Chapter 68

Mucosal Veterinary Vaccines: Comparative Vaccinology

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Chapter Outline

INTRODUCTION

Despite advances in nutrition, genetics, housing, and thera-peutics, diseases of the respiratory, reproductive, and gastrointestinal tracts of domestic animals and poultry continue to be major causes of morbidity and mortality. Although vaccines have been developed and licensed for prevention of many of these diseases, there is a need for improvement in vaccine efficacy and for new vaccines. Reasons for low vaccine efficacy include inappropriate, unstable, or outdated antigens in vaccine preparations (e.g., in vitro expressed antigens instead of in vivo expressed antigens), inappropriate immune responses (e.g., systemic instead of mucosal, or Th2 type instead of Th1 type or vise versa), and inappropriate use of otherwise efficacious vaccines (e.g., inappropriate timing of vaccination) (Tizard, 2013). An overview of some of the vaccines currently licensed for vaccination of domesticated animals and wildlife by mucosal routes is provided in Table 1. Many of these vaccines consist of pathogens attenuated by traditional methods, but engineered virus-vectored vaccines are now used extensively (by both mucosal and parenteral routes) in veterinary medicine.

The majority of current poultry vaccines are attenuated agents delivered in ovo, orally, intranasally (IN) or by other mucosal routes, for reasons of ease of administration, economy, and protective efficacy. In comparison, fewer vaccines for domestic mammals are delivered by mucosal routes. Management practices for mammals differ from those for poultry; mass vaccination techniques for mucosal delivery have been developed for poultry, but have not been pursued as zealously for mammals.

Recently however, a number of attenuated live vaccines for IN administration have been developed for respiratory tract infections, using traditional methodologies. Improved protective efficacy and rapid onset of immunity compared to killed vaccine products have led to widespread acceptance. Attenuated live oral vaccines for enteric diseases have also been marketed, but in many cases efficacy has been disappointing due to lack of potency in adults or interference by maternal antibodies in suckling animals. Better strategies for induction of immunity in the gastrointestinal tract are needed, especially for neonates in the presence of maternal antibodies. In contrast, effective parenteral vaccines for the most common diseases of the reproductive tract in veterinary species have been available for years, and there has been little motivation to develop mucosal vaccines.

Many of the diseases of the respiratory and gastrointestinal tracts are most devastating in the neonatal period.

For these diseases active immunization may not provide protection before natural exposure to the pathogen. Maternal vaccination to enhance passive immunity has been widely used in veterinary medicine, especially for control of enteric diseases. Practical difficulties arise, however, with diseases such as parvovirus enteritis in puppies in which a smooth transition must be made from protection by passive maternal antibodies to protection by active immunity, without permitting a window in between of disease susceptibility. This transition is difficult to achieve because induction of active immunity is commonly inhibited by maternal antibodies. Various strategies are used to address this problem, but improved vaccines, adjuvants, and antigen delivery systems would improve the reliability of neonatal immunization.

Although progress is being made in disease prevention in veterinary species, ever changing management practices (e.g., earlier weaning of piglets, larger animal operations) generate new patterns of disease, requiring new control strategies. The emergence of new pathogens (e.g., porcine reproductive and respiratory syndrome virus (PRRSV), porcine circovirus-2) provides new challenges for vaccine research before some of the older challenges have been met. In this chapter, we focus on mucosal veterinary vaccines and vaccine concepts related to selected pathogens of economic importance. Our intent is to highlight progress, to review existing and future vaccination strategies, and to acknowledge the unique contributions of this research to our understanding of mucosal vaccines and immunology.

RESPIRATORY VACCINES

Introduction

Respiratory tract infections are a major cause of morbidity and mortality in farm animals, poultry, and pets. Disease conditions are intensified by current management practices such as mixing of recently weaned, stressed beef calves from multiple sources in auction barns. Certain disease conditions, such as atrophic rhinitis of pigs, result from the interplay of several pathogens, and multiple agents must be represented in vaccines. Some respiratory pathogens such as *Mycoplasma hyopneumoniae* in young pigs are causing new patterns of disease as management practices change (e.g., weaning at an earlier age), requiring changes in vaccine strategies. Other pathogens such as PRRSV have only recently emerged and improved vaccines await advances in understanding of the agent and of disease pathogenesis. A discussion of respiratory vaccines for even the major pathogens of veterinary species is beyond the scope of the present review. This section

TABLE 1 Examples of Commercial Veterinary Vaccines Administered by Mucosal Routes[a]

Species	Agent[b]	Disease	Vaccine Type	Comments
Foxes, coyotes, raccoons	Rabies virus	Rabies	Vaccinia virus vectored—"ORV" (oral rabies vaccine)	Aerial baiting, with vaccine in coated sachets, or fishmeal polymer blocks; 8.7 million doses were used in the USA in 2010[c]
Horses	*Streptococcus equi* subsp. *equi*	"Strangles" pharyngitis with abscessation of lymph nodes	Live culture intranasal	Cases of lymphadenectasis and purpura hemorrhagica have been reported after vaccination
	Equine influenza virus	Influenza	Attenuated, cold-adapted, intranasal	Revaccination at 6-month intervals
Cattle	Bovine herpesvirus-1	Infectious bovine rhinotracheitis, abortion, infectious pustular vulvovaginitis	Attenuated intranasal	Vaccination with attenuated virus leads to latent infection[d]
	Dictyocaulus viviparus	Lungworm	Viable irradiated third-stage larvae	Oral administration—goal is controlled challenge
Dogs	*Bordetella bronchiseptica*	"Kennel cough" (tracheobronchitis)	Live culture—oral, intranasal	Intranasal vaccine also includes viral agents
Feline	Feline herpesvirus-1	Feline viral rhinotracheitis	Intranasal	Vaccine may also contain feline calicivirus
	Feline calicivirus	Respiratory tract infections, stomatitis	Intranasal	Vaccine may also contain herpesvirus-1
Embryonic chicks	Marek's disease virus (alphaherpesvirus)	Marek's disease (neurolymphomatosis)	Attenuated strains; increasing virulence of field strains has led to use of less attenuated vaccine strains	In ovo at embryonation day 18—automated systems process 50,000 eggs/hour; a hatchability of 95% can be attained; delivery of vaccine into amniotic fluid permits mucosal vaccination[e]
Chickens	Various species of *Eimeria*	Coccidiosis	Live oocysts	Live oocysts of *Eimeria* species administered in ovo, or to chicks at 1 day of age in a spray cabinet or sprayed on feed – goal is controlled exposure
	Infectious bursal disease (IBD) virus	IBD	Attenuated virus or herpesvirus of turkeys (HVT)-vectored vaccine	IBD in drinking water or HVT-vectored vaccine in ovo

[a]*For a more extensive summary of veterinary vaccines that includes vaccines given by parenteral routes, see the previous (3rd) edition of this book.*
[b]*Agents and vaccines have been selected as representative of common disease concerns and current commercial vaccines. Vaccine data have been collated from manufacturers' product monographs unless indicated otherwise.*
[c]*Available at: http://www.aphis.usda.gov/wildlife_damage/oral_rabies/downloads/NationalReport_2010.pdf (accessed 13.03.14).*
[d]*Nandi et al. (2009).*
[e]*Williams and Zedek (2010).*

will focus on vaccines for PRRSV infections in pigs and influenza in horses to illustrate general principles.

Porcine Reproductive and Respiratory Syndrome

Introduction

Porcine reproductive and respiratory syndrome (PRRS) is a chronic reproductive and respiratory disease of pigs caused by PRRSV, a member of the family Arteriviridae (Benfield et al., 1992). Signs associated with PRRS are anorexia, fever, lethargy, pneumonia, red/blue discoloration of the ears and vulva, delayed return of sows to estrus after weaning, abortion, fetal mummification, stillborn or weak born piglets, and high preweaning mortality (Mengeling et al., 1998). PRRSV is prevalent in swine-producing countries worldwide. Annual economic losses to the pork industry in the United States due to PRRS have been estimated at $664 million (Holtkamp and Kliebenstein, 2011). According to the American Animal and Plant Health Inspection Service (APHIS) report of January 2009, 49.8% of unvaccinated pigs in the United States are seropositive to PRRSV. Infected pigs excrete PRRSV in saliva, nasal secretions, urine, milk, colostrum, and feces at low levels or intermittently, and also in semen of infected boars (Rossow et al., 1994). In the absence of control measures, PRRSV is spread by aerosols, fomites, and personnel.

PRRSV is divided into two distinct genotypes, type I (European) and type II (North American). Each genotype contains subtypes and strains, which are genetically diverse and vary in virulence and pathogenicity (Kim et al., 2007). Immunity to one genotype of PRRSV may provide partial or no protection against reinfection, reflecting the complexity of PRRSV genetics and immunological variation among strains (Botner et al., 1997). Swine are the only known species susceptible to PRRSV. Alveolar macrophages (MΦs) are the primary permissive cells to PRRSV; MΦs present in pulmonary intravascular spaces, lymph nodes, thymus, heart, spleen, placenta, and umbilical cord may also be infected by the virus (Halbur et al., 1995).

Subversion of Immune Responses by PRRSV

The most efficient and rapid host response against viruses consists of production of type I interferons (IFNs) (IFNα and IFNβ). In PRRSV-infected pigs, innate IFNα secretion is significantly suppressed (Albina et al., 1998) and the virus dampens innate NK cell-mediated cytotoxicity as early as day 2 postinfection (Renukaradhya et al., 2010; Dwivedi et al., 2012).

In pigs, a significant correlation has been observed between the PRRSV infection and an increased expression of IL-10, associated with reduced expression of IFNα, IFNγ, IL-12, and TNFα (Gómez-Laguna et al., 2010). Induction of IL-10 is mediated by interaction between PRRSV proteins and MΦs/dendritic cells (DCs). The PRRSV nucleocapsid protein induces IL-10 production by peripheral blood mononuclear cells (PBMCs) and alveolar MΦs (Wongyanin et al., 2012), delaying the onset of protective cell-mediated immunity (CMI) to PRRSV (Dwivedi et al., 2011a, 2012). Dysregulated immune responses in infected pigs affect viral pathogenesis, disease severity, and susceptibility to secondary microbial infections (Thanawongnuwech et al., 2001; Renukaradhya et al., 2010). Immune responses against PRRSV are inadequate to completely clear the virus. Viremia disappears in 4–6 weeks, but the virus persists in the tonsils and lymphoid tissues for several months (Moyron-Quiroz et al., 2007). PRRSV infection in both germ-free and conventionally raised pigs is associated with polyclonal B cell activation, hypergammaglobulinemia, lymphoid adenopathy, renal lesions, and lymphoid hyperplasia (Cooper et al., 1997). Polyclonal lymphoplasia also occurs in mice infected with lactate dehydrogenase-elevating virus, a related virus (Grossmann et al., 1989). Primary antibody responses occur promptly after PRRSV infection (Loemba et al., 1996), but the majority of secreted antibodies are autoantibodies or PRRSV nonneutralizing antibodies (Lemke et al., 2004). The virus-neutralizing antibody (NA) response is weak, and delayed (Mateu et al., 2006).

