxylin.

Strategies for targeting vaccines to mucosal tissue sites

If, as mentioned in the previous section, the microenvironment of mucosal connective and lymphoid tissues is inherently conducive to the generation of a Th2-type response, delivering vaccines to mucosal surfaces should induce an appropriate mucosal response by default. Mucosal targeting can be achieved by incorporating vaccine antigens in bacterial or viral vectors that specifically infect mucosal tissues. Attempts have also been made to overcome safety concerns associated with live vaccines by the use of pseudoviruses or virus-like particles that do not contain any viral DNA/RNA but maintain immunogenicity and their ability to target mucosal tissues (Guerrero *et al*., 2001).

Other non-replicating delivery systems are designed to incorporate soluble vaccine antigen in a particular form and chemical composition that induces adherence and uptake by mucosal surfaces. These include micro-

https://doi.org/10.1079/AHR200470 Published online by Cambridge University Press

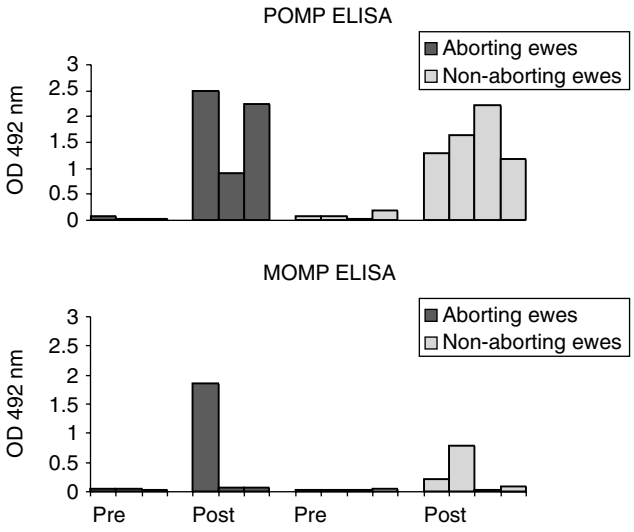

Fig. 3. Antibody to *Chlamydophila abortus* in sheep following experimental infection. Seven pregnant ewes were infected by subcutaneous injection of 2×10^6 inclusion forming units of *C. abortus* on day 70 gestation. Serum was collected 2 weeks before infection (Pre-inf) and 7 weeks after infection (Post-inf) and analysed for the presence of antibodies to the 90-kDa polymorphic outer membrane protein (POMP) and the major outer membrane protein (MOMP). Three of the infected ewes aborted during the final 3 weeks of gestation, and the four remaining ewes produced live lambs.

capsules, liposomes and immunostimulating complexes. While encouraging results have been obtained by oral immunization in mouse models, the different physiology and size in large animals has probably prevented a similar success in large animal species. Intranasal immunization, on the other hand, has been shown to be effective in inducing local and systemic antibody responses (Holmgren *et al*., 2003). Intranasal immunization is often more effective than oral immunization in inducing both mucosal and systemic immune responses and generally requires less antigen and adjuvant.

Intrarectal vaccination strategies have recently been considered in humans, in particular because of the importance for infection and persistence of HIV in this tissue (Berzofsky *et al*., 2001). Rectal immunization may also have possible applications in some veterinary species and mucosal infection systems. As far as we are aware, rectal immunization has so far only been attempted in sheep and cattle. In the sheep studies, depositing antigen on the rectal mucosal surface with cholera toxin was examined, as well as intramucosal injection of antigen with alum adjuvant (Jacobs *et al*., 1999; Premier, 2004). Injection of an experimental nematode antigen into the rectal tissue of sheep was shown to confer similar protection against infection of the abomasum as the antigen deposited on the rectal mucosal surface with cholera toxin (Jacobs *et al*., 1999). The injection of antigen with aluminium hydroxide adjuvant into intestinal tissue, in addition to inducing IgA, also effectively induced higher IgG2 responses compared with systemic or cholera toxin immunization (Premier, 2004). In cattle, DNA suppository-based vaccines prime the immune system for increased IgG and IgA in serum and IgA in nasal secretions (Babiuk *et al*., 2003).