Commercial PRRSV Vaccines

Both killed and modified live virus (MLV) PRRSV vaccines are available commercially for intramuscular or intradermal administration (Charerntantanakul, 2012).

Live PRRSV Vaccines

MLV vaccines confer protection against genetically homologous PRRSV, but incomplete protection against heterologous viruses (Mengeling et al., 2003). In both PRRSV-infected and MLV-PRRSV-vaccinated pigs, virus-specific cell-mediated immune responses are either delayed or dampened (Renukaradhya et al., 2012). Reversion to virulence is a major concern with modified live vaccines. There are numerous reports of reversion of PRRSV vaccine virus to virulence and of the presence of reverted vaccine strain PRRSV in unvaccinated sows and pigs (Nielsen et al., 2002). Vaccine-derived virus has been isolated from fetuses, stillborn, and dead piglets, indicating the spread of disease from vaccinated pigs. Identification of mutations in multiple vaccine-derived isolates at identical positions of the viral genome suggests a strong selective pressure on critical viral proteins in field situations.

Killed PRRSV Vaccines

Available killed PRRSV vaccines fail to protect even against homologous virus, do not elicit NA, and induce weak cell-mediated immune responses (Bassaganya-Riera et al., 2004). Pregnant sows and gilts and breeding boars are not protected from PRRSV due to a lack of safe and protective vaccines. Control of PRRSV in breeding stock is critical in preventing vertical and horizontal transmission of the virus. Early attempts to develop killed PRRSV vaccines using recombinant PRRSV proteins, plasmids expressing viral genes, and inactivated PRRSV administered with or without adjuvants have been unsuccessful (Charerntantanakul, 2009). However, recent studies show promise. Killed PRRSV vaccine coadministered IN with CpG oligodeoxynucleotide adjuvant (a TLR-9 ligand) induced antibodies, virus-specific T cells, and secretion of IFNγ and IL-6 (Zhang et al., 2007). In another study, PRRSV inactivated by UV light or binary ethylenimine induced NA and protected pigs against homologous viral challenge. The suppressive effect of live PRRSV on IFNα production was lost when the virus was inactivated by UV irradiation (Vanhee et al., 2009). An oral immunization strategy using PRRSV nucleocapsid protein genetically fused to cholera toxin (CT) stimulated PRRSV antibody responses in the intestines, but no detectable response in vaginal secretions (Hyland et al., 2004).

Lack of success in developing protective killed PRRSV vaccines may reflect an inability to present killed PRRSV antigens effectively to the pig immune system, or (like live PRRSV) killed virus may induce immunosuppressive effects. In addition, the antigenic mass used in killed vaccines may be insufficient, suggesting the need for potent adjuvants or novel delivery systems.

Experimental Systemic PRRSV Vaccines

Attempts have been made to improve MLV-PRRSV vaccines using adjuvants. In one study, pigs were injected with recombinant porcine IL-1, IL-6, IL-12, or CT within 1 week of intramuscular administration of MLV-PRRSV. IL-12 and CT enhanced IFNγ and GP5 antibody responses, respectively (Foss et al., 2002). However, use of these adjuvants did not reduce the severity of viremia. Unfortunately, PRRSV (NA) responses were not assayed, and protection against heterologous challenge was not assessed in this study.

Experimental Mucosal PRRSV Vaccines

PRRSV gains entry through respiratory and reproductive mucosal surfaces and causes disease primarily at mucosal sites. Therefore, a mucosal vaccine against PRRSV may be an effective strategy for controlling the disease. Until recently, attempts to elicit protective mucosal immunity against PRRSV have been unsuccessful, probably due to a lack of appropriate adjuvants. Because PRRSV infection rapidly subverts the host immune responses, an effective adjuvant must overcome immunosuppression caused by vaccine virus and simultaneously potentiate virus-specific adaptive immunity.

A panel of bacterial preparations derived from *Mycobacterium, Vibrio*, and *Streptococcus* species, which were potent adjuvants in rodent models, were tested IN with MLV-PRRSV (Bonavida et al., 1986; Barral and Brenner, 2007). Based on mucosal and systemic immune responses, three of the preparations, *Mycobacterium tuberculosis* whole cell lysate (*M. tb* WCL), CT B subunit, and OK-432 (a product of *Streptococcus pyogenes*), were selected for viral challenge trials. Only *M. tb* WCL significantly dampened PRRSV immunosuppressive effects, and enhanced virus-specific adaptive responses (Renukaradhya et al., 2010; Dwivedi et al., 2011b).

Historically, heat-killed mycobacteria are recognized to have as potent adjuvant effects as components of Freund's complete adjuvant (FCA) and have been used extensively in experimental animals. The use of FCA in humans and food animals is unacceptable due to severe granulomatous inflammatory reactions to toxic cell wall components (Bekierkunst, 1968). However, a nontoxic water-soluble purified fraction of *M. tb* WCL (Werner et al., 1975) has adjuvant properties in rodents, guinea pigs, and rabbits. Pigs inoculated IN with *M. tb* WCL have no detectable signs of toxicity locally or systemically (Dwivedi et al., 2011a,b). A recombinant vaccine (PRRSV GP5 and M expressed in Bacillus Calmette–Guerin (BCG)) is reported to reduce clinical signs of PRRS with decreased viremia and viral load in the tissues (Bastos et al., 2004).

Cross-protective immunity against PRRSV has been evaluated in pigs receiving MLV-PRRSV and *M. tb* WCL IN, by challenging with virulent heterologous PRRSV virus (Kim et al., 2007). Reduced clinical disease, viremia, and virus-mediated immunosuppression were noted. In addition, secretion of IFNγ and IL-12 by lung and blood lymphocytes in response to PRRSV M and nucleocapsid proteins was enhanced (Dwivedi et al., 2011a). Guillonneau et al. (2009) reported similar findings in mice vaccinated IN with adjuvanted influenza vaccines. Enhanced virus-specific cytotoxic T cell and memory responses to internal viral proteins were noted, as well as cross-protective immunity.

Studies of passive protection of pigs by PRRSV NA have indicated that modest NA titers ($\leq 1/32$) protect pregnant sows against reproductive failure, block placental transmission of infection, prevent viremia in piglets, and provide sterilizing immunity (Osorio et al., 2002). Pigs immunized IN with MLV-PRRSV alone or with *M. tb* WCL developed NA titers <1/16 and 1/32, respectively, postimmunization (Dwivedi et al., 2011b). In pigs immunized (MLV-PRRSV + *M. tb* WCL) and challenged with PRRSV MN184, an NA titer >1/16 persisted until PID 60

(Dwivedi et al., 2011a). Relatively low numbers of circulating virus-specific IFNγ secreting cells have been reported for pigs vaccinated IN with MLV-PRRSV without adjuvant, (50–100 cells per million PBMCs) (Dwivedi et al., 2011a). In contrast, Meier et al. (2003) have reported 200–300 IFNγ secreting cells per million PBMCs in pigs vaccinated against Aujeszky's disease virus. Pigs immunized IN with MLV-PRRSV with *M. tb* WCL and challenged with PRRSV had greater than 300 IFNγ secreting cells per million PBMCs, and more than a twofold higher frequency of CD4+CD8+ T cells (Dwivedi et al., 2011a).

Conclusion

The literature concerning mucosal immune responses in the pig respiratory tract is limited compared to studies of rodents and humans. Recent research highlights the advantages of activating the mucosal immune system using vaccines delivered with potent adjuvants. Induction of adequate immune responses in the respiratory and reproductive tract will be essential for control of PRRS in swine. IN-delivered, potent mucosal vaccines generate better cross-protective immunity against genetically variable PRRSV field viruses. Efforts to control PRRS outbreaks using conventional parenterally delivered vaccines have had limited success; development of an alternative approach of generating immunity in the respiratory tract should be a priority. In particular, mucosal adjuvants based on components of mycobacterial species (Renukaradhya et al., 2012) show promise.

Equine Influenza

Introduction

Respiratory tract disease affects virtually every aspect of equine husbandry, including working, pleasure, and race horses. Considerable efforts are expended to prevent epizootics of respiratory disease in stables, fairs, shows, and race tracks. Equine influenza virus will be discussed in detail as an example of past, current, and future vaccine approaches.

Equine Influenza Virus

Equine influenza virus causes epizootics of upper and lower respiratory tract disease almost worldwide. Until 2007 equine influenza was excluded from the continent of Australia through import restrictions and quarantine. In August of that year, importation of an infected horse led to an explosive epizootic of equine influenza with an estimated 70,000 horses infected over a 4-month period (Callinan, 2008), demonstrating the potential of the virus to spread in a naïve, unvaccinated population. Infection can occur in horses of all ages, but epizootics often involve younger animals (van Maanen and Cullinane, 2002). Clinical signs include high fever, a persistent dry cough, nasal discharge,

anorexia, and depression. Secondary bacterial pneumonia may complicate the clinical picture.

Equine influenza viruses are classified as type A influenza. Antigenic differences in the hemagglutinin (H) and neuraminidase (N) glycoproteins define the two recognized subtypes, A/equine/1 (H7N7) and A/equine/2 (H3N8) (Wilson, 1993). Two lineages of A/equine/2, American and European, have been identified; multiple virus strains are included in vaccines since protection against heterologous strains is incomplete (Yates and Mumford, 2000). Antigenic drift is sufficient to require regular reappraisal of strains included in vaccines (Mumford and Wood, 1993).

Natural infection induces IgA antibodies in nasal secretions, IgGa and IgGb antibodies in serum (Hannant et al., 1989; Nelson et al., 1998), and circulating cytotoxic T lymphocytes (Hannant and Mumford, 1989). Protection against reinfection persists for at least a year (Hannant et al., 1988). Vaccination with inactivated virus vaccines induces serum IgG (T) antibodies without detectable IgA in nasal secretions (Nelson et al., 1998) and without cytotoxic T cell activity (van Maanen and Cullinane, 2002). Two or three doses of vaccine are typically administered in the primary series, with booster doses at least once a year thereafter. More frequent vaccination is advised for horses at high risk of infection (Wilson, 1993). Protection is typically incomplete and of limited duration. Improved adjuvants can enhance the level and duration of antibody responses to inactivated virus vaccines (Mumford et al., 1994c). Suppressive effects of maternal antibodies on responses to inactivated vaccines have led to recommendations not to vaccinate foals before 6 months of age (Van Oirschot et al., 1991).

Immune Stimulating Complex Vaccines

A subunit equine influenza vaccine based on the immune stimulating complex (ISCOM) adjuvant technology has been licensed and marketed in Europe since 1987 (Newmark, 1988). Antibody responses to ISCOM-based vaccines typically are of higher titer and are more persistent than for conventional inactivated vaccines (Mumford et al., 1994a). Protection has been demonstrated against experimental challenge, 15 months after a three dose vaccination series (Mumford et al., 1994b). Protection may be due in part to the ability of ISCOM-adjuvanted vaccines to induce cytotoxic T lymphocytes (Morein et al., 1999). Although ISCOM-based vaccines can induce IgA antibody responses following IN administration (Hu et al., 2001), the commercial influenza ISCOM vaccine is administered parenterally.

IN Vaccination

IN administration of inactivated equine influenza virus with CT B has been reported to induce local IgA antibodies, and protection against experimental challenge (Hannant et al., 1998). A cold-adapted, temperature-sensitive live vaccine for IN administration is available commercially (Chambers

et al., 2001). Protection against experimental challenge has been demonstrated 6 months after a single vaccination (Townsend et al., 2001). This is a notable improvement in efficacy and practicality over conventional killed vaccines.

Plasmid DNA Vaccines

Experimental plasmid vaccines encoding the hemagglutinin gene of equine influenza have been examined in horses. Three doses of plasmid administered to the skin and mucosal sites (tongue, conjunctiva, and third eyelid) induced protection against clinical disease and partial protection against viral shedding. Protection against clinical disease was reduced if plasmid was administered only to the skin (Lunn et al., 1999).

Pox Virus Vectored Vaccines

A canarypox-vectored equine influenza vaccine, expressing hemagglutinins from two strains of H3N8 equine influenza, has been available commercially (intramuscular administration) since 2003 (Toulemonde et al., 2005). In the Australian epizootic of 2007, this vaccine was used extensively during the government program to eradicate equine influenza. The vectored vaccine was chosen for this program because of its DIVA (distinguishing infected from vaccinated animals) characteristics (Kirkland and Delbridge, 2011). Infected horses mount antibody responses to the viral nucleoprotein in addition to the viral hemagglutinin, whereas vaccinated horses respond only to the hemagglutinins expressed by the vaccine vector. A recent report indicates that adequate antibody responses are generated with as little as 14 days between primary and secondary vaccination (El-Hage et al., 2012). Protection against experimental challenge has also been noted 2 weeks after a single dose of vaccine in ponies (Toulemonde et al., 2005).

Future Needs

For some respiratory pathogens (e.g., *M. hyopneumoniae* in pigs) the critical antigens associated with protective immune responses have not been identified. For other pathogens (e.g., PRRSV) there is also a need to identify the appropriate type of immune response (Th1 or Th2) needed for protection. For some complex disease conditions (e.g., bovine respiratory disease complex) there is continuing uncertainty whether all of the relevant contributing pathogens have been identified. Although many parenteral vaccines are efficacious in reducing lower respiratory tract disease, there is a need to investigate whether induction of mucosal immunity in the upper airways, in combination with systemic immunity, can further reduce infection rates, transmission of pathogens, and economic losses. Finally, there is a need to devise and implement changes in management procedures to reduce disease exposure (by nonimmunological methods), and to optimize immune interventions by improved timing of vaccinations.

VACCINES FOR GENITAL INFECTIONS

Introduction

Vaccines to prevent reproductive tract disease have received much emphasis in veterinary medicine. This is especially true of food-producing animals because reproductive failure is a major economic problem. Although vaccines to prevent reproduction are of interest for abandoned pets or deer in areas of overpopulation, this section will deal only with vaccines designed to prevent infectious disease of the reproductive mucosa.

Infections causing adverse pregnancy outcome can be classified by route of infection: hematogenous or ascending. Several hematogenous infections have a predilection for the gravid uterus resulting in early to late abortions (Corbeil et al., 2001). These include leptospirosis, chlamydial infection, and brucellosis in several animal species, *Histophilus somni* infection in cattle and sheep and *Neospora caninum* in cattle. Although vaccines are available for several of these infections, the vaccine for brucellosis has been available since the 1940s and is the most well known. Several *Brucella* species cause abortion or epididymitis/orchitis in the primary hosts (*Brucella abortus* in cattle, *Brucella suis* in swine, *Brucella melitensis* in goats, *Brucella ovis* in sheep, *Brucella canis* in dogs, and *Brucella marinum* (or *Brucella delphini*) in marine mammals). Infection may be acquired via the gut mucosa or the conjunctiva/upper respiratory mucosa and localizes in the reticuloendothelial system and endometrium/placenta by systemic spread. Thus, systemic vaccines are effective. A modified live *B. abortus* vaccine, along with a "test and slaughter" eradication program, has been successful in controlling bovine brucellosis in North America. The modified live vaccine (*B. abortus* strain 19) is very effective in stimulating CMI that is critical for protection against this facultative intracellular pathogen. Strain 19 now has been largely replaced by a new attenuated strain, RB51, which does not stimulate an antibody response known to interfere with diagnostic serologic assays. There is considerable information on mechanisms of systemic immunity to brucellosis. *Neospora caninum* also causes abortion by a hematogenous route and a vaccine is available (Weston et al., 2012). *Histophilus somni* can infect either by the hematogenous route or by an ascending genital route to cause abortion or infertility and vaccines are available. Since the focus of this volume is mucosal immunity, no more will be said concerning protection against hematogenous infections of the genital tract.

Ascending local infections of the reproductive tract are usually transmitted sexually. The two best examples of vaccines for sexually transmitted infections of animals are *Campylobacter fetus* subsp. *venerealis* (formerly *Vibrio fetus* subsp. *venerealis*) and *Tritrichomonas foetus*. Both are host-specific bovine sexually transmitted diseases (STDs) that only infect the reproductive mucosa. Both are extracellular pathogens that do not invade the mucosa of the reproductive tract but may be found in the placenta and fetus. The localized nature of these infections and transmission limited to coitus suggest that mucosal immunity must be important. Because a vaccine has been available for *C. fetus* subsp. *venerealis* for several decades and its use has controlled the disease in developed countries, that vaccine will be discussed first.

Vibriosis

Vibriosis (or campylobacteriosis) is a chronic bacterial genital infection with no overt clinical signs other than reproductive failure (Corbeil et al., 1981). After months of infection, the uterus is cleared first, followed by the vagina. Convalescent immunity is partially protective for a limited time. Antibody is effective in protection against this extracellular pathogen as demonstrated by systemic passive immunization (Berg et al., 1979). The antibody response to infection is primarily IgA in the vagina and IgG in the uterus (Corbeil et al., 1981). Systemic immunization with a whole cell vaccine results in both IgG1 and IgG2 antibody responses to surface antigen in serum, and uterine and vaginal secretions (Corbeil et al., 1981). This response prevents infection and can rapidly clear infected cows (Corbeil and Winter, 1978). That is, the vaccine can be used prophylactically and even therapeutically. Immunization is efficacious even though surface antigenic variation occurs in the face of a local immune response (Corbeil and Winter, 1978). Presumably, immune clearance occurs when the dynamic interaction between protection and evasion is shifted in the favor of the host. This appears to occur earlier when the response is primarily IgG than when IgA predominates (Corbeil et al., 1981). This may be related to the ability of the IgG antibodies to mediate opsonization and intracellular killing of the bacterium, an ability which IgA antibodies lack (Corbeil and Winter, 1978). Although this work was done many years ago, it sets a precedent for systemic immunization for prophylaxis and therapy of reproductive mucosal infections.

Trichomoniasis

Trichomoniasis is a similar chronic genital mucosal infection of cattle. It is caused by the protozoan, *T. foetus,* and results in pregnancy loss. *Trichomonas vaginalis* causes a human STD also associated with adverse pregnancy outcome. Thus, bovine trichomoniasis serves as a model for immune prevention of both bovine and human reproductive mucosal infections. Because of the economic significance of bovine trichomoniasis and because no chemotherapy is approved, investigations have focused on immunoprophylaxis and immunotherapy. *T. foetus* colonizes the vaginal and uterine or preputial surfaces for months, like *C. fetus* subsp. *venerealis* infection. In fact, mature bulls are often infected for life whereas young bulls may clear the infection with time (Cobo et al., 2011). This is probably related to innate immunity. Trichomonads are anaerobic parasites and are found deep in uterine glands and epithelial crypts of the penis and prepuce (Rhyan et al., 1999) where the oxygen tension is probably lowest. Older bulls have deeper epithelial crypts, which may have lower oxygen tension. In order to understand protective acquired immune responses, monoclonal antibodies (mAbs) with putative protective functions were chosen for immunoaffinity purification of a highly glycosylated surface antigen (BonDurant et al., 1993; Corbeil et al., 2001). Analysis of many isolates of *T. foetus* indicated that two mAbs recognized different epitopes of the same antigen, which was conserved in all isolates tested. This glycosylated surface antigen was later shown to be a lipophosphoglycan (LPG)/protein complex. Systemic immunization with the immunoaffinity-purified LPG-containing surface antigen, followed by intravaginal challenge with *T. foetus*, resulted in significantly earlier clearance of the parasite from vaccinated animals than from controls (BonDurant et al., 1993; Corbeil et al., 2001). More importantly, clearance of immunized animals most often occurred before 7 weeks of infection. Parsonson et al. (1976) showed that significant inflammation accompanied by reproductive failure did not occur until after 7 weeks of infection, so the vaccine should protect against fetal loss. Analysis of vaccine-induced antibody responses demonstrated predominantly IgG1 responses in the serum and IgA plus IgG1 antibodies in secretions (Corbeil et al., 2001, 2005). IgE antibodies were also increased during infection. As IgE antibodies increased, mast cells degranulated and clearance of *T. foetus* occurred. These studies raised several questions. First, why is the systemic response to the LPG/protein complex skewed toward IgG1 (a Th2 response in cattle) and not IgG2 (a Th1 response)? This question is under investigation. Second, are IgG or IgA antibodies to the LPG-containing antigen most protective and how can that Ig isotype be enriched to enhance protection? To address the latter questions, preliminary studies were done in mice to determine the best routes and adjuvants to enrich for IgG or IgA in genital secretions (Corbeil et al., 2001). Subcutaneous priming with the immunoaffinity-purified LPG-containing surface antigen (called TF1.17 antigen) in Quil A adjuvant and subcutaneous boosting with whole cells enriched for IgG anti-TF1.17 antibodies in genital secretions whereas subcutaneous priming and intravaginal