There has been some success with intramuscular and intranasal delivery of plasmid DNA in turkeys using constructs encoding *Chlamydophila psittaci* MOMP (Vanrompay *et al*., 1999). Effectiveness of DNA vaccination for controlling chlamydial infections in ruminants is unknown at present and the two key factors that remain to be addressed are the priming and maintenance of immunological memory. First, it is recognized that although DNA vaccination works well in mice, responses in humans and large animals are poorer and require repeat vaccination and greater amounts of DNA to elicit strong cellular immunity (Scheerlinck, 2001; Kirman and Seder, 2003). Secondly, it is not known how robust infection-generated immune memory is, let alone vaccination-generated memory. Vaccines that induce large numbers of IFN-γ-producing cells are thought to be effective for inducing short-term but not long-term immunity against pathogens (Kirman and Seder, 2003). Thus, strategies for inducing stable protection with a subunit vaccine against *C. abortus* require careful thought.

Inducing mucosal immune responses through peripheral immunization

Several studies have reported the induction of mucosal immune responses by intradermal, subcutaneous or transcutaneous administration of antigen with various immunomodulatory substances (Bouvet *et al*., 2002). The mechanism of IgA induction has not been elucidated in these experiments, but studies in mice incorporating the steroid hormone precursor 1α,25-dihydroxy vitamin D3 [1,25$(OH)_2$D3] in a subcutaneous vaccine initially indicated that the microenvironment of the draining peripheral lymph node (PLN) was altered from a Th1 to a Th2, 'mucosal' phenotype (Daynes *et al*., 1996). Later studies by the same group reported similar results using cholera toxin, heat-labile enterotoxin and PT as adjuvants, but indicated that altered migration of skin dendritic cells to mucosal tissues may be responsible for SIgA induction rather than a change in the PLN environment (Enioutina *et al*., 2000).

We recently examined the effect of this hormone precursor on the generation of an immune response at the level of a single lymph node in sheep. Efferent lymphatics of both left and right prefemoral (peripheral) lymph nodes were cannulated in the same sheep and independent immune responses generated in the two nodes were compared after simultaneous injection of the draining tissues with Keyhole limpet hemocyanin-alum or KLH-alum-1,25$(OH)_2D_3$ on the contralateral sides of the same animal. The results (Fig. 4) indicated that 1,25$(OH)_2$ D3 enhanced the overall antibody levels to KLH and

https://doi.org/10.1079/AHR200470 Published online by Cambridge University Press

altered the ratios between KLH-specific IgG1 and IgG2. However, there was no significant alteration in the proportion of IgA antibodies in the lymph compared with other isotypes. These results were in contrast to other studies in pigs (Van der Stede *et al*., 2001) and cattle (Reinhardt *et al*., 1999), in which intramuscular coadministration of antigen with 1,25(OH)$_2$D3 significantly increased titers of antigen-specific IgA (and IgM) in serum and mucosal secretions, but similar to studies in humans in which incorporation of 1,25(OH)$_2$ D3 into the trivalent influenza vaccine did not induce enhancement of mucosal immunity when delivered in one dose to human subjects (Kriesel and Spruance, 1999). The apparent discrepancies observed in effector responses induced by the codelivery of 1,25(OH) $_2$D3 in these studies may be due to the fact that the enhanced antigen-specific IgA responses observed in the mice, pigs and cattle studies were measured following reimmunization after priming, while the vaccination trials performed in humans, and the sheep cannulation study reported here, measured antibody responses only after a primary dose of antigen, which may not have sufficiently matured to form antigen-specific IgA antibodies.