boosting greatly enriched for IgA antibodies in genital secretions. When cattle immunized by these two methods were challenged intravaginally with *T. foetus*, those with predominantly IgA or predominantly IgG1 anti-TF1.17 antibodies in genital secretions were equally protected. Later studies with similar IN immunizations showed that stimulation of the common mucosal immune system gave results similar to those of intravaginal immunization (Corbeil et al., 2001). This raised the question of inductive sites for local immune responses in the genital tract. Others have suggested that the genital tract is not an inductive site because M cells and mucosa-associated lymphoid tissue (MALT) are not present. This is true of cattle as well as mice and people. However, even though control cows did not have histologically demonstrable MALT in the uterus and vagina, cows experimentally infected with *T. foetus* did (Corbeil et al., 2001). Similar lymphoid nodules and follicles under a modified epithelium were detected in preputial and penial surfaces of bulls infected with *T. foetus* (Rhyan et al., 1999). Immunostaining of parallel sections with mAb to TF1.17 antigen indicated uptake of antigen by epithelial cells and large MΦ or DC under the basement membrane near the lymphoid follicles (Rhyan et al., 1999). Similar antigen uptake has been detected in the infected female uterine and vaginal mucosa (Corbeil et al., 2005). Thus, even though the parasite is noninvasive, released TF1.17 antigen appears to be taken up by epithelial cells. Rat uterine epithelial cells can present antigen to T helper cells (Wira and Rossol, 1995). Also, MΦ/DC positive for antigen should be antigen-presenting cells (APCs). Detection of IgG1 and IgA antibodies in genital secretions of infected bulls (Cobo et al., 2010; Rhyan et al., 1999) and cows (Corbeil et al., 2001) and the histologic demonstration of follicles and putative APCs suggests that inductive sites in the genital tract are formed in response to antigenic stimulation.

Like *C. fetus* subsp. *venerealis*, *T. foetus* has mechanisms for evasion of immune responses. These include coating of the surface with Ig nonspecifically (Corbeil et al., 1991), epitope variation (Ikeda et al., 1993), and cleavage of IgG1, IgG2, and complement component 3 by extracellular cysteine proteinase (Talbot et al., 1991; Kania et al., 2001). Studies with cysteine proteinase inhibitors demonstrated decreased cytotoxicity of *T. foetus* for bovine trophoblast cells and decreased infectivity in a mouse model, confirming the likely role of cysteine proteinases in pathogenesis (Cobo et al., 2012). Even with these mechanisms for immune evasion, as with *C. fetus*, it is clear that the dynamic interaction between host and parasite can be made to favor the host by systemic or local immunization. The usefulness of whole cell vaccines in preventing *T. foetus* infection and reproductive failure in cows has been demonstrated in clinical trials (Kvasnicka et al., 1992). First-generation whole cell vaccines are now commercially available for prevention of trichomoniasis in cows. Earlier, Clark et al. (1984) demonstrated efficacious

immunization of bulls with whole *T. foetus* cells or crude membrane glycoproteins that probably contained TF1.17 antigen. This study indicated that vaccination of bulls could both prevent infection and clear already established infection. So vaccines for bovine trichomoniasis are both immunoprophylactic and immunotherapeutic. Recent studies showed that vaccination of bulls with a commercially available whole cell killed *T. foetus* vaccine in oil adjuvant resulted in protection against challenge and high levels of IgG antibodies in serum and preputial secretions (Cobo et al., 2010). IgG1 antibodies to whole cell *T. foetus* antigens predominated but fairly high levels of IgG2 antibodies were also detected.

Summary

The above studies with *C. fetus* subsp. *venerealis* and *T. foetus* show that:

1. STDs can be prevented or even cured by systemic vaccination of both males and females.
2. At least for one STD, IgG and IgA of the same antigenic specificity are equally protective at the mucosal surface. Either systemic immunization or mucosal (IN or intravaginal) immunization with systemic boosting is protective.
3. Inductive sites are formed in the mucosa of infected male and female genital tracts even with noninvasive pathogens.
4. Strong and appropriate immune responses will clear microbial infection from the genital tract even when the microbe has multiple immune evasive mechanisms.
5. The fact that protection against two STDs has been demonstrated in the natural outbred host (cattle) has advantages over murine models of STD vaccines. In the latter, the human pathogen is usually inoculated into the unnatural murine host and the disease does not mimic the human infection. Furthermore, although inbred mice provide a homogeneous experimental animal, they do not represent the variation in immune responses seen in the outbred human population. The work on bovine vibriosis and trichomoniasis demonstrates protection under field conditions for two STDs that cause adverse pregnancy outcome in an outbred host. This is an encouraging precedent for control of human STDs and related adverse pregnancy outcomes.

Future Needs

Future needs include identification of the protective antigens for most STDs. For antibody-mediated protection of the genital mucosa, several questions have not yet been addressed. As far as we know, the role of IgE in the genital tract is largely unstudied even though it does seem to be involved in clearance of trichomonads from the bovine

genital tract. Lastly, manipulating genital immune responses to enhance Th1 or Th2 type responses is an unexplored research area.

ENTERIC VACCINES

Introduction

Enteric disease is a major cause of mortality and morbidity in animals. Agents causing diarrhea in animals include viruses (e.g., adenoviruses, pestiviruses, caliciviruses, coronaviruses, parvoviruses, rotaviruses, toroviruses), bacteria (e.g., *Campylobacter* spp., *Clostridium* spp., diarrheagenic *Escherichia coli*, *Salmonella* spp., *Yersinia* spp.), and parasites (e.g., *Coccidia* spp., *Cryptosporidium parvum*). These infections occur most commonly in suckling animals or in poultry under 3 weeks of age, but may also be common postweaning and in susceptible seronegative or stressed adult animals (Saif and Jackwood, 1990). Induction of local secretory IgA (S-IgA) antibodies (prevent attachment, invasion, and neutralize toxins or infectious agent) and mucosal cellular immune responses (against intracellular bacteria and viruses) by vaccines are essential for protection from enteric pathogens. Peyer's patches (PP) and mesenteric lymph nodes are two important organized gut-associated lymphoid tissues (GALT) in domestic animals and serve as the induction site for gut immune responses. Ileal PP in some domestic animals (sheep, cattle, pigs, dogs) differ from jejunal PP, serving as a primary lymphoid organ similar to the bursa of Fabricius in chickens, unlike in humans and rodents where PP are secondary lymphoid organs (Chu and Liu, 1984; Yasuda et al., 2006). Cryptopatches or clusters of lymphoid cells in the basal lamina propria occur in mice, but are absent in pigs (Pabst et al., 2005). Mucosal lymphoid tissues and lymphoid cells in domestic animals and humans differ in Toll-like receptor (TLR) expression and function (after binding their ligand) when compared to mice (Iwasaki and Medzhitov, 2004; Tohno et al., 2006; Guzylack-Piriou et al., 2004). These differences in immune components suggest that vaccine studies carried out in mice may fail to translate to domestic animals or humans.

Enteric pathogens have different characteristics related to their intestinal tropism and replication, requiring different vaccination strategies. Enteric viruses have predilections for replication in distinct vertical and longitudinal regions of the small or large intestines. Cytolytic infection of enterocytes leads to varying degrees of villous loss and fusion, reduced small intestinal absorptive capacity, and a malabsorptive, maldigestive diarrhea (Saif, 1999a). Secretory diarrhea is also induced by some viruses such as rotavirus (RV), which involves the viral enterotoxin, nonstructural protein 4 (NSP4), and/or stimulation of the enteric nervous system (Ball et al., 1996; Lundgren et al., 2000). Thus, viral diarrheas can be of variable severity and act via multiple mechanisms that differ from those of enteric bacteria, most of which cause secretory diarrhea mediated by enterotoxins (Fairbrother and Gyles, 2006).

Enteropathogenic viruses can be divided into three types (types I, II, and III) according to their preferred site of replication in the intestine (reviewed by Saif (1999a)). Type I (transmissible gastroenteritis virus (TGEV) and RV) and II virus infections can be prevented by inducing local intestinal immunity, whereas type III viruses (parvovirus), which infect crypt enterocytes basolaterally, can be prevented by inducing systemic immunity. The enteropathogenicity of bacteria is determined by their virulence factors including adhesion factors (fimbriae or pili) and enterotoxins; therefore, bacterial vaccines generally need to prevent attachment and toxin action within the intestine (Fairbrother and Gyles, 2006).

In the following sections, we will review vaccine strategies for these different types of enteric infections. TGEV and RV vaccines in pigs will be reviewed to illustrate findings using domestic outbred animals instead of inbred laboratory rodent models.

Vaccines to Induce Passive and Active Immunity to Type I Enteric Viruses Infecting Villous Enterocytes in Neonates

Passive Immunity

Neonates can be protected from enteric infections by providing passive lactogenic immunity, which can be achieved by immunizing mothers preparturition. Pioneering work done by Bohl and Saif in the early 1970s with live oral virulent TGEV in pigs was the foundation for the gut–mammary gland–S-IgA immunologic axis, which later became the basis for the concept of the common mucosal immune system. Their studies showed that in TGEV seronegative sows, only oral immunization with virulent TGEV induced high rates of protection in suckling neonates, which was associated with high titers of S-IgA antibodies in colostrum and milk, whereas systemic immunization induced mainly IgG antibodies (Bohl et al., 1972; Saif et al., 1972).

Active Immunity

Rotavirus is a major cause of dehydrating diarrhea in young livestock, infants, and poultry (Saif and Fernandez, 1996). Multiple RV serogroups, based on common inner capsid VP6 antigens (A–H), and multiple G (VP7, glycoprotein) and P (VP4, protease-sensitive) serotypes (based on neutralizing epitopes) or genotypes (based on sequence analysis) for group A RVs have been detected in humans, sheep, swine, cattle, horses, dogs, cats, poultry, and wildlife (Estes and Kapikian, 2007; Martella et al., 2010). Among the distinct RV serogroups and G/P serotypes/genotypes, cross-protection is limited. The antigenic divergence among different

sero/genotypes of RVs presents a challenge for the design of vaccines that are capable of inducing heterotypic protection.

Commercial modified live and killed RV vaccines for RV diarrhea in livestock and poultry are limited to group A RVs (Saif and Fernandez, 1996). Mebus et al. (1972) developed the first oral RV vaccine for calves in 1972 (1 year prior to the discovery of human RV) using a cell culture-adapted neonatal calf diarrhea RV strain. Although a significant reduction in morbidity and mortality was observed in a field trial among vaccinated calves, in the majority of herds (compared to previous years), subsequent field studies revealed variable efficacy. Experimental studies suggested that maternal antibodies interfered with live oral vaccine replication and suppressed development of active immunity (Saif and Fernandez, 1996).

The neonatal gnotobiotic pig model has been used to investigate immune responses to RV vaccines and infection for nearly three decades (Saif et al., 1996, 1997; Yuan and Saif, 2002; Gonzalez et al., 2008). Gnotobiotic pigs are free of maternal antibodies (placental transfer of Igs does not occur in swine), but they are immunocompetent at birth. They are maintained aseptically and are free of exposure to extraneous wildtype RVs, assuring that exposure to a single pathogen can be analyzed. Initial studies were conducted to mimic natural RV infection (Bohl et al., 1984) and to study immune correlates of protection (Saif et al., 1996; Gonzalez et al., 2008; Yuan et al., 2008). Understanding the sequence and kinetics of immune responses stimulated by virulent RVs allows for the determination of markers of protective immunity and pathogenicity, which can then be used to design vaccines that will stimulate protective immunity without inducing pathology. Gnotobiotic pigs orally inoculated with virulent porcine or human RVs were completely protected from homotypic but not heterotypic (distinct G/P types) RV challenge (Hoshino et al., 1988; Saif et al., 1997). Significant correlations were observed between the protection to RV-induced diarrhea and shedding and the following immune parameters: the number of intestinal IgA RV antibody-secreting cells (ASCs), serum and intestinal IgA RV antibody titers, and the frequency of intestinal RV-specific IFNγ producing CD4$^+$ and CD8$^+$ T cells (Saif et al., 1996; Yuan et al., 1996; To et al., 1998; Yuan and Saif, 2002; Yuan et al., 2008). These immune parameters were significantly higher in virulent RV inoculated pigs than in those inoculated with attenuated or inactivated RV (Ward et al., 1996b). Pigs inoculated with two or three doses of attenuated RV were moderately protected against diarrhea (62%-3 doses, 44%-2 doses) and virus shedding (67%-3 doses and 19%-2 doses) after homotypic challenge, suggesting a need for multiple doses and suitable mucosal adjuvants to enhance the efficacy of RV vaccines (Saif et al., 1996; Yuan et al., 1996, 2001; Yuan and Saif, 2002).

Gnotobiotic pigs inoculated with virulent RV developed an acute proinflammatory serum cytokine profile (IL-6, TNFα) coinciding with peak diarrhea and viremia, followed immediately by increased Th1 (IL-12, IFNγ) cytokines and convalescent Th2 (IL4) and Tr1 (IL-10) cytokine responses. Gnotobiotic pigs inoculated with one dose of attenuated RV showed lower early IFNγ and pro-inflammatory cytokine responses compared to virulent RV inoculated pigs. Both attenuated and virulent RV inoculated pigs developed Th1/Th2/Tr1 cytokine-secreting cell (CSC) responses at 2–4 weeks postinoculation; however, attenuated RV inoculated pigs developed lower intestinal IFNγ and higher intestinal and splenic IL-10 CSCs compared to virulent HRV inoculated pigs (Azevedo et al., 2006). Virulent RV inoculated pigs had significantly higher protection rates against RV challenge (87% and 100% against diarrhea and shedding, respectively) compared to one dose of attenuated RV inoculated pigs (0% and 14% against diarrhea and shedding, respectively) (Azevedo et al., 2006). These findings suggest that higher protection rates are associated with early proinflammatory and Th1 cytokine responses, which promote cytotoxic T cell activity and viral clearance, and later Th2 induced cytokines, which are important for protective S-IgA antibody responses. Thus, protection against RV in pigs requires balanced Th1/Th2/Treg responses (Azevedo et al., 2006; Gonzalez et al., 2008). Infection of gnotobiotic pigs with virulent RV causes early recruitment of innate APCs (monocytes/MΦ and DCs) and $\gamma\delta$ T cells into the ileum, and enhanced TLR2, TLR3, and TLR9 expression among APCs in spleen (Zhang et al., 2008c; Wen et al., 2009, 2011). In virulent RV-infected pigs, plasmacytoid DCs are major producers of serum IFNα and, along with other innate immune cells ($\gamma\delta$ T cells and APCs) and cytokines (TNFα, IFNγ and IL-12), are important for controlling early RV viral replication and subsequent development of protective adaptive immune responses (Gonzalez et al., 2010). Development of attenuated RV vaccines with mucosal adjuvants that mimic immune responses to virulent RV may improve existing vaccines.

Immunogenicity and protective efficacy of RV vaccine formulations (attenuated replicating virus, inactivated virus, and recombinant baculovirus-expressed virus-like particles (VLP)), administration routes, and adjuvants have also been evaluated using the gnotobiotic pig model (Bohl et al., 1984; Saif et al., 1996; Yuan and Saif, 2002; Gonzalez et al., 2008). Inactivated oral or intramuscular RV vaccines failed to protect against virulent RV challenge, despite high IgG antibody responses induced in serum and systemic lymphoid tissues. Serum IgG antibodies did not correlate with protection. However, a recent study showed that an inactivated reassortant RV strain (CDC-9 strain) formulated with aluminum phosphate and administered systemically in gnotobiotic pigs resulted in induction of serum IgG antibody titers, coinciding with partial protection against shedding and diarrhea, suggesting that adjuvant may have stimulated local specific (gut

IgA antibodies) or nonspecific immune responses, which were not assessed in this study (Wang et al., 2010). Rotavirus subunit vaccines consisting of double-layered VLP composed of RV inner capsid proteins VP2 and VP6 (2/6-VLPs) administered IN or orally with mutant heat-labile toxin of *E. coli* (mLT) or ISCOMs as adjuvants (Yuan and Saif, 2002) induced IgG ASC responses in systemic lymphoid tissues and low or no IgA ASC responses in intestinal lymphoid tissues and also failed to mediate protection, contrary to results in adult mouse studies (Yuan and Saif, 2002). The failure of IN or oral 2/6-VLP vaccines suggests that protective immunity to RV diarrhea in neonatal pigs requires mainly the presence of systemic or intestinal IgA antibodies to the outer capsid RV proteins, VP4 and VP7, each of which induces NA.

However, when 2/6-VLPs adjuvanted with mLT or ISCOM were used as IN or oral booster doses in pigs orally primed with attenuated RV, the protective efficacy increased significantly. An oral attenuated RV prime and IN 2/6-VLP-ISCOM boost regimen (AttRV/2/6-VLP) induced the highest numbers of intestinal IgA ASCs and serum and intestinal IgA antibody titers, and protection rates were similar to or higher than those induced by three oral doses of attenuated RV (Gonzalez et al., 2004, 2008; Azevedo et al., 2010). Interestingly, priming with two doses of 2/6-VLP (IN or oral) followed by live attenuated RV was ineffective for inducing IgA antibodies or protection. Thus the use of a replicating vaccine to prime at one inductive site (GALT) followed by boosting with a nonreplicating vaccine at a second mucosal inductive site (nasal-associated lymphoid tissue, NALT) is an effective strategy for stimulating protective mucosal immune responses, which can be applied to other enteric viral vaccines. Furthermore, efficacy of the prime/boost strategy (replicating/nonreplicating vaccine, AttRV/2/6-VLP) was examined in the presence of high and low titers of passive antibodies to mimic neonatal pigs receiving maternal antibodies (Nguyen et al., 2006a,b). Similar to AttRV/2/6-VLP prime/boost vaccine studies, plasmid DNA containing VP6 was used as a booster subsequent to oral attenuated RV vaccine priming. This regimen showed high protection against shedding, but poor efficacy against RV diarrhea (Yuan et al., 2005). Collectively these findings suggest that mucosal boosters are effective in enhancing IgA antibody titers to RVs in orally primed animals (Gonzalez et al., 2008).

Studies have shown that immunogenicity and efficacy of mucosal vaccines can be improved by the use of appropriate strains of probiotics that modulate mucosal and systemic immune responses, by interaction with epithelial cells and the underlying intestinal immune cells (Fukushima et al., 1998; Sanz and De Palma, 2009). Certain probiotic strains are reported to enhance immune responses to RV vaccines in children; others reduce RV diarrhea severity, but the mechanisms are not well defined (Fang et al., 2009; Holscher et al., 2012).

Supplementation with *Lactobacillus acidophilus* in attenuated RV vaccinated pigs is reported to enhance intestinal IFNγ-producing CD8^{+} T cells, intestinal IgA and IgG RV ASCs, and serum IgA and IgG RV antibody titers (Zhang et al., 2008b). These findings suggest that probiotics are an alternative, cheap, and safe adjuvant for enhancing efficacy of oral attenuated RV vaccines in animals and potentially in humans. Colonization of pigs by two different strains of lactic acid bacteria (LAB, *L. acidophilus and Lactobacillus reuteri*) and subsequent virulent RV infection resulted in higher and balanced Th1/Th2/Treg cytokine responses (IL-12, IFNγ, IL-4, IL-10, TGFβ), higher total intestinal IgA-secreting cells, total serum IgM, and intestinal IgM and IgG titers, although the numbers of IgA RV ASCs and serum and intestinal RV antibody titers did not differ compared to virulent RV-only infected pigs. No overall difference in protection rates was noted when compared to virulent RV-only inoculated pigs, which was likely because of the short interval (only 3 days) between LAB colonization and virulent RV challenge (Zhang et al., 2008a; Azevedo et al., 2012). Dual colonization of the aforementioned LAB strains also modulated innate immune components in virulent RV inoculated pigs as follows: (1) enhanced frequency of γδ T cells in the intestine and their distribution; (2) enhanced TLR2- and TLR9-expressing CD14^{+}APC, and TLR2-expressing CD14^{−} APCs in the blood, but reduced TLR3- and TLR9-expressing CD14^{−} APCs in the spleen, and (3) reduced frequency of MΦ and cDCs in the spleen. Collectively, these findings suggest that probiotics may reduce systemic inflammatory responses induced by virulent RV. Effects on TLR expression on APCs in the ileum were not determined (Zhang et al., 2008c; Wen et al., 2009, 2011). Probiotics may not only modulate immune responses to RVs or other enteric vaccines, but may also directly ameliorate diarrhea/infection by enhancing gut barrier integrity and maintaining intestinal permeability, by stimulating nonspecific immune responses, by changing gut microbial populations, and by aiding in the regulation and prevention of apoptosis (Madsen et al., 2001; Yan and Polk, 2002; Preidis et al., 2012).