Conclusion

Major advances in basic immunology have been made, challenging long-standing concepts in mucosal vaccination. In particular, it has been shown that mucosal immunity can be generated by dendritic cells present along the mucosal tissues and is not restricted to defined mucosal lymphoid structures or 'inductive sites' such as Peyer's patches. In addition, the realization that the mucosal environment is inherently biased towards and promotes the generation of a Th2-type mucosal response indicates that it may be more rewarding to concentrate on targeting vaccine antigens to mucosal tissue sites rather than developing 'mucosal adjuvants' *per se*. An important role for adjuvants in generating appropriate immune responses to isolated, non-stimulatory antigens does, however, persist for providing adequate danger or innate response signals for subunit vaccines. An important aspect of vaccine development, in particular for subunit vaccines, is to have an understanding of the host–pathogen system to be targeted. As exemplified by the *Chlamydia* studies, major differences exist in pathogen behavior in different hosts and even in closely related hosts, resulting in different immune response profiles and effector responses. It will therefore be crucial to refer studies of host–parasite immunity to the target species.

Acknowledgments

G.E. and S.W. are funded by the Scottish Executive Environment and Rural Affairs Department. We thank David Longbottom (Moredun Research Institute) for contributing serological data.

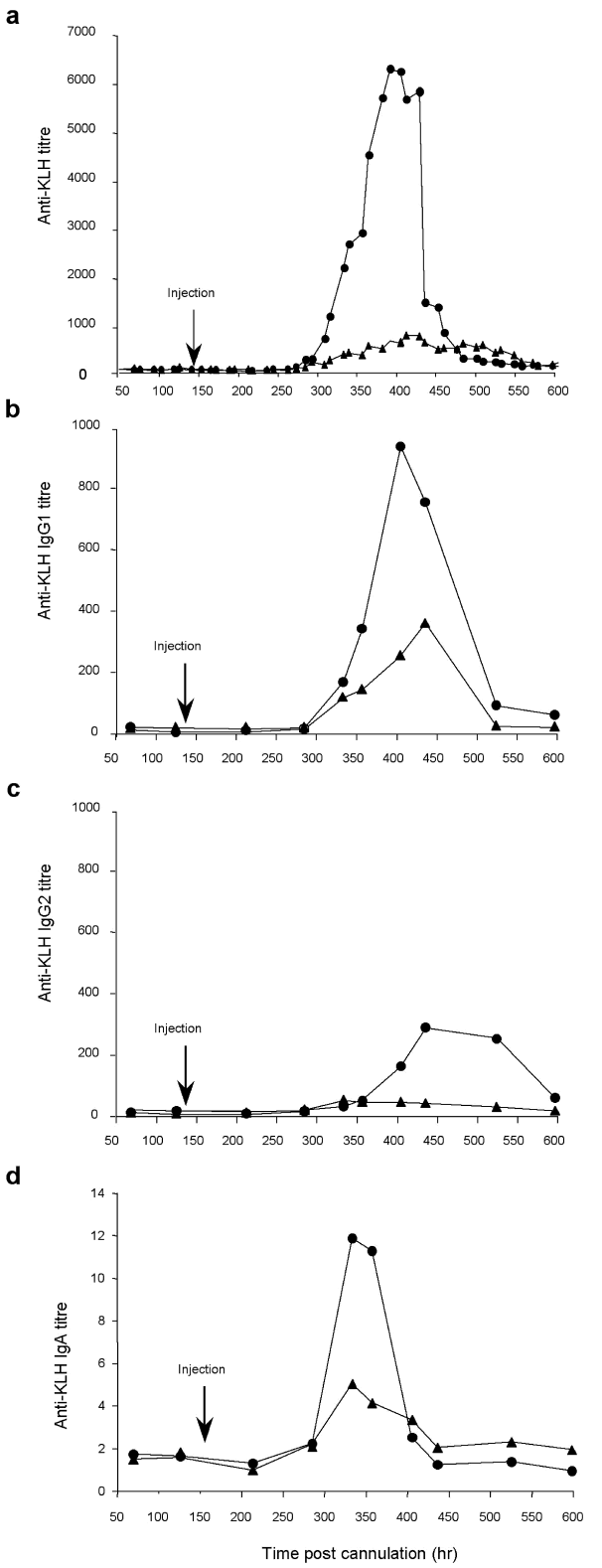

Fig. 4. Production of antibodies in lymph following systemic immunization with KLH-alum (triangles) and KLH-alum-1,25(OH)$_2$D3 (circles). Anti-KLH antibodies were measured by an enzyme-linked immunosorbent assay using isotype-specific reagents to measure total IgG (a), IgG1 (b), IgG2 (c) and IgA (d). The responses to both treatments were compared in lymph draining opposite sides of the same sheep, following cannulation of left and right efferent prefemoral lymphatics.

https://doi.org/10.1079/AHR200470 Published online by Cambridge University Press

References

Ayabe T, Satchell DP, Wilson CL, Parks WC, Selsted ME and Ouellette AJ (2000). Secretion of microbicidal alpha-defensins by intestinal Paneth cells in response to bacteria. *Nature Immunology* **1**: 99–100.