Using the TGEV model for evaluation of active protection against diarrhea in pigs, researchers also delineated compartmentalization in the common mucosal immune system and its impact on mucosal vaccine strategies and protection (VanCott et al., 1993, 1994). The natural occurrence of a deletion mutant of TGEV with exclusive respiratory tropism, referred to as porcine respiratory coronavirus (PRCV), provided a unique opportunity to study ASC responses and protective immunity to two antigenically related porcine coronaviruses with enteric (TGEV) versus respiratory (PRCV) tropism. Oral immunization of pigs with TGEV induced high numbers of IgA ASC in the intestine and provided complete protection against TGEV challenge, whereas IN immunization of pigs with PRCV induced mainly systemic responses (IgG ASC) and

provided only partial protection against TGEV challenge. Thus, the IN PRCV alone failed to elicit sufficient intestinal IgA ASC to provide full protection against the enteric pathogen, TGEV. Findings from this study and RV vaccine studies suggest that the use of multiple mucosal inductive sites in a prime/boost vaccination regimen may be an effective approach for overcoming compartmentalization in the common mucosal immune system.

Vaccines to Induce Active Immunity in Neonates to Type III Enteric Viruses Infecting Crypt Enterocytes

Canine parvovirus (CPV) infects crypt enterocytes causing hemorrhagic gastroenteritis in pups (Saif and Jackwood, 1990). Because CPV is likely disseminated to the basolateral surface of crypts by the hematogenous route, serum NA (derived maternally or actively produced) is protective against the disease. Pollock and Carmichael (1982) demonstrated that pups with hemagglutination inhibition (HI) serum antibody titers of >1:80 were immune to oronasal CPV type 2 challenge. CPV is highly stable in the environment and pups were susceptible to infection as soon as maternal antibodies declined to HI titers of 1:64–1:80. A maternal HI antibody titer as low as 1:20 severely affected the efficacy of a live low titer CPV vaccine (100–115th passage in culture) in generating active immune responses (Carmichael et al., 1983), which resulted in a window of susceptibility for pups. Others have shown that an HI titer of less than 1:20 in CPV-2 (low passage, high titer) vaccinated pups did not severely affect active antibody responses (Burtonboy et al., 1991; Hoare et al., 1997), suggesting that increasing the dose or reducing the attenuation of the virus may help to overcome inhibitory effects of maternal antibodies. Studies with modified live variant CPV-2b strain (low titer) have shown that these vaccines, when given either parenterally (40th passage) or IN (68th tissue culture passage), elicited almost 100% protection in pups with maternal antibody titers of 1:10 to 1:80 and even 60% protection in pups with antibody titers of 1:160. Higher efficacy of these vaccines can be attributed to either strong inherent immunogenicity of these new vaccines or antigenic differences among CPV-2 and CPV-2a and CPV-2b (Pratelli et al., 2000). Overall, during the past four decades of CPV vaccine development, modified live viruses have proven superior to inactivated intramuscular (IM) vaccines (Appel, 1999). In brief, various strategies such as the use of less attenuated virus (low serial passage), high titer vaccines, multiple doses, or the use of the IN route of immunization have been attempted to overcome inhibitory influences of maternal antibodies and to reduce the window of susceptibility in pups.

Recently new variant CPV-2c has emerged, which is highly pathogenic and causes more severe diarrhea in dogs.

Currently used vaccines (CPV-2 or CPV-2b strains) are effective in protecting dogs against challenge with CPV-2c virus under experimental conditions (Spibey et al., 2008), although efficacy in the field is unknown (reviewed by Decaro and Buonavoglia (2012)). Antigenic differences between original CPV-2 and its variants may reduce the efficacy of current CPV vaccines, which is supported by in vitro virus-neutralization tests conducted on vaccinated animals that showed low cross-reactivity between heterologous CPV variants (Cavalli et al., 2008). However, this may not represent actual cross-protection in clinical cases. There is a need not only to make current vaccines effective in the presence of maternal antibodies, but also to update them based on continuous epidemiological surveillance studies.

DNA plasmids expressing VP1 (Jiang et al., 1998), replicon-based CPV DNA vaccine expressing VP2 (Dahiya et al., 2011), B cell epitope (2L21 peptide of VP2) fused to CT B subunits expressed in transgenic tobacco chloroplasts (Molina et al., 2005), chimeric virus particles expressing CPV peptide (different prime/boost strategies) (Nicholas et al., 2003), and recombinant VLPs formed by baculovirus-expressed VP2 (Casal, 1999) have been evaluated in dogs or rodents without maternal antibodies and have demonstrated good immunogenicity and/or protective efficacy. Further efficacy tests in pups with maternal antibodies are needed to assess their commercial potential.

Vaccines to Induce Immunity against Enteric Bacterial Infections in Neonates

Oral vaccines for the induction of active immunity against bacterial diarrhea are not commonly used in livestock, although *E. coli* diarrhea is an important problem in neonatal and postweaning calves and pigs. F4 (K88) and F18 are the major fimbrial adhesins present on swine enterotoxigenic *E. coli* (ETEC) and are major targets for *E. coli* vaccines (Fairbrother et al., 2005). Whole bacteria vaccines are routinely administered parenterally to pregnant cattle, sheep, and swine to protect their suckling neonates against ETEC infections (Moon and Bunn, 1993). Such vaccines are practical and effective because: (1) fimbriae are required for the adhesion-colonization of bacteria early in the pathogenesis of the disease; (2) most fatal ETEC infections in farm animals occur in the neonatal period; (3) more than 90% of the ETEC in farm animals belong to a small family of fimbrial antigen types, (4) and mothers are seropositive to ETEC so booster responses are elicited. Recent vaccine studies have focused on administration of purified bacterial subunits (transgenically expressed adhesin of F4 fimbriae in plants) and mucosal adjuvants (Verdonck et al., 2005; Joensuu et al., 2006) and have shown promising results. Overall, the vaccine strategy used, parenteral vaccination of field-exposed seropositive mothers, to induce lactogenic immunity is the same as that shown to be effective for parenteral application of RV

vaccines in RV seropositive mothers (Bohl et al., 1984; Saif and Jackwood, 1990; Saif and Fernandez, 1996).

Summary

Studies of live oral enteric vaccines in animals have clarified the mechanisms of induction of protective immunity against enteric disease and contributed to our understanding of the common mucosal immune system. However, commercial live oral vaccines often have shown inadequate or inconsistent efficacy under field conditions (Saif and Jackwood, 1990; Saif and Fernandez, 1996). Major obstacles to improved efficacy of oral vaccines include: (1) maternal antibodies in the intestine of neonates (mainly colostrum and milk antibodies), which interfere with live vaccine replication; (2) qualitative and quantitative limitations in the neonatal immune system, although neonates are immunocompetent; (3) the inability of attenuated vaccine strains to adequately infect (too high attenuation) or stimulate S-IgA antibodies in the intestine; (4) the use of inappropriate (or unstable) antigens or route for subunit vaccines; (5) the lack of oral delivery vehicles or mucosal adjuvants for subunit vaccines; and (6) infection by pathogens prior to vaccination.

Differences in Veterinary Species and Mouse Models

Studies of neonatal pigs indicate that protection rates against RV diarrhea upon challenge correlate with the magnitude of IgA ASC and memory B cell responses in intestinal lymphoid tissues (Saif et al., 1996; Gonzalez et al., 2008; Yuan and Saif, 2002). Studies conducted in immunodeficient or specific gene knockout adult mice have shown that neither CD4$^+$ or CD8$^+$ T cells nor antibodies were essential for induction of protective immunity to RV infection, but usually one of these effectors (T or B cells) was necessary for elimination of primary RV infection (McNeal et al., 1995, 2002). In pigs, recent studies have shown that CD4$^+$ and CD8$^+$ IFNγ producing T cells play a role in protection against RV diarrhea and infection (Yuan et al., 2008), but it is difficult to create genetically modified pigs similar to knockout mice, to assess the contribution of B cells and T cells. The redundant nature of immune responses to RV in mice, the multiple immunologic and possibly nonimmunologic pathways to resolve RV infections (Franco and Greenberg, 2000; Ward, 2003), the age factor and host differences in RV pathogenesis in mice and pigs (Saif et al., 1996, 1997; Ward, 2008), and the use of inbred mouse strains contribute to the discrepancies seen between the adult mouse and the neonatal pig models.

Conclusion and Future Directions

The majority of pathogens enter and initiate infection at mucosal surfaces, making mucosal sites relevant targets for vaccines to prevent infection. To develop effective mucosal vaccines, it is important to determine correlates of protection against enteric pathogens. Generally for localized gut infections, S-IgA antibodies and intestinal T cells play an important role. Improved vaccines that induce high levels of intestinal IgA antibodies against the appropriate microbial antigens can be achieved by choosing the proper vaccine formulation and delivery method. Vaccines should also induce: (1) heterotypic protection; (2) active immunity in the presence of maternal antibodies; and, (3) long-lasting immunological memory. Novel vaccines (e.g., VLPs, transgenic plants), adjuvants (e.g., mLT, ISCOMs, TLR ligands (e.g., CpG), mycobacterial extracts, 1α 25-dihydroxyvitamin D3, retinoic acid, probiotics, and cytokines), and vaccine delivery systems (e.g., recombinant plant or animal viruses or bacterial vectors, genetically engineered probiotics, ISCOMs, liposomes, and nanoparticles) should be explored and evaluated in relevant animal models to further enhance the efficacy of current or new vaccines. Recent studies have shown that the targeting of antigens directly to APCs (via surface receptors) is an effective way to tailor immune responses to optimize protection and should be explored further in large animals (Trumpfheller et al., 2012).