Babiuk LA, Pontarollo R, Babiuk S, Loehr B and van Drunen Littel-van den Hurk S (2003). Induction of immune responses by DNA vaccines in large animals. *Vaccine* **21**: 649–658.

Berzofsky JA, Ahlers JD and Belyakov IM (2001). Strategies for designing and optimizing new generation vaccines. *Nature Reviews. Immunology* **1**: 209–219.

Bouvet JP, Decroix N and Pamonsinlapatham P (2002). Stimulation of local antibody production: parenteral or mucosal vaccination? *Trends in Immunology* **23**: 209–213.

Brown J, Howie SEM and Entrican G (2001). A role for tryptophan in immune control of chlamydial abortion in sheep. *Veterinary Immunology and Immunopathology* **82**: 107–119.

Constant S, Brogdon J, Piggott D, Herrick C, Visintin I, Ruddle N and Bottomly K (2002). Resident lung antigen-presenting cells have the capacity to promote Th2 T cell differentiation in situ. *Journal of Clinical Investigation* **110**: 1441–1448.

Cotter TW, Meng, Q, Shen, Z-L, Zhang, Y-X, Su H and Caldwell HD (1995). Protective efficacy of major outer protein-specific immunoglobulin A (IgA) and IgG monoclonal antibodies in a murine model of Chlamydia trachomatis genital tract infection. *Infection and Immunity* **63**: 4704–4714.

Crottet P and Corthesy B (1998). Secretory component delays the conversion of secretory IgA into antigen-binding competent F(ab')2: a possible implication for mucosal defense. *Journal of Immunology* **161**: 5445–5453.

Daynes R, Enioutina E, Butler S, Mu H-H, McGee Z and Araneo B (1996). Induction of common mucosal immunity by hormonally immunomodulated peripheral immunization. *Infection and Immunity* **64**: 1199–1109.

Enioutina E, Visic D and Daynes R (2000). The induction of systemic and mucosal immune responses to antigen-adjuvant compositions administered into the skin: alterations in the migratory properties of dendritic cells appears to be important for stimulating mucosal immunity. *Vaccine* **18**: 2753–2767.

Entrican G (2002). Immune control during pregnancy and host-pathogen interactions in infectious abortion. *Journal of Comparative Pathology* **126**: 79–94.

Entrican G, Brown J and Graham S (1998). Cytokines and the protective host immune response to Chlamydia psittaci. *Comparative Immunology, Microbiology and Infectious Diseases* **21**: 15–26.

Entrican G, Buxton D and Longbottom D (2001). Chlamydial infection in sheep: immune control versus fetal pathology. *Journal of the Royal Society of Medicine* **94**: 272–277.