PASSIVE IMMUNITY

Introduction

The passive transfer of maternal immunity provides essential protection in newborns. Although most neonatal immune systems are competent to mount primary immune responses against pathogens, primary responses often do not develop quickly enough to prevent disease. Maternal immunologic transfer provides a critical (though temporary) aid to survival for neonates.

The enhancement of passive immunity through vaccination of the mother has been a successful disease prevention strategy in domesticated animals. Vaccinated mothers develop higher levels of specific antibodies in colostrum and milk and increased levels of immunity in their offspring (Glezen, 2001; Saif and Fernandez, 1996). Passive immunity can also be enhanced by oral administration of immune milk or heterologous antibody preparations (e.g., chicken egg yolk IgY (Ikemori et al., 1992; Kuroki et al., 1994) or monoclonal antibodies) or by parenteral administration of hyperimmune plasma (Becu et al., 1997). Recent studies using monoclonal, single-chain antibodies (variable heavy domain (VHH) nanoantibodies) of llama origin open new possibilities for providing passive immunity to humans and animals (Garaicoechea et al., 2008). Immunoglobulins of the IgG2 and IgG3 isotypes of camelids consist of heavy chains without associated light chains (Hamers-Casterman et al., 1993). The distinctive biochemical characteristics and

binding qualities of VHH antibodies overcome some key limitations of conventional antibodies (see below).

Unfortunately, passive antibodies often interfere with active immunization of young animals and birds. Various vaccination strategies have been developed to minimize the suppressive effects of maternal antibodies, but improved adjuvants and antigen-delivery systems are needed to facilitate efficient induction of active immunity in the presence of maternal antibodies. This section will address past, current, and future approaches for enhancing passive immunity in veterinary species.

Transfer of Maternal Immunity

The transfer of systemic passive immunity from mother to offspring can occur prenatally, via the placenta or yolk sac, or postnasally via ingestion of colostrum and milk, depending on the species. The main Ig isotype transferred in most mammalian species is IgG. In avian species IgY, the functional equivalent of mammalian IgG, is transferred to the yolk to passively protect the developing chick (Kovacs-Nolan and Mine, 2012). In primates and rabbits, maternal IgG is transferred across the placenta to the fetus. In rodents, transplacental transfer occurs in combination with prolonged (16–21 days) postnatal transfer by means of colostrum and milk (Husband, 1998). In dogs and cats transfer of IgG occurs by a combination of prenatal and postnatal mechanisms, with 5% to 10% of total transfer occurring before birth (Tizard, 2013). In ruminants, horses, and pigs, offspring are born virtually agammaglobulinemic and transmission of Ig occurs only via colostrum for a limited time after birth (Tizard, 2013). After the transition from production of colostrum to milk, Ig are no longer absorbed from the intestines and only act locally in the gastrointestinal tract.

Immunoglobulin absorption in neonates of large domestic species is facilitated by the presence of protease inhibitors in colostrum and its efficiency declines rapidly after birth, with maximal absorption occurring in the first 4 h. The cessation of absorption of intact macromolecules is termed "gut closure," and occurs at different ages in different species. In calves and pigs closure normally occurs by 24–36 h after birth.

Failure of Passive Transfer in Domestic Large Animals

Absorption of colostrum Ig can be highly effective, supplying the newborn with serum Ig at concentrations similar to those of the dam. Failure of passive transfer (FPT) is a common problem, however, in newborn calves and foals (Besser and Gay, 1994; Tyler-McGowan et al., 1997). FPT may occur because of the production of low quantities of colostrum, because of production of colostrum with low concentrations of maternal antibodies, because of ingestion of low quantities of colostrum, or because of inefficient absorption (Quigley and Drewry, 1998). Colostrum supplements, colostrum replacers, and plasma products have been developed commercially to address this problem, with variable success. Vaccination of the dam in late pregnancy can enhance antibody titers in colostrum and after suckling in the serum of the offspring (Saif and Fernandez, 1996). The benefits of vaccination of the dam for enhancing passive immunity can be lost if colostrum is of low quality or if absorption of colostral Ig is inefficient.

Mechanisms of Clearance of Passively Acquired IgG

The half-life of Ig varies considerably among species of domestic animals and with the Ig isotype. Neonatal receptor for Fc of IgG (FcRn) is involved in homeostasis of serum levels of IgG in general, but distinct mechanisms may function in neonates. The main route of clearance of passively acquired maternal IgG1 in calves is transfer from the serum to the intestine (Besser et al., 1987). Approximately 70% of passively acquired IgG1 is eliminated by this route. If titers of passive circulating antibodies are high enough, the transfer of antibodies from the circulation to the intestinal lumen can mediate short-term protection against rotavirus diarrhea (Besser et al., 1988). The same mechanism may be functional in piglets (Saif and Wesley, 1999; Parreño et al., 1999; Ward et al., 1996a).

The persistence of titers of circulating maternal antibodies is generally considered in designing vaccination strategies for young animals because of suppressive effects of maternal antibodies on active immune responses. Induction of immune memory can occur in the absence of a detectable serum antibody response (Boersema et al., 1998; Parreño et al., 2004). The presence of passive antibodies in the intestines can interfere with mucosal immune responses to natural infection as well as to vaccination (Hodgins et al., 1999; Parreño et al., 2004; Nguyen et al., 2006a,b).

Passive Immunity in the Respiratory Tract

Experiments in colostrum-deprived lambs (Jones et al., 1989) and calves (Mosier et al., 1995) have demonstrated the ability of parenterally administered immune antisera to mediate protection following experimental challenge with *Mannheimia haemolytica*. Parenteral administration of hyperimmune plasma raised against *Rhodococcus equi* has been shown to protect against pneumonia in young foals in experimental (Hooper-McGrevy et al., 2001) and field studies (Becu et al., 1997). Hyperimmune plasma is available commercially for use in foals.

Prepartum vaccination of beef (Van Donkersgoed et al., 1995) and dairy (Hodgins and Shewen, 1996) cows has been demonstrated to increase antibody titers to *M. haemolytica* in their colostrum and in the serum of their calves.

Vaccination of broiler breeder chickens can be used to provide passive protection against respiratory/septicemic disease caused by avian pathogenic *E. coli* (Kariyawasam et al., 2004).

Passive Immunity in the Gastrointestinal Tract

Rodents have been a popular model for the study of passive protection by milk antibodies. However, rats and mice actively transport IgG from the gut into the circulation during the first 2–3 weeks of life. Thus, antibodies in ingested milk contribute to both local and systemic immunity in rodents, in contrast to the strictly local effects occurring in humans and most domestic animals.

In pigs, horses, dogs, and cats, IgG is the most abundant Ig in colostrum but IgA predominates in milk. Parenteral vaccination, by enhancing serum IgG antibody titers, contributes to IgG antibodies in colostrum but has limited effects on IgA antibodies in milk. Milk antibodies provide passive protection to the neonatal intestinal tract by immune exclusion preventing the attachment of viruses, bacteria, and parasites and by neutralizing viruses or enterotoxins. S-IgA antibodies, because of their resistance to cleavage by digestive enzymes, and higher levels in milk are more efficient in mediating protection in the gut of pigs and other monogastrics (Saif and Jackwood, 1990), but high persisting levels of passive IgG antibodies are also protective. In ruminants IgG1 antibodies, relatively resistant to proteolytic enzymes (Brock et al., 1977) and predominant in milk, have functions similar to those of S-IgA.

Numerous vaccines are marketed for vaccination of cows and sows to provide lactogenic immunity to rotavirus, coronavirus, and *E. coli* in suckling offspring. Vaccine efficacy has been variable. Ideally, suckling animals become subclinically infected with enteric pathogens while receiving adequate passive antibodies to prevent disease, and develop active immunity to prevent subsequent diarrhea. This balance between passive immunity and disease can be disrupted in intensive animal production systems by exposing animals to pathogens in confined, contaminated environments. Earlier weaning practices reduce intake of milk antibodies. Maternal enteric vaccines are commonly used in two populations of pregnant animals. To control epidemic infections, they are used in naïve, seronegative animals to induce primary immune responses. To control endemic infections (such as rotavirus and *E. coli*), booster vaccines are used in seropositive, field-exposed animals to stimulate anamnestic memory responses.

Virulent TGEV given to pregnant sows stimulates high levels of IgA antibodies in milk and passive protection (Saif and Jackwood, 1990). Oral attenuated TGEV vaccines, which replicate poorly in sows, induce lower IgA milk antibody titers and low or variable efficacy in the field (Moxley and Olson, 1989). Parenteral killed TGEV vaccines induce

only low IgG milk antibody titers and have the lowest protection rates. Attempts to develop maternal TGEV recombinant subunit vaccines based on the surface TGEV spike (S) protein that induces NA, or live vector vaccines expressing the S protein, have also been of limited success in TGEV seronegative swine (Saif and Wesley, 1999). However, prime/boost strategies such as IM administration of TGEV S protein following oral/IN priming with attenuated TGEV have shown promise as a means of enhancing IgA milk antibody titers (Park et al., 1998).

Booster vaccination strategies are required to enhance lactogenic immunity to endemic enteric pathogens, such as rotavirus and *E. coli,* because antibody titers in milk decline dramatically during lactation. Breast milk IgA antibodies are increased in women endemically exposed to cholera following parenteral boosting with a cholera vaccine (Svennerholm et al., 1977). In pigs infected with rotavirus, IgA memory B cells initially reside in the ileal PPs but are subsequently present in substantial numbers in spleen (Yuan et al., 2001). Systemic stimulation of such IgA memory B cells by parenteral booster vaccines can yield dimeric IgA antibodies in serum for transport to mucosal secretions via the polymeric Ig receptor.

Under field conditions, antibodies to endemic intestinal pathogens are also common in bovine colostrum and milk, but without the boosting effect of highly immunogenic vaccines, antibody titers are often too low to protect calves (Besser and Gay, 1994; Saif and Fernandez, 1996). Thus vaccines are marketed for prepartum vaccination of cows against rotavirus, coronavirus, and *E. coli* to enhance passive immunity in their calves, but the field efficacy of these vaccines has been questioned (Waltner-Toews et al., 1985). Vaccination of pregnant dairy cows with modified live or binary ethyleneamine inactivated rotavirus or recombinant 2/4/6/7 VLPs has been shown to increase IgG1 and virus NA titers to rotavirus in colostrum and milk and mediate passive protection in calves against oral rotavirus challenge (Saif and Fernandez, 1996; Kim et al., 2002).

Prepartum vaccination of cows and sows with bacterins prepared from enteropathogenic (EPEC) *E. coli* for prevention of diarrhea in their offspring is also commonly practiced. Under modern farming practices, dairy and veal calves rarely are fed whole milk from their dams for more than 1 or 2 days. Thus vaccine efficacy is based on antibodies absorbed from colostrum or retained temporarily in the gut, rather than on a continuing supply of immune milk. Piglets, in contrast, continue to receive immune milk until weaning at 2–3 weeks of age. The importance of a continuous supply of passive antibodies for protection against TGEV has been demonstrated (Saif and Wesley, 1999).

Numerous commercial Ig preparations with antibody activity against specific enteric pathogens have been marketed. Products intended for prevention of *E. coli* enteritis

in calves include dried bovine colostrum and whey, hyper-immune sera raised in horses, and mouse monoclonal anti-bodies to the K99 (F5) antigen of *E. coli*. These products are administered orally in the first 12 h of life to prevent adhesion of EPEC *E. coli*. Orally administered bovine colostral whey containing rotavirus antibodies also passively protects piglets against rotavirus (Schaller et al., 1992).

Oral Administration of Chicken Egg Yolk Antibodies

Immunization of chickens shows promise as an efficient method for producing polyclonal antibodies for passive protection. Specific antibodies of the IgY isotype are induced by vaccination and are concentrated in egg yolk. Laying hens can produce about 20 g of IgY per year. Yolk antibodies with virus NA provide calves with partial protection against diarrhea caused by rotavirus (Kuroki et al., 1994; Vega et al., 2011), and ETEC *E. coli* (Ikemori et al., 1992). In a recent study, supplementation of the milk ration of neonatal calves with egg yolk containing IgY antibodies to rotavirus for 14 days provided 80% protection against rotavirus diarrhea after challenge, and also enhanced mucosal ASC numbers in the duodenum (Vega et al., 2011). Egg yolk lacking rotavirus specific IgY did not provide clinical protection, but did enhance ASC responses, suggesting the presence of immune modulators in egg yolk.

Protective effects of yolk antibodies are dependent on antibody titers in oral preparations (Marquardt, 2000). Development of better means to protect yolk antibodies from digestive processes will improve both the efficacy and the economic viability of yolk antibodies for clinical applications (Kovacs-Nolan and Mine, 2012).

Induction of Active Immunity in the Presence of Maternal Antibodies

For many diseases of newborns and neonates, passive immunity is the only practical means of providing timely protection. Unfortunately, it is well documented that maternal antibodies can suppress active immune responses following vaccination. This effect has been observed with both live and nonreplicating vaccines, and for both systemic and mucosal responses (Siegrist et al., 1998; Hodgins et al., 1999; Parreño et al., 1999; Nguyen et al., 2006a,b). Antibody responses especially are affected; T-lymphocyte responses may not be suppressed (Siegrist et al., 1998). Titers of maternal antibodies are maximal for most species of interest in the first week of life and then decline gradually over the next few months, but variability of titers among individuals is high. With many vaccines, a "window of disease susceptibility" of variable duration occurs when titers of maternal antibodies are too low to mediate

protection, but are too high to permit effective vaccination. A number of strategies are used to cope with this problem.

Some veterinary vaccines for cattle are sold with the disclaimer that "animals vaccinated before 6 months of age should receive a booster dose of vaccine at 6 months of age." This provides little solace for the many diseases of cattle occurring in the first weeks or months of life. A common strategy for vaccines of dogs and cats is to administer a series of doses of vaccine from an early age (at which time only a few individuals will be responsive) and to continue vaccinating until an age at which virtually all can respond to vaccination. This strategy has economic disadvantages for the pet owner. Some manufacturers produce low passage, high virus titer vaccines especially for use in situations where high titers of maternal antibodies and high pathogen exposure are anticipated. This is similar to a strategy once (but no longer) approved by the World Health Organization for vaccination of children in developing countries against measles (Gellin and Katz, 1994). Preliminary evidence suggests that incorporation of vaccine antigens in highly structured ISCOMs or IN application of vaccines can enhance immune responses in the presence of maternal antibodies (Van Binnendijk et al., 1997; Brockmeier et al., 1997).

Variable Heavy Chain Fragment Recombinant Nanoantibodies

Immunoglobulin G in most mammalian species is composed of two heavy chains, covalently linked by disulfide bonds, and two light chains. "Conventional" heavy chains consist of one variable domain and three constant domains (CH1, CH2, and CH3); light chains are composed of a variable and constant domain. In contrast camelid species produce "heavy chain immunoglobulins" that lack light chains and the first constant heavy domain (Hamers-Casterman et al., 1993). The antigen-binding site of these unusual heavy chain antibodies is formed by a single domain, designated VHH in camelids. VHH are easily produced as recombinant proteins, designated single domain nanoantibodies or nanobodies® and represent the smallest molecule in nature capable of binding a specific antigen. The CDR3 region of these nanobodies has the capacity to form long loops that can extend into cavities on antigens, e.g., the active site crevice of enzymes. Other advantageous features of nanobodies include their high solubility, thermal stability (resist pasteurization), refolding capacity, and optimal tissue penetration in vivo (reviewed by Vanlandschoot et al. (2011)). Nanobodies have demonstrated efficacy as agents of passive immunity for infectious diseases. VHH specific for rotavirus inner capsid protein VP6 are able to broadly neutralize rotaviruses independently of serotype, and in mouse experiments provide passive protection against challenge with human rotavirus (Pant et al., 2006; Garaicoechea

et al., 2008). Nanobodies against other viral diseases with veterinary impact have been developed (foot-and-mouth disease, influenza, rabies (Vanlandschoot et al., 2011)) and represent a promising next-generation biologic platform for passive immunity.

Future Needs

Maternal vaccination to enhance passive immunity is already widely used in veterinary medicine. Some of these vaccines, especially vaccines against enteric viruses, have limited efficacy. New approaches to enhance immunogenicity are promising, but await commercial development. A clearer understanding of protective mechanisms and immune modulation mediated by passive antibodies would contribute to more effective interventions. There is an urgent need for adjuvants and delivery systems capable of reliably inducing active immunity in neonates despite the presence of maternal antibodies. The ability to provide continuity of immune protection from birth, by combining passive immunity with active immunization, would have a major impact on neonatal morbidity and mortality.

VACCINATION OF FARMED FISH

The rapid expansion of commercial fish farming (aquaculture) in many countries in recent decades has been accompanied by an urgent need to develop vaccines to prevent (infectious) fish diseases that were previously unknown or obscure. Added to the difficulties involved in identifying the pathogens responsible, there has been the challenge of delivering vaccine efficiently with minimal stress to very large numbers of fish. Some commercial vaccines for fish are administered by intraperitoneal or IM injection, but mucosal vaccines are also widely used (reviewed by Brudeseth et al. (2013)). Fish are routinely vaccinated by immersion in tanks of diluted bacterin, modified live bacteria or viruses, with exposure times ranging from 30 s up to 60 min, depending on the vaccine and age of the fish. It is unclear whether the main route of antigen uptake is oral or via the mucosal surface of the gills. Several commercial vaccines are now available that consist of recombinant viral proteins for mixing into the feed (Evensen and Leong, 2013); 10 days of feeding is recommended by the manufacturer.

CONCLUSIONS

Research on mucosal veterinary vaccines has contributed new concepts to the field of mucosal immunity. Investigations of pathogen–host interactions in outbred animals have illustrated the complexity of these interactions. Early studies of an enteric coronavirus infection of swine (TGEV) led to the concept of the gut–mammary gland S–IgA immunologic axis and provided part of the basic tenet for a common mucosal immune system. Later studies of TGEV and a deletion mutant of TGEV with respiratory tropism (PRCV) revealed functional compartmentalization within the common mucosal immune system whereby IN inoculation of pigs with PRCV failed to elicit sufficient intestinal IgA antibody to fully protect against the enteric pathogen TGEV (VanCott et al., 1993, 1994). Subsequent studies have explored new prime/boost mucosal immunization strategies to elicit intestinal immunity to rotavirus in naïve pigs (Saif, 1999b; Yuan and Saif, 2002; Gonzalez et al., 2008). In these studies only oral priming with attenuated virus led to successful IN booster responses using nonreplicating (VLP) vaccines combined with mucosal adjuvants such as ISCOM or mLT. Thus use of a replicating vaccine to prime lymphocytes at a major mucosal inductive site (GALT) followed by boosting with a nonreplicating vaccine at a second inductive site (NALT) effectively stimulated intestinal IgA antibodies and induced active protection against rotavirus diarrhea. Although there is progress in developing safe and effective nonreplicating vaccines to boost mucosal immune responses (Saif and Fernandez, 1996), the dilemma remains to develop effective vaccines to prime for mucosal immunity. Mucosal adjuvants (mLT, ISCOM, CpG, cytokines) and new delivery systems (replicating vectors, microparticles) have shown promise in animal studies reviewed in this chapter. However, their economical production and final evaluation under field conditions, including in the presence of maternal antibodies as relevant, are needed.

Considerable research effort has been devoted to development of vaccines for respiratory diseases of domestic animals. In some instances attenuated organisms delivered by mucosal routes have demonstrated improved efficacy over nonreplicating antigens given by systemic routes. For many respiratory diseases, however, further progress in the development of mucosal vaccines will have to await advances in understanding of disease pathogenesis and identification of protective antigens. In contrast, studies of ascending infections of the reproductive tract in cattle have demonstrated the efficacy of systemic vaccination to clear established infections, and highlight the possibility of therapeutic vaccines.

Finally it is important to realize that there are considerable species differences in mucosal immunity. For example, the primary Ig in mammary secretions of ruminants is IgG1 which is actively transported to the mammary gland from serum and provides effective passive immunity to the nursing offspring against enteric pathogens. Thus parenteral immunization of the dam stimulates passive immunity in ruminants against enteric pathogens. In contrast, in monogastrics, IgA predominates in milk and IgA lymphoblasts that traffic to the mammary gland originate in the intestine. Therefore oral vaccines in monogastrics may provide

a more effective vaccine strategy to induce IgA antibodies in milk (Saif and Fernandez, 1996). By applying the aforementioned vaccine concepts with new and effective mucosal adjuvants, delivery systems, and bioengineered vectors expressing appropriate microbial antigens, it is likely that a new generation of veterinary vaccines will emerge to better cope with existing and emerging mucosal pathogens.

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