Entrican G, Wattegedera S, Chui M, Oemar L, Rocchi M and McInnes C (2002). IFN-γ fails to induce expression of indolamine 2,3- dioxygenase and does not control the growth of Chlamydophila abortus in BeWo trophoblast cells. *Infection and Immunity* **70**: 2690–2693.

Fagarasan S and Honjo T (2003). Intestinal IgA synthesis: regulation of front-line body defences. *Nature Reviews. Immunology* **3**: 63–72.

Fujioka H, Emancipator S, Aikawa M, Huang D, Blatnik F, Karban T, DeFife K and Mazanec M (1998). Immunocytochemical colocalization of specific immunoglobulin A with sendai virus protein in infected polarized epithelium. *Journal of Experimental Medicine* **188**: 1223–1229.

Grohmann U, Fallarino F and Puccetti P (2003). Tolerance, DCs and tryptophan: much ado about IDO. *Trends in Immunology* **24**: 242–248.

Guerrero R, Ball J, Krater S, Pacheco S, Clements J and Estes M (2001). Recombinant Norwalk virus-like particles administered intranasally to mice induce systemic and mucosal (fecal and vaginal) immune responses. *Journal of Virology* **75**: 9713–9722.

Harandi A, Eriksson K and Holmgren J (2003). A protective role of locally administered immunostimulatory CpG oligodeoxynucleotide in a mouse model of genital herpes infection. *Journal of Virology* **77**: 953–962.

Holmgren J, Czerkinsky C, Eriksson K and Mharandi A (2003). Mucosal immunisation and adjuvants: a brief overview of recent advances and challenges. *Vaccine* **21** (Supplement 2): S89–S95.

Jacobs HJ, Wiltshire C, Ashman K and Meeusen EN (1999). Vaccination against the gastrointestinal nematode, Haemonchus contortus, using a purified larval surface antigen. *Vaccine* **17**: 362–368.

Kirman J and Seder R (2003). DNA vaccination: the answer to stable, protective T-cell memory. *Current Opinion in Immunology* **15**: 471–476.

Kriesel J and Spruance J (1999). Calcitriol (1,25-dihydroxy-vitamin D3) coadministered with influenza vaccine does not enhance humoral immunity in human volunteers. *Vaccine* **17**: 1883–1888.

Kutteh W, Hatch K, Blackwell R and Mestecky J (1988). Secretory immune system of the female reproductive tract: I. Immunoglobulin and secretory component-containing cells. *Obstetrics and Gynecology* **71**: 56–60.

Lamkhioued B, Gounni A, Gruart V, Pierce A, Capron A and Capron M (1995). Human eosinophils express a receptor for secretory component. Role in secretory IgA-dependent activation. *European Journal of Immunology* **25**: 117–125.

Longbottom D (2003). Chlamydial vaccine development. *Journal of Medical Microbiology* **52**: 537–540.

Longbottom D and Coulter LJ (2003). Animal chlamydioses and zoonotic implications. *Journal of Comparative Pathology* **128**: 217–244.

Macpherson A, Lamarre A, McCoy K, Harriman G, Odermatt B, Dougan G, Hengartner H and Zinkernagel R (2001). IgA production without mu or delta chain expression in developing B cells. *Nature Immunology* **2**: 625–631.

McSorley S, Asch S, Costalonga M, Reinhardt R and Jenkins M (2002). Tracking salmonella-specific CD4 T cells in vivo reveals a local mucosal response to a disseminated infection. *Immunity* **16**: 365–377.

Meeusen EN, Premier RR and Brandon MR (1996). Tissue-specific migration of lymphocytes: a key role for Th1 and Th2 cells? *Immunology Today* **17**: 421–4.

Meeusen EN, Bischof RJ and Lee CS (2001). Comparative T-cell responses during pregnancy in large animals and humans. *American Journal of Reproductive Immunology* **46**: 169–79.

Mowat A (2003). Anatomical basis of tolerance and immunity to intestinal antigens. *Nature Reviews. Immunology* **3**: 331–341.

Munn DH, Zhou M, Attwood JT, Bondarev I, Conway SJ, Marshall B, Brown C and Mellor AL (1998). Prevention of allogeneic fetal rejection by tryptophan catabolism. *Science* **281**: 1191–1193.

Nagler-Anderson C (2001). Man the barrier! Strategic defences in the intestinal mucosa. *Nature Reviews. Immunology* **1**: 59–67.

Phalipon A and Corthesy B (2003). Novel functions of the polymeric Ig receptor: well beyond transport of immunoglobulins. *Trends in Immunology* **24**: 55–58.

Podolsky DK (1999). Mucosal immunity and inflammation V. Innate mechanisms of mucosal defence and repair: the best offence is a good defense. *American Journal of Physiology* **277**: G495–G499.

Premier RR and Meeusen EN (1998). Lymphocyte surface marker and cytokine expression in peripheral and mucosal lymph nodes. *Immunology* **94**: 363–367.

Premier RR, Jacobs HJ, Lofthouse SA, Sedgmen BJ and Meeusen ENT (2004). Antibody isotype profiles in serum and circulating antibody-secreting cells following mucosal and peripheral immunisations of sheep. *Veterinary Immunology and Immunopathology* **98**: 77–84

Reinhardt T, Stabel J and Goff J (1999). 1,25-dihydroxyvitamin D3 enhances milk antibody titers to Escherichia coli J5 vaccine. *Journal of Dairy Science* **82**: 1904–1909.

Reinhardt R, Khoruts A, Merica R, Zell T and Jenkins M (2001). Visualizing the generation of memory CD4 T cells in the whole body. *Nature* **410**: 101–105.

Rescigno M, Urbano M, Valzasina B, Francolini M, Rotta G, Bonasio R, Granucci F, Kraehenbuhl J and Ricciardi-Castagnoli P (2001). Dendritic cells express tight junction proteins and penetrate gut epithelial monolayers to sample bacteria. *Nature Immunology* **2**: 361–367.

Rincheval-Arnold A, Belair L and Djiane J (2002). Developmental expression of pIgR gene in sheep mammary gland and hormonal regulation. *Journal of Dairy Research* **69**: 13–26.

Robinson J, Blanchard T, Levine A, Emancipator S and Lamm M (2001). A mucosal IgA-mediated excretory immune system in vivo. *Journal of Immunology* **166**: 3688–3692.

Scheerlinck J-PY (2001). Genetic adjuvants for DNA vaccines. *Vaccine* **19**: 2647–2656.

Sedgmen BJ, Lofthouse SA, Scheerlinck JP and Meeusen EN (2002). Cellular and molecular characterisation of the ovine rectal mucosal environment. *Veterinary Immunology and Immunopathology* **86**: 215–20.

Staats HF and Ennis FA Jr (1999). IL-1 is an effective adjuvant for mucosal and systemic immune responses when co-administered with protein immunogens. *Journal of Immunology* **162**: 6141–6147.

Stanley A, Huntley J, Jeffrey M and Buxton D (2001). Characterization of ovine nasal-associated lymphoid tissue and identification of M cells in the overlying follicle-associated epithelium. *Journal of Comparative Pathology* **125**: 262–270.

Stephens RS (2003). The cellular paradigm of chlamydial pathogenesis. *Trends in Microbiology* **11**: 44–51.

Van der Stede Y, Cox E, Van den Broeck W and Goddeeris B (2001). Enhanced induction of the IgA response in pigs by calcitriol after intramuscular immunization. *Vaccine* **19**: 1870–1878.

Vanrompay D, Cox E, Volckaert G and Goddeeris B (1999). Turkeys are protected from infection with Chlamydia psittaci by plasmid DNA vaccination against the major outer membrane protein. *Clinical and Experimental Immunology* **118**: 49–55.